

Inorganic solar filters incorporated to carnauba wax and incidence of citrus black spot on tangerine

Abstract – The objective of this work was to evaluate the effect of zinc (ZnO) and titanium (TiO₂) oxides, added to carnauba wax emulsions, on the incidence of citrus black spot (CBS) caused by *Phyllosticta citricarpa* and, consequently, on tangerine quality. Wax emulsions blended with ZnO or TiO₂ were sprayed up to concentrations of 8% on late-season tangerine cultivars. Three experiments were carried out in a randomized complete four-block design, with two control treatments – one with the application of only the carnauba wax emulsion and the other without the application of the emulsion. The spraying of ZnO and TiO₂ reduced the incidence of the disease on the harvested fruit; however, the increase in the concentrations of the oxides did not improve CBS control. Two consecutive applications of the oxides reduced the photosynthetic activity of the plants, negatively affecting yield, and caused damage to fruit peel. Tangerines infected artificially with 10⁵ conidia mL⁻¹ of *P. citricarpa*, before or after the sprayings of the photoprotective films, showed a reduced CBS incidence. The treatments with the application of only carnauba wax do not differ from those with films combined with ZnO and TiO₂ regarding the control of CBS incidence. The addition of inorganic oxides to the carnauba films limits the photosynthetic activity and reduces the yield of the plants, besides damaging the visual quality of the tangerines.

Index terms: *Citrus deliciosa*, false melanose, hard spot.

Filtros solares inorgânicos incorporados à cera de carnaúba e incidência de mancha preta em tangerinas

Resumo – O objetivo deste trabalho foi avaliar os efeitos dos óxidos de zinco (ZnO) e titânio (TiO₂), adicionados à emulsão de cera de carnaúba, sobre a incidência de mancha preta dos citros (MPC) causada por *Phyllosticta citricarpa* e, conseqüentemente, sobre a qualidade de tangerinas. Emulsões de cera com adição de ZnO ou TiO₂ foram aplicadas a concentrações de até 8% em cultivares tardias de tangerinas. Foram realizados três experimentos, em delineamento de quatro blocos completos ao acaso, com dois tratamentos controle – um com a aplicação apenas da emulsão de cera de carnaúba e outro sem a aplicação da emulsão. As pulverizações com ZnO e TiO₂ reduziram a incidência da doença em frutos colhidos; no entanto, os aumentos nas concentrações dos óxidos não melhoraram o controle da doença. Duas aplicações consecutivas dos óxidos inibiram a atividade fotossintética das plantas, tendo afetado negativamente a sua produção, e causaram danos na casca das frutas. Tangerinas infectadas artificialmente com a suspensão de 10⁵ conídios mL⁻¹ de *P. citricarpa*, antes ou depois da pulverização dos filmes,

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apresentaram menor incidência de MPC. Tratamentos com a aplicação apenas da emulsão de carnaúba não diferem dos com filmes preparados com ZnO e TiO₂ quanto ao controle da MPC. A adição de óxidos inorgânicos aos filmes de carnaúba limita a atividade fotossintética e reduz a produtividade das plantas, além de prejudicar a qualidade visual das tangerinas.

Termos para indexação: *Citrus deliciosa*, falsa melanose, mancha dura.

Introduction

Citrus production is of great economic and social importance for the Brazilian fruit industry; however, it is affected by a number of phytosanitary problems, among which citrus black spot (CBS) is one of the main diseases. Caused by the fungus *Phyllosticta citricarpa*, CBS devalues the fruit, mainly those destined for fresh consumption. CBS is responsible for increases of the production costs because of successive fungicide sprays required for its control (Silva Junior et al., 2016). The symptoms of the disease are restricted to the citrus flavedo and, typically, a higher incidence of lesions is observed on the sunlight-exposed area of the fruit. On fruit located in the highest part of the citrus tree canopy, the incidence of disease is three times higher than the incidence on fruit of the lower part of the canopy (Kotzé, 1981).

Some studies conducted in Brazil report the severity of CBS on the planting arrangement of the orchards. In a northeast-southwest orientation of the planting scheme in which the fruit are exposed to more direct solar incidence, plants were considerably more affected than that on fruit of a grove with north-south orientation, according to Andrade et al. (2009).

In vitro tests evidenced a larger production of pseudothecia and ascospore of *P. citricarpa* in Petri dishes incubated either under a 12-hour photoperiod, or continuous light, than in the incubation under complete darkness (Timossi et al., 2003). These data indicate that light affects the pathogen significantly and positively, in the infecting processes and symptom appearance. Therefore, the use of coatings capable to lessen the incidence of radiation on fruit surfaces could suppress the fungus and hold back the disease.

The film technology with inert particles is being used to reduce sunburn on fruit and, in some cases, has proven efficacy in the reduction of damages caused by

insects and pathogens (Sharma et al., 2015). Several commercial products available in foreign countries are formulated mainly on the basis of kaolin, such as Cocoon, Parasol, Purshade, Screen, Snow, and Surround WP. There are also wax-based formulations such as Raynox, a formulation of carnauba wax and organoclay (Schrader, 2011). This formulation is not yet available in the market for Brazilian fruit growers. However, there is a high availability of carnauba wax that has been used in formulation of emulsions as coating for fruits and vegetables, with positive effects on the shelf life extension and visual quality of the products (Pereira et al., 2014; Thu & Tanachai, 2016).

The organic photoprotecting capacity conferred by cinnamates of carnauba wax (Schrader, 2011) could be complemented with the addition of inorganic solar filters, such as zinc oxide or titanium dioxide. These molecules are used as skin photoprotectants because of their ability to absorb, reflect, and scatter visible and UV radiation (Flor et al., 2007; Sambandan & Ratner, 2011; Stiefel & Schwack, 2015). Besides, zinc oxide and titanium dioxide are also used as food additives (Boccio & Monteiro, 2004; Oliveira et al., 2006).

The objective of this work was to evaluate the effect of zinc (ZnO) or titanium (TiO₂) oxides, added to carnauba wax emulsions, on the incidence of citrus black spot and, consequently, on tangerine quality.

Materials and Methods

The influence of solar filters was investigated in field trials for late season tangerine cultivars, in an area of typical humid subtropical climate with hot summers, and average temperature at about 25°C. Two experiments were carried out for 'Rainha' tangerine (*Citrus deliciosa* Tenore) grafted onto *Poncirus trifoliata*, in an eight-year-old commercial orchard located in the municipality of Pareci Novo (29°37'S, 51°25'W), in the state of Rio Grande do Sul, Brazil. The area has a history of CBS incidence and it is managed under the conventional production system, in which diseases and pests are controlled by means of agrochemical sprays.

A third experiment was conducted in a 31-year-old experimental orchard of 'Montenegrina' (*C. deliciosa*), which was also grafted onto *P. trifoliata*, in the experimental agronomic station of Universidade Federal do Rio Grande do Sul (UFRGS). The area is

located in Eldorado do Sul (30°06'S, 51°39'W), about 75 km southwest of the first area, and it has also a history of CBS incidence. The plants did not receive phytosanitary treatments all over the experiment.

The evaluated treatments were the carnauba wax emulsions blended with inorganic filters; the controls were a treatment with the application of only carnauba wax emulsion and another without the application of the emulsion. The emulsion was prepared with a 15% oil phase, 10% of which was carnauba wax and 5% soybean oil. Tween 80 and Span 60 were used as emulsifiers and surfactants. Inorganic filters were added later to the emulsion, as follows: micronized zinc oxide (ZnO) (Merck, Darmstadt, Germany); nanoparticle of anatase titanium dioxide T (TiO₂-T) (Merck); titanium dioxide V Rutilite T-2000 (TiO₂-R), (Merck); and the formulated product Escalol (International Specialty Products, ISP, Tadworth, UK), which consists of 40% micronized titanium dioxide with the addition of dispersants. The inorganic filters were supplied by Fagron Tech (Jundiaí, SP, Brazil).

The first experiment was installed in the 2014/2015 growing season, and the sprays were applied when fruit were at 40 mm average diameter of (April 2015). The films were diluted in water and applied to fruit with hand sprayers until the point of run off. For each inorganic filter ZnO, TiO₂-T or TiO₂-R and Escalol Block at of 2, 4, 6, and 8% concentrations were tested. Control treatments were emulsion spraying of only carnauba wax or no emulsion spraying.

The experiment was carried out in a randomized complete block design, as follows: four blocks and one plant as experimental unit, from which 50 fruit were sprayed at random. The incidence of CBS on tangerines was determined at the time of harvest (August of the same year) by counting the symptomatic fruit. After harvest, symptomless fruit were stored at 3±1°C, and the upsurge of CBS symptoms was visually evaluated 30 days after.

The second experiment was conducted in the 2015/2016 growing season, in a randomized complete block design of four blocks and one plant as experimental unit. Photoprotective films were applied twice to plants. At the first time, spraying was performed when fruit were at 18 mm average diameter (on December 2015). The second round of sprays was applied when fruit had reached 50 mm diameter (on May 2016). Five treatments were applied: the controls

(no spray application to the plants and carnauba wax emulsions only), and the photoprotective films: carnauba wax emulsions complemented with either ZnO, TiO₂-T or TiO₂-R at a 2% (w/v) concentration.

The formulations were diluted in water and sprayed to the whole plants with a 20 L backpack sprayer until run off. Between the first and second spray treatments, the photosynthetic activity was determined on four well-developed leaves from every quadrant of the tangerine trees with a LI-6400XT portable photosynthesis system (Li-Cor, Lincoln, NE, USA), with a camera equipped with a LED light source and light density at 1,000 µmol m² s⁻¹. At harvest, in August, yields per treatment were quantified on samples of 50 fruit selected at random, and the CBS incidence was visually determined. These fruit were also evaluated for peel color and juice contents for soluble solids (SS) and titratable acidity (TA). The SS content (°Brix) was determined with a benchtop refractometer. The TA (% citric acid) was determined by titration up to pH 8.1 of 6 g juice, which was diluted in 50 mL distilled water with 0.1 mol L⁻¹ NaOH solution, on a pHmeter. Peel color was determined with a Konica/Minolta model CR 400 colormeter (Ramsey, NJ, USA), and the color index (CI) was calculated according to Vidal et al. (2013), using the coordinates L*, a* and b*, in the following equation:

$$CI = 1000 \times a^* / (L^* \times b^*)$$

The third experiment was carried out in the 2016/2017 growing season, in a completely randomized design with four replicates and one plant as experimental unit. The same treatments of the second experiment were applied to this last trial: carnauba wax emulsion only; carnauba wax emulsions complemented with either ZnO, TiO₂-T, or TiO₂-R at 2% (w/v) concentration; and a control treatment (no film sprayings). The formulations were diluted in water and sprayed directly onto fruit with hand sprayers, until run off, in the region with epidermal color change of the tangerines. The fruit harvest from each experimental unit occurred 30 days after the treatment applications, in September of the same year.

The curative and protective effects of the formulations were determined by artificially inoculating a suspension of *P. citricarpa* at 10⁵ conidia mL⁻¹ concentration (either two hours before or two hours after treatment sprays on the tangerines). Suspensions were prepared from ten-day colonies grown in PDA

(potato-dextrose-agar) in sterilized distilled water. Control fruit also underwent the fungus inoculation and received no further treatment. The fruit were evaluated for quality attributes, SS, TA, and peel color.

All data were subjected to the analysis of variance through the software Proc Mixed of SAS 9.4, at 5% probability. Orthogonal contrasts complemented the analyses. In the first trial, the following five contrasts were built: control vs. protective films; carnauba wax emulsion vs. inorganic filters; Escalol block vs. ZnO, TiO₂ (R) and TiO₂ (T); ZnO vs. TiO₂; and TiO₂ (R) vs. TiO₂ (T). For the last two experiments, the following four contrasts were arranged: control vs. protective films; carnauba wax emulsion vs. inorganic filters; ZnO vs. TiO₂; and TiO₂ (R) vs. TiO₂ (T).

Results and Discussion

In the first experiment, 'Rainha' tangerines – treated with photoprotective films on the basis of carnauba wax emulsion and different concentrations of inorganic filters – indicated two types of CBS symptoms: hard spot and false melanose. Hard spot is a lesion that has a depressed center of light color, with the most prominent edges of dark brown color; in the center of the lesions there are pycnidia. False melanose shows very small and smooth dark spots, without the formation of pycnidia (Feichtenberger et al., 2005).

The concentration increases of each inorganic filter was not enough to show an effect on the two types of CBS symptoms, and it was not possible to adjust it to the linear or polynomial regression. Therefore, an orthogonal contrast analysis was performed to compare the films and selections of inorganic filters. The results of the orthogonal contrast analysis (Table 1) indicate that spraying with photoprotective films resulted in the reduction of the symptoms of false melanose in comparison with the control. However, no effect on hard spot symptoms was observed on the total incidence of the disease. Furthermore, the analysis shows that the emulsion application of carnauba wax only has the ability to reduce the disease, and it does not differ statistically from the spraying of carnauba with the addition of inorganic filters.

Among the inorganic filters, the tangerine sprayed with ZnO had a higher incidence of false melanose symptoms and a lower incidence of hard spot than the fruit treated with the two different forms of TiO₂.

The comparison between both TiO₂ forms (rutile and anatase) showed a distinct response in CBS symptoms. The rutile form (TiO₂ - R) was more efficient in the reduction of hard spot.

The treatments did not affect titratable acidity and soluble solids contents of the tangerines (Table 2). The peel color index of fruit were not affected by the treatments as well, and varied between 5.1 and 6.5. These values represent an intense orange color, which is a desirable characteristic when harvesting tangerines intended for fresh consumption (Jomori et al., 2014). In a comprehensive review on coatings and films fortified with inorganic nanoparticles, such as TiO₂, and applied to fruits and vegetables, Xing et al. (2019) observed that coatings and films maintained the sensory attributes and showed no detrimental effects on a large array of fruit species.

In the analyses of contrasts (Table 3), the index of peel color of fruit treated with protective films did not differ from that of the control fruit, but when compared in between themselves, differences were observed. All treatments with the addition of inorganic filters showed higher values of peel color index than the treatment with sole carnauba wax spray. The distinction was the fruit treated with TiO₂ (T).

In the second experiment, protective films at 2% concentration were applied to tangerine trees, since higher concentrations of both ZnO and TiO₂ (tested in the first experiment) did not enhance the CBS control. The photoprotective films influenced the photosynthetic activity of plants (Table 4). The incidence lowering of the photosynthetically active

Table 1. Orthogonal contrast analysis for the different symptoms of citrus black spot incidence on 'Rainha' tangerine (*Citrus deliciosa*) subjected to the application of protective films, in the first experiment.

Treatment - contrast	Black spot symptom ⁽¹⁾	
	False melanose	Hard spot
Control vs. protective films	0.0135	0.4437
Carnauba wax emulsion vs. inorganic filters	0.8849	0.1756
Escalol Block vs. ZnO, TiO ₂ (R), and TiO ₂ (T)	0.1366	0.6418
ZnO vs. TiO ₂	0.0009	0.0010
TiO ₂ (R) vs. TiO ₂ (T)	0.0293	<0.0001
Analysis of variance (p)	0.0033	0.0099

⁽¹⁾Probability (p) of orthogonal contrasts for black spot incidence. Inorganic filters: ZnO, zinc oxide; TiO₂ R, rutile titanium dioxide; TiO₂ T, anatase titanium dioxide.

radiation onto leaf surfaces might be the reason for lower photosynthesis, since the stomatal conductance did not change due to the treatments. The reduction of photosynthetic activity in some treatments, such as the carnauba wax emulsion complemented with TiO₂ (T), resulted in lower yields of tangerine trees. Santos et al. (2021) also observed lower net photosynthesis and reduced stomatal conductance after applying kaolin-based films to clonal saplings of eucalyptus. Xi et al. (2022) have concluded that coatings on the basis of TiO₂, ZnO, and SiO₂ (silicon dioxide) may impact photosynthesis, they are applied to leaves.

Examining the orthogonal contrasts for photosynthetic activity, it becomes evident that the protective films significantly reduced the photosynthesis, in comparison with tangerine trees without the spraying treatments (Table 5). Complementing the carnauba wax emulsion with inorganic filters resulted in reduced yields of the sprayed trees. The trees treated with the formulation of carnauba wax emulsion with the addition of TiO₂ – T (anatase form) were the most affected.

The parameters juice contents, TA, and SS contents of 'Rainha' tangerines were not affected by the photoprotective film applications (Table 6). In contrast, Wang et al. (2020) concluded that spraying kaolin films resulted in better grape quality, as indicated by fruit higher contents of reducing sugars and lower titratable acidity. These authors also report that there were no symptoms on the visual quality of the treated grapes.

In the present experiment, it was not possible to observe a significant effect of photoprotective films on the incidence reduction of CBS, as the occurrence of damage to fruit of the treatment with TiO₂ (T) may have masked the result (Figure 1). Therefore, for a better evaluation of the treatment effects on the control of CBS, a third experiment was carried out with the artificial inoculation of conidia of *P. citricarpa* on 'Montenegrina' tangerine.

In infected 'Montenegrina' fruit, the timing of the inoculation either before or after spraying treatments did not impact the incidence of CBS (Figure 2). The photoprotective films reduced the disease incidence, in comparison to the control treatment, which was

Table 2. Application effects of carnauba wax emulsions complemented with two inorganic filters (ZnO, zinc oxide; TiO₂, titanium dioxide) on the titratable acidity (TA) and soluble solids (SS) of 'Rainha' tangerine (*Citrus deliciosa*), in the first experiment.

Treatment	Emulsion/filter (%)	At harvest		After 30 days	
		TA (%)	SS (°Brix)	TA (%)	SS (°Brix)
Control	0/0	0.754	10.13	0.613	9.31
Carnauba wax emulsion	20/0	0.844	10.13	0.696	9.19
Carnauba wax emulsion + ZnO	20/2	0.831	10.31	0.629	9.00
	20/4	0.868	10.50	0.599	9.50
	20/6	0.736	9.63	0.598	8.63
	20/8	0.848	11.88	0.706	8.69
Carnauba wax emulsion + TiO ₂ (rutile)	20/2	0.836	10.25	0.693	9.19
	20/4	0.775	9.75	0.463	8.75
	20/6	0.815	10.63	0.678	10.19
	20/8	0.775	9.56	0.502	9.75
Carnauba wax emulsion + TiO ₂ (anatase)	20/2	0.896	10.44	0.697	8.81
	20/4	0.920	10.88	0.513	8.88
	20/6	0.833	10.75	0.797	9.50
	20/8	0.853	10.00	0.653	9.31
Carnauba wax emulsion + Escalol Block	20/2	0.806	9.63	0.558	9.19
	20/4	0.823	9.63	0.646	10.00
	20/6	0.891	9.38	0.596	9.88
	20/8	0.944	10.19	0.599	9.33
Analysis of variance (p-value) ⁽¹⁾		0.1266 ^{ns}	0.4999 ^{ns}	0.8183 ^{ns}	0.1613 ^{ns}

⁽¹⁾Significant at 5% probability. ^{ns}Nonsignificant.

also subjected to inoculation; however, the control was not subjected to any compound application (Table 7). Photoprotective films on the basis of carnauba wax emulsions have the potential to reduce the incidence

Table 3. Analyses of contrast for peel color index of 'Rainha' tangerines (*Citrus deliciosa*) subjected to the application of photoprotective films, in the first experiment.

Contrast – treatment	Peel color index
Control vs. Photoprotective films	0.3871 ^{ns}
Carnauba wax emulsion vs. inorganic filters	0.0086**
Escalol Block vs. ZnO, TiO ₂ (R) and TiO ₂ (T)	0.0045**
ZnO vs. TiO ₂	<0.0001**
TiO ₂ (R) vs. TiO ₂ (T)	<0.0001**
Analysis of variance (p)	<0.0001

* and **Significant means at 5 and 1% probability, respectively, for the complementing orthogonal contrasts. ^{ns}Nonsignificant difference. Inorganic filters: ZnO, zinc oxide; TiO₂ R, rutile titanium dioxide; TiO₂ T, anatase titanium dioxide.

Table 4. Application effects of photoprotective films (based on carnauba wax emulsion (CWE) complemented with different inorganic filters) on the photosynthetic activity, stomatal conductance, and yield of 'Rainha' tangerine trees (*Citrus deliciosa*), in the second experiment.

Treatment	Photosynthesis ($\mu\text{mol m}^{-2} \text{s}^{-1}$)	Stomatal conductance ($\text{mol m}^{-2} \text{s}^{-1}$)	Yield (kg per tree)
Control	4.719	0.050	28.76
CWE	4.067	0.048	30.41
CWE + ZnO	3.163	0.040	25.41
CWE + TiO ₂ (R)	3.575	0.051	26.28
CWE + TiO ₂ (T)	2.395	0.035	14.09
Analysis of variance (p)	0.0347	0.0748 ^{ns}	0.0359

Inorganic filters: ZnO, zinc oxide; TiO₂ R, rutile titanium dioxide; TiO₂ T, anatase titanium dioxide. ^{ns}Nonsignificant difference.

Table 6. Application effects of carnauba wax emulsion complemented with inorganic filters on the quality of 'Rainha' tangerines (*Citrus deliciosa*), in the second experiment.

Treatment	TA (%)	SS (°Brix)	Juice content (%)	Peel color index
Control	1.13	11.38	65.25	6.43
Carnauba wax emulsion	1.07	10.75	63.29	6.62
Carnauba wax emulsion + ZnO	1.02	10.25	68.29	5.94
Carnauba wax emulsion + TiO ₂ (R)	1.15	11.56	62.37	6.52
Carnauba wax emulsion + TiO ₂ (T)	1.20	12.06	61.72	6.11
Analysis of variance (p)	0.0767 ^{ns}	0.0770 ^{ns}	0.1459 ^{ns}	0.3139 ^{ns}

Inorganic filters: ZnO, zinc oxide; TiO₂ R, rutile titanium dioxide rutile; TiO₂ T, anatase titanium dioxide. ^{ns}Nonsignificant difference.

of citrus black spot on tangerines. That response might be a consequence of the photoprotective film barriers to the incident radiation on the fruit surface; these barriers could stimulate the development of the fungus (Timossi et al., 2003).

The reduction of the CBS incidence observed in the last experiment, when artificial inoculation was performed, may be also due to the formation of a physical barrier caused by the application of treatments which prevent the development of the fungus (Sharma et al., 2015).

A pre-harvest Raynox spray to the Fuji Suprema and Pink Lady apple cultivars reduced the after harvest incidence of *Botryosphaeria dothidea* and *Cryptosporiopsis perennans* (Valdebenito-Sanhueza et al., 2016). Likewise, the authors presume that the reduction of decayed apples could be attributed to a physical barrier generated by the formulation. Another possibility referred to by Walters (2006) is that the film covering host tissue surfaces impede the pathogen to recognize that environment, deterring its germination and subsequent development.

Table 5. Orthogonal contrast analysis of photosynthesis and yield of 'Rainha' tangerine trees (*Citrus deliciosa*) subjected to the application of photoprotective films, carnauba wax emulsion (CWE), and inorganic filters, in the second experiment.

Contrast – treatment	Photosynthesis	Yield
Control vs. photoprotective films	0.0170	0.2320
CWE vs. inorganic filters	0.0774	0.0488
ZnO vs. TiO ₂	0.7570	0.2267
TiO ₂ (R) vs. TiO ₂ (T)	0.0938	0.0244

Inorganic filters: ZnO, zinc oxide; TiO₂R, rutile titanium dioxide rutile; TiO₂ T, anatase titanium dioxide.

When the formulations were sprayed only onto fruit surfaces (first experiment), the symptoms of false melanose of treated fruit were less severe, and no effects on fruit quality were determined. Fruit subjected to the inorganic filter TiO₂ (T) application showed higher peel color indices. However, when

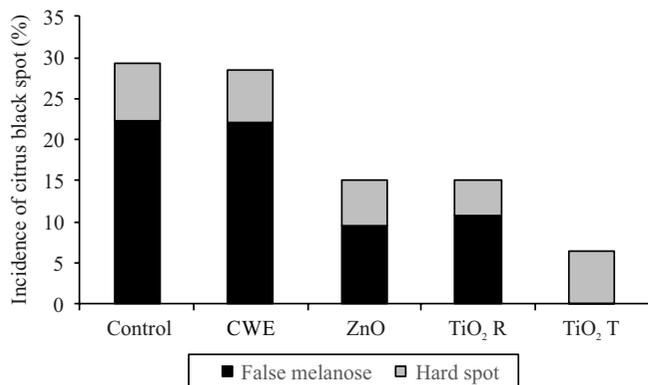


Figure 1. Application effects of carnauba wax emulsion (CWE) complemented with inorganic filters on symptoms caused by the incidence of citrus black spot on 'Rainha' tangerine (*Citrus deliciosa*). Inorganic filters: ZnO, zinc oxide; TiO₂ R, rutile titanium dioxide; TiO₂ T, anatase titanium dioxide. Analysis of variance (p): symptoms (0.0966); false melanose (0.0710); hard spot (0.9344).

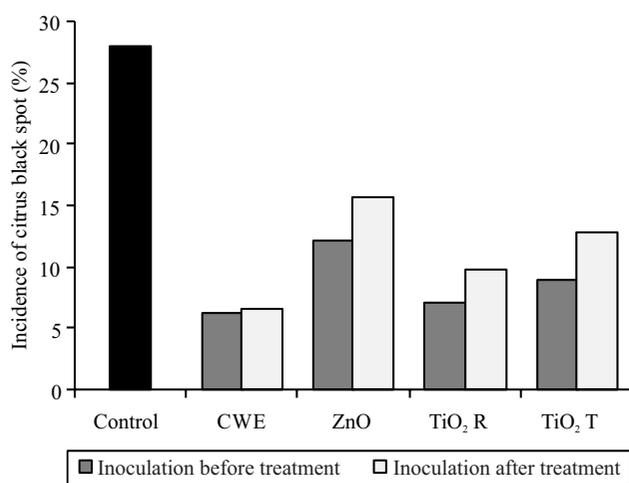


Figure 2. Application effects of carnauba wax emulsion (CWE) complemented with inorganic filters on the incidence of citrus black spot on 'Montenegrina' tangerine (*Citrus deliciosa*) artificially infected. Inorganic filters: ZnO, zinc oxide; TiO₂ R, rutile titanium dioxide; TiO₂ T, anatase titanium dioxide.

Table 7. Orthogonal contrast analysis for the incidence of citrus black spot on 'Montenegrina' tangerine (*Citrus deliciosa*) subjected to the application of photoprotective films and infected artificially with conidia suspension of *Phyllosticta citricarpa*, in the third experiment.

Contrast	p ⁽¹⁾
Previous inoculation vs. subsequent inoculation	0.2636
Control vs. photoprotective films	<0.0001
Carnauba wax emulsion vs. inorganic filters	0.0904
ZnO vs. TiO ₂	0.1425
TiO ₂ (R) vs. TiO ₂ (T)	0.4504
Analysis of variance (p)	0.0009

⁽¹⁾Probability of the orthogonal contrast for the incidence of citrus black spot. Inorganic filters: ZnO, zinc oxide; TiO₂ R, rutile titanium dioxide; TiO₂ T, anatase titanium dioxide.

that same formulation was sprayed twice to the whole plants, in the second experiment (when fruit had with 18 mm diameter, and when fruit reached 50 mm diameter), a significant reduction of the photosynthetic activity was determined and, consequently, there was a yield drop. Further studies are necessary to include carnauba wax emulsions in CBS control protocols, mainly with regards to wax concentration and most appropriate period to subject citrus trees to spraying.

Conclusions

1. Sprays of photoprotective films based on emulsions of carnauba wax only reduce the incidence of citrus black spot (CBS) on tangerine (*Citrus deliciosa*).
2. The addition of inorganic filters to photoprotective films, such as TiO₂ (T), may limit the photosynthetic activity in tangerine trees, damage the appearance of fruit, and reduce the production.
3. The photoprotective films do not affect the fruit quality attributes, such as the content of soluble solids and titratable acidity.

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