FINAL REPORT
WTFRC Project # AH-01-67

Project title: Portable meter to measure chlorophyll and nitrogen

PI: Leslie H. Fuchigami, Dept. of Horticulture, OSU, Corvallis, OR
Co-PI(s): Tom Plant, Dept. of Computer and Electrical Eng. , OSU, Corvallis, OR
Cooperators: Pinghai Ding, PhD Candidate, Dept. of Hort, OSU, Corvallis, OR
Shufu Dong, PARC, Agriculture and Agri-Food Canada, Summerland, BC
Lailiang Cheng, Dept. of Horticulture, Cornell Univ., Ithaca, NY
Guihong Bi, PhD Candidate, Dept. of Horticulture, OSU, Corvallis, OR
Dan Melende, MS Student, Dept. of Computer and Elec. Eng., OSU

Objectives:

1. Develop a portable (OSU) meter for determining chlorophyll and nitrogen in fresh leaves of apples and other fruit trees.
2. Test and compare the accuracy of the OSU prototype with the commercial meters.
3. Develop a second OSU prototype with better accuracy by eliminating factors that interfered with the wavelengths used to measure chlorophyll and nitrogen.
4. Develop a software for converting the meter readings from all the meters tested into chlorophyll and nitrogen content.
5. Develop the database for most important species and cultivars including apple, pear and cherry

Significant Findings:

1. Determined the major factors affecting the accuracy of the chlorophyll and nitrogen meters
2. Found the best wavelengths for measuring chlorophyll, nitrogen and other compounds by using the Foss Near Infrared (NIRsys-6500) equipment, regression analysis and other statistical techniques.
3. Results of field and lab tests showed that the OSU prototypes (OSU-I and OSU-II) were more accurate in determining chlorophyll and nitrogen content than all other meters tested.
4. The OSU-II is more accurate than OSU-I and all the other meters tested. It contains additional wavelengths to measure more factors and compounds. The OSU-II prototype also stores more information than the first prototype and the commercial meters, including the software for converting the meter readings to chlorophyll and nitrogen, database for some of the fruit varieties, and stores at least 1000 data sets.
5. Developed a Microsoft Windows based PC software for converting OSU and commercial meters readings directly to chlorophyll and nitrogen content. The software has been updated to second version with more functions.
6. Developed a database for storing the calibration information used for converting meter reading to chlorophyll and nitrogen contents of the most important species and cultivars including apple, pear and cherry.

Methods and Procedures:

1. Plant materials

The leaves containing different chlorophyll and nitrogen levels were sampled from the following plants throughout the summer of 2000 and 2003:

a. Bench-grafted Fuji/M26, 'Gala'/M26, 'Nonpareil' almond, and *Populus* spp were grown in pots and fertigated with six different levels of nitrogen each mixed with the modified Hoagland solution and applied at 3 days interval from June to September.

b. ‘Jonagold’/M26, ‘Fuji’/M26, ‘Gala’/M26, ‘Liberty’/M26, and ‘Braeburn’/M26 apple varieties growing at the Lewis-Brown horticulture farm at OSU.
c. ‘Bartlett’ and ‘Comice’ pear varieties, ‘Bing’, ‘Skeena’ and ‘Regina’ cherry varieties from Hood River OSU research station.
d. ‘Cameo’ apple growing at Fleming Orchard
e. Red and green leafed varieties of *Prunus spp*. growing on the OSU campus.
f. Eastern redbud and red maple growing on the OSU campus.

2. Method used for determining wavelengths

Fresh leaves from the above plants were harvested and immediately placed into plastic bags for transport to the laboratory. Leaf discs were taken with a 2.85cm^2 cork borers and used to immediately obtain data with the SPAD meter, Foss NIR System and spectroradiometer. The leaf discs were then placed in test tubes with 80% acetone to determine chlorophyll content. A portion of the same leaf was also used for Kjedahl nitrogen analysis. Multiple regression and statistical analysis methods were used to choose the best wavelength for measuring chlorophyll and nitrogen content in fresh leaves.

3. Software development and update

MS Visual Basic 6.0 SQL and Access were used to develop the software and related database for converting meter readings directly into chlorophyll and nitrogen contents. The method used for the development for developing the database included the following:

1) Selected fruit species and varieties
2) Determined the meter readings using the OSU prototypes and the commercial meters
3) Compared the meter readings with chemical methods for determining the chlorophyll, nitrogen concentrations and other compounds/factors and used the software developed for converting the meter readings to chlorophyll and nitrogen content.
4) Developed the software for the plants tested and related this to current standards.

Results and Discussion

1. Comparison between SPAD meter and NIR measurement:

In order to find the best wavelengths for determining nitrogen and chlorophyll; Fuji apple, poplar and almond plants were fertigated with a wide range of N throughout the growing season. Leaves were sampled from the plants and analyzed for nitrogen and chlorophyll with the SPAD meter, the laboratory Foss NIR equipment and chemical methods. Statistical methods were then used to analyze data to determine the R^2’s for the meter readings and the chlorophyll and nitrogen content. The results showed that the wavelength used by the SPAD meter was not accurate for determining chlorophyll and nitrogen in fresh leaf samples (Table 1).

Table 1. The relationship between instrument readings and chlorophyll and nitrogen content by chemical analysis (2001)

R^2 between	**Instrument**	**Fuji apple**	**Poplar**	**Almond**
Meter reading and chlorophyll content	SPAD	0.8676	0.9022	0.7443
	NIR Reflectance	0.9024	0.9390	0.8972
	NIR Transmission	0.9143	0.9252	0.8308
Meter reading and nitrogen content	SPAD	0.6698	0.9384	0.3828
	NIR Reflectance	0.7591	0.9653	0.5798
	NIR Transmission	0.7668	0.9533	0.6887

2. OSU-I prototype development and comparison with the commercial meters

Based on the optimum wavelengths for determining chlorophyll and nitrogen we obtained from our laboratory tests, Dr. Tom Plant and Dan Melende of the Electrical and Computer Engineering Department assisted us in producing the first OSU prototype (OSU-I). In order to test and verify the accuracy and reliability of the OSU-1 prototype, three commercial meters (SPAD, CCM-200 and CM-1000) were compared with the OSU-I prototype. The results showed that the OSU-I prototype was consistently more accurate than all other meters tested (higher R^2 than other meters) in measuring chlorophyll and nitrogen.

Table 2. The relationship between chlorophyll, nitrogen and the readings of CNDS, SPAD meter, CM-1000, CCM-200 (2001)

R^2 between	Instrument	Jonagold	Fuji	Cameo
Meter reading and chlorophyll content	OSU-I prototype	0.8952	0.9023	0.9486
	SPAD	0.8334	0.8990	0.9073
	CM-1000	0.6910	0.8257	0.8556
	CCM-200	0.7439		0.7996
Meter reading and nitrogen content	OSU-I	0.8922	0.8840	0.8841
	SPAD	0.8078	0.8478	0.7930
	CM-1000	0.6198	0.7756	0.7616
	CCM-200	0.7458		0.6050

Table 3. The R^2 between meter reading and chlorophyll content (2002)

Cultivar/ Varieties	**CM-1000**	**SPAD**	**CCM-200**	**OSU-I**
Cameo apple	0.5304	0.9257	0.8448	0.9370
Jonagold apple	0.7323	0.9487	0.9149	0.9457
Braeburn apple	0.7060	0.8900	0.8190	0.9006
Fuji apple	0.7451	0.9274	0.9107	0.9511
Red Maple	0.8771	0.9327	0.9467	0.924
Concord Pear	0.6795	0.8955	0.8566	0.9127
Popular	0.6977	0.8809	0.8896	0.9078

Table 4. The R^2 between meter reading and N content (2002)

Cultivar/ Varieties	**CM-1000**	**SPAD**	**CCM-200**	**OSU-I**
Cameo apple	0.5908	0.9055	0.8134	0.9147
Jonagold apple	0.7885	0.8772	0.8598	0.9146
Braeburn apple	0.6459	0.8188	0.7625	0.8848
Fuji apple	0.6118	0.8871	0.7967	0.9097
Red Maple	0.639	0.7826	0.7404	0.8745
Concord Pear	0.5892	0.8767	0.8265	0.8828
Poplar	0.8642	0.9017	0.8741	0.9206

3. Factors affecting the accuracy of the meters

(1)Relationship between the wavelengths and the accuracy of the meter

The most important parameter that affects the accuracy of the meter is the wavelengths of the LEDs used in the meters. The results showed that the wavelength of the LEDs used by the SPAD and other meters was not optimal for determining chlorophyll and nitrogen in fresh leaf samples. In addition to the wavelength, other factors such as the light source and sampling distance also affected the accuracy of some of the meters (Table 5).

Table 5. Relationship between structure and meter accuracy (2002)

Meter	SPAD Meter	CCM-200 Meter	OSU Prototype	CM-1000 Meter
Mechanism	Transmission	Transmission	Transmission	Reflectance
Light Source	Inside LEDs, Stable	Inside LEDs, Stable	Inside LEDs, Stable	Nature light, affected by direction, conditions
Sampling Distance	Constant	Constant	Constant	Not Stable
Accuracy	Accurate	Accurate	Accurate	Not accurate

(2) Effects of the physical and chemical properties of plants on the accuracy of the meters

Although the OSU-I prototype was more accurate than the commercial meters we found that the physical and chemical properties of the leaves, e.g. leaf texture and leaf water content, may also affect the accuracy of the meter. Figure1 showed that a decrease of leaf water content increased the readings of the SPAD, CCM-200 and OSU-I prototype.

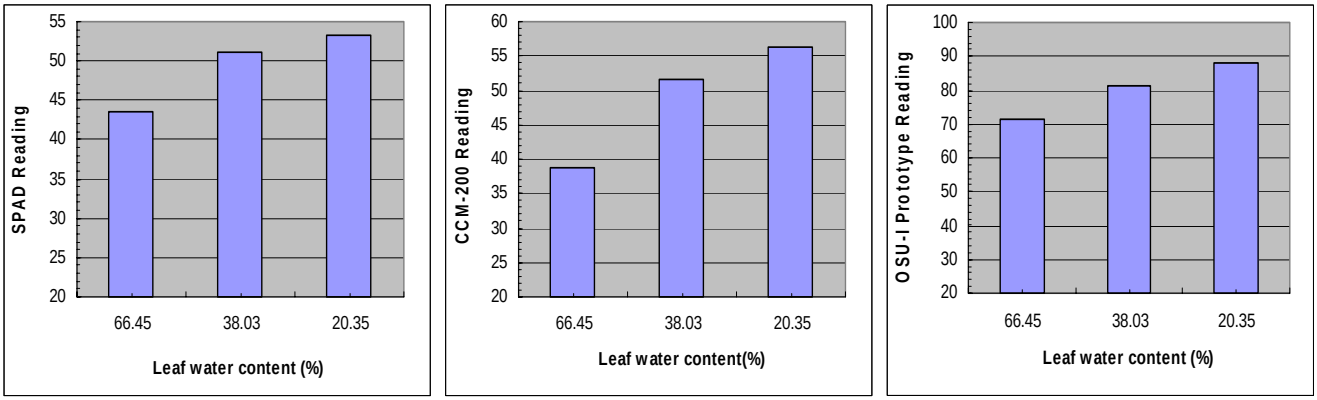

Fig.1 Effect of leaf water status on meter reading

(3) Effect of growth stage on meter reading

Leaf chlorophyll and nitrogen vary with changes in either growth stages or leaf age. The meters were compared at different growth stages and the results are shown in Fig2. The data shows that the readings of all meters taken at different growth stages throughout the growing period varied with growth stages. This suggests the importance of knowing the growth status of the plant when using the meter or other methods to determine chlorophyll and nitrogen content in fruit trees.

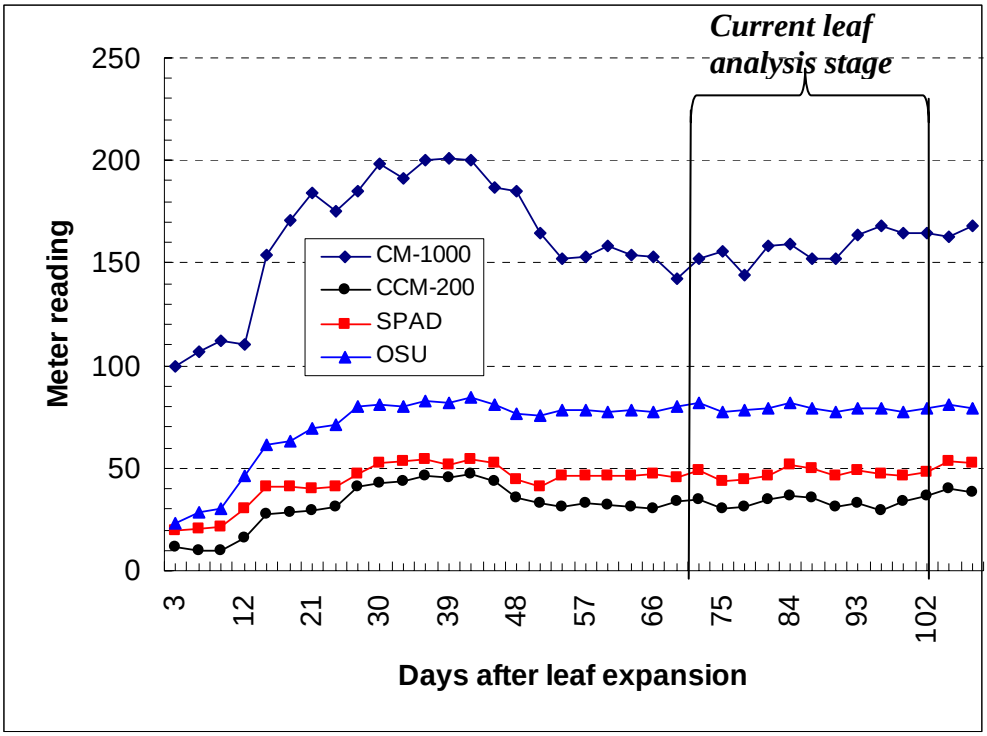

Fig2. Relationship between Gala leaf age and meter reading

4. OSU-II prototype development and its relationship with the other meters

Our laboratory analysis indicated that the accuracy of the meter could be greatly improved by including several wavelengths to determine chlorophyll, nitrogen, water, etc. Based on the results of the above studies the second OSU prototype (OSU-II) was developed with the assistance of Professor Tom Plant. The result of the laboratory tests with the OSU-II meter showed that the OSU-II prototype was more accurate than the OSU-I and other meters tested (e.g. higher R^2 than other meters) in measuring chlorophyll (Table 6). The tests with the new meter for nitrogen are still being analyzed but preliminary data shows that the OSU-II is more accurate than the other meters.

Table 6. The R^2 between meter reading and chlorophyll content (2002)

Cultivar/ Varieties	CM-1000	CCM-200	SPAD	OSU-I	OSU-II
Cameo apple	0.6790	0.8332	0.9046	0.9298	0.9231
Gala apple	0.8077	0.8661	0.9034	0.9080	0.9281
Fuji apple	0.8844	0.8343	0.9090	0.9295	0.9348
Bartlett pear	0.3981	0.8835	0.8802	0.902	0.9109
Comice pear	0.6488	0.9012	0.8233	0.9115	0.9214
Bing cherry	0.5613	0.7026	0.7343	0.7474	0.8338
Skeena cherry	0.7609	0.8739	0.8971	0.8904	0.9053
Regina cherry	0.6237	0.7443	0.7553	0.8571	0.9028
Pinot Noir Grape	0.8866	0.8726	0.9087	0.9171	0.9293
Poplar	0.8010	0.8743	0.8862	0.9085	0.9221

5. Software Development and update

In order to convert the meter readings into chlorophyll and nitrogen content, we developed a software called the **Chlorophyll and Nitrogen Diagnostic System (CNDS).** This software converts the meter reading into chlorophyll and nitrogen content. In addition to the OSU prototypes, the software was also developed to convert the readings of the SPAD, CM-1000 and CCM-200 meters directly into chlorophyll and nitrogen content.

The MS Windows based software was updated to version II in 2003. The new version software has many more functions than the previous version I and is much more user friendly. The software also includes the current leaf analysis standards in the database, which should help orchardist relate the meter readings to the plant health of the fruit crop by comparing the meter readings with the current leaf analysis standards.

The software has a database to store the calibrations of the various parameters for different species and cultivars/varieties. After calibrated for specific cultivar/variety of fruit trees, the grower can use the software to directly measure the chlorophyll and N contents of the specific tree fruit cultivar/variety. To use this Microsoft Windows based software, the data obtained from the OSU-I and other meters must be downloaded into a computer containing the software and database. In contrast, the OSU-II prototype has a smaller version of the software and database of some fruit tree varieties stored in it to make the conversions directly with the OSU-II. At least 1000 data sets can be stored in the OSU-II prototype.

6. Functions of the OSU-II prototype

The OSU-II prototype includes a smaller version of the CNDS software and database stored in it to convert the meter readings into chlorophyll and nitrogen concentration. With this database, it can directly convert the readings into chlorophyll and N content or display the readings of the different wavelength used in the meter. The schematics of the main functions and the usage of the OSU-II prototype are shown in Fig3. After the OSU-II is turned on, it will first display "CNDS 1000 meter ready", followed by the MODE selection. There are 6 main modes (Measure, View, Delete, Plant, Clear and Upload) marked as numbers 1 to 6. The contents of different mode are as follows:

(1) Measure mode:

This mode enables the user to calibrate the meter by closing the sampling head without any test sample. After calibration, the meter is ready to be used for measuring samples. In the "Measure" mode, the OSU-II will display the value for the test sample as the raw sample reading of different wavelengths rather than the chlorophyll and N contents. The meter will also show the current sample number "S#" and the available space as "SP#".

(2) View mode:

The view mode will display all the data that has been stored in the meter. It will also show the current viewing sample number "View#" and the total stored sample number as "T#". By pressing "A.NEXT" the user can view the stored data of the next sample. The user can view the previous sample's result by pressing "C.PREV" or return the meter back to the mode selection screen by pressing "B.MODE".

(3) Delete mode:

If the user is interested in deleting the most recent data taken he presses "Delete**"** mode, and the OSU-II will show the last data of the measurement, the sample number "View#" and the total stored sample number as "T#". Only the last data set (the displayed data) can be deleted from the meter by pressing "C. Delete". The data can be deleted one at a time only starting with the most recent data taken. The user can returning the meter to MODE selection at anytime by pressing the "B.MODE".

(4) Plant mode:

The OSU-II had a limited version of the database. If the user is interested in locating the database of a particular plant genotype the user would select the "Plant**"** mode which will display apple, pear, cherry or grape. Each major plant group may contain some specific plant variety. To use the OSU-II for measuring chlorophyll and nitrogen contents user need first select the major species, eg. Apple, then select the specific variety/cultivar (for example Fuji) to work with. As soon as the cultivar is selected, the OSU-II will let the user "Close the sampling head for calibration (No Sample)". After calibration, the meter can now be used for measuring the chlorophyll and nitrogen of 'Fuji' leaf samples by directly showing the chlorophyll, nitrogen and water contents.

5) Clear mode:

By selecting the "Clear**"** mode, the user will be "Cautioned" about losing the data. If the user presses "A. YES", the entire data stored ion the meter will be lost. If the user presses "B. NO", the meter will return to the state if mode selection.

(6) Upload mode:

By selecting the "Upload**"** mode, the OSU-II will show the status of the connection between the OSU-II and the PC, then upload the data to PC for future analysis or later use.

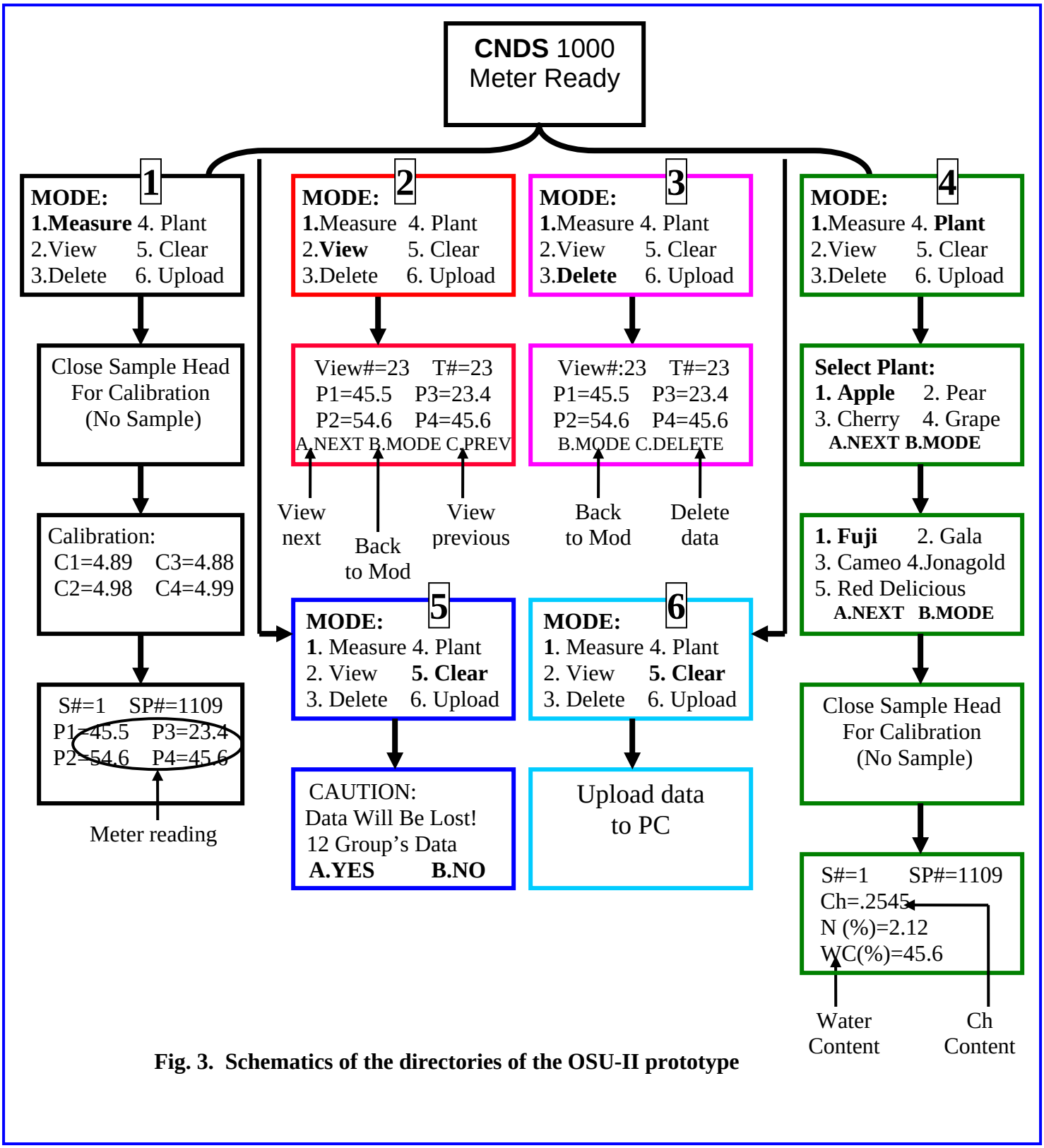

Fig. 3. Schematics of the directories of the OSU-II prototype

7. Limitation of the current OSU prototypes

The objectives of the 2001-2003 project was to develop a portable meter based on near-infrared technology to accurately measure the chlorophyll and nitrogen contents of tree fruit crop leaves. The OSU-I and OSU-II prototypes were found to be more accurate than all the commercial meters available. The software we developed can be used by both OSU prototypes and the commercial meters. To convert the meter readings from any of the meters the data is downloaded from the meter into a computer which then uses the software we have developed for each of the meters to convert the meter readings into the chlorophyll and nitrogen concentrations. In contrast to the other meters the OSU-II prototype has a small "computer" which has the ability to store the software for converting the meter readings into chlorophyll, nitrogen and water content. It also has the capability of storing a limited amount of the database for the tree fruit varieties/cultivars and about 1000 data sets. The OSU-II prototype can therefore either display the readings of each wavelength or directly display the actual chlorophyll and nitrogen concentrations of the test sample. Unfortunately the micro-computer and the storage function of the OSU-II prototype are quite limited. In order to increase the capabilities of the meter we are proposing to develop a new prototype by installing/attaching the sampling head of the OSU meter to a pocket computer (PDA) and combine this with a GPS hardware and software to produce a PDA + GPS system for the instantly diagnosing the chlorophyll, nitrogen and water content of fruit trees and at the same time identify the location of the test sample. The proposed system will utilize the computer and storage capacities of the PDA for storing the software, database and sample data sets which includes the identification of the sampling sites.

BUDGET

Project title: Portable meter to measure chlorophyll and nitrogen
PI: Leslie H. Fuchigami
Project duration: 2001-2003
Current year: 2003
Project total (3 years): **$**99,065
Current year request: **$**21,540

Year	Year 1 (2001)	Year 2 (2002)	**Year 3(2003)**
Total	52,525	25,000	**21,540**

Current year breakdown

Item	Year 1 (2001)	Year 2 (2002)	**Year 3(2003)**
Salaries	26,000	18,000	**18,000**
Benefits (%)	13,000	540	**540**
Equipment	3,150	0	**0**
Materials and Supplies	8,915*	5,000*	**2,000***
Travel	1,460**	1,460**	**1,000****
Total	52,525	25,000	**21,540**

*Nitrogen and chlorophyll analysis $10.00 per sample.

**Travel to Washington to collect samples and take readings with the chlorophyll and nitrogen meters.