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RELATIONSHIP BETWEEN CHLOROPHYLL CONTENT AND CANOPY REFLECTANCE IN WASHINGTON NAVEL ORANGE TREES (*CITRUS SINENSIS* (L.) OSBECK)

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Abstract

Understanding the spectral features of Washington navel trees is important to remotely measure and monitor the canopy properties and biochemical changes in plantations. Satellite and ground based passive remote sensing techniques have been widely used in many plant species. However, the use of these techniques in orange trees is limited. In this study, relationships between total chlorophyll (Chl) contents and canopy reflectances (RF) were used to determine the most suitable growth and development stage that could be used to measure and monitor the canopy properties and biochemical changes by remote sensing technologies in Washington navel orange trees (*Citrus sinensis* (L.) Osbeck). Plant materials consisted of four different age groups of 15, 20, 25 and 30 years old orange trees. Spectral measurements in the range of 450-900 nm were made at four different growth and development stages; viz., flowering, fruit setting, fruit maturity and dormancy using a portable spectroradiometer and total chlorophyll contents of the leaves at these stages were assessed with a spectrophotometer. The highest relationship between Chl and RF at blue and red bands was r^2= 0.845 and 0.860 at the 0.01 level, respectively, at the fruit setting stage of the 20 years old trees. Again at this stage, fruit setting, total Chl and RF values among the 40 orange trees were significantly correlated at the blue and red bands with the r^2 values of 0.787 and 0.812 at the 0.001 level, respectively. Results of the present study suggested that the most suitable growth and development stage was the fruit setting stage for passive remote sensing techniques to determine and monitor the canopy properties and biochemical changes of orange trees.

Introduction

Plant production is based on photosynthesis and in turn the process of photosynthesis is governed by the use and bio-processing of electromagnetic energy especially at blue and red wavelength of the spectrum. As a perennial plant, citrus trees occurring naturally in the Mediterranean region are important for human consumption, biodiversity and genetic resources. In the last decades several remote sensing techniques were developed to monitor and determine biological processes of living plants. These sensing techniques can provide an immediate, nondestructive and quantitative assessments and allow producers and buyers to make decisions with respect to crop management and pricing. It is well established that there are strong correlations between remotely sensed data and plant physiological status and photosynthetic functioning (Gausman, 1982; Poul *et al*., 1997; Ma *et al*., 2001). Remotely sensed data can also provide timely and spatial distributed information on the crop's conditions and these data can be incorporated into a farm management scheme (Maas, 1998). Applications of remotely sensed data to crops have been adopted for various purposes (Fernandez *et al*., 1994 and Cloutis *et al*., 1996). For instance, the input of reflectance data into yield production models have been shown

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to improve yield estimates, monitoring the plant diseases and stress conditions, and physiological status in the field (Clevers *et al*., 1994; Penuelas *et al*., 1994; Clevers, 1997 and Craig & Shih, 1998).

Reflectances in the visible red ranging from 550 to 675 nm and blue band ranging 450 to 550 nm have been commonly used to estimate green biomass, chlorophyll, carotenoid, nitrogen, dry matter and water content of leaves. Remotely sensed data have also been used in the accurate estimation of some other leaf pigments and as bioindicators of crop stress (Carter, 1994; Penuelas *et al*., 1994; Belanger *et al*., 1995; Filella *et al*., 1995; Yong & Ko, 1998; Luther & Caroll, 1999; Zarco-Tejada *et al*., 2001).

Knowledge of quantitative relationship between leaf chlorophyll content and canopy reflectance is an important step to develop effective indices for remote estimation in different crop development stages (Blazquez *et al*., 1996; Zarco-Tejada *et al*., 2003). Chlorophyll content in the precision agriculture serves as an indicator of photosynthesis activity, stress conditions, measure of the crop response to nitrogen application and many other plant biochemical aspects (Jacquemoud, 1993; Baret *et al*., 1994; Kupiec & Curran, 1995). It is well established that chlorophyll content of leaf is negatively proportional to spectral bands (at 450 nm blue and 670 nm red bands) and positively proportional to the point of maximum slope between 690-740 nm bands (Curran *et al*., 1991; Gitelson and Merzylak, 1997; Wells, 2001).

Although satellite and ground based remote sensing techniques have been widely used in a number of plant species, there is limited research on citrus species. In the present study, relationships between total leaf chlorophyll (Chl) contents and canopy reflectances (RF) were assessed to determine the most suitable growth and development stage that could be used to measure and monitor the canopy properties and biochemical changes by ground-based remote sensing in Washington navel orange trees (*Citrus sinensis* L. Osbeck).

Materials and Methods

Study site: The study site, the Akdeniz Citrus and Greenhouse Research Institute, is located on the Aksu Plains (30°53' E, 36°52' N), 20 km east of Mediterranean Basin of Antalya, Turkey. Four different experimental plots (orchards) planted with Washington navel (*Citrus sinensis*) trees were used in this study. Each orchard consisted of 10 randomly selected trees. The ages of the orange trees grown in each plot were 15, 20, 25 and 35 year-old. Orange trees growing in each plot received similar cultural practices. All the spectral reflectance measurements (at each of the four wavelength) and chlorophyll content values obtained from each plot were averaged and the averaged values were used for further analysis.

Reflectance measurements: Spectral reflectance measurements for each growth and development stages with three replications were made on randomly selected 10 trees for each plot. Each spectral measurement at 4 different wavelength (three visible and one NIR) was made for 10 trees grown in each plot and each growth and development stage. Spectral measurements of canopies were assessed on each tree from each orchard under cloudless conditions between 09:30 and 10:30 local standard time at the four different growth and developmental stages, viz., flowering (April, 2002), fruit setting (June, 2002), fruit maturity (November, 2002), and dormancy (February, 2003) stages according to Erickson (1968). Canopy reflectance (RF) measurements in the range of 450-900 nm were made using a portable spectroradiometer (Model 100AX Exotech Inc., USA) which was set to four spectral wavelength channels (bands). Wavelength of the channels termed as CH1 (blue band), CH2 (green band), CH3 (red band), and CH4 (near-infrared band, NIR) were 450-520, 520-600, 630-690, and 760-900 nm, respectively. Calibrations of the sunlight and canopy reflectance measurements for each orange tree at each orchard were made using the standard $BaSO_4$ panels according to Rudorf & Batista (1990), Jackson *et al*., (1992) and Zarco-Tejada *et al*., (2003).

Chlorophyll content measurements: Plant leaf samples from the sunlit parts of each individual canopy were clipped and immediately brought to the laboratory in a plastic bag. For the chlorophyll content analysis a total of 480 leaf samples with three replications (10 trees from each plot, 3 replicate, 4 different plots and 4 different growth and development stages) collected from each plot and each growth and development stage. Extraction of chlorophylls from the leaves of each canopy at the 4 different growth and development stages was carried out and total chlorophyll content (Chl) of these leaves were determined as mg/g using a spectrophotometer (Model UV-160A Shimadzu, Japan) as per Williams (1984).

Data analyses: Data were analyzed using two different steps. First, average of three replicated canopy reflectance readings and average of three replicated spectrophotometer chlorophyll readings obtained from each 10 individual trees at each plot were statistically analyzed to determine the relationship between age-dependent chlorophyll contents and canopy reflectances of Washington navel trees at four different growth and development stages (Table 1). In the second step, average of three replicated canopy reflectances and average of three replicated spectrophotometer chlorophyll readings collected and measured from 40 individual trees at all plots were statistically analyzed to determine the relationship between age-independent chlorophyll contents and canopy reflectances at different growth and development stages of Washington navel trees (Table 2).

Correlation coefficients between chlorophyll content and canopy reflectance of trees of varying ages and growth and development stages were calculated. A linear regression model was then performed to determine the relationship between reflectances and chlorophyll contents using a computer program Minitap Win version 13.

Results and Discussion

Understanding the spectral features of Washington navel orchard is important to monitor the seasonal canopy properties and biochemical changes in plantations. Like spectral reflectances of typical living plants (Rees, 1990; Gitelson & Merzylak, 1997), all the orange trees under the study showed lower reflectance values at the blue (CH1) and red (CH3) bands and significantly higher reflectance values at the green (CH2) and near-infrared (CH4) bands for all the growth and development stages. Relationships between canopy reflectances (RF) and chlorophyll (Chl) contents among the four different age groups and growth and development stages presented in Table 1. Analyzed reflectance values of visible (blue, red, and green) and near-infrared (NIR) wavelength bands of the electromagnetic spectrum (EMS) significantly differed among plants of varying ages, growth and development stages. There were also significant differences in the amount of chlorophyll content of the leaves in the four different growth and development stages and the age groups (Table 1). These observations are in agreement with the previous studies conducted on different plant species (Gausman, 1982; Sinclair *et al*., 1971; Yoder & Pettigrew-Crosby, 1995; Blackburn, 1998; Yang & Ko, 1998).

Table 1. Canopy reflectances, chlorophyll contents and analysis results of citrus trees of four different age groups at different growth and development stages grown in Mediterranean Basin of Turkey.

Tree ages	15 (n:10)		20 (n:10)		25 (n:10)		35 (n:10)	
	RF	r^2	RF	r^2	RF	r^2	RF	r^2
Flowering stage								
CH1	0.24	0.484*	0.23	0.762**	0.22	0.736**	0.27	0.426*
CH2	1.09	0.028 ns	1.21	0.004ns	1.17	0.075 ns	1.38	0.004 ns
CH3	0.69	0.432*	0.56	0.697**	0.56	0.506*	0.68	0.289 ns
CH4	9.50	0.000 ns	10.47	0.000 ns	10.24	0.000 ns	14.09	0.328 ns
Chl	1.91		1.72		1.71		1.77	
Fruit setting stage								
CH1	0.35	0.513*	0.32	0.845**	0.30	0.688**	0.19	0.488*
CH2	1.02	0.159 ns	1.18	0.021 ns	1.06	0.128 ns	0.86	0.195 ns
CH3	0.72	0.659**	0.70	0.860**	0.67	0.750**	0.50	0.648**
CH4	11.45	0.266 ns	13.40	0.728**	11.74	0.104 ns	10.23	0.369 ns
Chl	2.67		2.70		3.11		4.07	
Fruit maturity stage								
CH1	0.19	0.626**	0.17	0.532*	0.21	0.518*	0.14	0.357 ns
CH2	0.68	0.386 ns	0.61	0.488*	0.74	0.603**	0.56	0.292 ns
CH3	0.46	0.626**	0.41	0.404*	0.45	0.715**	0.32	0.744**
CH4	9.32	0.477*	8.97	0.257 ns	10.84	0.661**	5.18	0.365 ns
Chl	3.75		3.60		4.17		6.39	
Dormancy stage								
CH1	0.33	0.544*	0.14	0.926**	0.17	0.677**	0.19	0.530*
CH2	0.86	0.003 ns	0.68	0.243 ns	0.59	0.406*	0.75	0.495*
CH3	0.62	0.892**	0.45	0.747**	0.35	0.000 ns	0.42	0.817**
CH4	10.38	0.148 ns	9.13	0.405*	9.34	0.513*	9.61	0.308 ns
Chl	3.59		3.06		3.33		3.76	

**: Significant at P≤0.01.
*: Significant at P≤0.05.
ns: non-significant
CH1: Blue band (450-520 nm); CH2 green band; (520-600 nm), CH3 red band (630-690 nm), CH4, near-infrared band (760-900 nm) and Chl: total chlorophyll content (mg/g).

Table 2. Relations between canopy reflectances and chlorophyll contents of Washington navel leaves grown in four different orchards at different growth and developmental stages.

	Growth and development stages			
	Flowering (n:40)	**Fruit Setting (n:40)**	**Fruit Maturity (n:40)**	**Dormancy (n:40)**
CH1	0.344**	0.787**	0.414**	0.046 ns
CH2	0.014 ns	0.043 ns	0.000 ns	0.276**
CH3	0.104*	0.812**	0.594**	0.103*
CH4	0.009 ns	0.000 ns	0.215**	0.294**

**: Significant at P≤0.01.
*: Significant at P≤0.05.
ns: non-significant
CH1: Blue band (450-520 nm); CH2 green band; (520-600 nm), CH3 red band (630-690 nm), CH4, near-infrared band (760-900 nm).

Relationship between canopy reflectance and chlorophyll content at flowering stage: At flowering stage, the amount of chlorophyll content varied from 1.71 mg/g in the 25 year old trees to 1.91 mg/g in the 15 year-old trees. Chlorophyll content (Chl) of spring shoot leaves collected in April 2002 was relatively lower in comparison to the other three growth and development stages. This was most likely due to insufficient leaf area of the canopy and the compositions of immature leaves of which biochemical and biophysical composition differed from that of the mature leaves (Erickson, 1968; Penuelas *et al*., 1994; Moran *et al*., 1997; Karaca, 2001). At this stage the highest relationship between the Chl and RF was found to be $r^2 = 0.762$, $P \leq 0.01$ at the blue band in the 20 year old trees. On the other hand, the smallest correlation ($r^2 = 0.426$, $P \leq 0.05$) was observed between Chl and RF of the blue band and in the 35 year old trees. There was also significant correlation between chlorophyll contents and RF at the blue band $r^2 = 0.484$, $P \leq 0.05$ and $r^2 = 0.736$, $P \leq 0.01$ of other two age groups, namely 15 and 25 years old trees. At the red band with the exception of 35 year old trees, there were significant relationships between Chl and RF ($r^2 = 0.432$, $P \leq 0.05$, $r^2 = 0.697$, $P \leq 0.01$ and $r^2 = 0.506$, $P \leq 0.05$) for 15, 20 and 25 years old trees, respectively. This indicated that biomass (canopy surface area, leaf area and leaf density) and leaf maturity were much well developed in 20 and 25 years old trees and as shown in Table 1, the use of energy level in these trees were in relatively higher level. Photosynthetic activities of the middle-aged trees (20-25 year-old) were higher than those young and old (15 and 35 year-old) trees (Penuelas *et al*., 1994; Blazquez *et al*., 1996 and Moran *et al*., 1997). Nevertheless, we did not find any statistical relationship between Chl and RF in 35 year old trees at the blue and red bands. It was also noted that there were not statistically significant correlations between Chl and RF values at the CH2 and CH4 bands for the all four different age groups (Table 1).

The findings of this study indicated that there were statistically significant relationships between Chl and RF values among the 40 trees in the four different age groups at flowering stage at the blue and red bands ($r^2 = 0.344$, $P \leq 0.01$ and $r^2 = 0.104$, $P \leq 0.05$, respectively (Table 2). This indicated that at the flowering stage the linear relationship between Chl and RF could possibly be used to determine the biochemical features of the citrus leaves at the blue and red bands.

Relationship between canopy reflectance and chlorophyll content at fruit setting stage: Canopy reflectance and chlorophyll content measurements were made in June 2002 and the averages of evaluated results were shown in Table 1. At this stage, the amount of chlorophyll content varied from 2.67 mg/g in the 15 year old trees to 4.07 mg/g in the 35 year old trees. This is because at fruit setting stage the leaves were much mature and the canopy were well developed in comparison to the previous flowering stage (Erickson, 1968). We also noted that the chlorophyll content of the leaves showed a significant increase in comparison to that of the flowering stage (Table 1).

The results indicated that although chlorophyll contents of the leaves of 15, 20 and 25 years old trees increased at this stage absorbance at the blue and red bands were not increased with the exception of the leaves of the 35 year old trees in comparison to the flowering stage. This was most likely due to limited amount of plant nutrition and influence of some other stress conditions at this stage. This kind of phenomenon have already been reported by several studies in which plants were in the stress conditions for the nutrition and some other stress conditions (Demetriades-Shah *et al*., 1990; Carter, 1994; Penuelas *et al*., 1994; Craig & Shih, 1998). In the ranges of chlorophyll absorption regions, blue and red bands, trees at the age of 35 showed the smallest RF values probably due to the fact that they had a high amount of chlorophyll content or they were not affected by any other stress conditions. Results indicated that there were statistically significant relationships between chlorophyll content and canopy RF values at the blue (CH1) and red (CH3) bands. However, with the exception of trees at the age of 20, there were not statistically significant relationships between the Chl and RF values at the green (CH2) and NIR (CH4) bands (Table 1). Results of the present study indicated that in the first chlorophyll absorption region, there was the highest correlation between Chl and RF at blue band ($r^2 = 0.845$, $P \leq 0.01$) in the 20 year old trees. However, although trees at the age of 35 showed the highest level of Chl, the relationship between Chl and RF at the first absorption region was the smallest ($r^2 = 0.488$, $P \leq 0.05$). On the other hand, in the second chlorophyll absorption region the highest relationship, ($r^2 = 0.860$, $P \leq 0.01$), was observed between Chl and RF at the red band in the 20 year old trees while the lowest relationship ($r^2 = 0.648$, $P \leq 0.01$;) was detected in the 35 year old trees.

Further analyses indicated that there were significant relationships between Chl and RF values among the 40 trees in the four different age groups at fruit setting stage at the blue and red bands ($r^2 = 0.787$, $P \leq 0.01$, and $r^2 = 0.812$, $P \leq 0.01$, respectively (Table 2). These r^2 values were the highest among the four different growth and development stages in this study. At this stage, the high level of used energy observed at low reflectance values at the blue and red bands indicated that leaves were highly active during the fruit setting stage. Differing from the other three stages, at the fruit setting stage there were statistically significant and reliable relationships between Chl and RF values of the all four different age groups. The, Fig. 1 also demonstrated a linear regression at the blue and red bands indicating the strong relationship between Chl and RF independent from the age.

Relationship between canopy reflectance and chlorophyll content at maturity stage: At this stage, the chlorophyll contents of the leaves reached to the highest level varying from 4.17 mg/g in the 25 year old trees to 6.39 mg/g in the 35 year old trees. The leaves of the maturity stage are usually considered to be fully matured in orange trees (Erickson, 1968). Analyses of RF results indicated that the leaves at this stage used relatively the highest energy level for the photosynthesis in comparison to other three different growth and development stages (Table 1). This was most likely due to the fact that approximately 8 month long time interval from flowering to maturity stage resulted in an increase in the plant canopy, leaf density and area, consequently increasing the chlorophyll contents. Also the physiological stress factors probably decreased by the application of fertilizers, irrigations and other management practices through this period. As a consequence a linear relationship was observed between the chlorophyll content of mature leaves and spectral absorptions as previously reported on different plant species (Sinclair *et al*., 1971; Penuelas *et al*., 1994; Belanger *et al*., 1995; Cloutis *et al*., 1996). Results indicated that chlorophyll content of the mature leaves 30-34% increased and the reflectances at the blue and red bands 50-70% decreased in comparison to the previous stage, fruit setting. Thus, decrease in reflectances at blue and red bands demonstrated that there was a strong relationship between Chl and RF. This was not unexpected since the chlorophyll energy level used at this stage was also increased by the fruits which also contained chlorophylls (Erickson, 1968).

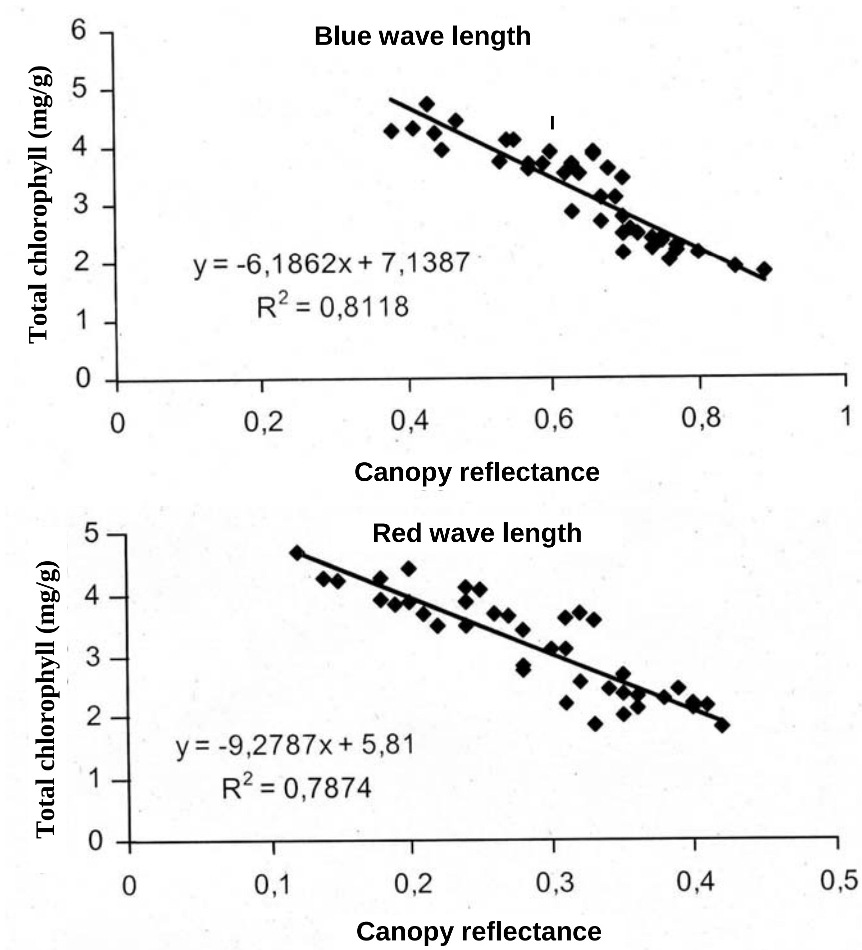

Fig. 1. The relationships between total chlorophyll contents (Chl) and canopy reflectances (RF) at the blue and red bands at fruit setting stage of the Washington navel trees (n: 40).

Analyses indicated that at maturity stage there were statistically significant relationships between Chl and RF values among the 40 trees in the four different age groups at the blue and red bands (r^2 = 0.414, P ≤ 0.01, and r^2 = 0.594, P ≤ 0.01, respectively (Table 2). Although the magnitude of the relationship observed at this stage was higher than at the flowering and dormancy stages, it was lower than fruit setting stage. At this stage statistically significant relationships between Chl and RF were probably due to chlorophyll contents of the fruits. However, this study did not determine the sign and magnitude of the direct and indirect effects of fruit chlorophyll content. Therefore, further studies may be helpful to investigate the interrelationship between the chlorophyll contents of leaves and fruits at this stage.

Relationship between canopy reflectance and chlorophyll content at dormancy stage: Spectral reflectance and chlorophyll measurements were made in February 2003 (Table 1). At this stage, although the chlorophyll contents of the leaves decreased in comparison to the previous stage, Chl values were relatively higher than that of the flowering and fruit setting stages. At dormancy stage, the amount of chlorophyll contents varied from 3.06 mg/g in the 20 year old trees to 3.76 mg/g in the 35 year-old trees. The decrease in the amount of chlorophyll contents suggested that the leaves were at their senescence and/or dormancy stage. During the process of senescence or dormancy, the composition of cell components of the leaves are progressively degraded (Erickson, 1968; Sinclair *et al*., 1971; Craig & Shih, 1998). However, the results of the present study indicated that there was not a significant decrease in the absorptions at blue and red bands in comparison to the fruit maturity stage. This also indicated that physiologic functions of the leaves continued at this stage and this was expected since the orange trees belong to the evergreen plants differing from the annual plant species. Physiologic functions of the leaves were proceeding to a certain extent to accumulate more biochemical substances that would be needed in the next stage i.e., flowering stage (Erickson, 1968). Results also demonstrated that at this stage 20 and 25 year old trees used more energy at CH1 and CH3 bands than 15 and 35 year old trees. At this stage there were non-linear relationships between Chl and RF among the four different age groups (Table 1). While there were statistically significant relationships between Chl and RF values at the blue ($r^2 = 0.926$, $P \leq 0.01$) and red bands ($r^2 = 0.747$, $P \leq 0.01$) in the leaves of 20 years old trees, this relationship was not significant for the leaves of 25 year old trees at the red band. On the other hand, relationships between Chl and RF values for the trees at the ages of 15, 20 and 35 were significant at the red band, ($r^2 = 0.892$, $r^2 = 0.747$, and $r^2 = 0.817$, at 0.01 level, respectively).

Further analyses also indicated that there was statistically significant relationship between Chl and RF among all the 40 trees in the four different age groups at the red band ($r^2 = 0.103$, $P \leq 0.05$, Table 2) at dormancy stage. At this stage, the relationship between Chl and RF was not statistically significant at the blue band in comparison to the that of the three stages. This indicated that Chl and RF values of the dormancy stage could not be used to measure and monitor the canopy properties and biochemical changes in Washington navel orange trees.

Conclusion

Studies reported that vegetation optical properties are the functions of plant internal and external structure, water content, and biomass (Curran *et al*., 1992; Fourty *et al*., 1996; Moran *et al*., 1997). Therefore, accurate results of remote sensing techniques are usually dependent on the age and growth and development stages of the trees in a plantation under the study. However, the effects of the ages of the plants are usually considered to be much less than growth and development stages for the remote sensing techniques (Sinclair *et al*., 1971; Yoder & Pettigrew-Crosby, 1995; Blackburn, 1998). Results of this study demonstrated that the relationships between Chl and RF were affected by the plant ages, growth and development stages. It was found that there was the highest relationship between Chl and RF at blue and red bands at the fruit setting stage of the 20 years old trees in comparison to the other ages, and growth and development stages. The relationship between the Chl and RF values among the 40 orange trees were also significantly correlated at the blue and red bands at fruit setting stage. Therefore, this stage may be considered as most suitable stage for remote sensing techniques to analyze the some physiological properties of the orange leaves. Our findings also indicated that the period between fruit setting and maturity stage (June to November) was quite long to determine the physiological characteristics of the orange trees. Thus, further studies are needed to determine the most suitable time interval that would reflect the best relationship between RF and physiological properties of the leaves in Washington navel trees.

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