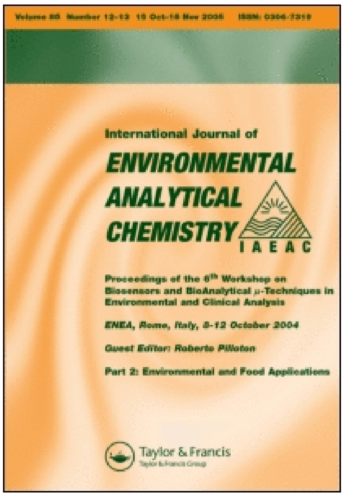

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An Investigation of the Effects of Seasonal Changes, Leaf Maturity, Nitrogen Deficiency and Leafhopper Injury on the Chlorophyll Content and Diffuse Reflectance Spectroscopic Properties of Orchard Leaves

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Diffuse reflectance spectroscopy was used to measure leaf surface reflectance and pigmentation to reveal any changes caused by seasonal variation, leaf maturation, nitrogen deficiency and feeding leafhoppers. Leaves were also analyzed for chlorophyll content. Significant changes in yellow-to-green leaf pigment ratios were observed between May and June readings for Red Delicious and McIntosh varieties and also between the July and August readings for Red Delicious. Significant differences were also noted for measurements of chlorophyll content and leaf surface reflectance for the two apple varieties between May and June observations. Leaves from apple trees infested with leafhoppers showed significant changes in pigment ratios and diffuse reflectance from controls and differed from results previously reported for damage caused by mite infestation. Nitrogen deficiency produced the greatest changes in chlorophyll content, reflectance and pigment measurements.

KEY WORDS: Diffuse reflectance spectroscopy, leafhoppers, nitrogen deficiency, insects, orchard.

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INTRODUCTION

In a previous study,[1] we demonstrated the application of diffuse reflectance spectroscopic measurements to the assessment of damage to leaves caused by infestation with European red mites, *Panonychus ulmi* (Koch). Changes caused in measured pigmentation at the leaf surface were interpreted in terms of overall changes in leaf chlorophyll content, as measured by chemical analyses. The use of the technique provided an alternative to leaf collection and counting the number of mites present per leaf.

A quantitative relationship between the chlorophyll content of soybean and Valencia orange leaves and their percent reflectance of light at 625 nm had been shown earlier by Benedict and Swindler.[2] Changes in the reflectance of leaves from soybeans, corn, wheat, oats, sorghum and Sudan-grass at three periods during the growing season were studied by Sinclair *et al.*[3] These authors found that the reflectance spectra of all fresh green leaves from these crops were very similar and that reflectance increased at all wavelengths as the crop matured and the leaves senesced. Wooley[4] reported that leaves lacking normal pigment content reflect increasing amounts of visible light. Studies by Al-Abbas *et al.*[5] on normal and nutrient-deficient maize leaves showed that both the physiological age of leaves and nutritional deficiencies produced changes in their reflectance of visible light, while Kanemasu[6] measured changes in the reflectance of these wavelengths over a growing season for foliage of sorghum, wheat and soybean. Thomas and Oerther[7] used diffuse reflectance spectroscopy to estimate the nitrogen content of sweet pepper leaves.

While more recent work has investigated similar applications using near infrared reflectance spectroscopy, such as the estimation of the nutrient content of dehydrated vegetables[8] and corn silage,[9] the ultraviolet-visible region of the spectrum yields useful information on field samples, as previously demonstrated.[1–7] In the study reported herein, experiments were conducted to monitor by reflectance spectroscopy the effects of seasonal changes, nitrogen deficiency and the presence of other leaf-feeding insects in producing changes which might correspond to those previously reported caused by feeding mites.[1]

MATERIALS AND METHODS

Experiments were conducted using test blocks in several orchards on the Summerland Research Station. Seasonal changes and differences between new and mature growth were assessed in a mixed planting of apple cultivars (block "A") which included 15 trees of each of the McIntosh, Red Delicious and Golden Delicious varieties.

For each sampling date, to assess seasonal changes, 30 leaves (6 per tree) were collected from 5 trees each of the Red Delicious and McIntosh varieties. Fifteen leaves were then randomly selected for individual reflectance analysis and the remaining 15 were analyzed as a composite sample for chlorophyll as previously described[1] to monitor changes during the growing season. The effect of leaf maturity on diffuse reflectance spectroscopic measurements and chlorophyll content was determined using 30-leaf samples of developing shoot leaves (new growth) and mature spur leaves collected in late June from all three varieties.

Damage caused by the white apple leafhopper, *Typhlocyba pomaria* McAtee, was measured using 30-leaf samples of mature spur leaves collected in late June from a separate planting of Red Delicious trees (block "B") where leafhopper populations had been allowed to build up. The effect of nitrogen deficiency was measured on 30-leaf samples (mature spur leaves) chosen in late June from a separate planting of Spartan variety apple trees (block "C") which exhibited symptoms of nitrogen deficiency. Controls were selected from other trees of this variety which did not show such symptoms.

Measurements for chlorophyll analyses were made with a Pye Unicam SP1800 spectrophotometer, following extraction with ethanol as previously described.[1] Reflectance analyses were conducted using a Hunterlab D25 Color and Color Difference Meter.

Parameters measured with a Hunterlab D25 Color and Color Difference Meter were the diffuse reflectance, R_d, and the color coordinates, a and b, which are an indication of leaf pigmentation. Positive values of a relate to red, while green is represented by negative a values. Yellow is indicated by positive b and blue is measured by negative b readings.

Tests for significant differences between populations with unequal standard deviations (t-test) were done according to Miller and Miller.[10]

RESULTS AND DISCUSSION

Seasonal changes

Measurements of reflectance, pigment ratio and chlorophyll content of leaves collected from Red Delicious and McIntosh trees in block "A" monthly from early May to late August are reported in Table I. The yellow-to-green pigment ratio ($b/-a$) reached maximum values in early June and had declined by August 20, the final date for measurements. Applying the null-hypothesis for populations with unequal standard deviations (t-test),[10] Red Delicious leaf pigment ratios differed significantly between the readings for May and June ($P=0.02$) and July and August ($P=0.05$), while significant differences ($P=0.01$) were observed for McIntosh leaf ratios between readings for May and June and June and July.

Chlorophyll levels followed a similar pattern to the pigment ratios for Red Delicious (Table I) and, with the exception of the June 6 sample, remained constant through the growing season for McIntosh after a drop from the May analysis. The very low value observed for the June analysis of McIntosh is attributed to a biased sampling of immature growth and may explain the finding of a significant difference in b/a ratio for McIntosh between June and July readings when a similar change was not observed for Red Delicious.

Measurements of the reflectance of the leaf surfaces, R_d, revealed

Table I Changes in chlorophyll content, reflectance R_d and pigment ratio ($b/-a$) and for leaves of two varieties of apple trees over a growing season

Date of sample	Chlorophyll content[a] mg/m^2	R_d[b]	$b/-a$[c]
Red Delicious			
May 10	315.4	5.94±0.19	0.15±0.16
June 6	343.0	5.77±0.35	0.32±0.19
July 25	321.2	5.73±0.23	0.20±0.19
August 20	267.9	5.91±0.25	0.04±0.20
McIntosh			
May 10	308.8	5.68±0.30	0.30±0.13
June 6	188.3	6.00±0.47	0.47±0.19
July 25	285.1	5.88±0.27	0.29±0.16
August 20	281.8	5.90±0.26	0.28±0.10

[a]Analysis of 15-leaf composite sample.
[b]Diffuse reflectance, mean value ± standard deviation.
[c]Yellow-to-green pigment ratio, mean value ± standard deviation.

that no significant differences were observed during the course of the growing season for Red Delicious. Reflectance values for McIntosh showed a significant difference ($P = 0.05$), as determined by the t-test,[10] between May and June readings, but this may be attributed to the sampling problem described above.

Leaf maturity

The stage of maturity of the sample collected had a pronounced effect on values for R_d and leaf pigmentation (b and a), as shown in Table II. Young leaves from new shoot growth had a lower chlorophyll content, which is also demonstrated by the b/a ratio, showing, by t-test, a significant increase ($P = 0.01$) in the yellow-to-green pigment relationship in young leaves. New growth leaves provided consistently higher reflectance readings than leaves sampled from mature spur growth ($P = 0.01$ for McIntosh and Red Delicious; $P = 0.02$ for Golden Delicious). The increase in R_d and b/a values for new growth leaves relative to mature growth leaves produced results equivalent to those previously observed for leaves subjected to a mite infestation,[1] and well in excess of those encountered from seasonal variations. Appropriate sampling of mature growth is therefore an important consideration in assessing the presence of destructive populations of mites as the growing season advances.

Damage by other feeding insects

Leafhopper infestation produced changes in the reflectance parameters measured, as well as in the chlorophyll content of the mature leaves in test block "B" (Table III). Both a and b coordinates were observed to increase with leafhopper injury, while mite-induced injury was previously observed to be marked by an increase in b and a decrease in a.[1] Applying the t-test, the b/a ratios showed significant differences ($P = 0.01$) between controls, moderate and severe infestations. The measured reflectance values R_d showed significant differences ($P = 0.01$) between the controls and leafhopper-infested leaves, but no difference statistically between the samples for moderate and severe infestations. Differentiation between leaf injury caused by leafhoppers and mites was possible, based on our results.

Table II Comparison of chlorophyll analyses and reflectance measurements for new shoot growth and mature spur leaves from three varieties of apple trees

Sample	Chlorophyll content[a] (mg/m^2)	R_d[b]	$-a$[c]	b[c]	$-b$[c]	$\frac{+b^c}{-a}$	$\frac{-b^c}{-a}$
Red Delicious (young)	191.8	6.32±0.37	2.67±0.24	2.06±0.60	—	0.77±0.24	—
Red Delicious (mature)	207.7	5.85±0.18	1.54±0.26	—	0.11±0.21	—	0.09±0.16
Golden Delicious (young)	183.6	6.50±0.35	2.70±0.56	1.84±0.86	—	0.64±0.21	—
Golden Delicious (mature)	245.1	6.07±0.14	2.06±0.25	0.62±0.25	—	0.30±0.09	—
McIntosh (young)	211.3	6.47±0.34	2.50±0.31	1.68±0.67	—	0.65±0.20	—
McIntosh (mature)	242.1	6.11±0.30	1.94±0.25	0.63±0.27	—	0.31±0.13	—

[a]Analysis of 15-leaf composite sample.
[b]Diffuse reflectance, mean ± standard deviation.
[c]Leaf pigmentation: $+a$, red; $-a$, green; $+b$, yellow; $-b$, blue; mean ± standard deviation.

Table III Comparison of chlorophyll analyses and reflectance measurements of leaf samples subject to leafhopper infestation and nitrogen deficiency

Injury	Chlorophyll content[a] (mg/m^2)	R_d[b]	$-a$[c]	b[c]	$-b$[c]	$b/-a$[c]	$\frac{-b}{-a}$[c]
Red Delicious (Block "B")							
Control	303.9	5.63 ±0.20	1.69 ±0.25	—	0.02 ±0.15	—	0.02 ±0.10
Leafhopper (moderate)	277.2	5.90 ±0.28	1.86 ±0.28	0.32 ±0.37	—	0.16 ±0.17	—
Leafhopper (severe)	228.8	5.99 ±0.12	2.04 ±0.17	0.79 ±0.35	—	0.38 ±0.15	—
Spartan (Block "C")							
Control	239.1	6.26 ±0.43	2.14 ±0.54	0.90 ±0.39	—	0.40 ±0.12	—
Nitrogen deficient	81.6	7.19 ±0.36	3.55 ±0.40	3.89 ±0.65	—	1.10 ±0.15	—

[a]Analysis of 15-leaf composite sample.
[b]Diffuse reflectance, mean ± standard deviation.
[c]Leaf pigmentation: $+a$, red; $-a$, green; $+b$, yellow; $-b$, blue; mean ± standard deviation.

Effect of nitrogen deficiency

The greatest changes in chlorophyll content, reflectance and pigmentation observed in these experiments (Table III) were found in measurements of nitrogen-deficient leaves relative to representative controls, as previously reported in a study of maize by Al-Abbas *et al.*[5] Using the *t*-test, significant differences ($P = 0.01$) were observed for comparisons of R_d and b/a for the nitrogen deficient leaves and the controls. The chlorophyll content of the leaves was reduced by 66%, with a corresponding increase in b/a ratio of 175%. Largest values of R_d observed for any leaves examined were also seen for nitrogen deficient leaves. The b values, related to yellow pigmentation, showed a 4-fold increase to values nearly double those found for any other leaves measured. A smaller, but corresponding, increase in observed values for a resulted in a less dramatic increase in the b/a ratio than would have been seen had a remained relatively constant, as previously reported for mite injury.[1] Such consistent and significant changes therefore made it possible to differentiate nitrogen deficiency from insect or mite injury to leaves.

CONCLUSIONS

It is possible to differentiate between injury caused to leaves by feeding mites and that caused by leafhoppers using diffuse reflectance spectroscopic measurements of the leaf surfaces. Nitrogen deficiency provides characteristically different results from those measured for changes caused by feeding mites. The stage of leaf maturity has a definite influence on observed measurements, so only mature spur growth with suitable equivalent controls should be sampled for testing. Observations should be interpreted relative to normally observed variations in leaf reflectance and pigmentation during the growing season. These results suggest that a field-portable reflectance analyzer would have practical applications in screening an orchard for mite and insect damage or nutritional deficiencies.

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