

RAPID TISSUE TESTS ON PECANS TO DETERMINE THE PRESENCE
OF THE THREE MAJOR ELEMENTS

By

CLARENCE RICHARD ROBERTS

Bachelor of Science

Oklahoma Agricultural and Mechanical College

Stillwater, Oklahoma

1949

Submitted to the Faculty of the Graduate School of
the Oklahoma Agricultural and Mechanical College
in Partial Fulfillment of the Requirements
for the Degree of
MASTER OF SCIENCE

1950

OKLAHOMA
AGRICULTURAL & MECHANICAL COLLEGE
LIBRARY

MAY 9 1951

RAPID TISSUE TESTS ON PECANS TO DETERMINE THE PRESENCE
OF THE THREE MAJOR ELEMENTS

CLARENCE RICHARD ROBERTS

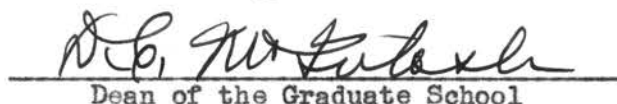
MASTER OF SCIENCE

1950

THESIS AND ABSTRACT APPROVED:


Thesis Adviser


Faculty Representative


Dean of the Graduate School

273808

Acknowledgement

The writer wishes to express his sincere thanks to Dr. Frank B. Cross and Professor Fred Lecrone of the Department of Horticulture, Oklahoma A. & M. College. The problem was investigated under their joint direction and their kind criticisms have been much appreciated.

Grateful acknowledgement is also made to Dr. R. W. MacVicar, Head of the Agricultural Chemistry Department, who arranged for leaf samples to be chemically analyzed, and to all others who aided in this work.

Table of Contents

Chapter	page
Introduction.	1
Review of Literature.	3
Methods and Materials	8
Soil Fertilizer Treatments	8
Rapid Plant Tissue Tests	11
Sampling	13
Nutrient Sprays.	15
Experimental Results and Discussion	17
Nitrates. Adapting a Rapid Nitrate Test to Pecan Trees and Comparing Results with Chemical Laboratory Analyses.	17
Inorganic Phosphates. Adapting a Rapid Phosphate Test to Pecan Trees and Comparing Results with Chemical Analyses	35
Potassium. Adapting a Rapid Potassium Test to Pecan Trees and Comparing Results with Chemical Laboratory Analyses.	40
Nutrient Solutions Applied to Pecan Foliage to Test Absorption of Nitrate Nitrogen Through the Foliage.	43
Summary and Conclusions	45
Literature Review	47

Introduction

Nitrogen, phosphorus and potassium are known to be required by plants in comparatively large quantities as compared to other mineral elements and are the three main variables of all soils. For this reason they have been called the three major elements.

The development of plant science has indicated a need for more information about the nutrient elements supplied through the soil which are required in the development of plant tissue. As a result of scientific investigations, various techniques and procedures have been partially or wholly worked out to determine the presence of the various elements within both plant tissues and the soil. Of the techniques developed, many are time consuming and require elaborate equipment and skilled technicians. These procedures are therefore poorly adapted for field use.

Since the history of agricultural science is largely a search for the simplest and most economical methods of bringing under control the factors related to agriculture, new methods of diagnosing the elemental makeup of both soils and plants have been developed. These are referred to as the quick test methods. Some of these tests are adapted for use in testing both soils and plant tissues while others contain only the reagents suitable for the testing of one or the other. The Purdue test kit is one which can be used for the testing of both plant tissues and soils.

There has been some question as to the reliability of the quick tests and some experimenters report that these tests are so inaccurate that they are unreliable. Other workers, however, support them.

The purpose of this investigation was to collect information comparing the Purdue test kit to other methods of testing as a measuring stick in determining the amounts of nitrate nitrogen, inorganic phosphates and potassium in

the tissues of pecan trees.

Members of the staff of the department of Horticulture, Oklahoma A. & M. College, suggested to the writer that an insufficient supply of nitrates for pecan trees might be the cause of alternate bearing, a common occurrence with pecans in Oklahoma. It has also been suggested by Hammar and Hunter (32) that prolific pecan varieties such as the Moore, which tend to produce very heavy crops in alternate years, lack an adequate supply of nutrient elements in their tissues for the production of annual crops. This probability was kept in mind throughout the studies.

Review of Literature

According to Brown (11) the use of plant tissue tests to measure soil fertility is becoming increasingly popular. It has been shown by various workers (11,17,37,46) that rapid plant tissue tests were an effective means of measuring certain mineral nutrient elements in tomatoes, corn, sugar beets, clover, soy beans, potatoes, wheat, barley and rye. Very little information concerning the use of quick tissue tests on woody perennials was to be found in the literature. Inciardi (38) ran tissue tests on peach trees grown in controlled nutrient solutions, using the Purdue tissue test kit. The tests he made indicated that nutrient conditions within peach leaves could not be as readily measured as in tomato plants. However, through correspondence (38) with Purdue University horticulture members it was learned that with certain modifications these tests could be used on peach trees. In a recent publication (29) a modification of the Purdue tissue test kit for potassium adapted for use on peach tree leaves for diagnostic purposes is reported.

Alben (2) reports numerous chemical laboratory analyses of leaf samples taken from pecan orchards in Louisiana and Texas. Most of the analyses have been made for nitrogen, phosphorus and potassium. According to Alben (2) this work has produced enough data to indicate when the amount of an element in pecan leaves is low, average or high. Results indicated that the phosphorus content of pecan leaves appeared to be comparatively stable. Total nutrient element determination by leaf analyses is time consuming and expensive and a quick test would be beneficial from the point of view of economy.

According to Smith (60) one cannot tell by soil analyses what the pecan tree is absorbing. Regardless of how well supplied with elements the soil may be, the tree may still suffer because of a lack of absorption of certain elements. Scarseth (51) reports similar information by stating that the mere

addition of an element to the soil is no assurance that it is entering the plant. Those elements that actually get into plant tissues are the ones effective in feeding plants. Thus it is of first importance to know if plants are absorbing the specific elements needed. Information on the nutritional status is therefore of vital importance.

It has been reported by Gossard (31) that pecan trees respond in yield, quality of nuts, amount of growth and in foliage condition to differences in soil moisture, soil fertility, fertilizer treatments, and cultural treatments that affect the mineral nutrient elements in the soil.

Krantz, Nelson and Burkhart (41) pointed out four objectives in determining the mineral nutrient status of plants as follows: first, to aid in determining the nutrient element supplying power of the soil; second, to aid in determining the effect of soil treatments on the mineral nutrient status of the plant; third, to study the relationships between the mineral nutrient status of the plant and crop performance as an aid in predicting fertilizer requirements; and fourth, to help lay the foundation for approaching new problems or for surveying unknown regions to determine where critical plant nutritional experimentation should be conducted.

Scarseth (50) concluded that since crop production is a dynamic process requiring constant study as to the fertility of the soil, it should be of advantage to a grower to study the performance of the growing crop with tissue tests. According to Scarseth (52) such tests would furnish information on the status of the nutrient element supply of plants at any time during the growing season. Bray (10) reports that plant tissue tests appear to be the means of solving nitrogen control in growing plants. A positive test for nitrate nitrogen in the tissues is an indication that the plant is receiving sufficient amounts of nitrates from the soil at the time that the test is made. With many plants,

a negative test means that sufficient nitrate nitrogen is not being supplied. According to Bray (10) within certain limits the yield and the protein content of the corn grain have been found to be related to the nitrate level in the plant.

Many factors must be considered in interpreting the results of nitrate tests according to Krantz, Nelson and Burkhart (41). One of these factors is the time of day at which the tests are made. According to Welch, referred to by Krantz, Nelson and Burkhart (41), the nitrate status of the corn plant was lowest in early morning. As the day advanced the nitrate level increased, reaching its peak from 11:00 A.M. to 3:00 P.M. after which it decreased to a level somewhat above that of early morning.

It was reported by Knowlton (39) that nitrate nitrogen was reduced and ready for plant use within twelve days after the nitrate nitrogen was applied to the soil. There was apparently little cross transfer of elements within plants. Auchter (4) concluded that the mineral nutrient elements absorbed by the roots on one side of a woody plant are translocated to and used by the trunk, main branches, twigs and foliage directly above them.

Thornton (66) stated that tests made on different parts of plants may give widely different values. Invariably a larger inorganic phosphorus content was found in the translocation and storage tissues than in the leaf tissues. The leaf phosphorus was also less sensitive to changes in conditions of fertility. An accumulation of inorganic phosphorus in the actively growing portions of plants makes the upper portions more suitable when testing for phosphates. (Thornton (66)). According to Ulrich (70) not only was it important to select the proper part of the plant for analyses, but the tissues must be taken from a definite position. This position differs with the crop sampled and also with the nutrient element assayed.

In working with peach trees Frear, Anthony, Haskins and Hewetson (28) found that leaves from the basal (oldest) portion of the current season's growth were more suitable for estimating the level of potassium supply available to the trees. According to Wood (73) various investigators have used whole leaves, have removed the petioles before analysis, have used old leaves, the growing tips or the skeleton of the leaves after a special treatment to remove parts of the tissue. Some workers have used specific leaves, such as the first mature leaf behind the growing tip in order to discover an index leaf that would eliminate the necessity of destroying the plant in diagnosis so as to indicate a cure before the plant was severely damaged. According to Thornton, Conner and Fraser (67) the main stems or leaf petioles were in general most satisfactory for nitrogen tests. These workers also reported that phosphorus tests should be made on the main stems or leaf petioles from actively growing portions of the plant and that potassium tests should be made on the main stem or leaf petiole.

Carolus (12) states that when the supply of all nutrient elements except nitrogen is sufficient for normal growth, the lack of an adequate amount of nitrogen usually results in an extremely high concentration of soluble phosphorus and a comparatively low concentration of both total soluble and nitrate nitrogen in the petioles of vegetable crops.

According to Scarseth (52) the Purdue plant tissue tests indicated a nutrient element deficiency before the plants showed starvation symptoms. Scarseth (52) states that in making a rapid chemical determination of the contents of the plant tissues with the Purdue plant tissue test method, the operator is in fact, looking at the conveyors (xylem) in the plant to observe if plenty of such essential elements as nitrates, inorganic phosphates, and potassium are entering the plant and passing to the areas where these elements

are needed. This test indicates the presence or absence of these elements in the conducting tissues of the plant in soluble unassimilated form. Scarseth (51) states that the assimilation of potassium is not understood. Therefore the potassium test does not show nutrients that have been converted into organic compounds.

Krantz, Nelson and Burkhart (41) state that plant tissue tests should supplement rather than replace other tests and measurements. As pointed out by Scarseth (51), in general, soil tests help to estimate the supply status, tissue tests help to indicate the first limiting nutritional element factor, and deficiency symptoms indicate extreme and damaging shortages in plant tissues.

In recent years various types of organic plant food materials such as "Ra-Pid-Gro" and "Nu-Green" have been developed as sprays for feeding plants through their foliage. This type of fertilization, if successful, would undoubtedly be of great benefit in correcting deficiencies or maintaining a proper nutrient balance, especially with tree crops. Foliage sprays supplying nitrogen in the form of Urea have been used to maintain an apparently satisfactory level of nitrogen nutrition in McIntosh apple trees by workers in New York (26). Pecan Rosette due to zinc deficiency has been controlled by foliar sprays of zinc sulfate. Inciardi (38) using tissue tests reported no observable nitrogen absorption in spinach when liquid nitrogen sprays were applied.

Methods and Materials

Pecan trees of various ages and sizes treated with varying types and amounts of fertilizers were used for sampling purposes.

Sixteen one year old seedling pecan trees were brought into the greenhouse on February 27th in an attempt to get an early growth of foliage. Two trees were placed in each of eight wooden tubs with a capacity of approximately 1.43 cubic feet. A sandy loam soil of average fertility was used and the tubs were placed in the warm section of the greenhouse at a temperature of approximately 80 degrees Farenheit. The tubs were moved outside into the open on May 4th.

Pecan trees both native and natives top worked to named varieties growing on the banks of Cow Creek, in the college grove, were used for fertilizer practices and sampling.

One year old seedling pecan trees growing in the college nursery were also used for testing various concentrations of "Ra-Pid-Gro" and "Nu-Green", foliar fertilizer sprays.

Other trees used for sampling were located in the R. C. Walker pecan groves in the Arkansas River flood plain near Bixby, in Tulsa County. The soil of this area is principally a Miller silty clay.

Soil Fertilizer Treatments.

Approximately 667 cc. of Ammonium nitrate solution equaling a fertilizer application of seventy pounds of soluble nitrogen per acre was added to each wooden tub containing two seedling pecan trees on April 27th. The object was to induce faster leaf development in order to secure material for testing.

On May 4th when these trees were moved outside, "Ra-Pid-Gro" nutrient solution was prepared and applied to the total leaf area of one tree in each tub with a camel hair brush. The solution was prepared according to the directions accompanying the materials. The rate was 1 pound/22 gallons of water.

The following treatments were applied to trees in each tub on June 2nd.

Table 1.

Tub No.	Treatment
1	Two liters of Ra-Pid-Gro solution to the soil. 1#/22 gal. H ₂ O.
2	226 grams of muriate of potash.
3	226 grams of NaNO ₃ .
4	226 grams of 4-12-4.
5	226 grams of NH ₄ NO ₃ .
6	226 grams of Ammo. Phos.
7	Check.
8	Ra-Pid-Gro 1#/22 gal. H ₂ O applied to leaves.

These tubs were fertilized in an attempt to provide trees that were known to be well supplied with nutrient elements.

The following trees growing along the banks of Cow Creek were used for testing.

Table 2.

Tree	Diameter of Tree	Treatment
Early Bird 5*	3.5"	1 lb. of NH_4NO_3 to each 1" diam. of the tree applied on 3/24/49 and again on 1/27/50.
Burkett 16*	9.0"	1 lb. of NH_4NO_3 to each 1" diam. of the tree applied on 3/24/49 and again on 1/27/50.
Love 12*	3.0"	2 lb. of Ammo. Phos. (16-20-0) to each 1" diam. of the tree was applied on 3/25/49 and 1/28/50.
Tree # 1 NaNO_3	6.0"	NaNO_3 applied around the base of tree at rate 1 lb. to 1" diam. of tree, 5/25/50 & 6/7/50.
Tree # 3 NaNO_3	12.0"	NaNO_3 applied around the base of tree at rate 1 lb. to 1" diam. of tree, 5/25/50 & 6/7/50.
Tree # 4	12.0"	No treatment. Used for comparison studies. Tree next to tree # 3 NaNO_3 .
Tree # 5	12.0"	No treatment. Used for comparison studies. In the same row as tree # 3 NaNO_3 and tree # 4.
Tree # 150	23.0"	No treatment.
Tree # 154	18.0"	No treatment.
Tree # 162	24.0"	No treatment.
Texas Prolific	18.0"	No treatment.
Stuart # 1	15.0"	No treatment.
Stuart # 2	18.0"	No treatment.
Halbert	12.0"	No treatment.

* Starred trees are those that had been fertilized during the 1949 growing season. All applications of fertilizers were made on the basis of the diameter of the tree trunks breast high.

In the above table, sodium nitrate was applied by digging several holes approximately 18 to 24 inches deep with a post hole digger around the outside perimeter of the trees and adding the fertilizer in these holes.

The trees listed in the following table are those used in the R. C. Walker grove southeast of Bixby. These were used to determine if possible the benefits derived from potassium fertilization, however, tests for all of the elements were made on all trees. The potassium fertilizer applied to these trees was broadcast under the branches during the 1949 season.

Table 3.

Tree	Diameter of Tree	Treatment
Tree # 3 San Saba Imp.	13-14"	100 lb. 25% potash applied 1949
Tree # 4 San Saba Imp.	14"	100 lb. 25% potash applied 1949
Tree # 5 Western Schley	12"	100 lb. 25% potash applied 1949
Tree # 6 Western Schley	12"	No fertilization

Rapid Plant Tissue Tests.

The Purdue tissue test kit was used for making quick tests on plant tissue. In testing for nitrates and phosphates the procedure recommended by Thornton, Conner and Fraser (67) was followed. A modification of the potassium test in the Purdue quick test kit was prepared according to the directions given by Garrad (29). The reagents for this test are essentially the same as those used in the first potassium test. The new test, however, consists of a new extracting solution of 15 percent sodium nitrite acidified to pH 5.0 with acetic acid. This reagent is practically the same as the potash reagent No. 1 in the Purdue kit except that it lacks the sodium cobaltinitrite. With the extra solution

and some carbon black for clarifying, the modified test has been satisfactory when used on peach tissue (29).

In connection with the Purdue rapid tissue test kit, Bray's nitrate powder described by Bray (9) was used. This is essentially a white powder which with plant tissue forms a pink dye through the interaction of nitrous acid with alpha-naphthylamine and sulfanilic acid in an acid medium. Manganous sulfate is used to prevent interference from chlorides and gives a more nearly quantitative reduction of nitrates to nitrites.

A quick test for potassium explained by Melsted (46) was also used in attempting to determine the presence of potassium in pecan tissues. It is the dipicrylamine spot test method, modified and adapted for field use. A drop of the test reagent is placed on a strip of filter paper and allowed to dry. Then a drop of plant sap is squeezed onto the test spot, allowed to react about thirty seconds, and then the paper is immersed in a solution of 0.5 normal hydrochloric acid for thirty seconds. If a reddish spot remains, potassium is present; otherwise, the test turns a lemon yellow color. Preparation of the test reagent is explained by Melsted (46).

Chemical analyses were made of the leaves and shoots from certain trees to determine the percent of total nitrogen, phosphorus and potassium present. Results of these analyses were compared to the Purdue quick tissue tests. In selecting tissue for chemical analysis the leaf samples were taken at random over the tree. The leaves most characteristic of an individual shoot were selected. Samples were analyzed in duplicate and the average taken using the Kjeldahl method for determining nitrogen and the ash analyses method for determining phosphorus and potassium.

Soil samples were taken and tested to determine if the amount of nutrient elements in the soil could be correlated with those being taken up by the trees

as indicated by the quick tests and the total chemical analyses made in the laboratory. Soil samples were analyzed in the soil laboratory. Rapid soil tests were made with the Simplex soil kit as well as the Purdue kit.

Sampling.

In sampling, various methods were investigated to determine the portion of the tree most desirable for testing and the best procedure to follow in gathering samples for tests.

Quick tests for nitrates using the Purdue tissue test method were made on diced petioles, the leaflets of the shoots, and the main shoots. The actual tests were made by cutting across the end of leaf petioles, or by making longitudinal cuts of the stems and applying nitrate reagent directly to the cut surface or to the base of the petioles where the leaf was snapped from the shoot. Leaves for sampling were taken from the lower branches of the tree starting at the trunk and proceeding outward to the outer perimeter of the tree.

Ten shoots were taken at random from tree 3 which after fertilization with nitrate of soda tested positive for nitrates with the Purdue kit. The petioles were taken from these ten shoots one at a time from each shoot and tested for nitrates in an attempt to determine which petiole on the shoot would be most reliable for the nitrate test. Krantz, Nelson and Burkhart (41) brought out the fact that nitrates in the plant varied with the time of day samples were taken. Following up the results of this work nitrate tests were made on two trees, at hourly intervals from sunup until sunset using the Purdue quick tissue test kit. Petioles were snapped off at random over the trees and tested. Several petioles were tested from each tree at each hourly interval.

In order to determine portions of the petiole that contained the nitrates free hand sections as thin as could be cut with a razor blade were mounted on slides. These sections were examined under the low power of a microscope after

adding one drop of the Purdue nitrate reagent. The tissue producing the blue color was thus observed.

Basal portions of petioles were killed and fixed in formaldehyde, alcohol and acetic acid solution (F.A.A.) and the n-butyl alcohol method of infiltrating and embedding described by Sass (49) was followed. Sections were made with the aid of a sliding microtome. A drawing was made of a cross section of the base of the petiole to show the region where nitrates accumulated.

Quick tests for inorganic phosphates were made by dicing leaf petioles, leaflets and the main stem of shoots. A phosphate test on petioles from the base of the shoot upward was also made to determine if a certain petiole was more dependable than others in testing for phosphates. Phosphate tests were made from sunup until sundown at two hour intervals in the same manner as the nitrate tests. It has been reported by Alben (2) however, that phosphorus in pecan leaves is fairly stable and varies little during the day.

In collecting tissue for potassium tests, samples were taken at random over a tree. Tests for potassium were made on diced leaflets, the leaf petioles and the main stem of shoots. Ten shoots were taken at random from tree 5. The basal petiole from each shoot was used, therefore the ten petioles collected were mixed in the final sample for each test. The other petioles likewise were used to make up the samples for testing the entire shoot.

Shoots representative of the majority of those on the tree were selected for testing in all cases.

Early in the season core samples from the trunks were taken from two trees with a one-fourth inch increment borer. The core was taken deep enough to include the (xylem) conductive tissue. Nitrate tests were made on these core samples to determine if nitrates were present in the conductive tissues of the trees as growth started in the spring.

The Purdue quick test kit was used to test for nitrate absorption from foliar sprays. "Ra-Pid-Gro" and "Nu-Green" two commercially prepared products were used in these tests. The guaranteed analyses of "Ra-Pid-Gro" is 23 percent nitrogen, 21 percent phosphoric acid and 17 percent potash. "Nu-Green" contains 44 percent nitrogen in the form of Urea.

Four different concentrations of "Ra-Pid-Gro" and "Nu-Green" were used at various times and upon various trees to test for the possibility of using such nitrogen solutions to correct nitrogen deficiencies. A test was made before spraying to determine if any nitrates were present.

Tree 4 (Table 2) was used for the testing of the four different concentrations of the two above mentioned foliage sprays. One branch was used for each concentration. Each solution was applied with a two and one-half gallon hand pump type sprayer. Two quarts of each solution were applied to each branch. The following table shows the various solutions prepared and the concentrations at which they were applied.

Table 4.

Treatment	Concentration of Treatment
Ra-Pid-Gro # 1	1 lb./22 gal. H ₂ O or 2 teaspoons to 2 qts. H ₂ O.
Ra-Pid-Gro # 2	2 lb./22 gal. H ₂ O or 4 teaspoons to 2 qts. H ₂ O.
Ra-Pid-Gro # 3	3 lb./22 gal. H ₂ O or 6 teaspoons to 2 qts. H ₂ O.
Ra-Pid-Gro # 4	4 lb./22 gal. H ₂ O or 8 teaspoons to 2 qts. H ₂ O.
Nu-Green # 1	1 lb./20 gal. H ₂ O or 6 teaspoons to 2 qts. H ₂ O.
Nu-Green # 2	2 lb./20 gal. H ₂ O or 12 teaspoons to 2 qts. H ₂ O.
Nu-Green # 3	3 lb./20 gal. H ₂ O or 18 teaspoons to 2 qts. H ₂ O.
Nu-Green # 4	4 lb./20 gal. H ₂ O or 24 teaspoons to 2 qts. H ₂ O.

The first branches were sprayed at 9:00 o'clock and the remainder as soon

as possible thereafter. Nitrate tests were made on various petioles from each shoot on the sprayed branches at 10:00 A.M., 11:15 A.M., and 5:00 P.M. of the same day. Other tests were made in succeeding days on the same branches at various times of the day.

Tree 75, a tree having no fertilizer treatment and showing no test for nitrates using the Purdue kit was also treated with Number 1 and Number 4 concentrations of "Ra-Pid-Gro" solutions. On this tree, however, various shoots were dipped in the solutions, tagged and tested at intervals to check for nitrogen absorption.

Experimental Results and Discussion

Nitrates. Adapting a rapid nitrate test to pecan trees and comparing results with chemical analyses.

Initial studies were conducted in February and March using small one year old seedling pecan trees. These had been transferred to the greenhouse in order to make preliminary investigations of the best sampling and testing procedures. Nitrate tests using the Purdue nitrate reagent were made on buds and diced leaflets of the seedling pecan trees as they started growth and before the fertilizers were applied. No blue coloring appeared as an indication of the presence of nitrates. The sulfuric acid of the nitrate reagent discolored and charred the tissue before the presence of color could be detected.

On June 6th, 1950, these same trees, which had been variously fertilized on June 2nd, were tested for nitrates using reagents of the Purdue test kit. The level and type of fertilizer applications and the results obtained are summarized in Table 5. These results indicate that under these conditions the test for nitrates using the Purdue kit was sufficiently obvious to overcome the charring. The test was positive at the basal region of the petiole. In applying the test reagent to the base of the petiole, one drop of reagent applied to the fresh tissue immediately after the petiole was snapped off and held in an upright position, developed in a few seconds the blue coloring indicative of the presence of nitrogen. A scale for use in classifying the amount of nitrate in a petiole was set up on the basis of the intensity of the blue coloring as follows:

- | | |
|--------------------------|----------------------------|
| 0 - No test | 5 - Medium test |
| 1 - Light trace | 6 - Medium to high test |
| 2 - Trace | 7 - High test |
| 3 - Light test | 8 - High to very high test |
| 4 - Light to medium test | 9 - Very high test |

Table 5. Rapid tissue tests applied to trees growing in tubs. June 6th.

Tub No.	Treatment	Tests
1	Ra-Pid-Gro 2 liters applied to soil.	NO ₃ - Light trace P ₂ O ₅ - Medium test K ₂ O - Test inadequate
2	Muriate of potash. 1 lb./1" diameter of tree.	NO ₃ - No test P ₂ O ₅ - Very low test K ₂ O - Very high test
3	Sodium nitrate. 1 lb./1" diameter of tree.	NO ₃ - Very high test P ₂ O ₅ - Medium test K ₂ O - Test inadequate
4	4 - 12 - 4 fertilizer	NO ₃ - High test P ₂ O ₅ - Medium test K ₂ O - Test inadequate
5	Ammonium nitrate. 1 lb./1" diameter of tree.	NO ₃ - Very high test P ₂ O ₅ - Very low test K ₂ O - Test inadequate
6	Ammo. Phos. (16-20-0)	NO ₃ - Very high test P ₂ O ₅ - Medium test K ₂ O - Test inadequate
7	Check	NO ₃ - Light test to no test P ₂ O ₅ - Medium test K ₂ O - Test inadequate
8	Ra-Pid-Gro applied to leaves.	NO ₃ - No test P ₂ O ₅ - K ₂ O -

Phosphorus and potassium tests were based on the color charts accompanying the Purdue kit.

Trees in the check tub showed a light trace of nitrates as they had been fertilized with ammonium nitrate earlier in an effort to speed up growth. Results of the tissue tests (Table 5) correlate fairly well with the results obtained with the Simplex soil kit (Table 6).

Bray's nitrate powder was also used in testing for the presence of nitrates. Variations in the pink color as an indication of high, low or medium nitrates and various intergradations between these could not be distinguished so well as in the case of the Purdue nitrate test. It was decided that the Purdue nitrate reagent was the more suitable of the two for this work and hence was used throughout the remainder of the investigations.

Trees growing in the pecan grove were tested by the several methods mentioned heretofore to determine a suitable procedure to follow in detecting nitrates.

By April 23rd, 1950, pecan trees growing out of doors were beginning to develop new leaves and shoots. Tests for nitrates, phosphates and potassium were made on a mature Mahan tree growing on the horticulture grounds in bermuda sod. The new leaflets and shoots were diced together and the tests made on this material according to the directions in the Purdue kit. No positive test was obtained for either of the three elements nitrogen, phosphorus and potassium. Efforts were made to detect a nitrate test for pecan tissue by dicing new shoot growth and young leaflets in a vial, a procedure which was found to give a satisfactory nitrate test by Inciardi (38) on tomatoes. This procedure with pecan tissues gave no positive results.

Table 6. Soil tests made on soil in tubs. June 19.

Tub No.	Laboratory Analyses	Rapid Soil Tests
1	pH - Basic P - 20 K - 102.4 ppm.	NO ₃ - Medium 25 ppm. P ₂ O ₅ - Low 1 ppm. K - Medium 10 ppm.
2	pH - Basic P - 20 K - 400 ppm	NO ₃ - Medium 10 ppm. P ₂ O ₅ - Medium to moderately high. 2-5 ppm. K - Very high. More than 20 ppm.
3	pH - Basic P - 20 K - 122.0 ppm.	NO ₃ - Very high P ₂ O ₅ - Moderately high. 5 ppm. K - Medium. 5-10 ppm.
4	pH - Slightly acid P - 20 K - 285.6	NO ₃ - Very high. 125 ppm. P ₂ O ₅ - Moderately high. 5 ppm. K - High. 20-25 ppm.
5	pH - Neutral P - 20 K - 108.8	NO ₃ - Very high. 250 ppm. P ₂ O ₅ - Moderately high. 5 ppm. K - Exceedingly high. above 25 ppm.
6	pH - Slightly basic P - 20 K - 112.0	NO ₃ - Medium. 10 ppm. P ₂ O ₅ - Moderately high. 5 ppm. K - High. Greater than 20 ppm.
7	pH - Basic P - 20 K - 108.8	NO ₃ - Low to medium P ₂ O ₅ - Low. 1 ppm. K - Medium. 10-20 ppm.
8	pH - Basic P - 20 K - 118.8	NO ₃ - 2 ppm. or less P ₂ O ₅ - Low. 1 ppm. K - Medium. 5-10 ppm.

On May 12th, 1950, trunk core samples were taken with a one-fourth inch increment core sampler from various trees both fertilized and unfertilized, to determine if nitrates were being transported upward through the conducting tissue in the tree trunks. Nitrate tests made on these samples gave no evidence that nitrates were present, however, a brownish discoloration of the tissue developed due to disintegration of the cells and may have covered up any blue coloring which indicates the presence of nitrates. On May 12th, trees: Early Bird 5 and Love 12 were tested for the presence of nitrates using various techniques. Both trees had been fertilized (Table 2). Leaf petioles were split and the nitrate reagent applied to the fresh longitudinal cut. No blue coloring was observed to indicate the presence of nitrates. Also petioles, leaflets and main shoots were diced into small pieces, placed in a vial and tested. The results were all negative.

On June 6th, nitrate tests were made on tree 1 and tree 3 both having been fertilized with nitrate of soda. Samples were prepared by cutting across the base of the leaf petioles with a razor blade and the fresh cut surface tested by applying one drop of the nitrate reagent from the Purdue kit. A light trace of nitrates was detected on both of these trees.

On June 7th nitrate tests were made again on the same two trees, but instead of cutting across the base of the leaf petioles at the place of attachment the petioles were snapped off and tested. It seemed that breaking petioles from the main shoot gave better results than cutting with a razor blade. A positive test was obtained for nitrates on tree 1 from the lower branches near the trunk of the tree. On tree 3 nitrate tests were obtained from samples taken near the base of the tree and also from those taken three-fourths of the way up in the tree. Above this point no positive tests were secured.

Having established a successful method by which pecan tree tissue could

be tested for the presence of nitrates, this method was used throughout the remainder of this study. After finding that nitrates were present in pecan tissue and could be detected with the Purdue nitrate reagent, free hand cross sections were made of the petioles and put under a microscope and one drop of nitrate reagent added to determine from which area the blue color developed. From all the observations made, indications were that the nitrates were present in the parenchyma cells of the pith. There was no indication of blue color coming from the conductive tissues (xylem). In one instance where a high test for nitrates was detected at the base of the petiole it was found that nitrates were present in the pith approximately three-fourths of the way up the length of the petiole. Figure 2A shows the area in which nitrates are to be found in pecan tissue. The arrows in Figures 1 and 3 point to the place where the petiole was snapped from the main shoot and Figure 4 shows the base of the petiole where the test for nitrates was obtained.

Since it was found that nitrate nitrogen does reach the upper regions of the pecan tree and is not all reduced in the roots before reaching the top, comparisons were made between the Purdue quick test and chemical analyses. In chemical analyses nitrogen was expressed as the percent of total nitrogen on a dry weight basis. In comparing data of these two tests any correlation between nitrate nitrogen and total nitrogen should be brought out. It was expected that perhaps a certain degree of color in the tissue test would agree with a level of total nitrogen expressed as percentage of dry weight. From the data in Table 7 it seems that there is a general trend in this direction. The nitrogen content of tissues giving a positive tissue test were generally higher in total nitrogen analysis. When such is not the case as in tree 1 it is possible to advance at least a partial explanation. This tree on May 31st and June 6th tested higher in total nitrogen than on July 22nd, while



Figure 1.

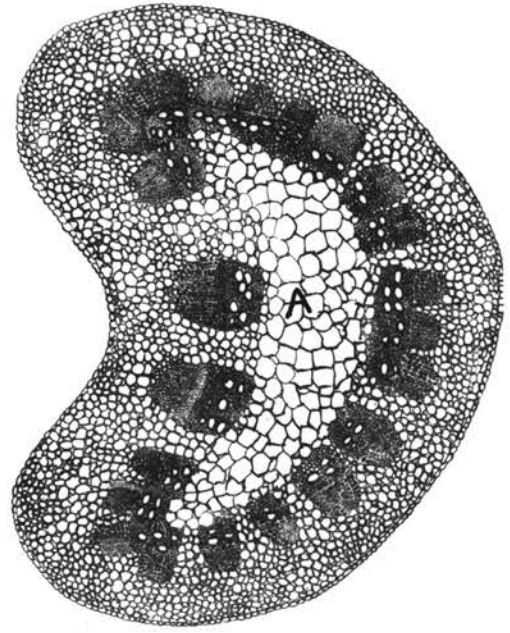


Figure 2.



Figure 3



Figure 4.

a high test was shown for nitrates by the Purdue kit on July 22nd and no test for nitrates on the other two dates. This can be explained by the fact that on May 25th, only twelve days before June 6th when samples were taken, this tree was fertilized with sodium nitrate; one pound to one inch diameter of the tree. From this data it would seem that at this time the nitrates had not yet reached the shoots. By July 22nd however, a comparatively large amount of nitrates had undoubtedly been absorbed and taken into the tree but had not yet been reduced to nitrite nitrogen. Also it seems possible that the leaf area was larger and the percentage of total nitrogen was less on the basis of the amount of tissue analyzed.

From the limited amount of data obtained it seems that the Purdue test cannot be used satisfactorily to determine the percentage of total nitrogen in a tree. These are only indications, however, and it seems necessary that many more tests be made before any definite conclusions are drawn.

Data from Table 7 gives evidence that some trees show a relatively high total nitrogen content and still show no test for nitrates. In other cases trees show a high total nitrogen content and also a high nitrate content. This seems to indicate that some trees require more nitrogen than others or have a better metabolic system than others, thus reducing nitrates as fast as they are taken up and allowing for no surplus to accumulate in the petioles.

According to a report by Shear, Crane and Meyers (54) considerable work has been done in attempt to correlate the nitrogen content of the leaves and the nutritional status of plants with respect to the element nitrogen in tung trees. This evidence indicates that the supply of nutrient elements other than nitrogen may often be more effective in altering the nitrogen content of leaves than the supply of nitrogen itself. From work with tung trees growing in nutrient solutions these workers found that when the nitrogen supply was kept constant

and phosphorus was supplied at various levels the nitrogen content of the leaves in percentage of dry matter increased in close proportion to the amount of phosphorus added.

From the data obtained from chemical analyses of pecan leaves there seems to be a correlation between phosphorus and nitrogen as shown by the data in Table 9.

It was pointed out by Shear, Crane and Meyers (54) that leaf analyses showing unusually high or low nitrogen content should immediately direct attention toward the determination of the nutritional imbalance responsible for this situation. This seems to apply to the results obtained here.

From the total nitrogen analyses data (Table 7), information comparable to that found by Hammar and Hunter (32) is shown. There is a definite peak of total nitrogen during the earlier part of the growing season. It then seems to decline gradually until the end of the season.

Table 10 shows results obtained with the Purdue nitrate tests at hourly intervals from sunup until sunset. The tests were made on two trees, one fertilized and one non-fertilized. Results indicate a high accumulation of nitrates in the tissues between 7:30 A.M. and 10:30 A.M. Another relatively high point is between 4:30 and 5:30 in the afternoon. Tree 5 showed a very high test at 5:30 A.M. while tree 3 showed a rise and decrease in nitrates an hour previous to tree 5. This would seem to indicate that tree 3 would have tested very high in nitrates at 4:30 A.M.

Table 11 shows the results obtained when petioles from ten different shoots were tested for nitrates in an attempt to determine if any one certain petiole on a shoot would be more reliable in testing for nitrates than the others. Each graph represents one shoot. Results indicate that from the ten shoots observed nitrates occurred forty percent of the time in the third and fifth petioles

upward from the base and fifty percent of the time in the fourth petiole upward from the base. The remaining petioles showed nitrates thirty percent of the time or less. Petioles three, four and five would therefore seem to be the best indicators of nitrates on an average. Tests were all made with the Purdue kit.

Table 7. Comparing total nitrogen of pecan tissues with nitrates obtained with the Purdue quick test.

Tree	Date Sampled	Total Analyses	Quick Tests
Texas Prolific	July 22	2.18%	Trace
	August 11	2.15	No NO ₃
	Sept. 22	2.03	Trace
Stuart # 1	July 22	2.54	No NO ₃
	August 11	2.79	No NO ₃
	Sept. 22	2.34	No NO ₃
Halbert	July 22	2.17	No NO ₃
	August 11	2.24	No NO ₃
	Sept. 22	2.05	No NO ₃
Stuart # 2	July 22	2.16	Light test
	August 11	2.14	No NO ₃
	Sept. 22	2.12	No NO ₃
Tree # 154	July 22	2.22	No NO ₃
	August 11	2.33	No NO ₃
	Sept. 22	2.57	No NO ₃
Tree # 150	July 22	2.28	No NO ₃
	August 11	2.42	Light test
	Sept. 22	2.19	No NO ₃
Love 12	July 22	2.14	No NO ₃
	August 11	2.19	Light test
	Sept. 22	1.88	No NO ₃
Early Bird 5	July 22	2.44	Light to medium
	August 11	2.29	No NO ₃
	Sept. 22	2.33	No NO ₃
Burkett 16	July 22	2.22	No NO ₃
	August 11	2.23	No NO ₃
	Sept. 22	2.14	No NO ₃
No. 1 NaNO ₃	May 31	3.02	No NO ₃
	June 6	2.96	No NO ₃
	July 22	2.39	High test
	August 11	2.31	High test
	Sept. 22	2.22	High to very high

Table 7. (cont'd)

Tree	Date Sampled	Total Analyses	Quick Tests
No. 3 NaNO ₃	June 6	2.97	Medium test
	July 22	2.27	Light test
	August 11	2.18	No NO ₃
	Sept. 22	2.24	Light trace
Tree # 3 Walker's	June 28	2.43	Light trace
	August 10	2.35	No NO ₃
Tree # 4 Walker's	June 28	2.23	No NO ₃
	August 10	2.18	No NO ₃
Tree # 5 Walker's	July 28	2.33	No NO ₃
	August 10	2.31	No NO ₃
Tree # 6 Walker's	July 28	2.08	No NO ₃
	August 10	2.18	No NO ₃
Tree # 4 West side	July 22	2.24	No NO ₃
	August 11	2.26	No NO ₃
	Sept. 22	2.23	No NO ₃
Tree # 5 West side	August 11	2.31	Very high
	Sept. 22	2.31	Light test

Table 8. Comparison of total nitrogen with nitrates on trees grouped together according to similar soil type and soil conditions.

Tree	Date Sampled	Nitrogen Analysis	Nitrate Tests
Early Bird 5	May 12	3.33	No NO ₃
Love 12	May 12	2.97	No NO ₃
No. 1 NaNO ₃	June 6	3.02	No NO ₃
No. 3 NaNO ₃	June 6	2.97	Light trace
Tree # 3 Walker's	June 28	2.43	Light trace
Tree # 4 Walker's	June 28	2.23	No NO ₃
Tree # 5 Walker's	June 28	2.33	No NO ₃
Tree # 6 Walker's	June 28	2.08	No NO ₃
Texas Prolific	July 22	2.18	Light trace
Halbert	July 22	2.17	No NO ₃
Stuart # 1	July 22	2.54	No NO ₃
Stuart # 2	July 22	2.16	Light test
Tree # 154	July 22	2.22	No NO ₃
Tree # 150	July 22	2.28	No NO ₃
Love 12	July 22	2.14	No NO ₃
Early Bird 5	July 22	2.44	Light to Medium
Burkett 16	July 22	2.22	No NO ₃
No. 1 NaNO ₃	July 22	2.39	High test
No. 3 NaNO ₃	July 22	2.27	Light test
Tree # 4 West side	July 22	2.24	No NO ₃
Texas Prolific	August 10-11	2.15	No NO ₃
Halbert	"	2.24	No NO ₃
Stuart # 1	"	2.79	No NO ₃
Stuart # 2	"	2.14	No NO ₃
Tree # 154	"	2.33	No NO ₃
Tree # 150	"	2.42	Light test
Tree # 162	"	2.40	No NO ₃
Love 12	"	2.19	Light Test
Early Bird 5	"	2.29	No NO ₃
Burkett 16	"	2.23	No NO ₃
No. 1 NaNO ₃	"	2.31	High test
No. 3 NaNO ₃	"	2.18	No NO ₃
Tree # 3 Walker's	"	2.35	No NO ₃
Tree # 4 Walker's	"	2.18	No NO ₃

Table 8 (cont'd).

Tree	Date Sampled	Nitrogen Analysis	Nitrate Tests
Tree # 5 Walker's	August 10-11	2.31	No NO ₃
Tree # 6 Walker's	"	2.18	No NO ₃
Tree # 4 West side	"	2.26	No NO ₃
Tree # 5 West side	"	2.31	Very high test
Early Bird 5	Sept. 22	2.33	No NO ₃
Love 12	Sept. 22	1.88	No NO ₃
Burkett 16	Sept. 22	2.14	No NO ₃
Tree # 1 Na NO ₃	Sept. 22	2.22	High to very high
Tree # 3 Na NO ₃	Sept. 22	2.24	Light trace
Tree # 4 West side	Sept. 22	2.23	No NO ₃
Tree # 5 West side	Sept. 22	2.31	Light test
Tree # 150	Sept. 22	2.19	No NO ₃
Tree # 154	Sept. 22	2.57	No NO ₃
Texas Prolific	Sept. 22	2.03	Trace
Halbert	Sept. 22	2.05	No NO ₃
Stuart # 1	Sept. 22	2.34	No NO ₃
Stuart # 2	Sept. 22	2.12	No NO ₃

Table 9. Correlating total phosphorus with total nitrogen content in pecan tissue.

Tree	Date sampled	Total Nitrogen	Total Phosphorus
Texas Prolific	May 5, 1950	3.60%	.370%
Texas Prolific	July 22, 1950	2.17	.121
Texas Prolific	August 11, 1950	2.15	.142
Texas Prolific	Sept. 22	2.03	.138
Stuart # 1	May 5, 1950	3.48	.416
Stuart # 1	July 22, 1950	2.54	.149
Stuart # 1	August 11, 1950	2.78	.133
Stuart # 1	Sept. 22, 1950	2.34	.165
Halbert	May 5, 1950	3.52	.374
Halbert	July 22, 1950	3.16	.130
Halbert	August 11, 1950	2.23	.120
Halbert	Sept. 22	2.05	.141
Stuart # 2	May 5, 1950	3.68	.421
Stuart # 2	July 22, 1950	2.15	.158
Stuart # 2	August 11, 1950	2.13	.116
Stuart # 2	Sept. 22, 1950	2.12	.147
Tree # 154	May 6, 1950	3.66	.423
Tree # 154	July 22, 1950	2.22	.156
Tree # 154	August 11, 1950	2.32	.145
Tree # 154	Sept. 22, 1950	2.57	
Tree # 158	May 6, 1950	3.15	.425
Tree # 150	May 6, 1950	3.72	.504
Tree # 150	May 31, 1950	2.79	.283
Tree # 150	July 22, 1950	2.28	.167
Tree # 150	August 11, 1950	2.41	.161
Tree # 150	Sept. 22, 1950	2.19	
Tree # 162	May 6, 1950	3.56	.472
Tree # 162	May 31, 1950	2.86	.259
Tree # 162	August 11, 1950	2.39	.145
Love 12	May 12, 1950	2.97	.300
Love 12	May 12, 1950	2.93	.305
Love 12	July 22, 1950	2.14	.144
Love 12	August 11, 1950	2.19	.119
Love 12	Sept. 22, 1950	1.88	.147
Early Bird 5	May 12, 1950	3.33	.354
Early Bird 5	May 12, 1950	3.44	.352
Early Bird 5	May 31, 1950	3.01	.224
Early Bird 5	July 22, 1950	2.44	.136
Early Bird 5	August 11, 1950	2.29	.109
Early Bird 5	Sept. 22, 1950	2.33	

Table 9 (cont'd).

Tree	Date sampled	Total Nitrogen	Total Phosphorus
Burkett 16	May 24, 1950	3.29	.411
Burkett 16	May 31, 1950	2.54	.206
Burkett 16	July 22, 1950	2.22	.120
Burkett 16	August 11, 1950	2.23	.125
Burkett 16	Sept. 22, 1950	2.14	.148
No. 1 NaNO ₃	May 31, 1950	3.02	.255
No 1 NaNO ₃	June 6, 1950	2.96	.245
No. 1 NaNO ₃	June 6, 1950	3.08	.313
No. 1 NaNO ₃	July 22, 1950	2.39	.140
No. 1 NaNO ₃	August 11, 1950	2.30	.137
No. 1 NaNO ₃	Sept. 22, 1950	2.22	.157
No. 3 NaNO ₃	May 31, 1950	2.53	.219
No. 3 NaNO ₃	June 6, 1950	2.97	.284
No. 3 NaNO ₃	June 14, 1950	---	---
No. 3 NaNO ₃	July 22, 1950	2.27	.144
No. 3 NaNO ₃	August 11, 1950	2.17	.147
No. 3 NaNO ₃	Sept. 22, 1950	2.24	.183
Tree # 3 Walker's	May 24, 1950	2.92	.251
Tree # 3 Walker's	June 28, 1950	2.43	.163
Tree # 3 Walker's	August 10, 1950	2.35	.141
Tree # 4 Walker's	May 24, 1950	2.64	.240
Tree # 4 Walker's	June 28, 1950	2.23	.156
Tree # 4 Walker's	August 10th, 1950	2.17	.135
Tree # 5 Walker's	May 24, 1950	2.80	.220
Tree # 5 Walker's	June 28, 1950	2.33	.189
Tree # 5 Walker's	August 10, 1950	2.30	.130
Tree # 6 Walker's	May 24, 1950	2.74	.191
Tree # 6 Walker's	June 28, 1950	2.08	.155
Tree # 6 Walker's	August 10, 1950	2.18	.118
Tree # 4 West side	July 22, 1950	2.24	.142
Tree # 4 West side	August 11, 1950	2.26	.157
Tree # 4 West side	Sept. 22, 1950	2.23	.185
Tree # 5 West side	August 11, 1950	2.30	.147
Tree # 5 West side	Sept. 22	2.31	.187

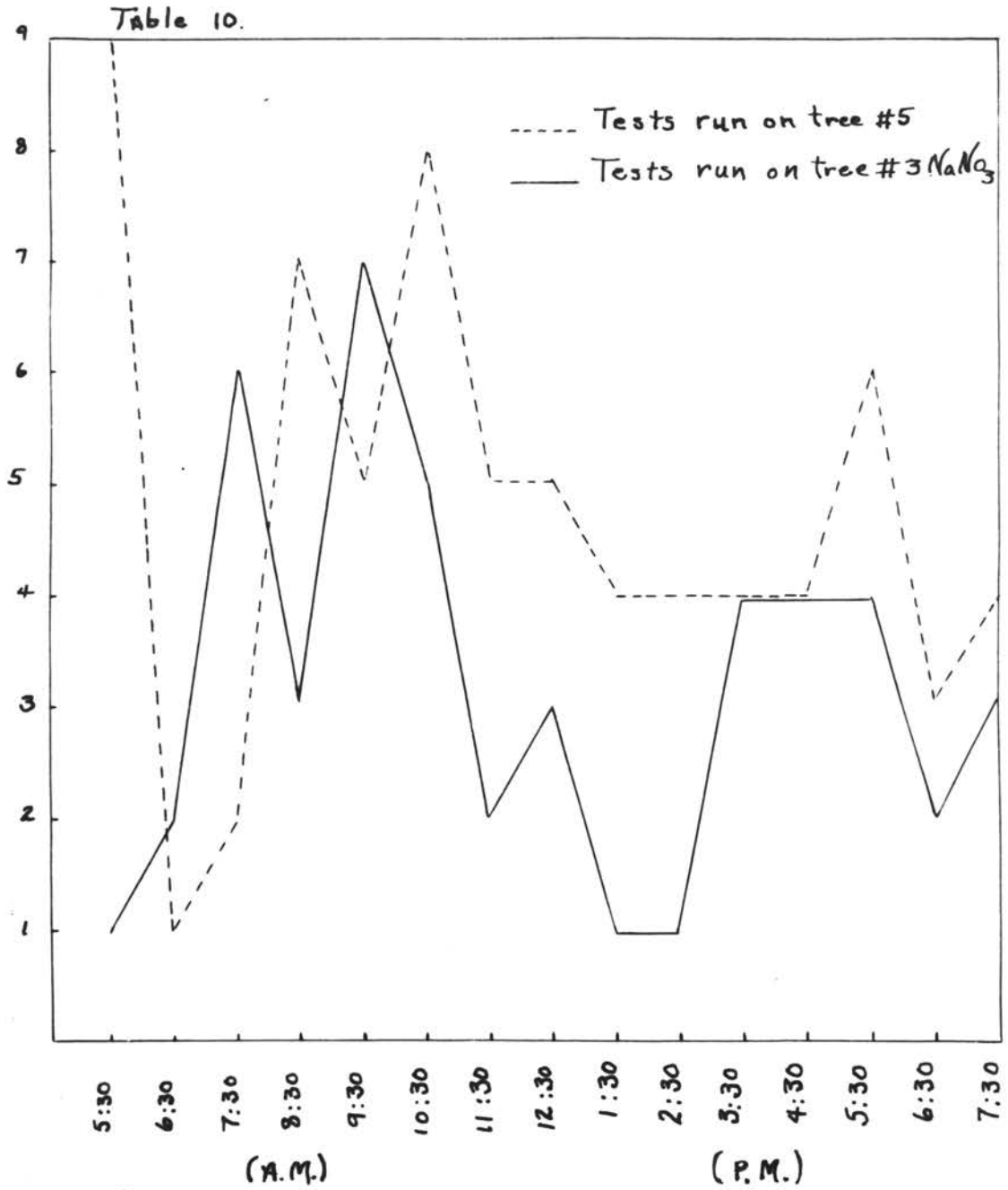
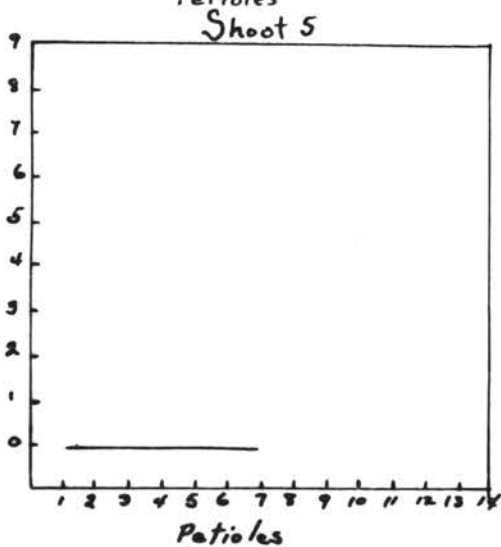
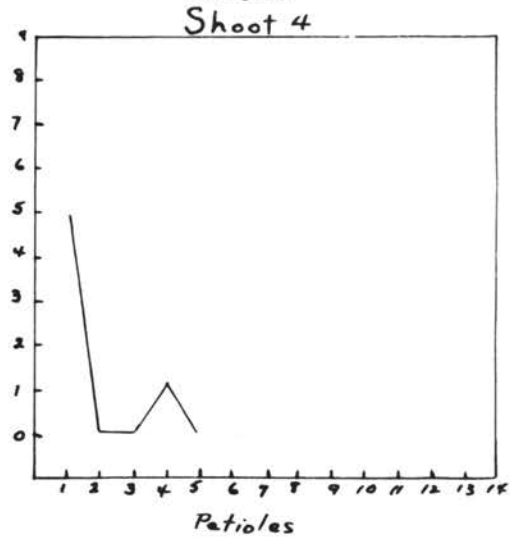
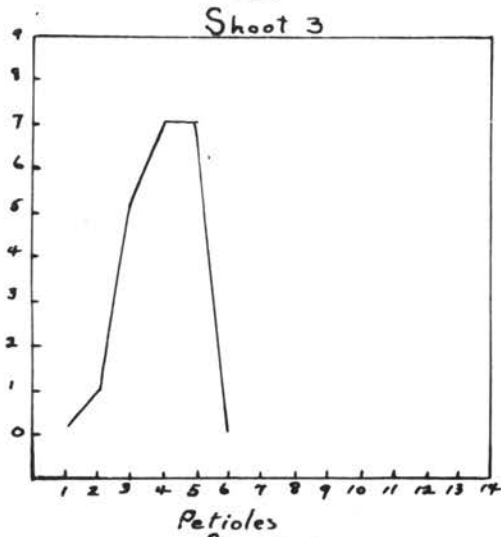
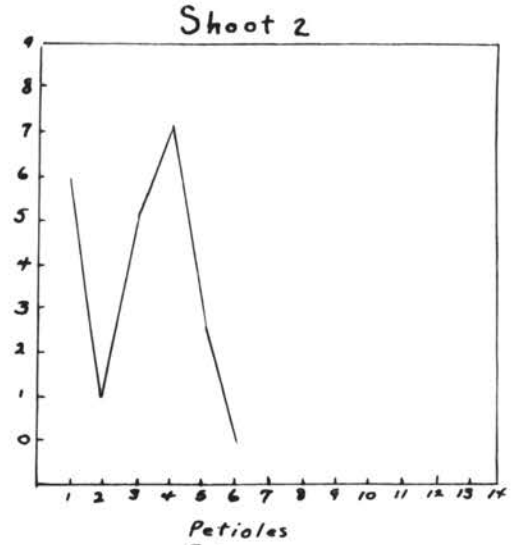
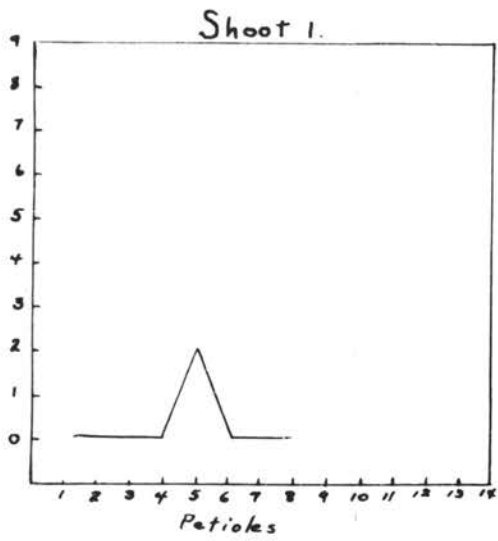


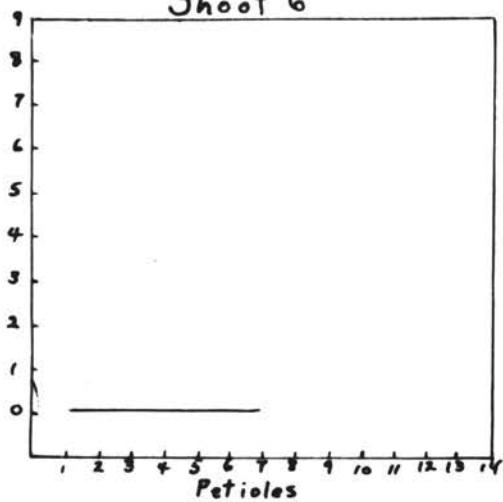
Table II.



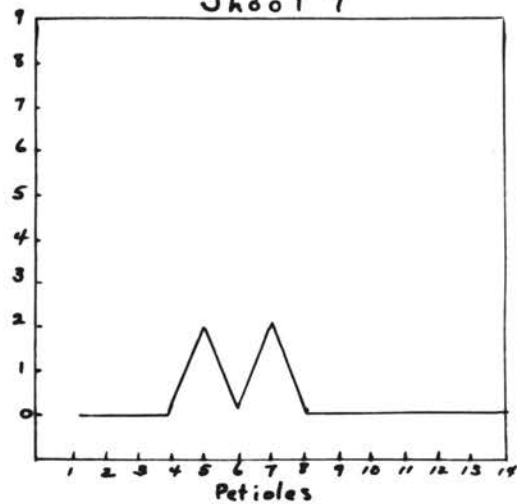
Key:

- 0 - No test
- 1 - Light trace
- 2 - Trace
- 3 - Light test
- 4 - Light to Medium test
- 5 - Medium test
- 6 - Medium to high
- 7 - High test
- 8 - High to Very high
- 9 - Very high test

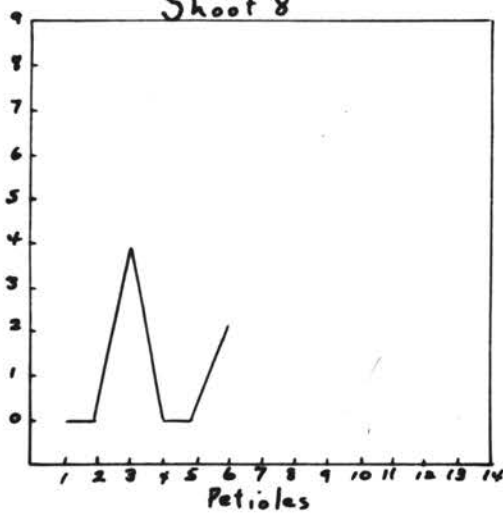
Table 11 (Cont.)
Shoot 6



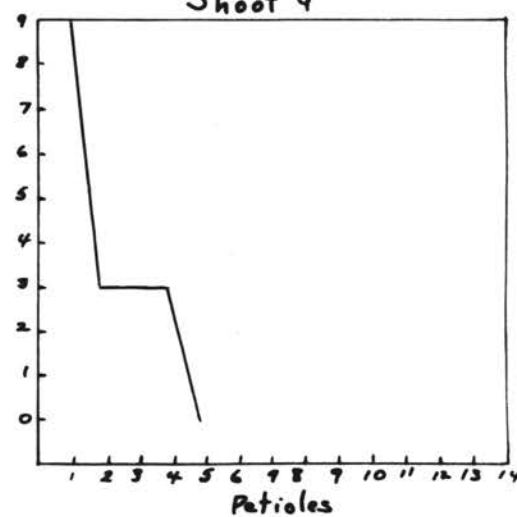
Shoot 7



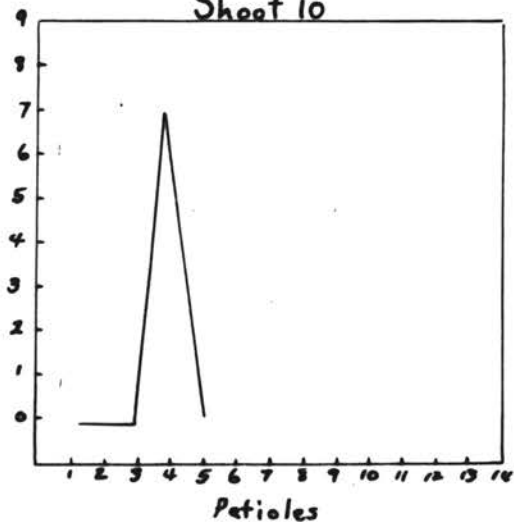
Shoot 8



Shoot 9



Shoot 10



Inorganic Phosphates. Adapting a rapid phosphate test to pecan trees and comparing results with chemical analyses.

On June 6th, 1950, trees in the wooden tubs that had been fertilized previously with various fertilizers were tested for inorganic phosphates using reagents of the Purdue tissue test kit. Table 5 shows results obtained for phosphates. At the time these tests were made there was still insufficient foliage to make separate tests on petioles and leaflets so leaflets and petioles were diced together to make one sample. No large differences were obtained here to indicate that the Purdue kit would detect a larger amount of phosphorus in a tree that had received a heavy application of phosphorus fertilizer. From information found later on, however, the leaflets seem to be less suitable for detecting the presence of inorganic phosphates than the leaf petioles. If sufficient material had been available for testing petioles it is believed that a more accurate test for phosphates could have been determined.

The color scale used for determining the amount of phosphates in a measured amount of fresh tissue was the one coming with the Purdue kit. No phosphate or potassium tests were made on the trees in tub 8 as they had been sprayed with "Ra-Pid-Gro" and it was believed impossible to get an accurate reading of what was actually in the plant because of the possibility that some of the material sprayed on the surface still remained there.

Testing Trees for Inorganic Phosphates.

New shoot and leaf growth was diced and measured amounts of this fresh tissue tested according to Purdue kit directions, as pecan trees came into foliage. There is apparently little to no inorganic phosphates in very young tissue as indicated by the Purdue test.

On May 12th tests for inorganic phosphates were made on two trees; Early Bird 5 and Love 12. Both petioles and main shoots of each tree gave a high

test for phosphates. Both trees had been fertilized (Table 2).

On June 9th, tree 3 which had been fertilized with nitrate of soda, was tested for inorganic phosphates. A high to very high test for phosphates was obtained from diced petioles and a medium to high test for diced leaflets. Leaf petioles were used in testing for phosphates throughout the remainder of this study.

From the data in Table 11 there appears to be no correlation between total phosphorus, as measured by chemical analyses, and inorganic phosphates as measured by the Purdue phosphate reagents.

Table 12 shows the results obtained when phosphate tests were made on petioles from the base of the shoot upwards to determine if a certain petiole were more indicative of deficiency than the others.

Table 13 shows evidence that inorganic phosphates vary little during the day in the petioles of pecans.

The data in Table 11 shows a gradual decline in total phosphorus in pecan leaves as the season progresses.

Table 11a. Comparing total phosphorus of pecan tissues with inorganic phosphates obtained with the Purdue quick test.

Tree	Date Sampled	Total Phosphorus	Inorganic Phosphates
Texas Prolific	July 22, 1950	.121%	High
	August 11	.142	High
Stuart # 1	May 5	.416	High
	July 22	.149	High
	August 11	.133	Medium
Halbert	July 22	.130	Medium to low
	August 11	.120	Medium
Stuart # 2	July 22	.158	High
	August 11	.116	Medium
Tree # 154	July 22	.156	High
	August 11	.145	Very high
Tree # 150	May 6	.504	High
	July 22	.167	Medium to high
	August 11	.161	Very high
Tree # 162	August 11	.145	Very high
Love 12	May 12	.300	High
	July 22	.144	High
	August 11	.119	Very high
Early Bird 5	May 12	.354	High
	July 22	.136	Medium
	August 11	.109	High
Burkett 16	July 22	.120	Very high
	August 11	.125	High
No. 1 NaNO ₃	May 31	.255	Low
	June 6	.245	High
	July 22	.140	High
	August 11	.137	Very high
No. 3 NaNO ₃	June 6	.284	Medium
	July 22	.144	High
	August 11	.147	High to very high
Tree # 3 Walker's	June 28	.163	Very high
	August 10	.141	Very high
Tree # 4 Walker's	June 28	.156	High to very high
	August 10	.135	Medium to high

Table 11^a (cont'd).

Tree	Date Sampled	Total Phosphorus	Inorganic Phosphates
Tree # 5 Walker's	June 28	.189	Very high
	August 10	.130	High to very high
Tree # 6 Walker's	June 28	.155	Very high
	August 10	.118	Medium to high
Tree # 4 West side	July 22	.142	Very high
	August 11	.157	Very high
Tree # 5 West side	August 11	.147	Very high

Table 12. Determining the petiole most satisfactory for inorganic Phosphate test.

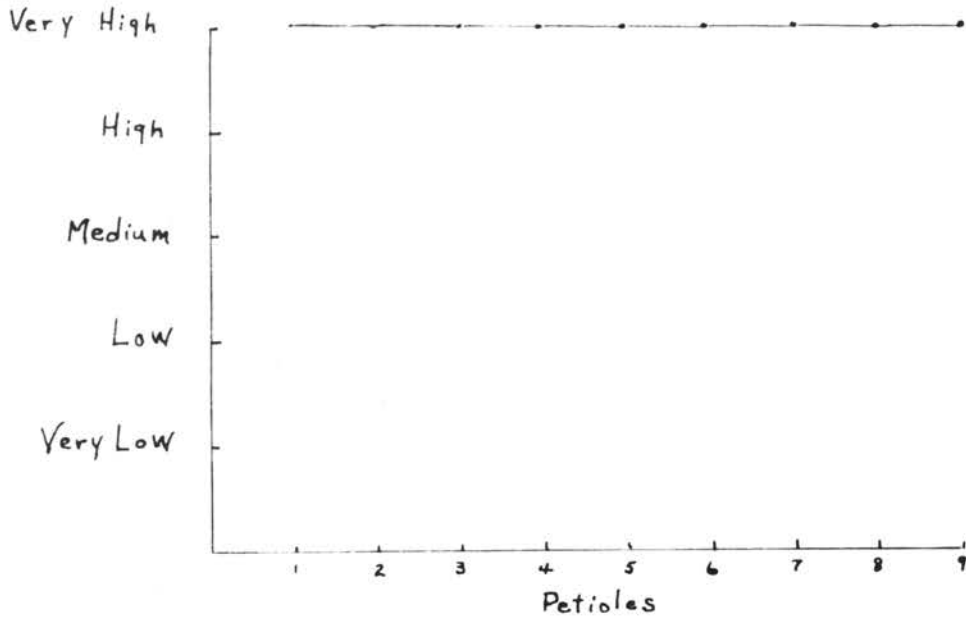
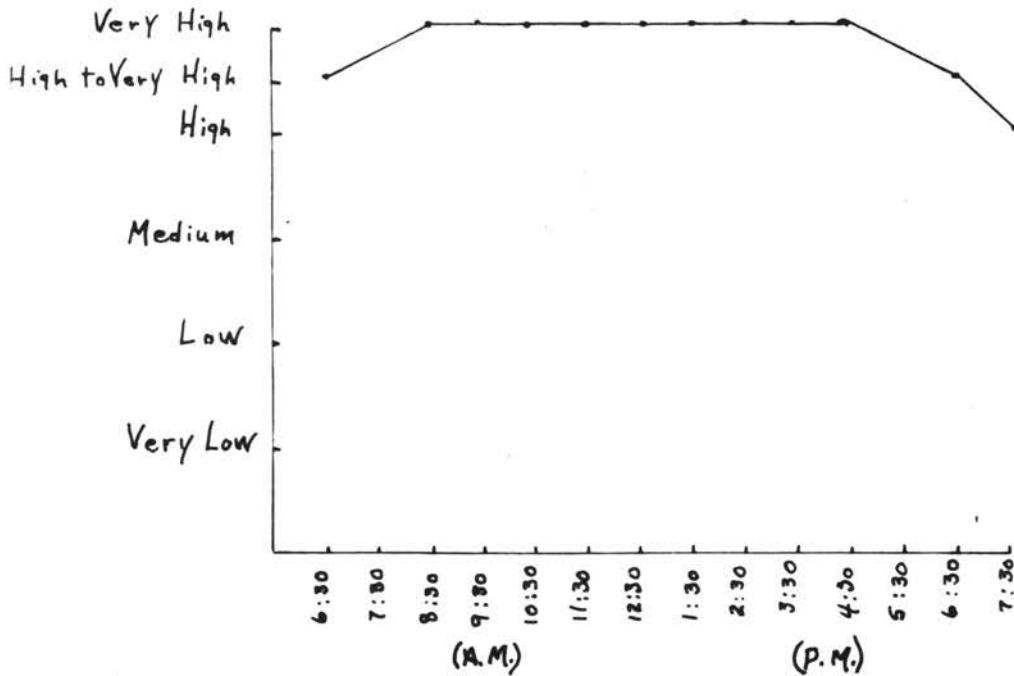


Table 13. Testing for phosphates in pecan petioles throughout the day.



Potassium. Adapting a rapid potassium test to pecan trees and comparing results with chemical analyses.

It was found that the Purdue potash test as recommended in the kit did not give satisfactory results when applied to pecan tissues. In order to have some means of testing for potassium, Melsted's potash spot test was used. This test will show if potassium is present up to 3000 parts per million, but for pecan tissue a modification of the test seems necessary to check higher concentrations.

On July 18th, a modification of the Purdue potassium test was prepared and used as recommended by Garrad (29). The test was found to give a clear color reading. It was found that diced petioles gave a higher test for potassium than did diced leaflets, therefore petioles were used in the remainder of the tests. Table 14 shows results of potassium tests. From this data there is evidence that the quick tests do not correlate with the chemical analyses tests. A slight correlation of tests seems to be shown with some trees, however.

Table 14 also shows evidence that total potassium in pecan leaves decreases as the season progresses, with a slight decrease on September 22nd when the last tests were made.

Table 14. Comparison of potassium of chemical analyses to potassium of the rapid tissue tests.

Tree	Date Sampled	Chemical Analyses	Quick Tests
Texas Prolific	May 5, 1950	1.97	Test inadequate
	July 22	.85	Very high
	August 11	.77	Medium
	Sept. 22	.92	Low
Stuart # 1	May 5	1.91	Test inadequate
	July 22	.98	High
	August 11	.87	Medium to high
	Sept. 22	.99	High to very high
Halbert	July 22	.979	Medium to high
	August 11	.62	Very high
	Sept. 22	.76	Very high
Stuart # 2	May 5	2.02	Test inadequate
	July 22	1.20	Very high
	August 11	.92	Very high
	Sept. 22	1.09	High
Tree # 154	July 22	.94	No test
	August 11	.85	Medium
	Sept. 22		Very high
Tree # 150	July 22	1.13	Medium to high
	August 11	1.11	Very high
	Sept. 22		Very high
Tree # 162	May 31	1.09	Test inadequate
	August 11	1.01	Low
Love 12	May 12	1.95	Test inadequate
	July 22	1.05	Very high
	August 11	1.07	Very high
	Sept. 22	1.23	Very high
Early Bird 5	May 12	2.09	Very high
	July 22	1.08	Very high
	August 11	.84	Low to very low
	Sept. 22		Very high
Burkett 16	July 22	1.11	Very high
	August 11	1.04	Low to medium
	Sept. 22	1.21	Very high
No. 1 NaNO ₃	May 31	1.10	Test inadequate
	June 6	1.02	Very high
	July 22	.672	Very high
	August 11	.64	Very low
	Sept. 22	.73	Very low

Table 14 (cont'd).

Tree	Date Sampled	Chemical Analyses	Quick Tests
No. 3 NaNO ₃	June 6	1.20	Very high
	July 22	.89	Very high
	August 11	.932	Low
	Sept. 22	1.04	Very high
Tree # 3 Walker's	June 28	1.11	No test
	August 10	1.08	Very low
Tree # 4 Walker's	June 28	1.42	No test
	August 10	1.24	Very low
Tree # 5 Walker's	June 28	1.43	No test
	August 10	1.08	Low
Tree # 6 Walker's	June 28	1.30	No test
	August 10	.99	Very low
Tree # 4 West side	July 22	.761	Very high
	August 11	.84	Very high
	Sept. 22	.82	Very high
Tree # 5 West side	August 11	.81	High
	Sept. 22	.79	Very high

Nutrient solutions applied to pecan foliage to test absorption of nitrate nitrogen through the foliage.

Nutrient sprays were applied to trees to test for evidence of absorption by pecan leaves. It was believed that if nitrogen is the regulating factor of nut production and nut filling that nutrient sprays applied to trees and absorbed through the leaves would allow a close regulation of the amount of growth made by trees, thus regulating the flowering, setting and later filling of the nuts. A spray application early in the spring to give lush growth and good flower set would be desirable.

The purpose of this experiment was to test two commercially advertised nutrient sprays for absorption through the leaves and to detect their effectiveness by using the Purdue nitrate reagent on the bases of leaf petioles as was found to give positive tests for nitrates in previous experiments.

Trees 75 and 4 in the college pecan grove were used for this part of the experiment. Tree 75 showed no test for nitrates at 10:15 A.M., just previous to the application of "Ra-Pid-Gro." Number 1 and Number 4 concentrations of "Ra-Pid-Gro" were applied to shoots of tree 75 by dipping one shoot in each of the two concentrations mentioned.

Tests for nitrates were made on the bases of snapped off petioles at 10:30 A.M., 11:00 A.M., and 3:00 P.M. of the same day. No tests for nitrates were obtained at any time. Tests were not made at later dates on tree 75, however.

To further continue the studies of the nutrient sprays, tree 4 was used for testing four varying concentrations of both "Ra-Pid-Gro" and "Nu-Green." Of all the tests made for nitrates on the petioles of tree 4 no test for nitrates was obtained in the inner tissues of the plant. Very high tests for nitrates were obtained on the outside of the leaves treated with "Ra-Pid-Gro," however

leaves sprayed with "Nu-Green" showed no test for nitrates on the outsides of the leaves indicating that nitrate nitrogen is not present in "Nu-Green." The Number 4 concentration of "Nu-Green" caused severe burning of the foliage. Tests were made at intervals during the first two days after the trees were sprayed.

Similar results were obtained in other tests on seedling pecan trees growing in the nursery row. These tests were also made at intervals during the first two days after spraying.

No positive test for nitrates was secured on those trees using the Purdue nitrate reagent.

Summary and Conclusions

1. A test for nitrates was found when the leaf petioles of pecan shoots were snapped from the main stem of the shoot and the basal area tested. A photograph showing the area of the petiole in which nitrates were found is presented. Diced petioles did not give a positive test for nitrates. There is some question here as to whether or not nitrates were present since the color indicating nitrates may have been masked by a charring of the tissues by the reagent before the color could be detected. It is also possible that in dicing the petioles the amount of nitrates in proportion to the total amount of tissue was reduced, therefore the concentration of the nitrates was not sufficiently high to give a positive test.
2. Core samples of pecan tree trunks failed to give a positive test for nitrates in the conducting tissues (xylem) at the time the tests were made.
3. There seemed to be little correlation between the tissue nitrate tests and the percentage of total nitrogen indicated by complete analyses.
4. Data presented shows a positive correlation between total phosphorus and total nitrogen.
5. Results obtained with the Purdue nitrate test indicate that there is a higher accumulation of nitrates in pecan petioles between 7:30 A.M. and 10:30 A.M. and again between 4:30 P.M. and 5:30 P.M.
6. The third, fourth and fifth petioles on a shoot gave the most consistent test for nitrates.
7. The Purdue kit gave positive tests for inorganic phosphates in pecan petioles, but there was no correlation between inorganic phosphates as determined by the Purdue kit and total phosphorus determined by complete analyses.
8. A modification of the Purdue potassium test as explained by Garrad (29) gave satisfactory tests on pecan tissue.

9. Data indicates that the tests for potassium obtained with the modified Purdue quick test do not correlate with chemical analyses tests.
10. A gradual seasonal decline in total potassium of pecan leaves is shown, as was found to be true with total nitrogen and total phosphorus.
11. Nitrate nitrogen was not detected in leaf petioles using the Purdue nitrate reagent, after foliage sprays of "Ra-Pid-Gro" and "Nu-Green" were applied.

Literature Review

1. Alben, Arthur O. Results of a fertilizer and cultural experiment on pecans growing in haymeadow and pasture. Proceedings of Annual Meeting of Oklahoma Pecan Growers' Association. pp. 55-61. Dec. 1948.
2. ----- Analyses of pecan leaves as an aid in fertility studies. Proceedings of the Annual Meeting of the Oklahoma Pecan Growers' Association. pp. 38-45. Dec. 9-10. 1947.
3. Atkinson, H. T. and Others. Plant tissue testing. Sci. Agri. 24:437-42. May 1944.
4. Auchter, E. C. Is there normally a cross transfer of foods, water and mineral nutrients in woody plants? Md. Agr. Exp. Sta. Bul. 257: 33-60. 1923.
5. Blackmon, G. H. Maintaining permanent soil fertility for the production of well filled pecan nuts. Proceedings Ga. Fla. Pecan Growers' Assoc. 23:12-16. 1929.
6. ----- and R. W. Ruprecht. Fertilizer experiments with pecans. Fla. Agr. Exp. Sta. Bul. 270. 1934.
7. Boynton, D. and A. B. Burrell. Effects of nitrogen fertilization on leaf nitrogen, fruit color and yield in two New York McIntosh apple orchards. 1942 and 1943. Proc. Amer. Soc. Hort. Sci. 44:25-30. 1944.
8. ----- and O. C. Compton. Leaf analysis in estimating the potassium, magnesium, and nitrogen needs of fruit trees. Soil Science 59:339-351. 1945.
9. Bray, R. H. Nitrate tests for soils and plant tissues. Soil Science 60: 2190221. 1945.
10. ----- Correlation of soil tests with crop response to added fertilizers and with fertilizer requirement. Diagnostic Techniques for Soils and Crops. Their value and use in estimating the fertility status of soils and nutritional requirements of crops. pp. 53-86. Publ. by the Amer. Potash Insti. Washington 6, D. C. 1948.
11. Brown, R. J. Sampling sugar beet petioles for measurement of soil fertility. Soil Science. 56:213-222. 1943.
12. Carolus, R. L. The use of rapid chemical plant nutrient tests in fertilizer deficiency diagnosis and vegetable crop research. Va. Truck Agr. Exp. Sta. Bul. 98:1531-1556. 1938.
13. Chapman, G. W. Leaf analysis and plant nutrition. Soil Science 52:63-81. 1941.
14. Crane, H. L. and Max B. Hardy. Interrelation between cultural treatment of pecan trees, the size and degree of filling of the nuts, and the composition of kernels. Jour. Agr. Res. 49:643-61. 1934.

15. Daniel, W. H. and L. M. Turk. Methods for determining the needs of peach trees for potash fertilizer. Reprint from Mich. Agr. Exp. Sta. Quarterly Bul. 32:199-205. No. 2. 1949.
16. Drosdoff, M. Fertilizing tung trees by leaf analyses. Better Crops With Plant Foods. 27 (4):9-11. 1943.
17. Emmert, E. M. Tests for phosphates, nitrates and soluble nitrogen in conducting tissue of tomato and lettuce plants, as indicators of availability and yield. Ky. Agr. Exp. Sta. Cir. 43:25-40. 1934.
18. -----. Plant tissue tests as a guide to fertilizer treatment of tomatoes. Ky. Agr. Exp. Bul. 430:1-48. 1942.
19. -----. Field method for estimating nitrates, phosphates, and potassium in plants. Plant Physiology 7:315-321. 1932.
20. -----. Plant tests as a guide to the fertilizer treatment of tomatoes. (Preliminary report). Proc. Amer. Soc. Hort. Sci. 38:621-622. 1941.
21. Finch, A. H. and C. W. Van Horn. The physiology and control of pecan nut filling and maturity. Univ. of Arizona Tech. Bul. No. 62. June, 1936.
22. ----- and H. L. Crane. Physiological factors associated with the productiveness of pecan shoots. Proc. Nat'l. Pecan Assoc. 30:98-105. 1931.
23. -----. Notes on pecan filling and maturity. Proc. Amer. Soc. Hort. Sci. 30:387-91. 1933.
24. -----. Further notes on pecan filling and maturity. Proc. Amer. Soc. Hort. Sci. 32:24-28. 1934.
25. Fiegl, F. Spot tests. Nordeman Pub. Co. Inc. New York. 1939.
26. Fischer, E., D. Boynton, and K. Skadvin. Nitrogen fertilization of the McIntosh apple with leaf sprays of urea. Proc. Amer. Soc. Hort. Sci. 51:23-33. 1948.
27. Fowler, E. D. A report on pecan fertilizers for the southeastern United States. Proc. Ga. Fla. Pecan Growers' Assoc. 25:6-10. 1931.
28. Frear, D. E., R. D. Anthony, A. L. Haskins, and F. H. Hewetson. Potassium content of various parts of the peach tree and their correlation with potassium fertilization. Proc. Amer. Soc. Hort. Sci. 52:61-74. 1948.
29. Garrad, H. L. Potash tissue test for peach leaves. Better Crops with Plant Food. pp. 17-27. June, July, 1950.
30. Goodal, D. W., Studies in the diagnosis of mineral deficiency III. the mineral composition of different types of leaves on apple trees in early summer. Jour. of Pom. and Hort. Sci. 21:90-102. 1945.
31. Gozzard, Atherton C. A study of methods of sampling pecan leaves for total nitrogen analyses. Proc. Amer. Soc. Hort. Sci. 42:109-114. 1943.

32. Hammar, H. E. and James H. Hunter. Influence of fertilizer treatment on the chemical composition of Moore pecan leaves during nut development. Plant Physio. 24:16-30. 1949.
33. Harrington, J. F. Some factors influencing the reliability of plant tissue testing. Proc. Amer. Soc. Hort. Sci. 45:313-17. 1944.
34. Hardy, Max B. and H. L. Crane. Can the composition of pecan nuts be changed by fertilizer or other cultural treatment? Proc. Nat'l Pecan Assoc. 31:110-14.
35. Hester, J. B. Soil and plant tests as aids in soil fertility programs. Commercial Fertilizer. 63:10-20. Nov., 1941.
36. Hill, H. Malnutrition symptoms and plant tissue test of vegetable crops. Better Crops 27:6-10. May, 1943.
37. Hoffer, G. N. Testing corn stalks chemically to aid in determining their plant food needs. Indiana Agr. Exp. Sta. Bul. 298:1-31. 1926.
38. Inciardi, N. F. Rapid plant tissue tests as a means of testing for mineral deficiencies in plants. Unpublished thesis in partial fulfillment of the requirements for the degree of Master of Science. Okla. A. & M. College. pp 1-48. 1949.
39. Knowlten, H. E. A preliminary experiment on half tree fertilization. Proc. Amer. Soc. Hort. Sci. pp 148-149. 1921.
40. Knobel, E. W. and O. H. Brensing. Soil Survey. Tulsa County, Oklahoma. Series 1935. No. 22. United States Dept. of Agri. Bureau of Plant Industry. pp 1-68. Feb., 1942.
41. Krantz, B. A., W. L. Nelson, and L. F. Burkhart. Plant tissue tests as a tool in agronomic research. Diagnostic techniques for soils and crops. pp 137-155. 1948.
42. Laustalot, A. J. and Felix S. Lagasse. A comparison of winter and early spring application of nitrogen to tung trees. Amer. Soc. Hort. Sci. 48:51-58. 1948.
43. Lilleland, O. and J. G. Brown. The potassium nutrition of fruit trees. II. Leaf analysis. Proc. Amer. Soc. Hort. Sci. 36:91-98. 1938.
44. Magness, J. R. Moisture supply as a factor in pecan production. Proc. Nat'l. Pecan Assoc. 30:18-23. 1931.
45. Marshall, H. L. and R. W. Ruprecht. Pecan analyses. Proc. Ga. Fla. Pecan Grower's Assoc. 24:19-22. 1930.
46. Melsted, S. W. A simplified field test for determining potassium in plant tissue. Better Crops With Plant Food. p 26. Jan. 1950.

47. Nicholas, D. J. D. The application of rapid chemical tests to the diagnosis of mineral deficiencies in horticultural crops.II. Crops grown at various centers. Jour. of Hort. Sci. 24:106-122. 1948.
48. Romberg, Louis D. Some characteristics of the Juvenile and the Bearing Pecan Tree. Amer. Soc. Hort. Sci. 44:255- 1944.
49. Sass, John E. Elements of Botanical Technique. McGraw-Hill Book Co. Inc. New York. 1940.
50. Scarseth, G. D. Soil and plant tissue tests as aids in determining fertilizer needs. Better Crops With Plant Food. 25:9-17. Mar. 1941.
51. ----- Value and limitations of methods of diagnosing plant nutrient needs. Better Crops With Plant Food. 27:11-15. May 1943.
52. ----- Plant tissue testing in diagnosis of the nutritional status of growing plants. Soil Sci. 55:113-120. 1943.
53. ----- What is the matter with your soil? One of a series of articles for general reading. Purdue Univ. Agri. Exp. Sta. 1945.
54. Shear, C. B., H. L. Crane, and A. T. Meyers. Nutrient element balance. Application of the concept of the interpretation of foliar analysis. Proc. Amer. Soc. Hort. Sci. 51:319-326. 1948.
55. ----- Plant tests versus soil tests for determining the availability of nutrients for tobacco. Va. Agr. Exp. Sta. Tech. Bul. 84:1-12. 1943.
56. Shinhart, D. V. Morphology and anatomy of the fruit of *Hicoria pecan*. Bot. Gaz. 93: 1-20. 1932.
57. Sitton, B. G. A preliminary report on the relation of foliage to filling of the pecan. Proc. Nat'l Pecan Assoc. 30:120-125. 1931.
58. Skinner, J. J. The work of the bureau of chemistry and soils with pecans. The relation of soil fertility and commercial fertilizers to pecan production. Proc. Nat'l Pecan Grower's Assoc. 26:52-63. 1927.
59. ----- and A. O. Alben. Fertilizing the pecan. Proc. Nat'l Pecan Grower's Assoc. 30:26-29. 1931.
60. Smith, C. L. Work of the federal station on pecans in Texas. Proc. Annual Meeting Okla. Pecan Grower's Assoc. Dec. 10, 11. 1946.
61. Taylor, R. W. The influence of fertilizer treatments on yield and quality of pecans. Proc. Nat'l. Pecan Assoc. 29:18-21. 1930.
62. Thomas, W. and W. B. Mack. Control of crop nutrition by the method of foliar analyses. Penn. Agr. Exp. Sta. Bul. 378:1-32. 1939.
63. ----- Present status of diagnosis of mineral requirements of plants by means of leaf analyses. Soil Sci. 59:353-374. 1945.
64. ----- Foliar diagnosis: Principles and practices. Plant Physiology. 12:571-599.

65. Thor, J. B. and C. L. Smith. A physiological study of seasonal changes in the composition of the pecan during fruit development. Jour. Agri. Research. 50:97-121. 1935.
66. Thornton, S. F. A field and laboratory test on plant material for diagnosing phosphorus deficiencies. Indiana Agr. Exp. Sta. Bul. 355:1-20. 1932.
67. -----, S. D. Conner, and R. R. Fraser. The use of rapid chemical tests on soils and plants as aids in determining fertilizer needs. Pur. Univ. Agr. Exp. Sta. Cir. 204 (Rev.) 1939.
68. Tynes, E. H. and J. R. Webb. The relation of corn yields to nutrient balance as revealed by leaf analysis. Jour. Amer. Soc. Agron. 38: 173-185. 1946.
69. Ulrich, A. Potassium content of grape leaf petioles and blades contrasted with soil analyses as an indicator of the potassium status of the plant. Amer. Soc. Hort. Sci. Proc. 46:204-212. 1942.
70. -----, Plant analyses as a diagnostic procedure. Soil Sci. 55:101-112. 1943.
71. Ulrich, A. Nitrate content of grape leaf petioles as an indicator of the nitrogen status of the plant. Proc. Amer. Soc. Hort. Sci. 41:213-218. 1942.
72. Wolf, B. Rapid determination of soluble nutrients in soil and plant extracts by means of a photoelectric colorimeter. Ind. and Chemical Eng. Anal. ed. 15:248-51. Apr. 1943.
73. Wood, L. K. Seasonal variation in leaf and soil potassium. Soil Sci. 63:305-314. 1947.

THESIS TITLE: RAPID TISSUE TESTS ON PECANS TO DETERMINE
THE PRESENCE OF THE THREE MAJOR ELEMENTS

NAME OF AUTHOR: CLARENCE RICHARD ROBERTS

THESIS ADVISER: FRANK B. CROSS

The content and form have been checked and approved by the author and thesis adviser. "Instructions for Typing and Arranging the Thesis" are available in the Graduate School office. Changes or corrections in the thesis are not made by the Graduate School office or by any committee. The copies are sent to the bindery just as they are approved by the author and faculty adviser.

NAME OF TYPIST: CAROL EALY