International Journal of Geosciences, 2022, 13, 681-694
https://www.scirp.org/journal/ijg
ISSN Online: 2156-8367
ISSN Print: 2156-8359

Combined Radiometric Analysis Related to Guava Leaf Phenology in Response to Soil Application of Paclobutrazol (PBZ)

Victor Manuel Rodríguez-Moreno[1]*, José Saúl Padilla-Ramírez[1], Guillermo Medina-García[2], Arturo Reyes-González[3]

[1]Experimental Field Station "Pabellón", Km 32.5, highway Ags-Zac, Pabellón de Arteaga, Aguascalientes, México
[2]Experimental Field Station "Zacatecas", Km 24.5 highway Zacatecas-Fresnillo, Calera de Victor Rosales, Zac., México
[3]Experimental Field Station "La Laguna", Boulevard José Santos Valdez 1200 Pte, Matamoros, Coahuila, México
Email: *rodriguez.victor@inifap.gob.mx, padilla.saul@inifap.gob.mx, medina.guillermo@inifap.gob.mx, reyes.arturo@inifap.gob.mx

How to cite this paper: Rodríguez-Moreno, V.M., Padilla-Ramírez, J.S., Medina-García, G. and Reyes-González, A. (2022) Combined Radiometric Analysis Related to Guava Leaf Phenology in Response to Soil Application of Paclobutrazol (PBZ). *International Journal of Geosciences*, **13**, 681-694.
https://doi.org/10.4236/ijg.2022.138036

Received: July 5, 2022
Accepted: August 27, 2022
Published: August 30, 2022

Abstract

The leaf-response to three-soil applied treatments of Paclobutrazol (PBZ; 1000, 2000 and 0 ppm-control) was studied in a high-density plantation of eight guava (*Psidium guajava*) genotypes trees. All materials were prunned in vase form, with two to three major branches, yearly prunning for triggering the annual production cycle, and average height of 2.0 m. The dataset comprises fourth radiometric indices highly related to plant physiological activities. The dataset model took into account data collection dates, guava genotypes, and the positional effect of sun radiation on leaves based on their proximity to the canopy level and downward to the base of the woody seasonal-branch. Unexpectedly, there were no significant differences (NS) in PBZ treatments for genotypes, leaf position and radiometric indices. Analysis of the radiometric indices data revealed that anthocyanin (ARI index) and chlorophyll (PRI index) have a strong inverse relationship. Significant differences ($P \leq 0.05$) were found between guava genotypes, and anthocyanin content; these results show that guava genotypes have varied responses, which could derive in their classification based-on drought resistance or low water requirements, however, it is important to note that additional research is required to determine the scope of these indications.

Keywords

Radiometric Indices, PBZ, Guava, High-Density Plantation, Climate Change

1. Introduction

Plant growth regulators are chemical substances, which govern all the factors of development and growth within plants [1]. Paclobutrazol (PBZ) [(2RS, 3RS)-1-(4-chlorophenyl)-4, 4-dimethyl-2-(1H-1, 2, 4-trizol-1-yl)-pentan-3-ol], is one of the members of triazole family having growth regulating properties. It is a systemic plant growth regulator that limits plants growth by inhibiting gibberellin biosynthesis [2], abscisic acid and cytokinins [1]. The effects of PBZ on plants have been reported on fruit bearing trees where it: 1) increases the quantity of floral buds; and 2) induces early and profuse production of flowers, early maturity of fruit, restriction of vegetative sprouting, while 3) increasing the quantity of fruit [3] [4].

Conventional approaches to obtain information on the exchange of leaves and atmosphere usually involve large serial datasets, field data collect embedded in strictly growing season period. Alternatively, the approach of utilizing remote sensing information has increasingly attracted attention, and may potentially be used for estimating plant physiological, biochemical, and biophysical properties [5] [6].

Mature leaves are the most representative organ to infer on plant's health status. The effects of shading, the balance of carbon assessment, biotic and abiotic stress, and the standard production practices on water management in cultivated species. Shade leaves that grow under weak irradiance must catch photons as efficiently as possible [7]. In this rate of efficiency the characteristics in leaf anatomy are crucial as much the associated with plant growth.

Plant growth and differentiating organs processes are controlled by phytohormones [8] along with its sensibility to environmental factors. Plant hormones are among the most important biochemical affecting plant growth and yield production, including stress [9]. Accordingly, the foliar surface reaction to sun radiation is in the same proportion as its optical properties, while absorbing, reflecting, or transmitting the outcome to another medium. Most photosynthetic pigments absorb strongly in the visible region (400 - 700 nm), but not in the infrared region (700 - 1200 nm). The ratio between wavelengths generates various indices that are representative of biomass production, and leaf efficiency for converting solar radiation to chemical energy.

The process of photosynthesis occurs in the presence of chlorophyll and other photosynthetic pigments, including carotenoids and anthocyanin. The concentration of these photosynthetic pigments determines leaf's efficiency in absorbing solar radiation and in protecting the photosynthetic apparatus from excessive radiant energy. Anthocyanin are water-soluble pigments in plants known for their photo protective role against photo inhibitory and photo oxidative damage under high light [10]; however, this hypothesis has not been proved because a number of studies found evidence that anthocyanin content is not advantageous for photosynthesis [11] [12]. The production of anthocyanin is opposed by that of chlorophyll and to obtain an estimated rate of its synthesis involves the same difficulties as those hindering carotenoid study [13]. To overcome this hurdle [14] propose obtaining indirect data for demonstrating the synthesis of the pigment of interest. For example, to determine the maximum content of anthocyanin in the leaf, the reflectance data of the first spectral band is used; then to eliminate the effect of absorption by other pigments (in the example, chlorophyll), the reflectance value in the second band is used, which is considered "sensitive" to the content of chlorophyll and "not sensitive" to anthocyanin. The difference between these two bands is called the Anthocyanin Reflectance Index (ARI): cf. Equation (1).

$$\text{ARI} = \frac{1}{550\text{ nm}} - \frac{1}{700\text{ nm}} \tag{1}$$

In Equation (1), the 550 nm is located at the visible green section, whilst the 700 nm is at the red section of the electromagnetic spectrum. ARI allowed an accurate estimation of anthocyanin accumulation (STD within 3.9 nmol/cm^2), even in minute amounts, in intact stressed and senescing leaves [15].

Another index is the Structure Insensitive Pigment Index (SIPI). The index is a reflectance measurement designed to maximize the sensitivity of the index to the ratio of bulk carotenoids (for example, alpha-carotene and beta-carotene) to chlorophyll while decreasing sensitivity to variation in canopy structure (for example, leaf area index) [16]; it also exhibits a correlation with yield (Equation (2)).

$$\text{SIPI} = \frac{\rho_{800} - \rho_{445}}{\rho_{800} - \rho_{680}} \tag{2}$$

Increases in SIPI are thought to indicate increased canopy stress (carotenoid pigment) [16]. Its major advantage is that it minimizes the effect of variation in canopy structure and leaf topology. It is useful for monitoring vegetative health and detecting plant physiological stress and crop production.

In Equation (2), ρ represents reflectance data and the subscript indicates the wavelength where the data was registered. The index value goes from 0 to 2. The common range for green vegetation is from 0.8 to 1.8 [16].

The Photochemical Reflectance Index (PRI; [17]). This index is a reflectance measurement that is sensitive to changes in carotenoid pigments (particularly xanthophyll pigments) in live foliage. Carotenoid pigments in leaves are indicative of photosynthetic light use efficiency, or the rate of carbon dioxide uptake by foliage per unit energy absorbed [16] [17]. As such, it is used in studies of vegetation productivity and stress. This index is used as a warning of non-biotic stress [18]. The index value goes from −1 to +1 (Equation (3)).

$$\text{PRI} = \frac{\rho_{531} - \rho_{570}}{\rho_{531} + \rho_{570}} \tag{3}$$

Applications include vegetation health in evergreen shrublands, forests, and agricultural crops prior to senescence.

The Plant Senescence Reflectance Index (PSRI; [19]) can be used to measure leaf and fruit senescence; it provides information about leaf mesophyll structure. How high the value (positive) is, provides an indication of how close the leaf is to senescence; a negative value is associated with chlorophyll content [20]. The index value goes from −1 to +1: cf. Equation (4). The common range for green vegetation is from −0.2 to +0.2 [19].

$$\text{PSRI} = \frac{\rho_{680} - \rho_{500}}{\rho_{750}} \tag{4}$$

The PSRI can be used to measure leaf and fruit senescence.

This study is not meant to replace laboratory-based leaf response analysis procedures. More accurate indicators of photosynthesis and respiration processes are produced using laboratory equipment, such as growth chambers, where the researcher has control over the time of insolation and the intensity of radiant energy. The goal of this manuscript is to use a dataset of non-destructive radiometric indices to assess the effect of the growth regulator PBZ on photosynthesis processes in guava leaves. Guava, is one of the main fruit crops in Mexico. The cultivated area is over than 21 thousand hectares and an annual production of around 300 thousand tons [21]. Most of guava orchards have a plant density of 200 to 400 ha^{-1} trees. However, to increase productivity of several fruit crops, it has been used high plant densities. [22] noted that increased guava yield per unit area is possible at high planting densities. Nonetheless, in high-density plant systems various aspects can affect fruit yield and quality because of plant competition for resources such as water, light and nutrients. Consequently, it is important to assess the physiological response of cultured guava to high plant densities and the use of plant regulators. Radiometric indices at the leaf scale will be used to investigate the gross relationship between phytohormone synthesis and production and the vegetative growth of the woody sections of the guava tree in one growing season. As previously indicated, the metrics of the extent of the link between indices and PBZ cannot be compared to those acquired using laboratory equipment; however, the results obtained here are sufficiently precise to support any evidence that may develop.

2. Materials and Methods

2.1. Experimental Design

The experimental design is a factor with three main independent variables: treatments (PBZ treatments), eight guava selections, and the position of the leaves in the seasonal hardwood branch. The PBZ treatments were labelled T1, T2, and T0. PBZ solution was incorporated into the soil; 1.0 ml of PBZ/tree (1000 ppm), 2.0 ml of PBZ/tree (2000 ppm) and control (0 ppm). The eight genotypes were from the Guava Arboretum Bank of the Instituto Nacional de Investigaciones Forestales, Agrícolas y Pecuarias (INIFAP) (**Table 1**). One mature leaf near the apex of the selected branch, but not the uttermost, was the first reading source; this data was labelled as "up". The second data was labelled as "down" and corresponded to the one leaf near the trunk. Following the recommendations

Table 1. Description of guava varieties and high-density batch selections. Tree formation: cup or vase, with two to three major branches. With annual pruning (pruning of all shoots around the tree) to induce the annual production cycle. Average height of the shaft 2.0 m.

Selection	Pulp Color	Shape of fruit	Transversal diameter (cm)	Number of seeds	°Brix
S11[a]	Cream	Ovoid	4.5 - 5.0	190 - 210	12.0 - 14.0
S12[b]	Cream	Ovoid	5.5 - 6.0	Unregistered	11.0 - 12.0
S20[b]	Cream	Ovoid	5.5 - 6.0	Unregistered	11.0 - 12.0
S45[c]	Cream	Ovoid	4.0 - 4.5	Unregistered	11.7
S46[c]	Cream	Ovoid	4.0 - 4.5	Unregistered	13.3
S47[c]	Cream	Ovoid	4.0 - 4.5	Unregistered	12.7
S54 [a]	Pink	Truncated pear	5.0 - 5.5	200 - 230	11.0 - 13.0
S106 [a]	White cream	Round	4.8 - 5.5	300 - 310	11.0 - 13.0

[a][23]; [b][24]; [c][25].

of [7], we chose the southern side of the tree, where the highest interceptions occur.

For data management and interpretation of results, the guava selections were coded as: S-11 (common name, Calvillo S-XXI), S-12, S-20, S-45, S-46, S-47, S-54 (common name, Hidrozac) and S-106 (common name, Caxcana). These individuals shares similar standards in morphological structure (root system and aerial part), growth habits, soil nutrients and water requirements. The data was collected of two mature leaves for this evaluation. The first was close to the seasonal guava branch and the second was located at 5 or 6 knots inside the same branch.

2.2. Field Data

The data collection dates correspond to the most representative phenological stages of the guava production cycle: budging, flowering, and fruiting. Spectral indices field data were collected on four dates from the end of July to the end of August: July 29th, August 5th, 12th, and 26th. The data collection time was about 10:00 AM during the period in order to avoid a potential bias in leaf response to solar radiation due to day. The measurement device was the CI-710 miniature leaf spectrometer. The CI-710 (CID Bio-Science) measures foliar transmission, absorption and reflection of light by albedo biological components over a wide range of wavelengths, from the visible to the near infrared.

The study design consists of independent categorical variables and numeric response variables. The categorical variables were PBZ treatments (three levels), the selected guava materials (eight levels), and the positon of data collection (two levels). There were fourth numeric response variables: the ARI, SIPI, PRI, and PSRI spectral indices. Multivariate analytical techniques and the Bonferroni post-hoc test for differences within homogeneous groups were used to analyze the dataset. Trough multivariate variance analysis, MANOVA can integrate several dependent and independent variables into the same model. This design provides a higher level of complexity and analytical capacity. To determine statistical significance, contrary to the statistical ANOVA F, MANOVA interprets the Wilk's Lambda value; decreases the value and increases the difference between the analyzed groups; a value of 1 means equality between groups. The post-hoc Bonferroni test evaluates all feasible pairs of independent variable combination. The test returns the difference between each group's means as well as a p value that indicates whether two groups differ significantly. This is the most extensively used test, according to [26] since it is very versatile, easy to calculate, and applicable to any statistical analysis approach.

3. Results and Discussion

3.1. Radiometric Indices

The ARI and PRI indices has the strongest significant connection ($\alpha = 0.05$). It was observed an inverse relationship between them (r = −0.82, Fig. 1A). PSRI (r = 0.73) was also found to have a strong relationship with the ARI index. According to our findings, an increase in anthocyanin content occurs when guava leaves are nearing senescence. The PSRI index was observed in two moderate relationships. SIPI-PSRI (r = 0.63) and PRI-PSRI (r = −0.63). The leaf is healthy and close to senescence in these connections, and carotenoid pigments are diminishing due to the closeness to senescence. The SIPI-ARI and SIPI-PRI indices had a low correlation (r = 0.45, r = −0.31, respectively).

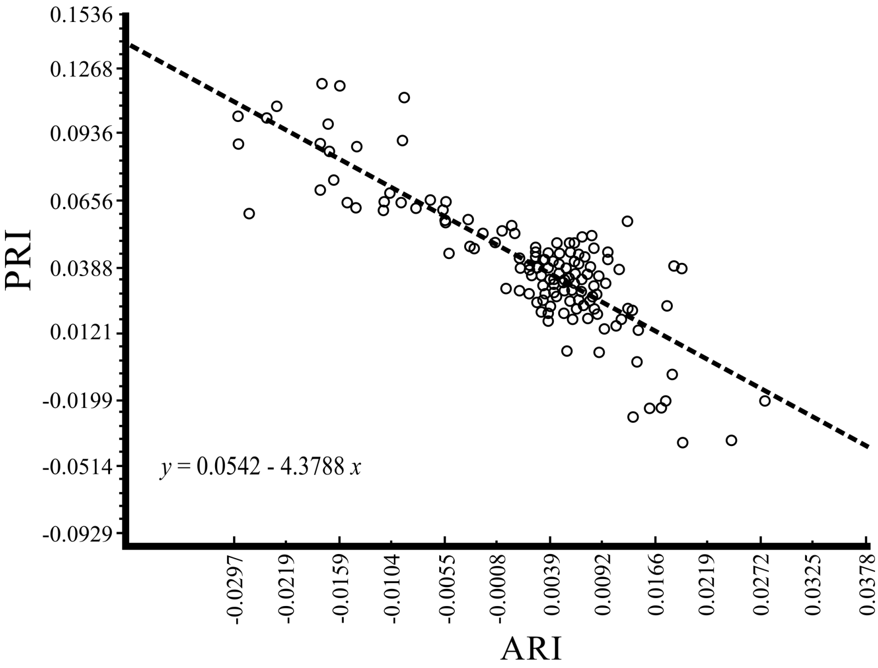

Figure 1. ARI and PRI dispersion chart. The obvious reverse relationship between maximum anthocyanin content and carotenoid content pigment changes in the leaf is characterized.

The light screen idea proposes that foliar anthocyanin concentration shields the photosynthetic machinery from excess light when senescence-related photosynthetic instabilities are compounded by additional stresses like as excessive temperature, low soil nutrition, and water availability. In autumn, when low and even freezing temperatures are widespread, the synthesis of anthocyanin in senescing leaves is selectively favored [12]. Because the concentrations of carotenoids and anthocyanin in guava leaves are diametrically opposed, previous research on other plants suggests that it may shield the leaves from light damage. The concentration of anthocyanin in leaves is related to its photosynthetic effectiveness; nonetheless, it is important to remember that the chlorophyll content in leaves is critical to the photosynthesis process [27] and the physiological status of the anthocyanin content in leaves [14].

Its presence in leaves reduces photo oxidative damage [28], but just a few studies [29] [30] have confirmed this assertion in guava trees or agricultural production systems. High leaf anthocyanin concentrations were associated with reduced photo inhibition in plants cultivated at the highest light level, but no indication of photo inhibition was found in plants grown at the intermediate light level [31].

3.2. Categorical Variables

Collection date, selections, and reading Position (Wilk's lambda = 0.0002, 0.016, and 0.40, respectively) showed statistical significance ($p \le 0.05$) in multivariate analysis; PBZ treatments (Wilk's lambda = 0.73) showed no significance ($p > 0.05$). As expected, the most variation was seen among sampling dates and selections, while the least variation was seen for Reading Position. The ARI index demonstrated significant findings for dates and selections among radiometric indicators (**Figure 2**).

The PRI index (**Figure 3**) revealed significant results for the three categorical factors when it came to carotenoid content. The inverse link between anthocyanin

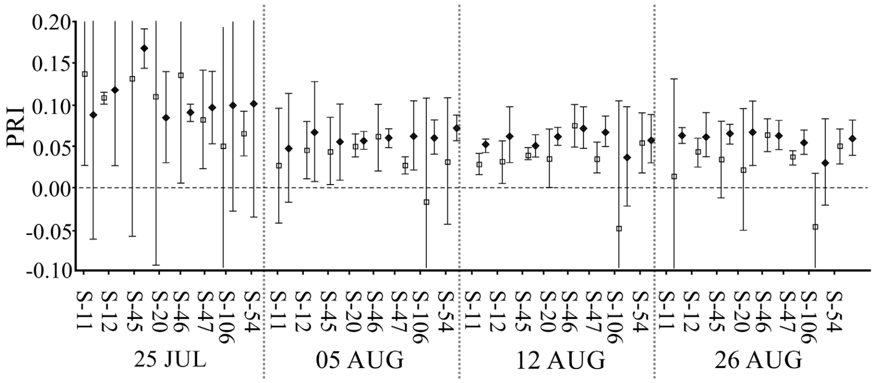

Figure 2. The ARI index showed differences during the field data collecting period. In this composite graph, the "down" reading position is represented by filled symbols square and diamond-shaped (▪ and ♦), whereas the "up" reading position is represented by an empty square symbol (☐). The average is displayed, with the bars representing a 0.95 confidence level. The vertical dotted line serves as a visual aid to separate Dates.

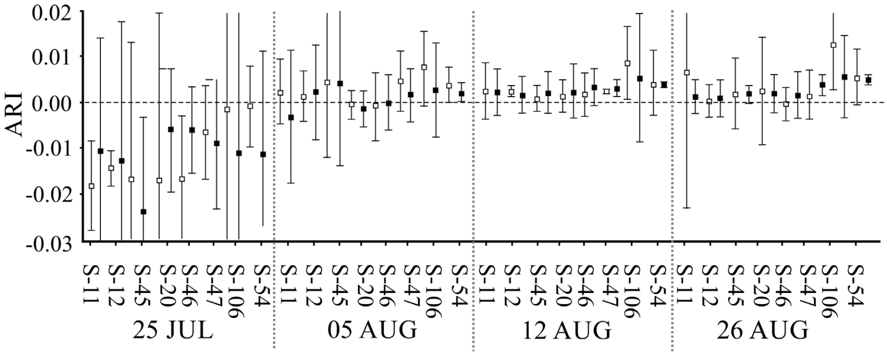

Figure 3. The PRI index showed differences during the field data collecting period as is showed in this composite graph. As in Figure 2, the "down" reading position is represented by filled symbols square and diamond-shaped (▪ and ♦), whereas the "up" reading position is represented by an empty square symbol (☐). The average is displayed, with the bars representing a 0.95 confidence level.

and carotenoids in leaves is confirmed by comparing Figure 2 and Figure 3.

The inverse link between the indices is seen in Figure 2. From the initial data collection (July 25th) to the last, ARI data show a trend of increasing (August 26th). The PRI index, on the other hand, showed that the mean value declined only from 25th to August 5th; the average value looked to be more or less stable for the last two dates. Three of the selections revealed an inverse association between the indices in regard to the reading position. The S-11, S-46, and the S-106 selections had the highest average mean value in the ARI on every date in the "up" reading position, while the PRI for the "down" reading position had the lowest value. These data suggest that anthocyanin concentration in guava leaves has a modest vertical gradient, which may be connected to the quantity of solar light received in the canopy. Furthermore, carotenoids are more concentrated in the lower canopy, according to the PRI; these leaves receive less direct solar flux, and their development is influenced by the shadowing effect generated by upper canopy leaves. Even if these findings appear to be ambiguous, they do hint to the need for further inquiry. There were no significant differences in the SIPI and PSRI.

On July 29th, extreme values in all indices were reported, according to the Bonferroni test for homogeneous groups (Table 2). The ARI and SIPI had the lowest average values (−0.0117 and 0.6780, respectively), whilst the PRI and PSRI had the highest average values (−0.0117 and 0.6780, respectively) (0.1045 and −0.1490, respectively), whereas the PRI and PSRI revealed their highest scores (0.1045 and −0.1490, respectively). For later dates, no significant changes were observed.

The Bonferroni test, which has been confirmed in previous investigations, reveals an inverse relationship between the ARI and PRI indices in leaves. The S-106 result stood out among the selections, with the highest estimated anthocyanin content and the lowest PRI index score-bold enhanced in Table 1. According to the reading Position results, the only difference between groups was

Table 2. Bonferroni test for homogeneous groups ($\alpha = 0.05$) by sampling date, selections, and reading position, with radiometric indices.

	ARI				SIPI				PRI				PSRI			
		MEAN	a	b		MEAN	a	b		MEAN	a	b		MEAN	a	b
DATE	**29 JUL**	**−0.0117**		*	**29 JUL**	**0.678**		*	26 AUG	0.0425	*		**29 JUL**	**−0.1490**		*
	5 AUG	0.0015	*		12 AUG	0.779	*		12 AUG	0.0431	*		5 AUG	−0.0143	*	
	12 AUG	0.0026	*		26 AUG	0.781	*		5 AUG	0.0470	*		26 AUG	−0.0139	*	
	26 AUG	0.0029	*		5 AUG	0.804	*		**29 JUL**	**0.1045**		*	12 AUG	−0.0092	*	
SELECTIONS	S-45	−0.0032	*		S-45	0.720	*		**S-106**	**0.0213**		*	S-45	−0.0786	*	
	S-12	−0.0022	*		S-11	0.731	*		S-11	0.0576	*		S-20	−0.0612	*	
	S-11	−0.0022	*		S-20	0.757	*		S-47	0.0583	*		S-11	−0.0604	*	
	S-46	−0.0021	*		S-106	0.758	*		S-54	0.0615	*		S-12	−0.0570	*	
	S-20	−0.0020	*		S-54	0.768	*		S-20	0.0618	*		S-46	−0.0402	*	
	S-54	−0.0014	*		S-12	0.771	*		S-12	0.0677	*		S-54	−0.0284	*	
	S-47	**0.0002**	*	*	S-47	0.788	*		S-46	0.0725	*		S-47	−0.0244	*	
	S-106	**0.0037**		*	S-46	0.792	*		S-45	0.0734	*		S-106	−0.0226	*	
POS	DOWN	−0.0014	*		UP	0.757	*		**UP**	**0.0474**	*		UP	−0.0516	*	
	UP	−0.0009	*		DOWN	0.764	*		**DOWN**	**0.0711**		*	DOWN	−0.0416	*	

the PRI index, which was lower in the "down" position than in the "up" position.

Variability in microclimate is driven by the amount of radiation and leaf exposition at the upper canopy, which decreases downward inside the shrub tree. The energy required by for basic physiological activities such as photosynthesis and respiration is provided by light interception, which is a decisive factor in crop development [32]. Lower leaves receive a tinny of direct radiation, while upper leaves receive both direct and diffuse sunlight. As diffuse radiation is absorbed, transmitted and reflected by leaves, and reflected by the soil surface, it becomes increasingly important for lower leaves. Plant canopies absorb, scatter and reflect solar radiation, lowering the amount of energy that reaches the soil and air beneath the canopy; a denser canopy should result in cooler air beneath the canopy [33]. Several components of the data analysis undertaken in this study revealed an inverse trend in the association between the ARI and PRI indices. These findings appear to support the hypothesis that anthocyanin accumulates in young leaves and declines in mature leaves as a result of anthocyanin's ability to shield leaves from UV light and oxidative damage, as well as plants' gradual accumulation of chlorophyll [34]. Nonetheless, caution appears warranted in this study, given the results presented in the majority of research studies on this topic were obtained in controlled environments through laboratory procedures, whereas this study relied on field data.

The inverse pattern in the relationship between ARI and PRI indices has been observed in several components of data analysis conducted in this study. These results seem to corroborate [34] that anthocyanin often accumulates in young leaves and degrades in mature leaves due to the fact that anthocyanin can protect leaves from UV light and oxidative damaging, and to the gradual accumulation of chlorophyll in plants. Nonetheless, in this study caution seems justified, because the results reported in most of the research articles treating this topic were provided under controlled environments through the execution of laboratory proceedings while this study approach relied on field data. This reflection is not intended to minimize the findings, but to emphasize the need to generate research investigation conscious to extend these kind of studies by including a larger number of production environments and extending the period of data collection to cover at least one full production season, unconventionally of the number of harvest events.

Temperature variation, day length period, and the amount of incoming short wave radiation are the main indicators of how climate change is expect to modify the production systems. Warmer temperatures favor crop growth, but at the same time, they decrease yield [35] and short their growing season. For guava species, the temperature increase causes a reduction in the rate of floral bud formation [36] and consequently affects the later extent of rate of tie up fruit. Another example to mention is that short cycle plants may not be able to discern flowering time and long cycle plants could sprout at a different time, forming a number of leaves, and then die without ever perceiving the appropriate time to bloom. Mitigating the impact of increasing temperatures on crops is a relevant issue; one important approach involves promoting the use of plant genetic materials, which are able to tolerate temperature variations. Additional measures for alleviating the impact of increasing temperatures involve updating the technologic package of production systems; these include improve the efficiency of water management, applying correct amounts of fertilizer, to promote the eco-friendly agronomic practices for soil recovery, and the use of sustainable practices. The results reported in this study along with the discovery of good agronomic characteristics among some of the evaluated guava selections indicate that additional studies are necessary to provide sufficient information for elaborating a robust technological package for its adoption.

Results obtained on PBZ were clearly unexpected. The PBZ has been associated with effects on the photosynthetic capacity of plants [37]. Since resulted non-significant for data collection dates, reading position, and selections, our study supports the asseveration that physiological processes of guava trees are not affected by this growth regulator. This prompts to suggest widening the as type data collection to the associated to plant morphometric, such as tree height, length of the season's branches, leaf width/length ratio, internode length, and leaf texture. [38] reported that a PBZ application of 500 ppm per tree increased the thickness of the season's branches by 34.95 %. They also documented other developments: an earlier start of flowering, an increased number of flowers, enhanced fruit attachment, and a reduced number of days to harvest. In view of these observations, it seems appropriate that further research endeavors are needed to study the residual effects of PBZ in soils and its mobility inside plant structures (leaves, trunk, branches, and fruit).

Radiometric indices are valuable because it can be used as a time/effectiveness indices to quick general diagnose of plants physiologic status. They are a non-destructive source of data that characterize the photosynthetic process and provide an estimate for leaf content of anthocyanin and chlorophyll. As shown in this study, the inverse relationship of these two pigments provides a glimpse of the usefulness of this type of technology; nonetheless, we are aware of its inability to detect the more than 500 different anthocyanins that differ not only for the glycosylation pattern of the scaffold, but also for the presence and position of aliphatic or aromatic carboxylates [39].

This usefulness of spectral indices is that provides information on plant stress and associates them with nutritional deficiencies and water availability. Nevertheless, it is important to mention that the radiometric indices are based on reflectance, transmission, and absorbance ratios on specific wavelengths, and hence carry an inherent bias that makes it difficult to compare with those obtained in laboratory facilities.

This bias is masked by equipment precision error, the anatomical characteristics of leaves (ruggedness, hairiness, and thickness), and the prevailing environmental conditions at the time of data collection. As the data collection period has been short, we estimate that the physiology of the response of guava plants to environmental conditions is maintained throughout and after any production cycle. However, this may also be considered a relevant bias for many readers, which calls into question the analysis of the data and the conclusions drawn in this manuscript.

4. Conclusions

In this leaf-level study, spectral indices were used to assess the response of guava genotypes to PBZ application. No significant differences were observed between the eight guava genotypes, neither among the spectral indices, nor in leaf position in the seasonal woody shoots.

There was a clear difference in the estimated photosynthetic pigment content of the leaves, anthocyanin and chlorophyll, based on field radiometric indices. The antagonist association between anthocyanin and chlorophyll was apparent in the guava leaves. There was also an evident effect of the position of the anthocyanin content of the leaves relative to their proximity to the canopy. The findings from this study suggest that more research is needed to provide enough information to develop a viable technical package that can be recommended to farmers. Variables linked to guava leaf shape, seasonal branch length, internode number, tree height, and PBZ traceability in leaves, branches, and fruits, as well as in the soil are indicated.

Acknowledgements

The authors would like to thank the INIFAP for the financial and technical support for this study. We are indebted to Don José, Don Jorge, Don Humberto, and Don Jesús, the field technicians at the Los Cañones experimental site. We also express our great recognition to the anonymous reviewers and the editorial staff for their time and attention.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Desta, B. and Amare, G. (2021) Paclobutrazol as a Plant Growth Regulator. *Chemical and Biological Technologies in Agriculture*, **8**, Article No. 1. https://doi.org/10.1186/s40538-020-00199-z

[2] Rademacher, W. (2000) Growth Retardants: Effects on Gibberellin Biosynthesis and Other Metabolic Pathways. *Annual Review of Plant Physiology and Plant Molecular Biology*, **51**, 501-531. https://doi.org/10.1146/annurev.arplant.51.1.501

[3] Cárdenas, K. and Rojas, E. (2003) Efecto del Paclobutrazol y los Nitratos de Potasio y Calcio Sobre el Desarrollo del Mango "Tommy Atkins". *Bioagro*, **15**, 83-90.

[4] Chorbadjian, R.A., Bonello, P. and Herms, D.A. (2011) Effect of the Growth Regulator Paclobutrazol and Fertilization on Defensive Chemistry and Herbivore Resistance of Austrian Pine (*Pinus nigra*) and Paper Birch (*Betula papyrifera*). *Arboriculture & Urban Forestry*, **37**, 278-287. https://doi.org/10.48044/jauf.2011.036

[5] Asner, G.P. (1998) Biophysical and Biochemical Sources of Variability in Canopy Reflectance. *Remote Sensing of Environment*, **64**, 234-253. https://doi.org/10.1016/S0034-4257(98)00014-5

[6] Doughty, C.E., Asner, G.P. and Martin, R.E. (2011) Predicting Tropical Plant Physiology from Leaf and Canopy Spectroscopy. *Oecologia*, **165**, 289-299. https://doi.org/10.1007/s00442-010-1800-4

[7] Gomes-Laranjo, J., Coutinho, J.P., Galhano, V. and Ferreira-Cardoso, J.V. (2008) Differences in Photosynthetic Apparatus of Leaves from Different Sides of the Chestnut Canopy. *Photosynthetica*, **46**, 63-72. https://doi.org/10.1007/s11099-008-0012-1

[8] Baca, B.E. and Elmerich, C. (2003) Microbial Production of Plant Hormones. In: Elmerich, C. and Newton W.E., Eds., *Associative and Endophytic Nitrogen-fixing Bacteria and Cyanobacterial Associations*, Vol. 5, Kluwer Academic Publishers, Dordrecht, 113-143. https://doi.org/10.1007/1-4020-3546-2_6

[9] Miransari, M. (2016) Soybeans and Plant Hormones. In: Miransari1, M., Ed., *Environmental Stresses in Soybean Production*, Vol. 2, Academic Press, Cambridge, 31-156. https://doi.org/10.1016/B978-0-12-801535-3.00006-1

[10] Zheng, X.T., Yu, Z.C., Tang, J.W., Cai, M.L., Chen, Y.L., Yang, C.W., Chow, W.S. and Peng, C.L. (2021) The major Photoprotective Role of Anthocyanins in Leaves of Arabidopsis thaliana Under Long-term High Light Treatment: Antioxidant or Light attenuator? *Photosynthesis Research*, **149**, 25-40. https://doi.org/10.1007/s11120-020-00761-8

[11] Burger, J. and Edwards, G.E. (1996) Photosynthetic Efficiency, and Photodamage by

UV and Visible Radiation, in Red versus Green Leaf Coleus Varieties. *Plant and Cell Physiology*, **37**, 395-399. https://doi.org/10.1093/oxfordjournals.pcp.a028959

[12] Lee, D.W. and Collins, T.M. (2001) Phylogenetic and Ontogenetic Influences on the Distribution of Anthocyanins and Betacyanins in Leaves of Tropical Plants. *International Journal of Plant Sciences*, **162**, 1141-1153. https://doi.org/10.1086/321926

[13] Sims, D.A. and Gamon, J.A. (2002) Relationships between Leaf Pigment Content and Spectral Reflectance across a Wide Range of Species, Leaf Structures and Developmental Stages. *Remote Sensing of Environment*, **81**, 337-354. https://doi.org/10.1016/S0034-4257(02)00010-X

[14] Gitelson, A.A., Chivkunova, O.B. and Merzlyak, M.N. (2009) Nondestructive Estimation of Anthocyanins and Chlorophylls in Anthocyanitic Leaves. *American Journal of Botany*, **96**, 1861-1868. https://doi.org/10.3732/ajb.0800395

[15] Henrich, V., Jung, A., Götze, C., Sandow, C., Thürkow, D. and Gläßer, C. (2009) Development of an Online Indices Database: Motivation, Concept and Implementation. 6*th EARSeL Imaging Spectroscopy SIG Workshop Innovative Tool for Scientific and Commercial Environment Applications*, Tel Aviv, 16-18 March 2009.

[16] Peñuelas, J., Baret, F. and Filella, I. (1995) Semi-Empirical Indices to Assess Carotenoids/Chlorophyll—A Ratio From Leaf Spectral Reflectance. *Photosynthetica*, **31**, 221-230.

[17] Gamon, J.A., Serrano, L. and Surfus, J.S. (1997) The Photochemical Reflectance Index: An Optical Indicator of Photosynthetic Radiation Use Efficiency across Species, Functional Types and Nutrient Levels. *Oecologia*, **112**, 492-501. https://doi.org/10.1007/s004420050337

[18] Shresta, S., Brück, H. and Asch, F. (2010) Diagnosis of Rice Leaf N Status by Photochemical Reflectance Index (PRI) and Chlorophyll Index (SPAD). http://www.asch-online.eu/downloads/FA-Shrestha-TT10-Poster.pdf

[19] Merzlyak, J.R., Gitelson, A.A., Chivkunova, O.B. and Rakitin, V.Y. (1999) Non-Destructive Optical Detection of Pigment Changes During Leaf Senescence and Fruit Ripening. *Physiologia Plantarum*, **106**, 135-141. https://doi.org/10.1034/j.1399-3054.1999.106119.x

[20] Castro-Esau, K.L., Sánchez-Azofeifa, A., Rivard, B., Wright, S.J. and Quesada, M. (2006) Variability in Leaf Optical Properties of Mesoamerican Trees and the Potential for Species Classification. *American Journal of Botany*, **93**, 517-530. https://doi.org/10.3732/ajb.93.4.517

[21] SIAP (Servicio de Información Agroalimentaria y Pesquera) (2021) Producción agrícola. https://www.gob.mx/siap/acciones-y-programas/produccion-agricola-33119

[22] Mitra, S.K., Sen, S.K., Maiti, S.C. and Bose, T.K. (1984) Effect of Plant Density on Growth, Yield and Fruit Quality in Guava. *Bangladesh Horticulture*, **12**, 7-9.

[23] Padilla-Ramírez, J.S., González-Gaona, E. and Perales de la Cruz, M.A. (2010) Nuevas Variedades de Guayaba (*Psidium guajava* L.). Folleto Técnico No. 42. INIFAP-CIRNOC-Campo Experimental Pabellón. Pabellón de Arteaga, Ags, México, 28 pp.

[24] Pérez-Barraza, M.H., Osuna-García, J.A., Padilla-Ramírez, J.S., Sánchez-Lucio, R., Nolasco-González, Y. and González-Gaona, E. (2015) Fenología, Productividad y Calidad de Fruto de Guayaba Pulpa Crema y Rosa en Clima Tropical en México. *Interciencia*, **40**, 198-203.

[25] Hernández-Delgado, S., Padilla-Ramírez, J.S., Nava-Cedillo, A. and Mayek-Pérez, N. (2007) Morphological and Genetic Diversity of Mexican Guava Germplasm. P*lant Genetic Resources: Characterization and Utilization*, **5**, 131-141.

https://doi.org/10.1017/S1479262107827055

[26] Keppel, G. and Wickens, T.D. (2004) Design and Analysis: A Researchers Handbook. 4th Edition, Pearson

[27] Curran, P.J., Dungan, J.L. and Gholz, H.L. (1990) Exploring the Relationship Between Reflectance Red Edge and Chlorophyll Content in Slash Pine. *Tree Physiology*, **7**, 33-48. https://doi.org/10.1093/treephys/7.1-2-3-4.33

[28] Gould, K.S. (2004) Nature's Swiss Army Knife: The Diverse Protective Roles of Anthocyanins in Leaves. *Journal of Biomedicine and Biotechnology*, 314-320.

[29] Kaliamoorthy, S. and Rao, A.S. (1994) Effect of Salinity on Anthocyanin Accumulation in the Root of Maize. *Indian Journal of Plant Physiology*, **37**, 169-170.

[30] Chalker-Scott, L. (1999) Environmental Significance of Anthocyanins in Plant Stress Responses. *Journal of Photochemistry and Photobiology*, **70**, 1-9. https://doi.org/10.1111/j.1751-1097.1999.tb01944.x

[31] Li, Y-C., Lin, T-C. and Martin, C.E. (2015) Leaf Anthocyanin, Photosynthetic Light-Use Efficiency, and Ecophysiology of the South African Succulent *Anacampseros rufescens* (Anacampserotaceae). South African Journal of Botany, **99**, 122-128. https://doi.org/10.1016/j.sajb.2015.04.001

[32] Villalobos, F.J., Mateos, L., Orgaz, F. and Fereres, E. (2002) Fitotecnia: Bases y Tecnologías de la Producción Agrícola. Editorial Mundi-Prensa, Madrid, 496 p

[33] Hardwick, S.R., Toumi, R., Pfeifer, M., Turner, E.C., Nilus, R. and Ewers, R.M. (2015) The Relationship Between Leaf Area Index and Microclimate in Tropical Forest and Oil Palm Plantation: Forest Disturbance Drives Changes in Microclimate. *Agricultural and Forest Meteorology*, **20**, 187-195. https://doi.org/10.1016/j.agrformet.2014.11.010

[34] Oren-shamir, M. (2009) Does Anthocyanin Degradation Play a Significant Role in Determining Pigment Concentration in Plants. *Plant Science*, **177**, 310-316. https://doi.org/10.1016/j.plantsci.2009.06.015

[35] Karl, T.R., Melillo, J.M. and Peterson, T.C. (eds.) (2009) Global Climate Change Impacts in the United States. United States Global Change Research Program, Cambridge University Press, New York, NY, USA

[36] Padilla-Ramírez, J.S., González-Gaona, E., Rodríguez-Moreno, V.M., Reyes-Muro, L., Osuna-Ceja, E.S. and Acosta-Díaz, E. (2014) Varianza Entre y Dentro e Índice de Repetitividad de Características Cuantitativas de Fruto de Guayaba. *Revista Mexicana de Ciencias Agrícolas*, **5**, 1423-1432. https://doi.org/10.29312/remexca.v5i8.820

[37] Xia, X., Tang, Y., Wei, M. and Zhao, D. (2018) Effect of Paclobutrazol Application on Plant Photosynthetic Performance and Leaf Greenness of Herbaceous Peony. *Horticulture*, **4**, Article No. 5. https://doi.org/10.3390/horticulturae4010005

[38] Jain, M.C. and Dashora, L.K. (2007) Growth, Flowering, Fruiting and Yield of Guava (*Psidium guajava* L.) cv. SARDAR as Influenced by Various Plant Growth Regulators. *International Journal of Agricultural Science*, **3**, 4-7.

[39] Mannino, G., Gentile, C., Ertani, A., Serio, G. and Bertea, C.M. (2021) Anthocyanins: Biosynthesis, Distribution, Ecological Role, and Use of Biostimulants to Increase Their Content in Plant Foods—A Review. *Agriculture*, **11**, Article No. 212. https://doi.org/10.3390/agriculture11030212