SÃO PAULO STATE UNIVERSITY – UNESP
CÂMPUS OF JABOTICABAL

COLOURED PLASTIC MULCHES IMPROVE THE GROWTH AND YIELD OF THE ‘MICRO-TOM’ TOMATO IN HIGH-DENSITY PLANTINGS

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TÍTULO DA DISSERTAÇÃO: COLOURED PLASTIC MULCHES IMPROVE THE GROWTH AND YIELD OF THE 'MICRO-TOM' TOMATO IN HIGH-DENSITY PLANTINGS

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CARLA CONSTANZA MANGANELLI, 24/07/1989 – She was born on July 24th, 1989 in San Miguel de Tucuman, Argentina. Her parents Marcela Maria Alicia Sal and Carlos Daniel Manganelli. In 2007 she started the bachelor degree in Agronomist Engineering at Faculty of Agronomy and Zootechny at the Tucuman National University (UNT), and finished in October, 2013. During the 2008 and 2009 she received the scholarship of "Scholarship National Program" and of "Bicentennial Scholarship National Program" in the years 2010 to 2013. In the second semester of 2011 she held an exchange at the University of Brasília with mobility scholarship of program MARCA. In the 2012 and 2013 she started her professional traineeship at the fruitculture department of the Agroindustrial Obispo Colombres Experimental Station, in which took part in projects related to the culture of citrus. At the same time, she worked as an intern at the Rio de Arena Vineyard, participating in activities of vineyard management. In August 2015, she started the Master's Degree in Agronomy (Plant Production) at the São Paulo State University (UNESP), campus of Jaboticabal. She works on the area of Plant Physiology area with a scholarship granted by the National Council for Scientific and Technological Development – CNPq, under the supervision of Dr. Rogério Falleiros Carvalho.

“Go confidently in the direction of your dreams. Live the life you have imagined”.

Henry David Thoreau

To my parents Marcela and Daniel for the love and confidence always deposited and for they always being present.

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COBERTURAS PLÁSTICAS COLORIDAS MELHORAM O CRESCIMENTO E O RENDIMENTO DO TOMATE "MICRO-TOM" EM PLANTIOS DE ALTA DENSIDADE

RESUMO – As coberturas plásticas têm diferentes propriedades térmicas e de radiação, e podem afetar o rendimento e a qualidade do tomate. Neste estudo, compararam-se a influência das coberturas plásticas coloridas (vermelho, azul, e cinza/preto) e a densidade de plantio no crescimento do tomateiro, o rendimento e a qualidade dos frutos com o da cobertura preta convencional e o solo descoberto (controle) no desenvolvimento do tomateiro ‘Micro-Tom’. Além disso, determinou-se a acumulação de pigmentos, a temperatura do solo e a água total aplicada. Os resultados indicaram que o peso seco e a área foliar das plantas cultivadas com cobertura azul, vermelha e cinza/preta foram significativamente maiores do que o tratamento controle em parcelas de alta densidade. Em comparação com o tratamento controle, o rendimento total, o peso do fruto e o peso total de frutos por planta foram consequentemente melhorados nas plantas crescidas com cobertura vermelha e cinza/preta em altas densidades. Contudo, o número de frutos não foi afetado. Além disso, o tamanho do fruto aumentou com cobertura vermelha em parcelas de baixa densidade. Por outro lado, as temperaturas do solo registradas sob a cobertura vermelha foram as mais adequadas para o tomateiro nessas condições climáticas. Em conclusão, os tratamentos com cobertura colorida afetaram positivamente o crescimento e o rendimento do tomateiro em parcelas de alta densidade, e a cobertura vermelha em particular poderia ser usada para melhorar o rendimento e o tamanho dos frutos de tomate ‘Micro-Tom’, devido à otimização da temperatura e o ambiente de luz.

Palavras-chave: ambiente de luz, coberturas coloridas, comprimento de onda, tomate

CHAPTER 1 - General considerations

1.1. INTRODUCTION

Soil mulching techniques have long been used in agriculture. Greater growth and yield in annual and perennial crops have been attributed to the improved water use efficiency, increased soil temperature, and inhibition of weed growth that result from using mulch (LIANG et al., 2000). For example, the effects of a plastic mulch could affect plant growth and yield as a result of changes in soil temperature (DÍAZ-PÉREZ;BATAL, 2002; LAMONT, 2005*;* IBARRA-JIMENEZ et al., 2006). This is determined primarily by the optical properties of the material (HAM;KLUITENBERG;LAMONT, 1993) and the degree of contact between the plastic and soil (LIAKATAS;CLARK;MONTEITH, 1986; HAM;KLUITENBERG, 1994). According to Tarara (2000), soil temperature is higher in mulched plots because the air gap between the soil and mulch acts as an insulating layer and reduces convective heat transfer. In addition, the plastics retain part of the longwave radiation emitted from the soil to the atmosphere, and this prevents the cooling of the evaporative layer in the mulched beds.

Higher soil temperatures change soil biological characteristics and fertility (CHEN et al., 1998; LIU;WANG;ZHENG et al., 2013). Studies have shown that plastic mulching accelerates nutrient transformations, such as ammonification and nitrification (WILSON;JEFFERIES, 1996), and provides a better root growth environment. Moreover, Mulumba and Lal (2008*)* observed that mulch application increases total porosity and moisture content at field moisture capacity, avoiding alterations in soil aggregation. The efficient use of water is a result of reduced evaporation from the soil and better availability of soil moisture (LI et al., 2013). At the same time, mulching adjusts the regulation of the vertical distribution of the soil water, which moves from deeper layers to the topsoil by capillarity and vapour transfer, and the water content of the topsoil therefore remains relatively stable (TIAN et al., 2003; WANG;HORTON;SHAO, 2003; XIUKANG;ZHANBINA;YINGYING, 2015).

Although the most commonly used plastic mulch is black, different coloured plastic mulches are also used and have been reported to improve the yield and quality of crops by various researchers. The use of coloured plastic mulch offers the same advantages as the use of black plastic mulches as well as additional benefits in terms of the distribution of light reflected to sun-grown plants, which is related to the quality and quantity of reflected light and modifies gene expression enough to improve the yield and nutrient content of some food crops (KASPERBAUER;LOUGHRIN, 2004). Moreover, studies have shown that coloured plastic mulches may be utilized in vegetable crops to repel insect pests, such as thrips (GREENOUGH;BLACK;BOND, 1990) and aphids (OROZCO-SANTOS et al., 1994; OROZCO-SANTOS;PÉREZ-ZAMORA;LÓPEZ-ARRIAGA, 1995), to modify soil temperatures (FARIAS-LARIOS;OROZCO;GUZMÁN, 1994; FARIAS-LARIOS;OROZCO-SANTOS, 1997) and to increase yield and quality of crops (LOUGHRIN;KASPERBAUER, 2002; LAMONT, 2005; MAY;HANSON;MOLINAR, 2005; IQBAL;GOHEER;KHAN, 2009; SING;KAMAL, 2012). Thus, plants have evolved an extensive collection of photoreceptors characterized as perceiving information about their light environment, or wavelengths reaching the plant surface, which initiate a sophisticated system in the light signal transduction pathways (SULLIVAN;DENG, 2003).

Light is a form of radiant energy of a portion of the electromagnetic spectrum categorized as wavelength (nm) and energy (photons or quanta) in the photobiology of plants, whilst the breaking of this energy into its wavelength components is termed spectral distribution or light quality. In this sense, previous studies have demonstrated that the application of coloured mulch can affect the plant microclimate sufficiently to alter the growth and improve the yield in some plants because, depending on the mulch colour, it changes the amount and spectral distribution of radiation reflected to the leaves (DECOTEAU;KASPERBEAUER;HUNT, 1989).

In this way, reflected light from mulches influences plant growth through photosynthesis and photomorphogenesis. Photosynthesis depends on the number of photons in the spectral range of 400-700 nm (red and blue light), and the responsible pigment is chlorophyll. In contrast, photomorphogenesis depends on light quality and involves the activation of various receptor systems in the perception of specific wavelengths. These pigment systems include phytochromes, which absorb red light (R) (660-680 nm) and far-red light (FR) (730-740 nm), cryptochromes, phototropins, the Ztz/cbvg system, which absorbs UV-A and blue light (400-500 nm), and a UV-B receptor (290 nm) termed UVR8 (FRAIKIN;STRAKHOVSKAYA;RUBIN, 2013; GALVÃO;FANKHAUSER, 2015; MAWPHLANG;KHARSHIING, 2017). Also important are the combinations of wavelengths, such as R/FR and R/B, absorbed by the receivers mentioned above. All of them work in the perception of specific light wavelengths as indicators of the plant environment and in the regulation and development of the plants (JOHKAN et al., 2010), detecting changes in the quality, quantity, duration and direction of the light around them. Then, the message is perceived by the plant, affecting cell metabolism and altering the expression of thousands of genes that at the same time generate the physiological responses (TEPPERMAN;HWANG;QUAIL, 2006; QUAIL, 2007).

Thus, alterations to photosynthate partitioning and morphological development (internode elongation, lateral shoot growth, root and shoot growth) and modification of the chemical composition in crops are photomorphogenic processes that have been attributed to differences in the plant light environment caused by the use of mulch (DECOTEAU;KASPERBAUER;HUNT, 1990; KASPERBAUER;LOUGHRIN;WANG, 2001), particularly to changes in the FR/R ratio. Consequently, the use of different coloured mulches could enhance plant productivity and the quality of the products obtained from them, starting from phytochrome regulation, resulting from the reflection of FR/R ratios (FRANQUERA, 2011). However, most papers that have addressed the effects of mulches on the physiological responses of plants approach the subject from the viewpoint of photosynthesis and overlook the role that phytochromes play in such responses. For this reason, the aim of this review is to document the current use of coloured plastic mulch in agriculture and to present multiple benefits in relation to the traditional black mulch, with special reference to the effects of the light environment on the yield and quality of plant products as a result of photomorphogenesis.

1.2. LITERATURE REVIEW

1.2.1. Tomato crops

Tomato (*Solanum lycopersicum* L.) is a plant belonging to the Solanaceae family (PERALTA;KNAPP;SPOONER, 2006) that originated in the Andean region of South America in countries such as Peru, Bolivia, Chile, and Ecuador. It is the second most produced and consumed vegetable crop in the world (FAO, 2017), and it is considered to be a functional food due to the high levels of vitamins A and C in addition to other multiple benefits.

In 2014, the total production of tomatoes around the world was over 170 million tonnes, which ranked first among all vegetables, with a total acreage of 5.2 million ha. China, India and the United States are the main producers, corresponding to approximately 49% of the world production. In addition, Brazil ranks ninth in world production, reaching 4.30 million tonnes in an area of 64.4 thousand ha (FAO, 2017). Currently, the agricultural production of tomato in Brazil is of greater importance in the Southeast and Central-West regions. Thus, the states of Goiás, Minas Gerais and São Paulo together account for approximately 57% of the total production in the country (IBGE, 2016), with an estimated productivity of 66,85 t/ha (FAO, 2017).

The tomato plant has become a model plant for cellular, biochemical, molecular and genetic studies because it has a short life cycle, is easy to manipulate and is easily grown. Therefore, the tomato is an excellent tool for improving knowledge regarding horticultural crops.

Biologically, it is a semi-perennial plant, suitable for living and producing fruits for several years. However, it is grown annually for economic and commercial reasons. The duration of the life cycle, from seedlings to harvesting, ranges from 95 to 125 days (MAROUELLI;SILVA;SILVA, 2012). The duration of each developmental stage depends mainly on genotype, health, nutrition and weather conditions. The ideal temperature range for tomato is 20 to 25 °C, with maximum of 30 °C during the day and 11 to 18 °C at night. In addition, the crop requires an optimal range of temperature in each phenological phase, ie: germination of 16 to 29 °C, vegetative period of 20 to 24 °C; flowering from 18 to 24 °C; fixation of fruits from 13 to 18 ° C at night and from 19 to 25 °C diurnal and fruit ripening from 20 to 24 ° C (SILVA;NASCIMENTO, 2007; FILGUEIRA, 2008). Additionally, it prefers dry growing conditions and can be grown in a variety of soil types, provided that they are well-drained and fertile.

Micro-Tom is a dwarf tomato cultivar that differs from standard tomato cultivars, mainly by two recessive genes, conferring the dwarf phenotype. It is used as a model system because of its small size, short life cycle (70-90 days after sowing until fruit ripening) and small genome (950 Mb) (MEISSNER et al., 1997).

1.2.2. Plant responses to the quality of light reflected from the mulch

In the horticulture industry, there has been extensive research on the use of systems that manipulate the light environment with the aim of enhancing desired traits, such as growth habit, foliage quality, and flower production, and facilitating pest and disease management (SHAHAK;GUSSAKOVSKY;GAL, 2004; DEVLIN;CHRISTIE;TERRY, 2007; HALIAPAS et al., 2008). Thus, in recent years, the importance of selecting the right colour of plastic mulch in vegetable production has been demonstrated, given that it promotes changes in the crop microclimate, modifying the quantity of light and spectral balance reaching plants, with effects on the distribution of photosynthates to shoots and roots and on the phenology growth, production and yield (DECOTEAU et al., 1988, DECOTEAU;KASPERBAUER;HUNT, 1990; DIAZ-PEREZ, 2010; GORDON et al., 2010; EL-ZOHIRI;SAMY, 2013). Therefore, plants grow in sunlight and under specific light wavelengths reflected from the surface colour, where through different types of photoreceptors, including phytochromes, this light will be absorbed, resulting in the desired plant responses (GALVÃO;FANKHAUSER, 2015; HUCHÉ-THÉLIER et al., 2016).

Initially, plastic mulches were primarily black, clear, and white, but since 1985, the role of the light reflecting off the coloured mulch, such as red, blue, silver, silver-black, yellow, green and brown (FRANQUERA, 2011) began to be considered, with each of them having different reflective characteristics, particularly in the reflected FR/R ratio (Tabela 1 and 2). Additionally, plants respond morphologically to various colours in controlled environments through the natural growth regulating system, with far-red, red, and blue being the most influential colours of light (ANTONIOUS;KASPERBAUER, 2002; KASPERBAUER;LOUGHRIN, 2004; SHIUKHY;RAEINI-SARJAZ;CHALAVI, 2015). Likewise, the R/FR ratio has a dramatic effect on the phytochrome photoequilibrium within the plant and is involved in chloroplast development, the regulation of stem elongation (FRANKLIN;QUAIL, 2010; CASAL, 2013), fruit quality (GONZÁLEZ et al., 2015), root elongation (SALISBURY et al., 2007; COSTIGAN et al., 2011) and tolerance to biotic and abiotic stressors (BALLARÉ et al., 2012; CARVALHO;CAMPOS;AZEVEDO, 2011). Other studies have shown that it influences apical dominance (CASAL;SANCHEZ;DEREGIBUS, 1986), flowering time (KIM et al., 2008; ADAMS;ALLEN;WHITELAM, 2009; NISHIDATE et al., 2012; KOHYAMA;WHITMAN;RUNKLE, 2014) and, especially, photosynthate partitioning among roots, shoots and fruit (KASPERBAUER, 1987). Indeed, previous studies have demonstrated that coloured mulch reflecting a FR/R ratio lower than the ratio in incoming light favours below-ground crops and, conversely, the reflection of a FR/R photon ratio higher than the ratio in incoming sunlight (at the same time and place) favours shoot crops (KASPERBAUER;LOUGHRIN;WANG, 2001; ANTONIOUS;KASPERBAUER, 2002).

On the other hand, it is known that the spectral distribution of reflected light can modify gene expression in a way that can also influence the chemical composition of the developing plant, which contributes to quality. Antioxidant activity, flavonoids, phenolics, anthocyanins, vitamins, tannins and other secondary metabolites may change by modifying the proportions of selected light wavelengths (TEGELBERG;JULKUNEN-TIITTO;APHALO, 2004, WU et al., 2007, PALLOZZI et al., 2013; TAULAVUORIA et al., 2016).

In the following sections, the modifications of the light spectrum caused by the reflection of light by the different colours of mulch and how the plants are affected by it will be presented.

1.2.3. Red mulch

The light reflected from red mulch has low R/FR and high R/B ratios, with more R and FR than blue and green surfaces (ANTONIOUS;KASPERBAUER, 2002). Thus, it has been demonstrated that red light is involved in the development of the photosynthetic apparatus and photomorphogenesis through its effect on phytochrome responses (CHEN et al., 2017). However, the development processes initiated by red light may be counteracted by far-red light, and the red to far-red ratio determines the activity of molecular, biochemical and morphological processes (QUAIL, 2002; DEVLIN;YANOVSKY;KAY, 2003; CHEN;LI;PAN, 2004; CASAL;YANOVSKY, 2005). Stem elongation, foliar expansion and flowering are processes that are often promoted by a low R/FR ratio, whereas branching is generally diminished, suggesting that plants respond to changes in the light environment induced by the light reflected from red films. On this subject, results have been reported in strawberry (NISHIYAMA;KANAHAMA, 2009) and lettuce (FRANQUERA;MABESA, 2016), where leaf production was promoted by light in the red range, and in red bell pepper plants, which showed a greater height when compared to treatment with mulches of other colours (DECOTEAU, 2008). Therefore, when grown with a reduced R:FR reflected from mulch, plants initiate a suite of developmental responses that are similar to those when grown in close proximity to neighbouring vegetation whereby growth is stimulated, and the plants become taller (MUTETWA;MTAITA, 2014). Such a response in stem elongation is due to greater internode elongation rather than a greater number of internodes (FRANKLIN;QUAIL, 2010; CASAL, 2013).

Likewise, leaves also perceive and respond to FR (LIBENSON et al., 2002; CASAL;SMITH, 1989). In addition, this response can vary from the promotion of leaf growth in dicotyledonous species to the inhibition of leaf expansion (CASAL;SMITH, 1989), which may in part result from competition for resources with the stem, the growth of which is stimulated under FR, as we saw. For example, in lettuce, the results from all the parameters showed that red mulch had the best performance compared with the other coloured mulches. Thus, the red wavelengths stimulated the uptake of carbon dioxide, which is needed to make glucose materials and could be attributed to the longer leaves of lettuce grown with red mulch (FRANQUERA, 2011).

In addition, red mulch increases the ratio of FR/R wavelengths in the light reflected to the canopy and acts through phytochrome in the natural growth regulatory system within the plants so that the allocation of photosynthates are more directed to the developing fruit, which results in larger fruits and a higher yield than when grown with black mulch (KASPERBAUER, 2000; LOCASCIO et al., 2005). In this regard, Decoteau, Kasperbauer and Hunt (1989) found that tomato plants grown with red and black mulch had the greatest fruit yields, while plants grown with white and silver-coloured mulch had more foliage. Similar results were obtained by Orzolek, Otjen and Fleck (2000).

Additionally, a red polythene mulch treatment was found to cause accelerated flowering in many crops in response to low R/FR ratios. However, the flowering in some species is insensitive to low R/FR ratios or is only partially affected (UGARTE et al., 2010). Flower induction is a complex process that requires the integration of environmental and endogenous factors (BÄURLE;DEAN, 2006; SRIKANTH;SCHMIDT, 2011). However, red mulch has been observed to be effective in extending the flowering and fruiting periods and to improve fruit set. This was demonstrated in strawberry, which showed a considerable increase in berry size and weight and fruit yield when compared to treatments with black plastic and no mulch (SHARMA;SHARMA;SPEHIA, 2013).

On the other hand, previous studies have demonstrated that the use of red mulch also results in a series of chemical modifications in fruits because of alterations to the sugar and organic acid contents, improving sweetness and flavour, as observed in strawberry (KASPERBAUER;LOUGHRIN;WANG, 2001). An increase in the activity of saccharose phosphate synthase (LOUGHRIN;KASPERBAUER, 2002) and the activity of the enzyme fructose-1,6-bisphosphatase could be the cause of these improved chemical characteristics (BASSON et al., 2010). Additionally, increasing concentrations of individual aroma compounds were observed compared with those fruits that had developed over black mulch, mainly aliphatic esters (SHIUKHY;RAEINI-SARJAZ;CHALAVI, 2015).

Additionally, anthocyanin and flavonoid biosynthetic pathways have been extensively studied in strawberry at the genetic, biochemical and molecular levels because anthocyanins are one of the principal bioactive components of strawberries that play a role in antioxidant activity (ALMEIDA et al., 2007; CARBONE et al., 2009; SCHAART et al., 2013). Light is one of the most important environmental factors affecting their biosynthesis in plants, so photoperiod and light intensity (quantity) and light quality (spectrum) influence anthocyanin biosynthesis in different ways (ZORATTI et al., 2014). On this subject, Shiukhy, Raeini-Sarjaz and Chalavi (2015) describe that differences in R, FR and the FR/R photon ratio reflected from the surface of red mulch increased the anthocyanin contents in strawberry in addition to increasing flavonoids and the average size of the fruits when compared to a black mulch treatment. Other phytochemicals, such as phenols, could also be enhanced with red mulch (LOUGHRIN;KASPERBAUER, 2002).

Further, the higher amounts of R, FR, and R/BL and FR/R photon ratios and the very small amounts of blue light reflected from red mulch can improve the distribution of photoassimilates to the above-ground portions of the plant, including seeds. For instance, in speckled butterbean plants (*Phaseolus lunatus* L.), treatment with red mulch resulted in the greatest yield of dry beans, the greatest amount of seed protein per plant and the greatest accumulation of anthocyanins in the speckled areas of seed coats (KASPERBAUER;LOUGHRIN, 2004). Such a positive response to mulch colour provides evidence for the action of the phytochrome pigment in strawberry plants.

Finally, it should be considered that the red mulch surface area must remain intact and be large enough to reflect morphogenic light to the developing parts of the plant, especially the fruit and nearby leaves. However, plant responses to different mulch colours are highly dependent on the species, on experimental conditions, and on the timing of leaf area sampling.

1.2.4. Blue mulch

Among the main characteristics of blue mulch are the high amounts of BL of the light reflected from it when compared to red and green surfaces and the low amounts of R and FR (ANTONIOUS;KASPERBAUER, 2002). This difference in the light quality that reaches the canopy of plants could cause some modifications to crop development because blue light interferes with photomorphogenesis, chlorophyll biosynthesis, and stomatal opening (URBONAVICIUTE et al., 2007; CHEN et al., 2017). In this way, depending on the species, higher proportions of blue in growth irradiance could result in reductions in stem elongation and an induction of biomass production (SARALA et al., 2007, SARALA et al., 2009; SARALA et al., 2013), unlike red light, which induces hypocotyl elongation and the expansion of area leaf (JOHKAN et al., 2010). Additionally, blue light increases the number of axillary buds that differentiate from the apical meristem (MULEO;MORINI;CASANO, 2001). However, exceptions may occur in some species, as was observed in a study carried out in Cascate palm, in which the results indicated that there was no growth inhibition induced by the blue light reflected from the mulch. In contrast, blue mulch stimulated an increase in leaf number and plant height (GEORGE et al., 2011).

On the other hand, biochemical characteristics may be altered by the reflection of light from some coloured mulch surfaces, as occurs in turnip. In this crop, the concentrations of photosynthetic pigments and leaf protein were influenced (BRADBURNE;KASPERBAUER;MATHIS, 1989) as well as the concentrations of b-glucosinolates and sugars (ANTONIOUS;KASPERBAUER;BYERS, 1996) and the quantity and composition of epicuticular waxes on leaves (KASPERBAUER;WILKINSON, 1995). In this sense, Antonious, Kasperbauer and Byers (1996) found that turnips grown with coloured mulches (black plastic mulch painted blue, white, or green) resulted in differential increases in total glucosinolates. In this study, the blue mulch treatment reflected the greatest amount of B among the mulch treatments and resulted in the highest concentrations of total glucosinolates, suggesting that B influenced an enzyme involved in its pathway of production from glucose.

In addition, some studies have shown that blue light increases the concentrations of phytochemicals in edible species (LI;KUBOTA, 2009; TAULAVUORI et al., 2013;

TAULAVUORI et al., 2017), such as flavonoids (EBISAWA et al., 2008), carotenoids and anthocyanins in lettuce (LI;KUBOTA, 2009) and anthocyanins in tomato (GILIBERTO et al., 2005). However, the effects of light quality on flavonoid and phenolic acid biosynthesis in plants is species dependent (TAULAVUORI et al., 2016), indicating that many plant traits are modified by the reflection of blue light from blue mulch, but many of them need to be explored regarding their agronomic importance.

1.2.5. Green and yellow mulch

Green mulches reflect a higher FR/R ratio than that present in sunlight, only approximately 5% of the BL and relatively low PPF (KASPERBAUER;LOUGHRIN, 2004), while yellow reflects much PPF and about half as much BL as white mulch. Nevertheless, the use of green, blue, and yellow mulches does not appreciably improve the physic-chemical properties of crops, possibly because the photoreceptors that capture this range of wavelengths are less effective than phytochromes in inducing photomorphogenic responses.

On the other hand, Matsuda et al. (2004) and Ohashi et al. (2006) suggested that the sensitivity to light quality depends on the plant species, at least with regard to biomass production and morphogenesis. Thus, in sangria watermelon, the first and total yield were higher with green and silver on black plastic mulch, but the fruit total soluble solids were not affected, nor the average fruit weight, diameter or length (ANDINO;MOTSENBOCKER, 2004).

In addition, in studies conducted in basil (*Ocimum basilicum* L.), exposure to different colours of reflected light during development were found to influence the levels of fragrant compounds, including volatile terpenoids, emitted by its leaves. Basil is an herb whose leaves are used in cooking to add aroma and flavour. Therefore, the yield and chemical composition of leaves are important. Thus, plants grown with yellow and green mulch produced higher concentrations of aromatic and phenolic compounds than those grown with white or blue mulch (LOUGHRIN;KASPERBAUER, 2001). Leaf area, weight per unit area, and moisture percentage of fresh basil were also different between the treatments. In addition, plants that had developed over a red surface had greater leaf area, moisture percentage, and fresh weight than those that had developed over black plastic mulch, possibly due to the higher R/BL and lower R/FR photon ratios received, since both green and red surfaces reflect a FR/R ratio that is higher than that present in sunlight without decreasing the incoming PPF.

Likewise, increasing the FR/R ratio can increase the amounts of volatile terpenoids that accumulate in leaves, which at the same time could increase the emission of insect-attracting terpenes from cotton. On this subject, Kasperbauer and Loughrin (2004) have shown that cotton plants of the cultivar SC-1 grown with green mulches contained the highest levels of total terpenes, followed by leaves that had developed over mulch red. In addition, the low efficiency obtained from white and yellow mulch reflects that an increase PPF was less effective in inducing the accumulation of leaf terpenes than was a low R/FR ratio. Consequently, the quality of reflected light from cover can also affect insect orientation to plants by affecting plant metabolism and boosting the available olfactory cues.

On the other hand, Miao et al. (2016) found that strawberries treated with red and yellow films presented a higher total anthocyanin content (TAC) than those treated with white film and that fruits treated with green and blue films presented a lower TAC. This could be due to the alteration of related enzymes and the regulation of the expression of the structural genes involved in the pathway of flavonoid biosynthesis resulting from the use of selective coloured plastic films. Thus, the yellow mulch decreases the R/FR ratio in the light reflected to the canopy, favouring the allocation of photosynthates to developing fruits, which is similar to red mulch, as previously seen (KASPERBAUER, 2000; LOCASCIO et al., 2005).

1.2.6. White and silver mulch

It has been demonstrated that lighter-coloured mulches reflect more total light but a higher ratio of R/FR than other coloured mulches. Therefore, white surfaces reflect approximately 40% of the B and PPF that reaches in them. This greater light intensity can affect plant development and yield through higher photosynthetic rates. On the other hand, the use of silver plastic mulch in cucumber resulted in a greater number of leaves due to a greater number of internodes, and, hence, the plants showed a greater photosynthetic surface area. Such characteristics affect photosynthate partitioning in the yield, improving the extent of fructification and increasing the yield (MUTETWA;MTAITA, 2014). Similar results were obtained in okra, where silver mulch was found to have a significantly greater effect on yield when compared with five other colours, followed by blue and red plastic mulch (GORDON et al., 2010).

Similarly, the higher amounts of blue light over white mulches might contribute to the regulation of plant growth, resulting in shorter stems with more axillary growth (TANADA,1984; KASPERBAUER;HUNT, 1987), suggesting that the composition of the light reflected from the plant surroundings influences how and where the photosynthates will be used. In fact, the light reflected from the soil surface can influence allocation among shoots and roots. Thus, a higher R/FR ratio could favour the below-ground parts of plants, whereas a lower R/FR ratio should favour increased shoot size and shoot-to-root biomass ratio (KASPERBAUER, 1987; 1988). For example, the light reflected from white mulch resulted in the partitioning of more photosynthate into the tubers of potato plants, thereby producing a higher yield and larger-sized marketable tubers (MATHENY;HUNT;KASPERBAUER, 1992). Additionally, in carrots, better results were also observed with white surfaces, and the concentrations of chemical compounds were modified, improving some characteristics, such as flavour and nutrition (ANTONIOUS;KASPERBAUER, 2002).

Moreover, Rajablariani, Hassankhan and Rafezi (2012) obtained the highest total and marketable yield in tomato with silver/black mulch and black mulch. In addition, in terms of total plant dry matter, the results were similar to those observed with blue plastic mulch. Nevertheless, in radicchio, lighter-coloured mulches (white, silver) produced higher yields and total number of harvested heads than red mulch and the darker mulches (black and blue) (RANGARAJAN;INGALL, 2001). Conversely, Casierra-Posada, Fonseca and Vaughan (2011) demonstrated that the excess UV light reflected from silver mulch could cause leaf burns, such as those that occur in strawberry, which negatively affect the fruit characteristics. Therefore, silver mulch could not always be beneficial in all crops.

Finally, silver mulch has also been reported to repel aphids and thrips from vegetable crops (CSIZINSZKY;SCHUSTER;KRING, 1995), which is an important factor that should be considered.

1.2.6.1. Reflective mulches in fruit crops

Light is an environmental parameter that is a determinant of fruit quality in fruiting species. Therefore, fruit tree orchard designs and management systems must be selected with the objective to optimize the management of this resource and so improve the canopy light environment, the production of high-quality fruit and high-yielding crops. For this reason, in fruit crops, selective light-reflecting mulch films are used to change the light quality of the microclimate for plants. This is done in apple orchards, where reflective films placed between the rows improve the surrounding microclimate and enhance the light environment of the other parts that are the most heavily shaded within and lower in the tree canopy (PRIVÉ;RUSSELL;LEBLANC, 2008) since the structure and optical properties of the canopy components (branches, leaves, and fruits) widely change the spectral distribution of sunlight when light penetrates and is scattered within the tree canopy. Therefore, reflective mulch film is a shiny bright white/silver plastic cover developed to cover the entire orchard floor, which maximizes the amount of light that can be reflected back into the tree to improve fruit yield and quality (MEINHOLD et al., 2010). In this manner, the reflected light within the tree canopy may be five times higher in trees grown over reflective film than in trees grown without reflective films (VANGDAL;MELAND;HJELTNES, 2007).

Since the 1970s, studies on this subject have documented that reflective films increase the light intensity and canopy absorption of photosynthetic photon flux (PPF) in the lower parts of the canopy by providing supplementary illumination (MILLER;GREENE, 2003), which improves photosynthetic activity, yield, fruit weight, and soluble solids concentration (JU et al., 1999; PALMER et al., 2003). Additionally, these mulches improve the final colour of the fruits (GUERRERO et al., 2002; MILLER;GREENE, 2003), which is the prime external quality parameter for consumer preference and the fruit sales; therefore, this practice has been widely applied in different fruits, including apples, pears and strawberries. Thus, mainly in apple, numerous studies have reported that fruits from trees with reflective foil had a greater percentage of red skin colouration than did fruit from trees without reflective foil (BLANKE, 2007, 2008; SOLOMAKHIN;BLANKE, 2007). Normally, fruits from the upper parts and/or exterior of trees have significantly more surface colour that fruits from the lower parts, but no such difference is found in trees grown with reflective films, or it is not very significant (VANGDAL;MELAND;HJELTNES, 2007; IGLESIAS;ALEGRE, 2009).

Further, fruit surface colour is determined by the concentrations of different pigments, such as anthocyanins, chlorophylls, and carotenoids, of which anthocyanins are responsible for the red colour (AWAD;WAGENMAKERS;JAGER, 2001; LAYNE;JIANG;RUSHING, 2001). These pigments have a light-dependent metabolism, and their biosynthesis and accumulation increase when irradiation is enhanced (BAKHASHI;ARAKAWA, 2006). On this subject, it has been demonstrated that simultaneous irradiation with white and UV-B light synergistically stimulated anthocyanin production in apple fruits (UBI et al., 2006), and higher UV-A irradiated caused greater anthocyanin accumulation in grape (KATAOKA;SUGIYAMA;BEPPU, 2003). In addition, longer wavelengths increased the intensity of the red colouration in apple, pear (FENG et al., 2013) and cranberry (ZHOU;SINGH, 2002). On the other hand, Knee, Hangarter and Knee (2000) concluded that increased far-red radiation in the light environment can improve ethylene action in maturation, which could enhance red colour development during apple maturation. However, others studies suggested that FR is less effective or even inhibitory. Nevertheless, it must be considered that, in addition to light, surface colouration is regulated by internal and environmental factors such as temperature, stress, pathogen attacks and mechanical lacerations (UBI, 2004; IGLESIAS et al., 2002).

On the other hand, generally reflective mulches are applied a little before harvest time, especially in the 2 weeks preceding, because the periods with the most anthocyanin accumulation are in the middle and at the end of the growing season, and the content increases continuously during fruit maturation, as was reported by Iglesias et al. (1999). However, substantial final colour development was obtained with the application of reflective film in the first half of the growing season, which suggests that a phytochrome-mediated signal may be generated in the early portion of the growing season with season-long effects.

Furthermore, increased fruit firmness is another benefit of this practice (FUNKE;BLANKE, 2006; SOLOMAKHIN;BLANKE, 2007), which results from the acceleration of starch breakdown, although some studies reported that the firmness was unaffected (OVERBECK;SCHMITZ-EIBERGER;BLANKE, 2013; BLANKE;KUNZ, 2016). Likewise, reflective ground covers could have an important effect on fruit size and yield, such as was demonstrated in kiwifruit (THORP;BARNETT;TOYE, 2001), which likely involves a phytochrome-mediated process due to greater FR reflection into the inner canopy, according to Glenn and Puterka (2007). This higher FR/R ratio affects the dry matter partitioning to the developing fruit, thereby increasing its weight (KASPERBAUER, 2000). Additionally, reflective mulch has an effect on promoting the fruit yield per tree. Thus, Grout, Beale and Johnson (2004) reported a 30 to 39% increase in fruit number per tree and a 39 to 42% increase in total yield per tree with the use of reflective groundcover in the apple orchard, which might be due to the effects of the film on flower bud formation (BERTELSEN, 2005). Nevertheless, this benefit is infrequently documented and may vary according to species and conditions, such as occurred in a study by Baraldi et al. (1998), in which the spectral light composition had no effect on flower bud burst or flower bud differentiation, suggesting that these events also depend on genetic factors.

On the other hand, increasing levels of light intercepted by trees also has the potential to improve internal fruit quality, such as soluble solids concentration, acidity, flavour and volatile composition. These parameters are generally used to establish the optimum ripening stage of fruits. Thus, studies have reported higher amounts of soluble solids in the fruits of peach (LAYNE;JIANG;RUSHING, 2001), grapevine (JAMSHIDIAN et al., 2010), plum (KIM et al., 2008) and apple (VANGDAL;MELAND;HJELTNES, 2007). However, in some cases, the soluble solids concentration or titratable acidity may not be significantly affected compared to the control (IGLESIAS;ALEGRE, 2009). In addition, the increased sunlight reflection from reflectance films in the inter-row spaces of orchards improves the vitamin C content in apple (SOLOMAKHIN;BLANKE, 2007), antioxidant content and activity, and accumulation of phenolics in both the skin and fruit pulp tissues of fruits (ANDREOTTI;RAVAGLIA;COSTA, 2009), particularly phenolic acids and proanthocyanidins.

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CHAPTER 2 - Coloured plastic mulches improve the growth and yield of the 'micro-tom' tomato in high-density plantings

Abstract

Plastic mulches have different thermal and radiation properties, and they can affect tomato fruit yield and quality. In this study, the influence of coloured plastic mulches (red, blue and gray on black) and planting density on tomato growth, fruit yield and quality was compared with that of conventional black mulch and uncovered soil (control) in developing 'Micro Tom' tomatoes. In addition, the pigment accumulation, soil temperature and total applied water were determined. The results indicated that the dry weight and leaf area of plants grown over blue, red and gray/black mulches were significantly higher than the control treatment in high-density plots. Compared to the control treatment, the total tomato yield, fruit weight and total fruit weight per plant were consequently improved in plants grown over red and gray/black mulches at high densities. However, the fruit number was not affected. In addition, the fruit size increased over red plastic mulch when planted at a low density. In addition, the soil temperatures registered under the red mulch were the most suitable for the tomatoes in these climatic conditions. In conclusion, treatments employing coloured mulch positively affected the growth and yield of tomatoes in high-density plots, and red mulch in particular could be used to improve the yield and fruit size of Micro-Tom tomatoes because of the optimization of the thermal and light environment.

Keywords: coloured mulches, light environmental, photomorphogenesis, *Solanum lycopersicum,* wavelength.

1. Introduction

The use of mulch is a widely used practice for several species because undesirable factors such as invasive plants and excessive water loss by evaporation from the soil can be avoided (Steinmetz et al., 2016). In addition, this type of agricultural practice facilitates harvest and commercialization because the product remains clean and healthy. For this purpose, dark mulches are commonly used because they are easily found and inexpensive. However, this type of material provides a lower quality of reflected light compared to the coloured mulches, especially red and blue mulches, because the spectral distribution of the light reflected by these colours is known to be better utilized for photosynthetic processes and photomorphogenesis (Antonious et al., 1996; Kasperbauer and Loughrin, 2004).

Thus, changes in the quality of the light spectrum promoted by coloured mulches are directly related to the proportions of the red (R)/red-end (FR) wavelengths as well as the blue light (B), which predominantly control the photomorphogenesis mediated by different photoreceptors (Kasperbauer 1987; Bradburne et al., 1989; Decoteau et al., 1989). Among the most frequently explored factors is the phytochrome, which is a component of signal transduction promoted by R, FR, and, less effectively, by B, although other pigments of equal importance can control photomorphogenesis through these spectrum bands (Kendrick and Kronenberg, 1986).

In many species, particularly in economically important vegetables, the use of coloured mulches has favoured several aspects of development, including nutraceuticals such as vitamin C, carotenoids and flavonoids, which are effective antioxidants (Bradburne et al. 1989, Kasperbauer et al., 2001; Loughrin and Kasperbauer, 2002; Kasperbauer and Loughrin, 2004; Lamont, 2005). For example, in tomato, which is one of the most frequently consumed species in the world, this practice has promoted obvious morphological changes when cultivated over a red cover, increasing fruit productivity (Decoteau et al., 1988, Kasperbauer and Hunt, 1998). Interestingly, this treatment provides more resistance to nematode attacks (Fortnum et al., 1997, 2000), indicating that the light reflected by this material may involve extensive photomorphogenic processes that are mediated by an intricate photoreceptor system.

Because to the better light environment induced by this type of coloured plastic mulch, an interesting question can be raised. Can plants grown at high densities on coloured mulch grow better compared to plants grown on soils that are devoid of these covers? Accordingly, when the plant density is very high, the shading is increased and results in a reduction in the light interception, especially in the lower basal leaves, resulting in lower carbon dioxide (CO_2) fixation (Law-Ogbomo and Egharevba, 2009; Amundson, 2012), which unconditionally affects important agroeconomic parameters. Also, the low light availability in high-density plots can alter the source-drain relationship, for example, inducing higher sowing and decreasing reproductive development. However, coloured covers might improve the light quality and consequently provide increased yields compared to the control.

Thus, the hypothesis of this proposal relates to the induction of better light conditions by coloured mulches under high-density cultivation. These conditions may represent a greater number of cultivated plants per square metre. Moreover, a high-density system could result in better water utilization, providing similar yields with smaller amounts of water than would be needed in a more widely spaced system.

In this study, we aimed to study the effect of the planting density and colour of plastic mulches on tomato (*Solanum lycopersicum* L.) growth and development, one of the most frequently consumed vegetables in the world. Tomato plants grown in plots with gray over black, black, red and blue polyethylene films have been used to analyse aspects of vegetative and reproductive development and fruit quality. In addition, the applied water sheets and yields were compared between the two planting densities.

2. Materials and methods

2.1. Plant materials and growth conditions

This experiment was conducted between September and December of 2016, at the São Paulo State University (UNESP), campus of Jaboticabal (latitude 21º 14 '05 "S, longitude 48º 17' 09" W, altitude: 615.01 m). Seeds of the Micro-Tom (MT) tomato (*S. lycopersicum L*.) cultivar were sown in boxes containing a mixture of commercial substrate (Bioplant®, Brazil) and expanded vermiculite (1:1, v:v), and they were maintained in the greenhouse until transplant, which was performed 16 days after sowing (DAS).

The experiment was developed in a subdivided plot scheme. Four mulch treatments (black, red, blue, and gray on black) were applied to 10-cm raised beds to provide different combinations of B, R and FR, together with a control (bare soil), with 28 plants each. In each treatment, two planting densities were used as follows: 100 plants m^{-2} (high-density) or 25 plants m^{-2} (low-density). The colour sequence was randomized within each plot.

The initial soil analysis showed a soil pH of 6,9, organic matter content of 18 g dm^{-3}, 75 mg dm^{-3} P, 2,0 $mmol_c$ dm^{-3} K, and a CEC of 66,4 $mmol_c$ dm^{-3}. The individual plants were fertilized three times during the cycle with granulate NPK (10:10:10), with applications of approximately 20%, 30% and 50% of a 5 g $plant^{-1}$ dose. In addition, insecticides and fungicides were applied to prevent insect and disease infestation.

Irrigation was provided through a drip irrigation system. The soil water content (SWC) was monitored during the growing season using a tensiometer, and the plants were watered when the SWC dropped to -20 KPa. At the end of the cycle, the amount of water applied in each density was measured (Table 1).

Table 1. Effect of the planting density on the total amount of water applied during the 'Micro-Tom' cycle.

Conduction	Area (m^2)	Spacing (cm)		Plants number	Emitter flow (L / h)	Spacing of emitters (m)	Drip tube spacing (m)	Application rate (mm / h)	Total irrigation applied (mm)
		Line	*Plants*						
Low-density	8.12	20.0	20.0	203	2.4	0.50	0.47	10.21	233,68
High-density	2.88	10.0	10.0	288	2.4	0.50	0.40	12.00	261,22

The soil temperature of each treatment was continuously measured under the mulch and above the mulch, and the soil temperatures were recorded at 5 centimetres under the ground using thermocouple sensors attached to a data logger, which registered the temperature every 30 seconds (for 24 hours). The leaf temperatures were measured at the third true leaf at 9 a.m. in plants that were under similar conditions between treatments.

Additionally, some phonologic stages such as the date at the beginning of flowering and the dates of fruiting and the end of fruiting were registered by monitoring the cultivation every two days.

Finally, the spectrum distribution and the amount of light (PPF) reflected from the mulch was measured at 10 cm above the surface of the plastic with a spectroradiometer (SpectraPen LM 500 - PSI). Measurements were taken at 10 a.m. on a sunny day. The resulting values were expressed as the percentage of natural light for each wavelength, which were B (450 nm), R (630 nm) and FR (740 nm). From the data about F and FR, the R/FR ratio reflected from each mulch was also calculated (Table 2).

Table 2. Characteristics upwardly reflected above the different coloured mulches relative to those of incoming sunlight at the same time and place, and the FR/R ratios at 10 cm above the soil surface.

Characteristics	Color on soil surface				
	Gray	Blue	Red	Black	Control
			%		
PPF (400-700 nm)*	13	59	31	6	18
Blue (B) (450 nm)	11	18	8	6	5
Red (R) (660 nm)	10	6	42	6	14
Far – red (FR) (730 nm)	12	8	43	6	17
			Ratio		
R/ FR	1.15	0.93	1.24	1.14	1.05

*PPF, Photosynthetic Photon flux.

2.2. Growth analyses

The plant height, root length, and leaf area were measured in five plants that were randomly selected from each replicate at 45 DAS. Measurements of the height and root length were performed with the aid of a ruler. Measurements were taken from the base of the stem to the insertion of the last leaf for the height, and the length of the primary root was also measured. Later, the roots were washed thoroughly to remove the soil.

To measure the leaf area, the leaves of each plant were digitized using QUANT software (Vale et al., 2002). To obtain the dry mass, both the roots and shoot were then placed separately in sachets and set out to dry for 3 days in a stove at 65 °C, and then they were weighed.

2.3. Chlorophyll fluorescence

The chlorophyll fluorescence was measured with a portable chlorophyll fluorometer. The maximum (Fm) and basal (F0) fluorescence yields were measured in dark-adapted leaves for 30 min in the morning to determine the potential quantum yield of photosystem II (PSII), which was calculated as follows: $Fv/Fm = (Fm - F_0)/Fm$. Light saturation curves were obtained using the light curve programme of the instrument for 4 min, with pulses saturating the irradiance that was applied every 30 seconds to obtain the chlorophyll fluorescence parameters. In addition, the maximum apparent photosynthetic electron transport rate values (ETRmax) and saturating photosynthetic photon flux density (PPFDsat) were calculated from light curves, according to Rascher et al. (2000).

2.4. Pigment contents

Leaf discs (0.6 cm^2) from the same leaves used during fluorescence measurements were collected to quantify the pigment contents, which were determined as described by Porra et al. (1989) with a spectrophotometer, with dimethylformamide as the solvent.

The equations used to determine the concentrations of chlorophyll *a* and *b* and the carotenoids in µg mL^{-1} were as follows:

$C_a = 11.65\ A_{664} - 2.69\ A_{647}$

$C_b = 20.81\ A_{647} - 4.53\ A_{664}$

$C_{x+c} = (1000A_{480} - 0.89C_a - 52.02C_b) / 245$

2.5. Fruit production and quality

Four plants per treatment and per replicate were taken at 90 DAS (120 plants). The number and total weights of the fruits per plant was recorded. In addition, five fruits per plant were randomly selected to be individually weighed on a balance that was sensitive to 0.1 g.

In relation to the fruit quality, the diameter, colour, soluble solids and titratable acidity were determined. Diameter measurements were taken from 20 randomly selected fruits of each replicate. To that end, the equatorial region of the fruit was measured with a Vernier caliper. The tomato colour was determined using a portable Minolta CR-400 colourimeter (Minolta Corp., Tokyo, Japan) with illuminant D65, an observer angle of 0 ° and calibration with a white extension using the CIE Lab system to obtain parameter L, which indicates the colour luminosity (0 = black, 100 = white), chromaticity (C *) and hue angle (H). Five fruits from each replicate were evaluated, and two readings per fruit were performed at two opposite points of the equatorial region.

In addition, ten fruits without seeds for each replicate were crushed with a portable food processor (MONDIAL Super Centrifuga Premium), and the obtained juice was analysed for soluble solids and titratable acidity. A manual digital refractometer (ATAGO Palette, PR-101) with a 0.1% accuracy was used to determine the total soluble solids, and the results were expressed as total soluble solids (%). The titratable acidity was estimated by titrating 10 grams of homogenized juice, which was diluted in 90 mL of distilled water with a standard solution of 0.1 N sodium hydroxide (NaOH). The turning point was a pH of 8.1, and the result was expressed in grams of citric acid per 100 g of juice. Finally, the ratio was determined to relate the soluble solids and titratable acidity values.

2.6. Statistical analysis

The experimental design was a randomized complete block with a factorial design of 2x5; there were 2 planting densities, 5 soil covers (4 mulches plus bare soil), with 3 replicates of 28 plants each one of them. The data were submitted to an analysis of variance (ANOVA) and the averages were compared by Tukey's test at a 5% probability using Sisvar 5.6 software.

3. Results

3.1. Tomato vegetative growth

Both the planting density and the mulch had no effect on the heights of the plants, and their values ranged between 6.22 and 5.38 cm. However, differences were observed in the roots only between cover treatments in high-density plots, in which the roots of the control treatments were found to be longer than they were in the red and gray treatments, at 46 and 52%, respectively (Fig. 1A). In addition, the roots of these treatments were also longer than the roots of the low-density control treatments, and they were the only colour treatments affected by the crop density.

However, in relation to the dry matter, a similar response to the previous figure was observed in the low-density treatments in which the coloured mulches did not affect the dry mass when compared to the black mulch and control treatments (Fig. 1B). This situation was observed in both the shoots and roots. By contrast, within the high-density treatment, plants grown over blue, red and gray/black mulches have shown high weights, surpassing the control by 145, 111 and 79%, respectively, while only red mulch had a better performance than black mulch. At the roots, the situation changes, and all the mulches, independent of their colour, influenced the accumulation of dry matter in the roots. These mulches promoted greater growth than the cover-free treatment, given that there were no significant differences between the covers. Regarding the effects of the plant density, only control plants were affected, and they presented low growth in the high-density plots.

In low-density treatments, there were no differences between the mulches in the leaf area (Fig. 1C), and the average between them was 49.90 cm^2. Nevertheless, the leaf area in the other spacing was greatly influenced by the mulches. Thus, the red mulch promoted a greater leaf area (61.10) than the black mulch (37.56) and the treatment without cover (27.18). Moreover, while the plants grown in bare soil presented a lower leaf area in the high-density plots, those that were grown with gray/black, red and blue mulch were not affected and exhibited more leaf area than the control plants.

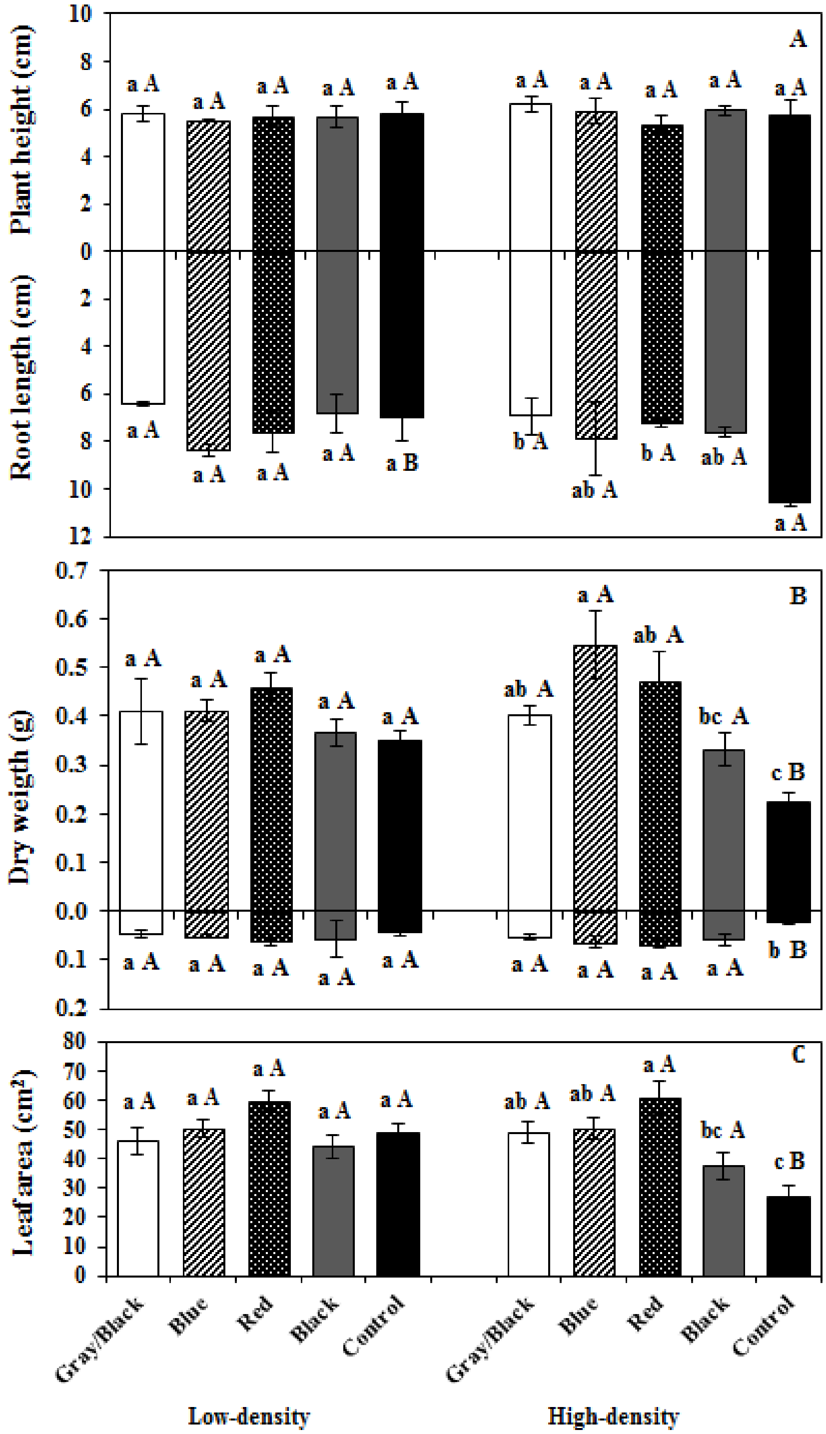

Fig. 1. Influence of the coloured covers and plant density on the vegetative growth. A: Plant height and root length; B: dry weights of shoots and roots; and C: leaf area. Lowercase letters indicate the significant difference between mulches; capital letters represent significant differences between planting densities (Tukey's test 5%).

3.2. Pigments

In general, an equal amount of chlorophylls was found in all the treatments, with the exception of chlorophyll B in the low-density treatments, which increased by 57.61% and 50.78% compared to the control (bare soil) in plants grown over blue film and black film, respectively, with differences between the two planting densities (Fig. 2). In addition, the carotenoid concentration was affected by the use of mulch and the density. The leaves developed over red mulch accumulated an equal amount of carotenoids as the control treatment, given that both had lower concentrations compared to the other mulch colours in the low-density plots. In the high-density treatment, the carotenoids in the mulch treatments did not differ from the control, and the leaves treated with gray/black and red mulches had carotenoid values lower than that of the black mulch.

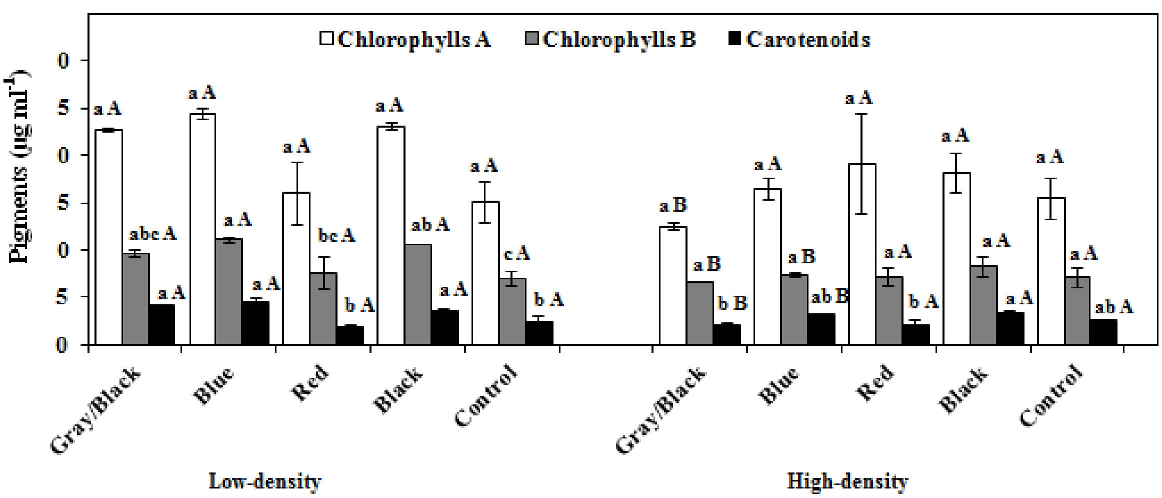

Fig. 2. Average content of chlorophylls and carotenoids for colour mulch treatments on tomato leaves. Lowercase letters indicate significant differences between mulches; capital letters represent significant differences between planting densities (Tukey's test, 5%).

Table 3 shows the PSII photochemical activity, which is expressed as a measurement of the Fv/Fm, and the apparent electron transport rate (ETR), both indicating the activity of the photochemical work.

The Fv/Fm indicates the extent of photoinhibition. When compared between cover treatments, the highest Fv/Fm values were determined for plants that were exposed to red mulch at both plant densities. Nevertheless, it was not possible to say that there was a decrease in a specific plant density, given that the results did not show a definite trend.

Table 3. The Fv/Fm, maximum apparent photosynthetic transport rate (ETRmax), and saturating photosynthetic photon flux density (PPFDsat) values resulting from the coloured mulches and plant density.

Mulch color	Fv/Fm		ETRmax (µmol m^{-2} s^{-1})		PPFDsat (µmol m^{-2} s^{-1})	
Low- density						
Control	0,45	b A	84,18	ab A	530,1	a A
Black	0,17	d B	65,78	c B	451,1	ab A
Red	0,58	a B	91,53	a B	421,6	ab A
Blue	0,33	c A	71,62	bc A	483,7	ab A
Gray/black	0,33	c B	64,52	c B	356,9	b A
High-density						
Control	0,16	d B	54,59	c B	589,9	a A
Black	0,43	b A	76,93	b A	465,6	ab A
Red	0,67	a A	103,43	a A	447,6	b A
Blue	0,28	c B	75,21	b A	436,0	b A
Gray/black	0,46	b A	83,84	b A	353,5	b A

Lowercase letters indicate a significant difference between mulches; capital letters represent significant differences between the planting densities (Tukey's test, 5%).

Moreover, plants grown over red mulch had higher ETRmax values than the other treatments, with maximum ETR values of 103.43 and 91.53 µmol m^{-2} s^{-1} for high-density and low-density treatments, respectively. In the high-density treatment, black-only, gray/black and red mulches presented values that were significantly higher than those in the low-density treatment. The opposite result occurred in the control treatment, and in blue mulch, significant differences were not detected in the ETRmax between the studied densities.

A PPFD analysis did not show significant differences between the density treatments. However, in both situations, the control treatment exerted a greater increase in the PPFD than the gray/black mulch and similar values when compared to black mulch (Table 3).

3.3. Soil temperature

During the growing period, the soil temperature at a 5 cm depth in the control treatment was lower than that of the mulch treatment. However, it started increasing during the last weeks of November. In fact, at the end of the crop cycle, the soil temperatures of red mulch and the control (bare soil) showed no difference, with an average of 2 °C below that of other treatments with mulch (Fig. 3A). Moreover, during the first period, the temperature above the soil was similar in all the treatments, with the exception of blue mulch, which was 1 °C lower on average. Nevertheless, in the middle of the cycle, the temperatures recorded in the treatment with red mulch decreased and remained 2 °C cooler than the other treatments until the harvest, while they did not differ between them and exceeded 34 °C (Fig. 3B).

The temperatures above the mulch showed a similar behaviour to that of the underground temperatures. Thus, the control treatment remained 1-3 °C lower than that of the mulch treatments, increasing only during the last two weeks. Additionally, during this period, the temperature was higher than that of the red mulch treatment, such as those in the other mulches, which reached temperatures of 33-36 °C at the end of the period (Fig. 3C).

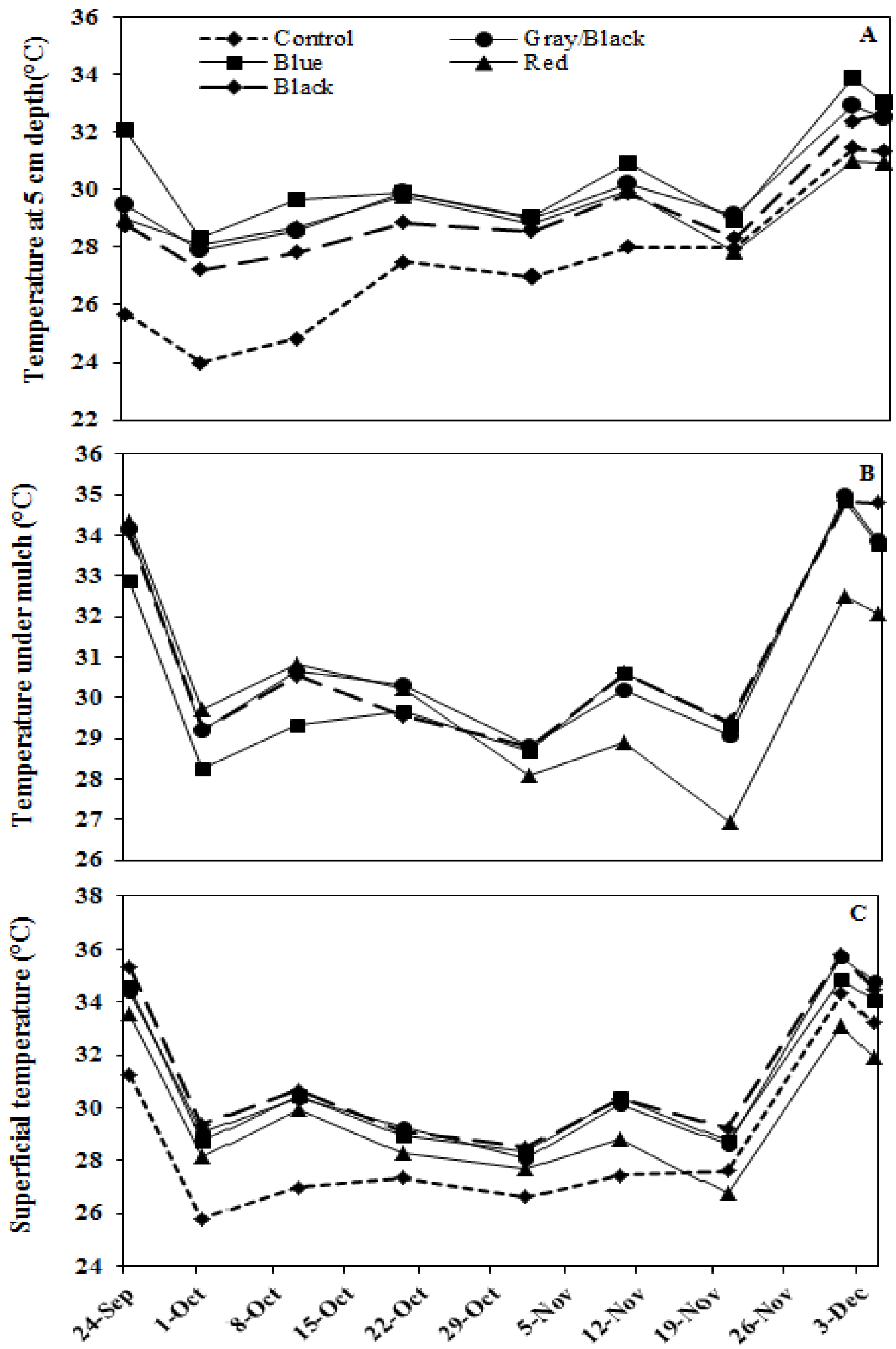

Fig. 3. Mean soil temperature (°C) during the plant growing period in the lower plant density treatments. A. Temperature at 5 cm depth; B. temperature under mulch; and C. temperature above mulch (superficial).

Clearly, the leaf temperature was affected by the use of coloured mulch (Fig. 4). The leaves of the control treatment had a lower temperature than the leaves of the treatments that included mulch, at an average of 23.33 °C. Treatments with gray/black and blue mulch were statistically similar to black mulch in the high-density plots. However, with the exception of gray/black mulch, the leaf temperature under black mulch was significantly higher than that of all the treatments in the low-density plots. The planting density had no influence on the leaf temperature values.

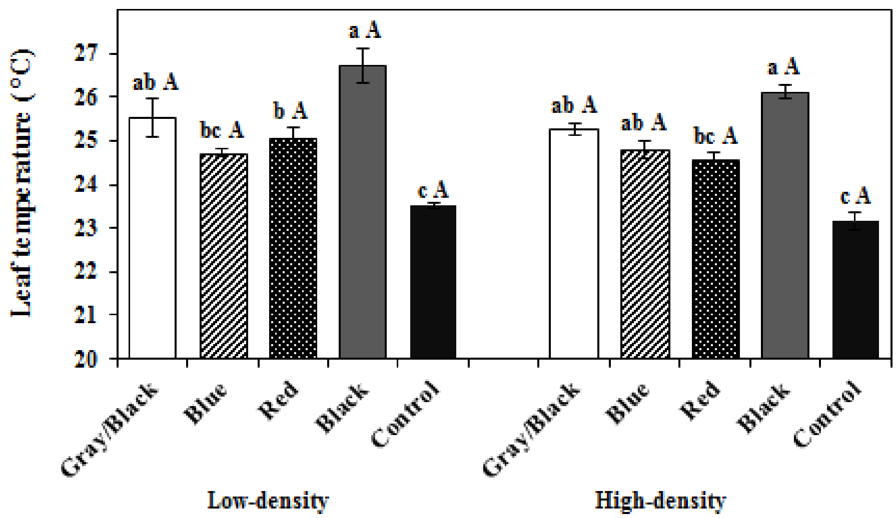

Fig. 4. Influence of the coloured mulch and planting density on the leaf temperature. Lowercase letters indicate the significant difference between mulches; capital letters represent significant differences between planting densities (Tukey's test, 5%).

3.4. Phenology, fruit yield and quality

The total fruit weight per plant and the fruit weights were significantly affected by the plastic mulch. Both the yield per plant and the fruit weight in the treatments with red mulch and gray mulch were higher under the control treatments at the two densities. The yield per plant in the high-density treatment showed no differences between red, gray/black and blue coverings, and they presented better performance compared to the control, with increases of 50, 52 and 69% in the fruit yields, respectively, despite the decreased yield per plant with red and gray/black mulches in high-density treatments when compared to the low-density treatment. A similar situation between mulches was observed in the weights of the fruits (Table 4), which were superior to the control with coloured mulches, and in addition, the use of red and blue covers resulted in heavier fruits than the black mulch treatment. In addition, there were no observed differences in the fruit weights when the densities were compared.

Table 4. Tomato yield (g per plant, number of fruits per plant, and g per fruit) over plastic mulches and the control treatment at both plant densities.

	Tomato yield					
Mulch colour	g per plant		n° fruits per plant		g per fruit	
Low-density						
Control	22.14	c A	8.75	ab A	3.31	b A
Black	27.45	bc A	8.58	b A	3.86	ab A
Red	48.10	a A	13.20	a A	4.59	a A
Blue	30.14	bc A	8.33	b A	4.30	a A
Gray/black	36.16	b A	10.30	ab A	4.04	a A
High-density						
Control	18.34	b A	7.00	a A	2.99	c A
Black	27.15	ab A	9.25	a A	3.44	bc A
Red	27.96	a B	8.33	a B	4.18	a A
Blue	31.05	a A	9.33	a A	4.20	a A
Gray/black	27.96	a B	8.50	a A	3.96	ab A

Lowercase letters indicate significant differences between mulches; capital letters represent significant differences between planting densities (Tukey's test, 5%).

However, the number of fruits in the control treatment was equal to those that had coverage in the low-density treatment, but the fruit number over red mulch was superior to that of black mulch. There were no observed differences between the mulches in the high-density treatment. Furthermore, for this variable, a difference between both densities was observed under the red treatment, and the highest value corresponded to the low-density treatment.

By contrast, the flowering date showed no significant change under the coloured mulch, but it significantly affected the fruiting date in the high-density plots (Fig. 5). Plants grown under the control treatment and blue mulch had similar behaviours, and both exhibit earlier fruiting under the gray/black mulch (approximately 4 days) than the same mulch colour under the low-density treatment. Additionally, coloured covers had no influence on the fruiting date in these plots. Finally, in the low-density treatments, plants grown with red and blue mulches experienced a reduced fruiting period, i.e., the number of fruits produced remained stable relative to the plants grown with black mulch, with durations of 17 days and 31 days, respectively.

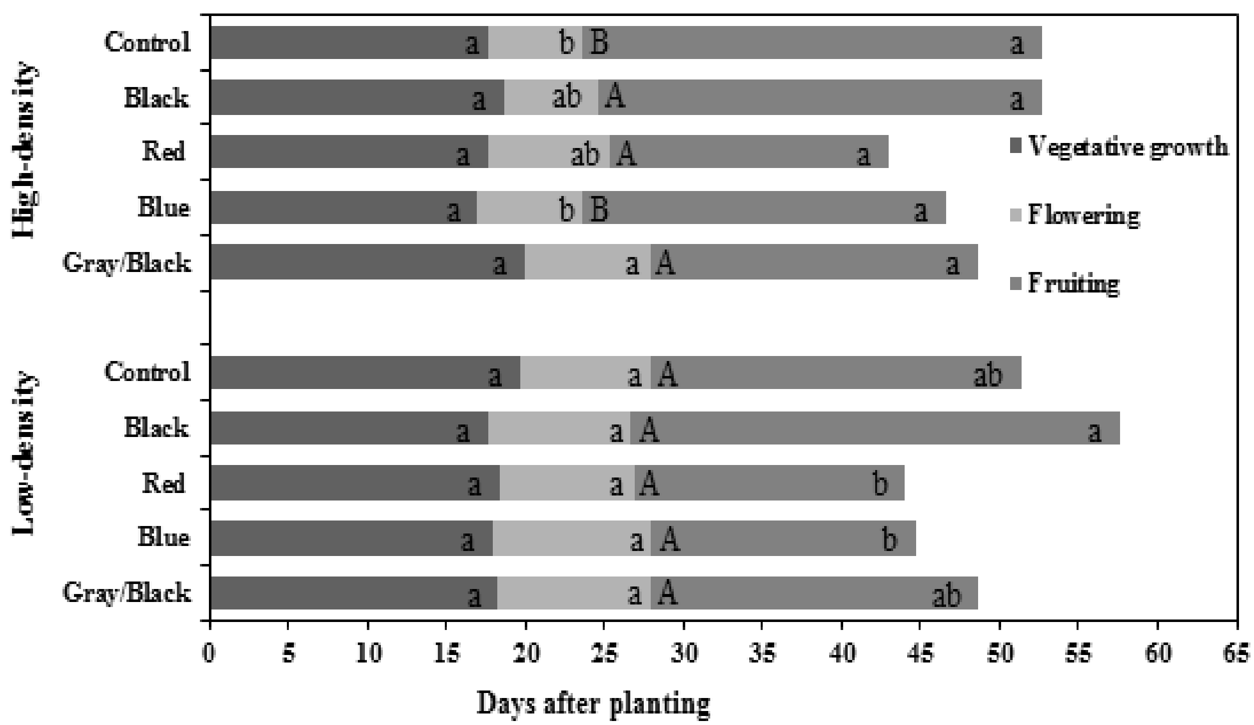

Fig. 5. Influence of the coloured mulch and planting density on the date of the first flowering, fruit initiation, and fruiting (appearance of the first fruit), and the end of fruiting (number of fruits stable). Lowercase letters indicate significant differences between mulches; capital letters represent significant differences between planting densities.

In general, the planting density did not influence the colour of the fruits, except for the chromaticity value in the control treatment, which showed a lower value in the high-density treatment when compared to the control treatment in the low-density treatment. In addition, the Hue value in the red mulch treatment was superior in the high-density treatment with respect to the other one, which indicates that the fruits had a red colouration with a tendency to become yellow when they were planted with greater spacing. Regarding the colour of the mulch, the fruits that developed on red and black mulches had greater chromaticity than the control. However, no significant differences were observed in the other colour variables (Table 5).

Table 5. Influence of coloured mulch and the density on the colour (L: luminosity; C: chromaticity; and H: Hue), soluble solids, acidity, ratio and fruit diameter.

Mulch colour	Colour variables			SST (°Brix)	TA (g cit ac/100ml)	Ratio (SST/AT)	Diameter (cm)
	L	C	H				
Low-density							
Control	43.7 a A	30.9 a A	34.6 a A	5.20 a A	0.83 a A	6.30 a B	1.8 b A
Black	44.2 a A	33.1 a A	33.9 a A	4.75 ab A	0.81 a A	5.90 a A	1.9 b A
Red	42.3 a A	30.5 a A	32.0 a B	4.55 b A	0.81 a A	5.62 a A	2.2 a A
Blue	44.0 a A	28.5 a A	36.5 a A	4.65 b A	0.71 a A	6.52 a A	2.0 b A
Gray/Black	44.5 a A	32.3 a A	35.6 a A	4.60 b A	0.84 a A	5.50 a A	2.0 b A
High-density							
Control	39.8 a A	26.3 b B	34.0 a A	5.35 a A	0.65 a B	8.24 a A	1.9 a A
Black	45.1 a A	33.1 a A	36.0 a A	4.85 ab A	0.81 a A	6.04 a A	1.9 a A
Red	44.5 a A	33.9 a A	37.7 a A	4.45 b A	0.78 a A	5.70 a A	2.1 a A
Blue	41.6 a A	31.1 ab A	39.1 a A	4.95 ab A	0.74 a A	6.74 a A	2.0 a A
Gray/Black	43.8 a A	28.8 ab A	36.3 a A	4.70 b A	0.75 a A	6.27 a A	2.0 a A

Lowercase letters indicate significant differences between mulches; capital letters represent significant differences between planting densities (Tukey's test, 5%).

However, there were significant differences in the °Brix caused by the mulch, with the control surpassing the red and gray treatments at both densities in addition to the blue mulch in the low planting density. However, there was no apparent influence by the mulches on the total acidity. In this variable, only the control treatment was affected by the density, in which the fruits were less acidic in the high-density treatment (0.65). The same situation occurred with the ratio, because the control treatment did not differ significantly from the treatments with coverings, and they did not differ between them. However, the ratio in the control group (8.24) was higher than that of the control in the low-density treatment (6.30) (Table 5).

In addition, with respect to the diameter, there were no significant differences between the mulches in the high-density treatment, with an average of 1.98 cm. However, the red treatment outperformed the control (22 %) and the other colours in the low-density treatment, indicating that the planting density did not influence the fruit diameter (Table 5).

4. Discussion

Many studies have shown that tomato fruit growth is a process that is determined by the interaction between the genetic potential of the species and the impact of the environment that the plant experiences during crop and fruit growth (Ortiz et al., 2007; Prudent et al., 2010). Thus, photoselective mulches are used in agriculture because they offer the possibility of combining the benefits of traditional mulch with their light properties, which regulate different processes such as photosynthesis, crop yield (Shiukhy et al., 2015) and the fruit phytochemical quality (Layne et al., 2001) through pigments, such as phytochromes.

In general terms, plant morphogenesis is strongly influenced by the quality of light that reaches the plants (Whitelam and Halliday, 2007). Many studies have demonstrated that a plant can adjust its carbon allocation patterns in a shade-avoidance response when neighbouring plants are detected, which occurs in high-density plots in which they become taller and more competitive for the light resource (Franklin and Whitelam, 2005). This can also occur in response to a low R/FR ratio in the light reflected from the mulches (Demotes-Minard et al., 2016). However, our results did not support that conclusion. The coloured mulches and plant density have both not affected the plant height, probably because the miniature 'Micro-Tom' tomato is a dwarfing tomato with determined growth (Fig. 1A).

In low-density treatments, differences in the vegetative growth have not been observed with the use of coloured mulches compared to black mulch treatments and control plants, which did not coincide with the results reported by Rajablariani et al. (2012). It was expected that the light reflected from the coloured mulches can act through the phytochrome system, leading to positive effects on the plant heights.

Although there was no difference in the vegetative growth of the low-density treatments, improvements in high-density plots were evidenced by increases in the leaf area and dry weights of plants grown with coloured plastic mulch. Thus, plants that were grown with red, blue, and gray/black mulches presented greater leaf areas than the control plants (Fig. 1C). The dry mass accumulation is directly related to radiation interception, so increases in the total light captured by leaves could have enhanced the photosynthesis process with repercussions for the dry weight, especially in plants grown over blue and red mulches (Fig. 1B). These mulches reflect higher percentages of blue and red light, respectively, and higher PPF values compared to the control and black mulch treatments, which reflect the low light percentage that reaches them (Table 2). In that way, both the red and blue wavelengths reflected from blue and red plastic mulches stimulate the absorption of carbon dioxide required to produce photoassimilates, and at a specific level, blue light can stimulate photosynthesis by inducing stomatal opening (Shimazaki et al., 2007) or through morphological adjustments, such as an increase in the leaf mass per area. Moreover, the higher reflected PPF values could increase the photosynthesis rate, although photosynthesis was not measured in this experiment. In high-density plots in which the light quality is normally diminished, coloured mulches offer the possibility of promoting growth and improving plant performance.

In addition, our study supports that reflected light from coloured plastic mulches can also influence the pigment concentrations in leaves (Fig. 2). Although red and blue mulches reflected higher amounts of PPF, the same chlorophyll A content in all the treatments leads one to believe that the light harvesting efficiency at the leaf level was the same. However, plants grown over blue mulch showed a higher accumulation of chlorophyll B and carotenoids, while the response in the red mulch treatment was similar to that of the control in the low-density plots (Fig. 2). Chlorophyll B and carotenoids are accessory pigments that aid in the absorption of light and in the transfer of radiant energy to the reaction centres, with a protective role towards the photosynthetic apparatus, preventing the photooxidation of chlorophylls (Domonkos et al., 2013; Esteban et al., 2015). This role would justify the finding in which the blue mulch treatment was not different from the control in the high-density treatment, in which the light competition is greater due to shading by neighbouring plants. According to Loreto et al. (2009), a high amount of blue light might be perceived as light stress; therefore, the greater amount of carotenoids in plants grown over blue mulch could be an advantage in terms of protecting the PSII against high irradiance and from blue light reflected from the blue cover when the plant spacing is high. Another important role of these pigments is to increase the light capture in shady environments (Wittmann et al., 2001; Valladares et al., 2003; Sánchez-Gómez et al., 2006), which might be considered as a stress-related response. Thus, this finding could explain the higher carotenoid content in plants grown over black mulch in both density situations (Fig. 2).

The Fv/Fm is considered a measure of the potential quantum yield of photosystem II after dark adaptation (Murchie and Lawson, 2013). This value should be close to 0.83 (Björkman and Demmig, 1987), and a lower value would indicate the possible photoinhibition of photosynthesis. In the present study, the Fv/Fm values suggested that all the treatments suffered significant stress from photoinhibition (Table 3). Moreover, plants grown over red mulch had a higher maximum apparent electron transport rate (ETRmax) when compared to other mulch colours and bare soil. The ETR value represents the relative quantity of electrons that pass through PSII in photosynthesis during steady-state (Tezara et al., 2003). Thus, this finding could suggest the greater activity of the photochemical work of plants (Table 3).

The probable reflected radiation is not the only factor contributing to the growth of tomato plants. Our data have demonstrated that plastic film mulch can increase the soil temperature (Fig. 3). Hence, this factor may be influencing the changes as well. Beyond their light properties, coloured mulches are also known to affect the microclimate around the plants, altering the temperature conditions by influencing the thermal properties (the reflectivity, absorptivity, or transmittance). In the present paper, a soil temperature to 5 centimetres deep under blue and gray mulches was similar to that of black mulches during the end of the experiment. However, this temperature was 2 - 3 °C higher than that of the soil without a cover and with red mulch (Fig. 3A). The temperature under red mulch was 2 °C lower than all the treatments that are able to reach a temperature of 35 °C (Fig. 3B). These data suggest that in summer crops, red mulch could modulate the root zone microclimates, keeping the soil temperatures within a more favourable range for plant growth compared with the conventional black mulch, which tends to warm the soil due to its high absorbance of visible light (Hochmuth et al., 2008).

At the same time, all the coloured mulch treatments exhibited temperatures that are more favourable for culturing during the early and middle periods over the mulch or soil surface (in the case of the control treatment), considering that the optimum root-zone temperature for tomato is 26 °C, while mean seasonal root-zone temperature >27 °C causes stress (Díaz-Pérez et al., 2007) (Fig. 3C). The root zone temperature has been found to affect the rates of biochemical processes and influences physiological processes in the roots such as the uptake of water, mineral nutrients, gas exchange, and the activity of various enzymes (Dodd et al., 2000; Ibarra-Jimenez et al., 2008; Subrahmaniyan and Zhou, 2008), which could indicate a positive effect by mulches on the microclimate in the root zone and crop yield compared with bare soil in cooler regions.

However, the effects on the leaf area from the manipulation of the microclimate light quality may affect early and late fruiting and extend the harvest season. Additionally, accelerated flowering occurs in response to low R/FR ratios (Whitelam and Halliday, 2007; Demotes-Minard et al., 2016), as demonstrated in some studies on tomatoes (Kasperbauer and Hunt, 1998; Decoteau, 2007). Nevertheless, our results have not reflected this situation clearly, perhaps because the differences between the R/FR ratios reflected from mulch have not been sufficiently higher than that of the control (Fig. 5).

Additionally, a higher yield is a benefit of using coloured mulch relative to bare soil (Table 4). Our data showed that fruit production on the red and gray/black covers was superior to that of the control treatment. However, in high-density plots, besides these findings, the blue mulch also improved the yield. Therefore, modifications to the plant light environment and/or root zone temperatures due to mulch have positive effects on the fruit production in high-density plots. In the case of red mulch, the higher reflected R/BL and R/FR photon ratio versus the standard black plastic mulch or control treatment could have influenced the allocation of relatively more photosynthates to fruits, since red light has by far the highest quantum yield for CO_2 fixation compared to other wavelengths in the photosynthetic active light spectrum (Hogewoning et al., 2012; Dueck et al., 2016). In addition, the red and blue light reflected from coloured mulches could have improved the fruit weight in comparison to black mulch due to the optimization of the photosynthesis process because the black mulch absorbs most of the light that reaches it.

Our results suggest that the variability in the yield primarily depended on the fruit weight, with the mean number of fruits being practically constant in the different treatments within both planting densities.

Conversely, our data showed that the fruit quality components are not enhanced by coloured plastic mulches, except for the fruit diameter, which was improved by red mulch (Table 5). Thus, the results of this study clearly showed that red plastic mulches positively affect the tomato fruit size and weight in the treatments with higher plant spacing. The increased fruit size is a benefit because consumers are attracted to big fruits, and hence the price on the market is higher.

Moreover, the bark colouration is the most important external characteristic for determining the degree of tomato ripening (Batu, 2004; Kim et al., 2015). Tomatoes with lower H and L indicate a red and darker colouration as a product of ripening, but differences have not been found between the covers and density treatments (Table 5). In relation to the chromaticity, differences have been observed between mulches in high-density treatments alone. High chromaticity values indicate saturation by the red colour, although this is not a good indicator of tomato ripening because, during the ripening, fruits present simultaneously different colours, which is the result of processes such as chlorophyll degradation and carotenoid syntheses (Lopez-Camelo and Gómez, 2004).

In addition, our results suggest that increased production could occur to the detriment of the soluble solids concentration because the control treatment showed lower fruit yields and more soluble solids than other treatments (Table 5). The soluble solid amount and type of sugars are important components of the post-harvest quality of tomatoes, affecting the fruit taste and overall quality, and their increase during the post-harvest period in tomatoes is related to the degradation of polysaccharides into simpler sugars, due to the ripening process. However, coloured mulches did not cause a significant difference in the titratable acidity between treatments (Table 5). In the same way, with the exception of the control treatment, the plants did not respond differently to the spacing. The total titratable acidity in the fruits indicates the amount of organic acids and astringency that are present (Ferreira et al., 2010). Thus, the desirability of the tomato taste will depend on the ratio of the sugars and acids present; thus, low sugar levels and high acid produced an acidic taste. High levels of sugars and low acid will give way to a softer flavour. When the sugars and acids are low, the result is an insipid tomato (Kader, 1986; Schouten et al., 2016). However, the ratio has not been affected by the treatments in our experiment (Table 5).

5. Conclusions

In the present study, we analysed the Micro-Tom tomato responses to four mulch treatments and two planting densities. The results demonstrated that coloured mulches enhance the plants growing in high-density plots, as primarily reflected by the dry weight and leaf area. In high-density plots, the total fruit yields per area of all the plastic mulch treatments were higher than the control, while differences have not been found between them. Clearly, coloured mulches affected the leaf area, enhancing the tomato yield and fruit weight, especially for red mulch when compared to traditional black mulch and bare soil. This finding is due to the modifications in the light environment of the plants and the more appropriate temperatures for conditions in this location, as achieved through the use of mulch, enabling the growth of a greater plant number per area.

6. Acknowledgements

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