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The effect of paclobutrazol treatment on root carbohydrate, macronutrient contents and fine root growth of rejuvenated coffee (*Coffea arabica* l.)

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Abstract

Thirty year old non-productive coffee (*Coffea arabica* L.) trees were treated with three levels of paclobutrazol (0 control, 0.5 g a.i and 1.0 g.a.i. PBZ /meter canopy span) using Cultar 1 and 2 months before rejuvenation pruning and the effects on root carbohydrate, macronutrient contents and fine root growth were assessed. Application of 1.0 g a.i. PBZ significantly increased root starch content but did not affect the root sugar content. Plants applied with PBZ 2 months prior to pruning had higher root P content compared to those applied 1 month prior to pruning. Root N, P, K, Ca and Mg contents did not differ with time of PBZ treatment. The timing of PBZ application had no significant effect on the root volume and dry weight but PBZ concentration significantly affected root volume with plants applied with 0.5 g a.i. PBZ per meter canopy span having 40% bigger fine roots volume relative to the non-PBZ treated control.

Key words: *Rejuvenation pruning, paclobutrazol, fine roots, macro nutrients, carbohydrates*

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Introduction

Among the many factors contributory to the decline in vigor and yield of old coffee trees is root dieback (Nutman,1933). He found that when a tree suffered die-back the current year, the tree had poor development of feeders especially in the subsoil due to loss of feeder bearers. Cannell (1971a) also found that if the supply of assimilates especially to the shoots is inadequate and the reserve carbohydrates have been used, the fine roots in bearing coffee trees die followed by the death of the heavily bearing shoots. Bragança (2005) reported that the growth of the root system of irrigated Conilon trees was strongly affected by the fruit-bearing capacity such that in a year of high fruit burden (12,000 kg dry beans ha^{-1}), dry matter of the root system (five-year-old plants) dropped from 3.30 kg $plant^{-1}$ in October (pinhead fruit stage) to 1.50 kg $plant^{-1}$ in January (bean-filling stage) and 1.60 kg $plant^{-1}$ in April (early ripening) indicating that heavy crops may jeopardize the function of the root system, particularly uptake of nutrients from the soil which ultimately could exacerbate the occurrence of die-back and tree degeneracy.

PBZ has been reported to increase root growth among treated trees (Watson, 1996; Watson and Himelick, 2004; Chaney, 2005). Watson (1996) reported an increase in fine root density in white oak and pin oak following PBZ treatment. Watson and Himelick, (2004) observed that soil injection of PBZ around the base of white and pin oaks caused fine root densities to be 60-80% higher near the trunk base. In rice, Yim et al. (1997) reported that PBZ-treated seedlings had higher root dry mass. In bean hypocotyl cuttings, Davis et al. (1985) also reported promotion of adventitious root formation following PBZ treatment. Watson (1996) attributed the increase in root densities brought by PBZ application in white and pink oak as either a direct effect of PBZ on root growth or just an indirect effect resulting from shoot growth modification and a shift in carbohydrate allocation to the roots

Aside from influencing root growth, PBZ was also reported to alter mineral uptake and hence tissue concentration among treated plants. Yelenosky et al. (1985) reported that leaves from PBZ-treated citrus seedlings had higher concentrations of N, Ca, B, and Fe. In apple seedlings, PBZ increased leaf concentrations of N, P, K, Ca, Mg, Mn, B and Zn without affecting the concentrations of Fe, Si and Pb (Wang et al., 1986).

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Steffens and Wang (1984) revealed that carbohydrate concentrations increased in fibrous roots of apple seedlings grown in nutrient solution containing PBZ. The high carbohydrate levels in fines roots among PBZ-treated seedlings was attributed either to improved portioning towards the root (Wang *et al*.,1985) or to reduced alpha-amylase activity caused by lowering of GA levels following PBZ application (Jacobsen *et al*., 1995).

Considering the ability of PBZ in promoting root growth and improving plant nutrition, and the root die back problem among old coffee trees aged 20 years and above, the potential of PBZ in improving the vigor and yield of rejuvenated coffee trees is worth investigating. The present study aimed to evaluate effect of PBZ treatment on the carbohydrate and macronutrient contents of the roots and on the fine root development of rejuvenated coffee trees.

Materials and Methods

The Experimental Trees

Thirty year old non-productive coffee trees planted at the Coffee Project of the Department of Horticulture, College of Agriculture and Food Science, Visayas State University, Visca, Baybay City, Leyte, were used. The trees were planted at 3 m x 3 m using triangular planting system and were under 30-35% shade provided by Thailand acacia (*Acacia spectabilis* A. Cunn. ex Benth) trees. The trees had an average height of 6.60 meters, base girth circumference of 65.52 cm and canopy span of 4.20 meters. The trees had multiple vertical stems due to non-removal of water sprouts. The lateral branches found mostly at upper part of the verticals were overcrowded, had already overlapped with lateral branches of the neighboring trees, and had only very few fruits mostly located near the tip of the lateral branches. Trees with more or less the same stand (height and spread) were selected as experimental samples.

Experimental Design and Treatments

The experiment was laid out in factorial randomized complete block design (RCBD) with three replications, each replicate with 3 sample trees. The treatments were as follows:

Factor A (Time of PBZ application)
- T_1 - 1 month before cutting
- T_2 - 2 months before cutting

Factor B (Level of PBZ application)
- L_1 - 0 (tap water, control)
- L_2 –0.5g.a.i.PBZ/ meter span of canopy or 2 ml Cultar/meter canopy span
- L_3 - 1.0g.a.i.PBZ/meter span of canopy or 4 ml Cultar/ meter canopy span

For L_2 and L_3 trees, a total of 8 and 16 ml Cultar per tree, respectively, was applied because their average canopy span was 4 meters.

Paclobutrazol Application

Commercial grade PBZ with 25% active ingredient (a.i.) (Trade name 'Cultar 25 SC', Syngenta UK Ltd.) was used. The required amount of Cultar was dissolved in one gallon water and was applied following the collar drench method (Figure 1). Furrows were made around the tree 30 cm away from the base and the PBZ solution was applied evenly along the furrows. For the untreated control, one gallon plain tap water was applied per plant.

Figure 1. PBZ formulation and application by collar drenching technique

Rejuvenation pruning

The procedures in heading back or rejuvenation pruning recommended by Cabangbang (1990) was followed. The main vertical stem was cut following a slanting cut 50 cm from the ground using sharp pruning saw. The cut surface was allowed to dry for 5 days and then was painted with coal tar (Figure 2).

Maintenance of the Experimental Trees

The rejuvenated plants were applied with 250 gram complete fertilizer (14-14-14) per plant one month after pruning following the holing method (six 5-cm deep holes around the stump at a distance of 50 cm).The base of experimental plants were ring weeded and were mulched with 5 cm thick rice hull. The areas in between rows were regularly underbrushed at three months interval. When the sprouts were about 10 cm tall, preliminary sprout selection was done by removing the weak sprouts and retaining the 3 most vigorous sprouts per stump. When the retained sprouts were about 30 cm tall, the 2 weakest sprouts were removed retaining the most vigorous sprout per stump. Three shoots (1 per stump) were allowed to grow per tree for 10 months (Figure 3). Subsequent shoots that emerged were regularly removed.

Figure 2. The rejuvenation pruning technique followed in rejuvenating old coffee trees.

Figure 3. The selection of sprouts to establish plants with 3 shoots (1 shoot per stump)

Measurement of Parameters

The fine root (1-3mm diameter) development was evaluated using the in-growth core technique (Person, 1983). Two opposite pits (6 cm diameter and 10 cm depth) were dug using improvised core sampler 50 cm from the base of the stump. The soil taken from the pits were placed in plastic bags and were brought to the laboratory for processing. The soils were freed of all foreign matters and were put back to the individual container. Fine net formed into receptacle was placed into each pit and then the soils (free of roots) were returned back to the pits where these were originally taken. To prevent possible contamination of the coffee roots with roots from other plants, the base of the coffee plant (including the pit) was mulched with 5 cm thick rice hull. After 10 months from cutting, the net containing the soil inside each pit was taken out and brought to the laboratory for the determination of root volume and dry weight.

Root composed of small and fine roots (1-3 mm diameter) were obtained from the base of sample trees, placed in properly labeled bag and were brought to the Crop Physiology Laboratory of the Department of Horticulture, washed to completely remove adhering soil and their volume was determined through displacement method. The roots were placed in properly labeled paper bags and were oven dried at 60-65°C for 5 days using forced draft oven, allowed to cool off and then were weighed to get the dry weight. The dried samples were ground using Willey mill, placed in labeled paper bags and submitted to Central Analytical Laboratory of the Philippine Rootcrop Research and Training Center for starch, sugar, N, P, K, Mg and Ca analyses.

Statistical Analysis

Data were analyzed by performing analysis of variance (ANOVA) and treatment means were compared by Least Significant Difference (LSD) test at 5% level of significance using the STAR, version 2.0.1 2014 Biometrics and Breeding Informatics, PBGB Division International Rice Research Institute, Los Banos, Laguna.

Results and Discussion

Effect on Root Carbohydrate Contents

The timing of application of PBZ did not significantly influenced the root starch and sugar contents. Roots of coffee plants applied with PBZ prior to cutting had only slightly higher starch and sugar contents relative to roots of plants applied with PBZ 2 months prior to cutting.

Table 1. Effect of timing and level of PBZ application on the root starch and sugar contents

Treatments	Starch (%)	Sugar (%)
Time of PBZ Application		
1 month before cutting	10.65	4.26
2 months before cutting	8.98	4.00
PBZ Concentration (ppm)		
0 (tap water, control)	8.04b	4.11
0.5 ga.i. PBZ m^{-1} canopy span	8.99b	4.41
1.0 g a.i. PBZ m^{-1} canopy span	12.41a	3.50
CV (%)	14.06	18.31

Mean separation within columns by LSD, 5%.

Regardless of the timing of PBZ application, PBZ concentration significantly affected root starch content but not the sugar content. Roots of plants applied with 1.0 g a.i.PBZ m^{-1} canopy span had significantly higher starch content than those applied with 0.5 g a.i.PBZ m^{-1} canopy span and the non-PBZ treated control. Such high root starch content among plants applied with 1.0 g ai PBZ m^{-1} canopy span can be partly attributed to the improved allocation of photo assimilates to the roots as a result of reduced top growth (Ramos and Acedo, 2013). Carbohydrate contents of various plant tissues can be increased by PBZ application (Wood, 1984; Wang *et al*.,1986;Yeshitela, 2004; Hua *et al*.,2014). Steffens *et al*. (1985) reported a 132% increase in total carbohydrates in fibrous roots of apple seedlings. Wang *et al*. (1985) attributed the increased in total non-structural carbohydrates among PBZ-treated apple seedlings to enhanced partitioning of assimilates to the stem, leaves and roots. The higher starch levels in PBZ- treated apple (Steffens et al, 1985) and bean (Upadhyaya *et al*., 1986), were attributed to reduced alpha-amylase activity caused by lowering of GA levels. GA stimulates the activity of alpha- amylase (Jacobsen *et al*., 1995).

Effect on Root Macro Nutrient Contents

PBZ significantly increased root P content but had no effect on total N, K, Mg and Ca contents (Table 2).

Table 2. Effect of timing and level of PBZ application on the root nutrient contents of rejuvenated coffee plants

Treatments	Total N (%)	Total P (mg/kg)	Total K (g/kg)	Total Cal (g/kg)	Total Mg (g/kg)
Time of PBZ Application					
1 month before cutting	1.57	221.18b	6.67	4.48	1.53
2 months before cutting	1.36	237.12a	7.01	5.42	1.99
PBZ Concentration					
0 (water, control)	1.16	225.61	6.10	5.31	1.78
0.5 ga.i. PBZ/m canopy span	1.56	232.98	7.06	4.75	1.78
1.0 g a.i. PBZ/m canopy span	1.53	229.37	7.36	5.34	1.79
CV (%)	25.73	6.65	27.69	25.23	25.52

Mean separation within columns by LSD, 5%.

Roots of plants applied with PBZ 2 months before cutting had higher total P than that of plants applied with PBZ 1 month before cutting. Such observed variations in P concentration could be partly attributed to the production of more fine roots (Table 3 and Figure 4) that could have improved P absorption particularly that the soil in the area was acidic (pH 4.5) and where P is less available. Surprisingly, this was not accompanied by increased absorption of other macronutrients which needs to be re-examined in follow-up studies.

PBZ application from 0.5 -1.0g a.i. per meter canopy span did not alter the root macronutrient contents relative to the control. Previous studies by Sadeghi-shoae *et al*. (2014) showed that the application of 150 and 300 ppm PBZ reduced root N and K contents of sugar beets. Swietlik and Miller (1983) reported that application of 0.8 and 1.6 kg a.i. PBZ ha^{-1} significantly increased root N but did not affect root K content of apple seedling.

Effect on Fine Root Production

The timing PBZ application had no significant effect on the root volume and dry weight (Table 3).

Table 3.Fine root production of rejuvenated coffee trees as influenced by the timing and levels of Paclobutrazol application 10 months after cutting

Treatments	Root Dry Weight (mg/283 cm^3 soil)	Root volume (ml/283 cm^3 soil))
Time of PBZ Application		
1 month before cutting	1.89	1.16
2 months before cutting	2.44	1.40
PBZ Concentration		
0 (tap water, control)	1.94	0.90b
0.5g.a.i. PBZ/m canopy span	2.43	1.51a
1.0g.a.i. PBZ/m canopy span	2.12	1.44a
CV (%)	33.8	17.38

Mean separation within columns by LSD, 5%.

However, the level of PBZ application had significant influence on the root volume. Plants applied with 0.5 g.a.i. and 1.0 g a.i. PBZ/m canopy span had bigger root volumes than the non-PBZ-treated control. Volume of roots produced by the plants treated with two levels of PBZ were just comparable. The bigger root volumes among the PBZ-treated plants was attributed to their production of more fines roots (Figure 4).

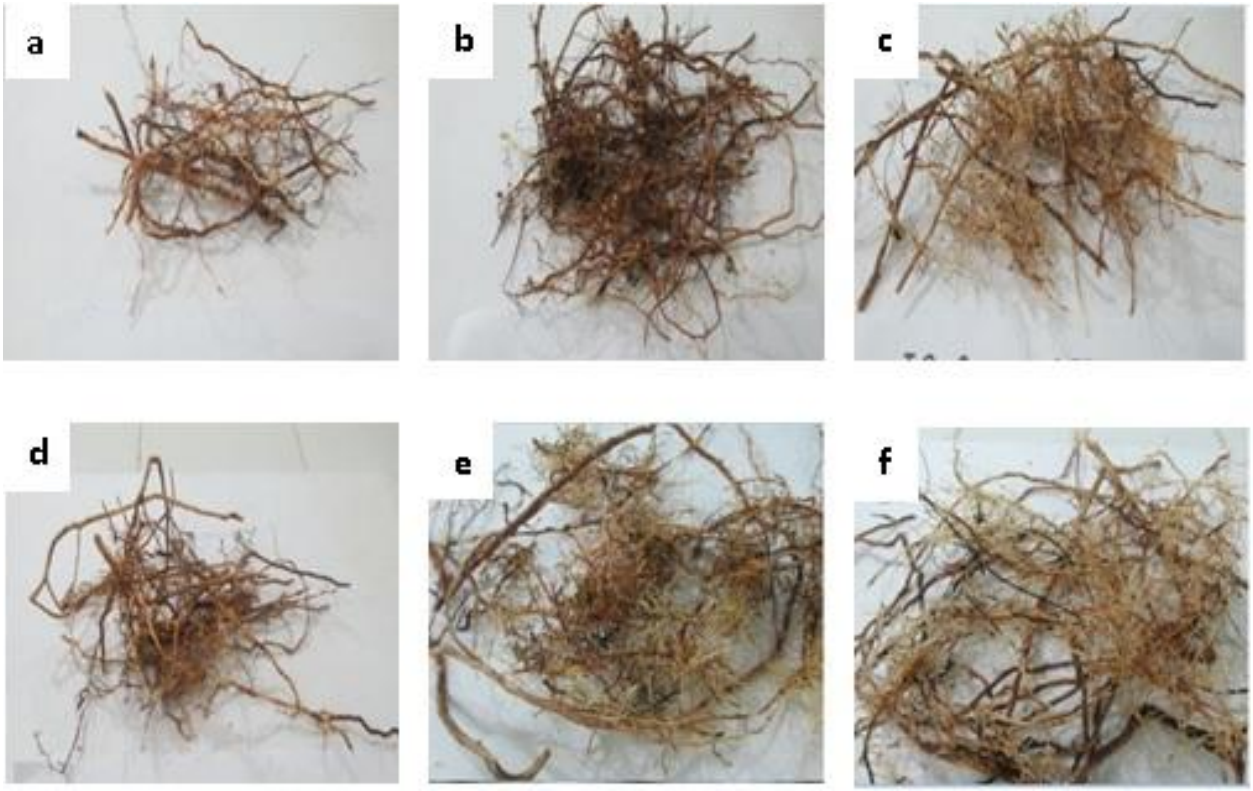

Figure 4. Fine root production of rejuvenated coffee trees applied with 0 g.a.i.PBZ (a), 0.5 g.a.i.PBZ (b) and 1.0 g a.i. PBZ (c) per meter canopy span 1 month before cutting and trees applied with 0 ga.i.PBZ (d), 0.5 ga.i.PBZ (e) and 1.0 g a.i. PBZ (f) per meter canopy span 2 months before cutting.

Chaney (2005) reported that in almost all cases the response in PBZ-treated trees was an increase in root to shoot ratio. Watson (1996) reported that soil injection of PBZ around the base of white and pink oaks caused fine root densities to be 60-80% higher near the trunk base than the non-PBZ treated trees. In rice seedlings, Yim et al. (1997) reported that PBZ treatment increased root dry mass and greater ability to produce new roots. Davis et al. (1985) also reported promotion of adventitious root formation by PBZ in bean hypocotyls cuttings. Symons et al. (1990) attributed the improved rooting/root formation to increased assimilate partitioning to the roots due to reduced demand in the shoots. In white and pink oak, Watson (1996) attributed the increase in root densities brought by PBZ application as either a direct effect of PBZ on root growth or just an indirect effect resulting from shoot growth modification and a shift in carbohydrate allocation to the roots.

Conclusion

The timing of PBZ application significantly affected root total P contents but did not influenced root total N, K, Ca, Mg, starch, sugar, root weight and volume. Regardless of concentration, application of PBZ 2 months prior to cutting significantly increased root total P contents. The level of PBZ application significantly affected root starch content and root volume but did not caused significant variations on root sugar and macro nutrient contents as well as fine root weight. Treating coffee plants with 1.0 g a.i. PBZ/m canopy span significantly increased root starch but not sugar content. Application of 0.5 g a.i. and 1.0 g a.i. PBZ/m canopy span significantly increased fine root volume of rejuvenated coffee.

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