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Growth effects of water excess on coffee seedlings (*Coffea arabica* L.)

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ABSTRACT. The objective of the present study was to evaluate the interference of water excess in soil on the growth of young coffee plants of the Mundo Novo and Catuaí cultivars.Plants were subjected to the following three different substrate water availability conditions: control (well-watered), continuous substrate waterlogged, and intermittent substrate waterlogged. Several growth-related traits were evaluated over the course of 19 weeks. Based on the number and quality of the affected variables from all forms of analyses, the Catuaí cultivar showed greater sensitivity to waterlogging. Both cultivars exhibited growth inhibition in response to substrate waterlogging stress, which was exacerbated by premature leaf dropping.

Keywords: soil waterlogging, hypoxia, mundo novo cultivar, catuaí cultivar.

Efeitos do excesso de água no crescimento de mudas de cafeeiro (*Coffea arabica* L.)

RESUMO. O presente trabalho visou avaliar a interferência do excesso hídrico do solo sobre o crescimento de plantas jovens de café, cultivares Mundo Novo e Catuaí. As mudas foram submetidas a três condições de disponibilidade de água no substrato: controle (mudas irrigadas), encharcamento contínuo do substrato e encharcamento intermitente do substrato. Diversas características de crescimento foram analisadas ao longo de 19 semanas. Baseado no número e qualidade das variáveis afetadas em todas as análises, a cultivar Catuaí mostrou maior sensibilidade ao encharcamento.. Ambas as cultivares de café apresentaram inibição do crescimento em resposta ao estresse por encharcamento do substrato, que foi agravado pela queda prematura das folhas.

Palavras-chave: encharcamento do solo, hipoxia, cultivar mundo novo, cultivar catuaí.

Introduction

Brazil currently produces large amounts of high quality coffee as a consequence of recent expansions in production. This expansion has been accomplished by using irrigation in regions that were previously considered to be marginal because of their extended periods of water deficiency (LEMOS FILHO et al., 2010). At the moment, the irrigation of coffee is a limiting factor in obtaining high yields, even in areas where it is performed only to supplement rainfall (PIMENTEL et al., 2010). Additionally, the global temperature is expected to increase by 2°C to 4.5°C with an increase of tropical rainfall, according to the latest report from the Intergovernmental Panel on Climate Change (IPCC, 2007). Based on these predictions, the areas that now border climatically ideal coffee cultivation regions such as Brazil will become unfavorable for growing coffee (ASSAD et al., 2004). As a result of the increased rainfall that was predicted by these authors, even the cultivars that are tolerant to an excess of water will approach their water stress limits. Moreover, there has been indiscriminate use of irrigation that contributes to an increase in the deficit of electricity and water, and can cause serious environmental problems such as water table contamination, soil salinization and erosion (OREN et al., 2004).

Brazil has experienced one of its most significant periods of rainfall in recent years(BACK, 2001; DANTAS et al., 2007). Excessive rains affect crop productivity, hamper the control of diseases and weeds, increase the lixiviation of nutrients and elevate the costs of cultivation practices. There are no studies that specifically address the effect of excess water on coffee crop development and production. Most related studies address the use of irrigation in the battery of problems that are related to the water deficit (ARRUDA; GRANDE, 2003; NASCIMENTO et al., 2008; SILVA et al., 2009; OLIVEIRA et al., 2010). These studies generally show a quadratic effect between crop production and applied water depth (AWD), especially during high-yield years. During low productivity years, irrigation treatments had a negative influence on production (SILVA et al., 2008).

Although Silva et al. (2008) do not associate the declines in coffee productivity with applying excess water to the soil, many other articles explain why most crops do not tolerate excess soil water (JACKSON; COLMER, 2005; IRFAN et al., 2010). Overall, one can say that flooding or waterlogging can provoke a condition of hypoxia or anoxia in the soil. In this environment, it is not possible to maintain the aerobic metabolism required to produce sufficient energy for the plant to grow adequately, even with a decline in the adenylate pool (IRFAN et al., 2010). As a result of low oxygen availability, the fermentative pathway is activated to produce acetaldehyde, ethanol and/or lactate, which result from anaerobic respiration and can only provide a minimal quantity of energy (VARTAPETIAN, 2006; ZABALZA et al., 2009). In some cases, reactive oxygen species such as superoxide anions ($O_2^{\cdot-}$), hydrogen peroxide (H_2O_2), hydroxyl radicals ($^{\cdot}OH$) and singlet oxygen ($^1O_2$) are formed, which can lead to plant death when present in combination (MITTLER et al., 2011).

Water excess generally reduces productivity and quality in the field, and it may also cause fruit ripening retardation, soluble nutrientlixiviation (mainly nitrogen and potassium), flower fall, a higher incidence of soil diseases and physiological disturbances, higher energy costs and wear on the irrigation system (FOLEGATTI et al., 2003). Even under nursery conditions, no protocol has been established for seedling irrigation. In general, coffee seedling nurseries irrigate from one to three times a day without attention to the amount of water that is applied. Because there is a high concentration of roots in a small volume of substrate growers often choose to add excess water with the assumption that more water is better.

The extent of soil waterlogging damage depends on several factors, including the duration of saturation, the developmental stage of the plant, the species and the environmental conditions (SILVA et al., 2006). From this list, we can see the importance of controlling irrigation scale through proper water management. For irrigation to be effective, it is essential to have adequate knowledge of the crop's water demand which is in turn regulated by the intrinsic characteristics of the plant, soil and climate of the region (PIMENTEL et al., 2010).

In this context, this study aims to evaluate the effects of excessive soil water on the growth of coffee seedlings from Mundo Novo IAC 379-19 and Red Catuaí IAC 44 cultivars.

Material and methods

Seedlings from Mundo Novo IAC 379-19 and Red Catuaí IAC 44 cultivars in the stage known as *orelha de onça* were provided by Foundation Procafé, Varginha Experimental Farm, and they were transferred to a nursery covered with black shading screens (for 30% shade). After they developed four pairs of fully expanded leaves, they were transplanted to perforated black polyethylene bags with dimensions of 15 x 25 cm (diameter x height) and a volume of 4.4 L. The substrate was made from 700 L of sieved subsoil, 300 L of sieved cattle manure, and 5 kg of simple superphosphate and 0.5 kg of potassium chloride (GUIMARÃES et al., 2002).

The experimental design was made up of randomized blocks, with four total blocks, three different conditions of water availability (FC, IW and CW) and two cultivars (Mundo Novo and Catuaí). Each experimental plot consisted of eight plants, for a total of 96 plants per cultivar.

After the development of eight pairs of fully expanded leaves, the seedlings were subjected to the following three different conditions of water availability in the substrate: (a) seedlings maintained with substrate moisture close to field capacity (FC), which were monitored by the direct method proposed by Souza et al. (2002); (b) continuous waterlogging of the substrate (CW), where the seedlings were placed in buckets with a permanent layer of water covering two-thirds of the height of the polyethylene bag; and (c) intermittent waterlogging of the substrate (IW), where the plants alternately remained for three days under continuous waterlogging and for four days under field capacity throughout the experimental period.

Data collection began after the first treatment and was conducted weekly for 19 weeks. Root system evaluation sampling took place at the beginning of the experiment and at two and five months, and the data were subjected to variance analysis. These evaluation times were chosen in response to the intensity of the symptoms observed in seedlings exposed to CW, with the second time defined as the onset of the yellowing of leaves and reduced seedling growth. The third time occurred at five months, when defoliation and increasingly poor symptoms were observed.

Plant height was measured as the distance between the stem base and the apical bud. Stem diameter was measured with a pachymeter in the stem base region. The total number of leaves and plagiotropic branches were obtained by direct counting. The total leaf area was determined with the formula AF = [(length x width) x 0.667] x the total number of leaves per plant

(BARROS et al., 1973). The dry root and shoot material (stem and leaf) was obtained from samples that were dried in a forced-air oven at 70ºC until they reached a stable weight.

To analyze the root system (in roots smaller than 3 mm in diameter), the plant roots were rinsed and stored in 5% methanol for subsequent collection and image processing. Images were captured using a HP Scanjet G2410 scanner (Regulatory model GRLYB-0209, HP, China) with an optical resolution of 1200 dpi, and with the aid of an acrylic tub. Image processing was performed using the software SAFIRA-an Analysis System for Fibers and Roots (JORGE; RODRIGUES, 2008). Software calibration was performed with an object of known length and diameter.

The dry matter and root system data were subjected to analysis of variance, and the means of the treatments were compared using the Scott-Knott test at a 0.05 probability level. The remaining data were subjected to regression analysis using the statistical program SISVAR 4.3 (System Analysis of Variance for Balanced Data) (FERREIRA, 2011). It is worth mentioning that the selection of regression models was performed with consideration of the following criteria: quality of fit (R^2), significance of the regression(p-value < 0.05) and the frequency goodness of fit test (p-value > 0.05).

Results and discussion

Waterlogged soil inhibited coffee seedling growth, and this inhibition was proportional to waterlogging intensity in that seedlings under CW were more affected than those under either IW or FC. When MundoNovo seedlings were submitted to IW, they showed a linear increase when taking height (Figure 1A), number of plagiotropic branches (Figure 2A), number of leaves (Figure 3A) and stem diameter (Figure 4A) into consideration.

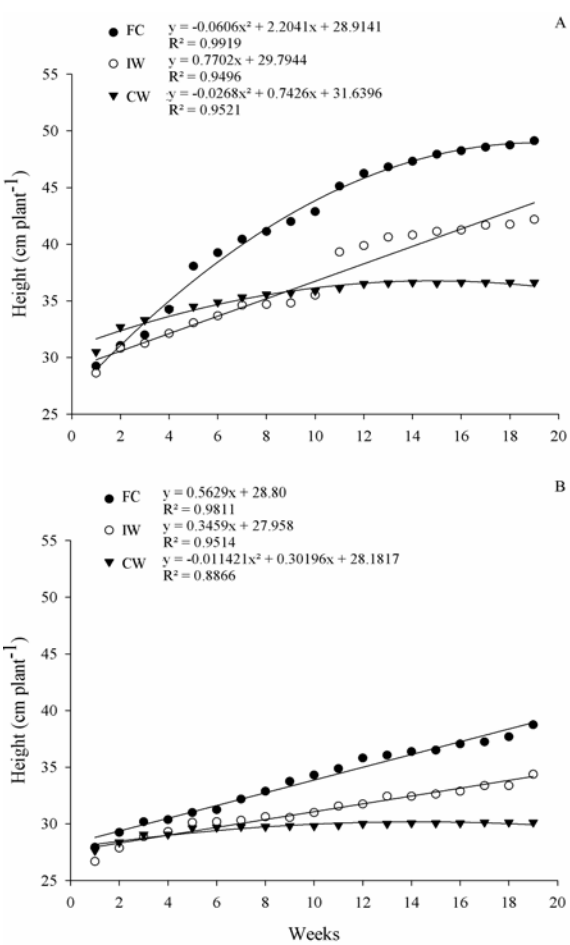

Figure 1. Coffee seedling heights of Mundo Novo (A) and Catuaí (B) that were subjected to the following three conditions of water availability in the substrate for 19 weeks: field capacity (FC), intermittent waterlogging (IW) and continuous waterlogging (CW).

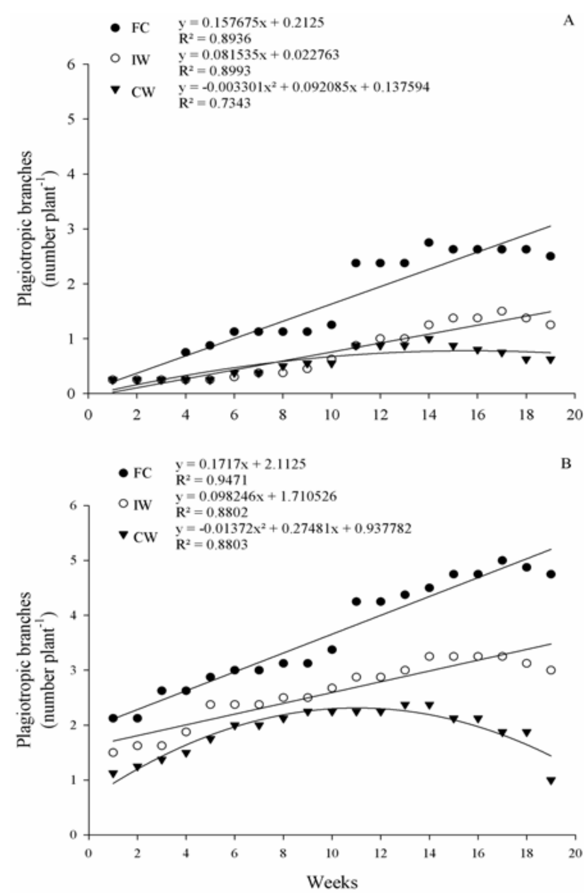

Figure 2. Number of plagiotropic branches on Mundo Novo (A) and Catuaí (B) coffee seedlings subjected to these three conditions of water availability in the substrate: field capacity (FC), intermittent waterlogging (IW) and continuous waterlogging (CW) for 19 weeks.

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Catuaí demonstrated the same behavior, except for the number of leaves that grew until the 16th week, which then began to fall (Figure 3B). For both cultivars, the increase in leaf area (Figure 5) followed a quadratic pattern, with a peak of 892.41 cm^2 for Mundo Novo and 1603.04 cm^2 for Catuaí, which were observed in the 12th and 11th weeks of treatment, respectively. After 19 weeks of evaluation, the growth of Mundo Novo seedlings was measured as a function of their height (Figure 1A), number of plagiotropic branches (Figure 2A), number of leaves (Figure 3A), stem diameter (Figure 4A) and leaf area (Figure 5A) under IW, which were 86, 50, 64, 73 and 54%, respectively, when compared to FC. For Catuaí, these differences were 89, 63, 58, 80 and 38% of FC, respectively.

In analyzing the growth of seedlings under CW during the experimental period, Mundo Novo seedlings showed linear behavior for the leaf numbers (Figure 3A) and stem diameters (Figure 4A). The other traits demonstrated quadratic behavior, with peaks observed for plant height (Figure 1A), number of plagiotropic branches (Figure 2A) and leaf area (Figure 5A) at the 14th, 14th and 7th weeks, respectively. Catuaí showed the same pattern, in that the peaks of maximum growth were seen in the 13th, 10th and 5th weeks, respectively, when considering plant height (Figure 1B), number of plagiotropic branches (Figure 2B) and leaf area (Figure 5B). In the last evaluation, the height increases (Figure 1), number of plagiotropic branches (Figure 2), number of leaves (Figure 3), stem diameter (Figure 4) and leaf area (Figure 5) for Mundo Novo seedlings under CW were 75, 25, 22, 63 and 19%, of their FC values, respectively. For Catuaí, these differences were 78, 21, 21, 68 and 24%, respectively.

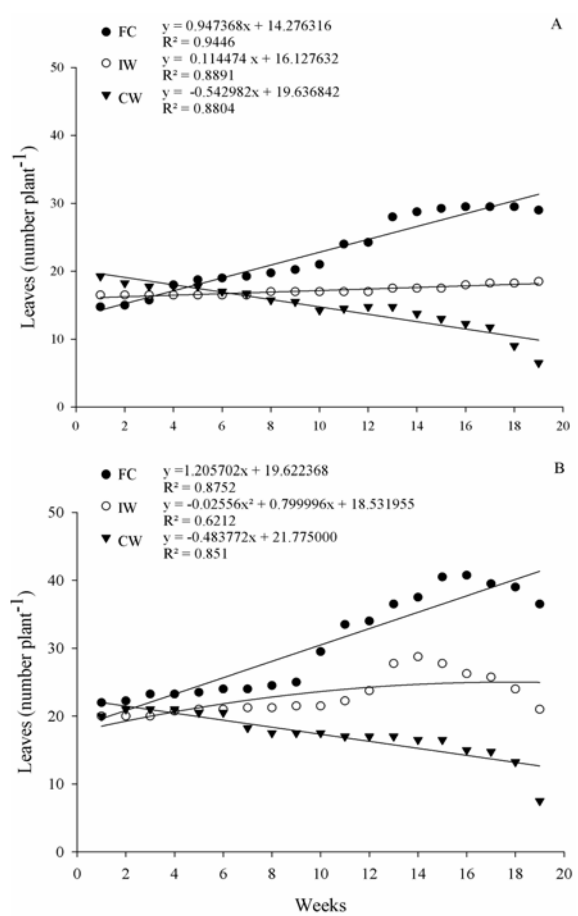

Figure 3. Number of leaves on Mundo Novo (A) and Catuaí (B) coffee seedlings subjected to these three conditions of water availability in the substrate: field capacity (FC), intermittent waterlogging (IW)

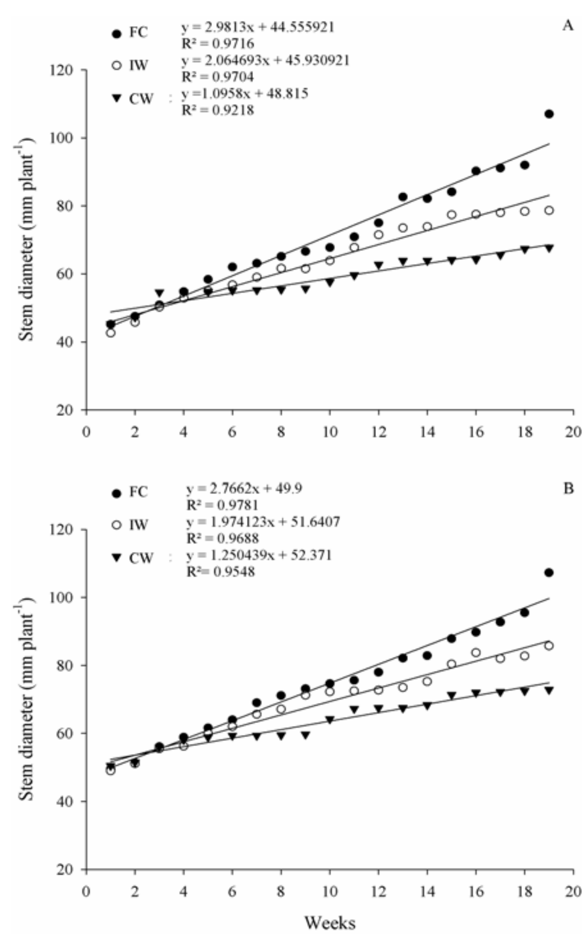

Figure 4. Stem diameter of Mundo Novo (A) and Catuaí (B) coffee seedlings subjected to these three conditions of water availability in the substrate: field capacity (FC), intermittent waterlogging (IW) and

and continuous waterlogging (CW) for 19 weeks.

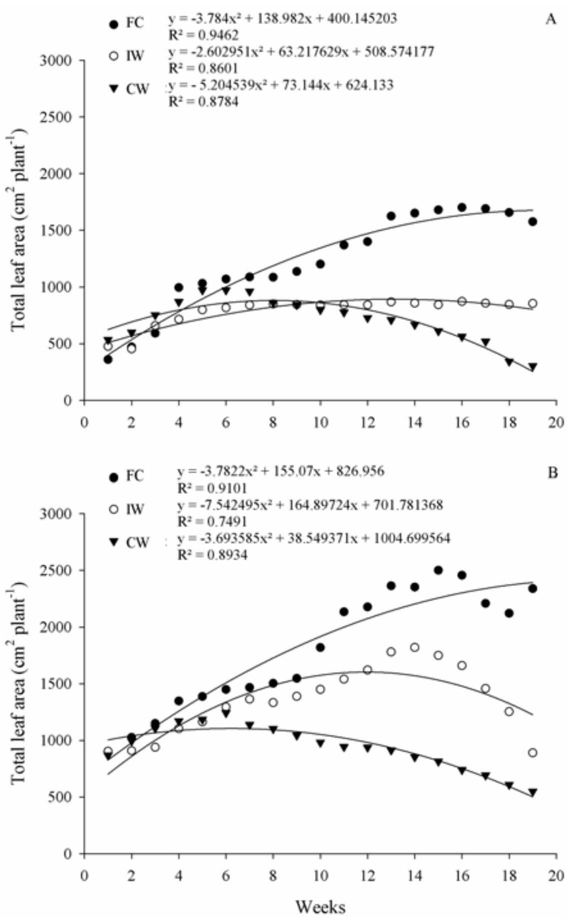

Figure 5. Total leaf area of Mundo Novo (A) and Catuaí (B) coffee seedlings subjected to these three conditions of water availability in the substrate: field capacity (FC), intermittent waterlogging (IW) and continuous waterlogging (CW) for 19 weeks.

Analysis of the dry leaves, stems and roots from both cultivars revealed that until the second month, there were no significant differences between the treatments (Figure 6). In the fifth month, soil waterlogging inhibited the growth of dry matter in the three organs that were analyzed, and this inhibition was proportional to the stress intensity. During this period, the growth inhibition for leaves, stems and roots of Mundo Novo under IW was 41, 22 and 65% of the FC value, respectively; under CW, the inhibition was 94, 71 and 79%, respectively. For Catuaí, the inhibition imposed by IW treatment, when compared to the FC, was 46, 28 and 66%, respectively. Under CW, the inhibition was 91, 58 and 81%, respectively.

For both cultivars, roots smaller than 3.0 mm in diameter were statisticallydifferent between treatments during the second month (Figure 7). On this occasion, the area of both cultivars under IW and CW decreased on average 39 and 61%, respectively in relation to the FC. At five months, the mean reduction was 49 and 70%, respectively. The difference between the areas of both cultivars that had experienced soil waterlogging in the second month was 37% on average, which was a favorable result for IW. At five months, the difference was already 30% for Mundo Novo and 51% for Catuaí.

continuous waterlogging (CW) for 19 weeks.

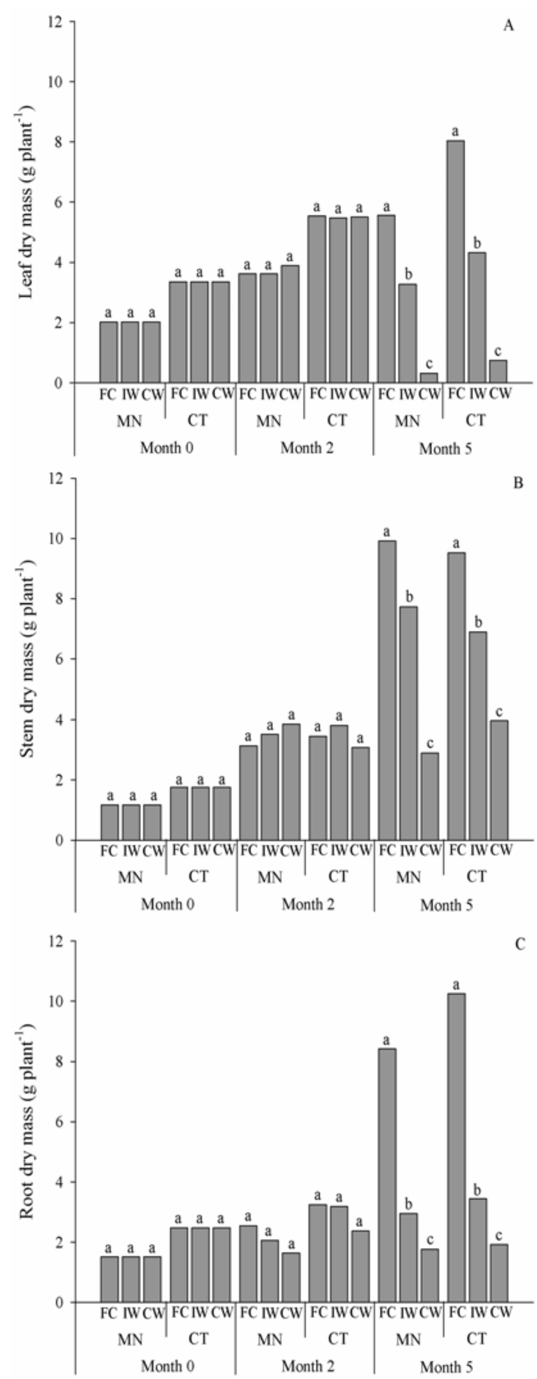

Figure 6. Leaf (A), stem (B) and root (C) dry mass of Mundo Novo (MN) and Catuaí (CT) cultivars were subjected to the following three water availability conditions in the substrate:

field capacity (FC), intermittent waterlogging (IW) and continuous waterlogging (CW). The letters compare the means between watering regimes during each stage for each cultivar, based on the Scott-Knott test ($p \leq 0.05$).

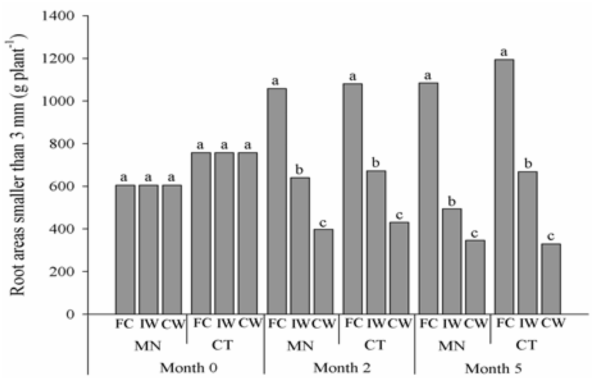

Figure 7. Roots with areas of less than 3 mm for both the Mundo Novo (MN) and Catuaí (CT) coffee cultivars were subjected to these three conditions of water availability in the substrate: field capacity (FC), intermittent waterlogging (IW) and continuous waterlogging (CW). Lowercase letters compare the means between different watering regimes during each stage for each cultivar, based on the Scott-Knott test ($p \leq 0.05$).

After the end of the stress period, the seedlings of both varieties were left at FC, and after 30 days all of them had recovered their growth and were able to produce new release leaves (data not shown). Of the five growth characteristics that were studied in the shoots of the Mundo Novo cultivar under IW, four (plant height, number of plagiotropic branches, leaf number and stem diameter) showed a positive linear growth pattern; the increase in leaf area followed a quadratic pattern, with a peak at the 12th week. In the Catuaí under IW, there were three growth characteristics with a positive linear growth pattern (plant height, number of plagiotropic branches and stem diameter) while leaf area increased until the 11th week and leaf numbers increased until the 16th week, at which time the leaves began to fall.

When Mundo Novo and Catuaí seedlings were subjected to CW, the stem diameter was the only characteristic with linear growth. For the Mundo Novo cultivar, the plant height, number of plagiotropic branches and leaf area increased until the 14th, 14th and 7th days, respectively, and thereafter decreased. For Catuaí, the peaks occurred at the 13th, 10th and 5th days, respectively. The only characteristic with a negative linear pattern was the number of leaves that decreased in relation to the duration of CW in both varieties.

Among all the traits studied in seedlings that were subjected to IW, the number of leaves for Catuaí and the leaf area in both varieties showed the greatest sensitivity to stress because at any given time they suffered inhibition. Under CW, the height, number of plagiotropic branches, number of leaves and leaf area followed a quadratic growth pattern and were therefore not able to maintain a positive growth pattern. In this case, Catuaí was prematurely affected after a period of continual stress.

These results show that seedlings were able to grow reasonably well for a period of 19 weeks under IW even though the growth occurred at lower rates than those of the control. This alternation between periods of hypoxia, waterlogging, and normoxia relieves the pressure of oxygen deficiency stress by returning the soil to field capacity, which causes aerobic respiration to return, which in turn restores the energy level that is necessary for plant growth.

Structured and well drained soils allow oxygen diffusion down to the deeper layers (YOUNG; CRAWFORD, 2004). Under this condition of normoxia, which was created for this study by suspending irrigation for three days after a similar period of waterlogging (IW), the roots had sufficient oxygen to maintain aerobic respiration (VOESENEK et al., 2006). During the periods of excess watering, oxygen diffusion in the soil becomes too low, creating a hypoxic environment (oxygen deficiency). When the root system experiences hypoxia, it redirects its respiration to become anaerobic and consequently has a drop of up to 18 times the production of adenosine triphosphate (ATP) as aerobic respiration, ATP being essential for plant growth and development (LIAO; LIN, 1995). To suppress the roots' energy use during relatively short periods of oxygen deficiency, tolerant species increase the rate of reserve polysaccharide mobilization and sugar translocation from the shoot to the root system, followed by an increase in the glycolytic rate of these tissues as a response to the large amount of sugars (KUMUTHA et al., 2008). This strategy ensures a low level of energy followed by limited growth, as observed when the seedlings were subjected to IW.

With stress that is represented here by continual waterlogging (CW), these cultivars suffered a variety of effects, depending on the characteristic under study, including inhibited growth that was exacerbated by the premature fall of leaves. Roots act as an important sensor of many environmental conditions such as a lack of water, soil compaction or flooding. One means by which roots are able to signal to the aerial parts of the plant is through ethylene-mediated changes in root growth and/or in the synthesis of 1-aminocyclopropane-1-carboxylate (ACC) that is then transported throughout the plant (IRFAN et al., 2010). Leaf abscission is a common response of avocado trees to root hypoxia, and it is presumably stimulated by increased ethylene concentration (GIL et al., 2009).It is possible that the smaller leaf area of these plants compromised the production of carbohydrates and thus the recovery of energy, even for the seedlings under IW conditions.

Data analysis reveals that under IW all the traits studied for Mundo Novo seedlings followed a linear pattern throughout the experimental period and that for Catuaí, the growth in leaf number and leaf area were negatively impacted beginning in the 16th and 11th weeks, respectively. When the waterlogging was continuous (CW), the peak for Mundo Novo seedling growth took place later than for Catuaí for all the variables that followed the quadratic pattern. This finding means that the Catuaí cultivar felt the harmful effects of waterlogging a few days before the Mundo Novo.

Based on the peak points that were determined for the shoot characteristics, Mundo Novo and Catuaí seedlings tolerate intermittent soil waterlogging for 12 and 11 weeks, respectively. Under CW, the tolerance period falls to seven weeks for Mundo Novo seedlings and to five weeks for Catuaí. However, the analysis of dry leaf, stem and root mass showed that, by the end of the second month of stress, the seedlings of both coffee cultivars had a high tolerance to soil waterlogging, as there were no significant differences when compared to the control. When comparing the variation in shoot and root dry mass from control seedlings with those that were under stress for five months, one realizes that the roots, followed by the leaves, were most affected. The sensitivity of these organs to waterlogging was a reflection of the decrease in the area of absorbent roots that had occurred three months before, i.e., after two months of waterlogging.

For these reasons, even though the Mundo Novo seedlings were believed to be more tolerant than Catuaí to excess soil watering, when we analyzed those growth variables with methods that were non-destructive to the shoots of both varieties after 19 weeks of stress, we did not observe any increase in dry matter at a proportion equal to that of seedlings under FC, whether they were exposed to an intermittent or continuous excess. This sensitivity was due to the death of fibrous roots after two months of stress. At any rate, the coffee seedlings showed a good ability to tolerate waterlogged soil, as both varieties recovered after a period of time when the soil was at field capacity.

Conclusion

The growth and development of Mundo Novo and Catuaí seedlings were affected by substrate waterlogging after 19 weeks of stress. Under these conditions they were unable to increase their dry mass to the same proportion of those that were at field capacity. In general, all growth characteristics exhibited losses in proportion to the intensity of stress, i.e., seedlings under continuous waterlogging were more affected than those under intermittent waterlogging. With the stress of continuous waterlogging, these cultivars exhibitedgrowth inhibition, which was exacerbated by the premature dropping of leaves along with a reduction in absorbent root area.

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