

AN EVALUATION OF SELECTED  
HARDINESS TESTS IN RELATION TO  
PEACH BREEDING AND MINIMIZATION  
OF NON-GENETIC WOOD HARDINESS  
VARIATION THROUGH SAMPLING

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## ABSTRACT

### AN EVALUATION OF SELECTED HARDINESS TESTS IN RELATION TO PEACH BREEDING AND MINIMIZATION OF NON-GENETIC WOOD HARDINESS VARIATION THROUGH SAMPLING

By

David Wayne Cain

This research deals with winter injury to woody tissues of peach trees. When developing a program to improve wood hardiness, it is necessary to develop satisfactory sampling techniques and viability evaluation methods. A single peach genotype (Prunus persica Batch. 'Redhaven') was used to study non-genetic hardiness variation and evaluate tissue browning, regrowth, and electrolytic conductance. The three evaluation methods were studied to determine their suitability as evaluation methods for handling large volumes of plant materials encountered in a breeding program for improving wood hardiness.

Electrolytic conductance was unsatisfactory for evaluating large amounts of material because experimental techniques could not be satisfactorily controlled when handling such large quantities. Tissue browning was more

closely correlated with regrowth than with electrolytic conductance. Both tissue browning and regrowth tests appear well suited to rapid evaluation of large volumes of plant material.

Each of several randomly chosen trees were divided into two parts; the upper southwest (sector 1) and the lower northeast (sector 2). Twigs were removed from each sector. They were frozen and divided into three sections; the basal, middle, and tip sections. Tissue browning was used to determine hardness.

An appropriate statistical model was devised which would separate the variance components of interest. Statistical analysis revealed significant hardness differences among twig sections, tree sectors, and between trees. Examination of variance components indicated that in most experiments trees and tree x sector interaction constitutes only a small portion of the total random variation. Twigs and residual error accounted for 57 percent to 95 percent of the total random variation. The browning rating system had a repeatability of .79. An appendix illustrating the browning rating is included.

Variance estimates were used to estimate sample sizes needed to detect hardness differences of a desired magnitude. It is suggested that sampling uniformly from one location within all trees and within one part of all twigs would eliminate a considerable amount of non-genetic variation. The upper southwest sector contains more twigs



David Wayne Cain

and is less variable than the lower northeast sector. The base twig sections were more differentiated and were easier to rate visually than were the middle or tip sections.

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## TABLE OF CONTENTS

	Page
LIST OF TABLES . . . . .	v
LIST OF FIGURES. . . . .	vii
INTRODUCTION. . . . .	1
LITERATURE REVIEW . . . . .	5
Hardiness Variability in Relation to Plant Breeding. . . . .	10
SECTION I: AN EVALUATION OF THE SUITABILITY OF TISSUE BROWNING, REGROWTH, AND ELECTROLYTIC CONDUCTANCE WOOD HARDINESS TESTS FOR PEACH BREEDING . . . . .	13
Introduction . . . . .	14
Materials and Methods . . . . .	15
Freezing Techniques . . . . .	16
Evaluation Methods . . . . .	17
Electrolytic Conductance . . . . .	17
Tissue Browning . . . . .	17
Regrowth. . . . .	18
Individual Experiments. . . . .	19
November 24, 1973 (Expt. 1) and December 15, 1973 (Expt. 2). . . . .	19
February 7, 1974 (Expt. 3). . . . .	19
March 20, 1974 (Expts. 4 and 5) . . . . .	20
Results and Discussion. . . . .	20



	Page
SECTION II: MINIMIZING NON-GENETIC WOOD HARDINESS VARIATION IN REDHAVEN PEACH ( <u>PRUNUS PERSICA</u> BATCH.) THROUGH SAMPLING . . . . .	32
Introduction . . . . .	33
Materials and Methods . . . . .	35
Results. . . . .	37
Discussion. . . . .	40
SUMMARY AND CONCLUSIONS . . . . .	48
APPENDIX . . . . .	52
BIBLIOGRAPHY. . . . .	62

## LIST OF TABLES

Table		Page
SECTION I		
1.	Overall experiment means for each evaluation method. . . . .	28
2.	Simple correlations between evaluation methods . . . . .	28
3.	Twig section means within each sector of the tree for experiments 1 and 2 . . . . .	29
4.	Twig section means within each sector of the tree for experiment 3 . . . . .	30
5.	Regrowth and conductance means at each browning level in experiment 3. . . . .	31
6.	Random variation in experiment 3 expressed as a percentage of the grand mean. . . . .	31
SECTION II		
1.	Analysis of variance table with the expected mean squares used to calculate F values and variance components . . . . .	44
2.	Analysis of variance table showing degrees of freedom, mean squares, and significance values of the F statistic for effects of interest in all experiments. . . . .	45
3.	Estimates of variance components in all experiments . . . . .	46

4. An estimate of the number of twigs ( $n$ )  
needed to detect a desired difference ( $\delta$ )  
where  $\alpha = .05$ ,  $p = .8$ , and  $t$  has infinite  
degrees of freedom. . . . . 47

## LIST OF FIGURES

Figure	Page
1. Regression of browning on wood weight for sector 1 twig section 1 and sector 2 twig section 3. . . . .	53
2. Photographs illustrating the 5 unit browning scale. A through E represent a rating of 1 through 5 respectively . . .	55
3. Illustration of a tree divided into sectors . . . . .	59
4. Illustration of a twig showing its division into twig sections . . . . .	61



## INTRODUCTION

Peaches are among the most cold susceptible perennial fruit crops able to be commercially grown in the northern U.S. Their range is much more restricted than apples, pears or other fruit trees, primarily because they are more subject to winter injury.

In the northcentral and northeastern United States much winter injury occurs in late fall or early winter, resulting from freeze injury to immature tissue before it has fully acclimated. Conversely, the southern peach growing regions receive much cold damage during late winter due to early deacclimation (Andersen, 1974).

In Michigan, boundaries of the peach producing regions can be delineated by winter low temperature isotherms (Kessler, 1971). Winter injury can include sunscald, black heart, root killing, death of flower buds and even death of the entire tree. Winter killing of flower buds causes losses to growers both by reducing crops and by over production during years when no injury occurs. These factors cause disruption of orderly marketing.

Winter injury to woody tissues is sometimes dramatic, killing thousands of trees in a single freeze (Bradford and Cardinell, 1952; Kessler, 1971). Often, however, winter injury to woody tissues is more subtle, causing injury to cambium and xylem which reduces tree vigor and makes the tree more susceptible to attacks by insects and diseases. This reduces the economic life of the orchard and increases production costs.

Bradford and Cardinell (1952) have surveyed winter injury occurring in Michigan from 1846 to 1926. They report numerous winters in which severe winter injury has occurred. Spectacular winter injury resulting in the death of thousands of peach trees throughout Michigan occurred after the winters of 1855-56, October 1906, 1917-18, and Thanksgiving Day 1950. Kessler (1971) reports that there were 12,500,000 peach trees in Michigan in 1889. In October 1906 a severe freeze killed 73 percent of the peach trees in Michigan after which the peach never regained its former prominence. In 1949 there were 3,603,800 peach trees in Michigan but again a severe freeze in November 1950 killed thousands and in 1972 there were 1,630,000 trees.

Thus, winter injury to the wood has limited peach production in Michigan to favorable sites located in a narrow strip of land protected by Lake Michigan. Even within this area winter injury problems often occur as the winters of 1971-72 and 1972-73 point out. Furthermore, as human population pressure eliminates many of these

favorable lakeside sites, peach production will be forced to move to less favorable land.

Improved wood hardiness is essential for the successful long-term commercial production of peaches in Michigan and should be of prime concern in breeding new peach varieties for Michigan.

In setting up a breeding project to identify hardy genotypes one of the first logical steps is to decide on a suitable method of injury evaluation. Any evaluation technique used in breeding must be adaptable to handling large sample sizes, have reasonable accuracy and precision, and must be able to evaluate a single plant without completely destroying that genotype.

When trying to identify genetic differences in hardiness it is important to know the amount of non-genetic variation which may be encountered. Knowing the magnitude and sources of non-genetic variation, sampling techniques and sample size estimates which allow detection of genetic differences of a given magnitude can be calculated.

This work was a preliminary step in development of a program for breeding peaches which would be winter hardy in climates similar to Michigan. There were three specific objectives to this thesis research. The first was to evaluate the suitability of tissue browning, tissue regrowth, and electrolytic conductance as possible techniques of injury evaluation when handling large volumes of plant materials. The second objective was to identify some

sources and obtain some estimates of the magnitude of non-genetic hardness variation occurring within a representative peach genotype. The third was the development of a satisfactory sampling scheme and estimation of a reasonable sample size needed for detection of hardness differences of a desired magnitude.



## LITERATURE REVIEW

Fruit growers and agricultural scientists have learned the importance of good cultural practices in limiting the extent of winter injury problems (Chandler, 1913; Bradford and Cardinell, 1913). Varietal hardiness differences have also been recognized (Chandler, 1913; Hedrick, 1916). These varietal differences showed up in field survival results after test winters. Such test winters have been widely used to study plant hardiness and many researchers have issued reports dealing with injury to fruit crops after such winters (Chandler, 1913; Dorsey and Strausbaugh, 1923; Dorsey and Bushnell, 1925; Potter, 1938; Lantz and Pickett, 1942; Cooper, 1953; Fogle and Overley, 1954). At most locations a good test winter may occur on an average of once in ten years (Levitt, 1972). Researchers realized that test winters alone occurred too infrequently to provide a continuous reliable means of determining hardiness of new genotypes. Therefore, they sought some method of artificially cold stressing plants.

Chandler (1913) described a salt ice bath in which a temperature of -12 to -15°C could be reached. This

system could not be programmed to control freezing rate. According to Levitt (1972) an artificial freezing chamber in which material could be quickly and quantitatively frozen was introduced by Harvey in 1918. Edgerton (1960) described an antifreeze bath freezer which is programmable. Meader (1945) described a modified ice cream freezer using an ethanol mixture for coolant. Weaver et al. (1968) have used a cryostat chamber. Weaver et al. (1969) have also used a liquid nitrogen system. Scott and Spangelo (1964) described a portable freezing chamber for use in the field. Weiser (1970) had used insulated thermos bottles placed in a Revco freezer. To give the best research information the system must allow controlled freezing at a predetermined rate.

The evaluation of injury to buds is relatively easy. Live buds remain green and healthy while dead buds quickly become water soaked, turn brown, and eventually dry up. Number dead versus number alive can be expressed as a percent live or dead buds (Chandler, 1913; Edgerton, 1960). The percentage of live or dead buds over a range of temperatures can be expressed graphically and the  $LT_{50}$  can be estimated (Proebsting and Fogle, 1956). Bittenbender and Howell (1974) have recently adapted the Spearman-Kärber method to estimate the  $LT_{50}$  using an equation rather than a graph. This equation also provides information on the slope of the curve through the  $LT_{50}$  point. Evaluation of woody parts is difficult because the

injury ratings are a continuous gradient rather than discrete in nature.

Some methods try to measure a plant character which appears to be correlated with differences in plant hardness. Some characters which have been so measured include: osmotic pressure (Chandler, 1913), cell size (Wiegand, 1906), moisture content (Hildreth, 1926), various carbohydrates (Cooper, 1953), fatty acids (Ketchie, 1966), and protein content (Siminovitch and Briggs, 1949; Craker et al., 1966).

One of the oldest and most widely used methods is to rate the survival of whole plants or plant parts on an arbitrary scale. These subjective ratings may be expressed verbally (Hildreth, 1926; Fogle and Overly, 1954; Brierly and Landon, 1954), or as a percentage of live versus dead tissue (Watkins and Spangelo, 1970; Ketchie et al., 1972), or the ratings may be given a numerical rating on an arbitrary scale (Lantz and Pickett, 1942; Lapins, 1962a, 1962b). Numerical data can be analyzed using analysis of variance procedures providing the assumptions of homogeneous variance and normal distribution of residual deviations are not violated. Often such data are skewed toward one end of the rating scale. In such cases some type of scaling procedure can be used to give approximate solutions (Snell, 1964) or some type of nonparametric statistic such as Friedman's two-way analysis of variance can be used (Steel and Torrie, 1960). It should be noted that

regrowth does not directly measure injury. It describes the ability of the plant to survive injury and is closely related to the amount of injury induced but injury and recovery are two separate phenomena.

Another subjective rating system involves visual estimation of tissue damage via tissue browning. Usually the wood or cambium tissue is given a numerical rating describing the severity of browning (Lapins, 1962a, 1962b; Blazich, 1974). The data are then handled in the same manner as for recovery ratings.

Regrowth and browning ratings have been considered reliable but they have been criticized for being slow (Wilner, 1955; Stushnoff, 1972; Blazich, 1974). Individual bias can influence tissue browning results (Steponkus, 1967). Workers searched for evaluation techniques which would be simple, fast, quantitative, and free from individual bias. Many methods have been developed, each having its own advantages and disadvantages.

Cell plasmalysis and neutral red staining methods have been used to determine cell viability (Siminovitch and Briggs, 1953; Lumis et al., 1972). Stepokus (1967) has described a refinement of the triphenyl tetrazolium chloride (TTC) method of determining cold injury involving measurement of the reduced TTC using spectrophotometric techniques. Staining techniques, however, are not well adapted to handling large volumes of plant materials.



Heat is given off when supercooled liquid water suddenly freezes. This heat can be measured as an exotherm. Quamme et al. (1972a) have associated exotherm analysis with seasonal changes in hardness of apple xylem. In blueberry stems exotherms were associated only with xylem injury which was not as critical for survival as the bark tissues (Quamme et al., 1972). Stergios and Howell (1973) found that exotherm analysis worked well for some species but not for others and the method was not adapted to quantitative analysis.

Electrical resistance or impedance has been used to measure plant hardness in situ (Wilner, 1960a). This method involves placing the tissue to be tested between two electrodes and passing a small electrical current through the tissue and measuring the resistance. Results of this method are closely correlated to results from electrolytic conductance methods (Wilner, 1961). Blazich et al. (1974) found that electrolytic conductance was more closely associated with tissue browning than was electrical impedance. Evert and Weiser (1971) using electrical measurements at two frequencies found that stem sections exposed to lethal temperatures could not consistently be separated from sections exposed to non-lethal temperatures when tested immediately after thawing.

Dexter et al. (1930) described a method of studying injury by measuring electrolytic conductance of water leachates of frozen tissues. This method has been used by

a large number of researchers (Emmert and Howlett, 1953; Wilner, 1955; Edgerton, 1960; Lapins, 1962a, 1962b; Ketchie et al, 1972). Results are usually expressed as absolute conductance or as a percentage of the initial leachates in relation to the total amount of leachates in a boiled sample (Wilner, 1960b). Flint et al. (1966) have expressed electrolytic conductance as an index of injury where an unfrozen sample has a value of zero and a heat-killed sample has a value of one hundred. The injury is expressed as a relation between the frozen sample minus the unfrozen sample, and the heat-killed sample minus the unfrozen sample.

Wilner (1955) states that electrolytic conductance is more desirable than the more tedious and time-consuming examination of sectioned tissues. Lapins (1962b), however, found that recovery tests were much more sensitive in differentiating plant hardiness. He also found a higher correlation between recovery and browning than between either recovery or browning and electrolytic conductance.

#### Hardiness Variability in Relation to Plant Breeding

Dorsey and Bushnell (1925) found that winter hardiness in plums was controlled by a multiple allelic series. Watkins and Spangelo (1970), using apple, investigated the polygenetic trait called plant survival. Thus, winter hardiness can be considered a quantitative trait. Such traits are often involved in development of new varieties.

Breeding new superior varieties of asexual propagated plants involves two distinct aspects. One involves selection and asexual propagation of commercially acceptable seedlings which are superior to and are expected to replace a standard variety. The other involves selection of seedlings with some characteristics superior to the old variety but are themselves of little commercial value. These plants constitute parents of the next breeding cycle. They are used in an effort to recombine their different favorable genes into a single genotype.

The progress which can be made for both aspects of variety development is a function of the heritability of the characters concerned. Broad sense heritability is the genetic variance as a fraction of the phenotypic variance. If genotypes are randomly placed into the environment, the total phenotypic variance can be expressed as:  $\sigma_p^2 = \sigma_y^2 + \sigma_e^2$  where  $\sigma_p^2$  is the phenotypic variance,  $\sigma_y^2$  is the total genetic variance and  $\sigma_e^2$  is the environmental variance (Comstock and Robinson, 1948). The environmental variation is a nuisance and masks the genetic differences of interest. Therefore, any reduction in environmental variation will result in the genetic variation contributing proportionately more to the total phenotypic variation. This increases the heritability of the trait in question.

Differences in wood hardness within twigs, between twigs within a tree, and among trees of the same genotype have been found (Dorsey and Strausbaugh, 1923;

Dorsey and Bushnell, 1925; Fogle and Overlay, 1954; Wilner, 1960; Wilner, 1961; Lapins, 1962). Using an appropriate experimental design, such differences can be measured on a quantitative scale and the data analyzed via standard analysis of variance techniques (Steel and Torrie, 1960; Sokal and Rohlf, 1969). The identified sources of variance and the estimates of their magnitude can then be used to develop optimum sampling procedures to reduce this nuisance variation (Marcuse, 1949; Schultz, 1955). Sample sizes necessary to detect differences of a desired magnitude can be calculated using the variance estimates (Sokal and Rohlf, 1969; Schultz, 1955).

## **SECTION I**

**AN EVALUATION OF THE SUITABILITY OF TISSUE  
BROWNING, REGROWTH, AND ELECTROLYTIC  
CONDUCTANCE WOOD HARDINESS  
TESTS FOR PEACH BREEDING**

## Introduction

In hardiness research some method of injury evaluation must be devised. Methods suitable for physiological research on wood hardiness may not be well adapted as mass screening techniques for identifying wood hardy plants (Lapins, 1962a, 1962b; Stergios and Howell, 1973; Stushnoff, 1972). While an evaluation method used as a screening technique need not yield immediate results, it must be able to handle large amounts of plant materials with ease. Tissue browning, regrowth, and electrolytic conductance have been widely used and are well suited as evaluation techniques for mass screening (Lapins, 1962a, 1962b; Wilner, 1960b). Tissue browning and regrowth have been used to standardize other tests but have been criticized as being qualitative and subject to individual bias (Ketchie et al., 1972; Lapins, 1962a; Stergios and Howell, 1973).

Electrolytic conductance of leachates provides a qualitative test which has correlated well with regrowth and visual methods (Blazich et al., 1974; Ketchie et al., 1972; Wilner, 1960b). Some workers have claimed that while browning and regrowth were qualitative, they provided

a better estimate of injury than conductance (Lapins, 1962a, 1962b; Stergios and Howell, 1973).

Most studies which have compared different methods utilized relatively small sample sizes (Blazich et al., 1974; Stergios and Howell, 1973; Wilner, 1960b; Wilner, 1961). This study was included as part of a larger experiment to study wood hardness variation occurring within a single peach genotype. Tissue browning, regrowth, and electrolytic conductance methods were studied to determine their suitability as evaluation methods for handling large volumes of plant materials similar to the amounts which would be encountered in a breeding project for improving wood hardness.

#### Materials and Methods

Estimates of wood hardness were determined on November 24, 1973; December 15, 1973; February 7, 1974; and March 20, 1974. Plant material was collected near Hartford, Michigan, from a typical commercial orchard of four-year old Redhaven peach trees grafted to Siberian C rootstocks. Trees selected at random represented a wide range of orchard elevations differing by over 9m. Variation in elevation, fertility, moisture, soil, and tree vigor as well as the effects of the seedling rootstocks contributed to microenvironmental variation which would be expected to lead to random differences in wood hardness among trees. Different trees were used at each sampling date.

Each tree was divided into eight sectors. Hardiness of the current years twig growth in the upper southwest and the lower northeast sectors was evaluated (see Figure 3). They were chosen because they exhibit maximum differences in the interception of solar radiation. A number of twigs were taken at random from within each sector. Each twig was divided into three equal parts and the test sections were removed from within each of these parts (see Figure 4).

#### Freezing Techniques

All twigs from within each sector were taped together and properly labeled. The strips of whole twigs were wrapped in several layers of aluminum foil and foam rubber to allow uniform removal of heat during the freezing process. Three such packages were frozen to different test temperatures at each date to be sure at least one received the desired stress.

Temperature was monitored by inserting 26 gauge copper-constantan thermocouples into the pith of several twigs in the bundle. The thermocouples were connected to a 24 point recorder. All bundles were placed in a Revco freezer and temperature reduction was maintained at approximately 3°C/hr. Each bundle was removed immediately upon reaching the desired test temperature and was allowed to thaw completely at room temperature. Upon thawing, stressed twigs were unwrapped and stored under humid conditions



until they were evaluated for injury. Conductivity tests were performed within twenty-four hours after thawing and browning was usually done within seventy-two hours.

### Evaluation Methods

#### Electrolytic Conductance

An electrolytic conductance test of leachates, similar to the technique described by Wilner was used (Wilner, 1960a, 1960b; Wilner, 1961). Each twig section was weighed then cut into approximately 1 cm sections. The sections were placed into individual test tubes and a volume of water equal to seven times the wood weight was added to each tube. All tubes were stored at room temperature for approximately twenty-four hours.

After twenty-four hours initial conductance was taken using a solu-bridge soil tester (Model RD-15XI Industrial Instruments, Inc.). The solu-bridge had 1 cm<sup>2</sup> platinum electrodes enclosed in a glass bulb. After recording initial readings, all tubes were autoclaved for twenty minutes. The final conductance was taken after another twenty-four hours. Injury was expressed as:

$$\frac{\text{Initial Conductance}}{\text{Final Conductance}} \times 100 = \% \text{ specific conductivity}$$

#### Tissue Browning

A visual estimation of injury was made by examining a cross section of each twig section under a binocular

microscope (see Figure 2). The extent of tissue injury was expressed on the following 1 to 5 tissue browning scale:

- 1--No injury. All tissues appear bright green and alive.
- 2--Primary phloem fibers brown but surrounding tissue still green, giving a circle of brown spots surrounding the cambium area. These were judged fully capable of recovery.
- 3--Primary phloem fibers brown and some browning in the secondary phloem forming a continuous ring between the cortex and cambium areas. It is considered alive but with moderate injury.
- 4--All phloem cells brown and the cambium area showing some discoloration. Viability is questionable.
- 5--Completely brown and discolored with only the cortex possibly being green. These were judged to be dead.

On the basis of regrowth tests, browning ratings of 1 to 3 were considered to be alive and able to recover from injuries. A 4 rating was considered very severe injury and recovery was uncertain. A 5 was considered dead.

#### Regrowth

Materials for the regrowth tests were properly labeled and taped together, then placed in flats of moist peat moss. The flats were incubated in a 26°C greenhouse for fourteen to twenty-one days to allow development of callus formation. The following rating system was used:

- 1--No injury, very prolific callusing covering the entire cut surface.
- 2--Callus protruding from the cut surface around the entire cambium area.

3--Moderate callus formation not protruding above the cut surface and not necessarily forming a continuous ring around the entire twig section.

4--No callus formation but the twig bark appeared green and intact with no or only slight degeneration.

5--No callus formation with the outer bark brown and showing extensive tissue degeneration and often being invaded by saprophytic fungi.

A regrowth rating of 1 to 3 was considered alive and fully capable of recovery. Twigs with a 4 rating were considered severely injured and chance of recovery was considered very low. A rating of 5 indicated death.

#### Individual Experiments

November 24, 1973 (Expt. 1) and  
December 15, 1974 (Expt. 2)

Twelve twigs were removed from each of the two sectors in each of six trees. Each twig was divided into three equal parts and the tip section discarded. A 5 cm portion was removed from the middle of both remaining sections. The distal 2 cm of each section was used in the browning test. Regrowth data were incomplete and were not used. The artificially induced test temperature in both experiments was  $-28^{\circ}\text{C}$ .

February 7, 1974 (Expt. 3)

Fifteen twigs from each sector within eight trees were used. Twigs were divided into three equal parts. A 9 cm twig section was removed from the middle of each part. The distal 2 cm portion was utilized for the

browning and regrowth tests. A thin cross-section slice was removed from this piece and evaluated for browning, the remaining portion was placed in moist peat moss. The remaining 7 cm portion was used in the conductance test. Twigs were frozen to  $-34^{\circ}\text{C}$ .

March 20, 1974 (Expt. 4 and 5)

The sampling procedure was the same as in experiment 3 except no conductance tests were performed and the whole 9 cm twig section was used in the browning and regrowth tests. Twigs in experiment 4 were frozen to  $-25^{\circ}\text{C}$  while those in experiment 5 were frozen to  $-27^{\circ}\text{C}$ .

Results and Discussion

For statistical and discrimination purposes it was desirable to induce an intermediate level of injury in the plant material. This level would be represented by a 3 on the browning and regrowth scales. This was desired because it would produce a normal distribution about the mean. Either very little or very severe injury would produce a skewed distribution because of the finite scale. This could seriously distort assumptions underlying the analysis of variance, biasing, results, making application of nonparametric statistics necessary.

Table 1 shows the overall experiment means for each evaluation method. Experiments 2, 3, and 4 have mean browning ratings very close to the desired 3.0, while experiments 1 and 5 show more injury than desired. The

regrowth means indicate more severe injury than the browning means. However, as the browning ratings increase, regrowth ratings increase in a similar magnitude.

Browning tests indicate twigs in experiment 1 were more severely injured than those in experiments 2 or 3 but the percent conductance did not indicate any substantial change in injury.

Simple correlations between the three methods (Table 2) indicate a much closer relationship between browning and regrowth than between either browning or regrowth and electrolytic conductance. Experiment 1 indicates no relationship between browning and electrolytic conductance while experiments 2 and 3 indicate a low but highly significant (testing the null hypothesis of zero correlation) negative correlation of about  $-.25$ . The correlation between regrowth and conductance ( $-.29$ ) is similar to that of browning and conductance. These negative correlations indicate that as browning and regrowth rating increase, indicating more severe injury, the percent conductance decreases indicating decreased injury. This conflicts with results obtained by other researchers using electrolytic conductance (Blazich et al., 1974; Ketchie et al., 1972; Lapins, 1962a, 1962b; Wilner, 1960a, 1960b; Wilner, 1961). The highest correlation between browning and regrowth was  $.67$  (Expt. 4). This is not an extremely high correlation but it is good considering the subjectivity of both rating systems.



In experiments 1 and 2, tissue browning reveals significant differences among twig sections within a given sector as well as between sectors (Table 3). Twig sections in sector 1 are hardier than comparable sections in sector 2. The base sections (section 1) are hardier than the middle sections of the twigs. The electrolytic conductance test in experiment 1 indicates that only the bases of the twigs in sector 2 were less injured than the tip sections in sector 2. In experiment 2 conductance fails to reveal any differences in injury. This conflicts with results of the browning tests.

In experiment 3 browning and regrowth means (Table 4) indicate a significant hardness gradient exists within the twigs from both sectors, the basal sections being hardest and the tip sections being least hardy. Browning and regrowth also indicate that twig sections in sector 1 are hardier than comparable twig sections in sector 2.

Electrolytic conductance tests in experiment 3 (Table 4) indicate that within a sector basal twig sections have higher conductance values than tip sections. The basal sections in sector 1 have a higher conductance than those in sector 2 while other twig sections show no differences from one sector to the other. The conductance means in Table 4 show an inverse relationship to both the browning and regrowth means so that those twig sections having the severest injury according to browning and

regrowth show the lowest conductance values. This disagrees with conclusions reached by other researchers (Blazich et al., 1974; Ketchie et al., 1972; Lapins, 1962a, 1962b; Wilner, 1960a, 1960b; Wilner, 1961).

Table 5 shows regrowth and conductance means for all the cases of a given browning rating in experiment 3. While regrowth ratings indicate more severe injury to the twig sections than browning ratings, both methods increase in a similar manner. The conductance means are again shown to decrease as browning and regrowth ratings indicate more severe injury.

The results show that browning and regrowth yield similar results even though regrowth often indicates more severe injury than browning. One explanation of this discrepancy is that control of regrowth conditions in the greenhouse was not ideal. Fungi and bacteria infected many of the cuttings and spread to adjacent twig sections even though a fungicide was used. This caused some bias in the estimate of injury in some trees and sectors because twig pieces were taped together according to tree and sector. Even healthy twigs were often invaded and killed by microorganisms. There was also some evidence that callus formation occurred more readily after the rest period had been satisfied.

Electrolytic conductance proved to be a very unsatisfactory evaluation method. It is not understood why the results from conductance tests show an inverse relationship



to browning and regrowth or why they disagree with other researchers' conclusions (Blazich et al., Ketchie et al., 1972; Lapins, 1962a, 1962b; Wilner, 1960a, 1960b; Wilner, 1961). Some factors which may have contributed to unusual conductance results are listed here. The xylem was injured much more severely than the phloem and cambium areas. The relatively large amount of leachates released by the xylem could have largely masked any relatively small differences caused by release of leachates from a small number of injured cells in the phloem and cambium. Lapins (1962b) indicated that this is a problem with the conductance method. The solu-bridge used to make the readings was not as accurate as desired and led to a reduction in the ability to detect small differences. The small amount of material used (3 or 7 cm sections) and the small amount of water in which they were immersed led to relatively large measurement errors which further reduced the ability to detect small differences.

Control of experimental conditions when such a large number of samples was involved (n=720 samples) was very difficult. The time delay between reading the first sample and the 720th was several hours and led to increased error.

The large random variation about the grand mean for sectors (51.34%) shown in Table 6 is the result of having all twig sections from one sector of one tree placed together in one test tube rack and thus being

analyzed as a group. This resulted in large random differences between racks which increased experimental error. The large random variation for sectors in the regrowth test (Table 6) is also a result of not having complete randomization of all twig sections.

The time involved in preparing samples and calculating conductance values combined with the difficulty in controlling experimental error effectively eliminates electrolytic conductance as a method of mass screening large numbers of seedlings in a breeding program.

Tissue browning proved to be the most satisfactory evaluation method. About thirty-six hours after thawing tissue browning was well developed and changed very little over the next several days as long as the twigs were not allowed to dessicate. This provides some flexibility concerning time of evaluating injury. Browning is much faster and less technically involved than conductance. The time involved in determining browning value is no greater than the time involved in rating the amount of regrowth, but browning eliminates the disease control problems associated with the regrowth test. Another advantage of browning is that it permits multiple observations on one twig section.

A major criticism of tissue browning is the subjectivity and individual bias which may be associated with the test (Blazich et al., 1974; Lapins, 1962a; Stergios and Howell, 1973). In experiments 4 and 5, two browning

observations were made on each twig section. To eliminate any memory bias each of the 720 twig sections were rated once before the second rating on any section was performed. A determination of the repeatability of the browning test was then made by using estimates of variance components in the following equation:

$$\text{Repeatability of determinations} = \frac{\hat{\sigma}^2_{\text{trees}} + \hat{\sigma}^2_{\text{trees} \times \text{sectors}} + \hat{\sigma}^2_{\text{twigs}} + \hat{\sigma}^2_{\text{error}}}{\hat{\sigma}^2_{\text{trees}} + \hat{\sigma}^2_{\text{trees} \times \text{sectors}} + \hat{\sigma}^2_{\text{twigs}} + \hat{\sigma}^2_{\text{error}} + \hat{\sigma}^2_{\text{determinations}}}$$

As the  $\hat{\sigma}^2_{\text{determinations}}$  increases the repeatability decreases from unity. This gave a repeatability of .76 for experiment 4 and .82 for experiment 5, giving an average repeatability of .79 for 2880 observations. This suggests that the determination error was as large as any of the other components but it is still small enough to be controlled reasonably well through proper experimental design. It should be noted that this expresses nothing about the accuracy of the method.

By observing browning of specific tissues rather than overall intensity of browning, subjectivity of the test is reduced. The possibility exists that killing temperature of specific cell types which cause only partial injury are not a true reflex of the ultimate killing

temperature of the whole twig involved. The close relationship between browning and regrowth minimizes this danger.

Tissue browning and regrowth tests appear well suited to rapid evaluation of large volumes of materials involved in preliminary screening of large numbers of seedlings in a hardiness breeding program.

Table 1.--Overall experiment means for each evaluation method.

Experiment Number	Test Temperature °C	Browning	Regrowth	Percent Conductance
1	-28	4.09±.05		23.87±.25
2	-28	2.86±.06		23.29±.25
3	-34	2.84±.03	3.62±.03	22.85±.17
4	-25	3.10±.03	3.77±.05	
5	-27	3.59±.03	4.20±.04	

Table 2.--Simple correlations between evaluation methods.

Experiment Number	Browning Conductance	P	Browning Regrowth	P	Regrowth Conductance	P
1	.05	N.S.				
2	-.25	<.001				
3	-.26	<.001	.54	<.001	-.29	<.001
4			.67	<.001		
5			.64	<.001		

n = 720 observations in each experiment.

P = Significance level of the null hypothesis of zero correlation.

Table 3.--Twig section means within each sector of the tree for experiments 1 and 2.

Sector	Twig Section	Experiment 1		Experiment 2	
		Browning	Conductance	Browning	Conductance
1	1	3.31	23.21	2.17	24.63
1	2	4.19	23.68	3.01	24.62
2	1	4.07	23.45	2.75	22.05
2	2	4.79	25.15	3.53	21.87
LSD <sup>z</sup> .05		.17	1.35	.23	.76
LSD <sup>y</sup> .05		.38	4.22	.45	3.59

<sup>z</sup>LSD value used to compare twig sections within a sector.

<sup>y</sup>LSD value used to compare twig sections in different sectors.

Table 4.--Twig section means within each sector of the tree for experiment 3.

Sector	Twig Section	Browning	Regrowth	Conductance
1	1	2.26	2.83	26.34
1	2	2.60	3.32	23.87
1	3	2.94	3.77	21.05
2	1	2.58	3.63	22.72
2	2	3.02	3.91	22.91
2	3	3.63	4.29	20.16
LSD .05 <sup>z</sup>		.11	.14	.81
LSD .05 <sup>y</sup>		.15	.28	2.19

<sup>z</sup>LSD value used to compare twig sections within a sector.

<sup>y</sup>LSD value used to compare twig sections in different sectors.

Table 5.--Regrowth and conductance means at each browning level in experiment 3.

Browning Rating	Regrowth	Percent Conductance	Number of Cases
1	2.5 ± .50	25.94 ± 1.45	4
2	3.06 ± .06	25.34 ± .33	226
3	3.77 ± .03	22.41 ± .22	400
4	4.37 ± .07	20.96 ± .55	60
5	4.83 ± .06	20.90 ± .84	30

Table 6.--Random variation in experiment 3 expressed as a percentage of the grand mean.

	Browning	Regrowth	Conductance
Trees	29.06	26.64	20.65
Sectors	24.87	39.55	51.34
Twig Sections	15.86	15.70	14.08



## SECTION II

MINIMIZING NON-GENETIC WOOD HARDINESS

VARIATION IN REDHAVEN PEACH

(PRUNUS PERSICA BATCH.)

THROUGH SAMPLING

## Introduction

Lack of winter hardiness is a major limiting factor in fruit production in northern latitudes (Bradford and Cardinell, 1926; Chandler, 1912). Stushnoff (1972) has recently reviewed cold hardiness breeding and previous efforts have sought to select hardy genotypes either after test winters (Dorsey and Strausbaugh, 1923; Dorsey and Bushnell, 1925; Fogle and Overley, 1954; Lantz and Pickett, 1942) or by use of artificial freezing techniques (Lapins, 1962a, 1962b; Watkins and Spangelo, 1970; Wilner, 1960b, 1961).

Researchers have often used excised twigs of the previous season's growth as their experimental material to evaluate wood hardiness of the entire plant (Lapins, 1962a, 1962b; Wilner, 1960b, 1961). Twigs provide large amount of material for sampling and a means of testing wood hardiness of seedlings without destroying entire plants.

Differences in wood hardiness over the length of a twig, between twigs within a tree, and among trees of the same genotype have been found (Dorsey and Strausbaugh, 1923; Dorsey and Bushnell, 1925; Fogle and Overley, 1954; Lantz and Pickett, 1942; Lapins, 1962a, 1962b; Watkins and

Spangelo, 1970; Wilner, 1960b, 1961), but, few attempts have been made to systematically measure the sources and magnitude of this non-genetic variation. Lapins (1962a, 1962b) has stressed the use of uniform material for evaluating wood hardness. He gave estimates of sample sizes needed to select specific percentages of hardy seedlings from a population of apple seedlings (Lapins, 1962b)

Detection of genetic wood hardness differences of a given magnitude among seedlings in a breeding program has been the ultimate goal. A knowledge of sources of non-genetic wood hardness variation would be important if an efficient sampling method was to be developed. An estimate of the amount of non-genetic variation would allow determination of sample sizes needed for detection of genetic wood hardness differences of a desired magnitude.

This study was undertaken to identify sources of wood hardness variability and to ascertain the extent of variation within and among trees of a single peach genotype. This information would permit development of sampling methods to reduce non-genetic wood hardness differences. It would also provide estimates of the magnitude of random variation. These would be used to develop sample size estimates needed to detect differences of a desired magnitude in future experiments.

### Materials and Methods

Twigs of the previous season's growth were removed from the upper southwest (sector 1) and lower northeast (sector 2) sectors (see Figure 3) of four-year old peach (Prunus persica Batch. 'Redhaven') trees being grown on a good commercial peach site at Hartford, Michigan.

All twigs were wrapped in several layers of aluminum foil and foam rubber to allow slow uniform heat removal. They were frozen to a predetermined test temperature designed to induce the proper stress and were immediately removed upon reaching the desired temperature. Each twig was cut into three equal length sections. From within the middle of each of these twig pieces, a uniform length twig section was removed and used as the basic experimental unit (see Figure 4). A thin cross section of each twig section was microscopically examined for tissue discoloration. Extent of injury was based on an arbitrary 1 to 5 browning scale (see Figure 2).

In experiments 1 and 2 twelve twigs were removed from each sector of six randomly selected trees. Data from only the base and middle sections (twig sections 1 and 2) were analyzed. Fifteen twigs from the southwest and northwest sectors of eight trees were evaluated in experiments 3, 4, and 5. All three twig sections were examined. In experiments 4 and 5 two browning observations were taken on each twig section. More details of materials and methods are given in section I.

An appropriate statistical model was devised which would separate the variance components of interest. In the model the value of an individual observation ( $Y_{ijkl}$ ) can be described as:

$$Y_{ijkl} = \mu + T_i + S_j + (TS)_{ij} + A_{(ij)k} + B_l + (SB)_{jl} + E_{(ijkl)}$$

Where:

T--symbolizes Trees

S--symbolizes Sectors of the tree

A--symbolizes twig sections

E--symbolizes residual error

And:

$\mu$  = the true population mean which is a constant

$i = 1, 2 \dots t$  where  $i$  refers to the random effect of the  $i$ th tree

$j = 1, 2 \dots 5$  where  $j$  refers to the fixed effect of the  $j$ th sector of the tree

$k = 1, 2 \dots a$  where  $k$  refers to the random effect of the  $k$ th twig within the  $j$ th sector of the  $i$ th tree

$l = 1, 2 \dots b$  where  $l$  refers to the fixed effect of the  $l$ th twig section

The model is mixed. It also contains nested and crossed factors. The statistical implications of a mixed model and nested elements have been discussed in detail (Henderson, 1953; Marcuse, 1949; Schultz, 1955; Sokal and Rohlf, 1969).

Table 1 shows the analysis of variance table with the expected mean squares. Observed mean squares were equated to their expectation and the proper F test for each effect of interest was determined from Table 1. The residual error term is composed of higher order interactions as well as several two-way and three-way interactions which were not of interest.

In experiments 4 and 5 two browning observations were taken on each twig section. This effect was denoted as: determinations. It was symbolized in the model as  $F_{(ijkl)m}$  where  $m = 1, 2$ . It had an expected mean square of  $\sigma_F^2$ . It was not shown in Table 1 but its effect would be added to all higher effects in the table. This effect gave an estimate of the variance due to inaccuracies in the rating system.

### Results

Table 2 shows the experimentally obtained estimates of mean squares with their associated degrees of freedom plus the significance level of the F statistic. Significant differences among trees were found in all experiments except experiment 2. The two sectors of the tree also differed significantly. Because of the significant tree by sector interaction, sector differences should be examined separately within each tree. Closer examination revealed that although the magnitude of differences between sectors

changes from tree to tree, only three trees out of thirty-six examined in all five experiments showed sector 2 to be hardier than sector 1.

In every experiment twig sections showed a highly significant gradient exists within them. The highly significant sector by twig section interaction in experiments 3, 4, and 5 indicated that differences among twig sections were not consistent from one sector to the other. Such an interaction is of interest only if the order of hardness ranking of twig sections is changed from one sector to another. Relative magnitudes of differences between twig sections were not of interest. Closer examination showed that the gradient was less pronounced in twigs from sector 1 than in those from sector 2. However, in all cases the basal section of the twig was the least injured while the tip section always exhibited the most injury.

The variance components have been listed in Table 3. Values are expressed as the actual estimate of random variation due to the component and as a percent of the total random variation. It can be seen that differences among twigs and residual error accounted for a major portion of the total random variation while trees and the tree by sector interaction make up a relatively small proportion of the total random variation. Both trees and the tree by sector interaction showed increased magnitude in the March 20 experiments (expts. 4, 5). This may indicate an

increase in variability within and among trees as they begin to deacclimate in the spring.

Multiple browning determinations on the same twig section contributed about 20 percent of the total random variation in experiments 4 and 5. This gives an average repeatability of .79 (see Section I). This variability arises because different browning ratings are assigned to a single twig section when multiple observations are made. These inconsistencies appear at random throughout the entire experiment so blocking cannot be used to eliminate this source of error. In many instances where the second observation was different, it was obvious that it was a border-line case which could possibly fall into either of two categories. If such difficult to rate twig sections were discarded, a large part of the 20 percent of total random variation due to poor repeatability would be eliminated. This would decrease the determination variance component and increase the repeatability of the rating system.

The weight of a 7 cm portion of each twig section in experiment 3 was used as an estimate of the twig section size. Using this as the independent variable and tissue browning as the dependent variable a regression analysis was performed separately for each twig section within a sector. Figure 1 shows the regression slopes and their 95 percent confidence belts for sector 1 twig section 1 and sector 2 twig section 3. It can be seen that for a



given change in wood weight the browning values in the tip sections of sector 2 show a greater change than the base sections of twigs in sector 1. Twig size is much less critical if samples are removed from sector 1 twig section 1. Other sector-twig section combinations are not shown but have intermediate regression slopes.

### Discussion

Previous research has indicated that wood hardness is a complex genetic trait (Dorsey and Bushnell, 1925; Watkins and Spangelo, 1970). Its expression is controlled by many physiological and environmental processes (Brierley and Landon, 1954; Cooper, 1953; Craker et al., 1969; Ketchie, 1966; Levitt, 1972; Siminovitch and Briggs, 1949). When breeding for a quantitatively inherited trait, it is important to obtain an estimate of the trait's heritability. Broad sense heritability specifies the portion of total variation caused by genetic influences and that portion due mainly to environmental influences. Environmental and experimental variation may arise from a whole array of causes which are non-genetic in nature. Some, such as sampling technique, may be controlled by the experimenter while others such as microclimate effects cannot. It was desirable to reduce this non-genetic variation as much as possible because environmentally caused mistakes in identification of hardy plants reduce heritability and the amount of genetic progress which can be made.

A single peach genotype was used to eliminate any genetic variation due to scion variety. All variation obtained, therefore, should be non-genetic except that caused by genetic variation amongst seedling rootstocks. This variation was further broken down into variation which can be minimized by the experimenter through proper experimental design and sampling procedures and that which is beyond control of the experimenter.

Since there are significant wood hardness differences between sectors of the tree and between twig sections it is important to remove samples from equivalent areas of all trees. If samples were removed at random from any sector and section, the sector and twig section hardness differences are more likely to mask genetic differences. The other favorable effect of sampling one twig section in one sector of all trees is that the residual error component, which accounts for about 50 percent of the total random variation in the first three experiments (Table 3), is largely eliminated. This is due to the residual error being composed of two-way, three-way and higher order interactions not partitioned elsewhere in the analysis. Uniform sampling of a standardized sector and twig section would eliminate these interactions.

The base sections of uniform sized twigs removed from the upper southwest sector appear to be best suited to use. Figure 1 showed that differences in twig size were much less critical in sector 1-section 1 than for

those twigs in sector 2-section 3. The base of the twigs showed more uniformity in maturity and differentiation of tissues making it easier to rate the amount of injury in this part of the twigs. Finally, the standard deviation of the base twig sections in the upper southwest sector were generally smaller than the standard deviation of other sector-twig section combinations.

Variance estimates obtained by summation of twig, error, and determination components of Table 3 are shown in Table 4. These variance estimates can be used to calculate the sample size necessary to detect a given size difference between means in a future experiment. The sample size can be calculated using the equation (Sokal and Rohlf, 1969):

$$n \geq 2 \left( \frac{\hat{\sigma}}{\delta} \right)^2 \left( t_{\alpha, v} + t_{(1-p), v} \right)^2$$

where:  $\hat{\sigma}$  is an estimate of the variability

$\delta$  is the difference to be detected

$t$  is students  $t$  value

$\alpha$  is the desired probability level

$p$  is power of the test

$n$  is the estimated sample size, and

$v$  is the degree of freedom of the sample

In any program where seedlings are being screened for wood hardness there will probably be at least 120

observations so  $t$  has infinite degrees of freedom and no iteration is required.

Table 4 shows some estimates of the sample size needed to detect a given size difference ( $\delta$ ) between browning means. Using a reasonable sampling size of five to twenty twigs per seedling, differences between .5 and 1.0 browning units should be detectable. It is not known exactly what difference in stress tolerance one browning unit equals but preliminary estimates indicate that one browning unit may denote a 2 to 4°C difference in hardness. The scale is not linear throughout its range. These sample size estimates are slightly larger than estimates developed by Lapins (1962b) using apple seedlings but the two estimates are not directly comparable.

The stated sampling method and sample size estimates do not take into account the variation among trees. Since a wood hardness breeding program would involve many seedlings it would be economically unsound to replicate each genotype. Since trees cannot be replicated over time or space, microenvironmental differences unique to a given spatiotemporal arrangement can never be exactly duplicated. Such differences give rise to variation which will be confounded with genotypic differences. Good experimental design including blocking and treating all seedlings in as similar a manner as possible will minimize this nuisance variation.

Table 1.--Analysis of variance table with the expected mean squares used to calculate F values and variance components.

Source	d.f.	Expected Mean Square
(T) Trees	t-1	$\sigma_E^2 + b\sigma_A^2 + sab\sigma_T^2$
(S) Sector	s-1	$\sigma_E^2 + b\sigma_A^2 + ab\sigma_{TS}^2 + tab\Sigma S_j^2$
TS	(t-1)(s-1)	$\sigma_E^2 + b\sigma_A^2 + ab\sigma_{TS}^2$
(A) Twigs	ts(a-1)	$\sigma_E^2 + b\sigma_A^2$
(B) Twig Sections	b-1	$\sigma_E^2 + tsa\Sigma B_1^2/(b-1)$
SB	(s-1)(b-1)	$\sigma_E^2 + ta\Sigma (SB)_{j1}^2/(b-1)$
(E) Residual Error	$[(tsab-1)-(t-1)-(s-1)-ts]$ $[-ts(a-1)-(b-1)-sb]$	$\sigma_E^2$
Total	tsab-1	

Table 2.--Analysis of variance table showing degrees of freedom, mean squares, and significance values of the F statistic for effects of interest in all experiments.

EXPERIMENT Source	1			2			3			4			5		
	d.f.	M.S.	$\alpha$	d.f.	M.S.	$\alpha$	d.f.	M.S.	$\alpha$	d.f.	M.S.	$\alpha$	d.f.	M.S.	$\alpha$
(T) Trees	5	1.7722	.025	5	.5896	.75	7	2.1428	.005	7	12.7333	.001	7	12.7619	.001
(S) Sectors	1	33.3472	.01	1	21.6706	.025	1	41.0889	.001	1	117.8779	.005	1	98.1778	.025
TS	5	1.4722	.05	5	1.8951	.10	7	.4984	.75	7	7.9428	.001	7	11.2286	.001
(A) Twigs	132	.6218		132	1.0161		224	.6804		224	1.9201		224	1.9213	
(B) Twig Sections	1	46.7222	.001	1	47.5312	.001	2	45.2347	.001	2	255.5444	.001	2	277.3778	.001
SB	1	.5000	.25	1	.0868	.75	2	2.1847	.001	2	2.4111	.005	2	4.2861	.001
(E) Residual	142	.2590		142	.5062		476	.2027		476	.2027		476	.3915	
(F) Determinations	-			-			-			720	.1583		720	.1180	

$\alpha$  = significance level of F statistic.

Table 3.--Estimate of variance components in all experiments.

EXPERIMENT	1	2	3	4	5					
Source of Component	Component	% <sup>z</sup> Component	% Component	% Component	% Component					
Trees	.0240	4.80	0.0 <sup>y</sup>	0.0	.0162	4.29	.0601	8.92	.0602	18.94
TS	.0354	7.80	.0366	3.48	0.0	0.0	.0669	9.94	.1034	15.36
Twigs	.1814	36.29	<del>.2550</del> .5098	48.43	.1592	42.09	.2466	36.64	.2550	37.86
Error	.2590	51.82	.5062	48.09	.2027	53.62	.1412	20.97	.1367	20.30
Determinations	-	-	-	-	-	-	.1583	23.54	.1180	17.53

<sup>y</sup>negative numbers were assumed to be zero as the most reasonable estimate.

<sup>z</sup>% = the percent of the total random variation.

Table 4.--An estimate of the number of twigs (n) needed to detect a desired difference ( $\delta$ ) where  $\alpha = .05$ ,  $p = .8$ , and t has infinite degrees of freedom.

Experiment	$\hat{\sigma}$	$\delta$	$n$
1	<del>.6636</del> <del>.4404</del>	.1	<del>400</del> 691
		.5	<del>16</del> 28
		.75	<del>7</del> 12
		1.0	<del>4</del> 7
2	<del>.8725</del> <del>1.016</del>	.1	<del>2170</del> 1195
		.5	<del>87</del> 48
		.75	<del>39</del> 21
		1.0	<del>22</del> 12
3	<del>.6016</del> <del>.3619</del>	.1	<del>275</del> 568
		.5	<del>11</del> 23
		.75	<del>5</del> 10
		1.0	<del>3</del> 6
4	<del>1.7390</del> <del>.5097</del>	.1	<del>546</del> 858
		.5	<del>22</del> 34
		.75	<del>10</del> 16
		1.0	<del>5</del> 9
5	<del>.7139</del> <del>.5461</del>	.1	<del>626</del> 800
		.5	<del>25</del> 32
		.75	<del>11</del> 14
		1.0	<del>6</del> 8



## SUMMARY AND CONCLUSIONS

A single representative peach genotype was used to study non-genetic wood hardness variability and to evaluate tissue browning, regrowth and electrolytic conductance. The three methods were studied to evaluate their suitability as viability tests for use in a breeding program in which large amounts of plant material would be handled.

The electrolytic conductance tests involved many measurements of water extracts from the frozen twigs. The procedure was too involved to be well adapted to large volumes of plant materials. Careful experimental technique needed to obtain accurate and reliable conductance values could not be achieved because of the difficulty of handling large numbers of samples. The use of blocking procedures might have eliminated some of the variability associated with experimental techniques but it probably would not wholly eliminate the problem.

Tissue browning ratings on a 5 unit scale were made by microscopic examination of thin cross sections of the twigs. Regrowth ratings were based on a 5 unit scale dependent on the amount of callus developed at the cut ends.

Browning and regrowth were more highly correlated ( $R^2=.61$ ) than either browning and conductance ( $R^2=-.15$ ) or regrowth and conductance ( $R^2=.29$ ). Problems of controlling fungi and bacteria were associated with the regrowth test. Also, callus formation appeared to occur more readily after the cold requirement had been satisfied. Careful control of standardized regrowth conditions is needed to produce satisfactory results.

Tissue browning had a repeatability of .79. This value is high enough to give reasonable precision if this source of error is considered when establishing a sampling procedure.

One alternative approach to determine the feasibility of using these evaluation methods with large sample sizes would be extrapolation from a small, more carefully controlled experiment. A small timed experiment would permit more careful laboratory technique. Knowing the time needed to analyze a small number of samples, the time needed for a large number of samples could be estimated. Feasibility of the technique based on time and results obtained could then be determined.

The approach used in this study may not have allowed as careful control of experimental techniques as alternative methods. It did, however, provide a practical test of the techniques under conditions expected to be encountered in a breeding project with limited available manpower. This approach also provided an estimate of

problems and sources of error which might not arise and could not be measured in a smaller more controllable experiment.

Other sources of non-genetic hardiness variation were also identified using an appropriate statistical model. Using tissue browning to assess injury, significant differences were found among trees in all five experiments. This indicates the need for uniform field conditions. Significant differences between sectors of the tree and between sections of the twig were also found. In thirty-three of the thirty-six trees examined the upper southwest sector (sector 1) was hardier than the lower northeast sector (sector 2). In all cases a pronounced injury gradient was found to exist over the length of the twig. The bases of the twigs were injured least while the tips exhibited the most injury. This gradient was less pronounced in twigs located in sector 1 as opposed to those in sector 2.

Variance components were used to estimate the amount of variability contributed by each of the sources. Trees and the tree by sector interaction made up a relatively small portion of the total variation. Twigs and the residual error accounted for the largest portion. Uniform sampling from a given sector and twig section would eliminate much of the residual error by eliminating many of the interactions composing it. The upper southwest sector should be used because twig size was much less

critical in this section and more twigs were available for sampling. The base twig sections were more mature and differentiation of tissue made these sections easier to observe and rate. Finally, the standard deviation of sector 1-twig section 1 was generally smaller than other sector-twig section combinations. Thus, it is recommended that samples be removed from this area of the tree.

Variance estimates obtained by summation of twig, error and determination components were used to calculate sample sizes needed to detect differences of a desired size. Using a reasonable sample size of between five and twenty twigs, differences of .5 to 1.0 browning units could be expected to be detected.

## **APPENDIX**

Figure 1. Regression of browning on wood weight for sector 1 twig section 1 and sector 2 twig section 3 in experiment 3.

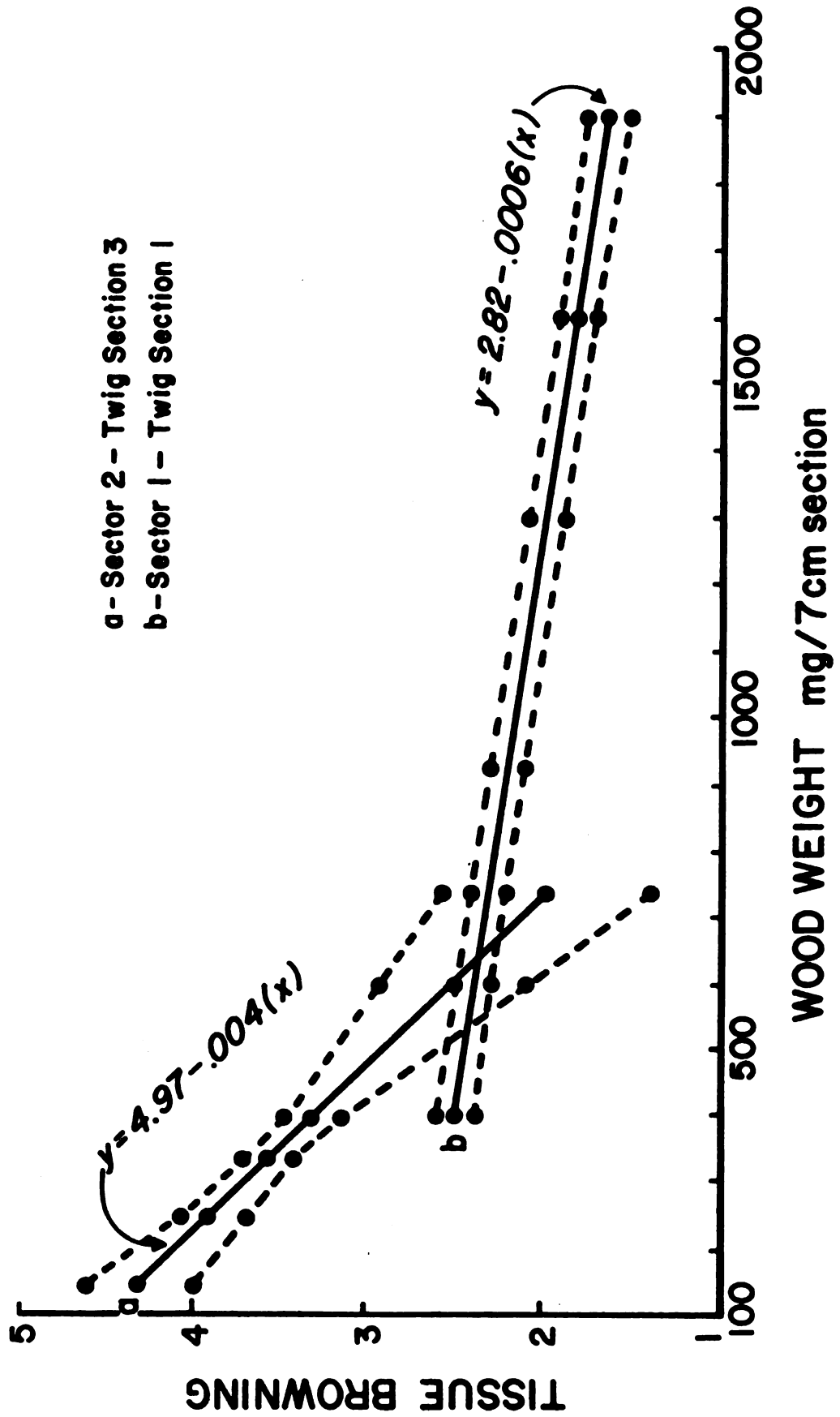
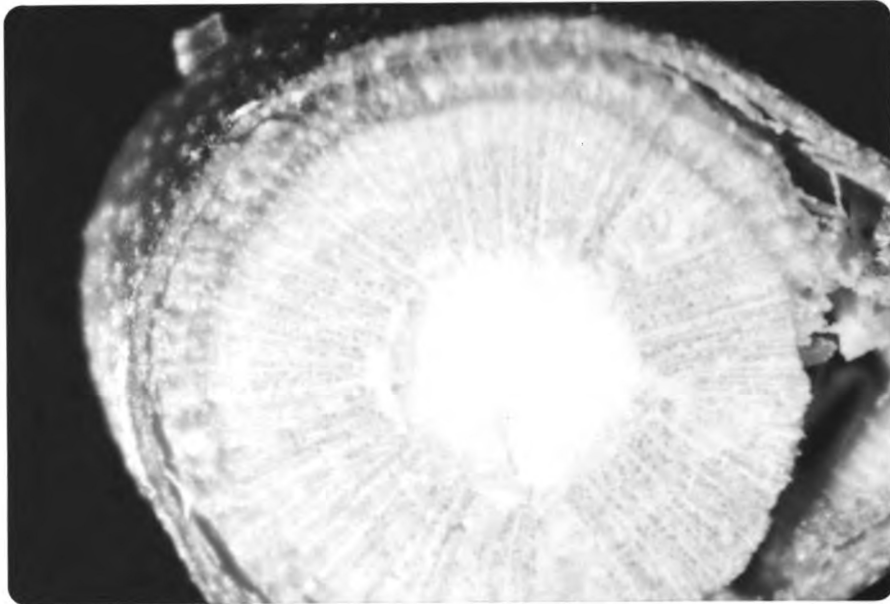
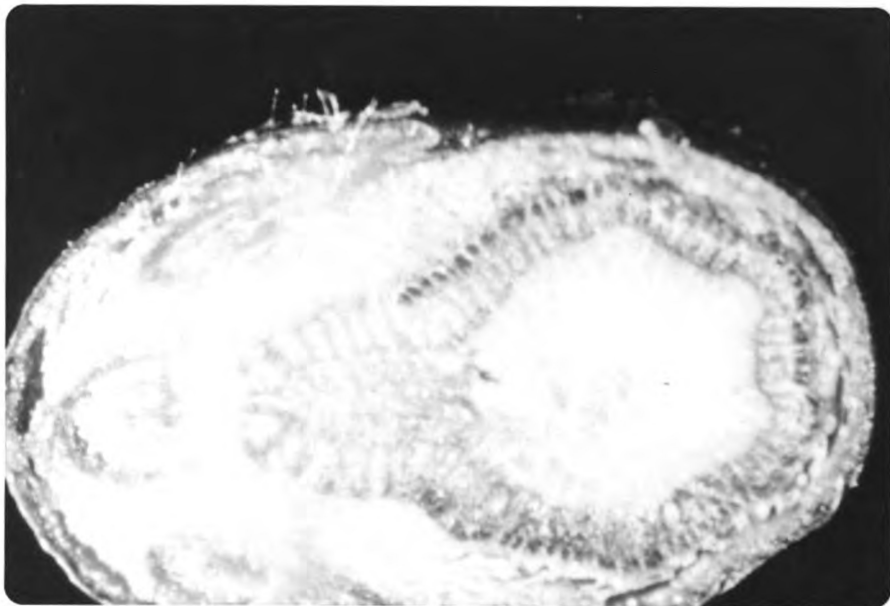


Figure 2. Photographs illustrating the 5 unit browning scale. A through E represent a rating of 1 through 5 respectively.

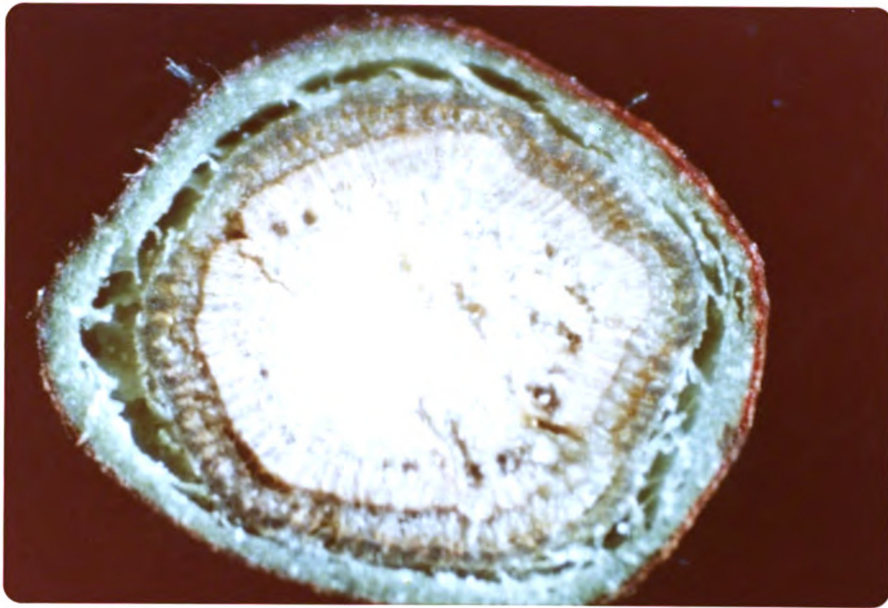




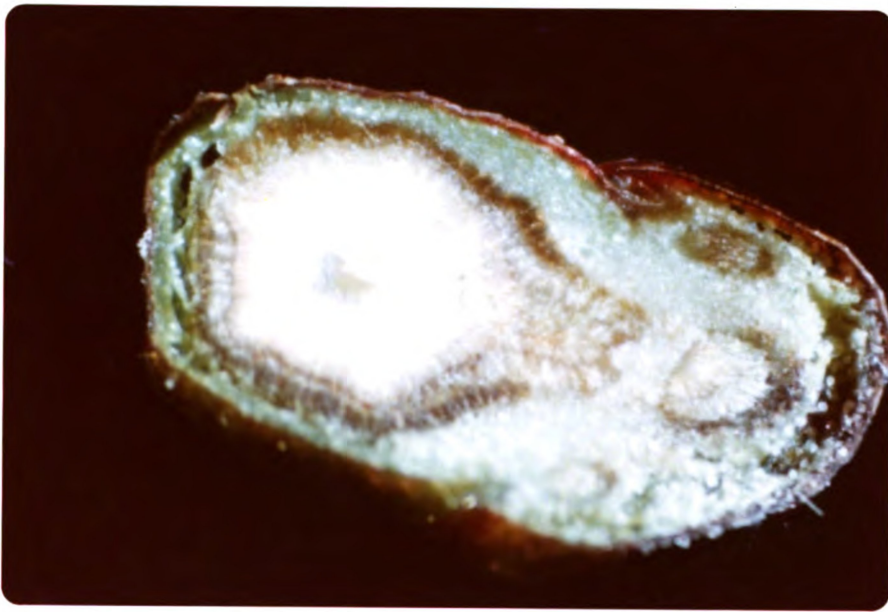
A



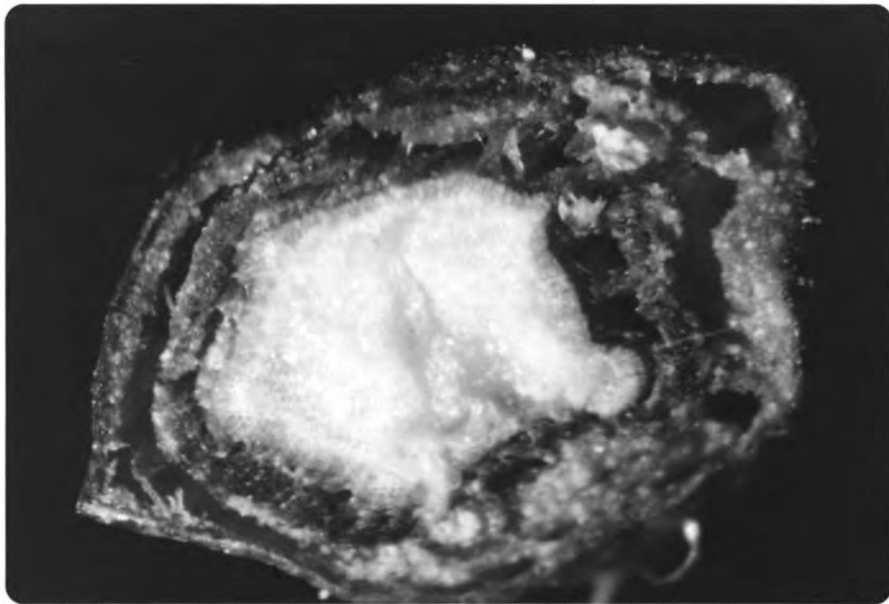
B



C



D



E

**Figure 3. Illustration of a tree divided into sectors.**

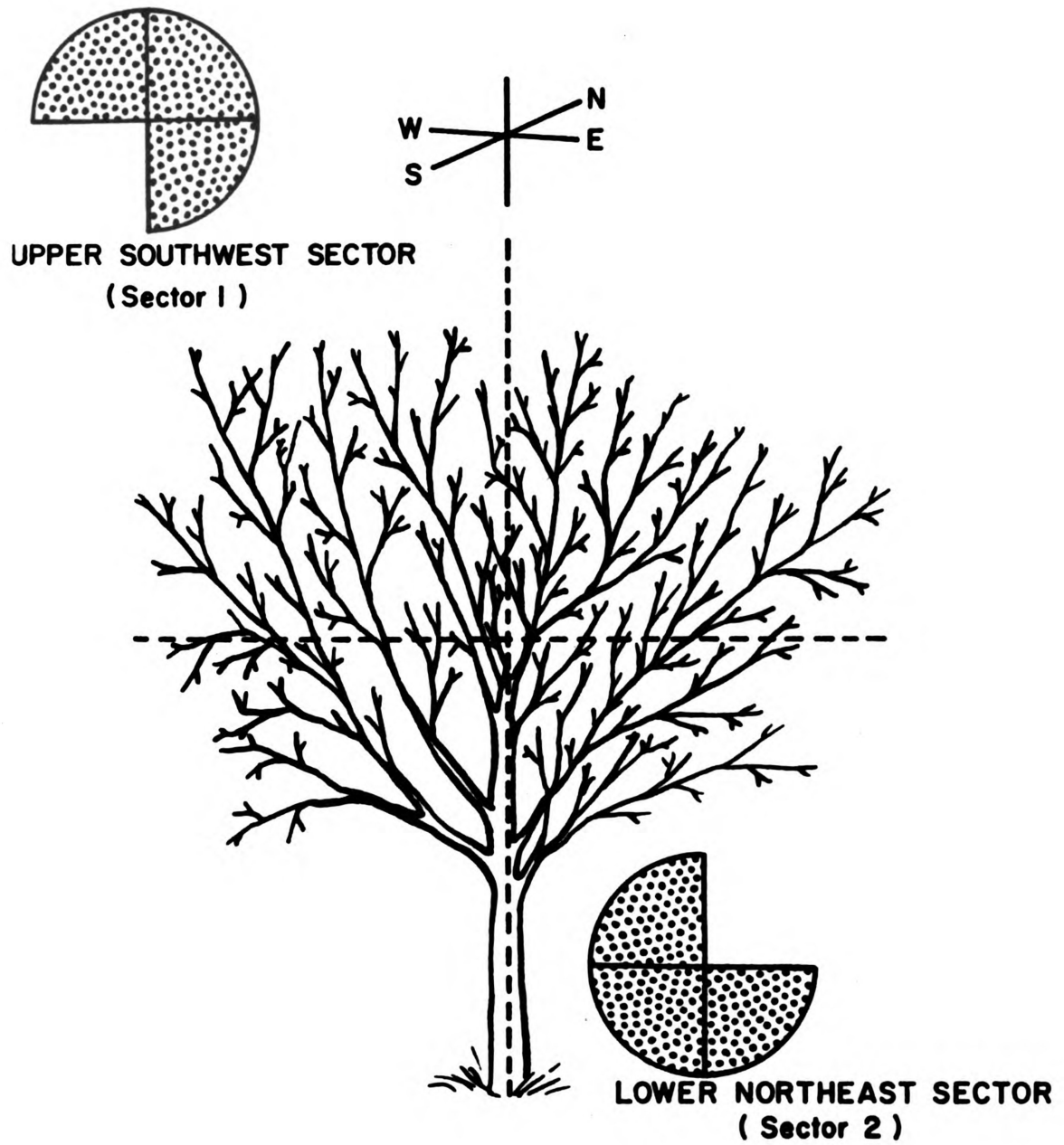
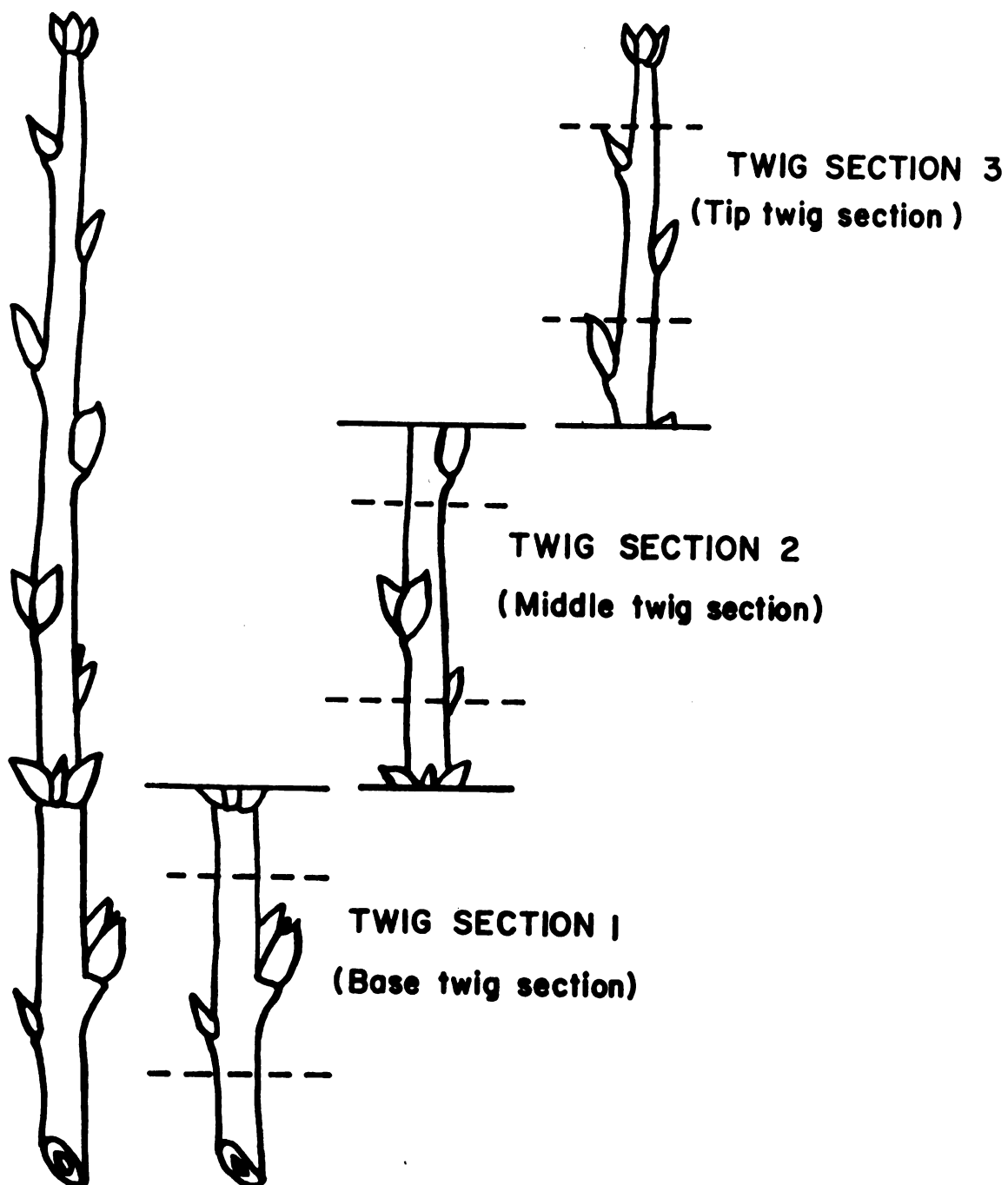


Figure 4. Illustration of a twig showing its division into twig sections.



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