providing access to the groves utilized in this study was greatly appreciated.

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# DIFFERENT TECHNIQUES AND VIEWS CONCERNING LEAF TISSUE ANALYSIS

JAMES R. ILEY
*Applied Agricultural Research, Inc.,*
*1305 East Main Street,*
*Lakeland, Florida 33801*

*Abstract.* **The sampling period of the present leaf analysis system is oriented more toward applying corrections to next year's crop. A proposed system, still under investigation, is described to make corrections on the present crop by moving the sampling date back to approximately the first half of April. This month lies within a period of great leaf activity and is not normally sampled; however, an attempt has been made and shows promise. With the use of a fixed leaf surface area as a basis rather than dry weight, much of the laboratory work can be eliminated and sampling can be simplified. At the same time, sample transportation to the laboratory is made easier and laboratory turn-around time is greatly reduced. The ideas, techniques and data are presented since others may have interest in this system or parts thereof.**

Most of the research work on leaf tissue analyses has been compiled and published (3, 6). These publications give instructions for sampling and standard values with which analytical results can be compared to determine if they are in a satisfactory range or not. Adaptions (5) have been made to fit local conditions and needs.

Leaf tissue analyses can be a very important tool but appears restricted by the present system of employment; therefore, this work is an exploration in ideas and techniques in search of a greater use that may reveal some of the unknown potential. The areas of exploration are:
1) To simplify the sampling procedure so that a selected field worker at the grove may be taught to sample with reliability.
2) To investigate different sampling periods where results may be more advantageous, so if needed, corrective action can be taken.
3) To simplify the laboratory techniques so that laboratory turn-around time is minimized and results can be readily obtained.

An underlying concept of these explorations is that the simplification will reduce sample cost which should induce more usage and result in a greater understanding of the use of a leaf tissue analysis. Leaf tissue analysis cannot replace soil analysis, nor was it intended to do so, but it can be used to develop a more efficient fertility program.

## Sampling

Modifications of the sampling procedure includes the selection of trees, the number of leaves taken per sample, the selection of different age leaves and the removal of a portion of the leaf tissue to be analyzed by the laboratory.

Samples would be from 10 trees, in line, perpendicular to the direction in which the grove is fertilized rather than the recommended 20 randomly selected trees. If a tree is in poor condition, an adjacent tree in that row is used. The location of these trees should be within the grove, not on the very edge, and should be recorded since the same trees should be used in future sampling of the area. If trees are located in blocks or large beds, with deep ditches on each side, and there are only 10 rows per block, the outside rows are excluded and 5 rows are sampled, two trees per row.

From each of these trees, one dry leaf is collected from a non-fruiting terminal about chest high, and from the side of the tree exposed to sunlight for a total of 10 leaves per sample. Newly expanded leaves of a flush may appear to be uniform but in fact may be a complex of three flushes possibly a week apart and can only be separated through feeling the difference in the leaves. All the leaves of a sample should be of the same age, and comparable samples also should be of the same age. In sampling old leaves behind new flushes it is difficult to determine the age of the leaf; therefore, it has been assumed that leaves of this old age group react similarly with respect to new growth emerging.

From young leaves, just fully expanded, 3 disks are punched from each side of the leaf for a total of 60 disks. Some laboratories may be able to use 30 disks from the young leaves, but if 60 are used it should be stated so values can be converted back to a standard 30-disk sample. A 6.35-mm hand paper punch is used with the holes evenly spaced along the leaf, the initial hole being halfway up the leaf length. Details of the procedure and comparisons to the conventional procedure have been published (4). All paper punches are not the same, and some can be easily modified with a small plastic receptacle to collect the 30 small disks.

From older leaves only 3 disks are punched from one side of the leaf. A small container for these disks can easily be constructed from a plastic sandwich bag and scotch tape. The important thing to remember is that all of the disks must be used in the analysis, 59 or 29 will not be acceptable. These small samples can be sent through the mail to the laboratory. They should be mailed on a Monday, so if taken later in the week, store in the refrigerator until the following Monday.

This procedure is a modification of the present recommendation of 100 leaves per sample. The immediate response is that 10 is not a sufficient number of leaves, but this statement is not justified without knowing the situation and conditions, and in most cases 10 leaves will be sufficient. If there is any question, the area can quickly be sampled again.

### Time of Sampling

Presently, the sampling period for leaves 4 or 5 months of age (5) or 4 to 7 months (6) for the spring flush is July through September. It has been reported (6) that the June flush also could be used, which would be sampled later in the year, but results should not be compared to values for spring cycle leaves.

Time of sampling is modified in this proposed system by suggesting two additional sampling periods, early April and late June-early July. The sampling at these periods would be of the newly expanded young leaves and the old leaves behind this new growth. These would be separate samples, and care should be exercised so that the new leaves are not contaminated from spray residues on the old leaves. This can be accomplished by handling the leaves at the stem when sampling. If verification of age is needed by feeling, use a leaf adjacent to the one to be sampled.

The young leaves taken at this time show promise of revealing the nutritional status of the micronutrients manganese (Mn), copper (Cu), zinc (Zn), iron (Fe) and boron (B); and from the April sampling, a decision could be made as to which of these elements would need to be applied in the postbloom spray. This tissue is clean and has not been sprayed. After a leaf has been sprayed with the micronutrients there is doubt as to what the actual values are.

The sample of older leaves may be used in evaluating the status of phosphorus (P) and magnesium (Mg), and as more confidence is gained in this system, the April sampling may be used to determine if these two elements are to be applied in the fertilizer program.

Fig. 1 shows the distribution of the P content of older leaves over a large grove with values ranging from 0.04 to 0.11%. Assuming that a P value below 0.08% in the old leaves could be expected to require the addition of P, areas with lower values are delineated in this figure with a broken line. It has been found that old leaves of P deficient trees contained 0.05% P while leaves from healthy trees contained 0.11% (1). This same work reported that young leaves of the same P deficient trees were the only part of the plant not to show very low levels of P. The age of the young leaves in Fig. 1 was approximately 2 to 3 months.

As has been emphasized, the April sampling of young and old leaf tissue appears to be the most logical one with regards to usefulness of leaf tissue analysis. The other sampling dates may be useful to the production man in evaluating his fertility program, especially the use of potash (K) since there seems to be various ideas as to what quantities should be applied and when.

N on the young and old leaves can be observed over a number of years with different sampling periods to better understand how they vary with a particular fertilizer program. N needs to be particularly high during the bloom and fruit set period (2); however, in the winter the old leaf weight increases, and analysis using dry weight basis may be misleading with respect to total N within the leaf, as seen later.

| | | | | | |
|---|---|---|---|---|---|
| .09 Val | | .09 Val | .07 PA | | .09 Val |
| .08 Val | .10 Val | | | .09 PA | .09 Val |
| .07 Dun | | .06 Red | | .06 PA | .10 Val |
| .07 Red | .04 MS | | | .08 Q | .11 Val |
| .06 MS | | .06 MS | .09 MS | | .09 Val |
| .06 MS | .07 PA | | | .10 Val | .10 Val |
| .06 MS | | .07 PA | .09 Val | | .10 Val |
| .07 MS | .07 PA | | | .08 Val | .09 Val |

Fig. 1. Distribution of % phosphorus (dry wt.) in old leaves over 1920-acre block showing area with lower P.

### Simplification of Laboratory Techniques

The basis for analytical results has always been the dry weight. If 100 g of dried plant material were placed in a furnace and burned to an ash, resulting in an ash weight of 1 g, then based on the plant material dry weight, the ash would be 1%. Likewise the elements, calcium (Cu), Mg, N, etc., also are based on the dry weight. The exception being that elements present in very small amounts such as Mn, Fe, Cu, Zn and B, are based on the weight of a million g, and the term used to express their value is parts per million (ppm).

With this proposed system no weights will be taken. The element content will be expressed as the amount of each particular element within this area covered by the combined thirty 6.35 mm diameter disks.

The initial reason for not weighing these disks is that it was another time consuming factor in the analytical process, and laboratory time is a cost item. Weighing also would increase the turn-around time in the laboratory, and may necessitate washing to remove debris that would alter the true tissue weight.

By using this proposed surface area system, laboratory tasks of washing, drying, grinding and weighing are eliminated; resulting in considerable savings in the analytical process.

The leaf is a dynamic organ which can change weight rather unexpectedly and the standard recommended leaf sampling period was established because of this leaf "activity." This "activity" appears mainly to be the changing weight of the leaf. This changing of weight is complicated, and appears to be influenced by the fertility program (4) of the grower. It may be a useful tool in the future. The N content of a fixed surface area is usually associated with the leaf weight and will give an indication of the leaf weight status.

It has been noted that leaf weight and N, expressed on the absolute basis of mg per leaf (7), builds up in the winter and drops as the new growth emerges. This also can be seen in Fig. 2 where the total N per unit area is highest during the winter. Sampling was terminated just before the new growth emerged but it can be assumed that the downward trend will be as cited in the literature (7). Fig. 2 shows that based on dry weight, N is lowest at that time of the year. This data indicates why sampling and analysis on a dry weight basis is not recommended at this time; however, N in mg per 30 disks may be an indicator of the amount of new growth that may be expected but at least 3 samples at 2-week intervals may be required since there are variable leaf weight changes during this period.

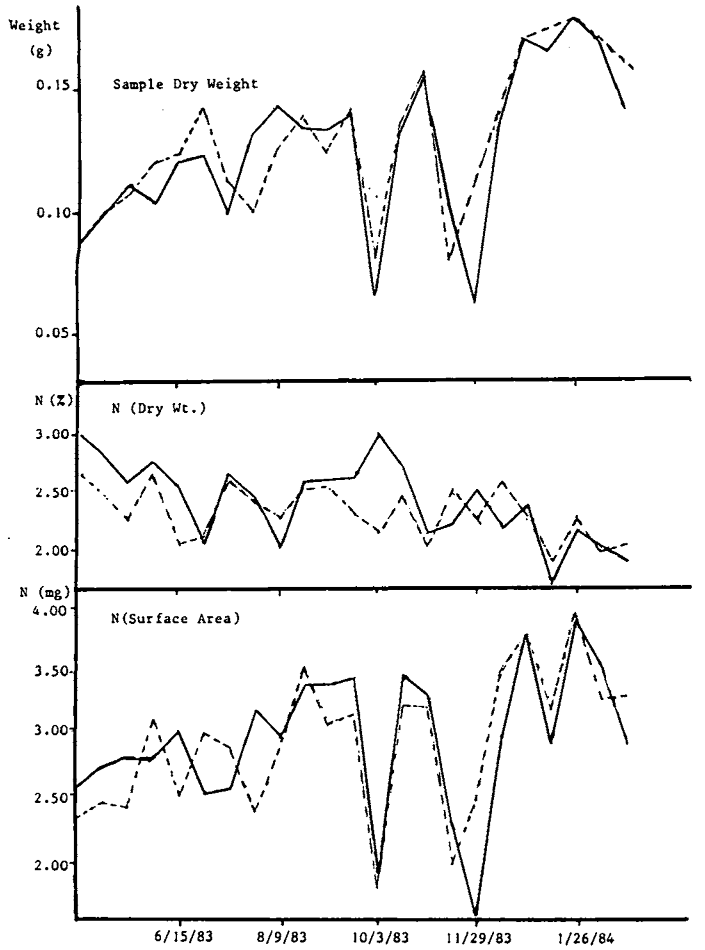


Fig. 2. Relationship between leaf dry weight and nitrogen determined on a dry weight basis and a surface area basis using 40 6.35-mm disk with two separate samples of 10 leaves each, one leaf per tree.

If the rapid drop in leaf weight and N in the fall and winter as seen in Fig. 2 is not due to the emergence of new growth, it may be the translocation of materials to other parts of the tree.

The data presented in Fig. 2 is not an indication that all leaf weights vary as shown, but that general trends can be seen due to grove practices and weather. Each tree is an individual which is reflected in the leaf tissue, so some may be similar and others may vary.

## Tentative Values As An Aid In Evaluating The Proposed Program

*Dry weight basis.* At present, tentative threshold values for Mn, Cu and Fe in newly expanded leaves that may be used by growers in evaluating this proposed system are 16, 6 and 40 ppm (parts per million) dry weight, respectively. Zn appears variable, so possibly a threshold value of 30 ppm may be more appropriate. For B, the present lower limit of 40 ppm (5) can be used, but extreme caution should be followed in making adjustments for B especially with young trees. This system should be used for monitoring B values at first, and only if very low values are found should adjustments cautiously be made after reviewing the present program. If the upper limit of 100 ppm (5) is found at this early age, it would be unwise to add more B.

The trend for Mg is to translocate to the newly emerged leaf. A tentative threshold limit of 0.25% in old leaves may be an acceptable value for evaluating this system. In general, values higher than 0.25% in old leaves may not require additional Mg for that crop. When values are from 0.16 to 0.24%, one should continue to use Mg in the fertilizer, possibly with adjustments if increases are needed. Values of 0.15% and lower, and values near 0.15% that do not show a response from ground applications also should receive foliar applied Mg. Table 2 shows two areas within a large grove where one area does not need to continue with additional Mg until the leaf values fall.

Table 1. Mg content of old and young leaves from same twig in 2 areas of the same grove showing that Area 2 does not need additional magnesium.

| P (% of dry weight) | | | |
|---|---|---|---|
| Area 1 | | Area 2 | |
| Old | Young | Old | Young |
| 0.24 | 0.34 | 0.45 | 0.41 |
| 0.25 | 0.37 | 0.50 | 0.55 |
| 0.20 | 0.39 | 0.33 | 0.41 |
| 0.10 | 0.23 | 0.40 | 0.42 |
| 0.24 | 0.32 | 0.43 | 0.43 |

P values in old leaves should be at least 0.10% dry weight. If values are lower, then P should be applied in the fertilizer.

*Fixed surface area of 30 6.35-mm disks.* Since this is a new method it should be cautiously applied because there are many items to be considered which are presently not well known.

The same precautions as presented above, especially for B, should be observed. The corresponding values for a fixed surface area of 30 6.35-mm disk are:

| | | |
|---|---|---|
| <u>Young Leaf</u> | | |
| Manganese | 1.0 | $\mu g$ |
| Copper | 0.4 | $\mu g$ |
| Iron | 2.4 | $\mu g$ |
| Zinc | 1.8 | $\mu g$ |
| Boron | 2.4 | $\mu g$ |
| <u>Old Leaf</u> | | |
| Phosphorus | 0.10 | mg |
| Magnesium | 0.25 | mg and above, may not need added Mg. |
| | 0.16-0.24 | mg, need Mg on fertilizer, adjust for amount needed. |
| | 0.15 | mg, and below may also need foliar application. |

### Summary

A leaf tissue analysis system has been proposed, employing different sampling and laboratory techniques. Emphasis has been placed on an early sampling of young and old leaf tissue, possibly April when the leaf has just fully expanded. Data could be used for making a decision as to which microelements may be applied in the post-bloom spray, and with a little experience, when to use of P and Mg in the fertilizer program. Data on K and N for different age samples, and sampling periods, can be

used to evaluate different programs with respect to the quantities of these elements and when applied.

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# THE ROLE OF MAPS, AERIAL PHOTOGRAPHY, AND IMAGE ANALYSIS IN CITRUS GROVE SURVEILLANCE AND APPRAISAL

C. H. Blazquez, G. J. Edwards, and R. P. Muraro
*University of Florida, IFAS,*
*Citrus Research and Education Center,*
*700 Experiment Station Road,*
*Lake Alfred, FL 33850*

*Additional index words.* tree inventory, production estimates, damage assessment, wetlands distribution, property appraisals.

*Abstract.* **Properly prepared maps of citrus groves can play an important role in showing topographical, geological, and geomorphological anomalies that might affect grove management. Sequential aerial photographs taken at periodic intervals (biannually at least) with black and white (B & W) or aerial color infrared (ACIR) film, can reveal tree development (size), poor growth condition, and topographical characteristics. Aerial photographs, in combination with accurate grove maps, provide an excellent data base for comparisons with surveillance of the grove at desired intervals by grove managers or field inspectors. A sequential procedure is described whereby a citrus grove was located on a U.S.G.S. Quadrangle Sheet to clearly outline to the photographic plane the area to be covered in the photographic mission. Additional uses of the aerial photographs are described. When photographs were used in combination with maps, it was possible to measure productive and non-productive area, conduct tree counts, and make reliable yield estimates. Better and more accurate appraisals and management decisions were made with more available precise information.**

Among the many ways of keeping records in the production and management of agricultural crops, including citrus, one of the most used procedures involves use of grove sketches, maps, or diagrams (1, 2, 4, 10, 12, 13). These records are so common that not a great deal of thought is given to potential improvements or additional uses for them (3). Maps are an integral part of property records, deeds, and plat books of counties where agricultural areas such as citrus groves are recorded for assessing property values (11, 13). Included in these maps are property boundaries (3), wetlands areas, creeks, sinkholes, natural forests, and planting dates of citrus groves (2). Rootstocks and scions and other ancillary information is recorded in grove maps which can be used to determine how much has been invested in a particular block over a given period of time (2, 6, 7).

In recent years, the advent of aerial photography, aerial surveillance, and image analysis (5) have made grove sketches, maps, and aerial photographs more useful to those making management decisions (5, 8, 9). It is important for citrus grove owners and managers to be familiar with the impact that various types of maps have on the extraction of information from aerial photography and reconnaissance (6, 7, 8, 9). ACIR is also useful in appraising the value of groves and can reduce the time which an appraiser must spend in the field. The purpose of this study was to describe the need for various types of maps in locating a grove, why it is essential to outline an area in a U.S.G.S. Quadrangle Sheet, the use of image analysis of photographs, and how tree counts are useful in determining the dollar value of a grove.

## Materials and Methods

*Maps of test site.* A grove test site was selected in Polk County for the study because it has a variety of topographic characteristics such as wet and dry areas, ponds, creeks, and good citrus producing areas. Maps were obtained from: 1) Polk County Plat Book, 2) Florida General Highway Map, State Road Department, and 3) U.S. Geological Survey 7.5 minute Quadrangle Sheet Collection.

*Aerial and digitizing photography.* The experimental site was photographed by an aerial photographic company using a Wild RC-8 camera with a 6-inch focal length lens. Kodak Aerochrome 2443 Color Infrared film was exposed at a lens opening of f 5.6 and a shutter speed of 1/300th of a second. The Aerial Color Infrared (ACIR) film was processed by a commercial firm using a standard automated system and developed to positive transparencies (Fig. 1A).

Copies were made with black and white (B & W) 3.9 x 4.7-inch Panchromatic film and developed at the Photographic Laboratory of the Citrus Research and Education Center. Photographic prints were enlarged to 7.9 x 9.8 inches using glossy F2 Kodakbromide paper. A Linear Measuring Set System (LMS) (8) was used to digitize the entire Section 12 in the ACIR transparency and determine the productive (planted in citrus) and non-productive (fallow, wetlands, forest, housing, highways and railroads) areas observed in the transparency (Fig. 1B).

*Ground verification survey map.* A ground verification