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PROGRESS REPORT FOR 2000-2001

Title: Development of a portable non-destructive NIR system and related software for determining nitrogen and chlorophyll content of fresh leaf samples in fruit trees.

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Significant Findings: In order to develop a technique for orchardist to monitor their tree health during the growing season, the Near Infrared (NIR) technique and standard wet chemical analysis was analyzed statistically to determine the best wavelength for determining chlorophyll and nitrogen content. The information obtained from these studies was used to develop a prototype that is capable of measuring the chlorophyll and nitrogen content of leaves. Professor Tom Plante and his student, Dan Melende, took the information obtained by Dr. Ding and produced the first OSU prototype. The OSU prototype was then compared with four other chlorophyll and plant health meters: 1) Spad meter by Minolta; 2) Observer by Spectrum Technologies; 3) CCM-200 Chlorophyll Content Meter by OptiSciences) and some other prototypes developed by other companies that was sent to our lab for evaluation. In addition to the OSU prototype we also developed a software called the Chlorophyll and Nitrogen Diagnostic System (CNDS) that converts the readings from all the meters tested to chlorophyll and nitrogen content. Without this software the readings displayed by the meters is not related to anything.

The results of field tests comparing all the meters showed that the first OSU prototype meter was more accurate in determining chlorophyll and nitrogen content than all other meters tested. By comparing these meters with the Foss NIR system we were able to determine the cause of the inaccuracies of the meters tested, including the OSU prototype. We are currently working on a second OSU prototype that should be considerably better than the previous prtototype and the other meters tested. The second prototype should be more accurate and will contain additional hardware to measure other factors and store more data than the previous prototype.

The current software includes a few major apple varieties and other fruit crops. Our objective will be to work with orchardists and nurserymen to expand the data base to include most of the major fruit crops produced in the Northwest. In addition our goal is to also develop a diagnostic standard for fresh leaves that is related to the current leaf analysis standards for the major fruit crops. We also propose to determine the chlorophyll and nitrogen content of leaves throughout the growing season in relations to some of the tests we are currently investigating and develop a model for relating these measurements to the health and productiveness of fruit trees.

Objectives:

- Develop a portable hand held meter for determining chlorophyll and nitrogen content in apple leaves.
- Test the OSU prototype under field conditions with commercial and other chlorophyll and plant health meters.

- Develop a software for converting the readings from all the meters tested to chlorophyll and nitrogen content.

PROCEDURES:

(1) Plant materials

The leaves from the following plants were sampled throughout the summer of 1999 and 2000:

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

b. Field grown red and green leaf varieties of *Prunus growing on the OSU campus*;

c. Field grown 'Jonagold'/M26, 'Fuji'/M26, 'Gala'/M26, 'Liberty'/M26, and 'Braeburn'/M26 apple varieties growing at the Lewis-Brown horticulture farm at OSU.

(2) Method used for choosing wavelength

Fresh leaves from the above plants were harvested and immediately placed into plastic bags and a portable ice chest for transport to the laboratory. Individual leaves were removed from the bag as necessary. Leaf disks were then taken with a 2.85cm^2 cork borer and used immediately for the measurement in the SPAD meter, the Foss NIR System for spectral scanning from 400 to 2500nm to determine the absorptance measurements, and the spectroradiometer for scanning from 300 to 1100nm to determine both the reflectance and absorptance measurements. The leaf disks were then cut into small pieces and placed in test tubed with 80% acetone to determine chlorophyll A and B, and total chlorophyll. A portion of the same leaf was also used for Kjedahl chemical nitrogen analysis.

Stepwise regression statistical analysis was then used to determine the best wavelength for determining chlorophyll and nitrogen content from fresh leaves.

(3) Method used for writing software

Microsoft Visual Basic 6.0 was used to write the following software: Chlorophyll and Nitrogen Diagnosis System (CNDS).

(4) Development of the database for fruit tree cultivars.

Used fertigation studies to create a wide range of chlorophyll and nitrogen content for some of the species tested in 2000 and/or directly selected leaf samples from plants growing at the OSU Lewis-Brown Horticulture Farm and OSU campus.

RESULTS AND DISCUSSION:

Comparing the Minolta Spad Meter with the Foss NIR system and Spectroradiometer:

The R^2 's obtaine from simple linear regression analysis in Table 1 shows the results of testing the SPAD meter with the laboratory Foss NIR System and Spectroradiometer. (The higher the R^2 values the more accurate the instrument.) The data shows that the Spad meter values are consistently smaller (therefore less accurate) than the reflectance and transmission readings obtained from both the Foss NIR and spectroradiometer in all plants tested for chlorophyll a, chlorophyll b and chlorophyll a + chlorophyll b (Table 1) and nitrogen (Table 2). The wavelength selected for determining the Foss NIR and Spectroradiometer measurements was determined by regression analysis of the scannings from both instruments.

The results of this study indicated that the light source used by the SPAD meter is not ideal for determining chlorophyll and nitrogen in fresh leaf samples. The SPAD meter uses transmission at 650nm to measure the green color of leaves.

Table 1. The relationship between chlorophyll and the readings of SPAD meter, NIR reflectance and spectroradiometer transmission

Species or cultivar	Instrument	Chlorophyll a		Chlorophyll b		Chlorophyll a + b	
		Equation	**R^2**	**Equation**	**R^2**	**Equation**	**R^2**
Fuji Apple	SPAD	Y = -5.4578 + 0.1828 X	0.8751	Y = -1.2899 +0.0468X	0.8751	Y = -6.7477+0.2296X	0.8676
	Reflectance	Y=-10.1718 +25.2563X	0.9151	Y=-2.4485 + 6.3851X	0.9635	Y=-12.6203 + 31.6414X	0.9024
	Transmission	Y=11.5783 - 55.1205 X	0.9217	Y=3.3605 - 12.5411 X	0.9509	Y=16.0263 - 64.0910 X	0.9143
Poplar	SPAD	Y = -0.7134 + 0.0824X	0.9445	Y= - 0.3191+0.0236X	0.6718	Y=-1.0325+ 0.106X	0.9022
	Reflectance	Y=-4.2320 + 10.3092X	0.9635	Y=-2.9209 + 7.8964X	0.7662	Y=-6.6089 + 17.1035X	0.9390
	Transmission	Y=3.9993 -17.6341 X	0.9183	Y=1.3452 - 6.2274 X	0.6669	Y=5.3293-23.8653 X	0.9252
Almond	SPAD	Y = -2.6891 + 0.1506X	0.7486	Y= - 0.5600+0.0359X	0.6866	Y = - 3.2491+0.1865X	0.7443
	Reflectance	Y=-6.6310 + 15.5071X	0.8932	Y=-1.5557 + 3.7968 X	0.8629	Y=-8.1867 + 19.3039X	0.8972
	Transmission	Y=7.0467 -22.5193 X	0.8360	Y=1.7605 - 5.3629 X	0.7642	Y=8.8072-27.8822 X	0.8308

Table 2. The relationship between nitrogen and the readings of SPAD meter, NIR reflectance and spectroradiometer transmission

Instrument	Fuji apple		Poplar		Almond	
	Equation	R^2	**Equation**	R^2	**Equation**	R^2
SPAD	Y= 31.337+ 8.8376X	0.6698	Y =- 0.4297+0.0547X	0.9384	Y =-1.8484 +0.1242X	0.3828
Reflectance	Y=- 3.7546+10.9016X	0.7591	Y=-3.2834+ 8.7816X	0.9653	Y=- 5.8261+13.5161X	0.5798
Transmission	Y=3.9508 - 22.6870X	0.7668	Y=2.7392 - 11.8836X	0.9533	Y=6.7633- 30.6836X	0.6887

Development of the OSU prototype

Dr. Tom Plante and Dan Melende from the Computer and Electrical Engineering Department helped us build the first OSU prototype (OSUP) based on the results of laboratory tests on the best wavelength to determine chlorophyll and nitrogen content in fresh leaves of fruit trees. The first prototype was produced in the summer of 2000.

Comparison of OSU prototype with the commercial chlorophyll and plant health meters and prototype of chlorophyll meters from other companies

In order to test and verify the accuracy and reliability of OSU prototype, apple leaf samples of 'Cameo' (sampled from Fleming's Orchard), 'Fuji', 'Jonagold', and 'Gala' (Sampled from the Lewis-Brown Horticulture Farm at OSU) were measured with the meters shown in Table 3 and 4. The data shows that the OSU prototype was consistently more accurate than all other meters tested (e.g. had higher R^2 than other meters) for both chlorophyll and nitrogen content in fresh leaves (Table 3 and 4, respectively).

Table 3. . The relationship between chlorophyll and the readings of CNDS, SPAD meter, Observer, CCM-200

Apple Cultivar	Instrument	Equation	R^2
Cameo	OSUP	$y = 0.0044e^{0.0314x}$	0.9486
	SPAD	$y = 0.0049e^{0.0467x}$	0.9073
	Observer	$y = 0.0025e^{0.0146x}$	0.8556
	CCM-200	$y = 0.0149e^{0.032x}$	0.7996
Jonagold	OSUP	$y = 0.0036e^{0.0342x}$	0.8952
	SPAD	$y = 0.0047e^{0.0494x}$	0.8334
	Observer	$y = 0.0057e^{0.0107x}$	0.6910
	CCM-200	$y = 0.017e^{0.0303x}$	0.7439
Fuji	OSUP	$y = 0.0041e^{0.0334x}$	0.9023
	SPAD	$y = 0.0041e^{0.0511x}$	0.8990
	Observer	$y = 0.0035e^{0.0102x}$	0.8257
Gala	OSUP	$y = 0.0489e^{0.0258x}$	0.9640
	SPAD	$y = 0.0273e^{0.0549x}$	0.8922
	SPAD	$y = 0.0423e^{0.0408x}$	0.9025

Table 4. . The relationship between nitrogen and the readings of CNDS, SPAD meter, Observer, CCM-200

Apple Cultivar	Instrument	Equation	R^2
Cameo	OSUP	$y = 1.1024e^{0.0086x}$	0.8841
	SPAD	$y = 1.1623e^{0.0124x}$	0.7930
	Observer	$y = 0.9592e^{0.0039x}$	0.7616
	CCM-200	$y = 1.5919e^{0.0079x}$	0.6050
Jonagold	OSUP	$y = 0.819e^{0.0117x}$	0.8922
	SPAD	$y = 0.9105e^{0.0167x}$	0.8078
	Observer	$y = 0.9949e^{0.0035x}$	0.6198
	CCM-200	$y = 1.3994e^{0.0104x}$	0.7458
Fuji	CNDS	$y = 0.6037e^{0.0215x}$	0.8840
	OSUP	$y = 0.6208e^{0.0326x}$	0.8478
	Observer	$y = 0.5678e^{0.0064x}$	0.7756
Gala	OSUP	$y = 1.0191e^{0.0125x}$	0.9185
	SPAD	$y = 0.7812e^{0.026x}$	0.8945

Predicted results: comparison of OSU prototype with other meters

In order to use the meter reading of fresj leaves and predict the chlorophyll and nitrogen content based on the reading and the equation for the various meters, 10 leaves from 'Fuji' and 'Gala' were measured and compared with chemical analysis for chlorophyll and nitrogen

(Table 5). These results showed that the OSU prototype was more accurate in predicting chlorophyll and nitrogen (Table 5).

Table 5. Predicted and chemical result of chlorophyll and nitrogen of different meters

Apple Cultivar	Substance	Chemical Result	Predicted result		
			CNDS	SPAD	Observer
Fuji	Chlorophyll (mg/cm^2)	0.0534b	0.0528b	0.0489bc	0.0653a
	Nitrogen (%)	2.59b	2.56b	2.04c	3.19 a

*Values within a column followed by different letters differ significantly at *P=0.05*.

Software Development

In order to convert the meter readings into chlorophyll and nitrogen content, we wrote a software called the **Chlorophyll and Nitrogen Diagnosis System (CNDS).** This software has many functions. It can directly convert the meter reading into chlorophyll and nitrogen content without chemical analysis. In addition to the OSU prototype, this software also computes the chlorophyll and nitrogen content from the readings obtained from the SPAD, Observer , and CCM-200 meters. None of the meters come with a software to convert the meter readings to chlorophyll or nitrogen content.

CONCLUSIONS:

The OSU prototype (OSUP) meter for determining chlorophyll and nitrogen content of fresh leaves of fruit crops was developed by OSU Horticulturists (Ding and Fuchigami) and Computer and Electrical Engineers (Plante and Melende). This OSUP Meter was consistently more accurate than commercial chlorophyll and plant health meters and other prototypes produced by other companies. None of the meters produced commercially converts the meter readings to chlorophyll or nitrogen content. Dr. Ding has developed a software from Visual Basic to quickly and accurately convert the readings from the OSUP and other meters to chlorophyll and nitrogen content of several fruit tree varieties.