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Non-Structural Carbohydrates, Foliar Nutrients, Yield Components and Oxidative Metabolism in Pecan Trees in Response to Foliar Applications of Growth Regulators

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Abstract: Foliar sprays of growth regulators have commercial potential for improving the performance of some of the parameters associated with alternate bearing in pecan trees. The objective was to evaluate the behaviour of alternate bearing through analysis of seasonal variations in buds and leaflets of non-structural carbohydrates (glucose, fructose, sucrose, and starch), mineral nutrients (N-total, P, K^+, Ca^{2+}, Mg^{2+}, Fe^{2+}, Cu^{2+}, Mn^{2+} and Zn^{2+}), yield components (nut weight per kilogram and kernel percentage) and oxidative metabolism (superoxide dismutase, hydrogen peroxide, catalase, guaiacol peroxidase and antioxidant capacity) in cv. Wichita pecan trees in response to foliar applications of gibberellic acid (50 mg L^{-1}), calcium prohexadione (500 mg L^{-1}) or thidiazuron (10 mg L^{-1}). The experiment was of a completely randomized experimental design with five replicates. Foliar growth regulator (GRs) sprays help maintain the concentration of non-structural carbohydrates in the leaflets and buds between the evaluation years. With the exception of K^+ (12.9 and 10.9 g kg^{-1}) and Zn^{2+} (45.1 and 30.5 mg kg^{-1}), the GRs did not show any effects on the concentrations of the foliar mineral nutrients. The results suggest foliar sprays of gibberellic acid improve the performance of parameters associated with alternate bearing, including oxidative metabolism.

Keywords: alternate bearing; antioxidant capacity; *Carya illinoinensis*; gibberellic acid; prohexadione calcium; starch; thidiazuron

1. Introduction

Alternate bearing (AB) is a physiological phenomenon that affects both fruit yield and fruit quality in many perennial tree-fruit species, including in pecan [*Carya illinoinensis* (Wangenh.) K. Koch.]. In pecan, AB affects the number, size of the fruits and kernel percentage [1]. External factors, such as inadequate winter chill, inadequate light intensity, too long or too short a photoperiod, pruning, drought and soil mineral nutrient availability. Similarly, internal factors, such as tree age, cultivar, reserve carbohydrates and the activities of various endogenous growth regulators (GRs), all contribute to the occurrence of AB and in turn determine the induction of flower buds [2–4]. The incidence and severity of AB results in significant reductions in economic return (c. 30%) for growers and marketers of pecan nuts [5]. Among the pecan cultivars most suited to, and most grown in, the United States and Mexico are 'Western Schley', 'Wichita', 'Pawnee' and 'Stuart' [6]. These cultivars are differently susceptible to AB. Maintenance of optimal levels of yield and nut quality between production cycles requires appropriate agronomic managements,

in particular with regard to pruning, fertilisation, irrigation and pathogen control [3,7]. However, modification of concentrations of GRs may also be a key to minimising the negative effects of AB [8].

The use of exogenous GRs can have significant effects on transitions in the floral development phases of fruit trees [9]. Thus, studies with foliar applications of the GRs: gibberellic acid (GA_3), calcium prohexadione (3-oxido-4-propionyl-5-oxo-3cyclohexene-carboxylate) (PCa) and thidiazuron [1-phenyl-3-(1,2,3 thidiazol-5-yl) urea] (TDZ) have allowed modification of floral induction and fruit size and fruit number in avocado (*Persea americana* L.), volkamer lemon (*Citrus x volkameriana* L.), cherry (*Prunus cerasus* L.), mango (*Mangifera indica* L.), apple (*Malus domestica* Borkh) and pecan (*Carya illinoinensis*) ("Western Schley", "Sumner", "Oconee" and "Pawnee") [2,7,8,10,11]. Likewise, in pecan, exogenous GRs have also affected parameters associated with AB, including mineral nutrient concentrations, levels of non-structural carbohydrates (NSC), and nut yield and quality [10,11]. However, such studies with pecan are relatively few and the results have not always been consistent, perhaps due to variations in tree age, in cultivar, in the diversity of agronomic managements and in the phenological stages when the GR applications were made, the GR dose and the number of GR applications [12].

High availabilities of glucose, fructose, sucrose and starch prior to floral induction help to minimise AB [13]. In addition, levels of these non-structural carbohydrates play fundamental roles in vegetative growth and sprouting after dormancy [14]. Multiple studies have concluded that the crop load in an 'on' year is a factor that can deplete the tree's carbohydrate and/or mineral nutrient reserves, thus reducing the numbers of flower buds, and shoots, and leaflet area in the following 'off' year [15]. In addition to these effects, molecular mechanisms involved in floral induction, and initiation include a complex and interrelated network that in turn is affected by external factors (such as photoperiod, light quality and air temperature) and internal factors (such as competition between organs, carbohydrate concentrations and GR levels) [2,5,6]. The external (foliar) application of mineral nutrients and GRs can promote the synthesis and accumulation of additional reserve carbohydrates [11,16].

Under conditions of high abiotic stress, plants suffer reductions in growth and productivity, being targets of oxidative damage and cell death due to the generation and accumulation of reactive oxygen species (ROS), these directly increase the incidence of AB [17,18]. Under natural conditions, production of ROS, such as hydrogen peroxide (H_2O_2), superoxide anions ($O_2^{\bullet -}$), hydroxyl radicals ($OH^{\bullet}$) and singlet oxygen occurs during photosynthesis and cellular respiration [19]. The generation of ROS can be increased by factors such as drought, nutrient deficiency and pruning. Among the best-known mechanisms for the reduction in/elimination of these damaging molecules are those that make up the enzymatic defence systems including: superoxide dismutase (SOD) (EC. 1.15.1.1), catalase (CAT) (EC. 1.11.1.6) and guaiacol peroxidase (GPx) (EC. 1.11.1.7) and various antioxidants including: phenolic compounds, vitamins and carotenoids [20,21]. Monitoring the activities of all these enzymes and antioxidant compounds may help elucidate the behaviour of AB in pecan.

Despite advances in agricultural methods to minimise AB, it remains a significant industry problem in pecan production [3,22]. Pecan is currently considered the most valuable nut produced in North America and its cultivation is expanding, driven by this demand potential. However, AB remains a significant risk factor for the industry [1,11]. New information may help minimise the negative effects of AB. Among the management strategies for mitigating AB is increasing the concentration of endogenous hormones, through foliar applications of GRs, because these have been shown to affect the number and size of the fruit and kernel percentage in some pecan cultivars. The aim of this study was to evaluate relationships between the incidence of AB and the seasonal variations in these NSCs in buds and leaflets, foliar mineral nutrients and yield components and oxidative metabolism in the pecan cultivar Wichita in response to foliar applications of GA_3, PCa or TDZ.

2. Materials and Methods

2.1. Plant Materials, Orchard Management and Experimental Design

Partial results for cv. Western Schley have been published previously [11,12]. This study was conducted over two consecutive years (2017–2018) in an orchard established in Chihuahua, Mexico (28°57′1.44″ N, 106°14′2.73″ W) at an altitude of 1440 m, average annual rainfall 366.5 mm and average annual temperature 17.8 °C. The soil is characterised as having a sandy-crumb texture, 0.95% organic matter, pH 7.6 and electrical conductivity 2.5 dS m^{-1}. Soil nutrient concentrations were (mg kg^{-1}) 18 N-Total, 8 P, 275 K^+ and (mg kg^{-1}) 5.41 Ca^{2+}, 320 Mg^{2+}, 139 Fe^{2+}, 180 Mn^{2+}, 13 Zn^{2+} and 4 Cu^{2+}. The soil analysis was performed from a composite sample (10 sub-samples), collected in "Zig-Zag" in the experimental area. The plant material was 10-year-old pecan trees cv. Wichita grafted on a native rootstock. The planting density was 139 trees ha^{-1} (6 × 12 m).

The trees were fertilised in the first week of March with an N-P-K fertiliser of the general formula 150-100-100. The sources were $(NH_4)_2SO_4$ (20.5% N and 24% S), H_3PO_4 (49% P_2O_5) and K_2SO_4 (50% K_2O). At 80% sprouting, six applications of Zn (17% $ZnNO_3$) were made using the product GoZinc 17® (Gowan, Mexicali, BC, México). Screwworm (*Acrobasis nuxvorella* Neunzig) control was carried out with 0.5 L ha^{-1} of Intrepid™ (Dow AgroSciences®, Zionsville, IN, USA). Standard commercial practices for weed control and irrigation scheduling were followed throughout the experiment.

The experiment was established in a completely randomized experimental design with five replications, where the experimental unit consisted of a single tree. Tree height was 10 ± 0.5 m and a trunk girth 65 ± 10 cm. The three products (treatments, T1 . . . T3 and a water control (T4) were applied via foliar sprays: T1: GA_3 (50 mg L^{-1}) (ProGibb®, Bayer Crop Science®, Zionsville, IN, USA), T2: PCa (500 mg L^{-1}) (Apogee®, BASF®, Florham Park, NJ, USA), (T3: TDZ (10 mg L^{-1}) (Revent® 500 SC, Bayer Crop Science, Mason, MI, USA). To facilitate adhesion and penetration, 1 mL L^{-1} mL·L^{-1} of INEX-A® (Cosmocel S.A., Monterrey, NL, Mexico) and urea (1%) were applied. The GRs were applied using a 25 L motorized backpack fertilizer applicator, between 0600 and 0900 h. In the evaluation years, these treatments were applied at 0, 56, 70 and 84 days after flowering.

2.2. Sampling of Leaflets, Buds and Nuts

Collection of leaflets and buds was carried out in mid-July each year of evaluation. Materials were without obvious mechanical damage, pests or diseases. In all, 120 pairs of leaflets and 100 buds per treatment were collected from branches in the middle part of the canopy and from all four cardinal points. Fruit collection was carried out in the last week of November using mechanical vibration of the trees.

2.3. Non-Structural Carbohydrates (Leaflets and Buds)

Extraction and quantification of glucose, fructose, sucrose and starch were carried out according to the method described by Sánchez et al. [23], slightly modified. A sample of 0.5 g of fresh tissue was taken and homogenised twice, first with 5 mL of 95% aqueous ethanol (*v*:*v*) and second with 70% aqueous ethanol (*v*:*v*). The mixture was centrifuged at 5500× *g* for 10 min at 4 °C. Next, 0.1 mL of the supernatant was taken and 3 mL of anthrone solution was added. The mixture was placed in a water bath min at 4 °C for 10 min and, after cooling, the absorbance was measured at 650 nm. For the determination of starch, the dry residue of the extraction was taken and incubated in acetate buffer (4.5 M), 0.5% α-glucoamylase (*w*:*v*) and water at 37 °C for 48 h. The results are expressed in mg g^{-1}.

2.4. Leaf Mineral Nutrients

Extraction and quantification of nutrients was carried out using the method described by Orozco-Meléndez et al. [11] and Cruz-Alvarez et al. [24]. Briefly, the leaflets were washed at room temperature with tap water, then HCl solution (4 M) and then deionised water. Surface moisture was completely removed by blotting and the leaflets dried to constant weight at 70 °C for 24 h in a ventilated oven (Heratherm VCA 230®, Thermo

Scientific, Waltham, MA, USA). The samples were then homogenised in a mill (Wiley®, Culver City, CA, USA) with a 1 mm mesh. The extraction and quantification of total-N and P were determined by the Kjeldhal method (Novatech®, Nashville, TN, USA and Micro Kjeldahl, Labconco®, Kansas City, MO, USA and by the ammonium metavanadate method (NH_4VO_3) (Thermo Scientific™, Waltham, MA, USA), respectively. The extraction of K, Ca, Mn, Mg, Fe, Cu and Zn employed the triacid digestion method (HNO_3:$HClO_4$:H_2SO_4, 10:10:25, *v*:*v*:*v*) using 25 mL of the acid mixture on a hot plate under fume-hood. Analyte quantifications were carried out using an Analyst 100® atomic absorption spectrophotometer (PerkinElmer®, Waltham, MA, USA). Results are reported as g kg^{-1} (macronutrients) and mg kg^{-1} (micronutrients).

2.5. Yield Components (Yield and Nut Quality)

The performance components were determined according to the method described by the Mexican Standard NMX-FF-084-SCFI-2009 [25]. The weight of the harvested nuts (fruits) was obtained using a Combo-Rhino-122 scale (Rhino Maquinaria S.A. de C.V., Lomas de Atizapan, MEX, México) with a sensitivity of 0.1 g. Yield data are expressed as kg·$tree^{-1}$. For the number of nuts per kg, 1 kg of nuts was randomly selected and counted. Next, 300 g of nuts was selected as a sub-sample and the shells were removed and discarded. The kernels (the edible parts) were weighed. Kernel percentage was obtained as the ration of kernel weight divided by sub-sample weight × 100.

2.6. Oxidative Metabolism (Enzymatic Activity and Antioxidant Capacity)

Superoxide dismutase (SOD EC 1.15.1.1) activity was assayed by monitoring the inhibition of photochemical reduction of nitroblue tetrazolium (NBT), according to the method described by Giannopolitis and Ries [26] and modified by Sanchez et al. [21]. The activity was determined in a 5 mL reaction mixture containing 50 mmolM HEPES at pH 7.6, 0.1 mmolM EDTA, 50 mmolM Na_2CO_3 (pH 10), 13 mmolM methionine, 0.25% Triton X-100 (p/v), 63 µM NBT, 1.3 µmolM riboflavin and an appropriate aliquot of enzyme extract. Enzyme activity was reported as units/min/g, where one unit of SOD activity corresponded to the amount of enzyme required to cause a 50% inhibition of NBT reduction evaluated at 560 nm. The extraction and analysis of total H_2O_2, employed a colorimetric method [21]. The results are reported in µmol H_2O_2 min^{-1} g^{-1} (total peroxides). Last, the extraction and determination of enzymatic activity of the enzyme catalase and guaiacol peroxidase were determined by the method described by Sanchez et al. [21]. The results are expressed as mol H_2O_2 min^{-1} g^{-1} and nmol GSH min^{-1} g^{-1}, respectively.

Antioxidant capacity (AC) was determined using the 2,2-diphenyl-1-picrylhydraill (DPPH•) method. Aqueous ethanol (80%) was used for sample preparation. The DPPH• assay was carried out in accordance with Brand-Williams et al. [27]. Briefly, 0.3 mL of extract and 5.7 mL of the compound DPPH (2,2-diphenyl-1-picrylhydrazyl) were mixed at a concentration of 0.0375 g L^{-1}). The mixture was kept in the dark conditions for 30 min. The decrease in the DPPH radical was measured at 515 nm. The results are expressed as % inhibition of DPPH.

2.7. Statistical Analyses

Prior to statistical analysis, the normal distribution of the data was confirmed by the Shapiro–Wilk test ($p \leq 0.05$) [28]. Statistical analysis consisted of a general linear model with year and treatment effects. The comparison of means was carried out with a multiple comparison of means with Tukey's test ($p \leq 0.05$). The data were analysed with statistical package SAS/STAT | SAS (Statistical Analysis System Institute), version 9.3 (SAS Institute Inc., Cary, NC, USA).

3. Results

Foliar applications of GRs showed significant interactions ($p \leq 0.05$) between years and treatments on NSC (Tables 1–3, Figures 1–3).

Table 1. Concentration of NSC in cv. Wichita pecan leaflets and buds treated with GR.

Plant Organ	GR (mg L^{-1})	Fructose [1]		Glucose		Sucrose		Starch	
		2017	2018	2017	2018	2017	2018	2017	2018
Leaflets	GA_3 (50)	40.9 a	42.4 a	43.5 a	46.9 a	40.4 a	44.7 a	36.9 a	40.7 a
	PCa (500)	38.1 a	40.9 a	41.6 a	42.6 a	37.7 a	41.7 a	35.9 a	39.3 a
	TDZ (10)	38.5 a	41.8 a	41.9 a	46.2 a	40.4 a	44.2 a	37.0 a	40.3 a
	Control	33.1 a	35.6 b	33.6 a	37.6 b	25.7 a	38.7 b	23.4 a	28.3 b
Buds	GA_3 (50)	41.7 a	45.9 a	40.5 a	44.3 a	43.9 a	46.2 a	43.6 a	45.8 a
	PCa (500)	40.2 a	45.1 a	38.8 a	41.4 a	41.1 a	44.7 a	41.0 a	45.5 a
	TDZ (10)	41.73 a	45.2 a	38.6 a	42.6 a	41.6 a	44.5 a	42.5 a	45.1 a
	Control	30.3 a	35.9 b	28.9 a	33.8 b	31.9 a	35.7 a	31.6 a	34.9 b

[1] GR: Growth regulators; GA_3: gibberellic acid; PCa: prohexadione calcium and TDZ: thidiazuron. Values with the different letters within columns represent significant differences (Tukey's test, $p \leq 0.05$). Data are expressed on a fresh-weight basis (mg g^{-1}).

Table 2. Concentration of nutrients in pecan leaflets cv. "Wichita" with applications of GR.

GR [1]	g kg^{-1}									
	N-Total		P		K^+		Ca^{2+}		Mg^{2+}	
	2017	2018	2017	2018	2017	2018	2017	2018	2017	2018
GA_3	28.4 a	23.1 a	1.1 a	1.5 a	9.9 a	10.5 a	34.1 a	31.9 a	3.3 a	3.5 a
PCa	26.8 a	24.9 a	1.8 a	1.9 a	12.9 a	10.9 b	31.6 a	31.8 a	3.1 a	3.2 a
TDZ	20.7 a	19.0 a	1.7 a	2.0 a	11.4 a	11.9 a	31.2 a	32.4 a	3.1 a	3.1 a
Control	23.8 a	22.9 a	1.8 a	1.7 a	9.3 a	9.4 a	26.9 a	26.9 a	2.9 a	3.1 a
GR	mg kg^{-1}									
	Fe^{2+}		Cu^{2+}		Mn^{2+}		Zn^{2+}			
	2017	2018	2017	2018	2017	2018	2017	2018		
GA_3	142.0 a	138.3 a	6.2 a	6.4 a	229.7 a	236.3 a	40.5 a	38.1 a		
PCa	138.0 a	142.3	6.3 a	6.2 a	236.3 a	237.4 a	41.5 a	37.3 a		
TDZ	151.7 a	147.3 a	6.8 a	6.3 a	244.7 a	234.3 a	45.1 a	30.5 ab		
Control	123.8 a	114.3 a	5.6 a	5.6 a	217.0 a	205.6 a	31.2 a	28.2 b		

[1] GR: growth bioregulators; GA_3: gibberellic acid (50 mg L^{-1}); PCa: prohexadione calcium (500 mg L^{-1}) and TDZ: thidiazuron (10 mg L^{-1}). Values with the different letters within columns represent significant differences (Tukey's test, $p \leq 0.05$). Data are expressed on a fresh-weight basis.

Table 3. Application of GR to in cv. "Wichita" pecan trees and the inter-annual effect on nut yield and quality.

GR (mg L^{-1})	Yield (kg $tree^{-1}$)		Nut Quality			
			Nut Weight Per kg (g)		(%) Kernel	
	2017 [1]	2018	2017	2018	2017	2018
GA_3 (50)	14.53 a	16.7 a	6.7 a	6.1 a	61.8 a	59.9 a
PCa (500)	11.3 a	13.1 b	6.4 a	5.6 b	60.7 a	54.3 b
TDZ (10)	13.5 a	15.7 b	7.2 a	5.9 b	59.5 a	53.9 b
Control	6.1 a	9.9 b	5.5 a	5.0 b	58.6 a	55.1 b

[1] GR: growth regulators, GA_3: gibberellic acid; PCa: prohexadione calcium and TDZ: thidiazuron. Values with different letters within a column are significantly different (Tukey's test, $p \leq 0.05$).

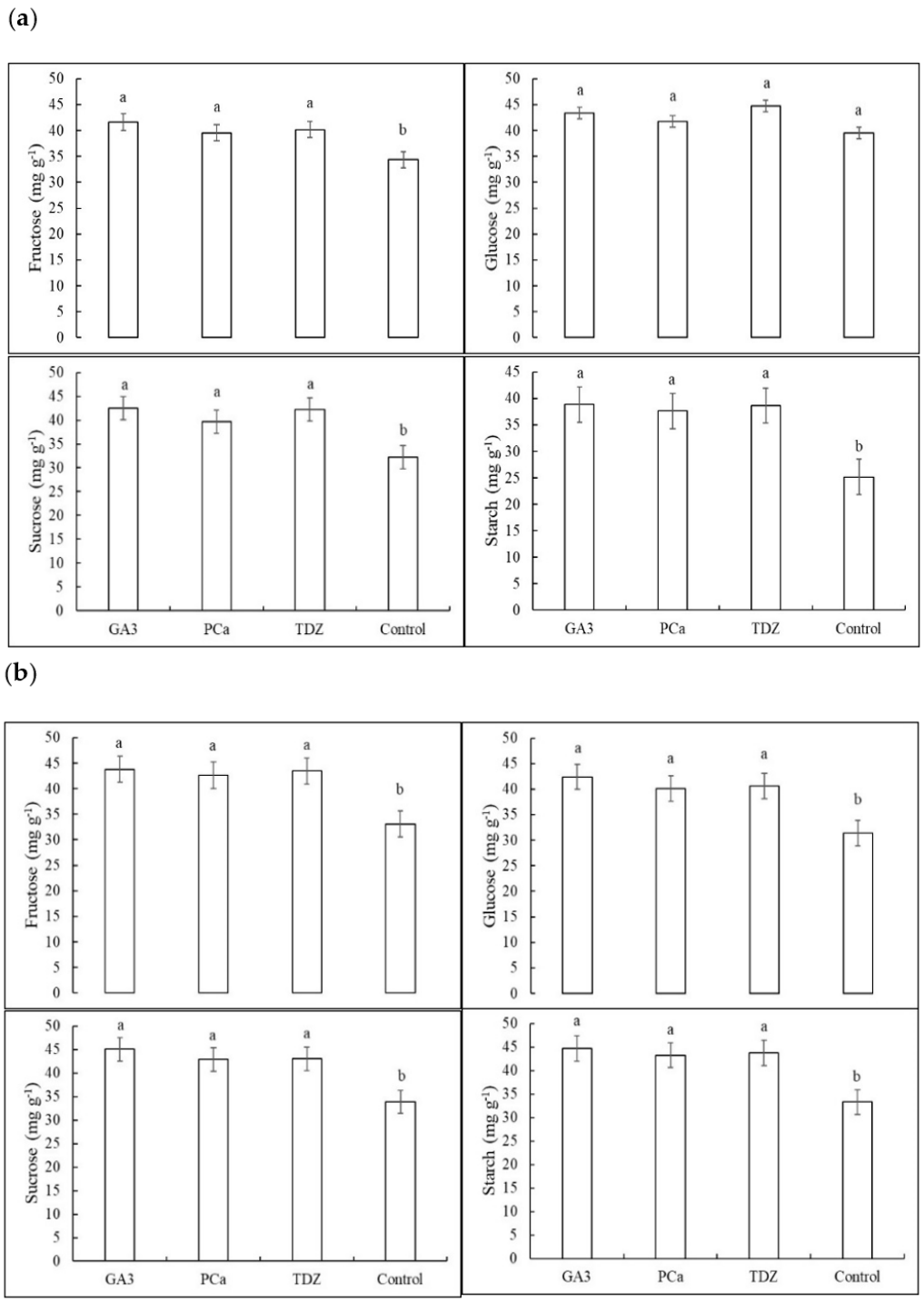

Figure 1. Concentrations of NSC in the leaflets (**a**) and buds (**b**) of pecan cv. Wichita treated with GR. The data are the means by treatment over two years (2017 and 2018). GA_3: gibberellic acid (50 mg L^{-1}); PCa: prohexadione calcium (100 mg L^{-1}) and TDZ: thidiazuron (500 mg L^{-1}). Bars with the same letter are not different according to Tukey's test ($p \leq 0.05$). Error bars represent standard deviations ($n = 5$).

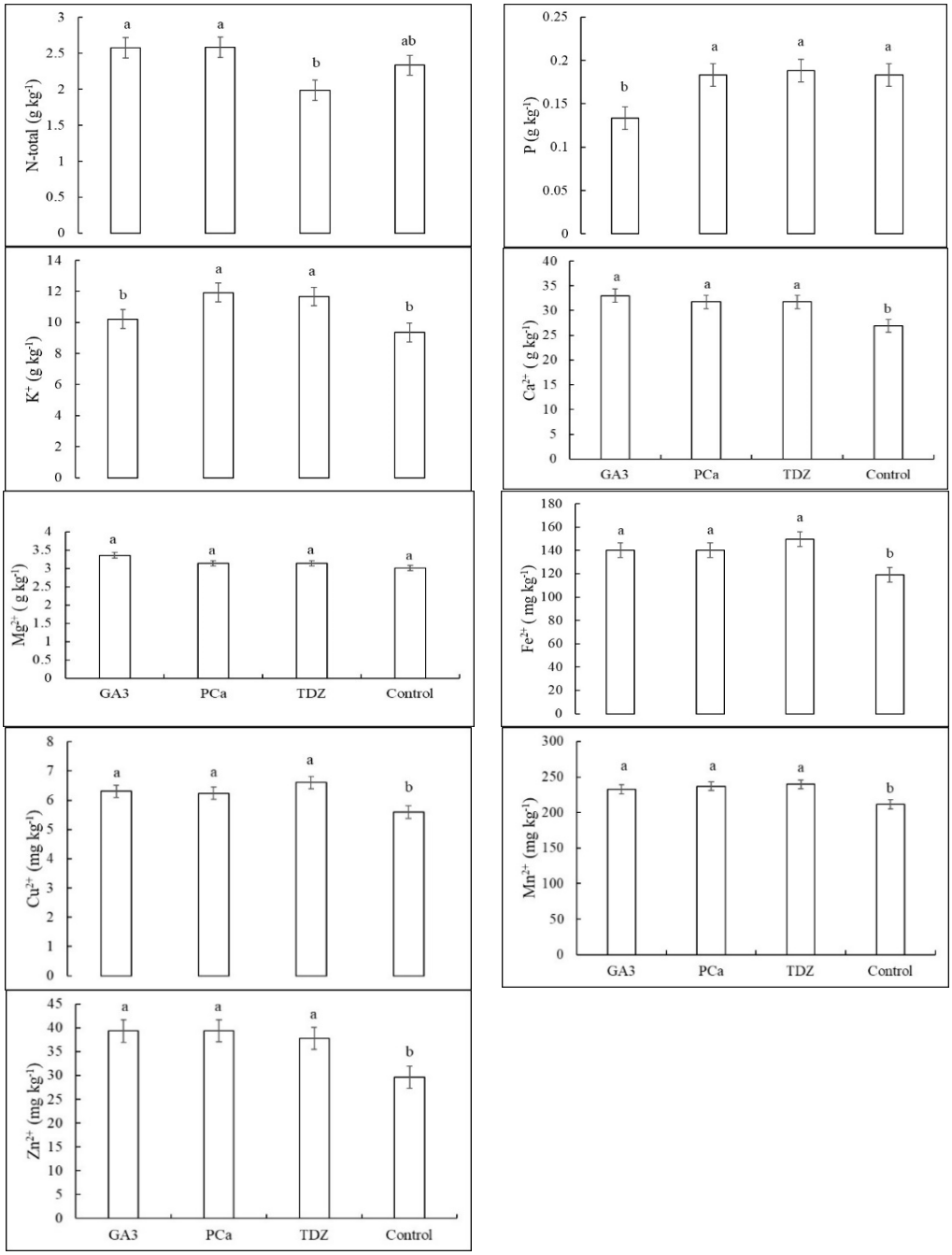

Figure 2. Concentrations of nutrients in pecan trees cv. Wichita after applying foliar growth regulator treatments. The results are the means per treatment over two seasons (2017 and 2018). GA_3: gibberellic acid (50 mg L^{-1}); PCa: prohexadione calcium (500 mg L^{-1}) and TDZ: thidiazuron (10 mg L^{-1}) and a water control. Bars with the same letter are not significantly different based on Tukey's test ($p \leq 0.05$). Error bars represent standard deviations ($n = 5$).

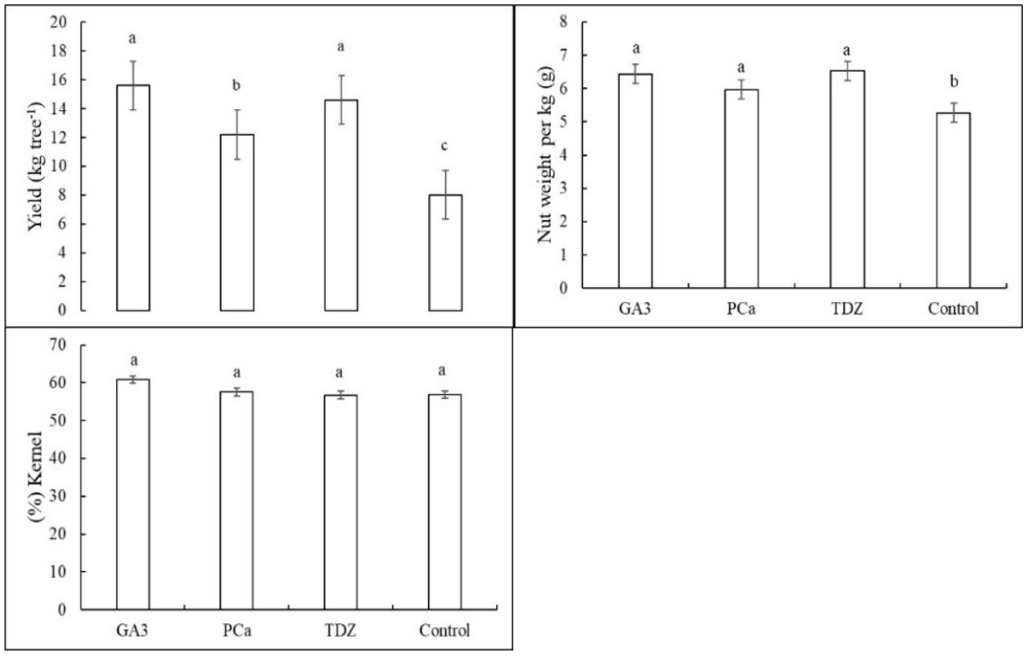

Figure 3. Yield, nut weight and kernel percentage in cv. Wichita pecan trees with the application of foliar treatments of growth regulators. The data are means of treatments in 2017 and 2018. GA_3: gibberellic acid (50 mg L^{-1}); PCa: prohexadione calcium (500 mg L^{-1}) and TDZ: thidiazuron (10 mg L^{-1}) and control. Bars with the same letter are not significantly different according to Tukey's test ($p \leq 0.05$). Error bars represent standard deviations ($n = 5$).

3.1. Non-Structural Carbohydrates in cv. Wichita Pecan Leaflets and Buds

The underlying causes of AB in pecan are not known but seem to be closely linked with the length of the dormant season, and to crop load and to the stored carbohydrate pool [22]. Here, the applications of GA_3, PCa or TDZ maintained the concentration of NSC in the leaflets and buds between the evaluation years (Table 1).

The leaflet and bud NSC concentration results by treatment are presented in Figure 1a,b, respectively. Foliar applications of GR increased ($p \leq 0.05$) the concentration of NSC in the leaflets and buds with respect to the water control.

Between treatments, the application of GA_3 shows a tendency for a higher concentration of carbohydrates between years, which increases the number of flower buds. Likewise, among the reasons for the behaviour observed in NSC, it can be associated with the sampling date, age of the tree and agronomic management [15,16,29].

3.2. Concentrations of Nutrients in Pecan Leaflets

To help understand the physiological aspects of AB, it is necessary to know the seasonal variations in leaflet mineral nutrient concentrations [22,30]. On the other hand, the seasonal pattern of nutrient uptake and partitioning is a key component of fertiliser management [31]. In our study, with the exception of K^+ and Zn^{2+}, the application of GR did not show any effects on the concentrations of the foliar mineral nutrients (Table 2).

In general, the application of the treatments with GRs (GA_3, PCa and TDZ) presented the highest concentration of mineral nutrients in the leaflets; however, in the case of P and K^+ only PCa and TDZ were outstanding and, finally, Mg^{2+} did not show changes (Figure 2).

3.3. Yield Components (Yield and Nut Quality)

Of great commercial importance are the yield and quality of the nut, as these are directly linked to AB and they affect the economic value of this deciduous fruit tree [5,29]. With the exception of GA_3, the yield and nut quality showed variation between years (Table 3).

Yield is the main parameter related to AB [5]. With the exception of PCa, the GR applications showed significant effects on yield (Figure 3).

3.4. Oxidative Metabolism and Antioxidant Capacity

Most of the pecan orchards in northern Mexico are established on calcareous soils with a pH between 7.5 and 8.5. These adverse conditions can cause abiotic stress including overproduction of ROS and thus can adversely affect productivity [18,19]. In this study, we explored the behaviour of parameters associated with oxidative metabolism in response to GR treatments; the results are presented in Table 4.

Table 4. Application of GRs in cv. Wichita pecan trees and their effects on oxidative metabolism and antioxidant capacity.

GR (mg L^{-1})	SOD [1]	H_2O_2	CAT	GPx	AC
GA_3 (50)	1.29 b	0.34 b	2.43 b	4.35 b	60.00 c
PCa (500)	1.18 c	0.35 b	2.31 b	4.20 b	71.09 b
TDZ (10)	1.44 ab	0.31 b	2.33 b	3.22 c	75.15 a
Control	1.60 a	0.42 a	3.20 a	5.46 a	76.78 a

[1] GR: growth bioregulators; GA_3: gibberellic acid; PCa: prohexadione calcium and TDZ—thidiazuron; SOD: superoxide dismutase (units min^{-1} g^{-1}); H_2O_2: hydrogen peroxide (μmol g^{-1}); CAT: catalase (nmol GSH min^{-1} g^{-1}); GPx: guaiacol peroxidase (nmol GSH min^{-1} g^{-1}) and AC—antioxidant capacity (% of DPPH inhibition). Values with different letters within columns are significantly different (Tukey's test, $p \leq 0.05$).

4. Discussion

It is well known that variations in reserve carbohydrate concentration affect the intensity of AB, and in pecan cv. Wichita this is affected by the tree age, being less variable in young trees [3]. Pecan is reported to show irregular AB—that is, pecan can present with a behaviour in which it has two consecutive 'on' years or 'off' years. This behaviour seems to be related to random external factors, including cold damage, hail, wind, solar radiation, and excess soil water [30]. In line with these results, similar values of NSC in leaflets of cv. Western Schley pecan trees treated with GA_3, PCa and TDZ were reported by Orozco-Melendez et al. [11]. On the other hand, Nzima et al. [29] in a study with pistachio (*Pistacia vera* L.) cv. Kerman reported significant variations between 'on' and 'off' years in the concentrations of soluble sugars and starch in the leaves. These results may suggest an attenuation in AB, by increasing of the internal concentration of GR. In addition, the intensity of the AB is much affected by tree age [16]. In our study, the trees were relatively young (about 10 yr), characterised by high leaf area and efficient root management, perhaps explaining the mild AB [29].

Cultivated plants can vary in their nutrient composition and still show optimal growth and development [32]. Here, with the exception of the leaflets sprayed with TDZ in 2018, the values found for N-total and P were in the 'normal' range for this species [33]. The leaflets treated with PCa showed variation between years (12.9 and 10.9 g kg^{-1}); however, these values were both in the range of sufficiency, and so should not present a problem maintaining good nut yield and good nut quality [24,33,34]. On the other hand, mineral nutrients such as Ca^{2+} and Mg^{2+} were highly variable and were found in both the 'high' and the 'low' ranges (15.7–24.2 and 3.9–5.8 g kg^{-1}, respectively), according to Pond et al. [33]. However, our trees did not show symptoms of marginal necrosis in leaflets, related to high concentrations of calcium.

The concentrations of Fe^{2+}, Cu^{2+} and Mn^{2+} were in the sufficiency range indicated for pecan [34,35]. However, Zn^{2+} was found to be deficient, as indicated by Jones et al. [35] (50–100 mg kg^{-1}), Robinson et al. [34] (50–100 mg kg^{-1}) and Pond et al. [33] (86–256 mg kg^{-1}). In this sense, Zn^{2+} is the second most important nutrient for pecan production, surpassed in importance only by N [24]. The deficiency of Zn^{2+} is very common in pecan orchards on alkaline and calcareous soils—our soils were pH 7.6 [19]. These authors suggest Zn^{2+} concentrations between 20 and 50 mg kg^{-1} are sufficient for normal pecan tree growth.

In general, AB is linked to alternate 'on' and 'off' cycles, where the concentrations of carbohydrates and mineral nutrients in the leaflets and buds are similar. In terms of fruit-bearing, pecan trees between 1 and 10 years old are in transition from the immature stage to the mature one [36]. The pecan cv. "Wichita" is reported as particularly prone to AB [3,37]. It is well known that pecan differs from some other AB tree species in that some individuals show high or moderate yields just prior to an 'off' year [38].

The concentration of macro nutrients in the leaflets varied between treatments (Figure 1a,b). The values of total-N and Ca^{2+} were higher in the leaflets sprayed with GA_3 and PCa; however, the level of Ca^{2+} was similar to TDZ. In the case of P and K^+, the application of PCa and TDZ stands out. However, Mg^{2+} did not show variation. On the other hand, the concentration of Fe^{2+}, Cu^{2+}, Mn^{2+} and Zn^{2+} in the leaflets presented a similar behaviour between the treatments, that is, a significant effect to the application of GR. With the exception of Mg^{2+} and Zn^{2+}, similar behaviours are reported in nine-year-old pecan trees of cv. Western Schley by Orozco-Meléndez et al. [11]. The foliar concentrations of mineral nutrients affect the synthesis and accumulation of carbohydrates, so nutrients play important roles in photosynthesis, leaf area increase and fruit set [22].

The cv. Wichita has been reported as having an intermediate tendency for AB with young trees having AB values of 0.50, which represent a 50% reduction in yield in 'off' years, compared with 'on' years. This value rises to 0.67 as the trees mature [3]. Similarly, the presence of fruits in the 'on' years, inhibits the formation of floral buds and reduces the number of shoots, causing a reduction in growth of the tree [31]. A previous study with applications of GA_3, PCa and TDZ (50, 500 and 10 mg L^{-1}) to pecan cv. Western Schley trees by Orozco-Meléndez et al. [11] reported significant values with GA_3 between 12.4 and 15.3 kg $tree^{-1}$. This result confirms the role of GA_3 in reducing the number of pistillate flowers, increasing crop load and mitigating AB in pecan trees [8].

The GA_3 treatment helped maintain nut weight (6.7 and 6.1 g) and the kernel percentage (61.8 and 59.9%). The nuts harvested from trees treated with GA_3 were of a weight allowing them to be classified as 'excellent' according to Nmx-Ff-084-Scfi-2009 [25]. A similar GA_3 response was reported by Orozco-Meléndez et al. [12] in cv. Western Schley trees but their nut weight and kernel percentage were lower (between 5.8 $\pm$ 0.13 and 5.5 $\pm$ 0.20 g and 59.4 $\pm$ 4 and 58.9 $\pm$ 1.20%, respectively). Thus, gibberellins can be considered inhibitors of flowering, so reducing the fruit number but increasing fruit weight [7]. In this way, increased accumulation of carbohydrates directly affects nut quality, which is also affected by genetic, edaphoclimatic and agronomic factors [15]. In pecan, flowering behaviour is modified by AB and other factors, including the temporal separation between floral induction and floral differentiation [38].

In a previous study on mature cv. Western pecan trees [38], treatment with GA_3 (50 mg L^{-1}) increased the number of flowers per new shoot by 125%. The responses to GA_3 and TDZ in our study are similar to that in cv. Western Schley pecan trees by Orozco-Meléndez et al. [12].

Significant differences between GR treatments were observed in the weight of the nut with respect to the control; however, the kernel percentage did not show variation. In contrast, Orozco-Meléndez et al. [11] found no significant differences for these parameters, when GA_3, PCa or TDZ (50, 500 and 10 mg L^{-1}) were applied to nine-year-old trees of cv. Western Schley pecan. For flower development, the initial requirement is for a reprogramming of the shoot meristem to transition from vegetative to floral [6]. The molecular mechanisms involved in flower initiation are complex and respond to a number of signalling pathways (photoperiodic, temperature, light quality, concentration of hormones and carbohydrates) [2,9].

The production of ROS, including of hydrogen peroxide, remained low, and significant reductions in the activities of SOD, CAT and GPx can be also seen in the GR-treated trees, and the TDZ treatment was similar statistically for the values of SOD with respect to the control. These results confirm the observation for NSC of mineral nutrients in buds and leaflets. With the exception of TDZ, the applications of GA_3 and PCa showed significant

reductions in CA, assuming all trees were subjected to the best production practices during the AB cycle [8,38].

5. Conclusions

Foliar sprays of growth regulators helped maintain the concentrations of non-structural carbohydrates in leaflets and buds and of mineral nutrients in leaflets. To minimise the effects of alternate bearing, GA_3 could be applied as a foliar spray at 50 mg L^{-1}. The use of growth regulators, in particular of GA_3, is a commercially viable way to improve the performance of yield parameters in pecan associated with alternate bearing, including of oxidative metabolism. However, it is necessary to conduct a more in-depth evaluation of the effect caused by growth regulators on the physiology and biochemistry in more varieties of this deciduous fruit tree, in order to improve the understanding of alternate bearing.

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Abbreviations

AB: Alternate bearing; GR: growth regulators; GA_3: gibberellic acid, PCa: prohexadione calcium (3-oxido-4-propionyl-5-oxo-3cyclohexene-carboxylate); TDZ: Thidiazuron [1-phenyl-3-(1,2,3 thidiazol-5-yl) urea]; NSC: non-structural carbohydrates; ROS: reactive oxygen species; SOD: superoxide dismutase, CAT: catalase; GPx: guaiacol peroxidase; AC: antioxidant capacity.

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