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ORIGINAL ARTICLE

Efficiency of cover materials in preventing evaporation in drought-stressed soybeans grown in pots

Eficiência de materiais de cobertura na prevenção da evaporação em vasos cultivados com soja submetida à seca

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ABSTRACT: There are few studies in the specific literature on the use of cover materials to control evaporation in drought-stressed plants grown in pots under greenhouse conditions. The use of these materials is of great importance to ensure that water loss occurs only through transpiration. Thus this study aimed to investigate the efficiency of different cover materials – polyethylene, marble, polystyrene, and polyvinyl chloride (PVC) – in controlling evaporation in black and in silver pots cultivated with the soybean cultivar BR 16 and subjected to drought under greenhouse conditions. The plants were kept at 100% field capacity until they reached the V_3 stage. The different cover materials were then applied to the substrate surface, irrigation was suspended, and the plants were evaluated with regard to water loss and temperature of leaf and substrate for nine consecutive days. The experiment was repeated without plants to assess evaporation. Substrate water potential was measured on the last day in both experiments. Although all the cover materials showed uniformity between the replicates with respect to water loss, polyethylene and PVC presented higher substrate water potential and leaf turgor after nine days of suspended irrigation; however, PVC led to soil compactions, restricting its use. Therefore, among the materials tested, polyethylene is the most suitable to control evaporation in pots cultivated with soybean plants subjected to drought, with no influence of pot color.

RESUMO: *Na literatura, existem poucos relatos sobre o uso de materiais de cobertura para controle da evaporação em vasos durante estudos de plantas submetidas à seca, sob condições de casa de vegetação. O uso destes materiais tem grande importância para assegurar que a perda de água ocorra somente pela transpiração. Assim, o presente estudo objetivou investigar a eficiência de diferentes materiais de cobertura – polietileno, mármore, isopor e policloreto de vinila (PVC) – para o controle da evaporação em vasos pretos e prateados, cultivados com a cultivar de soja BR 16 submetida à seca sob condições de casa de vegetação. As plantas foram mantidas a 100% da capacidade de campo até o estádio V3. Em seguida, os diferentes materiais foram aplicados à superfície do substrato, a irrigação foi suspensa e as plantas foram avaliadas quanto à perda de água e às temperaturas foliar e do substrato, durante nove dias consecutivos. O experimento foi repetido sem as plantas para avaliação da evaporação e, em ambos os experimentos, o potencial hídrico do substrato foi medido no último dia. Embora todos os materiais tenham mostrado uniformidade entre as repetições em termos de perda de água, o polietileno e o PVC levaram ao maior potencial hídrico do substrato e à maior turgescência foliar após nove dias de irrigação suspensa; entretanto, o PVC levou à compactação do solo, o que restringe seu uso. Verificou-se que, dentre os materiais testados, o polietileno é o mais adequado para controlar a evaporação em vasos cultivados com plantas de soja submetidas à seca, sem influência da cor do vaso.*

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1 Introduction

The rate of water uptake in root systems is influenced by factors such as temperature, presence of O_2 and CO_2, and soil moisture (Costa, 2001). In the soil, the evaporation rate changes according to texture, density, thermal profile, evaporative demand, and depth (Allen et al., 2005). These aspects are relevant because soil water availability has been considered the most influential climatic factor on crop productivity (Fioreze et al., 2011).

In this sense, drought stress usually leads to stomatal closing and a consequent increase in leaf temperature (Sdoodee & Kaewkong, 2006). Under drought conditions, leaf temperature is higher than air temperature, which results in an increased leaf/air temperature ratio (Mendes et al., 2007). Therefore, leaf temperature can be used to monitor the water status of plants (Jimenez-Bello et al., 2011).

Previously published studies have reported the effect of mulch on soil evaporation reduction and water storage (Gregory et al., 2000; Ji & Unger, 2001; Monzon et al., 2006), but there are few reports on the use of cover materials to prevent water loss through evaporation in substrates used to fill pots for the study of drought-stressed plants under greenhouse conditions. The few studies available report the use of different materials such as polystyrene spheres (Saint Pierre et al., 2012) and polyethylene bags (Bhatnagar-Mathur et al., 2007; Rolla et al., 2013), but these reports have not tested or compared the effectiveness of these materials. Furthermore, studies on the evaluation of drought effect under greenhouse conditions have generally described pot volume, but information about pot material or pot external color is rare.

All these aspects are of great importance for plants grown in pots, especially under well-watered conditions (controls), to prevent evaporation, thus ensuring constant substrate moisture, or in plants grown in pots subjected to water stress, to ensure that water loss occurs only through transpiration. It should be emphasized that the cover material must not present great variation between replicates with respect to water loss through evaporation to ensure an accurate evaluation of different traits, including physiological, molecular and biochemical properties. Another fact to be considered is the importance of calculating the transpiration efficiency (*TE*)/water-use efficiency (*WUE*) in studies involving water deficit. The *TE* is an important component of *WUE* and a major source of variation under water deficit in many crops (Passioura, 2012). *TE* is related to *WUE* through the formula *WUE* (biomass) = *TE*/(1+*Es*/*T*) proposed by Richards (1991), where *Es* is the water lost through evaporation from the soil surface and *T* is the water lost through transpiration. When pots are covered to prevent evaporation from the soil surface, *Es* is null and *TE* = *WUE* (Passioura, 2012).

Thus the current study aimed to investigate the efficiency of different cover materials – polyethylene bags, marble pebbles, polystyrene foam cubes, and polyvinyl chloride (PVC) discs – to prevent water loss through evaporation in the substrate used to fill polyethylene pots cultivated with the Brazilian soybean (*Glycine max* L. Merrill) cultivar BR 16 subjected to drought under greenhouse conditions. This cultivar is drought-sensitive (Oya et al., 2004; Stolf et al., 2009). The effect of the external color of pots – black or silver – was also investigated with respect to water loss through evaporation.

2 Materials and Methods

The experiments were conducted under greenhouse conditions. Soybean cultivar BR 16 seeds were previously treated with carboxin + thiram (200 g L^{-1}) and phypronyl (250 g L^{-1}) and then allowed to germinate for 96 h at 25 °C on Germitest® paper moistened with water (2.5-fold volume relative to the weight of the dry paper) (Brasil, 2009). Thereafter, the seedlings were inoculated with *Bradyrhyzobium japonicum* (Nitro-Super F-45 Premium®) and planted in 1 L pots (dimensions: 15 cm external diameter × 10 cm base × 11 cm height) filled with substrate (soil/sand/organic compounds, 3:2:2), so that each pot contained one seedling. The greenhouse temperature was set at 28 ± 1 °C, with cooling system actuation when temperature reached 29 °C, and heating system actuation when temperature reached values below 16 °C. The internal temperature and relative humidity (RH) were recorded in a thermohygrograph (Hobo U14-002, Onset®) every 5 min. The vapor pressure deficit (*VPD*) was obtained from the Equation 1:

$$VPD = e_s - e_a \tag{1}$$

The saturated vapor pressure (e_s) was calculated using the psychrometric chart available at http://physics.holsoft.nl/physics/ocmain.htm. The partial vapor pressure (e_a) was obtained according to Rodrigues et al. (2011) from the Equation 2:

$$e_a = \frac{\mathrm{RH}\, x\, e_s}{100} \tag{2}$$

The experimental design was completely randomized with a 2 × 5 (pot color × cover material) factorial arrangement and ten replicates, totaling 100 plots. The external pot color corresponded to black (original color) or silver (painted pots). Both colors were used to test the possibility of decreased substrate warming in silver pots. For the cover materials, polyethylene bags, marble pebbles, PVC discs, and polystyrene foam cubes were tested, in addition to the controls (uncovered pots). Polyethylene bags were adhered to the pots using adhesive tape and string to prevent contact with the stem. The marble pebbles (5.0 mm diameter, 14.0 mg mm^{-3} density, 186.2 g/pot) were washed beforehand under tap water and then dried in ambient air. The amount of polystyrene foam cubes (1.5 cm edges, 44.0 mg cm^{-3} density) was equal to 1.4 g/pot. The PVC discs corresponded to white semicircles (14.0 cm external diameter × 5.0 cm internal diameter) joined by silver tape. In all pots, each stem was wrapped with a sponge disc (5 cm diameter, 1.2 cm thickness, 3.08×10^{-5} g mm^{-3} density) at the substrate surface to prevent evaporation through the central region.

The substrate was kept at 100% field capacity until the plants reached the V_3 developmental stage. The substrate surface was then scarified, watered for the last time, and covered with the respective test materials. From this moment (day 0), irrigation was suspended. The substrate and leaf temperatures were measured for nine consecutive days. In addition, the pots were weighed daily to obtain the water loss (g/pot d^{-1}) value.

All evaluations were always performed at the same time of day (9:00 a.m.). The pots were randomly shuffled twice a week during the period of continuous irrigation (until V_3 stage), and daily during the period of suspended irrigation.

Substrate temperature was obtained by means of a digital thermometer (TD-800D model 9610, Icel-Gubintec) inserted to a depth of 6.0 cm. Leaf temperature was measured in the central leaflet of the third fully expanded trifoliate, using a thermal infrared sensor (InfraPro®, Oakton®). Plants were considered under stress when the leaf temperature was higher than the air temperature, whereas the opposite corresponded to unstressed conditions. The pots were weighed over ten consecutive days, always at the same time of day (9:00 a.m.) in a BEL Engineering SRL® (model ALBIL001) scale, with 0.1g readability. Water loss (g/pot d^{-1}) was calculated by the difference in pot weight between the consecutive days for each pot. On the last day of evaluation (day 9), in each pot, after plant removal, the substrate was homogenized on a tray, and then an aliquot was collected and placed into aluminum pots, which were immediately capped, sealed with tape, and taken to the lab for water potential measurement using a dew-point potentiometer (model WP4C, Decagon®). Subsequently, the experiment was repeated under the same conditions, but without the presence of the plants, in order to obtain only the water loss of the substrate, that is, the evaporation measured through the weighing of pots for nine consecutive days of suspended irrigation. The substrate water potential was also measured on the last day of evaluation (day 9) using the same procedure previously described. Respectively, in each experiment, the total transpiration and total evaporation were calculated by the sum of water mass lost after nine days of suspended irrigation.

As the data for total water loss, substrate water potential, and total evaporation presented normal distribution according to the Shapiro–Wilk test, they were subjected to statistical analysis using analysis of variance (ANOVA), the F-test, and their means were compared by the Tukey's test ($p \leq 0.05$) using the SISVAR software, version 5.3 (Ferreira, 2010). However, normal distribution was not observed for the substrate water potential data evaluated from the second experiment (evaporation measurements), according to the Shapiro–Wilk test. Therefore, they were subjected to the nonparametric Kruskal-Wallis test and the means were compared by the Dunn's test ($p \leq 0.05$) using the BioEstat software, version 5.0 (Ayres et al., 2007).

3 Results

There was no influence of pot color (Figure 1) with respect to water loss. Thus plants grown in either black (Figure 1A) or silver (Figure 1B) pots presented higher water loss in the first days (days 0-1, 1-2) in controls (uncovered pots), followed by the plants grown in pots covered with polystyrene foam cubes and marble pebbles, and by those grown in pots covered

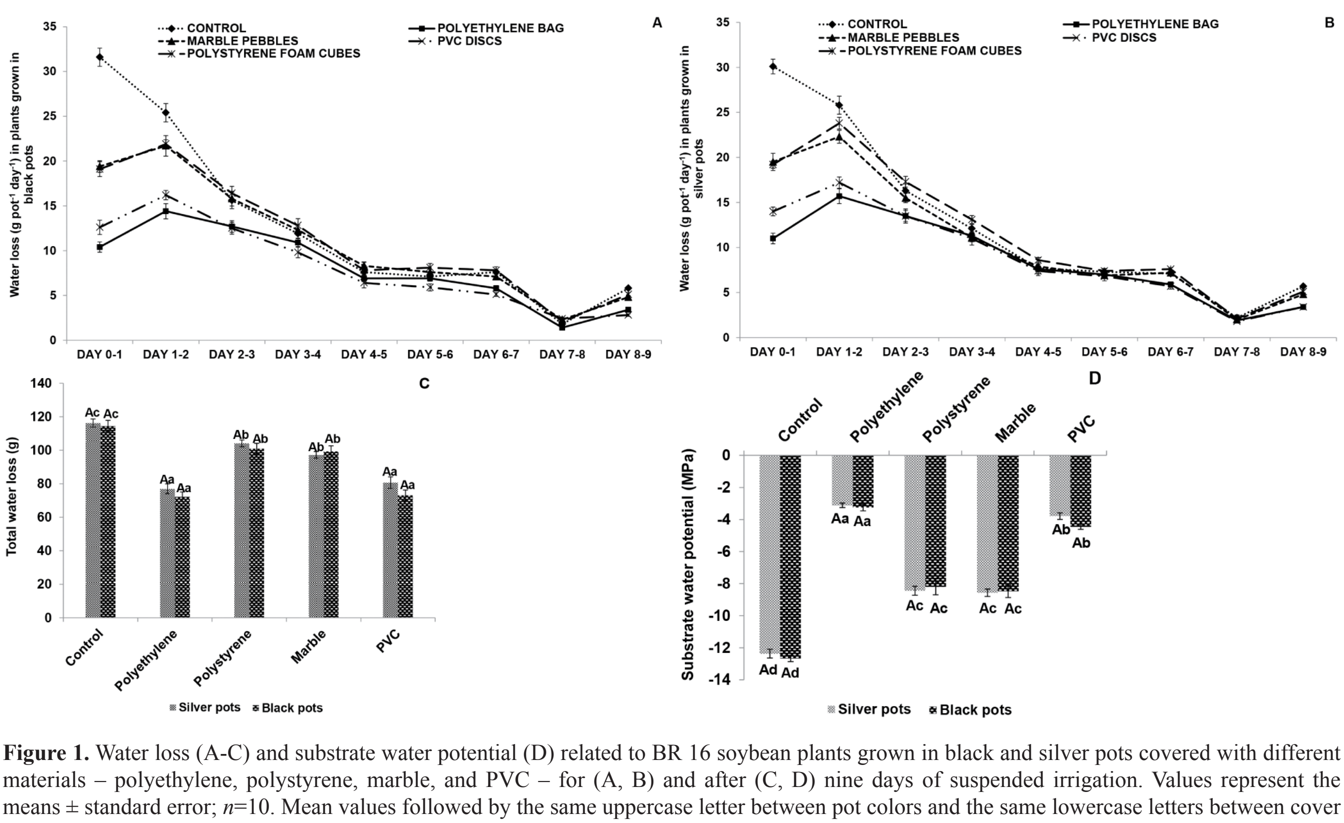

Figure 1. Water loss (A-C) and substrate water potential (D) related to BR 16 soybean plants grown in black and silver pots covered with different materials – polyethylene, polystyrene, marble, and PVC – for (A, B) and after (C, D) nine days of suspended irrigation. Values represent the means ± standard error; n=10. Mean values followed by the same uppercase letter between pot colors and the same lowercase letters between cover materials do not differ by the Tukey's test ($p \leq 0.05$).

Figura 1. Perda de água (A-C) e potencial hídrico do substrato (D) em plantas de soja BR 16 cultivadas em vasos pretos e prateados cobertos com diferentes materiais – polietileno, poliestireno, mármore e PVC – durante (A, B) e após (C, D) nove dias de irrigação suspensa. Valores representam médias ± erro padrão; n=10. Médias seguidas de mesma letra maiúscula entre cores dos vasos e mesmas letras minúsculas entre materiais de cobertura não diferem entre si pelo teste de Tukey ($p \leq 0.05$).

with PVC discs and polyethylene bags, which therefore led to greater substrate water retention. These differences between the cover materials with respect to water loss in the first days of evaluation resulted in lower total water loss in the pots covered with polyethylene bags and PVC discs, followed by those covered with polystyrene foam cubes and marble pebbles, and by those of the controls after nine days of suspended irrigation (Figure 1C). Consequently, the opposite was detected for substrate water potential, with higher values for the pots covered with polyethylene bags, followed by those covered with PVC discs, polystyrene foam cubes and marble pebbles, and by those of the controls (Figure 1D).

The differences observed between the materials, regarding substrate water retention, demonstrated an influence on the visual aspect of the plants, which showed early leaf wilting in the controls, followed by those grown in pots covered with polystyrene foam cubes and marble pebbles, and by those grown in pots covered with PVC discs and polyethylene bags, in both black and silver pots (Figure 2A-D).

The fact that plants grown in pots covered with PVC presented slightly greater leaf wilting than those in grown in pots covered with polyethylene bags was not expected, because the PVC discs remain in contact with the substrate and allow water vapor to condense in drops adhered to their inner surface until they drain into the soil or grow big enough and drop off, falling to the soil surface, keeping the condensed water available to the plants (Figure 2E). In contrast, although polyethylene bags also allow water vapor to condense on the inner surface, the water molecules will not necessarily be available to the plants because the polyethylene bags were not kept directly in contact with the substrate surface (Figure 2F).

Although the substrate temperature tended to be higher in the black and silver pots covered with polyethylene bags (Figure 3A, B), this material did not lead to a higher total water loss (Figure 1C). Specifically, the substrate temperature decreased abruptly in all pots on day 8 (27 August 2013) owing to the low mean air temperature (17 °C) recorded over the evaluations on that date.

Heat-stressed plants were detected both in black pots and silver pots, regardless of the cover material, as of the sixth day of suspended irrigation, based on the leaf and air temperatures (Figure 3C, D). As previously mentioned, there was an abrupt decrease in the mean air temperature on the day 8, which remained at 17 °C, whereas the leaf temperature ranged between 24 and 26 °C on that date. Although a higher mean air temperature (23.2 °C) was detected on the last day of suspended irrigation (day 9; 28 August 2013), in general, it remained lower than the leaf temperatures measured on the same day. It must be emphasized that the *VPD* demonstrated behavior similar to that of air temperature, with lower values between days 5 and 8 (23-26 August 2013) and a posterior increase on day 9, whereas the opposite was observed for the *RH* over the evaluation time range (9:00 a.m. to 10:30 a.m.) (Figure 4).

A greater level of evaporation was detected in the controls, followed by pots covered with polystyrene foam cubes and marble pebbles, and by those covered with PVC discs and polyethylene bags, both in black (Figure 5A) and in silver (Figure 5B) pots, over time. A similar pattern was observed for the total evaporation (Figure 5C) and the respective substrate water potential (Figure 5D) after nine days of suspended irrigation. Over the period of time relative to the daily measurements of evaporation (9:00 a.m. to 10:30 a.m.), the mean air temperature

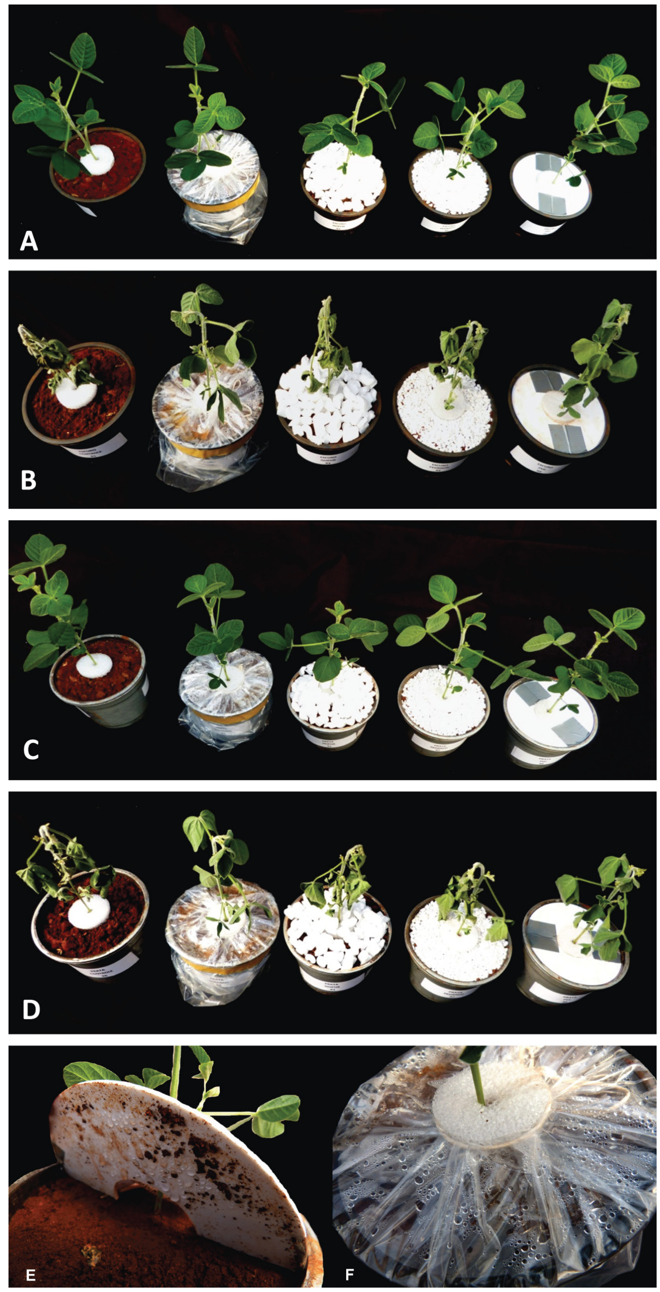

Figure 2. BR 16 soybean plants grown in black (A, B) and silver (C, D) pots under well-watered conditions (A, C) and after nine days of suspended irrigation (B, D). From left to right, uncovered pots (controls) and pots covered with polyethylene bags, polystyrene foam cubes, marble pebbles, and PVC discs. Condensation of water vapor is shown on the undersurface of a PVC disc (E) and a polyethylene bag (F).

Figura 2. Plantas de soja BR 16 cultivadas em vasos pretos (A, B) e prateados (C, D) sob condições bem-irrigadas (A, C) e após nove dias de irrigação suspensa (B, D). Da esquerda para a direita, vasos descobertos (controles) e vasos cobertos com polietileno, poliestireno, mármore e PVC. A condensação do vapor d’água é mostrada na superfície inferior dos discos de PVC (E) e do saco de polietileno (F).

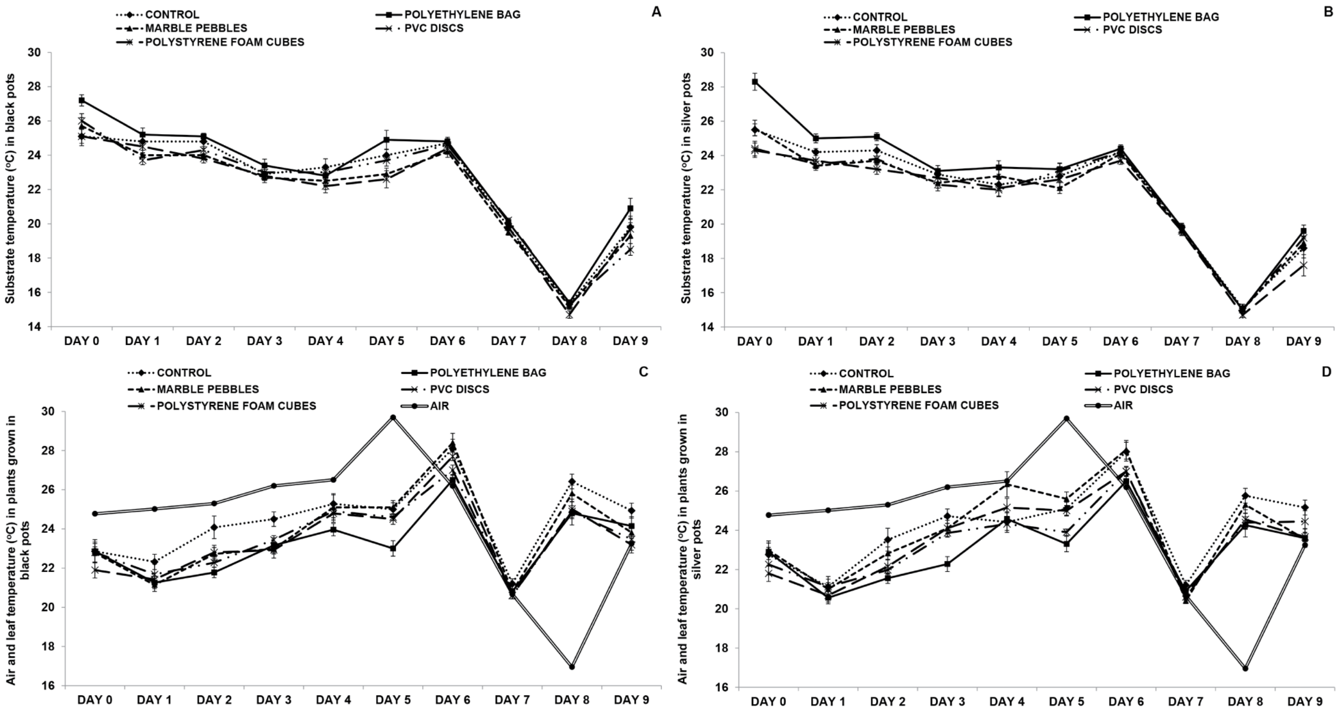

Figure 3. Substrate, air, and leaf temperatures in BR 16 soybean plants grown in black (A, C) and silver (B, D) pots covered with different materials – polyethylene, polystyrene, marble, and PVC – and subjected to suspended irrigation for nine days. Values represent the means ± standard error; *n*=10.

Figura 3. Temperaturas foliares, do substrato e do ar em plantas de soja BR 16 cultivadas em vasos pretos (A, C) e prateados (B, D) cobertos com diferentes materiais – polietileno, poliestireno, mármore e PVC – e submetidas à irrigação suspensa por nove dias. Valores representam médias ± erro padrão; *n*=10.

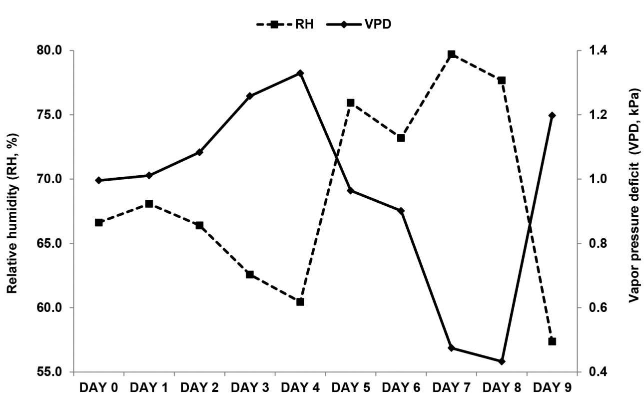

Figure 4. Relative humidity and vapor pressure deficit during water loss trial in pots covered with different materials – polyethylene, polystyrene, marble, and PVC – and subjected to nine days of suspended irrigation. Data correspond to the range between 9:00 a.m. and 10:30 a.m..

Figura 4. Umidade relativa e déficit de pressão de vapor durante a avaliação da perda de água em vasos cobertos com diferentes materiais –polietileno, poliestireno, mármore e PVC – e submetidos a nove dias de irrigação suspensa. Os dados correspondem ao período entre 9:00 e 10:30.

remained at approximately 26 °C until day 5, when there was an abrupt decrease (day 6; 20.8 °C) followed by a continuous increase until day 9. In contrast, the *RH* was kept at 50-60% until day 5, with high values on day 6 (90.8%) and a continuous decrease until day 9 (Figure 6).

4 Discussion

In the current study, four different cover materials – polyethylene bags, marble pebbles, polystyrene foam cubes, and PVC discs – were tested in order to identify the most efficient material in preventing water loss through evaporation in the substrate (soil/sand/organic compounds, 3:2:2) used to fill (originally) black or silver-painted pots cultivated with BR 16 soybean plants subjected to suspended irrigation for nine consecutive days under greenhouse conditions. Evaluations consisted of measurements of water loss, substrate temperature, and leaf temperature for nine days under suspended irrigation. On the last day, the substrate water potential was evaluated. Afterwards, the experiment was repeated under the same conditions, but with the absence of plants, to measure the evaporation for nine consecutive days under suspended irrigation. Substrate water potential was once again measured on the last day.

There are advantages and disadvantages between the different materials evaluated. Polyethylene bags present the advantage of better evaporation control. However, according to previous tests, this material leads to increased substrate temperature. Therefore, although polyethylene bags prevent evaporation, substrate cooling is hindered. Nevertheless, it must be emphasized that soil heating caused by the use of polyethylene can be advantageous; for example, in studies involving solarization, a method of heating moist soil by covering it with transparent polyethylene sheets to trap solar

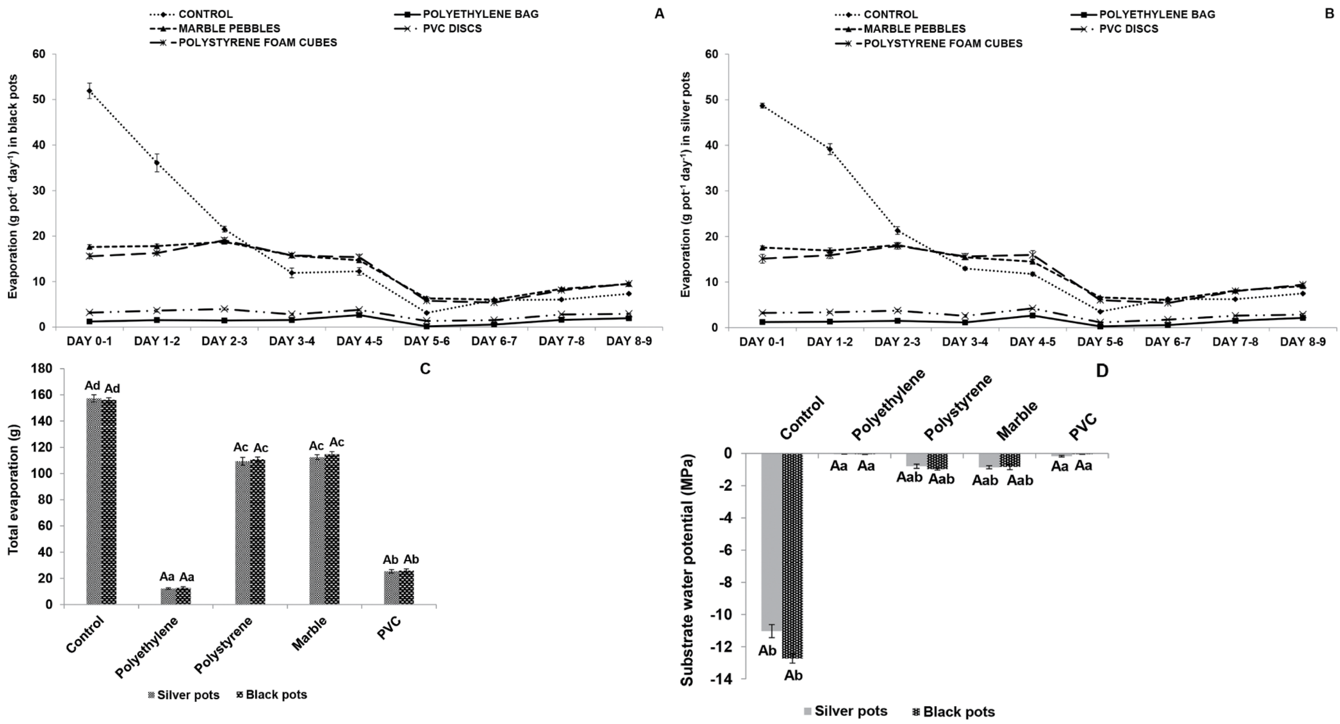

Figure 5. Evaporation (A-C) and substrate water potential (D) in black and silver pots covered with different materials – polyethylene, polystyrene, marble, and PVC – for (A, B) and after (C, D) nine days of suspended irrigation. Values represent the means ± standard error; *n*=10 (A-C) and *n*=6 (D). Mean values followed by the same uppercase letter between pot colors and the same lowercase letters between cover materials do not differ by the Tukey's test for (C) and by the Dunn's test for (D).

Figura 5. Evaporação (A-C) e potencial hídrico do substrato (D) em vasos pretos e prateados cobertos com diferentes materiais – polietileno, poliestireno, mármore e PVC – durante (A, B) e após (C, D) nove dias de irrigação suspensa. Valores representam médias ± erro padrão; *n*=10 (A-C) e 6 (D). Médias seguidas de mesmas letras maiúsculas entre cores dos vasos e mesmas letras minúsculas entre materiais de cobertura não diferem entre si pelo teste de Tukey (C) e pelo teste de Dunn (D).

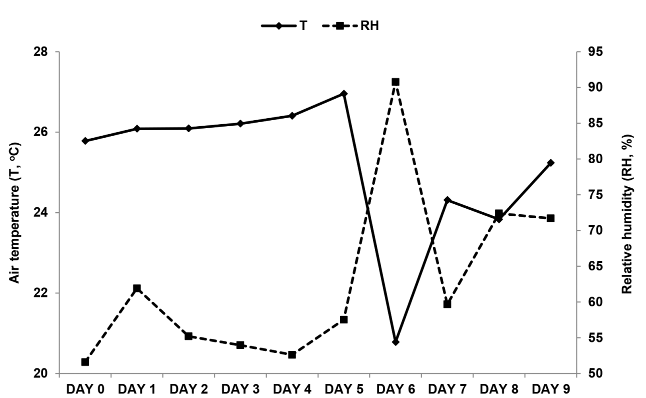

Figure 6. Air temperature and relative humidity during evaporation trial in pots covered with different materials – polyethylene, polystyrene, marble, and PVC – and subjected to nine days of suspended irrigation. Data correspond to the range between 9:00 a.m. and 10:30 a.m..

Figura 6. Temperatura do ar e umidade relativa durante a avaliação da evaporação em vasos cobertos com diferentes materiais – polietileno, poliestireno, mármore e PVC – e submetidos a nove dias de irrigação suspensa. Os dados correspondem ao período entre 9:00 e 10:30.

radiation, thus involving the use of heat as a lethal agent for soil-borne pathogens (Al-Kayssi, 2009).

Polyethylene also leads to greater water vaporization. The water vapor condenses on the inner surface of the polyethylene bags, mainly at night, so that in the next morning, such a volume of water adhered to the polyethylene is included in the water mass weighing, but this water is not available to the plants by absorption because the polyethylene bag does not remain in contact with the substrate surface. The mean weight of water adhered to the inner surface of polyethylene bags corresponded to 1.50 g (2.07% total water loss) and 1.55 g (2.01% total water loss) in black and silver pots, respectively, after nine days of suspended irrigation for water loss measurements. These quantities of water that are unavailable to the plants did not result in early onset of leaf-wilting symptoms.

Hence, even considering all the possible disadvantages of polyethylene bags, this material still led to the greatest water storage in the substrate (Figure 1D) and, consequently, resulted in higher leaf turgor after nine days of suspended irrigation (Figure 2A-D). When only evaporation was evaluated, that is, in pots with no plants, the mean weight of water adhered to the inner surface of the polyethylene bags corresponded to 3.3 g (26.08% total evaporation) and 3.2 g (26.16% total evaporation) in black and silver pots, respectively, after nine days of suspended irrigation. Therefore, in the absence of plants, there was greater water volume condensed on the inner surface of the polyethylene bags compared with that of the pots with plants.

In preliminary studies, we observed that cover provided by polystyrene foam cubes and marble pebbles have the advantage of keeping a reflective surface, thus contributing to avoid substrate overheating, as well as leading to a higher rate of gas exchange between the substrate and the ambient air compared with that provided by polyethylene bags. However, polystyrene and marble pebbles tend to cool the substrate surface because they mitigate the incidence of sunlight and prevent evaporation only partially.

Thus the most suitable material for covering the pots aiming at the greatest substrate water-content maintenance should be opaque, prevent heat absorption, allow the substrate to cool, allow gases to diffuse between substrate and ambient air, and most importantly, be impermeable to water vapor, thus preventing evaporation completely. Therefore, white PVC discs were tested in an attempt to reach these objectives. The PVC discs were more efficient than the polystyrene foam cubes or marble pebbles in maintaining substrate water content (Figure 1D), but they resulted in substrate compaction, mainly in the central region of the pots, that is, around the root system. This might have contributed to the increased loss of leaf turgor in the plants grown in pots covered with PVC discs compared with those covered with polyethylene bags after nine days of suspended irrigation. Soil compaction has been considered harmful to plant growth and agricultural yields, because it results in increased mechanical resistance to root penetration, thus hampering soil exploitation by plants. Furthermore, soil compaction may decrease the soil matric potential, which results in less water availability to the plants, aggravating the effects of drought (Alameda et al., 2012).

In the present study, plants grown in black or in silver pots covered with polyethylene bags presented, in general, lower leaf temperature; however, the substrate temperature was higher compared with that resulting from the use of other cover materials. Thus suspension of irrigation led to lower stress levels in those plants, presenting higher leaf turgor. In addition, pots covered with polyethylene bags presented, in general, higher substrate water potential. However, polystyrene foam cubes and marble pebbles allowed the substrate to cool, but these materials were inefficient in the maintenance of water and, therefore, resulted in a more rapid loss of leaf turgor over the assessment period.

5 Conclusions

Among the materials tested in the current study, polyethylene bags are the most efficient in preventing water loss through evaporation in pots cultivated with the Brazilian soybean cultivar BR 16 under greenhouse conditions. Both pot colors (black and silver) studied have no influence on water loss. There are few reports on the use of cover materials to prevent water loss through evaporation in substrates used to fill pots. Also, information about the external color of the pots used for such finality is scarce. Considering the importance of understanding the response of drought-stressed soybean plants under these conditions, our findings may contribute to further studies involving conventional and transgenic plants for the selection of more promising drought-tolerant genotypes.

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