UNIVERSIDADE ESTADUAL DO SUDOESTE DA BAHIA
PROGRAMA DE PÓS-GRADUAÇÃO EM AGRONOMIA
ÁREA DE CONCENTRAÇÃO: FITOTECNIA

EFFECTS OF FILMS OF MINERAL PARTICLES AND BIOMATERIALS ON OVIPOSITION OF *Anastrepha obliqua* AND *Ceratitis capitata* AND ON PARASITISM BY *Diachasmimorpha longicaudata*

DANIELA RIBEIRO DA COSTA

VITÓRIA DA CONQUISTA
BAHIA – BRASIL
2021

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Thesis presented to the State University of Southwest Bahia, as part of the requirements of the Graduate Program in Agronomy, area of concentration in Crop Science, to obtain the title of Doctor.

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Coadvisor: Profa. Dra. Iara Sordi Joaquim-Bravo

VITÓRIA DA CONQUISTA
BAHIA-BRASIL
2021

C871e

Costa, Daniela Ribeiro da.

Effects of films of mineral particles and biomaterials on oviposition of *Anastrepha obliqua* and *Ceratitis capitata* and on parasitism by *Diachasmimorpha longicaudata*. / Daniela Ribeiro da Costa, 2021.

146f. : il.

Advisor (a): D. Sc. Maria Aparecida Castellani.
Thesis (Doctor) – Universidade Estadual do Sudoeste da Bahia, Program in Agronomy, Area of concentration in Crop Science, Vitória da Conquista, 2021.
References: F. 86 - 91.

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CDD. 634.1

Catalogação na fonte: Juliana Teixeira de Assunção – CRB 5/1890

UESB- Campus Vitória da Conquista – Ba.

UNIVERSIDADE ESTADUAL DO SUDOESTE DA BAHIA
PROGRAMA DE PÓS-GRADUAÇÃO EM AGRONOMIA
Área de Concentração em Fitotecnia

Campus de Vitória da Conquista, BA

DECLARAÇÃO DE APROVAÇÃO

Título: Effects of films of mineral particles and biomaterials on oviposition of *Anastrepha obliqua* and *Ceratitis capitata* and on parasitism by *Diachasmimorpha longicaudata*.

Autora: Daniela Ribeiro da Costa

Aprovado como parte das exigências para obtenção do Título de DOUTORA EM AGRONOMIA, ÁREA DE CONCENTRAÇÃO EM FITOTECNIA, pela seguinte Banca Examinadora:

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Data de realização: 30 de julho de 2021.

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ACKNOWLEDGEMENTS

I thank God for the life, strength and faith to be able to overcome all the obstacles that arose during this stage of my life;

To the State University of Southwestern Bahia, in particular, to the Graduate Program in Agronomy, for the opportunity.

To the Foundation for Research Support of the State of Bahia (FAPESB), and CAPES Foundation (Brazilian Ministry of Education), for the concession of the scholarship doctorate

To Prof. Maria Aparecida Castellani for orientation, friendship and confidence during these years, since graduate until master and doctorate degrees. The choice a competent advisor is a vital aspect for the formation of a young researcher, I will take their teachings along of the my career. Thanks for the opportunity!

To Prof. Iara Sordi Joachim-Bravo, my coadvisor for her availability, teachings and support in the conduction the work. Thank you!

To Profs. Carlos Alberto Domingues da Silva, Raquel Perez-Maluf and Quelmo Silva de Novaes, for their valuable contributions to this work.

To my parents (Delzuito and Norma), and brothers (Danilo and Delson) for their love and support. Especially my mother, I will never have enough words to thank for all the care and affection that has for my daughter.

To my husband Ailton, for his love, dedication, support and patience, I love you.

To my children, my angel Daniel (in memoriam) for having chosen me as mother, and for giving me strength to overcome his passing, and Maria, love without equal in my life. Nothing better than arriving home and being received with your happiness, I love you my daughter.

To Aldenise and Selminha for their friendship, affection and support in every moment. My gratitude!

To Suzany, Beatriz, Jaqueline and Virgiane for friendship, laughs, fights, support and

help in the maintenance of creations, execution and revision of the work. Thank you!
To friends conquered during the work: Raul, Matheus and Mikaela. Thank you for your support and fun as stickers models.

To all the teachers of the Graduate Program in Agronomy, for the knowledge transmitted.

To the future master, Mateus Pereira dos Santos, always available to assist me with statistical analyses. Thank you!

To colleagues from the Graduate Program in Agronomy and graduation students: Ana Carolina, Thamires Francisca and Micaela for their support carrying out the work.

To everyone who joined the Entomology Laboratory and helped me somehow to accomplish this work.

The companies Brasilminas and NovaSource for donating the kaolin samples.

To teacher Alcebíades Rebouças São José, for make available Biofactory to equipment use, and student Ranyelly Coutrim, for their assist in the physical-chemical analysis of the fruits. Thank you!

To everyone who somehow contributed for the realization of this work. Thank you!

GENERAL ABSTRACT

COSTA, D. C. **EFFECTS OF FILMS OF MINERAL PARTICLES AND BIOMATERIALS ON OVIPOSITION OF *Anastrepha obliqua* AND *Ceratitis capitata* AND ON PARASITISM BY *Diachasmimorpha longicaudata*.** Vitória da Conquista – BA, UESB, 2021. 146p. (Thesis: Doctor Science in Agronomy; Area of Concentration: Crop Science)[*]

Fruit flies (Diptera: Tephritidae) are the main pests of the world fruit industry and the use of low impact population suppression methods is an increasingly strong demand in the consumer market. Particle film technology, mainly through the use of kaolin, may represent a promising alternative to conventional insecticides for the management of these tephritids. However, the impact of kaolin applications on natural enemies is little understood. Thus, the work was organized into four articles aiming to achieve the following objectives; 1) to evaluate the effect of mineral and natural films on the physicochemical properties of grape (*Vitis vinifera* L.) and oviposition behaviour of *Ceratitis capitata* (Wiedemann) in the laboratory; 2) to evaluate the influence of mineral particles and biomaterial films on the coloring of guava fruits and their implications for the oviposition of *Anastrepha obliqua* (Macquart) in laboratory; 3) to evaluate the influence of mineral particle films on the physical characteristics of grape and their effects on the oviposition behavior of *Diachasmimorpha longicaudata* (Ashmead) in the laboratory; and 4) to evaluate the interference of mineral particles and biomaterials films in the interactions of the tritrophic complex in grape, *C. capitata* and *D. longicaudata* in the field cages. Results obtained in this study are promising because films of mineral particles such as kaolin (Surround® WP, 607, 608 and 611), changed the firmness, luminosity, chroma and hue angle of grapes and reduce the oviposition of *C. capitata*. The studied natural polymers seem to stimulate oviposition in *C. capitata*. Mineral films and biomaterials interfered with the color of guavas inhibiting the oviposition of *A. obliqua*. In laboratory, the females of *D. longicaudata* were recognized to perform all the behaviors in treated and untreated grape, except buccal contact, which was not accomplished on the kaolin fruits. A variation was found in the quantity and/or time of behavior landing, inspection, oviposition, and fruit rest between treatments, resulting in smaller success of parasitism with kaolin application. In field cage bioassays, kaolin treatments showed to be promising for fruit protection and reduced oviposition in *C. capitata* without affecting the parasitism capacity of *D. longicaudata*.

Keywords: *Anastrepha obliqua*, *Ceratitis capitata*, *Diachasmimorpha longicaudata*, Particle films, Oviposition.

[*]**Advisor**: Profa. Dra. Maria Aparecida Castellani, UESB and **Coadvisor**: Profa. Dra. Iara Sordi Joachim-Bravo, UFBA.

RESUMO GERAL

COSTA, D.R. da. **EFEITO DE FILMES DE PARTÍCULAS MINERAIS E BIOMATERIAIS NA OVIPOSIÇÃO DE *Anastrepha obliqua* E *Ceratitis capitata* E NO PARASITISMO POR *Diachasmimorpha longicaudata***. Vitória da Conquista - BA, UESB, 2021. 146p. (Tese: Doutorado em Agronomia; Área de Concentração: Fitotecnia)[*]

As moscas-das-frutas (Diptera: Tephritidae) são as principais pragas da fruticultura mundial e a utilização de métodos de supressão populacional de baixo impacto é uma exigência cada vez mais forte do mercado consumidor. A tecnologia do filme de partículas, principalmente pelo uso do caulim, pode representar uma alternativa promissora aos inseticidas convencionais para o manejo desses tefritídeos. Contudo, o impacto das aplicações de caulim sobre os inimigos naturais é pouco conhecido. Assim, o trabalho foi organizado em quatro artigos visando os seguintes objetivos: 1) avaliar o efeito de filmes de partículas minerais e naturais sobre as propriedades físico-químicas de uvas (*Vitis vinifera* L.) e no comportamento de oviposição de *Ceratitis capitata* (Wiedemann), em laboratório; 2) avaliar a influência dos filmes de partículas minerais e de biomateriais na coloração de frutos de goiaba (*Psidium guajava* L.) e suas implicações na oviposição de *Anastrepha obliqua* (Macquart) em laboratório; 3) avaliar a influência dos filmes de partículas minerais nas características físicas de uvas e seus efeitos no comportamento de oviposição de *Diachasmimorpha longicaudata* (Ashmead) em laboratório; e 4) avaliar a interferência de filmes de partículas minerais e de biomateriais nas interações do complexo tritrófico uva, *C. capitata* e *D. longicaudata* em gaiolas de campo; Os resultados obtidos neste estudo são promissores, pois filmes de partículas minerais, como caulim (Surround® WP, 607, 608 e 611) alteram a firmeza, luminosidade, croma e ângulo de cor dos frutos e reduzem a oviposição de *C. capitata*. Os polímeros naturais estudados parecem estimular a oviposição de *C. capitata*. Filmes minerais e biomateriais interferem na cor das goiabas inibindo a oviposição de *A. obliqua*. Em laboratório, fêmeas de *D. longicaudata* realizaram todos os comportamentos em uva tratada e não tratada, exceto contato bucal, não realizado nos frutos com caulim. Houve variação na quantidade e/ou tempo dos comportamentos de pouso, inspeção, oviposição e descanso no fruto entre tratamentos, resultando em menor sucesso de parasitismo com aplicação de caulim. Em bioensaios em gaiola de campo, os caulins mostraram-se promissores para proteção dos frutos, reduzindo a oviposição de *C. capitata* sem afetar a capacidade de parasitismo de *D. longicaudata*.

Palavras-chave: *Anastrepha obliqua*, *Ceratitis capitata*, *Diachasmimorpha longicaudata*, Filme de partículas, Oviposição.

[*] **Orientadora**: Profa. Dra. Maria Aparecida Castellani, UESB e **Coorientadora**: Profa. Dra. Iara Sordi Joachim-Bravo, UFBA.

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LIST OF ABBREVIATIONS, INITIALS AND SYMBOLS

°C	Degree celsius
$g\ L^{-1}$	Grams per liter
GLM	Generalized Linear Models
IP	Parasitism index
KMO	Kaiser-Meyer-Olkin
N	Newton
NaOH	Sodium hydroxide
PCA	Principal Component Analysis
TA	Titratable Acidity
TSS	Total Soluble Solids
VL	Larval Viability
VP	Pupal Viability

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GENERAL INTRODUCTION

The Brazilian fruit growing is one of the most diversified of the world and the cultivated area in the country overcome 2 million hectares, with annual production higher the 40 million tons, staying ago only of the China and India (Kist et al., 2021). However, it exports only 2% of its production, and the occurrence of fruit flies (Diptera: Tephritidae) is one of the barriers that affect the production and commercialization of fruit in natura for determined markets (ABRAFRUTAS, 2020). Although the presence of fruit flies do not the only obstacle to exports, is without doubts the main challenge that must be overcome to increase the quality of the produced fruits and their commercialization in the external market (ABRAFRUTAS, 2020).

The fruit flies of economic and quarantine importance in Brazil are *Ceratitis capitata* (Wiedemann, 1824), acquaintanceas Medfly, detected in the early 20th century and, currently, with 94 confirmed hosts and distributed in 27 botanical families; *Anastrepha* Schiner, with about 121 species in the country, being the more polyphagous *Anastrepha fraterculus* (Wiedmann, 1830) and *Anastrepha obliqua* (Macquart, 1835); and *Bactrocera carambolae* Drew & Hancock, 1994, originally from Asia, with confirmation of its presence in the states of Amapá, Pará and Roraima (Zucchi and Moraes, 2012). According to European Union Enforcement Directive 2019/523, published in March 21, 2019, non-European Tephritidae species are now of quarantine importance for the export of citrus and mango fruits (European Union, 2019). In the case of Brazil, these species include *A. fraterculus* and *A. obliqua*.

The direct damage caused by fruit flies are represented by puncture accomplished by female, at the moment of oviposition, and/or by development of the larva interior of the fruit, making them unsuitable for *in natura* consumption and industrialization (Paranhos, 2008).

The management more adequate of fruit flies is done through toxic baits, containing a mixture of food attractant (hydrolyzed protein) and a lethal agent (insecticide molecule) (Raga and Souza-Filho, 2021). However, the intensive use of toxic baits, such as spinosad insecticide, can cause serious biological imbalances in fruit orchards by selecting resistant populations of this pest (Kakani et al., 2010). The use of chemical insecticides has been each less used to manage this pest, especially, with the change in the consumer profile, which requires foods with reduced levels or exempt from pesticide residues and the population awareness about environmental risks caused

by such products, and the programs of integrated pest management have encouraged the use of various control methods and tactics (Dias et al., 2018).

Particle film technology may represent a promising alternative to conventional insecticides to control fruit fly infestation (Palma et al., 2020), since not contaminate the environment and not leave toxic residues harmful to man and animal in the treated products (D'aquino et al., 2011; Lo verde et al., 2011).

Kaolin is the main component of particle film technology, composed of aluminosilicate, chemically inert, white, formulated for use in plants (Puterka et al., 2000). The mechanisms action of kaolin against pest insects include repellency, tactile or visual interference, compromise or interruption of oviposition and feeding activity, and decreased longevity and survival (Glenn and Puterka, 2005).

Beyond of the kaolin, the particle films to base of biomaterials have been also used to protect cultivated plants due to their high availability, biocompatibility, low toxicity and biodegradability (Kaushik et al., 2016; Gomes et al., 2017). In agriculture, these biomaterials are mainly used in coating and preserving fruits before and after harvest (Gomes et al., 2017; Ambore et al., 2013).

Particle films to base have been studied in the management of fruit flies, with promising results in reducing oviposition in diverse fruitful (Mazor and Erez, 2004; Lemoyne et al., 2008; Leskey et al., 2010; D'aquino et al., 2011; Yee, 2012; Palma et al., 2020). This is due, mainly, by the changes of fruit coloring provided by the films influencing oviposition behavior of fruit flies (Costa et al., 2021; Da Costa et al., 2021). In addition to reducing fruit fly oviposition, kaolin protects plants against various pest insects, as beetles (Showler, 2002; Silva; Ramalho, 2013), aphids (Barker et al., 2007; Alavo et al., 2011; Nateghi et al., 2013), caterpillars (Knight et al. 2000; Showler, 2003; Barker et al. 2006; Alavo et al., 2010; Gonçalves et al., 2015; Tacoli et al., 2019), leafhoppers (Tacoli et al., 2017a, 2017b) and psyllids (Daniel et al., 2005; Puterka et al., 2005; Peng et al., 2011).

The impact of mineral particle films and biomaterials on populations of different beneficial species is little known. In bees, for example, kaolin increases cuticular water loss, reducing the survival of these insects (Karise et al., 2015). Thus for the use of chemical products for population suppression of pest insects, be the product synthetic or natural, is very important which take into account the selectivity to natural enemies. Many studies on particle films only evaluate the pest related effects, disregarding sublethal effects to beneficial organisms (Mazor and Erez, 2004; Braham et al., 2007;

Lemoyne et al., 2008; D'Aquino et al., 2011; Yee, 2012; Ourique et al., 2019; D'Aquino et al., 2021).

The biological control is an excellent option to be used together with other management strategies, because it leaves no residues, does not disturb nontarget pests, and can be permanent if the natural enemy establishes itself in the field (Paranhos et al., 2019). Due to great importance of biological control, studies about the effect of kaolin on tritrophic complex interactions are necessary.

The parasitoid *Diachasmimorpha longicaudata* (Ashmead, 1905) (Hymenoptera: Braconidae) is a of the most important biological control agents of fruit flies used in augmentative releases, can be used in conjunction with other management strategies (Montoya et al., 2000). In Brazil, this parasitoid was introduced in the 1990s, by Embrapa, through the National Center for Research on Cassava and Tropical Fruit - CNPMF and the National Center for Environmental Monitoring - CNPMA, stemming of Flórida (EUA) (Carvalho & Nascimento, 2002). According to Garcia and Ricalde, (2013) is the most effective parasitoid for use in augmentative biological control programs of *Anastrepha* spp. and *C. capitata*, mainly due the facility of creation in laboratory.

During the host localization process, studies indicate that female parasitoids respond to different stimuli (Vinson, 1976; Segura et al., 2007; Sharma et al., 2019). As the kaolin particle film alter the coloring of surface plant tissues to white color, impairs insect movement, feeding and oviposition, and creates a hostile and unknown environment (Bürgel et al., 2005). According to Sackett et al. (2007), kaolin can affect host location strategies and larval parasitoid habits, affecting parasitism rates.

Before possible interference of these films in the acceptance and oviposition of fruit flies, and the lack of knowledge of the impact of kaolin applications on natural enemies, the hypothesis can be raised that mineral and biomaterial films reduce the use of fruits by *Anastrepha obliqua* and *Ceratitis capitata* for oviposition, reducing the infestation of these pests in the field, mayed alter the parasitism of *D. longicaudata* on *C. capitata* larvae.

Thus, the objectives of the present study were (1) to evaluate the effect of mineral film particles and biomaterials on the physicochemical properties of grapes (*V. vinifera* L.), cultivar Itália and on the oviposition behavior of *C. capitata* in laboratory; (2) to evaluate of the influence of mineral particles and biomaterials films on the coloring of guava fruits and their implications for oviposition of *A. obliqua* in the

laboratory; (3) to evaluate the influence of mineral particle films on the physical characteristics of grapes and their effects on the oviposition behavior of *D. longicaudata* in the laboratory, and (4) to evaluate the interference of films of mineral particles and biomaterials in the interactions of the fruit (grape), fruit fly (*C. capitata*) and parasitoid (*D. longicaudata*) tritrophic complex in field cages.

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Periodíco Científico: Bulletin of Entomological Research – A2

ARTICLE I

Mineral and natural films change the physical–chemical properties of grapes and modulate oviposition behaviour of *Ceratitis capitata* Wiedemann (Diptera: Tephritidae)[*]

[*] **Situation:** Published (Annex I)

Article

Mineral and natural films change the physical–chemical properties of grapes and modulate oviposition behaviour of *Ceratitis capitata* Wiedemann (Diptera: Tephritidae

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Received: 18 March 2020; Accepted: 12 March 2021; Published: 16 April 2021

Abstract: The Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), is one of the main pests of fruit, worldwide, and the use of population suppression method with low environmental impact is an increasingly strong requirement of the consumer market. The aim of this study was to evaluate the effect of mineral and natural films on the physical–chemical properties of grapes (*Vitis vinifera* L.), cultivar Itália, and oviposition behaviour of *C. capitata*. Fruits were immersed in suspensions (100 and 200 g L^{-1}) of mineral (kaolin Surround®WP, kaolin 607, kaolin 608, kaolin 611 and talc) and natural films (chitosan, cassava starch, potato starch and guar gum 5.0 g L^{-1}) and distilled water (control). After drying, fruits were exposed to *C. capitata* pairs of males and females for 24 h in choice and non-choice tests; the number of punctures with and without eggs, eggs per fruit and behavioural response of fly to treated and untreated fruits were recorded. Results obtained in this study are promising, given the scientific evidence that films of mineral particles such as kaolin (Surround®, 607, 608 and 611) changed the firmness, luminosity, chroma and hue angle of grapes and reduced the oviposition of *C. capitata*. In addition, our results also showed that natural polymers do not deter *C. capitata* females, but rather seem to stimulate oviposition**.**

Keywords: chitosan; fruits flies; kaolin; oviposition; puncture

Introduction

Among the main phytosanitary problems that affect the production and commercialization of fresh fruits, for certain markets, the occurrence of fruit flies (Diptera: Tephritidae) is one of the main obstacles. Fruit flies of economic and quarantine importance in Brazil are *Ceratitis capitata* (Wiedemann, 1824), known as Medfly, discovered at the beginning of the 20th century, and currently has 94 confirmed hosts and distributed in 27 botanical families; *Anastrepha* Schiner, with about 121 species in the country, the most polyphagous being *A. fraterculus* (Wiedmann, 1830) and *A. obliqua* (Macquart, 1835); and *Bactrocera carambolae* Drew & Hancock, 1994, originally from Asia, but its presence has been confirmed in the states of Amapá, Pará, and Roraima (Zucchi and Moraes, 2012). Based on a European Union Execution Directive 2019/523, published on 21 March 2019, non-European Tephritidae species are now of quarantine importance for the export of citrus and mango fruits (European Union, 2019).

Ceratitis capitata is considered as the main quarantine pest of the world fruit and in Brazil, it mainly infests exotic fruits in 23 states of the 26 Brazilian states, beyond the Federal District (Zucchi and Moraes, 2012), there was no record only in three states Amapá, Amazonas, and Sergipe (Zucchi and Moraes, 2012).

The control of these tephritids is mainly performed through the use of toxic baits, containing a lethal agent (insecticide molecule) mixed with a food-based attractant (Arioli *et al*., 2018). Insecticide spinosad has been used in fruit fly control programs in several countries. In Brazil, spinosad is available in a concentrated suspension formulation and as a ready-for-use toxic bait (Harter *et al*., 2015). However, the extensive use of spinosad for controlling olive fruit fly and other tephritids can cause problems related to the selection of populations resistant to this insecticide (Kakani *et al*., 2010).

The continued use of insecticides has an increasing limitation, mainly consumer pressure, owing to the presence of residues in fruits; thus, it is necessary to evaluate other control strategies for inclusion in the management of fruit flies (Dias *et al*., 2018).

The use of mineral and natural particle films may be a viable alternative to the use of insecticide, mainly because they do not contaminate the environment or leave toxic residues that are harmful to humans and animals in treated products. Kaolin, the main component of the technology the particle film, is a white, non-abrasive, and chemically inert aluminosilicate mineral formulated for use in plants (Puterka *et al*., 2000).

The use of kaolin for pest management is based on the interruption of the insect in recognizing its host plant, alteration in the texture of leaves or fruits, and masking of leaves or fruits by their light-reflective properties (Showler, 2002). Thus, one of the first modes of action of particle films is host camouflage, which makes plants unrecognizable by pests. Particle films have been used to control fruit flies in apple (Mazor and Erez, 2004; Leskey *et al*., 2010), nectarine (Mazor and Erez, 2004; D'aquino et al., 2011), cherry (Yee, 2012), blueberry (Lemoyne *et al*., 2008) and citrus and peach (D'aquino *et al*., 2011).

In addition to mineral polymers, natural polymers have wide applicability in several areas owing to their high availability and properties, such as biocompatibility and biodegradability, and they are used in agriculture as a coat in the preservation of fruits before and after harvest (Kaushik *et al*., 2016; Gomes *et al*., 2017). Cellulose, agar, starch, pectin, guar gum, alginates, carrageenans, xanthan gum, chitin, and chitosan are among the most well-known and used natural polymers. Among them, chitin and chitosan have been used as natural seed treatment agents, growth stimulators, and in the control of plant diseases (Kulkarni *et al*., 2012; Ambore *et al*., 2013; Casemiro *et al*., 2019). Besides the reduction of the ripening process of mango fruits subjected to the hydrothermal process, chitosan can also inhibit the development of eggs and larvae of *A. ludens* (Salvador-Figueroa *et al*., 2011, 2013).

Most of the species of fruit flies have stereotypical oviposition behaviour that comprises stages of arrival on fruit, inspection, aculeus insertion, egg deposition, aculeus cleaning, and in most species, aculeus dragging (Díaz-Fleischer *et al*., 2000). Moreover, films can constitute barriers to oviposition, causing interference to the host, mainly in colour and penetrability (Aluja and Mangan, 2008).

Owing to the possible effects of these films on the physical–chemical characteristics of fruits and oviposition of fruit flies, we hypothesize that particle films can reduce the use of grape by *C. capitata* for oviposition, changing their behaviour, and consequently decreasing their infestation in fields.

Therefore, the aim of this study was to evaluate the effect of mineral and natural films on the physical–chemical properties of grapes (*V. vinifera* L.), cultivar Itália and oviposition behaviour of *C. capitata*.

Materials and Methods

Origin of C. capitata and fruits used in bioassays

Studies were conducted at the Laboratory of Fruit Flies, State University of Southwestern Bahia-UESB, campus of Vitória da Conquista, Bahia, Brazil, from June to December 2019.

The *C. capitata* flies used in this study were reared at the Fruit Flies Laboratory of the UESB. With the aim of obtaining larvae, eggs were collected daily, sterilized, and subjected to the diet containing oat bran, sugar, beer yeast, soybean meal and distilled water, in addition to preservatives, as adapted from Tanaka *et al*. (1969). Approximately ten days after larvae hatched, formed pupae were collected and placed in plastic containers with vermiculite until adults emerged. The adults were transported to cages, suitable for breeding, mating, and oviposition, and fed a diet based on sugar and yeast extract (Bionis YE MF) (Silva Neto *et al*., 2012), offered on filter paper. Cages were kept in an air-conditioned room at an average temperature of 25 ± 2°C and relative humidity of 70%. All bioassays used six-day-old *C. capitata* pairs of males and females, and flies were exchanged after 24 h of exposure to treatments. The mature grapes (*V. vinifera* L.), cultivar Itália, used in this experimente were obtained in open markets. They were selected on the basis of uniform maturity, size, and absence of fruit fly punctures.

Fruit characterization

Fruit uniformity was determined by assessing some physicochemical characteristics of grapes, such as length, diameter, firmness, colour, total soluble solids (TSSs) content, and titratable acidity (TA). Fruit uniformity was determined in order to confirm the uniformity of the substrate used for oviposition. Grape weight (grams) was determined using a precision semi-analytical scale. Grape diameter and length in millimetres (mm) were obtained with the aid of a digital calliper. Firmness was determined using a TR penetrator (model WA68, Italy), with 8mm diameter tip. TSS content was obtained through a direct reading of the berry pulp extract in a digital refractometer and results expressed in °Brix. TA was determined by titration, with a 0.1 N sodium hydroxide (NaOH), and expressed in grams of tartaric acid per 100 ml of juice. pH was determined using a Mars pH meter (model MB-10), with readings directly made on the sample with 100ml of fruit juice. Three replicates of ten grapes (N = 30) were used for each evaluated parameter: firmness, TSS, and TA, and each group of grapes came from a bunch.

Fruit colour was measured before and after the application of treatments, resulting in

two measurements per fruit on the same position (opposite sides), thus, four fruits per treatment were used in each bioassay (N = 40). Changes in colour were determined using colorimeter CR-400 (Minolta®). The device was calibrated using white ceramic plate and D65 illuminant (z = 85.7; x = 0.3175; y = 0.3253). Luminosity (L), ranging from 0 to 100 (black/white), red/green intensity (+/−) (a), and yellow/blue intensity (+/−) (b) values were determined. In addition to these colour coordinates, colour parameters such as chroma value [$C = (a^2 + b^2)1/2$], which represents colour purity and angle measurement (Hue) [$H = tg^{-1}$ (b/a)], which represents colour tone (Lemoyne *et al*., 2008) were also determined. After the application of the highest suspension of treatments, the second analysis of fruits was also performed in relation to firmness to detect possible changes that could influence oviposition.

Oviposition: non-choice test (bioassays 1 and 2)

To assess oviposition in non-choice test, a completely randomized design with ten treatments and four repetitions was used, with three replicates on consecutive days. Treatment componentes were: T1-kaolin Surround® WP; T2-kaolin 607 cream; T3-kaolin 608 white; T4-kaolin 611 grey; T5-talc 657; T6-chitosan; T7-cassava starch; T8-potato starch; T9-guar gum and T10-control (distilled water). All the treatment components were dissolved in distilled water at 100 g L^{-1} (bioassay 1) and 200 g L^{-1} (bioassay 2), except for T9-guar gum, which was dissolved in water at 5.0 g L^{-1}, as it was added as a thickener in the same amount to all treatments. Guar gum acts as a thickener, improving the viscosity and stability of formulations, being commonly used in chemical and biological insecticide formulations, including nanoemulsions (Campos *et al*., 2015; Gao *et al*., 2020).

The chitosan used in the bioassays was obtained from the shell of crustaceans; it was also dissolved in distilled water, and the mixture maintained under constant agitation. Kaolin Surround® WP was obtained from NovaSource company; kaolin 607, 608 and 611 and talc were purchased from Brasil Minas company and natural polymers from 'Mercadão Natural'.

Plot consisted of a cage containing treated grapes and *C. capitata* pairs of males and females. Fruits were tied on pieces of plastic tape; subsequently, they were individually immersed for 10 s in a beaker containing 60 ml of a suspension that correspond to each treatment. After treatment, fruits were left at 25 ± 2°C a temperature for 1 h to dry. Subsequently, a single fruit was hung from the top of each cage using an adhesive tape,

following the methods outlined by Silva et al. (2015), which was adapted for this trial. Bioassays were maintained in the laboratory at 25 ± 2°C and 70% relative humidity. Fruits were removed after 24 h of exposure to flies, and the number of eggs per fruit and punctures with and without eggs were recorded.

Oviposition: choice test (bioassays 3 and 4)

Bioassays with choice were similar to those of non-choice, however, two fruits per cage were exposed: one was treated, the other was a control (distilled water). Bioassays were conducted in a completely randomized design with nine treatments and four repetitions, with three replicates on consecutive days. The treatments and procedures used were the same as those described in bioassay 1, except for control treatment (T10), which was offered together with the other treatments in the same plot. The treatments were dissolved in distilled water at 100 g L^{-1} (bioassay 3) and 200 g L^{-1} (bioassay 4). After immersion and drying, fruits (treated and control) were placed 10 cm apart and hung from the top of each cage using adhesive tape, following the methods outlined by Silva *et al*. (2015), which was adapted for this trial. Bioassays were kept under the same conditions as bioassay 1 with 24-hour exposure, and the same variables recorded.

Behavioural response of C. capitata to treated and untreated fruits

The design was completely randomized comprising kaolin Surround®, kaolin 607, kaolin 608, kaolin 611, and guar gum suspensions. These suspensions (200 g L^{-1}) resulted in better oviposition responses in bioassays choice and non-choice, in addition to control (water) and chitosan treatment that stimulated oviposition. The experimental plot consisted of a cage with two six-day-old fertile *C. capitata* females and a fruit (grape). Eight (8) flies were used per treatment, lower than in other studies (McDonald and Mclnnis, 1985; Jang *et al*., 1999; Yee, 2012), but sufficient to observe all expected behaviours as indicated in preliminary tests. Fruits were immersed in treatments for ~10 s and soon after, dried at room temperature to remove excess moisture. The fruit was hung from the top of each cage and flies released with the help of a sucker.

Evaluations were carried out with the same fruits and flies for two consecutive days, from 8:00 am to 12:00 pm, following the method adapted from Lemoyne *et al*. (2008) and Yee (2012). After the two days period of exposure, another cage was prepared, with another flies and fruit for observation, totalling 16 hours of observation for each treatment. The following behavioural parameters were evaluated: arrival at the fruit

(landing), search, puncture, aculeus dragging and cleaning, time of first landing, number of landings and time landed on the host, number and time of fruit searching, time and number of punctures, number and time for aculeus dragging, and time and number for aculeus cleaning.

Statistical analyses

The parameters firmness, TSS, and TA were not statistically analysed because they were only used to characterize the fruits before immersing them in suspensions. In addition, it was only in bioassays with 200 g L^{-1} suspensions that firmness was determined, after the immersion of fruits in suspensions. Paired t-test in the R software version 3.6.1 (R Development Core Team, 2019) was used to compare the average values of luminosity, chroma and hue angle before and after applying the suspensions of 100 and 200 g L^{-1}.

For oviposition non-choice tests (bioassays 1 and 2), data obtained for the behavioural response of *C. capitata* to treated and untreated fruits and the physical characteristics (weight, length, diameter, luminosity, chroma and hue angle) of fruits were subjected to Bartlett and Shapiro-Wilk tests for evaluation of homoscedasticity assumptions of treatment variances and normality of residues, respectively. In case of violation of these assumptions, data were transformed into $x\sqrt{}$ or $x + 1\sqrt{}$ and subsequently subjected to analysis of variance (ANOVA) for comparison of means using the Tukey test ($P < 0.05$) in the R software version 3.6.1 (R Development Core Team, 2019). For the number of eggs in bioassay 1, treatments were compared using the generalized linear models (GLMs) of the R software 'nlme' (Pinheiro *et al*., 2020) and 'lsmeans' (Lenth, 2016) packages.

The oviposition data obtained with choice tests (bioassays 3 and 4) did not meet ANOVA premises, thus, a Monte Carlo type randomization was carried out, with 1000 simulations to guarantee 95% probability. To confirm significant diferences among treatments, a priori orthogonal contrast was performed using the R software version 3.6.1 (R Development Core Team, 2019).

Data on the behavioural response (time of first landing, number of landings, search time, number of searches, puncture time, number of punctures, aculeus dragging time and number of aculeus dragging) and pulp firmness were transformed into log (x + 10). For variables such as time of first landing and puncture time, Poisson distribution was used for the variables time to first landing and time to puncture. It was used GLM,

considering each parameter separately and the Poisson error distribution with a log-binding function (as the data were not normally distributed), whit α set at 0.05. All of the analyses were performed utilizing the statistical program R (R Core Team, 2018), the statistical procedure also used by other authors in works with fruit flies, such as *A. fraterculus* (Proença, 2019), *A. obliqua* and *C. capitata* (Silva *et al*., 2020).

Results

Fruit characterization

Grapes showed an average pulp firmness of 5.4 N, TSS content of 18.1 °Brix, TA of 1.3 and pH of 3.7. Among the variables analysed (weight, length, diameter, luminosity, chroma and hue angle), significant differences were observed only for diameter and luminosity, indicating slight variations in characteristics of fruits used as a substrate for oviposition in the various bioassays. The mean values for weight ($F = 1.0573$; $df = 9, 39$; $P = 0.42075$) and length ($F = 1.587$; $df = 9, 39$; $P = 0.16428$) ranged from 8.76 ± 0.61 to 10.50 ± 0.55 g and 27.20 ± 0.77 to 30.12 ± 1.05 mm, respectively. The diameter of grapes in all treatments was equal to the diameter of control fruits, however, significant differences were found only for the diameter of grapes used in T1 (kaolin Surround®) and T6 (chitosan) treatments ($F = 3.2634$; $df = 9, 39$; $P < 0.001$) (table 1). Regarding luminosity of fruits before treatments, fruits immersed in potato and cassava starches and guar gum films were the same as those immersed in other treatments; their values were higher than that of the control ($F = 3.0522$; $df = 9, 39$; $P = 0.0102$). Regarding the two other factors related to colour, chroma or purity ($F = 1.3576$; $df = 9, 39$; $P = 0.25062$) and hue angle ($F = 1.0598$; $df = 9, 39$; $P = 0.41904$), fruits were uniform as there was no significant difference between them; their values ranged between 10.14 ± 0.50–11.39 ± 0.93 and 1.10 ± 0.02–1.15 ± 0.02, respectively (table 1).

Films suspension at 100 g L^{-1} had effects on luminosity ($t = 4.0613$; $df = 39$; $P < 0.001$), chroma ($t = 8.6448$; $df = 39$; $P < 0.001$) and hue angle ($t = 12.456$; $df = 39$; $P < 0.001$) of fruits. A comparison of luminosity values before (table 1) and after immersion in suspension at 100 g L^{-1} (table 2) shows that all films increased fruit luminosity after treatment, indicating that fruits immersed in mineral films had higher values than those in control.

For treatments at 100 g L^{-1}, significant differences were observed between the following parameters: luminosity ($F = 42.885$; $df = 9, 39$; $P < 0.001$), chroma ($F = 93.96$; $df = 9, 39$; $P < 0.001$), and hue angle ($F = 32.536$; $df = 9, 39$; $P < 0.001$).

Table 1. Weight (g), length (mm) and diameter (mm), luminosity, chroma and hue angle (mean ± standard deviation) of the grapes of the cultivar Italy used in the treatments before immersion in suspensions.

Treatments	Weight (g)	Lengt (mm)	Diameter (mm)	Luminosity	Chroma	Hue angle
T1-Kaolin Surround® WP	9.71 ± 0.41 a	28.10 ± 0.96 a	22.70 ± 0.42 b	37.89 ± 1.84 ab	10.28 ± 0.53 a	113 ± 1.5 a
T2- Kaolin 607 cream	9.95 ± 1.27 a	28.51 ± 0.69 a	23.01 ± 1.18 ab	38.63 ± 1.48 ab	10.95 ± 0.75 a	115 ± 1.5 a
T3- Kaolin 608 white	10.50 ± 0.55 a	30.12 ± 1.05 a	23.35 ± 0.50 ab	38.33 ± 0.60 ab	10.14 ± 0.50 a	114 ± 1.63 a
T4- Kaolin 611 grey	10.0 ± 2.52 a	28.11 ± 2.63 a	22.87 ± 2.34 ab	38.14 ± 1.29 ab	10.17 ± 0.59 a	112 ± 0.95 a
T5-Talc 657	8.96 ± 1.52 a	28.05 ± 1.72 a	21.66 ± 0.61 b	38.38 ± 1.53 ab	10.31 ± 1.06 a	113 ± 0.95 a
T6-Chitosan	10.46 ± 1.50 a	28.66 ± 0.70 a	25.33 ± 0.87 a	37.41 ± 1.86 ab	10.57 ± 0.58 a	113 ± 0.95 a
T7- Cassava starch	9.05 ± 0.80 a	27.25 ± 0.28 a	23.10 ±1. 27 ab	39.35 ± 0.80 a	11.17 ± 0.91 a	110 ± 1.5 a
T8- Potato starch	8.76 ± -0.61 a	27.20 ± 0.77 a	22.47 ±0.58 b	40.37 ± 0.45 a	11.39 ± 0.93 a	115 ± 0.81 a
T9-Guar gum	9.12 ± 1.16 a	27.62 ± 2.19 a	22.53 ± 0.74 b	39.31 ± 1.53a	11.09 ± 1.12 a	111 ± 0.95 a
T10- Distelled water	10.10 ± 0.44 a	27.33 ± 0.36 a	23.73 ± 0.74ab	35.92 ± 1.58 b	10.26 ± 0.53 a	112 ± 1.0 a
C.V (%)	12.92	4.85	4.65	3.6	7.37	3.64

Mean ± SD values in the same column followed by the same letter do not differ significantly at $P < 0.05$ (Tukey's test)

Luminosity, which can vary from 0 (black) to 100 (white), was significantly higher in fruits immersed in kaolin Surround® (76.28 ± 5.47, close to white) compared to that of fruits in all other treatments, including that of control (29.32 ± 2.88). Chroma values obtained before (table 1) and after immersion of grapes in suspensions (table 2) showed that there was a general reduction in all treatments, however, this reduction was less pronounced in fruits treated with potato starch, guar gum film, and water. In addition, immersion in suspensions significantly altered the hue angle of fruits. There was an increase in the hue angle of fruits treated with Kaolin 607 and a reduction in those treated with kaolin Surround® and 608, which were different from other treatments (table 2).

Films suspension at 200 g L^{-1} also affected luminosity (t= 10.712, df = 39, P < 0.001), chroma (t= 5.0254, df = 39, P < 0.001) and hue angle (t = 4.1679, df = 39, P < 0.001) (table 2). Luminosity values before (table 1) and after immersion at 200 g L^{-1} (table 2) showed that all films increased fruit luminosity after treatment, that is, fruits treated with mineral films had higher values compared to those in control.

Similar results were obtained for fruits immersed in suspensions at 200 g L^{-1}; particle films had effects on luminosity (F = 718.89; df = 9, 39; P < 0.001), chroma (F = 248.9; df = 9, 39; P < 0.001) and hue angle (F = 9.39; df = 9, 39; P < 0.001). It was observed that the luminosity values of fruits immersed in suspensions at 200 g L^{-1} were higher than those in suspensions at 100 g L^{-1}, and the average values of all treatments, except for guar gum, differed from that of control, almost reaching White colour in fruits immersed in kaolin Surround® (94.62 ± 0.82). Chroma values ranged from 2.41 ± 0.41 (cassava starch) to 15.70 ± 0.26 (kaolin 607), the highest average was observed in fruits treated with kaolin cream (15.70 ± 0.26). Hue angle ranged from 116 ± 3.10 (guar gum) to 156 ± 0.58 (kaolin 607), and only kaolin 608, talc and chitosan did not differ from control in hue angle.

Mineral films (kaolin Surround®, 607, 608 and 611 and talc) and cassava starch increased pulp firmness than control (F = 4.3069; df = 9, 39; P < 0.001) (table 3).

Oviposition: non-choice tests (bioassays 1 and 2)

In bioassay 1, which is characterized by the immersion of fruits in 100 g L^{-1} film suspensions, increase in punctures with eggs in kaolin (607 and 608), chitosan and starch (cassava and potato) treatments was observed, and their average values were significantly higher than those in distilled water treatment (F = 3.1682; df = 9, 39; P =

Table 2. Luminosity, chroma and hue angle (mean ± standard deviation) of the grapes after immersion in suspensions at 100 e 200 g L^{-1}.

Treatments	Suspension of 100 g L^{-1}			Suspension of 200 g L^{-1}		
	Luminosity	Chroma	Hue angle	Luminosity	Chroma	Hue angle
T1-Kaolin Surround® WP	76.28 ± 5.47 a	2.87 ± 0.28 e	45 ± 9.88 d	94.62 ± 0.82 a	3.73 ± 0.15 f	140 ± 2.89 b
T2- Kaolin 607 cream	57.61 ± 6.76 bc	8.00 ± 0.59 b	127 ± 6.85 a	83.64 ± 0.30 c	15.70 ± 0.26 a	156 ± 0.58 a
T3- Kaolin 608 white	64.33 ± 2.92 b	3.29 ± 0.17 e	69 ± 2.16 c	89.06 ± 0.92 b	3.65 ± 0.52 f	125 ± 6.23 c
T4- Kaolin 611 grey	49.63 ± 3.15 cd	5.94 ± 0.40 cd	108 ± 2.5 b	80.75 ± 1.85 d	7.79 ± 0.15 d	143 ± 1.63 b
T5-Talc 657	50.58 ± 3.72 cd	5.40 ± 0.40 d	112 ± 1.41 b	80.31 ± 0.52 d	6.08 ± 0.15 e	131 ± 1.29 c
T6-Chitosan	36.23 ± 6.07 ef	8.10 ± 0.35 b	117 ± 2.52 b	58.15 ± 0.65 f	8.28 ± 0.43 d	129 ± 2.21 c
T7- Cassava starch	45.94 ± 3.74 de	6.84 ± 0.91 bc	110 ± 2.21 b	79.4 6± 1.20 d	2.41 ± 0.15 g	118 ± 10.01 d
T8- Potato starch	37.49 ± 4.51 ef	10.02 ± 0.75 a	120 ± 2.21 a	72.55 ± 2.83 e	3.90 ± 0.44 f	118 ± 4.03 d
T9- Guar gum	32.42 ± 4.59 f	10.70 ± 0.75 a	109 ± 5.77 b	36.28 ± 2.41 g	10.15 ± 0.87 c	116 ± 3.10 d
T10- Distilled Water	29.32 ± 2.88 f	10.21 ± 0.68 a	112 ± 1.71 b	38.07 ± 1.47 g	11.40 ± 1.13 b	129 ± 10.80 c
C.V (%)	9.52	8.07	2.86	2.14	3.64	4.22

Mean ± SD values in the same column followed by the same letter do not differ significantly at $P < 0.05$ (Tukey's test).

Table 3. Firmness of grapes (mean ± standard deviation) subjected suspensions at 200 g L^{-1}.

Treatments	Firmess of grape (N)*
T1-Kaolin Surround® WP	6.37 ± 0.25 a
T2- Kaolin 607 cream	6.40 ± 0.19 a
T3- Kaolin 608 white	6.75 ± 0.94 a
T4- Kaolin 611 grey	6.42 ± 0.86 a
T5-Talc 657	6.13 ± 0.56 a
T6-Chitosan	5.85 ± 0.16 ab
T7- Cassava starch	6.36 ± 0.47 a
T8- Potato starch	5.88 ± 0.41 ab
T9-Guar gum	5.40 ± 0.41ab
T10-Distilled water (Control)	4.99 ± 0.32 b
C.V (%)	8.57

Mean ± SD values in the same column followed by the same letter do not differ significantly at $P < 0.05$ (Tukey's test).
*Data transformed into log (x + 10).

0.0083067) (table 4). As for the number of punctures without eggs, significant differences were observed (F = 3.5728; df = 9, 39; P = 0.004027), and only chitosan differed from control with 3.58 ± 0.96 punctures. Regarding the number of eggs, only chitosan, with the highest average number of eggs (30.25 ± 6.08), differed from control (F = 2.4247; df = 9, 39; P = 0.033221).

At the highest suspension (200 g L^{-1} – bioassay 2), all mineral films (kaolin Surround®, 607, 608 and 611 and talc) and guar gum treatments resulted in the lower average number of punctures with eggs compared to control, whereas the other treatments (chitosan and cassava and potato starches) did not have any effect on this variable (F = 3.0753; df = 9, 39; P = 0.0098394) (table 4). Regarding the number of punctures without eggs, there were no significant differences among treatments and control (F = 9.7759; df = 9, 39; P = 8.4543), with average values ranging from 1.0 ± 0 to 1.63 ± 0.16.

For the average number of eggs, it was observed that no treatment differed from control; however, significant differences were found between kaolin Surround®, 607 and 611 and chitosan and potato starch (F = 4.3264; df = 9, 39; P = 0.0011156), with fruits treated with kaolin having lower average values (table 4).

Table 4. Puncture with and without eggs and eggs (mean ± standard deviation) of *C. capitata* in grapes, submitted to suspensions in bioassays 1 and 2 (non-choice).

Treatments	Bioassay 1: 100 g L^{-1}			Bioassay 2: 200 g L^{-1}		
	Punctures with eggs (N°)	*Punctures without eggs (N°)	Eggs (N°)	Punctures with eggs (N°)	*Punctures without eggs (N°)	Eggs (N°)
T1-Kaolin Surround® WP	2.67 ± 0.47 b	0.41 ± 0.42 b	24.33 ± 6.00 ab	0.33 ± 0.26 c	1.14 ± 0.16 a	6.41 ± 7.81b
T2- Kaolin 607 cream	3.66 ± 0.60 a	0.66 ± 0.77 b	26.33 ± 5.40 ab	0.75 ± 0.50 c	1.0 ± 0.0 a	12.08 ± 9.24 b
T3- Kaolin 608 white	3.67 ± 1.27 a	0.41 ± 0.42 b	24.33 ± 10.05 ab	1.41 ± 0.79 c	1.28 ± 0.19 a	21.58 ± 14.95 ab
T4- Kaolin 611 grey	1.91 ± 1.25 b	0.25 ± 0.16 b	15.25 ± 10.07 ab	0.58 ± 0.32 c	1.14 ± 0.16 a	13.83 ± 7.71 b
T5-Talc 657	2.66 ± 1.27 b	0.66 ± 1.33 b	22.16 ± 6.02 ab	1.49 ± 0.88 b	1.0 ± 0.0 a	34.08 ± 21.51ab
T6-Chitosan	4.83 ± 0.88 a	3.58 ± 0.96 a	30.25 ± 6.08 a	5.08 ± 1.85 a	1.59 ± 0.43 a	46.33 ± 4.72 a
T7- Cassava starch	3.33 ± 0.71 a	0.74 ± 0.42 b	24.00 ± 3.12 ab	2.75 ± 1.78 a	1.42 ± 0.16 a	35.33 ± 23.26 ab
T8- Potato starch	3.5 ± 1.37 a	0.33 ± 0.27 b	20.42 ± 9.31 ab	4.50 ± 1.82 a	1.63 ± 0.16 a	43.25 ± 6.45 a
T9-Guar gum	2.33 ± 0.67 b	0.16 ± 0.33 b	17.50 ± 4.64 ab	1.83 ± 0.64 b	1.34 ± 0.31 a	22.08 ± 5.68 ab
T10-Distilled water	2.25 ± 0.83 b	1.66 ± 1.46 b	12.5 ± 7.35 b	4.90 ± 2.60 a	1.61 ± 0.71 a	30.25 ± 12.43 ab
C.V (%)	32.02	72.71	32.52	28.88	26.49	48.92

Mean ± SD values in the same column followed by the same letter do not differ significantly at $P < 0.05$ (Tukey's test)
* Data transformed in $\sqrt{x} + 1$

Oviposition: choice tests (bioassays 3 and 4)

In bioassay 3 (suspension of 100 g L^{-1}), significant diferences were observed among treatments for punctures with eggs ($F = 4.9854$; df = 8, 35; $P < 0.0001$) and number of eggs ($F = 8.7221$; df = 8, 35; $P < 0.0001$), but were not observed for punctures without eggs ($F = 0.9853$; df = 8, 35; $P = 0.4628$) (fig. 1). Kaolin Surround® was the only treatment that reduced the number of punctures with eggs, whereas others, except for guar gum treatment, increased the average values of this variable (fig. 1a). However, the reduction in the number of punctures with eggs by kaolin Surround® did not result in the lower average number of eggs in the same treatment (fig. 1c).

For bioassay 4 (immersion at 200 g L^{-1}), responses of flies to treated and untreated fruits were different compared to those in bioassay 3, with a significant reduction in the average number of punctures with eggs ($F = 6.9519$; df = 8, 35; $P < 0.00001$) by kaolin Surround®, 607, 608 and 611 and guar gum treatments, and a significant increase in the same variables by other treatments (fig. 2a). Similar responses occurred for the number of eggs ($F = 3.4768$; df = 8, 35; $P = 0.0026$), except for kaolin 607, which resulted in a higher average number of eggs compared to control (fig. 2c). Treatments did not affect the number of punctures without eggs ($F = 2.0896$; df = 8, 35; $P = 0.05282$) (fig. 2b).

Behavioural response of C. capitata to treated and untreated fruits

Time of first landing on fruit did not differ among treatments and control ($F = 14.143$; df = 6; $P > 0.05$; coefficient of variation (C.V) = 28.62%, with values ranging from 1.68 ± 0.216 (kaolin Surround®) to 2.12 ± 0.173 s (guar gum), (fig. 3a); however, for number of landings, kaolin Surround® treatment resulted in the lowest number of landings (2.43 ± 0.094) compared to control ($F = 0.73892$; df = 6; $P < 0.01$; C.V = 6.77%) (fig. 3b). Search time for all treatments did not differ from that of control ($F = 20.564$; df = 6; $P = 0.388$; C.V = 19.22%), however, kaolin Surround® treatment (3.72 ± 0495 s) and chitosan (6.11 ± 0495 s) were significantly different between each other, with shorter search time recorded for kaolin Surround® (fig. 3c).

Regarding the average number of searches, differences were found only between kaolin Surround® (2.49 ± 0.107) and kaolin 608 (2.94 ± 0.107) (fig. 3d) ($F = 0.97042$, df = 6, $P = 0.0811$, C.V = 7.82%). Time for aculeus insertion in fruits (puncture) did not differ among treatments ($F = 4.3002$, df = 6, $P = 0.162$, C.V = 20.64%) (fig. 3e); however, differences in the number of punctures were observed only between kaolin 607 (2.43 ± 0.081) and kaolin 611 (2.78 ± 0.081) ($F = 0.55152$, df = 6, $P < 0.05$, C.V =

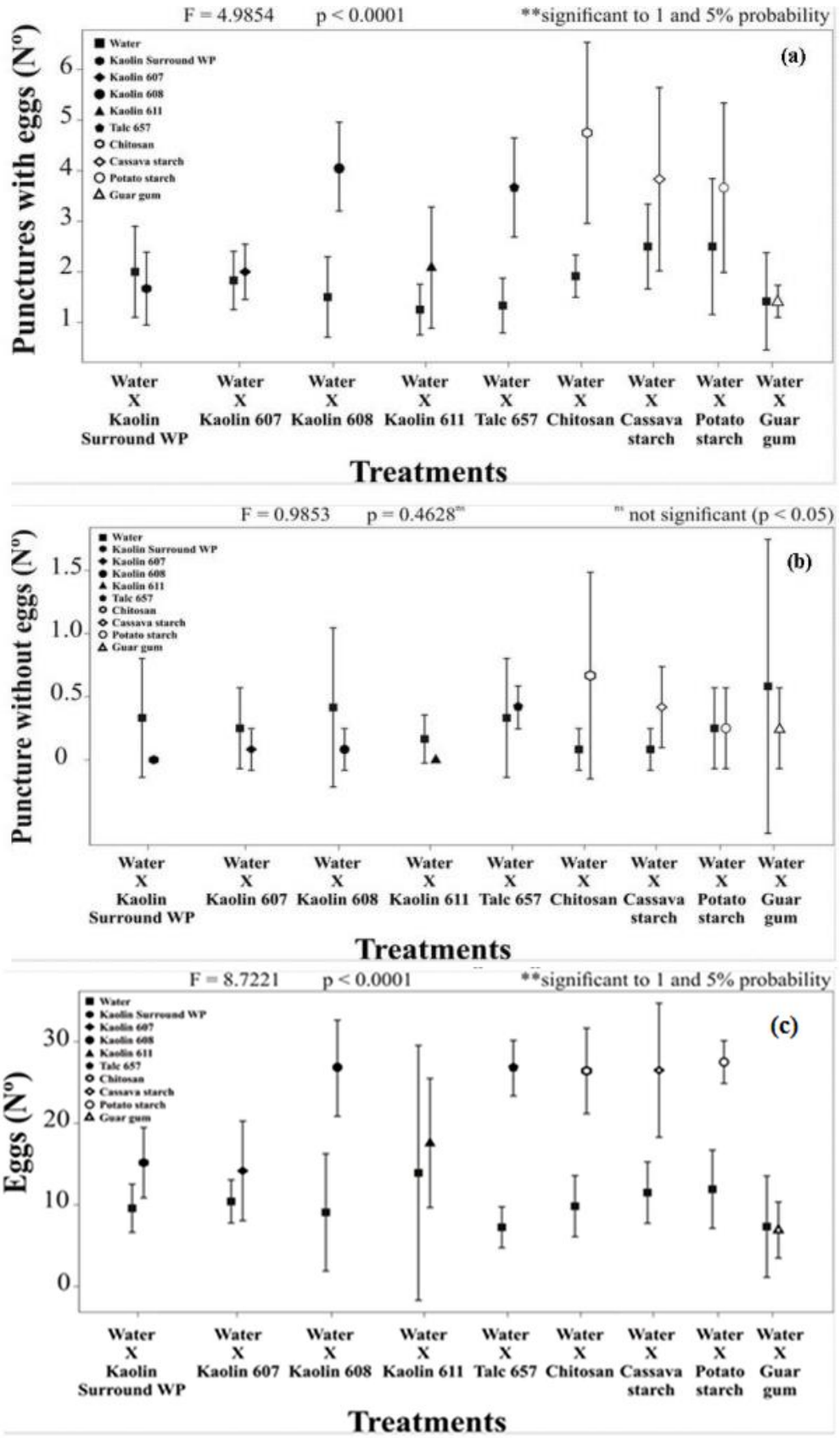

Figure 1. Punctures with (a) and without eggs (b) and eggs (c) (mean number ± standard deviation) of *C. capitata* in grapes, submitted to mineral and natural films, at 100 g L^{-1}, obtained in the bioassay 3 (choice test).

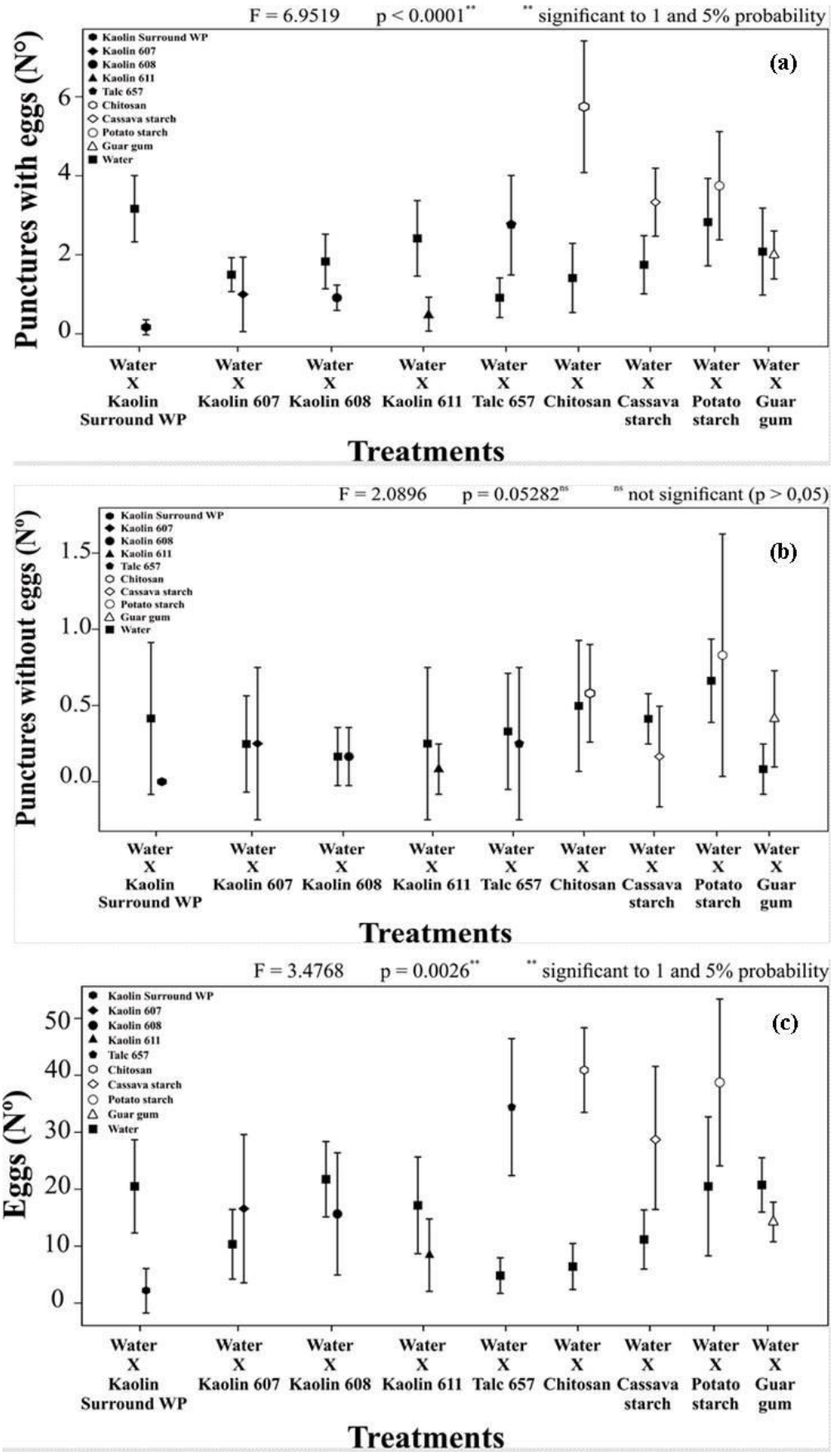

Figure 2. Punctures with (a) and without eggs (b) and eggs (c) (mean ± standard deviation) of *C. capitata* in grapes, submitted to mineral and natural films, at 200 g L^{-1}, obtained in bioassay 4 (choice test).

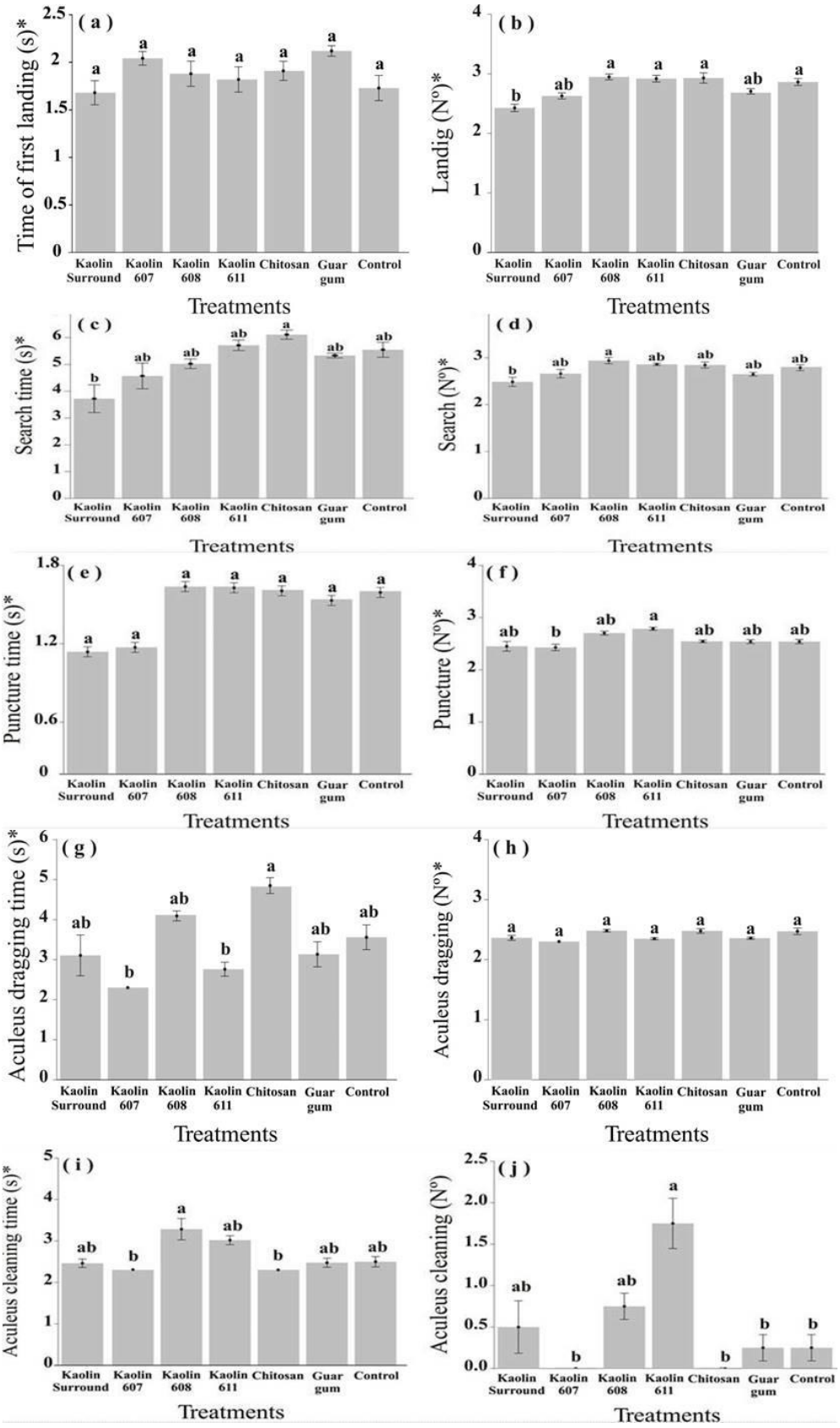

Figure 3. Oviposition behaviour (number mean ± standard deviation) of *C. capitata* in grapes, submitted the suspensions at 200 g L^{-1}. Time of first landing (a) Number of landings (b) Search time (c) Number of search (d) Puncture time (e) Number of punctures (f) Aculeus dragging time (g) Number of aculeus dragging (i) Cleaning time of the aculeus (j) (Number of cleaning of the aculeus). * Data transformed into log (x + 10).

6.31%) (fig. 3f). Time for aculeus dragging on fruit surface after oviposition differed only between kaolin (607 and 611) and chitosan ($F = 16.126$, $df = 6$, $P < 0.001$, C.V = 25.76%); (fig. 3g). The difference found in the average number of ovipositor aculeus dragging was not significant among treatments ($F = 0.21976$, $df = 6$, $P = 0.3748$, C.V = 4.26%) (fig. 3h). Regarding the time for aculeus cleaning, treatments did not differ from control ($F = 3.4687$, $df = 6$, $P = 0.5003$, C.V = 15.51%), however, diferences were found between kaolin 608 (3.28 ± 0.203 s), kaolin 607 (2.30 ± 0.203 s), and chitosan (2.30 ± 0.203 s) (fig. 3i). Regarding the number of times aculeus cleaning behaviour was performed, treatments did not differ from control ($F = 8$, $df = 6$, $P = 0.5728$, C.V = 123.44%), except for kaolin 611, which resulted in the greater number of times (1.75 ± 0.309 times) (fig. 3j).

Discussion

Studies were developed using grape as a substrate for *C. capitata* oviposition owing to its economic importance for export and the easy visualization of punctures and eggs, which help in minimizing experimental errors. The grapes used in the bioassays of this study were within the commercial standards reported in Normative Instruction No. 1 of 1 February 2002 (BRAZIL, 2002), which stated that fine table grapes should have a minimum soluble solids equal to 14° Brix and TA < 1.5 (Carvalho and Chitarra, 1984). In this study, the values obtained for mass, length and diameter of grapes can be considered within comercial standards (Mascarenhas *et al*., 2010, 2013). Before bioassays, grapes were uniform in terms of weight, length, chroma and hue angle, with variations only in diameter and luminosity values (table 1), indicating good fruit uniformity.

Variations in the diameter values of grapes did not interfere with the responses of females. According to Corrêa *et al*. (2018), grapes of different varieties and diameters did not influence the oviposition of *C. capitata* and *A. fraterculus*. Regarding the luminosity values obtained in grapes before applying treatments, differences were observed only between potato and cassava starches and guar gum and control, however, they were statistically equal to the values of grapes used in other treatments.

Thus, this factor alone probably did not influence females in choosing between fruits treated with different films (table 1). In general, it is considered that grapes had good uniformity for use in bioassays, and it could be inferred that variations in responses of

flies to oviposition were only due to treatments applied.

Regardless of the method used (choice and non-choice tests), studies with mineral and natural films indicated that suspension at 100 g L^{-1} does not protect grapes from *C. capitata* oviposition (table 4 and fig. 1), but even increases oviposition variables (punctures with eggs and number of eggs). The only exception was Surround® treatment in choice test, which resulted in a lower average number of egg punctures (fig. 1a), however, it did not result in fewer eggs on grapes (fig. 1c). These results differ from that recorded in some laboratory, where there was a reduction in punctures of *C. capitata* oviposition in citrus (D'aquino *et al*., 2011) and nectarine treated with Surround® at 30 g L^{-1} and 60 g L^{-1}, respectively; flies avoided landing on treated fruits, resulting in no infestation (Mazor and Erez, 2004); and reduction in punctures of *Rhagoletis mendax* Curran fly oviposition in blueberry treated with Surround® at 60 g L^{-1} (Lemoyne *et al*., 2008). In the field, kaolin sprays at 50 g L^{-1} in citrus (Braham *et al*., 2007; Lo Verde *et al*., 2011) and apple plants (Villanueva and Walgenbach, 2007) resulted in a significant reduction in the number of damaged fruits, indicating negative effects on oviposition.

For suspension at 200 g L^{-1}, the reduction of *C. capitata* oviposition in grapes was evidenced in treatments with mineral films and guar gum in the choice test of hosts by fly (bioassay 2). In this case, Surround® reduced the number of punctures with eggs and the number of eggs by ∼15 and 5 times, respectively (table 4). In bioassay 4, where flies had a choice for treated or untreated fruits, flies discriminated the treatments in two groups: oviposition inhibitors (Surround®, kaolin 608, kaolin 611 and guar gum) and stimulants (kaolin 607, talc, chitosan and potato and cassava starches). In this case, the greatest inhibition was achieved with Surround®, ∼19 and 9 times the number of punctures with eggs and number of eggs, respectively. In a suspension at 200 g L^{-1}, kaolin and liquid limestone applied to apple and mango fruits resulted in an inhibition of *C. capitata* oviposition (Ourique *et al*., 2017). The average number of punctures in apples and mangoes was 7 to 8 times and 3 times lower, respectively, when treated with both products.

Few ripe fruit species are white in colour and white can be considered a very neutral surface, reflecting a range of wavelengths within the visible spectrum of tephritids. According to Díaz-Fleischer *et al*. (2000), in laboratory experiments, females such as *A. fraterculus, A. ludens* and *C. capitata* generally show little or no discrimination between white spheres (substrate for oviposition) and spheres of other colours. With the use of suspension at 200 g L^{-1}, fruits from T1, T2, T3 and T4 treatments showed whitish

colour, evidenced by luminosity values ≥80. Surround® and kaolin 607 reduced the oviposition of *C. capitata* and both showed high luminosity value of 94.62 ± 0.82 and 83.64 ± 0.30, respectively, which also indicates reflectance. The colour change resulting from the effects of these films probably impaired the perception of host, a fact already reported by Katsoyannos *et al*. (1986) for wild *C. capitata* flies. In the laboratory, the authors found that flies preferred to oviposit in spheres coloured in black, blue and red than in those coloured in yellow and white, which received smaller number of eggs. The preference observed for certain colours depends on both colour tone and intensity of total light reflected (brightness) and white spheres showed 100% reflectance (Katsoyannos *et al*., 1986).

In all bioassays, when fruits were dissected for egg counting, it was observed that grapes with mineral films had punctures with eggs, but had a reduced number of eggs; however, smaller number of punctures with greater amount of eggs was observed under the fruit pedicel. Perhaps, this behaviour is owed to the perception that flies had towards the films in fruit, making them search for a more appropriate place without foreign substances for oviposition. It was observed that fruits with films had changed colour but did not prevent *C. capitata* from finding and accepting the host. However, the changed colour somehow prevented flies from having prolonged direct contact with foreign substances, causing them to look for alternative places in the fruit to oviposit.

According to Mazor and Erez (2004), kaolin-treated fruits are visually recognized by flies as host, but their colour does not match what not expect something appropriate for oviposition. Even in inappropriate hosts, in an attempt to leave offspring, fruit flies can oviposit on these substrates (Aluja and Mangan, 2008). In the absence of a primary host, *C. capitata* searches for an alternative host, such as *Opuntia ficus-indica* (L.) Mill and *Pereskia bahiensis* Gürke, to ensure offspring survival, even though they are poorly suited hosts for larval development (Leite *et al*., 2017; Leite *et al*., 2019).

Natural polymers have wide applicability in several study áreas owing to their properties such as biocompatibility, biodegradability, high availability and non-toxicity (Azevedo *et al*., 2007). The use of natural films at both suspension rates did not reduce Medfly ovipositions. This result was not expected, mainly owing to the colour change provided by these films. Chitosan affected the posture of *C. capitata*, with a consequent increase in the number of eggs; this result may have an application in bio-factories for massal rearing of fly, especially when aiming to sterile insect technique.

Regarding oviposition behaviour, *C. capitata* took the same time to recognize fruits

with and without films (fig. 3a). It was observed that the average number of landings was lower in treatment with Surround® (2.43 ± 0.094) compared to that in control (2.92 ± 0.094). These results are in accordance with those obtained by Mazor and Erez (2004) in studies of *C. capitata* oviposition in nectarine, in which average landing was 0.05 in kaolin treated fruits and 4.95 in untreated fruits. The authors attributed their results to the whitish colour left by the film on fruits, impairing the detection of hosts by flies (Mazor and Erez, 2004). In the present study, the number of *C. capitata* landings on fruits treated with Surround® was five times lower than that in untreated fruits (taking into account original unprocessed data). Probably, the particle films masked the volatile emission of fruits, interfering in the oviposition behaviour of fly. Studies using other films on 'Golden Delicious' apple fruits confirm that volatile compounds can be inhibited by up to 75% (Saftner, 1999) for this type of coverage. However, in the present study, the determination of volatiles by means of chromatographic analysis would be necessary to confirm this hypothesis.

Mineral films form a physical barrier over fruit, which is evidenced by the change in pulp firmness (table 3); however, this barrier did not influence the duration of aculeus insertion (puncture) (fig. 3e). Mineral films resulted in an increase in pulp firmness compared to control, which may have negatively affected oviposition at the highest suspension. *Ceratitis capitata* females prefer to oviposit on grape fruits with more advanced physiological development stage, that is, with lower firmness, lower TA and higher content of TSS (Gómez *et al*., 2019). The same fact has already been observed by Jang and Light (1991) for *Bactrocera* (*Dacus*) *dorsalis* Hendel in papaya.

Some fruits also possess epicarps that show resistance so that some species with short aculeus, like *C. capitata*, are unable to make punctures and deposit eggs (Aluja and Mangan, 2008). According to Saour and Makee (2004), mineral particles make fruit surface rough and may make them unsuitable for oviposition. Among the variables determined or observed in this study, the number of punctures without eggs occurred in all bioassays and in all treatments, but without significant difference. This resistance, mainly provided by minerals films, may influence flies to make punctures without depositing eggs on fruits. Films should also inhibit this behaviour, since, for certain thin-skinned fruits, the injury caused by puncture also results in microorganism contamination (Engelbrecht *et al*., 2004). It is observed that films resulted in a reduction in the number of landings of fly on fruits, but did not prevent them from recognizing and puncturing the treated grapes; this fact was also reported for blueberry fruits treated

with Surround® and exposed to the fly *R. mendax* (Lemoyne *et al*., 2008). The interference of films in colour (brightness, chroma and hue angle) and, probably, in the dispersion of volatiles, made it difficult for the females to recognize the fruits while the firmness may have acted directly in oviposition. *Ceratitis capitata* has short aculeus, smaller than other tefritids and usually selects fruits in more advanced maturation stages to oviposit.

After the puncture, flies exhibit the behaviour of circulating the fruit and occasionally dragging ovipositor to deposit marking pheromone (Díaz-Fleischer *et al*., 2000). All treatments showed this behaviour, without significant difference. According to Díaz-Fleischer *et al*. (2000) flies clean aculeus to disperse marking pheromone and remove fruit pieces that are attached to the aculeus. It was observed that this cleaning was not mandatory, and in kaolin 607 and chitosan treatments, flies did not perform this procedure (fig. 3j). The absence of aculeus cleaning behaviour reinforces the hypothesis that flies did not recognize chitosan as an inappropriate substrate for oviposition, otherwise, an increase in oviposition regardless of suspension and type of test (in choice and non-choice) would have been observed. Such a hypothesis can be made because, in kaolin-treated blueberry fruits, *R. mendax* females made relatively short walks, followed by frequent cleaning sessions, suggesting that some fragment in the film would have hindered the perception of stimuli (chemical compounds on the surface, blocked or absorbed by the particle film) needed to assess the suitability of hosts (Lemoyne *et al*., 2008).

The results obtained in this study are promising, given the scientific evidence that films of mineral particles such as kaolin (Surround®, 607, 608 and 611) change the firmness, luminosity, chroma and hue angle of fruits and reduce the oviposition of *C. capitata*. In addition, we also observed that natural polymers do not deter *C. capitata* females, but rather seems to stimulate oviposition.

Acknowledgments: We are grateful to the Foundation for Research Support of the State of Bahia (FAPESB) and the CAPES Foundation (Brazilian Ministry of Education; Finance Code 001) for financial support. The companies Brasilminas and NovaSource for donating the kaolin samples.

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Periodíco Científico: Insects – A2

ARTICLE II

Influence of mineral films and biomaterials on the coloring of guava fruits and implications for the oviposition of *Anastrepha obliqua* (Macquart) (Diptera: Tephritidae)*

* **Situation:** Published (Annex II)

Influence of mineral films and biomaterials on the coloring of guava fruits and implications for the oviposition of *Anastrepha obliqua* (Macquart) (Diptera: Tephritidae)

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Short title: Influence of films on the oviposition of *Anastrepha obliqua*

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Received 11 March 2021; Revised: 12 April 2021; Accepted: 16 April 2021; Published: 21 April 2021

Simple Summary: Among the main phytosanitary problems that affect the production and commercialization of fresh fruits, the occurrence of fruit flies (Diptera: Tephritidae) is one of the main obstacles. The control of these tephritids is mainly performed through the use of toxic baits. The use of mineral films and biomaterials may constitute a viable alternative in relation to the traditional insecticide method, mainly because they do not contaminate the environment and do not leave toxic residues harmful to humans and animals in treated products. Therefore, by modifying the color and texture of the fruit cuticle that covers the plant tissues, kaolin affects the perception of arthropod pests, impairing the localization process and acceptance of the host plant and, consequently, its feeding and oviposition. In this study, we hypothesized that the color changes of guava fruits because of mineral particle films and biomaterials can affect the oviposition of fruit flies. The results obtained are promising and show that mineral films and biomaterials interfering with the color of guavas inhibited the oviposition of *A. obliqua*. Therefore, they can be used to protect guava fruits from the damage caused by this pest.

Abstract

Anastrepha obliqua (Macquart, 1835) is an important pest of tropical fruits, especially Anacardiaceae and Myrtaceae, in the Americas. The objective of this study was to evaluate the influence of mineral films and biomaterials on the coloring of guava fruits (*Psidium guajava* L.) and implications for the oviposition of *A. obliqua*. Before the bioassays, color, firmness characteristics, total soluble solids, pH, and titratable acidity were determined to characterize the maturation stage of the fruits. Pieces of guava fruit covered in aluminum foil were immersed in suspensions of mineral particles (kaolins Surround® WP; 605, 607, 608, and 611; and talc) and biomaterials (chitosan, cassava and potato starch, and guar gum) and distilled water (control). After drying, the fruits were exposed to two *A. obliqua* pairs for 48 h in choice and non-choice tests, and the numbers of eggs per fruit were counted. Mineral films (kaolins Surround® WP, and 605, 607, 608, and 611) and biomaterials (cassava and potato starch) interfered with the color of guava (luminosity, chroma, and hue angle), inhibiting the oviposition of *A. obliqua*. Talc, chitosan, and guar gum did not influence the oviposition of *A. obliqua i*n guava.

Key words: chitosan; eggs; fruit flies; kaolin; luminosity

1. Introduction

Brazil is the world's largest red guava (*Psidium guajava* L.) producer, reaching 578,600 tons in 2019, of which 34% was exported [1,2]. Among the most cultivated guava varieties, "Paluma and Pedro Sato" have a dual aptitude, for consumption in natura and processing industries [3].

The valorization of guava trees as raw material for the food industry and the increased consumption of in natura fruit are proportional to changes in the production system and commercialization. This is particularly true concerning the quality of the fruits produced, which can be affected by phytosanitary problems [4].

Guava is one of the fruits most affected by fruit flies (Diptera: Tephritidae) in Brazil [5]. Fruit fly larvae cause serious damage to fruit growth because they feed on the fruit pulp, making the fruit unsuitable for consumption in natura or industrialization [6]. Several factors, such as climate, altitude, geographical location, hosts, and adjacent orchards, can influence the diversity and dominance of fruit fly species in orchards [7]. Among these species, *Anastrepha obliqua* (Macquart, 1835) is an important pest of tropical fruits in the Americas, with great genetic variability among its populations and a wide geographical distribution, from northern Mexico to southeastern Brazil [8]. The most common hosts of *A. obliqua* are fruits of the family Anacardiaceae, such as the mango (*Mangifera indica* L.), the genus *Spondias* [9,10], and within the Myrtaceae family, mainly fruits of guava [11]. *Anastrepha obliqua* reach the peak of oviposition between 15 and 25 days, producing an average of 137 eggs per female, depositing one egg per oviposition [12,13].

To locate the host plant, female fruit flies can select oviposition sites based on the host plant species, size, color, odor, flavor, and maturation stage of the fruits, and avoid fruits previously oviposited [14]. Chemical stimuli, nutritional and inhibitory substances, or food stimulants also affect resource localization [15]. Fruit flies respond negatively to visual stimuli with high reflectance and wavelengths less than 520 nm, reducing oviposition and the capture of adults in traps [16–18].

The population suppression of fruit flies via behavioral manipulation using toxic baits (a mixture of attractive food and lethal agents) has become an important component of integrated pest management (IPM) programs worldwide [19–27]. However, the intensive use of toxic baits, such as the insecticide spinosad, can cause serious biological imbalances in fruit orchards by selecting resistant populations of this

pest [28]. In addition, spinosad could also affect useful Arthropodofauna [29]. Thus, chemical insecticides are being used less to manage this pest, mainly because of pressure from consumers who prefer fresh fruits without residues, making it necessary to evaluate alternative strategies to manage this pest [30].

Mineral kaolin particle films and biomaterials are viable options for use in the replacement of synthetic chemical insecticides to avoid environmental contamination and the spread of toxic residues to humans and animals in the treated products [31,32].

Kaolin is an aluminosilicate mineral that is chemically inert, white, and formulated for use in plants [33]. The mechanisms of action of kaolin against insect pests include repellent, tactile, or visual interference, committed or interrupted oviposition and feeding activity, and decreased longevity and survival [34]. Therefore, by modifying the color and texture of the fruit cuticle that covers the plant tissues, kaolin affects the perception of arthropod pests, impairing the localization process and acceptance of the host plant and, consequently, its feeding and oviposition [35–37]. Unlike traditional agricultural chemicals, mineral kaolin particle films are inert and have no biochemical or physiological effects on plants or arthropod pests [38]. Thus, kaolin used in isolation does not cause fruit fly mortality [39,40], affect fruit fly attachment capacity on substrates treated with kaolin, or interfere with female oviposition behavior [41]; however, it can interfere with oviposition behavior [42]. When associated with entomopathogenic fungi, this product can cause insect pest mortality [43]. In addition to kaolin, biomaterial-based particle films have been used to protect cultivated plants because of their high availability, biodegradability and biocompatibility, and low toxicity [44,45]. In agriculture, these biomaterials are used mainly for the coating and preservation of fruits before and after harvest [46,47]. Cellulose, agar, starch, pectin, guar gum, alginates, carrageenan, xanthan gum, chitin, and chitosan are among the most commonly used natural polymers [47]. For example, chitosan is used to treat seeds, stimulate plant growth, and control phytopathogens [46,48]. When encapsulated in nanoparticles, chitosan is released gradually [46,47,49,50]. Chitosan also delays the fruit ripening process and inhibits the development of eggs and larvae of the *Anastrepha ludens* (Loew) [51,52].

Particle films based on minerals and biomaterials have been studied as important tools for the management of fruit flies in apples [53,54], nectarines [31,53], cherries [42], blueberries [40], citrus and peaches [31], and grapes [55]. Therefore, we hypothesized that the color changes of guava fruits, because of mineral particle films

and biomaterials, can affect the oviposition of fruit flies, reducing their infestation in the field.

The objective of the present study was to evaluate the influence of mineral particles and biomaterial films on the coloring of guava fruits and their implications for the oviposition of *A. obliqua* in the laboratory.

2. Materials and Methods

2.1. Origin of Anastrepha obliqua and fruits used in bioassays

Adults of *A. obliqua* fruit flies were obtained from Embrapa Mandioca and Fruticultura and maintained in an air-conditioned room of the Entomology Laboratory at the State University of Southwest Bahia in acrylic cages (30 × 30 × 30 cm). They were fed daily with a Bionis-based diet®, sugar (proportion 1:3) [56] and water and maintained at 25 ± 2 °C and 70 ± 10% relative humidity. Guava fruits of the Pedro Sato variety were offered to adult *A. obliqua* every two days for oviposition, and posteriorly removed and placed in plastic trays containing vermiculite to obtain larvae and pupae. The pupae were placed in 500 mL plastic pots containing a thin layer of vermiculite covered with paper towels until adult emergence.

The guava fruits (*Psidium guajava* L.) Pedro Sato variety with red colored pulp were obtained from the local fresh fruit trade and selected at maturation stage 2, based on the description by Azzolini et al. [57]. The use of guava fruits with red pulp in the presente *A. obliqua* oviposition study facilitated the visualization of eggs and minimized possible experimental errors because of the contrast of the white color of the eggs of *A. obliqua* compared to the red color of the guava pulp.

Fruits were selected based on the light green color of the epicarp (peel), color uniformity, hue angle (between 116 and 113 h), and absence of oviposition orifices of fruit flies.

The guavas were washed with 1% hypochlorite and cut in the part median, in average into 2 × 2 × 1 cm pieces (length, width, and height, respectively) (6 pieces). Based on the methodology described by Joachim-Bravo et al. [58], the pieces of guava were packaged in aluminum foil, such that only the peels were exposed for oviposition, and they were subsequently used in bioassays.

Before starting the bioassays, the physicochemical characteristics of the guava fruits, including firmness, color, total soluble solids (TSS), pH, and titratable acidity (TA),

were determined to characterize their ripening stage. Firmness was evaluated using a penetrometer (model WA68, Italy) with an 8 mm diameter tip. Two readings were taken per fruit on opposite sides in the equatorial region, on 20 fruits, with results expressed in Newtons.

The TSS content was determined by direct readings on a digital refractometer (Reichert, model r2 mini, Porto, Portugal); the results were expressed in ◦Brix, and the TA was determined by titrimetry [59], with results expressed as the % of citric acid per 100 g of pulp. The pH of 100 mL of guava juice was determined by direct readings using a digital potentiometer (Mars, model MB-10, São Paulo).

The color of the guava was determined previously and after applying the treatments on each piece of fruit, immediately after drying, using a colorimeter (CR-400, Minolta, Osaka, Japan). The apparatus was calibrated on a white ceramic plate using a D65 illuminant (z = 85.7; x = 0.3175; y = 0.3253). The luminosity values (L) were determined, which varied from 0 to 100 (black/white) and intensities of red/green (+/- (a) and yellow/blue (+/) (b). Additionally, the color parameters were estimated as chroma C = $(a^2 + b^2)$ 1/2, which represents the color purity, and the hue angle (Hue) H = tg^{-1} (b/a), which representes the color tone [40].

2.2. Oviposition: Non-Choice Tests

Two non-choice tests were performed to evaluate the effect of fruit acceptance of treated guava pieces as oviposition substrates. A completely random design was used with 11 treatments and four repetitions, evaluated on three consecutive days (one repetition every 48 h). Each non-choice test was performed using either a 100 or 200 g L^{-1} concentration of the tested mineral particle films or biomaterials. The treatments were as follows: T1, Surround® WP kaolin; T2, kaolin 605 white; T3, kaolin 607 cream; T4, kaolin 608 white; T5, kaolin 611 grey; T6, talc 657; T7, chitosan; T8, cassava starch; T9, potato starch; T10, guar gum; and T11, control (distilled water). The particle films were dispersed in distilled water at concentrations of 100 and 200 g L^{-1} and guar gum was added to these suspensions at 5 g L^{-1}, guar gum was used because it improves the viscosity and stability of formulations [60,61] except in the treatment T11 (control). These two concentrations were used because in preliminary tests with lower concentrations there was no verified effect on oviposition by the fruit fly. In the treatment with guar gum at 200 g L^{-1}, the concentration of this substance in distilled water was also doubled (10 g L^{-1}) to verify the effects of increasing the concentration.

Chitosan was obtained from the shells of crustaceans, dissolved in distilled water, and maintained under agitation for 2 min. Surround® WP kaolin was obtained from NovaSource (Phoenix, AZ, USA), and kaolins 605, 607, 608, and 611, and talc were acquired from Brasilminas (Guarulhos, SP, Brazil). Biomaterial particle films were obtained from a natural product market (Indianópolis, SP, Brazil).

The bioassays were performed in the laboratory at 25 ± 2 ºC and 70% relative humidity, with a 12 h photophase. The plot consisted of a plastic cage with a capacity of 3.5 L, containing a piece of treated guava and two pairs of 15-day-old naive *A. obliqua*, with 8 females per treatment, totaling 88 females. The pieces of guava were individually immersed for 10 s in 60 mL of each solution in a beaker. After immersion, the guava pieces were dried at 25 ± 2 ºC for 1 h. Subsequently, a piece of guava was randomly selected and exposed to the fruit flies for 48 h in each cage over a disposable plastic cup with a capacity of 50 mL and subsequently removed to determine the number of eggs.

2.3. Oviposition: Choice Tests

The bioassay of choice was developed with an experimental design similar to that described in the previous section, with 10 combined treatments and 8 females per treatment, totaling 80 females/replica and 240 females in total (3 replicates). The difference was that in this bioassay, two pieces of guava were offered to the fruit flies by cage: one was treated with mineral film or biomaterial film, and the other was untreated and immersed in distilled water (control).

The methodology was the same as described in the previous bioassay, except for the control offered to the fruit flies jointly with the other treatments. The mineral particle films and biomaterials were mixed in distilled water at a concentration of 100 g L^{-1} and 200 g L^{-1} , respectively. Guar gum was added to all treatments at a concentration of 5 g L^{-1}, except for 200 g L^{-1}, in which guar gum was used at a concentration of 10 g L^{-1}. After immersion and drying, the pieces of guava (treated and untreated (control)) were separated by 10 cm and placed on plastic cups with a 50 mL capacity, in the lower part of each cage, containing one pair of fruit flies.

2.4. Statistical Analyses

The oviposition data of the non-choice test and color of the fruits (luminosity, chroma, and hue angle) were subjected to Bartlett and Shapiro–Wilk tests to evaluate the presence of homoscedasticity of variances of the treatments and the normality of the

residues, respectively. When these assumptions were violated, the hue angle data after applying 100 and 200 g L^{-1} treatments and the number of eggs were transformed by $\sqrt{x + 1}$. Then, the data were compared using general linear models in the R software package "nlme" [62] and "lsmeans" [63]. A paired t-test was used to compare the average values of luminosity, chroma, and hue angle before and after applying the suspensions of 100 and 200 g L^{-1} [64].

The oviposition data obtained in the choice tests did not fit the assumptions of the analysis of variance, making it necessary to utilize randomization-type Monte Carlo simulations, with thousands of simulations to guarantee a 95% probability. To verify differences between treatments, a priori orthogonal contrasts were performed using R version 3.6.1 [64].

3. Results

3.1 Fruit Characterization

Before immersion in the treatments, guavas presented average values of TSS, TA, and pH were 7.0 ± 0.17 ºBrix, 0.52 ± 0.01, and 3.40 ± 0.52, respectively. The average firmness of guava pulp was 45 ± 0.91 N. The color of the guavas before treatments at a concentrations of 100 g L^{-1} differed only in the chroma parameter ($F = 82.101$; $df = 10, 43$; $p < 0.001$), ranging from 37.73 ± 1.82 (kaolin 607) to 40.01 ± 0.32 (Surround® WP kaolin); however, they did not differ from the control. The luminosity ($F = 1.7272$; $df = 10, 43$; $p = 0.11583$) and color angle ($F = 1.2427$; d f= 10, 43; $p = 0.3017$) did not differ between treatments (Table 1).

Film suspensions at 100 g L^{-1} affected the luminosity ($t = 11.454$; $df = 43$; $p < 0.001$), chroma ($t = 9.9953$; $df = 43$; $p < 0.001$), and hue angle ($t = -8.0453$; $df = 39$; $p < 0.001$). A comparison of the luminosity values before and after immersion in the 100 g L^{-1} suspension showed that all films increased the luminosity and hue angle, with a decrease in the chroma of the fruits, indicating immersion in mineral films and biomaterials influenced the change of guavas color (Table 1).

Differences were observed between treatments in luminosity ($F = 49.405$; $df = 10, 43$; $p < 0.001$), chroma ($F = 480.53$; $df = 10, 43$; $p < 0.001$), and hue angle ($F = 187.934$; $df = 10, 43$; $p < 0.001$) (Table 1) after immersion in 100 g L^{-1} suspensions. The luminosity and hue angles of the guava fruits before immersion in the suspensions were consistently lower than those after immersion in all treatments. Luminosity varied

Table 1. Luminosity, chroma and hue angle (mean ± standard deviation) of the guavas before and after immersion in suspensions at 100 g L^{-1}.

Treatments	Before immersion in suspension at 100 g L^{-1}			After immersion in suspension at 100 g L^{-1}		
	Luminosity	Chroma	Hue angle	Luminosity	Chroma	Hue angle
T1-Kaolin Surround® WP	54.71 ± 0.12 a	40.01 ± 0.32 a	113.78 ± 1.11 a	86.55 ± 1.73 a	2.87 ± 0.07 e	123.00 ± 0.0 e
T2- Kaolin 605 white	55.94 ± 1.15 a	39.01 ± 0.63 ab	114.32 ± 1.70 a	83.39 ± 1.72 a	3.45 ± 0.38 e	138.25 ± 2.63 bc
T3- Kaolin 607 cream	53.86 ± 1.91 a	37.73 ± 1.82 b	114.17 ± 1.00 a	74.12 ± 2.36 b	20.40 ± 1.61c	152.75 ± 0.5 a
T4- Kaolin 608 white	55.05 ± 1.01 a	38.36 ± 0.42 ab	115.6 1 ± 2.67 a	70.41 ± 4.80 bc	2.73 ± 0.18 e	126.75 ± 5.62 de
T5- Kaolin 611 grey	53.04 ± 1.35 a	37.96 ± 0.47 ab	114.25 ± 0.95 a	70.99 ± 3.00 bc	13.80 ± 1.03 d	143.5 ± 1.91 b
T6- Talc 657	56.14 ± 1.52 a	38.36 ± 1.32 ab	116.45 ± 1.31 a	73.42 ± 2.25 b	11.59 ± 1.41d	137.25 ± 2.36 c
T7- Chitosan	55.44 ± 1.54 a	39.09 ± 0.60 ab	115.10 ± 1.16 a	64.69 ±0.98 cd	28.41 ± 1.38 b	124.75 ± 4.03 de
T8- Cassava starch	56.13 ± 2.10 a	39.41 ± 0.55 ab	115.51 ± 1.68 a	68.71 ± 3.51 bcd	22.19 ± 1.37 c	129.75 ± 0.96 d
T9- Potato starch	55.70 ±1.98 a	39.53 ± 1.27 ab	114.06 ± 1.96 a	62.73 ± 2.83 de	30.12 ± 1.85 b	136.25 ± 2.87 c
T10- Guar gum	54.08 ± 1.78 a	39.08 ± 1.44 ab	113.69 ±1.68 a	58.01 ± 2.61 ef	40.63 ± 0.89 a	112.00 ± 0.82 f
T11- Distilled water	55.74 ± 1.77 a	39.70 ± 0.41 ab	114.94 ± 1.33 a	55.77 ± 2.06 f	40.20 ± 2.08 a	112.25 ± 1.70 f
Coefficient Variation (%)	2.86	2.5	1.37	3.89	6.54	2.05

Means followed by the same lowercase letter in the column are not different by the Tukey test ($P < 0.05$). Four repetitions per treatment were used.

from 0 (black) to 100 (white), and the guavas after treatments had values between 55.77 ± 2.06 and 86.55 ± 1.73. The highest luminosities were observed in the fruits treated with Surround® WP kaolin and kaolin 605, and the lowest was in the fruits treated with distilled water, followed by guar gum. In contrast, the largest hue angle was observed in fruits treated with kaolin 607, and the smallest was in those treated with distilled water and guar gum, with values ranging from 112 ± 0.82 to 152.75 ± 0.5.

Except for the control and guar gum, the chroma or purity of the color of the guava fruits before immersion in the suspensions was always lower than those after immersion in all treatments, with values ranging from 2.73 ± 0.18 to 40.63 ± 0.89 (Table 1). The highest chroma values were observed in fruits with treatments of guar gum and the control, and the lowest was in treatments with kaolins Surround® WP, 605 and 608.

Guavas immersed in the 200 g L^{-1} suspension differed in luminosity ($t = -11.293$; $df = 43$; $p < 0.001$), chroma ($t = 13.794$; $df = 43$; $p < 0.001$), and hue angle ($t = 235.42$; $df = 43$; $p < 0.001$) (Table 2), compared to guavas before immersion (Table 2). The color values of the guavas after immersion at 200 g L^{-1} were different from those of guavas before immersion in the suspensions, demonstrating that all films modified this parameter.

There were no differences in luminosity ($F = 1.4729$; $df = 10, 43$; $p = 19.36$), chroma ($F = 2.0251$; $df = 10, 43$; $p = 0.6254$), or hue angle ($F = 0.53799$; $df = 10, 43$; $p = 0.85047$) in guava fruits before immersion in 200 g L^{-1} suspensions (Table 2). However, differences in luminosity ($F = 718.89$; $df = 10, 43$; $p < 0.001$), chroma ($F = 248.9$; $df = 10, 43$; $p < 0.001$), and hue angle ($F = 21.179$; $df = 10, 43$; $p < 0.001$), (Table 2) were observed in fruits after immersion. The highest luminosities and lowest chroma of the guava fruits after immersion in the suspensions were observed in the kaolins Surround® WP and 605 treatments, respectively. However, the lowest luminosities and the highest chroma were observed in fruits treated with distilled water and guar gum, respectively. The major hue angle was observed in fruits treated with kaolin 607 and the smallest in those treated with kaolin Surround® WP, with values of 154.84 ± 1.49 (kaolin 607) and 98.44 ± 4.02 (kaolin Surround® WP).

The luminosities of the fruits immersed in the 200 g L^{-1} suspensions were always greater than those of the fruits immersed in the 100 g L^{-1} suspensions ($t = 4.9029$; $df = 43$; $p < 0.0001$), except for chitosan (Tables 1 and 2).

Table 2. Luminosity, chroma and hue angle (mean ± standard deviation) of the guavas before and after immersion in suspensions at 200 g L^{-1}.

Treatments	Before immersion in suspension at 200 g L^{-1}			After immersion in suspension at 200 g L^{-1}		
	Luminosity	Chroma	Hue angle	Luminosity	Chroma	Hue angle
T1-Kaolin Surround® WP	53.60± 5.3 a	40.07±2.09 a	113.77±2.40 a	91.08±2.98 a	3.52±0.21 h	98.44±4.02 d
T2- Kaolin 605 white	52.96±6.38 a	41.86±1.87 a	114.34±2.50 a	91.18±0.75 a	4.57±0.52 gh	106.27±10.18 cd
T3- Kaolin 607 cream	54.52±4.24 a	39.58±1.78 a	116.95±3.29 a	79.59±4.26 bc	14.09±0.94 d	154.84±1.49 a
T4- Kaolin 608 white	55.19±3.68 a	43.13±1.29 a	116.44±4.57 a	72,69±1.75 c	6.24±0.68 efg	134.09±1.01 b
T5- Kaolin 611 grey	49,63±3.39 a	39.14±3.57 a	114.06±2.41 a	75.47±2.12 c	7.98±0.40 e	133.04±1.22 b
T6- Talc 657	49,72±4.80 a	39.34±3.66 a	116.26±5.07 a	84.60±1.68 ab	6.92±0.23 ef	127.05±2.25 b
T7- Chitosan	55,86±2.71 a	41.95±1.71 a	114.48±2.14 a	58.07±1.86 d	18.95±0.98 c	110.94±2.61 cd
T8- Cassava starch	58.62±2.34 a	42.96±1.10 a	116.85±1.98 a	79.79±1.23 bc	5.49±0.30 fg	110.14±4.36 cd
T9- Potato starch	57.28±2.26 a	39.35±1.49 a	116.76±5.84 a	73.97±3.82 c	7.08±0.68 ef	106.36±1.88 cd
T10- Guar gum	51.21±2.21 a	40.90±1.21 a	114.46±2.59 a	57.47±6.04 d	37.70±1.10 b	114.14±1.04 c
T11- Distilled water	51.69±0.72 a	40.25±0.41 a	114.86±2.14 a	55.84±2.84 d	39.68±1.18 a	115.67±2.57 c
Coefficient Variation (%)	7.18	5.09	2.97	4.09	5.34	3.26

Means followed by the same lowercase letter in the column are not different by the Tukey test ($P < 0.05$). Four repetitions per treatment were used.

3.2 Oviposition: Non-Choice Tests

The number of eggs deposited by *A. obliqua* females in the pieces of guava immersed in the 100 g L^{-1} (AIC = 120.38; df = 43) and 200 g L^{-1} suspensions (AIC = 112.7; df = 43) varied between treatments in the non-choice test (Table 3). A small number of eggs were deposited by females of *A. obliqua* in the pieces of fruit treated with kaolins Surround® WP and 608 at 100 g L^{-1} concentration and the highest were in those treated with chitosan at the same concentration.

However, in the 200 g L^{-1} concentration, a small number of eggs was deposited by *A. obliqua* females into pieces of fruit treated with kaolins Surround® WP; 605, 607, 608, and 611; potato starch; and talc. The largest was for that treated with distilled water.

3.3 Oviposition: Choice Tests

In the choice bioassays, the number of eggs deposited by *A. obliqua* females in pieces of guava immersed in concentrations of 100 g L^{-1} ($F = 6.424$; df = 10; $p < 0.0001$) and 200 g L^{-1} ($F = 2.006$; df = 10; $p = 0.048$) varied between treatments (Figure 1).

Except for fruits treated with talc and chitosan at 100 g L^{-1}, guar gum at 5 g L^{-1} (Figure 1a), and those treated with chitosan at 200 g L^{-1} (Figure 1b), a small number of postures of *A. obliqua* occurred in the other treatments with films of mineral particles of kaolin and biomaterials based on potato and cassava starch (Figure 1a). Talc applied at a 200 g L^{-1} concentration decreased the number of eggs deposited by *A. obliqua* females in the guava pieces. However, the observed variations in the standard deviation of the means were consistent with the small numbers of eggs deposited by *A. obliqua* in fruits treated with kaolins Surround® WP, and 611, cassava, and potato starch at 100 g L^{-1} concentration and only those treated with kaolins 605 and 608 at a concentration of 200 g L^{-1}.

4. Discussion

The similarity in luminosity and hue angle of the peel between the guava fruits used in the bioassays before applying the suspensions of mineral particle films and biomaterials confirmed that they were in a similar stage of maturation, with small variations in chroma (Table 1). These results corroborate those obtained by Azzolini et al. [57], who

Table 3. Estimates for GLM parameters with model Gaussian for the number of eggs (mean ± SE) of *A. obliqua* in guavas, subjected to suspensions at 100 and 200 g L^{-1} no-choice tests.

Treatments	Suspension at 100 g L^{-1}					Suspension at 200 g L^{-1}				
	Estimate	Error Standard	Z-Value	*p*-Value	Eggs (N°)[1]	Estimate	Error Standard	Z-Value	*p*-Value	Eggs (N°)[1]
(Intercept)	0.707	0.4177	0.0999	0.0999	-	-4.017	0.000	0.000	1.0000	-
T1-Kaolin Surround® WP	-	-	-	-	0.70 ± 0.42 a	-	-	-	-	0.0 ± 0.38 a
T2- Kaolin 605 white	0.539	0.5907	0.3690	0.3682	1.25 ± 0.42 ab	3.231	0.000	0.597	0.5547	0.32 ± 0.38 a
T3- Kaolin 607 cream	0.323	0.5907	0.5884	0.5884	1.03 ± 0.42 ab	4.228	0.000	0.000	1.0000	0.0 ± 0.38 a
T4- Kaolin 608 white	0.161	0.5907	0.7863	0.7863	0.87 ± 0.42 a	1.436	0.000	0.265	0.7924	0.14 ± 0.38 a
T5- Kaolin 611 grey	0.730	0.5907	0.2249	0.2249	1.44 ± 0.42 ab	3.677	0.000	0.000	1.0000	0.0 ± 0.38 a
T6- Talc 657	0.515	0.5907	0.3896	0.3896	1.22 ± 0.42 ab	-6.206	0.000	0.000	1.0000	0.0 ± 0.38 a
T7- Chitosan	2.109	0.5907	0.0011**	0.0011**	2.85 ± 0.42 b	5.590	0.000	1.033	0.3092	0.56 ± 0.38 ab
T8- Cassava starch	0.871	0.5907	0.1498	0.1499	1.58 ± 0.42 ab	5.403	0.000	0.998	0.3255	1.17 ± 0.38 ab
T9- Potato starch	1.840	0.5907	0.0038**	0.0038**	2.55 ± 0.42 b	2.046	0.000	0.378	0.7078	0.17 ± 0.38 a
T10- Guar gum	0.865	0.5907	0.1524	0.1524	1.57 ± 0.42 ab	1.500	0.000	2.771	0.0091**	1.50 ± 0.38 b
T11- Distilled Water	1.677	0.5907	0.0077**	0.0077**	2.38 ± 0.42 b	2.175	0.000	4.017	0.0003***	2.17 ± 0.38 b
AIC					120.38					112.7

** $p \leq 0.01$, ***$p \leq 0.00$; [1] Data transformed in $\sqrt{x}+1$. Mean± SD values in the same column followed by the same letter do not differ significantly at $p < 0.01$

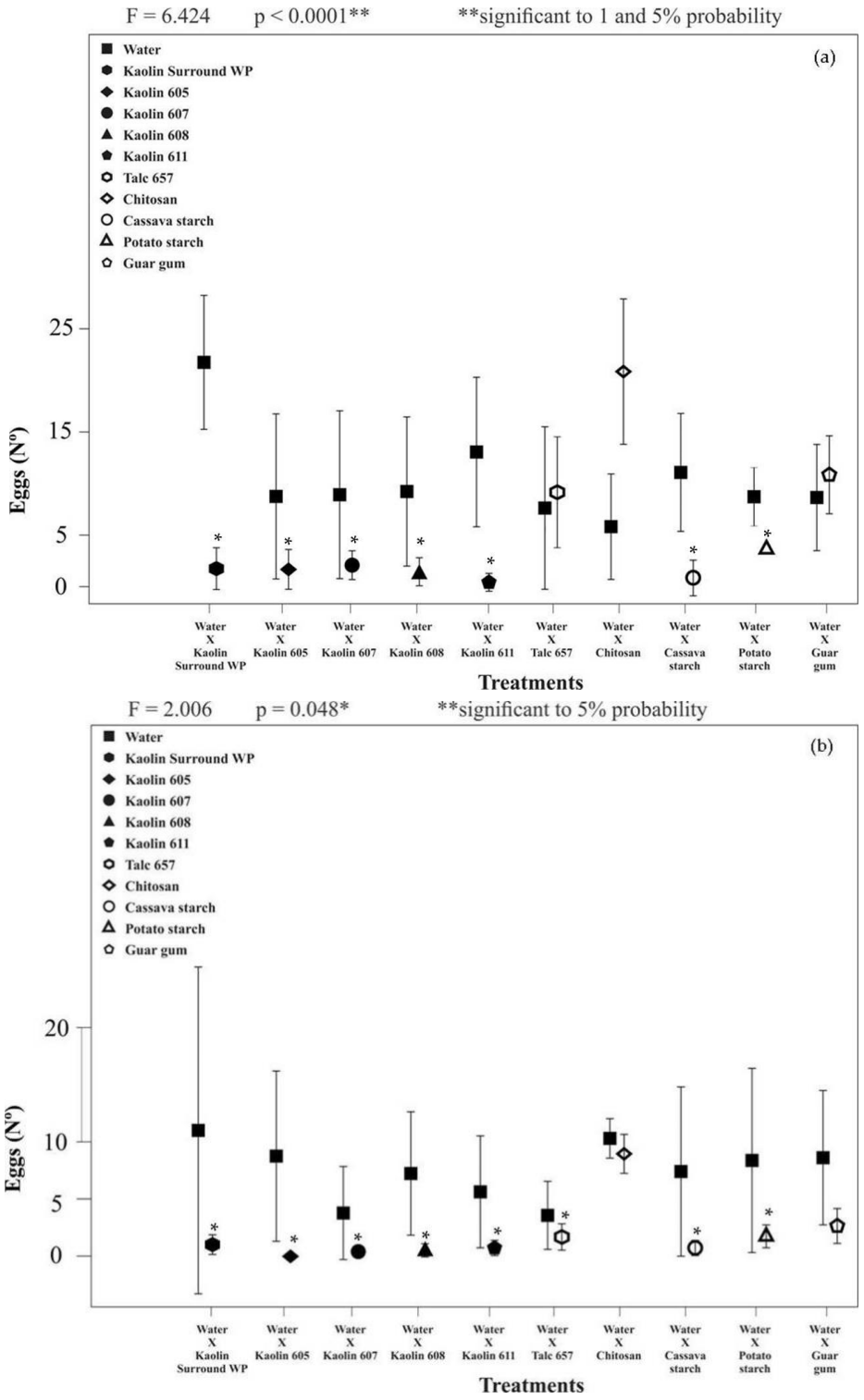

Figure 1. Number (N^o) of *A. obliqua* eggs (mean ± standard deviation) in guavas, submitted the suspensions mineral and biomaterials at 100 g L^{-1} (a) and 200 g L^{-1} (b). Four repetitions per treatment were used.

characterized maturity stage 2. This is important because the insertion of the aculeus of the flies in the fruits depends on several factors, including the type of host (primary or secondary), evidence of previous use by conspecifics (presence of pheromone marking), and quality of the fruit (i.e., degree maturation) [15]. Visual and tactile stimuli influence the recognition and acceptance of fruit as places of oviposition, making it difficult to location of oviposition sites and/ or the fixation of females on coated fruits [41]. In present study, the reduction in the oviposition of *A. obliqua* may not have been caused by the difficulty in locating the fruit due to the color change (visual stimulus) and the change in the texture of the skin due to the presence of the films (tactile stimulus).

The small number of eggs deposited by *A. obliqua* females in the pieces of fruit treated with kaolins Surround® WP and 608 at a 100 g L^{-1} concentration and in those treated with kaolins Surround® WP, 605, 607, 608, and 611; and potato starch and talc at 200 g L^{-1} in the non-choice test indicated that the mineral particle films used at the minor concentration were more suitable for protecting guava fruits than those of biomaterials. These results corroborate those of studies on kaolin applications that inhibited the oviposition of *C. capitata* in apples [54] and citrus fruits [31] at a concentration of 30 g L^{-1} in the laboratory and with those conducted in citrus orchards [32,65] and apples [66] sprayed with 50 g L^{-1} Surround®. However, the increase in the number of treatments with fewer postures of *A. obliqua*, both for mineral particles and for biomaterials in the fruits treated at a concentration of 200 g L^{-1} can be attributed to the uniform coating of the fruits provided by the higher concentration of these products [67].

In the non-choice test, when the treated and untreated fruits were offered simultaneously to laying *A. obliqua* females, an effect of the mineral particles and biomaterial films was observed regardless of concentration (100 g L^{-1} or 200 g L^{-1}). All mineral films and biomaterials based on potato and cassava starch and guar gum reduced *A. obliqua* oviposition. The preference of some tefrithids for certain colors depends on both color tone (chroma) and the intensity of the total reflected light (luminosity) [68]. For example, *A. obliqua* is attracted by wavelengths ranging from 340 nm to 670 nm, with a peak of attraction between 380 and 570 nm, corresponding to the electromagnetic spectrum where ultraviolet and visible light occur [18].

Therefore, the change of the natural green color of the guava fruit peel to the white color of the films of mineral particles or biomaterials probably impaired the perception of the *A. obliqua* females. Studies have shown that fruits or spheres covered with white coating reduce the oviposition of fruit flies [16,18,68]. The white color has a high reflectance and is less visually attractive to fruit flies, as demonstrated for *C. capitata* [68,69], *Bactrocera dorsalis* (Hendel) [70], and *A. obliqua* [18].

In general, it was verified that the 200 g L^{-1} suspension inhibited oviposition in choice and non-choice tests. Inhibition of oviposition of *C. capitata* was also obtained with the use of kaolin (Inducal®) and calcareous liquid, applied at the same concentration, in apple and mango fruits [71]. However, it was observed that 50% of the particle film-based biomaterials in the choice and non-choice tests did not protect the fruits from oviposition by *A. obliqua*. The exceptions were for potato starch, applied at a concentration of 200 g L^{-1}, which reduced the oviposition of flies in the bioassays of choice and non-choice, and cassava starch in the choice bioassay at the two concentrations tested. Several studies have been conducted with particle films based on edible biomaterials, such as starches, for post-harvest protection of fruits [72–75].

In the present study, potato and cassava starches were demonstrated to be promising for the protection of guava fruits because, in addition to preserving the color of the peel, they protected the fruit pulp from *A. obliqua* oviposition after 48 h of exposure to the insects. However, further studies in the laboratory and field should be conducted because with increased concentrations, the starch base films became brittle, exposing the fruit to flies. This is a common result, particularly in treatments with higher concentrations of this product [74,75].

The chitosan base film did not differ from the control in both bioassays for the number of eggs deposited by *A. obliqua*. This was because the product formed a semitransparent film, which delayed the ripening of the guava fruits and maintained them at the same color as the maturation stage 2 peel, similar to that of the control fruits. The maintenance of peel integrity and delaying the ripening of guava fruits are effects of chitosan, as observed by Hong et al. [76]. When applied to grapes, chitosan did not inhibit *C. capitata* but stimulated oviposition by this fruit fly [54]. Studies conducted after oviposition revealed that chitosan inhibited the development of eggs and larvae of *A. ludens* and *A. obliqua* in mangos [52,77].

Guar gum added to all suspensions of mineral particles and biomaterial films did not affect the oviposition of *A. obliqua*, except in the choice bioassay, when it was used at

10 g L^{-1}. Guar gum acts as a thickener, improving the viscosity and stability of formulations, and is commonly used in chemical and biological insecticide formulations [60,61] and as a diet for the mass production of the fruit flies and parasitoids [78]. In a similar study, guar gum, when used as a thickener in suspensions of mineral films and biomaterials, did not affect the inhibition of oviposition by *C. capitata* [55].

5. Conclusions

The results obtained in the present study are promising and show that mineral films (kaolins Surround®, 605, 607, 608, and 611) and biomaterials (cassava and potato starch) changed the color of guavas (luminosity, chroma, and hue angle), inhibiting the oviposition of *A. obliqua*. Therefore, they can be used to protect guava fruits from the damage caused by this pest. Additionally, different species of fruit flies vary their oviposition behavior in fruits treated with the studied particles. New studies should test films of mineral particles and biomaterials in other hosts for females of species of economic importance, since the oviposition behavior of fruit flies is probably regulated by an interaction of factors. Finally, it demonstrates the potential of biomaterials to protect fruits against attack by fruit flies, mainly because they are edible and rapidly degrade.

Author Contributions: Conceptualization, D.R.d.C., M.A.C., C.A.D.d.S. and I.S.J.-B.; methodology, D.R.d.C. and M.A.C.; software, D.R.d.C. and M.P.d.S.; formal analysis, D.R.d.C., M.P.d.S. and M.A.C.; investigation, D.R.d.C. and M.A.C.; resources, D.R.d.C. and M.A.C.; writing—original draft prepara- tion, D.R.d.C. and M.A.C.; writing—review and editing, D.R.d.C., S.A.L., M.P.d.S., B.S.C., A.A.M., C.A.D.d.S., I.S.J.-B. and M.A.C. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Foundation for Research Support of the State of Bahia (FAPESB) and CAPES Foundation (Brazilian Ministry of Education) Finance Code 001.

Institutional Review Board Statement: Not applicable.

Data Availability Statement: Data is contained within the article

Funding: This research was funded by Foundation for Research Support of the State of Bahia

(FAPESB) and CAPES Foundation (Brazilian Ministry of Education) Finance Code 001. Institutional Review Board Statement: Not applicable. Data Availability Statement: Data is ontained within the article.

Acknowledgments: We are grateful to the Foundation for Research Support of the State of Bahia (FAPESB) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) (Brazilian Ministry of Education; Finance Code 001) for scholarships and financial support. The companies Brasilminas and NovaSource for donating the kaolin samples. Conflicts of Interest: The authors declare no conflict of interest.

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Periodíco Científico: Biological Control – A2

ARTICLE III

Do particle films alter the parasitism of *Diachasmimorpha longicaudata* on medfly larvae?[*]

[*] **Situation:** Submitted.

Do particle films alter the parasitism of *Diachasmimorpha longicaudata* on medfly larvae?

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Abstract

The parasitoid, *Diachasmimorpha longicaudata* (Ashmead, 1905) (Hymenoptera: Braconidae), is one most important agents for the biological control of fruit flies. The majority of the studies assessing the effects of particle films focus the insect pest, leaving gaps in knowledge about the extent to which these films affect natural enemies. Thus, the objectives of this study were to evaluate the influence of mineral particle films on the oviposition behavior of *D. longicaudata* and determine the success of parasitism in Medfly (*Ceratitis capitata* Wiedmann, 1824) (Diptera, Tephritidae) using grape as substrate. Before the bioassays, the color characteristics, firmness, total soluble solid content, pH, and titratable acidity of the fruits were determined. Grapes were immersed in suspensions at 200 g L^{-1} of kaolin Surround® WP, kaolin 607, kaolin 608, and distilled water (control); thereafter, they were perforated, and two third instar larvae of *C. capitata* were inserted into the orifice. The grapes were then exposed to a female parasitoid. The frequency and duration of the following behavioral parameters of *D. longicaudata* were evaluated: landing on fruit, inspection, buccal contact, oviposition, cleaning, resting on fruit, and resting on cage. Mineral particle films altered the color and firmness of the grapes. The females of *D. longicaudata* performed all the behaviors in treated and untreated grapes, except buccal contact, which was not done on the kaolin fruits. A variation was found in the frequency and duration of behavior landing, inspection, oviposition, and fruit rest between treatments, resulting in smaller success of parasitism with kaolin application. This indicates that the effects of the particle films applied to plant organs and plant species in laboratory can affect the behavior of their natural enemies.

Keywords: color, fruit flies, kaolin, luminosity, oviposition

Introduction

Kaolin is the main component of the particle film technology and is composed of chemically inert white aluminosilicate, formulated for use in plants (Puterka et al., 2000). The mechanisms of action of kaolin against pest insects include repellency, tactile or visual interference, interruption of oviposition or feeding activity, and decreased longevity and survival (Glenn and Puterka, 2005). Plants covered with the films are altered from a visual and tactile point of view, harming the process of localization and acceptance of the host plant by insects, thereby reducing their infestation (Showler, 2002; Silva and Ramalho, 2013; Gonçalves et al., 2015). The color changes in fruits relative to the particle films reduce the oviposition of the fruit flies (Costa et al., 2021; Da Costa et al., 2021). In recent studies on fruit flies, the color change of the fruits caused by particle films significantly reduced the oviposition of females in the treated fruits (Costa et al., 2021; Da Costa et al., 2021). Several investigations, both in the laboratory and the field, highlighted that the use of films with kaolin is an important tool for the management of apple fruit flies (Mazor and Erez, 2004; Leskey et al., 2010), nectarine (Mazor and Erez, 2004; D'aquino et al., 2011), blueberry (Lemoyne et al., 2008), citrus and peach (D'aquino et al., 2011), cherry (Yee, 2012), guava (Costa et al., 2021), and grapes (Da Costa et al., 2021). However, little is known about the effects of kaolin on the oviposition behavior in parasitoids of fruit flies.

The parasitoid *Diachasmimorpha longicaudata* (Ashmead, 1905) (Hymenoptera: Braconidae), is one of the most important biological control agents for fruit flies. It is used in augmentative releases and can be used in conjunction with other management strategies (Montoya et al., 2000). This parasitoid was brought to Brazil from Florida in the 1990s by Embrapa, through the Center National Research for Cassava and Tropical Fruit and the Center National for Environmental Monitoring (Carvalho et al., 2002) and was released in the Recôncavo Baiano region (Carvalho, 2005) and the states of Minas Gerais (Alvarenga et al., 2005) and Rio de Janeiro (Leal et al., 2008).

During the process of locating the host, studies indicate that females of parasitoids respond to chemical, visual, and mechanical stimuli (Vinson, 1976; Segura et al., 2007; Quilici and Rousse, 2012; Blassioli-Moraes et al., 2016; Sharma et al., 2019). Some researchers report that female parasitoids do not discriminate or do not have preference for any host color (Leyva et al., 1991; Messing and Jang, 1992; Benelli and Canali,

2012). Color is only important for females with previous experience and is thus a result of an associative learning mechanism (Segura et al., 2007; Benelli and Canali, 2012). The kaolin particle film dyes the surface of plant tissues white and impairs the movement, feed, and oviposition of insects, creating a hostile environment for these organisms (Glenn and Puterka, 2005), which can also affect the behavior of predators and parasitoids (Vincent et al., 2003). Laboratory studies on blueberry fruits demonstrated that kaolin affects the parasitism of *Rhagoletis mendax* Curran (Diptera: Tephritidae) by *Diachasma alloeum* (Muesebeck) (Hymenoptera: Braconidae) (Stelinski et al., 2006). Although the parasitism of *Bactrocera oleae* (Rossi) (Diptera: Tephritidae) by *Psyttalia concolor* (Szèpligeti) (Hymenoptera: Braconidae) in olive fruits was not affected, when the females could choose between parasitising through a kaolin-treated surface and a water-treated, there was a slight reduction in the percentage of parasitised hosts for kaolin (Bengochea et al., 2014).

We hypothesized that changes in the physicochemical characteristics of the grape due to the kaolin particle film treatment may affect the parasitism behavior of *D. longicaudata* on *C. capitata* larvae.

Thus, the objectives of this study were to evaluate the influence of mineral particle films on the oviposition behavior of *D. longicaudata* and determine the success of parasitism in Medfly (*Ceratitis capitata* Wiedmann, 1824) (Diptera, Tephritidae) using grape as substrate.

Materials and methods

Origin of D. longicaudata and the fruits used in the bioassays

Specimens of the fruit fly, *C. capitata*, were obtained from the rearing colony located at the Laboratory Fruit Flies of the State University of Southwest Bahia-UESB, Vitória da Conquista, Bahia, Brazil. The adults were maintained in wooden cages (50 × 45 × 40 cm), with the sides covered with voile fabric for oviposition and manipulation of insects. The eggs laid by *C. capitata* on the side of the cage were collected daily and transferred to plastic pots containing an artificial larval diet adapted from Zucoloto (1987); this setup was maintained until pupariation (approximately 10 days). The pupae were collected and placed in 500 mL plastic containers with vermiculite until the adults emerged. Subsequently, couples of *C. capitata* were transferred to cages for mating and oviposition, and fed water and diet based on sugar and yeast extract Bionis® at a ratio of

3:1 (Silva-Neto et al., 2012). The cages were maintained in an acclimatized room with an average temperature of 25 ± 2 ºC, relative humidity of 70%, and photophase of 12 h.

The rearing colony of *D. longicaudata* was established from pupae parasitized by *C. capitata* obtained from the Fruit Flies Laboratory of the Embrapa Cassava and Tropical Fruit (Embrapa/CNPMF). The parasitoids were reared in acrylic cages (30 × 30 × 30 cm) on third-instar larvae of *C. capitata* according to Carvalho et al. (1998). The larvae of *C. capitata* were offered to parasitoids in parasitism units, composed of groups of 100 host larvae, packed in voile fabric, and hanged on the top of the cage. The larvae contained in the parasitism unit were exposed to *D. longicaudata* females for 1 h and transferred to 500 mL plastic containers containing vermiculite for pupariation and emergence of adult parasitoids. Adults were maintained in an acrylic cage containing water and artificial diet based on distilled water, honey, agar-agar, ascorbic acid, and nipagin (Carvalho and Nascimento, 2002).

For the experiments, grapes (*Vitis vinifera* L. 'Italia') were used as substrate. The fruits were obtained from fresh fruit trade and, subsequently, selected for maturity uniformity, size, and absence of fruit fly oviposition.

Fruit characterization

The physicochemical characteristics, such as mass, length, diameter, firmness, color, total soluble solids (TSS) content, and titratable acidity (TA) of the grapes were determined to ensure uniformity before the start of the bioassay and exposure of the fruits to adult parasitoids for oviposition.

The berry mass (grams) was determined using an analytical balance (Shimadzu - AUY 220), with a precision of 0.1 mg. The diameter and length of the berry in millimeters (mm) were obtained using a digital pachymeter (Model MPD-200, Metrotools, São Paulo, Brazil) with an accuracy of ± 0.02 mm.

To correct for possible changes in the fruits that could influence the oviposition of *D. longicaudata*, we determined the firmness of the fruits before and after the application of the suspensions (sample with 20 fruits) of kaolin, using a penetrometer (model WA68, Italy) with an 8 mm diameter tip. The SST content was determined by directly reading the extract of the pulp of the berry using a digital field refractometer (model Reichert r² mini, Porto, Portugal). The TA was measured by titration with sodium hydroxide (NaOH) at 0.1 N and expressed in grams of tartaric acid per 100 mL of juice. The pH was determined using a digital potentiometer (model MB-10, Mars, São Paulo,

Brazil), with readings obtained directly from a sample containing 100 mL of fruit juice. For each evaluated parameter, three repetitions of 10 berries were used, with each repetition derived from a single grape bunch.

Color was determined twice in each fruit, before and after applying the treatments, always in the same position (opposite sides), using 20 fruits per treatment. Changes in the fruit color were determined using a colorimeter (CR-400; Minolta®, Osaka, Japan). The calibration of the device was performed using a white ceramic plate and a D65 illuminant (z = 85.7; x = 0.3175; y = 0.3253). The luminosity values (L), ranging from 0 to 100 (black/white), the intensity of red/green (+/-) (a), and intensity of yellow/blue (+/-) (b) were determined. Beyond these color parameters, the chroma values $C = (a^2 + b^2)$ 1/2, which represent the color purity, and the hue angle (Hue) $H = tg^{-1}$ (b / a), which represents the color tone, were determined (Lemoyne et al., 2008).

Behavioral response of D. longicaudata to treated and untreated fruits

To evaluate parasitoid oviposition behavior, a completely randomized design was used, with four treatments and 20 repetitions. The treatments were as follows: T1, kaolin Surround® WP; T2, kaolin 607 cream; T3, kaolin 608 white; and T4, control (distilled water). The kaolin particles were dispersed in distilled water at a concentration of 200 g L^{-1}. Guar gum was added to all treatments, except for T4, at a concentration of 5 g L^{-1} to improve the viscosity and stability of the suspensions (Campos et al., 2015; Gao et al., 2020; Costa et al. 2021; Da Costa et al., 2021). The plot consisted of a transparent plastic cage (3.5 L capacity) containing a fertile female *D. longicaudata* (five days old) and a single grape containing two third instar larvae of *C. capitata*. Before starting the bioassay, the grapes were sanitized with sodium hypochlorite (0.5%) for 30 min. Subsequently, the grapes were immersed in the suspensions of kaolin or water (control) for 10 s and left to air dry at room temperature. After drying, the grapes were artificially infested with larvae of *C. capitata* using the methodology adapted from Pires et al. (2021). Briefly, the grapes were carefully perforated with a needle measuring 1.5 mm in diameter, at a depth of 1.5 cm, and the orifice was unobstructed. Two third instar larvae of *C. capitata* were inserted into the orifice of each grape with a fine paintbrush. Subsequently, the orifice was closed with a small cotton ball. An infested grape was hung on top of each cage, and a female parasitoid was released with the help of a buccal aspirator.

After the exposure of the fruits to female *D. longicaudata*, behavioral evaluations

were performed for 1 h, according to Altafini et al. (2019). The behavioral parameters evaluated were as follows: 1) landing (the parasitoid lands on the fruit); (2) inspection (the parasitoid walks on the fruit, vibrating its antennae and touching the oviposition substrate); (3) buccal contact (the parasitoid stops walking, leans, and touches the buccal apparatus in the substrate; (4) attempts to oviposition (the parasitoid inserts the ovipositor in the fruit); (5) cleaning (the parasitoid cleans its wings, legs, ovipositor, or buccal apparatus); (6) resting on fruit (the parasitoid remained resting on fruit, without performing any of the behaviors described above); and (7) resting on cage (the parasitoid does not land on the fruit and remains on the walls of the cage). The duration (in seconds) and frequency of each behavioral parameter were recorded and evaluated for each parasitoid.

After the evaluations, the fruits were dissected, and the larvae were removed and stored in plastic containers containing a thin layer of vermiculite to facilitate the emergence of adult parasitoids or hosts. The number of emerged parasitoids or hosts was quantified and the larval viability (VL% = number of pupae of the parasitoid × 100 / total number of fly larvae), pupal viability (VP% = number of emerged parasitoids + number of emerged flies × 100/total pupae of the fly), and parasitism index (IP% = number of emerged parasitoids × 100/number of emerged flies + number of emerged parasitoids) were calculated (Matrangolo et al., 1998).

Statistical analyses

Data on the physicochemical characteristics of the fruits and behavior of the parasitoid oviposition were subjeced to Bartlett and Shapiro-Wilk tests to evaluate the assumptions of homoscedasticity of variances and normality of the residues, respectively. In case of violation of these assumptions, the data of luminosity, hue angle after applying the treatments at 200 g L^{-1}, firmness, and the number of parasitoids were transformed into $\sqrt{x}$. Thereafter, the treatments were compared using generalized linear models (GLM) performed in R with the *nlme* (Pinheiro et al., 2020) and *lsmeans* (Lenth, 2016) packages. The paired t-test was used to compare the average values of luminosity, chroma, and hue angle before and after applying the suspension. Principal component analysis (PCA) was performed to group the variables firmness, luminosity, number of parasitoids, and number of flies using the R package *factoextra* (Kassambara and Mundt, 2017), applying the selected variables to transform data from a broad spectrum to a restricted spectrum PCA was carried out using the correlation matrix for each

variable to deduce the eigenvector and eigenvalue. The eigenvector indicates the direction of the main axis with the largest variation, and the eigenvalue indicates the magnitude of the variability of the secondary axis with the next variance. The Bartlett test was used to verify the measure of the correlation matrix and the identity matrix to indicate the existence of a relationship between the variables evaluated. The Kaiser-Meyer-Olkin (KMO) test was employed to measure the adequacy of the data for PCA (Cruz-Jesus et al., 2016). All analyses were performed using R software (version 3.6.1; R Core Team, 2019).

Results

Fruit characterization

The grapes used in the bioassay had average pulp firmness of 5.4 N; total soluble solids (TSS) of 12.8 ºBrix; TA of 1.2; and pH of 3.4.

There is no difference in mass ($F = 1.52$; $df = 3.79$; $P = 0.22$), length ($F = 1.47$; $df = 3.79$; $P = 0.22$), diameter ($F = 1.55$; $df = 3.79$, $P = 0.21$), luminosity ($F = 0.80$; $df = 3.79$, $P = 0.50$), chroma ($F = 1.84$; $df = 3.79$; $P = 0.15$), and hue angle ($F = 0.11$; $df = 3.79$; $P = 0.95$) between treated and untreated fruits, indicating uniformity of the fruits selected for the bioassay (Table 1).

In general, immersion of the grapes in the mineral suspensions revealed their effects on luminosity ($t = -14.66$; $df = 79$; $P < 0.01$), chroma ($t = 6.55$; $df = 79$; $P < 0.01$), and hue angle ($t = 1.77$; $df = 79$; $P < 0.08$), relative to the grapes before immersion (Table 2). The luminosities and hue angles of the grapes before immersion in the suspensions were always lower than those after immersion in kaolin. After immersion, differences were observed between treatments, revealing the effects on luminosity (AIC = 86.34; df = 79), chroma (AIC = 240.66; df = 79), and hue angle (AIC = 238.96; df = 79) (Table 2). Luminosity is a parameter that varies from zero (black) to 100 (white). After the application of the treatments, the grapes presented values between 36.90 ± 1.36 and 86.08 ± 2.01. The highest luminosities were observed for fruits treated with kaolin Surround® WP and kaolin 607 while the lowest luminosities were observed for fruits treated with distilled water. In contrast, a greater hue angle was observed for fruits treated with 607 kaolin while a lower hue angle was found for those treated with distilled water, kaolin Surround® WP, and kaolin 608, with values varying from 117 ± 3.76 to 124.9 ± 23.72.

All Mineral films increased the peel firmness of treated fruits compared to the

control group (AIC = 190.92; df = 79) (Table 2).

Behavioural response of D. longicaudata to treated and untreated fruits

Females only avoided landing on fruits coated with kaolin 607 (0.15 ± 0.09) (AIC = 103.1; df = 79) (Table 3). After landing, the time (AIC = 86.34; df = 79) and number of inspections (AIC = 109.53; df = 79) differed between treatments; grapes treated with kaolin 607 resulted in, on average, shorter time (1.14 ± 0.725 s) and lower number of inspections (1.14 ± 0.107) (Table 3). Buccal contact time was shorter after the kaolin treatments (AIC = 227.57; df = 79), varying from 0.00 ± 0.220 (kaolins Surround® WP, 607 and 608) to 1.51 ± 0.220 s (control - distilled water); this is because females did not display this behavior on fruits with the films and the treatments did not affect the number of times this behavior was displayed (AIC = 129.4; df = 79) (Table 3). Treatment effects were observed in relation to time (AIC = 335.48; df = 79) and number of oviposition attempts, with kaolin 607 providing a shorter time (0.43 ± 0.447 s) and lower number of attempts to oviposition (0.43 ± 0.156), with no difference compared to the product, Surround® WP, in terms of the quantity of attempts to oviposition (1.06 ± 0.23) (Table 3). The resting time on the fruit varied between treatments; the parasitoids remained longer on the fruits treated with kaolin 608 (4.50 ± 0.706 s); however, the number of times the female rested on the fruit was not affected by treatments (AIC = 128.5; df = 79) (Table 3). The resting in the cage time (AIC = 303.68; df = 79) and the number (AIC = 105.8; df = 79) did not vary between treatments.

A total of 188 pupae were obtained, of which 153 emerged. In total, 125 were flies and 28 were parasitoids, with high rates of viability for larvae (94.0%) and pupae (81.0%). The total parasitism index was 18.30%, varying from 1.96% in the treatments with kaolin to 47.5% in the control. In relation to the emergence of parasitoids, all kaolins decreased the number of parasitoids (AIC = 112.9; df = 79) (Table 3).

Kaolin was the main factor responsible for the alteration in the physical characteristics of the fruits, interfering with parasitism (Figure 1).

The variables presented in Table 4 and Figure 1 provide the total components and proportion of variance, indicating the total variation of the main component. The results indicated positive linear correlations for luminosity, firmness, and number of flies, and negative correlations for the number of parasitoids (Table 4). Four distinct axes were obtained for these components. The first two components (PC1 and PC2) explained 86.49% of the total variance observed (Figure 1). PC1 is a component of the physical

characteristics of luminosity (72.36) and firmness (16.70); these variables had a higher contribution to the construction of the component, with an effect on the emergence of flies and parasitoids. In relation to PC2, there was a positive correlation only for the number of flies, while the other characteristics were negatively correlated. However, this component explained only 20.7% of the variation in the data, and the largest contribution was to the number of flies (Flies = 20.81).

Discussion

Before the bioassays, the grapes were uniform in terms of all physical characteristics. The values of the mass, length, and diameter of the berries can be considered to be within the commercial standard (Brasil, 2002; Mascarenhas et al., 2013). The total soluble solids of 12.8 °Brix indicate that the grapes were found in the initial stage of maturation, as mature grapes presented contents equal to or greater than 14 °Brix (Brasil, 2002). In addition, the stage of maturation of the grape did not influence results, so the parasitoid *D. longicaudata* is able locate its host, not only in fruits mature infested in the canopy of the plant but those fallen in the soil in advanced stage of maturation (Harbi et al., 2018).

The oviposition behavior of female *D. longicaudata* was not affected by changes in the color of grapes after the application of the suspensions (Table 2); this is because female parasitoids could locate their host in most treatments. Grapes infested by *C. capitata* and dyed white by the kaolins Surround® WP and 608, attracted female *D. longicaudata* as much as grapes of natural color, except for the fruits coated with kaolin 607. In these cream-dyed fruits, the females avoided landing. According to Leyva et al. (1991), the parasitism of *Anastrepha ludens* (Loew) (Diptera: Tephritidae) by *D. longicaudata* females was not influenced by the color of grape (*Citrus paradisi* Macf.), mango (*Mangifera indica* L.), and peach (*Prunus persica* L.). Females of *D. longicaudata* discriminate fewer visual stimuli than males; olfactory stimuli predominate in the search for the host (Messing and Jang, 1992).

The lower number of inspections on fruits for a short period of time by female *D. longicaudata* on grapes treated with kaolins Surround® WP and 607 (Table 3) indicates that the particle film affected the behavior of this parasitoid. This finding might be due to the thickening of the pericarp of the grapes covered by the kaolin film; the physical characteristics of the fruits, such as slender pericarp and fleshy mesocarp, can facilitate the detection and oviposition of *D. longicaudata* in the larvae of *A. fraterculus* (Ovruski

et al., 2007).

The mean time of attempts to oviposition by female *D. longicaudata* was lower in the grapes treated with kaolin 607 (8.85 ± 17.08), with a lower frequency of attempts in those treated with the kaolins Surround® WP and 607; this can be attributed to changes in the peel texture of grapes, which is promoted by kaolin. Female *D. longicaudata* apparently had difficulty inserting their ovipositor in fruits coated with kaolin, folding against their own body. These results agree with those observed for the parasitoid, *D. alloeum*, whose blueberry fruits treated with kaolin prejudiced the oviposition of this parasitoid on *R. mendax* (Stelinski et al., 2006). In the present study, fruits treated with kaolin presented firmer peels (Table 2), which may have hindered the penetration of the ovipositor of the female *D. longicaudata*, reducing the time of attempts oviposition in the grapes. Female *D. longicaudata* are attracted to volatile compounds in decomposing fruits (Greany et al., 1977; Jang et al., 2000). These fruits softened the pericarp and mesocarp due to their advanced stage of ripeness, which can facilitate the penetration of the ovipositor of females (Greany et al., 1977; Silva et al., 2007). In addition, during the decomposition process, the fruits dehydrate and reduce, approaching the host larvae of the surface, facilitating the parasitism (Leyva et al., 1991).

The methodology of artificial infestation of grapes with Medfly larvae was adequate for this type of study, as the survival of the larvae and pupae phases was greater than that reported for mass rearing of *C. capitata* (FAO, 2019). The small cotton boll used to close the artificial orifice created for the infestation of *C. capitata* larvae in grapes absorbed the excess humidity and may have contributed to the increase in the survival of the immature stages of moscamed. In preliminary tests, attempts to close artificial orifices with other materials, such as paraffin and adhesive tape, resulted in high mortality of the larvae owing to liquid accumulation in the orifices, originating from the pulp residues left after the perforation of the fruits.

The total parasitism index of 18.30% for female *D. longicaudata* was similar to that obtained for artificial infestation of apples with larvae of second and third instars of *C. capitata*, in greenhouses (Harbi et al., 2018), as well as that for apple and orange in laboratory conditions (Harbi et al., 2019) with parasitism of less than 20%. However, this index can be considered low compared to that of other studies (Pires et al., 2021), which can be attributed to interference of the kaolin particle film, particularly during the insertion of the ovipositor in the fruits by the parasitoid. Limiting the exposure time of parasitoids to treatment can also hinder the parasitism of all available hosts (Harbi et al.,

2018).

All fruits coated with kaolin had decreased emergence of *D. longicaudata*. These results agree with those found for females of the parasitoid *P. concolor* which preferred the parasitized larvae of *B. oleae* in olive fruits without kaolin in choice tests (Bengochea et al., 2014).

Kaolin applied to grapes resulted in changes in the color and firmness of the berry, modifying the oviposition behavior of *D. longicaudata*, which reduced the rate of parasitism on *C. capitata* larvae. This finding indicates that the effects of the kaolin particle film applied to plant organs and plant species can affect the behavior of their natural enemies. However, the form of application and uniformity of the coverage of grapes by the film of the kaolin particles treated under field conditions might be different and smaller, respectively, relative to that in laboratory conditions, which may favor the parasitism of *D. longicaudata* under field conditions. Therefore, the location of the plant and the vegetable species to be treated with the kaolin particle film, as well as the cost benefit of the application must be considered, as different responses may be achieved depending on the considered agro-ecosystem (Silva and Ramalho, 2013).

Acknowledgements

We are grateful to the Foundation for Research Support of the State of Bahia (FAPESB) and the CAPES Foundation (Brazilian Ministry of Education; Finance Code 001) for providing financial support to our study. The companies Brasilminas and NovaSource donated the kaolin samples.

Author Contributions

Conceptualization, D.R.d.C., M.A.C., I.S.J.B., and C.A.D.S.; methodology, D.R.d.C. and M.A.C.; software, D.R.d.C. and M.P.d.S.; formal analysis, D.R.d.C., M.P.d.S., and M.A.C.; investigation, D.R.d.C. and M.A.C.; resources, D.R.d.C. and M.A.C.; writing—original draft preparation, D.R.d.C. and M.A.C.; writing—review and editing, D.R.d.C., S.A.L., M.P.d.S., C.A.D.d.S., I.S.J.B., B.S.C., R.P.M., and M.A.C. All authors have read and agreed to the published version of the manuscript.

Funding

This research was funded by the Foundation for Research Support of the State of Bahia (FAPESB) and CAPES Foundation (Brazilian Ministry of Education) Finance Code

001.

Conflict of interest

The authors declare no conflict of interest.

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Figure legends

Figure 1. Graph of the matrix of correlations between variables: luminosity, firmness, parasitoids, and flies.

Table 1. Weight (g), length (mm), diameter (mm), luminosity, chroma and hue angle (mean ± standard deviation) of the grapes used in the treatments before immersion in suspension.

Treatments	Weight (g)	Lengt (mm)	Diameter (mm)	Luminosity	Chroma	Hue Angle
T1-Kaolin Surround® WP	8.93 ± 0.70 a	27.26 ± 0.67a	22.56 ± 1.10a	37.74 ± 1.42a	9.36 ± 0.44 a	116.28 ± 0.05a
T2- Kaolin 607 cream	8.95 ± 0.80a	27.47 ± 0.88a	22.67 ± 0.94a	37.80 ± 0.87a	9.64 ± 0.66 a	116.30 ± 0.04a
T3- Kaolin 608 white	8.51 ± 0.67a	27.22 ± 0.95a	22.42 ± 0.87a	37.38 ± 1.26a	9.69 ± 0.69 a	115.53 ± 0.07a
T4-Distilled water (Control)	8.77 ± 0.76a	26.94 ± 0.69a	23.0 3 ± 0.83a	37.94 ± 1.17a	9.79 ± 0.62 a	116.14 ± 0.03a
coefficient of variation (%)	3.34	2.96	4.18	3.18	6.39	4.22

Means followed by the same letter in the column, do not differ statistically from each other by Tukey test (P<0.05).

Table 2. Estimates for GLM parameters with Gaussian model for the luminosity, chroma, hue angle, and firmness (mean ± standard deviation) of the grapes after immersion in suspensions.

Treatments	Standard Error	Z-Value	*p*-Value	Luminosity
Intercept	0.03040	292.706	<0.0001***	-
T1-Kaolin Surround® WP	0.04299	10.272	<0.0001***	86.08 ± 2.01 c
T2- Kaolin 607 cream	-	-	-	79.22 ± 3.49 b
T3- Kaolin 608 white	0.04299	10.272	<0.0001***	87.25 ± 1.86 c
T4-Distilled water (Control)	0.04299	-65.716	<0.0001***	36.90 ± 1.36 a
AIC				86.34
Treatments	Standard Error	Z-Value	*p*-Value	Chroma
Intercept	0.2347	50.730	<0.0001***	-
T1-Kaolin Surround® WP	0.3319	-25.239	<0.0001***	3.52 ± 0.32 a
T2- Kaolin 607 cream	-	-	-	11.90 ± 1.82 c
T3- Kaolin 608 white	0.3319	-26.309	<0.0001***	3.17 ± 0.59 a
T4-Distilled water (Control)	0.3319	-9.472	<0.0001***	8.76 ± 0.79 b
AIC				240.66
Treatments	Standard Error	Z-Value	*p*-Value	Hue Angle
Intercept	0.2322	53.374	<0.0001***	-
T1-Kaolin Surround® WP	0.3284	-4.393	<0.0001***	122.45 ± 28.96 a
T2- Kaolin 607 cream	-	-	-	153.64 ± 2.16 b
T3- Kaolin 608 white	0.3284	-3.916	<0.0001***	124.9 ± 23.72 a
T4-Distilled water (Control)	0.3284	-4.686	<0.0001***	117.76 ± 3.76 a
AIC				238.96
Treatments	Standard Error	Z-Value	*p*-Value	Firmness
Intercept	0.1720	17.766	<0.0001***	-
T1-Kaolin Surround® WP	0.2432		<0.0001***	18.36 ± 7.25 a
T2- Kaolin 607 cream	0.2432	5.173	<0.0001***	19.31 ± 7.98 a
T3- Kaolin 608 white	0.2432	5.311	<0.0001***	19.41 ± 6.84 a
T4-Distilled water (Control)	-	-		9.73 ± 3.75 b
AIC				190.92

Mean ± SD values in the same column followed by the same letter do not differ significantly (Tukey test, $P < 0.05$).
* $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$.

Table 3. Estimates for GLM parameters with Poisson model for the oviposition behaviour of *Diachasmimorpha longicaudata* in grapes, subjected to suspensions.

Treatments	Standard Error	Z-Value	*p*-Value	Inspection time (s)	Standard Error	Z-Value	*p*-Value	Inspection (N°)
Intercept	0.4288	8.576	0.0001***	-	0.1043	15.130	0.0001***	-
T1-Kaolin Surround® WP	0.5280	-3.367	0.00076***	1.90 ± 0.308 b	0.1474	-1.821	0.07260*	1.31 ± 0.104 bc
T2- Kaolin 607 cream	0.4768	-5.979	0.0001***	0.83 ± 0.209 c	0.1494	-2.930	000449**	1.14 ± 0.107 c
T3- Kaolin 608 white	0.6138	0.294	0.768735	3.86 ± 0.439 a	0.1474	1.144	0.25621	1.75 ± 0.104 a
T4- Distilled water	-	-	-	3.68 ± 0.429 a	-	-	-	1.58 ± 1.58 ab
AIC								109.53
Treatments	Standard Error	Z-Value	*p*-Value	Buccal Contact Time	Standard Error	Z-Value	*p*-Value	Buccal Contact (N°)
Intercept	0.2201	6.842	0.0001***	-	0.1182	2.738	0.0072**	-
T1-Kaolin Surround® WP	0.3112	-4.838	0.0001***	0.00 ± 0.220 b	0.1672	-0.679	0.49951	0.210 ± 0.118 a
T2- Kaolin 607 cream	0.3153	-4.775	0.0001***	0.00 ± 0.226 b	0.1694	-1.911	0.05984*	0.00 ± 0.121 a
T3- Kaolin 608 white	0.3112	-4.838	0.0001***	0.00 ± 0.220 b	0.1672	-1.936	0.05665*	0.00 ± 118 a
T4- Distilled water	-	-	-	1.51 ± 0.220 a	-	-	-	0.324 ± 0.118 a
AIC				227.57				129.4
Treatments	Standard Error	Z-Value	*p*-Value	Attempts to oviposition Time (s)	Standard Error	Z-Value	*p*-Value	Attempts to oviposition (N°)
Intercept	0.4357	5.610	0.0001***	-	0.3496	6.991	0.0001***	-
T1-Kaolin Surround® WP	0.6161	-2.242	0.0279*	1.06 ± 0.436 ab	0.4187	-3.298	0.0001***	1.06 ± 0.231b
T2- Kaolin 607 cream	0.6242	-3.171	0.0022**	0.43 ± 0.447 b	0.3830	-5.168	0.0001***	0.46 ± 0.156 b
T3- Kaolin 608 white	0.6161	0.182	0.8562	2.56 ± 0.436 a	0.500	0.224	0.822716	2.56 ± 0.357 a
T4- Distilled water	-	-	-	2.44 ± 0.436 a	-	-	-	2.44 ± 0.350 a
AIC				335.48				335.5
Treatments	Standard Error	Z-Value	*p*-Value	Cleaning Time (s)	Standard Error	Z-Value	*p*-Value	Cleaning (N°)
Intercept	0.38135	2.498	0.0147*	-	0.093082	1.862	0.0627*	-
T1-Kaolin Surround® WP	0.53931	0.080	0.9362	0.996 ± 0.381 a	0.179209	1.650	0.0989*	0.469 ± 0.1531a
T2- Kaolin 607 cream	0.54636	-0.099	0.9216	0.898 ± 0.391 a	0.132166	-0.046	0.9637	0.167 ± 0.0938a
T3- Kaolin 608 white	0.53931	0.093	0.9259	1.003 ± 0.381 a	0.139126	0.291	0.7707	0.214 ± 0.1034a
T4- Distilled water	-	-	-	0.952 ± 0.381 a	-	-	-	0.173 ± 0.0931a
AIC				314.43				194.9

Treatments	Standard Error	Z-Value	*p*-Value	Resting on Fruit Time (s)	Standard Error	Z-Value	*p*-Value	Resting on Fruit (N°)
Intercept	2.63730	3.734	0.000365***	-	0.50225	3.169	0.000153**	-
T1-Kaolin Surround® WP	0.05272	0.053	0.958050	2.69 ± 0.706 ab	-0.14979	-0.725	0.46872	1.31 ± 0.104 a
T2- Kaolin 607 cream	-1.49570	-1.478	0.143618	1.14 ± 0.725 b	-0.30810	-1.639	0.10119	0.352 ± 0.133 a
T3- Kaolin 608 white	1.86257	1.864	0.066168*	4.50 ± 0.706 a	0.02939	0.129	0.89715	0.532 ± 0.104 a
T4- Distilled water	-	-	-	2.64 ± 0.706 ab	-	-	-	0.502 ± 1.58 a
AIC								128.5
Treatments	Standard Error	Z-Value	*p*-Value	Resting on Cage Time	Standard Error	Z-Value	*p*-Value	Resting on Cage (N°)
Intercept	0.35625	19.621	0.0001***	-	1.2000	11.786	0.0001***	-
T1-Kaolin Surround® WP	0.50381	1.681	0.0969*	7.84 ± 0.356 a	-0.1000	-0.694	0.490	1.10 ± 0.102 a
T2- Kaolin 607 cream	0.51040	1.944	0.0556*	7.98 ± 0.366 a	-0.2000	-1.371	0.174	1.00 ± 0.104 a
T3- Kaolin 608 white	0.50381	-0.102	0.9189	6.94 ± 0.356 a	-0.0500	-0.347	0.729	1.15 ± 0.102 a
T4- Distilled water	-		-	6.99 ± 0.356 a	-	-	-	1.20 ± 0.102 a
AIC				303.68				105.8
Treatments	Standard Error	Z-Value	*p*-Value	Parasitoids (N°)	Standard Error	Z-Value	*p*-Value	Flies (N°)
Intercept	0.2179	4.359	0.0001***	-	0.2291	4.583	0.0001***	-
T1-Kaolin Surround® WP	-	-	-	0.15 ± 0.09 b	0.3708	1.753	0.0796	1.70 ± 0.292 a
T2- Kaolin 607 cream	0.2345	3.411	0.0001***	0.15 ± 0.09 b	0.3742	1.871	0.0614	1.75 ± 0.296 a
T3- Kaolin 608 white	0.2345	3.411	0.0001***	0.15 ± 0.09 b	0.3808	2.101	0.0356 *	1.85 ± 0.304 a
T4- Distilled water	0.2345	3.411	0.0001***	0.95 ± 0.2 a	-	-	-	1.05 ± 0.229 a
AIC				112.9				216.8
Treatments	Standard Error	Z-Value	*p*-Value	Landings (N°)				
Intercept	0.0001	3.873	0.0001***	-				
T1-Kaolin Surround® WP	0.0001	-1.225	0.2207	0.75 ± 0.194 a				
T2- Kaolin 607 cream	0.0001	-2.828	0.004**	0.15 ± 0.087 b				
T3- Kaolin 608 white	0.0001	0.000	1.000	0.45 ± 0.150 ab				
T4- Distilled water	-	-	-	0.75 ± 0.194 a				
AIC				103.1				

Mean ± SD values in the same column followed by the same letter do not differ significantly (Tukey test, $P < 0.05$).
* $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$.

Table 4. Principal components, eigenvalues, proportion of explained variance, and proportion accumulated by components for luminosity, firmness, parasitoids, and flies.

Component	Eigenvalue	Proportion (%)	Cumulative (%)
PC1	1.62	65.84	65.84
PC2	0.90	20.65	86.49
PC3	0.69	11.97	98.46
PC4	0.24	1.54	100

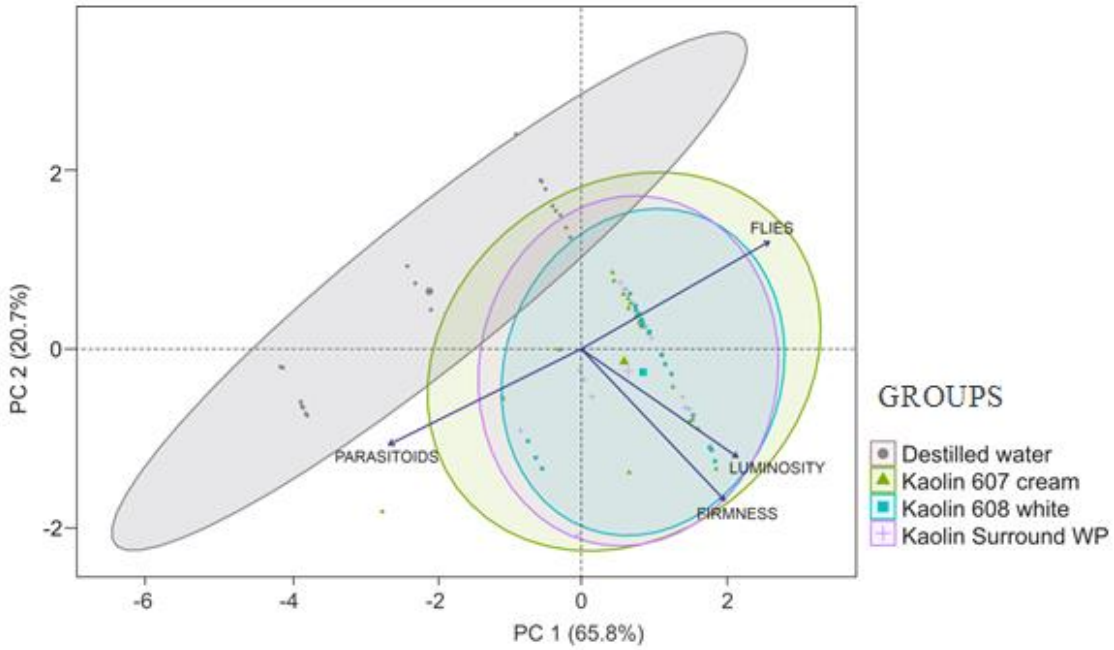
2
0
-2
PC 2 (20.7%)
-6
-4
-2
0
2
PC 1 (65.8%)
FLIES
PARASITOIDS
LUMINOSITY
FIRMNESS
GROUPS
Destilled water
Kaolin 607 cream
Kaolin 608 white
Kaolin Surround WP

Figure 1.

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ARTICLE IV

Interference of tritrophic (grape × medfly × parasitoid) interactions by mineral and biomaterial films[*]

[*] **Situation:** Submitted.

Interference of tritrophic (grape × medfly × parasitoid) interactions by mineral and biomaterial films

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ABSTRACT

Fruit flies (Diptera: Tephritidae) are considered one of the main obstacles to the exportation of fresh fruit. However, films of mineral particles and biomaterials have the potential to protect fruits against tephritid infestation and have been investigated for their effects on rates of fruit infestation rates by medfly (*Ceratitis capitata* Wiedemann) and on the parasitism of medfly larvae by *Diachasmimorpha longicaudata* (Ashmead) (Hymenoptera: Braconidae). The present study evaluated the effects of particle films on the tritrophic interactions of grape (*Vitis vinifera* L.), the fruit fly *C. capitata*, and the parasitoid *D. longicaudata* under semi-field conditions. Grapes were biometrically characterized (i.e., color, firmness, mass, length, and diameter), treated with mineral particles (kaolin: Surround WP, 605, 607, 608, and 611), biomaterials (cassava and potato starch), or distilled water (control), and then used in oviposition and parasitism bioassays. In the oviposition bioassay, the treated grapes were exposed to 50 *C. capitata* pairs in field cages, and after 48 h, the punctures and eggs on each fruit were counted. In the parasitism bioassay, treated grapes were artificially infested with third-instar *C. capitata* larvae (two per fruit), exposed (2 h) to 50 *D. longicaudata* pairs in field cages to determine parasitism index, larval and pupal viabilities, and number of flies and parasitoids emerged. Treatment with the mineral film affected fruit color and reduced C. capitata oviposition but failed to significantly affect the parasitism capacity of *D. longicaudata*. The ability of the parasitoid to locate and parasitize *C. capitata* larvae in kaolin-coated fruits suggests that kaolin films could be used in conjunction with biological agents to control fruit fly pests in organic agriculture operations.

Keywords: *Ceratitis capitata*, kaolin, oviposition, parasitism, particle film technology

Introduction

The Mediterranean fruit fly, or Medfly, *Ceratitis capitata* Wiedemann (Diptera: Tephritidae) is a major quarantine pest across the globe (Silva et al., 2011), and in Brazil alone, pest management costs summed to the production and commercialization losses due to damage by fruit flies are estimated to reach about US$ 34 million per year (MAPA, 2015). Control of *C. capitata* mainly involves the use of toxic baits that include both a lethal agent (insecticide molecule) and a food attractant (Arioli et al., 2018). However, the continued use of insecticides is becoming increasingly limited because of problems related to the selection of resistant populations (Kakani et al., 2010) and consumer pressure for chemical-free food. Thus, the evaluation of alternative fruit fly management strategies is greatly needed flies (Dias et al., 2018).

Particle film technology is one such alternative to conventional insecticides for controlling infestation by *C. capitata* (Palma et al., 2020) and is especially promising because it neither contaminates the environment nor leaves toxic residues in treated products (D'aquino et al., 2011; Lo verde et al., 2011). Particle film technology is based on the properties of kaolin (Glenn & Puterka, 2005), which is a mineral mainly composed of aluminum silicate that, when suspended in water, rapidly forms a chemically inert and non-expanding solution with white color and porous texture (Puterka et al., 2000). Abrasive mineral particles, such as kaolin, change the color of host plants, thereby repelling pests and disrupting their feeding and oviposition (Showler, 2002). For example, the application of mineral particle films to guava and grape significantly reduced the oviposition of fruit fly pests *Anastrepha obliqua* Macquart and *C. capitata* under laboratory conditions (Costa et al., 2021; Da Costa et al., 2021).

Most studies that assess the efficacy of particle films focus on bitrophic interactions (Mazor & Erez, 2004; Lemoyne et al., 2008; Leskey et al., 2010; D'aquino et al., 2011; Yee, 2012 D'aquino et al., 2021), and the extent to which the films affect natural enemies, such as predators and parasitoids, remain much less understood. However, detailed knowledge of the lethal and sublethal effects of kaolin on non-pest arthropods is needed before the mineral can be used in integrated pest management programs. For example, Bengochea et al. (2014) assessed the lethal and non-lethal effects of kaolin on olive trees, the fruit fly *Bactrocera oleae* (Rossi), and the parasitoid *Psyttalia concolor* (Szèpligeti).

Among the biological agents used to control fruit flies, parasitoid wasps of the

Braconidae family are the most thoroughly studied (Montoya et al., 2000; Montoya et al., 2007), and the braconid parasitoid *Diachasmimorpha longicaudata* (Ashmead, 1905) is currently one of the most important biological control agents for fruit flies (Montoya et al., 2000).

During the host localization process, female parasitoids respond to chemical, visual, and mechanical stimuli (Vinson, 1976; Segura et al., 2007; Sharma et al., 2019). When applied to crops, kaolin particle films form a protective barrier that creates a hostile environment for insects, makes plants visually or tactually unrecognizable, and prevents the oviposition of pest insects (Glenn et al. 1999; Bürgel et al., 2005), what can affect also the behavior of predators and parasitoids (Vincent et al., 2003). We hypothesize that films of mineral particles and biomaterials can change the physical characteristics of grapes and that such changes will reduce oviposition by *C. capitata*, as well as the parasitism of *C. capitata* larvae by *D. longicaudata*. The objective of the present study was to evaluate the effects of mineral particle and biomaterial films on the tritrophic interactions of grape, the fruit fly *C. capitata*, and the natural enemy *D. longicaudata*.

Material and methods

Origin of C. capitata, D. longicaudata and fruits used in the bioassays

Fruit fly (*C. capitata*) specimens were obtained from the colony maintained at the Fruit Fly Laboratory of the State University of Southwest Bahia, campus of Vitória da Conquista, Bahia, Brazil. Routine colony procedures included the maintenance of adults in wooden cages (50 × 45 × 40 cm) in which two sides were lined with voile fabric, one inclined for oviposition and the other for insect manipulation. Eggs laid on the side of the cage were collected daily, cleaned, transferred, and maintained in plastic pots that contained an artificial diet that was adapted from Zucoloto (1987) for larval development and pupation (~10 d). Pupae were collected, arranged in plastic containers (500 mL) with vermiculite, and maintained until the emergence of adults. Paired adults were then transferred to cages for aimed mating and oviposition and were provided water and a sugar- and yeast-based diet (3:1 proportion; Silva-Neto et al., 2012). The cages were maintained in a climatized room at 25 ± 2 ºC, relative humidity of 70%, and 12-h photoperiod.

Meanwhile, a *D. longicaudata* colony was established from parasitized *C. capitata* puparia that were obtained from the Entomology Laboratory of Embrapa Cassava and Tropical Fruit farming (Embrapa/CNPMF). The parasitoid colony was maintained as

described by Carvalho et al. (1998). Briefly, third-instar *C. capitata* larvae were offered to adult wasps in "parasitism units", which each included 100 *C. capitata* larvae packed in organza fabric and were attached to the top of acrylic cages (30 × 30 × 30 cm) that contained the parasitoids. Parasitism units were periodically exposed (1 h) to 5-d-old parasitoids, and the exposed larvae were placed in plastic containers (500 mL) that contained vermiculite for pupation and, subsequently, the emergence of adults. Adult parasitoids were maintained in an acrylic cage (30 × 30 × 30 cm) that contained water and a diet made using distilled water, honey, agar-agar, ascorbic acid, and nipagin.

The grapes (*Vitis vinifera* L. 'Italia') used in the bioassays were obtained from fresh fruit markets and, posteriorly, selected for uniformity of maturation, size, and lack of punctures by fruit flies.

Fruit characterization

The biometrical and physical characteristics of the grapes (i.e., mass, length, diameter, and color) were measured before conducting the bioassays, and both color and firmness, the latter of which requires destructive sampling, were also measured at 24 h after the initiation of the bioassays. Grape mass was determined using an analytical balance (AUY 220; Shimadzu), with a precision of 0.1 mg, and both the diameter and length of the grapes were measured using a digital pachymeter (Model MPD-200; Metrotools, São Paulo, Brazil), with a precision of ± 0.02 mm. Fruit firmness was measured using a penetrometer (model WA68; TR, Italy), with an 8-mm-diameter, after the treatments were applied (n = 20), and for each grape, color was measured twice (CR-400 colorimeter; Minolta, Osaka, Japan), once before and once after treatment, always in the same position (opposite sides), using four fruits per treatment. The colorimeter was calibrated using a white ceramic plate with D65 illuminant (z = 85.7; x = 0.3175; y = 0.3253). To evaluate fruit color, luminosity (L), which varies from 0 to 100 (black/white), red/green intensity (+/-) (a), and yellow/blue intensity (+/-) (b) were measured, and both chroma ($C = (a^2+b^2)1/2$), which represents color purity, and hue angle ($H = tg^{-1}(b/a)$), which represents color tonality (Lemoyne et al., 2008), were also measured.

Oviposition bioassay

The experiments were performed using a completely randomized design with eight treatments and four replicates conducted over three consecutive days. The treatments

included T1 - Surround WP (NovaSource, Phoenix, AZ, USA), T2 - kaolin 605 white (BrasilMinas, Guarulhos, SP, Brazil), T3 - kaolin 607 cream (BrasilMinas), T4 - kaolin 608 white (BrasilMinas), T5- kaolin 611 gray (BrasilMinas), T6 - cassava starch, T7 - potato starch, and T8 - control (distilled water). The particles were dispersed in distilled water (200 g L^{-1}) with guar gum (~5 g L^{-1}) to improve formulation viscosity and stability (Campos et al., 2015; Gao et al., 2020; Costa et al., 2021; Da Costa et al., 2021). The kaolin and biomaterial concentrations were based on previous studies (Costa et al., 2021; Da Costa et al., 2021). The biomaterial particles (cassava starch, potato starch and guar gum) were obtained from a natural products market in Indianópolis (SP, Brazil). Before starting the bioassays, the grapes were sanitized for 30 min in sodium hypochlorite (0.5%) and then individually immersed for 10 sec in a beaker that contained 60 mL of the corresponding treatment solution. After immersion, the grapes were dried at 25 ± 2°C for 1 h.

The plot was composed per a field cage (2 × 2 × 2 m) manufactured with metal structure and nylon fabric, in which was stored a seedling *Spondias tuberosa* L., with ~1.20 cm height and radius canopy around 30 cm. Eight treated grapes, one each from the eight treatments, were hung on top of the field cage, with 33 cm between, and then exposed to 50 pairs of 7-d-old *C. capitata* for 48 h. After exposure, each of the grapes was dissected to count total number of fruit fly eggs, number of punctures with eggs, and number of punctures without eggs. During the 15-h bioassay, the cage conditions were maintained at a temperature of 27.08 ± 1.5°C (min and max of 13.6 and 37.4°C, respectively), relative humidity of 51.6 ± 5.85 (min and max of 29.8 and 78.8%, respectively), and luminosity of 19.894 lux.

Parasitism bioassay

The parasitism of *D. longicaudata* on *C. capitata* larvae was evaluated using choice tests with a completely randomized design, eight treatments, and four replicates that were conducted over three consecutive days. The treatments were the same as those used in the oviposition bioassay. Before starting the bioassays, the grapes were sanitized for 30 min in sodium hypochlorite (0.5%) and then individually immersed for 10 sec in a beaker that contained 60 mL of the corresponding treatment solution. After drying at room temperature, the treated grapes were artificially infested with *C. capitata* larvae using methodology adapted from Pires et al. (2021). Briefly, the grapes were perforated to a depth of 1.5 cm using a 1.5-mm-diameter needle, and any pulp residue formed

during the penetration was removed to prevent orifice obstruction. Two third-instar *C. capitata* larvae were then inserted into the orifice of each grape using a fine-tipped brush tool, and the orifice was closed using a small cotton ball. After 1 h, the grapes were finally exposed to the parasitoids.

Similar to the oviposition bioassay, the parasitism bioassays were performed in field cages (2 × 2 × 2 m), each containing a potted plant. For each bioassay, eight artificially infested grapes were treated with particle suspensions or water and arranged as previously described. Then, 50 pairs of 5-d-old *D. longicaudata* were released into the field cage. The grapes were removed after 2 h of parasitoid exposure, and in the lab, the larvae were removed from the grapes and kept in plastic containers that contained a layer of vermiculite until adult emergence. The numbers of emerging parasitoids and flies, larval viability (VL% = no. parasitoid pupae × 100 / total fly larvae), pupal viability (VP% = no. emerged parasitoids + no. emerged flies × 100 / total fly pupae), and parasitism index (IP% = no. emerged parasitoids × 100 / no. emerged flies + no. emerged parasitoids) were determined (Matrangolo et al., 1998).

The bioassays were performed at a temperature of 22 ± 1.5°C (min and max of 17.1 and 33.9°C, respectively), relative humidity of 51 ± 8.5% (m min and max of 51 and max 80%, respectively), and luminosity of 13.586 lux measured during set up of the bioassay (8:00 am).

Statistical analyses

The homoscedasticity and normality of data for the biometrical and physical characteristics of the grapes and oviposition of *C. capitata* and *D. longicaudata* were evaluated using Bartlett and Shapiro-Wilk tests, respectively. Datasets that violated these assumptions (e.g., luminosity, post-treatment hug angle after, firmness, and number of parasitoids) were square root-transformed and, subsequently, analyzed using a generalized linear model (GLM) with a Poisson distribution. The GLMs were established using the nlme (Pinheiro et al., 2020) and lsmeans (Lenth, 2016) packages in R. Paired t-tests were used to compare the mean values of pre- and post-treatment luminosity, chroma, and hue angle. All analyses were performed using R software (version 3.6.1; R Core Team, 2019).

Results

Effect on fruit characteristics

The grapes used for the treatment groups exhibited no significant differences in regards to mass ($F = 0.22303$; $df = 7.31$; $P = 0.97605$), length ($F= 0.6665$; $df = 7.31$; $P = 0.70095$), diameter ($F = 0.20034$; $df = 7.31$ $P = 0.9823$), luminosity ($F = 1.0555$; $df = 7.31$; $P = 0.42077$), chroma ($F = 1.1042$; $df = 7.31$; $P = 0.39$), and hue angle ($F = 0.5303$; $df = 7.31$; $P = 0.80286$; Table 1).

The immersion of grapes in mineral and biomaterial suspensions affected both luminosity ($t = -11,795$; $df = 31$; $P < 0.0001$) and chroma ($t = 7.9406$; $df = 31$; $P <0.0001$), and the different immersion treatments resulted in significantly different luminosity ($F = 1258.1$; $df = 7.31$; $P < 0.0001$), chroma ($F = 183.69$; $df = 7.31$; $P < 0.0001$), and hue angle ($F = 188.71$; $df = 7.31$; $P < 0.0001$; Table 2). Grape luminosity was always increased by the kaolin and starch treatments, was highest in grapes treated with Surround WP and kaolin 605, and was lowest in control grapes. In contrast, chroma values were always decreased by immersion in the suspensions, and hue angle was lower in the Surround WP- and kaolin 605-treated grapes than in the control group. All the mineral films and starches increased fruit firmness ($F = 28.554$; $df = 7.31$; $P < 0.0001$).

Effect on C. capitata oviposition

Treatment had no effect on the number of punctures without eggs ($AIC = 20.63$; $df = 31$) but did significantly affect number of punctures with eggs ($AIC = 29.58$; $df = 31$) and number of eggs ($AIC = 94.31$; $df = 31$; Table 3). Briefly, both kaolin and cassava starch reduced the number of egg punctures, with fewer eggs in fruits treated with Surround WP, kaolin 605, and kaolin 608, whereas treatment with potato starch yielded the highest mean egg number (3.18 ± 0.46).

Effect on D. longicaudata parasitism

One hundred, fifty-seven (157) of the 172 puparia yielded adult insects (69 fruit flies, 88 parasitoids), with larval and pupal viabilities of 89.6 and 91.3%, respectively. The total parasitism index was 56%, ranging from 30% in the potato starch treatment to 69.6% in the control. Treatments had no effect on the numbers of parasitoids ($AIC = 42.35$, $df = 31$) or flies ($AIC = 35.78$; $df = 31$).

Discussion

The grapes, which were evaluated before being used in the bioassays, exhibited good

fruit uniformity, thereby preventing the possibility that fruit characteristics could account for any differences observed in the study's dependent variables, as suggested by Da Costa et al. (2021).

The application of mineral and biomaterial films to the grapes had no effect on number of punctures without eggs, thereby confirming the laboratory-based findings of Da Costa et al. (2021). It is possible that the resistance provided by the films discouraged flies from ovipositing in the fruit after puncturing it. However, films should ideally inhibit both oviposition and fruit puncturing, since puncture injuries, in some fruits (e.g., apples), can facilitate the entry of fungi and bacteria (Santos et al., 2008).

Both the kaolins and cassava starch reduced number of punctures with eggs, with fewer eggs in grapes treated with Surround WP, kaolin 605, and kaolin 608, which was similar to results reported by Costa et al. (2021) and Da Costa et al. (2021). In previously reported laboratory studies, kaolin reduced fruit fly oviposition in bitrophic interactions of grape × *C. capitata* (Da Costa et al., 2021) and guava (*Psidium guajava* L.) × *A. obliqua* (Costa et al., 2021) and number of punctures in apple (*Malus domestica* L.) × *C. capitata* (Leskey et al., 2010; Ourique et al., 2017), mango (*Mangifera indica* L.) × *C. capitata* (Ourique et al., 2017), and citrus × *C. capitata* (D'aquino et al., 2011). Kaolin has also been reported to reduce fruit fly landing and oviposition in field studies of citrus × *C. capitata* (Braham et al., 2007; Lo Verde et al., 2011), apple × *Rhagoletis pomonella* (Walsh) (Villanueva & Walgenbach, 2007), and cactus (*Opuntia ficus-indica* 'Gialla') × *C. capitata*. In contrast, the biomaterials failed to protect the fruits from oviposition, and the potato starch treatment yielded the highest mean egg number, appearing to actually stimulate oviposition. These findings were in agreement with previous laboratory-based studies (Da Costa et al., 2021), although it is important to note that potato starch was reported to preserve guava peel color and to protect guava fruits from oviposition by *A. obliqua* (Costa et al., 2021).

It is likely that the reduced oviposition of *C. capitata* in kaolin-coated grapes was due to changes in fruit color and firmness. More specifically, it is possible that the effects of the white mineral particles on the grape peels' natural green color interfered with host identification by *C. capitata* females. Indeed, some studies have demonstrated that fruits or spheres coated with white substances experience reduced fruit fly oviposition (Cytrynowicz et al., 1982; Katsoyannos et al., 1986; López-Guillén, et al., 2009; Costa et al., 2021; Da Costa et al., 2021). The high reflectance of white surfaces is visually less attractive to fruit flies, as demonstrated in *C. capitata* (Nakagawa et al.,

1978; Katsoyannos et al., 1986), *Bactrocera dorsalis* (Hendel) (Wu et al., 2007), and *A. obliqua* (López-Guillén et al., 2009). In addition, the films formed a physical barrier that affected fruit firmness. The epicarp of some fruits provides natural resistance that prevents some species of flies with short aculea (e.g., *C. capitata*) from puncturing or depositing eggs (Aluja & Mangan, 2008), preferring to oviposit in fruits with maturation stage more advanced, that is, with less firmness (Gómez et al., 2019). Mineral particles also make the surface of the fruit rough, making it inadequate for oviposition (Saour & Maker, 2004). Second Salermo et al. (2020), the kaolin also reduces the insect adhesion to artificial and natural substrates characterized by different surface features, and these studies can contribute to the development of new physical control methods, such as physical barriers that protect crops from pest infestation.

In the present study, the effect of mineral particle and biomaterial films on grape color did not affect the parasitism capacity of *D. longicaudata*, and as such, parasitoid females were able locate *C. capitata* larvae in all treatments (Table 4). Messing & Jang (1992) reported that *D. longicaudata* females respond to fewer visual stimuli than males, since olfactory stimuli (e.g., larvae kairomones) play a more important role in host localization (Carrasco et al., 2005), and Benelli & Canali (2012) reported that naive *P. concolor* females show no color preferences. These findings agree with those of Bengochea et al. (2010), who investigated the effectiveness of kaolin against *Bactrocera oleae* in olive groves, as well as the effect of kaolin on the parasitoid *Psyttalia concolor*, and found that the parasitism capacity of *P. concolor* was unaffected by kaolin treatment. Additional laboratory and semi-field studies have also reported that kaolin is harmless to the fruit fly parasitoid *P. concolor* (Adán et al., 2007; Bengochea et al., 2010). According to Bengochea et al. (2010), the use of kaolin in olive crops is promising because it affects beneficial arthropods to a lesser extent than other commonly used compounds, such as dimethoate. However, these findings also contradict those of Bengochea et al. (2014), who reported that kaolin treatment reduced the rate of parasitism by *P. concolor*.

Together, these findings support the conclusion that, although mineral films do not completely prevent damage by fruit flies, they do not interfere with the parasitism of *C. capitata* by *D. longicaudata*, thereby promoting their use in integrated pest management schemes. The ability of the parasitoid to locate and parasitize *C. capitata* larvae in kaolin-coated fruits suggests that kaolin films could be used in conjunction with biological agents to control fruit fly pests in organic agriculture operations.

Acknowledgements

We are grateful to the Foundation for Research Support of the State of Bahia (FAPESB) and the CAPES Foundation (Brazilian Ministry of Education; Finance Code 001) for providing financial support. The companies, Brasilminas and NovaSource, donated the kaolin samples.

Author Contributions

Daniela R. Costa and Maria A. Castellani conceived research. Daniela R. Costa, Beatriz S. Coelho and Maria A. Castellani conducted experiments. Daniela R. Costa and Mateus P. Santos, Suzany A. Leite, Maria A. Castellani analysed data and conducted statistical analyses. Daniela R. Costa, Iara S. Joachim- Bravo, Pablo Montoya, Suzany A. Leite, Vanessa S. Dias and Maria A. Castellani wrote the manuscript. Daniela R. Costa and Maria A. Castellani secured funding. All authors read and approved the manuscript.

Conflict of interest

The authors declare no conflict of interest.

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Table 1. Weight (g), length (mm), diameter (mm), luminosity, chroma and hue angle (mean ± standard deviation) of the grapes used in the treatments before immersion in suspension.

Treatments	Weight (g)	Lengt (mm)	Diameter (mm)	Luminosity	Chroma	Hue angle
T1-Kaolin Surround® WP	11.86 ± 0.47 a	28.16 ± 1.03 a	25.35 ± 1.03 a	36.50 ± 0.88 a	8.18 ± 0.81a	113.20 ± 3.16 a
T2- Kaolin 605 white	11.61 ± 1.11 a	28.12 ± 1.27 a	25.38 ± 1.21 a	36.48 ± 0.20 a	8.57 ± 0.13a	111.21 ± 1.86 a
T3- Kaolin 607 cream	11.54 ± 0.97 a	28.83 ± 1.97 a	25.44 ± 0.67 a	36.91 ± 0.75 a	8.75 ± 0.70 a	114.03 ± 1.97 a
T4- Kaolin 608 white	11.49 ± 1.09 a	29.35 ± 0.97 a	27.71 ± 2.01 a	36.59 ± 0.56 a	8.48 ± 0.33 a	113.63 ± 1.71a
T5- Kaolin 611 grey	11.32 ± 0.45 a	28.30 ± 0.72 a	24.99 ± 0.95 a	36.88 ± 0.58 a	8.97 ± 0.38 a	115.42 ± 3.40 a
T6- Cassava starch	11.48 ± 0.75 a	28.66 ± 1.10 a	25.10 ± 1.34 a	37.26 ± 0.20 a	8.60 ± 0.88 a	113.13 ± 5.91a
T7- Potato starch	11.18 ± 0.86 a	28.12 ± 0.41 a	25.03 ± 0.56 a	37.09 ± 0.4 a	8.38 ± 0.41a	112.73 ± 4.62 a
T8-Distilled water (Control)	11.69 ± 1.14 a	28.26 ± 0.30 a	25.40 ± 0.83 a	37.57 ± 0.53 a	9.08 ± 0.46 a	114.24 ± 1.67 a
Coefficient of variation (%)	7.76	3.83	4.59	2.04	6.58	2.97

Mean ± SD values in the same column followed by the same letter do not differ significantly at $P < 0.05$ (Tukey's test).

Table 2. Estimates for GLM parameters with Gaussian model for the luminosity, chroma, hue angle, and firmness (mean ± standard deviation) of the grapes after immersion in suspensions.

Treatments	Luminosity	Chroma	Hue angle	Firmness (N)
T1-Kaolin Surround® WP	87.98 ± 1.72 a	1.28 ± 0.27 d	22.47 ± 2.09 d	6.43 ± 0.16 ab
T2- Kaolin 605 white	87.06 ± 0.84 ab	1.47 ± 0.23 d	67.07 ± 9.39 c	6.20 ± 0.11b
T3- Kaolin 607 cream	85.57 ± 0.61 b	9.48 ± 0.31 a	147.90 ± 3.46 a	6.47 ± 0.09 ab
T4- Kaolin 608 white	77.11 ± 1.10 c	2.27 ± 0.31 cd	113.46 ± 11.17 b	6.82 ± 0.19 a
T5- Kaolin 611 grey	76.89 ± 0.51 c	5.46 ± 0.16 b	136.12 ± 4.48 a	6.42 ± 0.41 ab
T6- Cassava starch	71.81 ± 0.58 d	2.75 ± 0.07 c	119.13 ± 3.89 b	6.33 ± 0.20 ab
T7- Potato starch	56.36 ± 1.21 e	5.29 ± 1.05 b	116.05 ± 3.24 b	6.04 ± 0.27 b
T8-Distilled water (Control)	37.32 ± 0.74 f	8.55 ± 0.53 a	112.87 ± 1.93 b	5.05 ± 0.07 c
Coefficient of variation (%)	1.37	10.23	5.66	3.44

Mean ± SD values in the same column followed by the same letter do not differ significantly at $P < 0.05$ (Tukey's test).
*Data transformed into √x.

Table 3. Estimates for GLM parameters with Gaussian model for the number of puncture with and without eggs and eggs (mean ± SE) of *C. capitata* in grapes exposed in field cage conditions.

Treatments	Standard Error	Z-Value	*p*-Value	Punctures with eggs (N°)	Standard Error	Z-Value	*p*-Value	Punctures without eggs (N°)	Standard Error	Z-Value	*p*-Value	Eggs (N°)
Intercept	0.1674	8.945	0.000***	-	0.1456	4.002	0.000***	-	0.4603	5.193	0.000***	-
T1-Kaolin Surround® WP	0.2368	-5.977	0.000***	0.0825 ± 0.167a	0.2058	-2.830	0.009**	0.0 ± 0.146c	0.6509	-3.129	0.004**	0.354 ± 0.46c
T2- Kaolin 605 white	0.2368	-6.325	0.000***	0 ± 0.167a	0.2058	-2.830	0.009**	0.0 ± 146c	0.6509	-3.672	0.001**	0.0 ± 0.46c
T3- Kaolin 607 cream	0.2368	-4.921	0.000***	0.3325 ± 0.167a	0.2058	-2.830	0.009**	0.0 ± 146c	0.6509	-2.024	0.05*	1.07 ± 0.46bc
T4- Kaolin 608 white	0.2368	-6.325	0.000***	0 ± 0.167a	0.2058	-2.830	0.009**	0.0 ± 146c	0.6509	-3.672	0.001**	0.0 ± 0.46c
T5- Kaolin 611 grey	0.2368	-5.628	0.000***	0.1650 ± 0.167a	0.2058	-1.615	0.1193	0.0 ± 146c	0.6509	-2.493	0.01*	0.77 ± 0.46bc
T6- Cassava starch	0.2368	-3.854	0.000***	0.5850 ± 0.167a	0.2058	-1.615	0.1193	0.25 ± 146bc	0.6509	-0.885	0.384	1.81 ± 0.46 abc
T7- Potato starch	0.2368	-1.045	0.3065	1.250 ± 0.167a	0.2058	-1.214	0.2364	0.33 ± 146ab	0.6509	1.216	0.236	3.18 ± 0.46a
T8-Distilled water (Control)	-	-	-	1.497 ± 0.167a	0.2058	-	-	0.58± 146a	0.6509	-		2.39 ± 0.46b
AIC				29.58				20.63				94.31

Mean ± SD values in the same column followed by the same letter do not differ significantly (Tukey's test, $P < 0.05$).

Table 4. Estimates for GLM parameters with model Poisson for the number of parasitoids, and number flies, and parasitism index in grapes exposed at field cage conditions.

Treatments	Standard Error	Z-Value	*p*-Value	Parasitoids (N°)	Standard Error	Z-Value	*p*-Value	Flies (N°)	Parasitism index (%)
Intercept	0.577	2.307	0.0211	-	0.382	1.530	0.126	-	-
T1-Kaolin Surround® WP	0.735	-0.677	0.4986	0.833 ± 0.456a	0.596	0.420	0.675	0.835 ± 0.457a	49.9
T2- Kaolin 605 white	0.763	-0.432	0.665	1.000 ± 0.500a	0.500	-0.340	0.734	0.415 ± 0.322a	70.6
T3- Kaolin 607 cream	0.735	-0.677	0.4986	0.833 ± 0.456a	0.596	0.420	0.675	0.835 ± 0.457a	49.9
T4- Kaolin 608 white	0.763	-0.432	0.665	1.000 ± 0.500a	0.540	-0.005	0.996	0.583 ± 0.382a	63.2
T5- Kaolin 611 grey	0.763	-0.206	0.8371	1.167 ± 0.540a	0.521	-0.163	0.870	0.500 ± 0.354a	70.0
T6- Cassava starch	0.706	-0.942	0.3464	0.665 ± 0.408a	0.595	0.416	0.678	0.833 ± 0.456a	44.4
T7- Potato starch	0.676	-1.227	0.2198	0.500 ± 0.354a	0.662	0.880	0.379	1.167 ± 0.540a	30.0
T8-Distilled water (Control)	-	-	-	1.330 ± 0.577a	-	-	-	0.585 ± 0.382a	69.6
AIC				42.35				35.78	

Mean ± SD values in the same column followed by the same letter do not differ significantly (Tukey's test, $P < 0.05$).

FINAL CONSIDERATIONS

World agriculture haved major transformations and production systems must, increasingly, approximate of the sustainability, with aggregation of value to products and respecting changes in consumer habits. Integrated pest management is a fundamental item in the context of the Agriculture of the future, whose path is the search for strategies with less environmental impact, as genetic, cultural and biological, mainly, what ensure product quality and consumer health due to absence of residues of pesticides.

Considering this scenario, the present work aimed to contribute for increase the knowledge on the use of particle film technology in the protection of fruits against infestations of two tephritids of quarantine importance, *Ceratitis capitata* and *Anastrepha obliqua*, also worryed with likely interference of technology in the parasitism rates of these pests by the parasitoid *Diachasmimorpha longicaudata*.

The particle films used provided distinct effects on the oviposition of the species fruit fly studied. Mineral particles (Kaolins 607, 608, 609, 611 and Surround® WP) reduced oviposition of the two species of flies; while biomaterials to base of potato and cassava starches reduced oviposition only for *A. obliqua*. This reinforces the fact that it is not possible to generalize the responses of this group of insects to technology studied, and that for same culture under pressure from a tephritid community, protection may fail for some species. Thus, studies on the effect of kaolin on other bitrophic interactions are needed.

For most particle films, the satisfactory concentration was 200 g L^{-1}, had need, however, study concentrations the end verify the question economic of the technology. Furthermore, only kaolin Surround® WP is a commercial formulation; for the other kaolins, are necessary studies related to formulation and to costs involved in sense of to assess the viability of use.

Another important aspect to be considered is the effect of the films on the physicochemical characteristics of the fruits after application. These acquire a whitish coloration that could be rejected by consumers; treatments in the *packing house*, aiming at removing of the products, especially kaolin, perhaps become necessary.

An innovative aspect of the work is the use of potato and cassava starches for fruit protection, and with potential for use in guava crops where *A. obliqua* populations are generally expressive. An obstacle to be resolved for the use of these materials is the

brittle aspect of the coating with increasing concentrations, exposing parts of the fruit to infestation by flies. The increase of glycerin to solutions could be studied with this finality. Finally, considered that the use of these biomaterials probably be more accepted by the consumer market por are edible.

A positive aspect of this technology was not affect the parasitism capacity of the parasitoid of fruit fly *D. longicaudata* in field cage conditions.

Finally, can be affirm that despite the many promising results obtained, field studies for commercial fruit crops conditions are necessary to confirmation of the viability technical and economic of particle film technology in the protection against tephritids.

ANNEX I

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Mineral and natural films change the physical–chemical properties of grapes and modulate oviposition behaviour of *Ceratitis capitata* Wiedemann (Diptera: Tephritidae)

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Research Paper

Cite this article: da Costa DR, Leite SA, Moreira AA, Perez-Maluf R, Novaes QS, dos Santos MP, dos Santos MM, Joachim-Bravo IS, Castellani MA (2021). Mineral and natural films change the physical–chemical properties of grapes and modulate oviposition behaviour of *Ceratitis capitata* Wiedemann (Diptera: Tephritidae). *Bulletin of Entomological Research* 1–11. https://doi.org/10.1017/S0007485321000250

Received: 18 March 2020
Revised: 8 September 2020
Accepted: 12 March 2021

Keywords:
chitosan; fruits flies; kaolin; oviposition; puncture

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Abstract

The Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), is one of the main pests of fruit, worldwide, and the use of population suppression method with low environmental impact is an increasingly strong requirement of the consumer market. The aim of this study was to evaluate the effect of mineral and natural films on the physical–chemical properties of grapes (*Vitis vinifera* L.), cultivar Itália, and oviposition behaviour of *C. capitata*. Fruits were immersed in suspensions (100 and 200 g L^{-1}) of mineral (kaolin Surround®WP, kaolin 607, kaolin 608, kaolin 611 and talc) and natural films (chitosan, cassava starch, potato starch and guar gum 5.0 g L^{-1}) and distilled water (control). After drying, fruits were exposed to *C. capitata* pairs of males and females for 24 h in choice and non-choice tests; the number of punctures with and without eggs, eggs per fruit and behavioural response of fly to treated and untreated fruits were recorded. Results obtained in this study are promising, given the scientific evidence that films of mineral particles such as kaolin (Surround®, 607, 608 and 611) changed the firmness, luminosity, chroma and hue angle of grapes and reduced the oviposition of *C. capitata*. In addition, our results also showed that natural polymers do not deter *C. capitata* females, but rather seem to stimulate oviposition.

Introduction

Among the main phytosanitary problems that affect the production and commercialization of fresh fruits, for certain markets, the occurrence of fruit flies (Diptera: Tephritidae) is one of the main obstacles. Fruit flies of economic and quarantine importance in Brazil are *Ceratitis capitata* (Wiedemann, 1824), known as Medfly, discovered at the beginning of the 20th century, and currently has 94 confirmed hosts and distributed in 27 botanical families; *Anastrepha* Schiner, with about 121 species in the country, the most polyphagous being *A. fraterculus* (Wiedmann, 1830) and *A. obliqua* (Macquart, 1835); and *Bactrocera carambolae* Drew & Hancock, 1994, originally from Asia, but its presence has been confirmed in the states of Amapá, Pará, and Roraima (Zucchi and Moraes, 2012). Based on a European Union Execution Directive 2019/523, published on 21 March 2019, non-European Tephritidae species are now of quarantine importance for the export of citrus and mango fruits (European Union, 2019).

Ceratitis capitata is considered as the main quarantine pest of the world fruit and in Brazil, it mainly infests exotic fruits in 23 states of the 26 Brazilian states, beyond the Federal District (Zucchi and Moraes, 2012), there was no record only in three states Amapá, Amazonas, and Sergipe (Zucchi and Moraes, 2012).

The control of these tephritids is mainly performed through the use of toxic baits, containing a lethal agent (insecticide molecule) mixed with a food-based attractant (Arioli *et al.*, 2018). Insecticide spinosad has been used in fruit fly control programs in several countries. In Brazil, spinosad is available in a concentrated suspension formulation and as a ready-for-use toxic bait (Harter *et al.*, 2015). However, the extensive use of spinosad for controlling olive fruit fly and other tephritids can cause problems related to the selection of populations resistant to this insecticide (Kakani *et al.*, 2010).

The continued use of insecticides has an increasing limitation, mainly consumer pressure, owing to the presence of residues in fruits; thus, it is necessary to evaluate other control strategies for inclusion in the management of fruit flies (Dias *et al.*, 2018).

The use of mineral and natural particle films may be a viable alternative to the use of insecticide, mainly because they do not contaminate the environment or leave toxic residues

that are harmful to humans and animals in treated products. Kaolin, the main component of the technology the particle film, is a white, non-abrasive, and chemically inert aluminosilicate mineral formulated for use in plants (Puterka *et al.*, 2000).

The use of kaolin for pest management is based on the interruption of the insect in recognizing its host plant, alteration in the texture of leaves or fruits, and masking of leaves or fruits by their light-reflective properties (Showler, 2002). Thus, one of the first modes of action of particle films is host camouflage, which makes plants unrecognizable by pests. Particle films have been used to control fruit flies in apple (Mazor and Erez, 2004; Leskey *et al.*, 2010), nectarine (Mazor and Erez, 2004; D'aquino *et al.*, 2011), cherry (Yee, 2012), blueberry (Lemoyne *et al.*, 2008) and citrus and peach (D'aquino *et al.*, 2011).

In addition to mineral polymers, natural polymers have wide applicability in several areas owing to their high availability and properties, such as biocompatibility and biodegradability, and they are used in agriculture as a coat in the preservation of fruits before and after harvest (Kaushik *et al.*, 2016; Gomes *et al.*, 2017). Cellulose, agar, starch, pectin, guar gum, alginates, carrageenans, xanthan gum, chitin, and chitosan are among the most well-known and used natural polymers. Among them, chitin and chitosan have been used as natural seed treatment agents, growth stimulators, and in the control of plant diseases (Kulkarni *et al.*, 2012; Ambore *et al.*, 2013; Casemiro *et al.*, 2019). Besides the reduction of the ripening process of mango fruits subjected to the hydrothermal process, chitosan can also inhibit the development of eggs and larvae of *A. ludens* (Salvador-Figueroa *et al.*, 2011, 2013).

Most of the species of fruit flies have stereotypical oviposition behaviour that comprises stages of arrival on fruit, inspection, aculeus insertion, egg deposition, aculeus cleaning, and in most species, aculeus dragging (Díaz-Fleischer *et al.*, 2000). Moreover, films can constitute barriers to oviposition, causing interference to the host, mainly in colour and penetrability (Aluja and Mangan, 2008).

Owing to the possible effects of these films on the physical–chemical characteristics of fruits and oviposition of fruit flies, we hypothesize that particle films can reduce the use of grape by *C. capitata* for oviposition, changing their behaviour, and consequently decreasing their infestation in fields.

Therefore, the aim of this study was to evaluate the effect of mineral and natural films on the physical–chemical properties of grapes (*V. vinifera* L.), cultivar Itália and oviposition behaviour of *C. capitata*.

Material and methods

Origin of C. capitata *and fruits used in bioassays*

Studies were conducted at the Laboratory of Fruit Flies, State University of Southwestern Bahia-UESB, *campus* of Vitória da Conquista, Bahia, Brazil, from June to December 2019.

The *C. capitata* flies used in this study were reared at the Fruit Flies Laboratory of the State University of Southwest Bahia. With the aim of obtaining larvae, eggs were collected daily, sterilized, and subjected to the diet containing oat bran, sugar, beer yeast, soybean meal and distilled water, in addition to preservatives, as adapted from Tanaka *et al.* (1969). Approximately ten days after larvae hatched, formed pupae were collected and placed in plastic containers with vermiculite until adults emerged. The adults were transported to cages, suitable for breeding, mating, and oviposition, and fed a diet based on sugar and yeast extract (Bionis YE MF) (Silva Neto *et al.*, 2012), offered on filter paper. Cages were kept in an air-conditioned room at an average temperature of 25 ± 2°C and relative humidity of 70%. All bioassays used six-day-old *C. capitata* pairs of males and females, and flies were exchanged after 24 h of exposure to treatments. The mature grapes (*V. vinifera* L.), cultivar Itália, used in this experiment were obtained in open markets. They were selected on the basis of uniform maturity, size, and absence of fruit fly punctures.

Fruit characterization

Fruit uniformity was determined by assessing some physico-chemical characteristics of grapes, such as length, diameter, firmness, colour, total soluble solids (TSSs) content, and titratable acidity (TA). Fruit uniformity was determined in order to confirm the uniformity of the substrate used for oviposition. Grape weight (grams) was determined using a precision semi-analytical scale. Grape diameter and length in millimetres (mm) were obtained with the aid of a digital calliper. Firmness was determined using a TR penetrator (model WA68, Italy), with 8 mm diameter tip. TSS content was obtained through a direct reading of the berry pulp extract in a digital refractometer and results expressed in °Brix. TA was determined by titration, with a 0.1 N sodium hydroxide (NaOH), and expressed in grams of tartaric acid per 100 ml of juice. pH was determined using a Mars pH meter (model MB-10), with readings directly made on the sample with 100 ml of fruit juice. Three replicates of ten grapes ($N = 30$) were used for each evaluated parameter: firmness, TSS, and TA, and each group of grapes came from a bunch.

Fruit colour was measured before and after the application of treatments, resulting in two measurements per fruit on the same position (opposite sides), thus, four fruits per treatment were used in each bioassay ($N = 40$). Changes in colour were determined using colorimeter CR-400 (Minolta®). The device was calibrated using white ceramic plate and D65 illuminant ($z = 85.7$; $x = 0.3175$; $y = 0.3253$). Luminosity (L), ranging from 0 to 100 (black/white), red/green intensity (+/−) (a), and yellow/blue intensity (+/−) (b) values were determined. In addition to these colour coordinates, colour parameters such as chroma value [$C = (a^2 + b^2)1/2$], which represents colour purity and angle measurement (Hue) [$H = \text{tg}-1 (b/a)$], which represents colour tone (Lemoyne *et al.*, 2008) were also determined. After the application of the highest suspension of treatments, the second analysis of fruits was also performed in relation to firmness to detect possible changes that could influence oviposition.

Oviposition: non-choice test (bioassays 1 and 2)

To assess oviposition in non-choice test, a completely randomized design with ten treatments and four repetitions was used, with three replicates on consecutive days. Treatment components were: T1-kaolin Surround® WP; T2-kaolin 607 cream; T3- kaolin 608 white; T4-kaolin 611 grey; T5-talc 657; T6-chitosan; T7-cassava starch; T8-potato starch; T9-guar gum and T10-control (distilled water). All the treatment components were dissolved in distilled water at 100 g L^{-1} (bioassay 1) and 200 g L^{-1} (bioassay 2), except for T9-guar gum, which was dissolved in water at 5.0 g L^{-1}, as it was added as a thickener in the same amount to all treatments. Guar gum acts as a thickener, improving the viscosity and stability of formulations, being commonly used in chemical and

https://doi.org/10.1017/S0007485321000250

biological insecticide formulations, including nanoemulsions (Campos *et al.*, 2015; Gao *et al.*, 2020).

The chitosan used in the bioassays was obtained from the shell of crustaceans; it was also dissolved in distilled water, and the mixture maintained under constant agitation. Kaolin Surround® WP was obtained from NovaSource company; kaolin 607, 608 and 611 and talc were purchased from Brasil Minas company and natural polymers from 'Mercadão Natural.'

Plot consisted of a cage containing treated grapes and *C. capitata* pairs of males and females. Fruits were tied on pieces of plastic tape; subsequently, they were individually immersed for 10 s in a beaker containing 60 ml of a suspension that correspond to each treatment. After treatment, fruits were left at 25° ± 2°C a temperature for 1 h to dry. Subsequently, a single fruit was hung from the top of each cage using an adhesive tape, following the methods outlined by Silva *et al.* (2015), which was adapted for this trial. Bioassays were maintained in the laboratory at 25 ± 2°C and 70% relative humidity. Fruits were removed after 24 h of exposure to flies, and the number of eggs per fruit and punctures with and without eggs were recorded.

Oviposition: choice test (bioassays 3 and 4)

Bioassays with choice were similar to those of non-choice, however, two fruits per cage were exposed: one was treated, the other was a control (distilled water). Bioassays were conducted in a completely randomized design with nine treatments and four repetitions, with three replicates on consecutive days. The treatments and procedures used were the same as those described in bioassay 1, except for control treatment (T10), which was offered together with the other treatments in the same plot. The treatments were dissolved in distilled water at 100 g L^{-1} (bioassay 3) and 200 g L^{-1} (bioassay 4). After immersion and drying, fruits (treated and control) were placed 10 cm apart and hung from the top of each cage using adhesive tape, following the methods outlined by Silva *et al.* (2015), which was adapted for this trial. Bioassays were kept under the same conditions as bioassay 1 with 24-hour exposure, and the same variables recorded.

Behavioural response of C. capitata to treated and untreated fruits

The design was completely randomized comprising kaolin Surround®, kaolin 607, kaolin 608, kaolin 611, and guar gum suspensions. These suspensions (200 g L^{-1}) resulted in better oviposition responses in bioassays choice and non-choice, in addition to control (water) and chitosan treatment that stimulated oviposition. The experimental plot consisted of a cage with two six-day-old fertile *C. capitata* females and a fruit (grape). Eight (8) flies were used per treatment, lower than in other studies (McDonald and Mclnnis, 1985; Jang *et al.*, 1999; Yee, 2012), but sufficient to observe all expected behaviours as indicated in preliminary tests. Fruits were immersed in treatments for ∼10 s and soon after, dried at room temperature to remove excess moisture. The fruit was hung from the top of each cage and flies released with the help of a sucker.

Evaluations were carried out with the same fruits and flies for two consecutive days, from 8:00 am to 12:00 pm, following the method adapted from Lemoyne *et al.* (2008) and Yee (2012). After the two days period of exposure, another cage was prepared, with another flies and fruit for observation, totalling 16 hours of observation for each treatment. The following behavioural parameters were evaluated: arrival at the fruit (landing), search, puncture, aculeus dragging and cleaning, time of first landing, number of landings and time landed on the host, number and time of fruit searching, time and number of punctures, number and time for aculeus dragging, and time and number for aculeus cleaning.

Statistical analyses

The parameters firmness, TSS, and TA were not statistically analysed because they were only used to characterize the fruits before immersing them in suspensions. In addition, it was only in bioassays with 200 g L^{-1} suspensions that firmness was determined, after the immersion of fruits in suspensions. Paired *t*-test in the R software version 3.6.1 (R Development Core Team, 2019) was used to compare the average values of luminosity, chroma and hue angle before and after applying the suspensions of 100 and 200 g L^{-1}.

For oviposition non-choice tests (bioassays 1 and 2), data obtained for the behavioural response of *C. capitata* to treated and untreated fruits and the physical characteristics (weight, length, diameter, luminosity, chroma and hue angle) of fruits were subjected to Bartlett and Shapiro-Wilk tests for evaluation of homoscedasticity assumptions of treatment variances and normality of residues, respectively. In the absence of these assumptions, data were transformed into $\sqrt{x}$ or $\sqrt{x+1}$ and subsequently subjected to analysis of variance (ANOVA) for comparison of means using the Tukey test ($P < 0.05$) in the R software version 3.6.1 (R Development Core Team, 2019). For the number of eggs in bioassay 1, treatments were compared using the generalized linear models (GLMs) of the R software 'nlme' (Pinheiro *et al.*, 2020) and 'lsmeans' (Lenth, 2016) packages.

The oviposition data obtained with choice tests (bioassays 3 and 4) did not meet ANOVA premises, thus, a Monte Carlo type randomization was carried out, with 1000 simulations to guarantee 95% probability. To confirm significant differences among treatments, *a priori* orthogonal contrast was performed using the R software version 3.6.1 (R Development Core Team, 2019).

Data on the behavioural response (time of first landing, number of landings, search time, number of searches, puncture time, number of punctures, aculeus dragging time and number of aculeus dragging) and pulp firmness were transformed into log ($x + 10$). For variables such as time of first landing and puncture time, Poisson distribution was used for the variables time to first landing and time to puncture. It was used GLM, considering each parameter separately and the Poisson error distribution with a log-binding function (as the data were not normally distributed), whit α set at 0.05. All of the analyses were performed utilizing the statistical program R (R Core Team, 2018), the statistical procedure also used by other authors in works with fruit flies, such as *A. fraterculus* (Proença, 2019), *A. obliqua* and *C. capitata* (Silva *et al.*, 2020).

Results

Fruit characterization

Grapes showed an average pulp firmness of 5.4 N, TSS content of 18.1 °Brix, TA of 1.3 and pH of 3.7. Among the variables analysed (weight, length, diameter, luminosity, chroma and hue angle),

https://doi.org/10.1017/S0007485321000250

Table 1. Weight (g), length (mm) and diameter (mm), luminosity, chroma and hue angle (mean ± standard deviation) of the grapes of the variety Italy used in the treatments before immersion in suspensions.

Treatments	Weight (g)	Length (mm)	Diameter (mm)	Luminosity	Chroma	Hue angle
T1-Kaolin Surround® WP	9.71 ± 0.41a	28.10 ± 0.96a	22.70 ± 0.42b	37.89 ± 1.84ab	10.28 ± 0.53a	113 ± 1.5a
T2-Kaolin 607 cream	9.95 ± 1.27a	28.51 ± 0.69a	23.01 ± 1.18ab	38.63 ± 1.48ab	10.95 ± 0.75a	115 ± 1.5a
T3-Kaolin 608 white	10.50 ± 0.55a	30.12 ± 1.05a	23.35 ± 0.50ab	38.33 ± 0.60ab	10.14 ± 0.50a	114 ± 1.63a
T4-Kaolin 611 grey	10.0 ± 2.52a	28.11 ± 2.63a	22.87 ± 2.34ab	38.14 ± 1.29ab	10.17 ± 0.59a	112 ± 0.95a
T5-Talc 657	8.96 ± 1.52a	28.05 ± 1.72a	21.66 ± 0.61b	38.38 ± 1.53ab	10.31 ± 1.06a	113 ± 0.95a
T6-Chitosan	10.46 ± 1.50a	28.66 ± 0.70a	25.33 ± 0.87a	37.41 ± 1.86ab	10.57 ± 0.58a	113 ± 0.95a
T7-Cassava starch	9.05 ± 0.80a	27.25 ± 0.28a	23.10 ± 1. 27ab	39.35 ± 0.80a	11.17 ± 0.91a	110 ± 1.5a
T8-Potato starch	8.76 ± -0.61a	27.20 ± 0.77a	22.47 ± 0.58b	40.37 ± 0.45a	11.39 ± 0.93a	115 ± 0.81a
T9-Guar gum	9.12 ± 1.16a	27.62 ± 2.19a	22.53 ± 0.74b	39.31 ± 1.53a	11.09 ± 1.12a	111 ± 0.95a
T10-Distelled water	10.10 ± 0.44a	27.33 ± 0.36a	23.73 ± 0.74ab	35.92 ± 1.58b	10.26 ± 0.53a	112 ± 1.0a
C.V (%)	12.92	4.85	4.65	3.6	7.37	3.64

Mean ± SD values in the same column followed by the same letter do not differ significantly at $P < 0.05$ (Tukey's test).

significant differences were observed only for diameter and luminosity, indicating slight variations in characteristics of fruits used as a substrate for oviposition in the various bioassays. The mean values for weight ($F = 1.0573$; df = 9, 39; $P = 0.42075$) and length ($F = 1.587$; df = 9, 39; $P = 0.16428$) ranged from 8.76 ± 0.61 to 10.50 ± 0.55 g and 27.20 ± 0.77 to 30.12 ± 1.05 mm, respectively. The diameter of grapes in all treatments was equal to the diameter of control fruits, however, significant differences were found only for the diameter of grapes used in T1 (kaolin Surround®) and T6 (chitosan) treatments ($F = 3.2634$; df = 9, 39; $P < 0.001$) (table 1). Regarding luminosity of fruits before treatments, fruits immersed in potato and cassava starches and guar gum films were the same as those immersed in other treatments; their values were higher than that of the control ($F = 3.0522$; df = 9, 39; $P = 0.0102$). Regarding the two other factors related to colour, chroma or purity ($F = 1.3576$; df = 9, 39; $P = 0.25062$) and hue angle ($F = 1.0598$; df = 9, 39; $P = 0.41904$), fruits were uniform as there was no significant difference between them; their values ranged between 10.14 ± 0.50–11.39 ± 0.93 and 1.10 ± 0.02–1.15 ± 0.02, respectively (table 1).

Films suspension at 100 g L^{-1} had effects on luminosity ($t = 4.0613$; df = 39; $P < 0.001$), chroma ($t = 8.6448$; df = 39; $P < 0.001$) and hue angle ($t = 12.456$; df = 39; $P < 0.001$) of fruits. A comparison of luminosity values before (table 1) and after immersion in suspension at 100 g L^{-1} (table 2) shows that all films increased fruit luminosity after treatment, indicating that fruits immersed in mineral films had higher values than those in control.

For treatments at 100 g L^{-1}, significant differences were observed between the following parameters: luminosity ($F = 42.885$; df = 9, 39; $P < 0.001$), chroma ($F = 93.96$; df = 9, 39; $P < 0.001$), and hue angle ($F = 32.536$; df = 9, 39; $P < 0.001$). Luminosity, which can vary from 0 (black) to 100 (white), was significantly higher in fruits immersed in kaolin Surround® (76.28 ± 5.47, close to white) compared to that of fruits in all other treatments, including that of control (29.32 ± 2.88). Chroma values obtained before (table 1) and after immersion of grapes in suspensions (table 2) showed that there was a general reduction in all treatments, however, this reduction was less pronounced in fruits treated with potato starch, guar gum film, and water. In addition, immersion in suspensions significantly altered the hue angle of fruits. There was an increase in the hue angle of fruits treated with Kaolin 607 and a reduction in those treated with kaolin Surround® and 608, which were different from other treatments (table 2).

Films suspension at 200 g L^{-1} also affected luminosity (t= 10.712, df = 39, $P < 0.001$), chroma (t= 5.0254, df = 39, $P < 0.001$) and hue angle ($t = 4.1679$, df = 39, $P < 0.001$) (table 2). Luminosity values before (table 1) and after immersion at 200 g L^{-1} (table 2) showed that all films increased fruit luminosity after treatment, that is, fruits treated with mineral films had higher values compared to those in control.

Similar results were obtained for fruits immersed in suspensions at 200 g L^{-1}; particle films had effects on luminosity ($F = 718.89$; df = 9, 39; $P < 0.001$), chroma ($F = 248.9$; df = 9, 39; $P < 0.001$) and hue angle ($F = 9.39$; df = 9, 39; $P < 0.001$). It was observed that the luminosity values of fruits immersed in suspensions at 200 g L^{-1} were higher than those in suspensions at 100 g L^{-1}, and the average values of all treatments, except for guar gum, differed from that of control, almost reaching white colour in fruits immersed in kaolin Surround® (94.62 ± 0.82). Chroma values ranged from 2.41 ± 0.41 (cassava starch) to 15.70 ± 0.26 (kaolin 607), the highest average was observed in fruits treated with Kaolin cream (15.70 ± 0.26). Hue angle ranged from 116 ± 3.10 (guar gum) to 156 ± 0.58 (kaolin 607), and only kaolin 608, talc and chitosan did not differ from control in hue angle.

Mineral films (kaolin Surround®, 607, 608 and 611 and talc) and cassava starch increased pulp firmness than control ($F = 4.3069$; df = 9, 39; $P < 0.001$) (table 3).

Oviposition: non-choice tests (bioassays 1 and 2)

In bioassay 1, which is characterized by the immersion of fruits in 100 g L^{-1} film suspensions, increase in punctures with eggs in kaolin (607 and 608), chitosan and starch (cassava and potato) treatments was observed, and their average values were significantly higher than those in distilled water treatment ($F = 3.1682$; df = 9, 39; $P = 0.0083067$) (table 4). As for the number of punctures without eggs, significant differences were observed ($F = 3.5728$;

https://doi.org/10.1017/S0007485321000250

Table 2. Luminosity, chroma and hue angle (mean ± standard deviation) of the grapes after immersion in suspensions at 100 and 200 g l^{-1}.

	Suspension of 100 g L^{-1}			Suspension of 200 g L^{-1}		
Treatments	Luminosity	Chroma	Hue angle	Luminosity	Chroma	Hue angle
T1-Kaolin Surround® WP	76.28 ± 5.47a	2.87 ± 0.28e	45 ± 9.88d	94.62 ± 0.82a	3.73 ± 0.15f	140 ± 2.89b
T2-Kaolin 607 cream	57.61 ± 6.76bc	8.00 ± 0.59b	127 ± 6.85a	83.64 ± 0.30c	15.70 ± 0.26a	156 ± 0.58a
T3-Kaolin 608 white	64.33 ± 2.92b	3.29 ± 0.17e	69 ± 2.16c	89.06 ± 0.92b	3.65 ± 0.52f	125 ± 6.23c
T4-Kaolin 611 grey	49.63 ± 3.15cd	5.94 ± 0.40cd	108 ± 2.5b	80.75 ± 1.85d	7.79 ± 0.15d	143 ± 1.63b
T5-Talc 657	50.58 ± 3.72cd	5.40 ± 0.40d	112 ± 1.41b	80.31 ± 0.52d	6.08 ± 0.15e	131 ± 1.29c
T6-Chitosan	36.23 ± 6.07ef	8.10 ± 0.35b	117 ± 2.52b	58.15 ± 0.65f	8.28 ± 0.43d	129 ± 2.21c
T7-Cassava starch	45.94 ± 3.74de	6.84 ± 0.91bc	110 ± 2.21b	79.46 ± 1.20d	2.41 ± 0.15g	118 ± 10.01d
T8-Potato starch	37.49 ± 4.51ef	10.02 ± 0.75a	120 ± 2.21a	72.55 ± 2.83e	3.90 ± 0.44f	118 ± 4.03d
T9-Guar gum	32.42 ± 4.59f	10.70 ± 0.75a	109 ± 5.77b	36.28 ± 2.41g	10.15 ± 0.87c	116 ± 3.10d
T10-distilled Water	29.32 ± 2.88f	10.21 ± 0.68a	112 ± 1.71b	38.07 ± 1.47g	11.40 ± 1.13b	129 ± 10.80c
C.V (%)	9.52	8.07	2.86	2.14	3.64	4.22

Mean ± SD values in the same column followed by the same letter do not differ significantly at $P < 0.05$ (Tukey's test).

Table 3. Firmness of grapes (mean ± standard deviation) subjected suspensions at 200 g L^{-1}.

Treatments	Firmess of grape (N)[a]
T1-Kaolim Surround® WP	6.37 ± 0.25a
T2-Kaolim 607 cream	6.40 ± 0.19a
T3-Kaolim 608 white	6.75 ± 0.94a
T4-Kaolim 611 grey	6.42 ± 0.86a
T5-Talc 657	6.13 ± 0.56a
T6-Chitosan	5.85 ± 0.16ab
T7-Cassava starch	6.36 ± 0.47a
T8-Potato starch	5.88 ± 0.41ab
T9-Guar gum	5.40 ± 0.41ab
T10-Distilled water (Control)	4.99 ± 0.32b
C.V (%)	8.57

Mean ± SD values in the same column followed by the same letter do not differ significantly at $P < 0.05$ (Tukey's test).
[a]Data transformed into log ($x + 10$).

df = 9, 39; $P = 0.004027$), and only chitosan differed from control with 3.58 ± 0.96 punctures. Regarding the number of eggs, only chitosan, with the highest average number of eggs (30.25 ± 6.08), differed from control ($F = 2.4247$; df = 9, 39; $P = 0.033221$).

At the highest suspension (200 g L^{-1} – bioassay 2), all mineral films (kaolin Surround®, 607, 608 and 611 and talc) and guar gum treatments resulted in the lower average number of punctures with eggs compared to control, whereas the other treatments (chitosan and cassava and potato starches) did not have any effect on this variable ($F = 3.0753$; df = 9, 39; $P = 0.0098394$) (table 4). Regarding the number of punctures without eggs, there were no significant differences among treatments and control ($F = 9.7759$; df = 9, 39; $P = 8.4543$), with average values ranging from 1.0 ± 0 to 1.63 ± 0.16.

For the average number of eggs, it was observed that no treatment differed from control; however, significant differences were found between kaolin Surround®, 607 and 611 and chitosan and potato starch ($F = 4.3264$; df = 9, 39; $P = 0.0011156$), with fruits treated with kaolin having lower average values (table 4).

Oviposition: choice tests (bioassays 3 and 4)

In bioassay 3 (suspension of 100 g L^{-1}), significant differences were observed among treatments for punctures with eggs ($F = 4.9854$; df = 8, 35; $P < 0.0001$) and number of eggs ($F = 8.7221$; df = 8, 35; $P < 0.0001$), but were not observed for punctures without eggs ($F = 0.9853$; df = 8, 35; $P = 0.4628$) (fig. 1). Kaolin Surround® was the only treatment that reduced the number of punctures with eggs, whereas others, except for guar gum treatment, increased the average values of this variable (fig. 1a). However, the reduction in the number of punctures with eggs by kaolin Surround® did not result in the lower average number of eggs in the same treatment (fig. 1c).

For bioassay 4 (immersion at 200 g L^{-1}), responses of flies to treated and untreated fruits were different compared to those in bioassay 3, with a significant reduction in the average number of punctures with eggs ($F = 6.9519$; df = 8, 35; $P < 0.00001$) by kaolin Surround®, 607, 608 and 611 and guar gum treatments, and a significant increase in the same variables by other treatments (fig. 2a). Similar responses occurred for the number of eggs ($F = 3.4768$; df = 8, 35; $P = 0.0026$), except for kaolin 607, which resulted in a higher average number of eggs compared to control (fig. 2c). Treatments did not affect the number of punctures without eggs ($F = 2.0896$; df = 8, 35; $P = 0.05282$) (fig. 2b).

Behavioural response of C. capitata *to treated and untreated fruits*

Time of first landing on fruit did not differ among treatments and control ($F = 14.143$; df = 6; $P > 0.05$; coefficient of variation (C.V) = 28.62%), with values ranging from 1.68 ± 0.216 (kaolin Surround®) to 2.12 ± 0.173 s (guar gum), (fig. 3a); however, for number of landings, kaolin Surround® treatment resulted in the lowest number of landings (2.43 ± 0.094) compared to control ($F = 0.73892$; df = 6; $P < 0.01$; C.V = 6.77%) (fig. 3b). Search time

https://doi.org/10.1017/S0007485321000250

Table 4. Puncture with and without eggs and eggs (mean ± standard deviation) of *C. capitata* in grapes, submitted to suspensions in bioassays 1 and 2 (non-choice).

	Bioassay 1: 100 g L^{-1}			Bioassay 2: 200 g L^{-1}		
Treatments	Punctures with eggs (No)	[a]Punctures without eggs (No)	Eggs (No)	Punctures with eggs (No)	[a]Punctures without eggs (No)	Eggs (No)
T1-Kaolim Surround® WP	2.67 ± 0.47b	0.41 ± 0.42b	24.33 ± 6.00ab	0.33 ± 0.26c	1.14 ± 0.16a	6.41 ± 7.81b
T2-Kaolim 607 cream	3.66 ± 0.60a	0.66 ± 0.77b	26.33 ± 5.40ab	0.75 ± 0.50c	1.0 ± 0a	12.08 ± 9.24b
T3-Kaolim 608 white	3.67 ± 1.27a	0.41 ± 0.42b	24.33 ± 10.05ab	1.41 ± 0.79c	1.28 ± 0.19a	21.58 ± 14.95ab
T4-Kaolim 611 grey	1.91 ± 1.25b	0.25 ± 0.16b	15.25 ± 10.07ab	0.58 ± 0.32c	1.14 ± 0.16a	13.83 ± 7.71b
T5-Talc 657	2.66 ± 1.27b	0.66 ± 1.33b	22.16 ± 6.02ab	1.49 ± 0.88b	1.0 ± 0a	34.08 ± 21.51ab
T6-Chitosan	4.83 ± 0.88a	3,58 ± 0.96a	30.25 ± 6.08a	5.08 ± 1.85a	1.59 ± 0.43a	46.33 ± 4.72a
T7-Cassava starch	3.33 ± 0.71a	0.74 ± 0.42b	24.00 ± 3.12ab	2.75 ± 1.78a	1.42 ± 0.16a	35.33 ± 23.26ab
T8-Potato starch	3.5 ± 1.37a	0.33 ± 0.27b	20.42 ± 9.31ab	4.50 ± 1.82a	1.63 ± 0.16a	43.25 ± 6.45a
T9-Guar gum	2.33 ± 0.67b	0.16 ± 0.33b	17.50 ± 4.64ab	1.83 ± 0.64b	1.34 ± 0.31a	22.08 ± 5.68ab
T10-Distilled water	2.25 ± 0.83b	1.66 ± 1.46b	12.5 ± 7.35b	4.90 ± 2.60a	1.61 ± 0.71a	30.25 ± 12.43ab
C.V (%)	32.02	72.71	32.52	28.88	26.49	48.92

Mean ± SD values in the same column followed by the same letter do not differ significantly at $P < 0.05$ (Tukey's test).
[a]Data transformed in $\sqrt{x+1}$.

for all treatments did not differ from that of control ($F = 20.564$; df = 6; $P = 0.388$; C.V = 19.22%), however, kaolin Surround® treatment (3.72 ± 0495 s) and chitosan (6.11 ± 0495 s) were significantly different between each other, with shorter search time recorded for kaolin Surround® (fig. 3c).

Regarding the average number of searches, differences were found only between kaolin Surround® (2.49 ± 0.107) and kaolin 608 (2.94 ± 0.107) (fig. 3d) ($F = 0.97042$, df = 6, $P = 0.0811$, C.V = 7.82%). Time for aculeus insertion in fruits (puncture) did not differ among treatments ($F = 4.3002$, df = 6, $P = 0.162$, C.V = 20.64%) (fig. 3e); however, differences in the number of punctures were observed only between kaolin 607 (2.43 ± 0.081) and kaolin 611 (2.78 ± 0.081) ($F = 0.55152$, df = 6, $P < 0.05$, C.V = 6.31%) (fig. 3f). Time for aculeus dragging on fruit surface after oviposition differed only between kaolin (607 and 611) and chitosan ($F = 16.126$, df = 6, $P < 0.001$, C.V = 25.76%); (fig. 3g). The difference found in the average number of ovipositor aculeus dragging was not significant among treatments ($F = 0.21976$, df = 6, $P = 0.3748$, C.V = 4.26%) (fig. 3h). Regarding the time for aculeus cleaning, treatments did not differ from control ($F = 3.4687$, df = 6, $P = 0.5003$, C.V = 15.51%), however, differences were found between kaolin 608 (3.28 ± 0.203 s), kaolin 607 (2.30 ± 0.203 s), and chitosan (2.30 ± 0.203 s) (fig. 3i). Regarding the number of times aculeus cleaning behaviour was performed, treatments did not differ from control ($F = 8$, df = 6, $P = 0.5728$, C.V = 123.44%), except for kaolin 611, which resulted in the greater number of times (1.75 ± 0.309 times) (fig. 3j).

Discussion

Studies were developed using grape as a substrate for *C. capitata* oviposition owing to its economic importance for export and the easy visualization of punctures and eggs, which help in minimizing experimental errors. The grapes used in the bioassays of this study were within the commercial standards reported in Normative Instruction No. 1 of 1 February 2002 (BRAZIL, 2002), which stated that fine table grapes should have a minimum soluble solids equal to 14° Brix and TA <1.5 (Carvalho and Chitarra, 1984). In this study, the values obtained for mass, length and diameter of grapes can be considered to be within commercial standards (Mascarenhas *et al.*, 2010, 2013). Before bioassays, grapes were uniform in terms of weight, length, chroma and hue angle, with variations only in diameter and luminosity values (table 1), indicating good fruit uniformity.

Variations in the diameter values of grapes did not interfere with the responses of females. According to Corrêa *et al.* (2018), grapes of different varieties and diameters did not influence the oviposition of *C. capitata* and *A. fraterculus*. Regarding the luminosity values obtained in grapes before applying treatments, differences were observed only between potato and cassava starches and guar gum and control, however, they were statistically equal to the values of grapes used in other treatments.

Thus, this factor alone probably did not influence females in choosing between fruits treated with different films (table 1). In general, it is considered that grapes had good uniformity for use in bioassays, and it could be inferred that variations in responses of flies to oviposition were only due to treatments applied.

Regardless of the method used (choice and non-choice tests), studies with mineral and natural films indicated that suspension at 100 g L^{-1} does not protect grapes from *C. capitata* oviposition (table 4 and fig. 1), but even increases oviposition variables (punctures with eggs and number of eggs). The only exception was Surround® treatment in choice test, which resulted in a lower average number of egg punctures (fig. 1a), however, it did not result in fewer eggs on grapes (fig. 1c). These results differ

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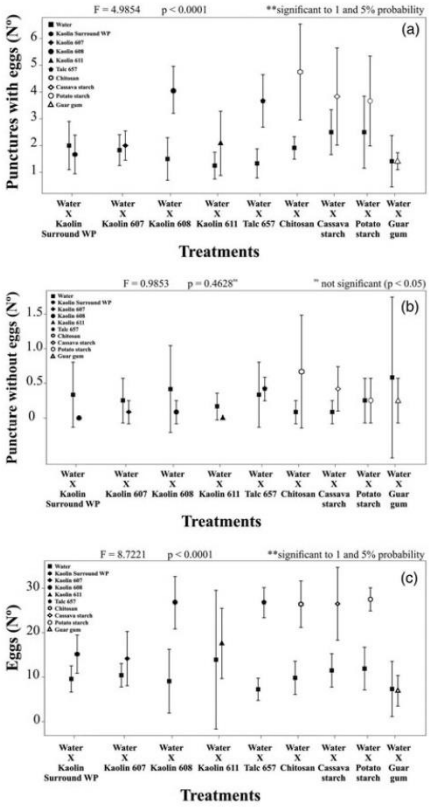

Figure 1. Punctures with eggs (a) and punctures without eggs (b) and eggs (c) (mean number ± standard deviation) of *C. capitata* in grapes, submitted to mineral and natural films, at 100 g L^{-1}, obtained in the bioassay 3 (choice test).

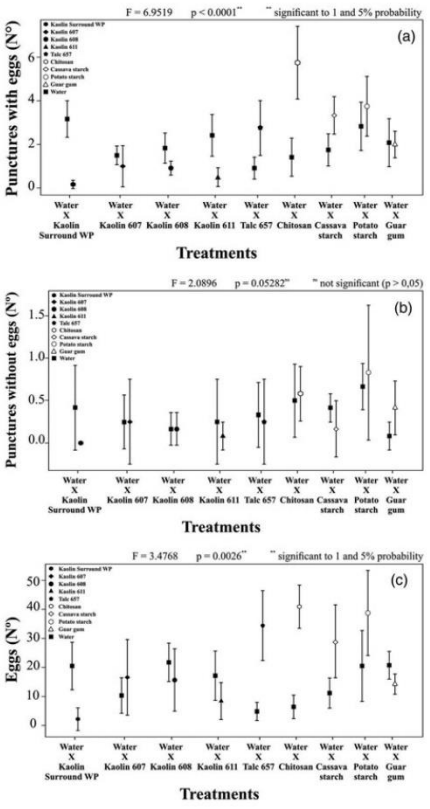

Figure 2. Punctures with eggs (a) and punctures without eggs (b) and eggs (c) (mean ± standard deviation) of *C. capitata* in grapes, submitted to mineral and natural films, at 200 g L^{-1}, obtained in bioassay 4 (choice test).

from that recorded in some laboratory, where there was a reduction in punctures of *C. capitata* oviposition in citrus (D'aquino *et al.*, 2011) and nectarine treated with Surround® at 30 g L^{-1} and 60 g L^{-1}, respectively; flies avoided landing on treated fruits, resulting in no infestation (Mazor and Erez, 2004); and reduction in punctures of *Rhagoletis mendax* Curran fly oviposition in blueberry treated with Surround® at 60 g l^{-1} (Lemoyne *et al.*, 2008). In the field, kaolin sprays at 50 g L^{-1} in citrus (Braham *et al.*, 2007; Lo Verde *et al.*, 2011) and apple plants (Villanueva and Walgenbach, 2007) resulted in a significant reduction in the number of damaged fruits, indicating negative effects on oviposition.

For suspension at 200 g L^{-1}, the reduction of *C. capitata* oviposition in grapes was evidenced in treatments with mineral films and guar gum in the choice test of hosts by fly (bioassay 2). In this case, Surround® reduced the number of punctures with eggs and the number of eggs by ∼15 and 5 times, respectively (table 4). In bioassay 4, where flies had a choice for treated or untreated fruits, flies discriminated the treatments in two groups: oviposition inhibitors (Surround®, kaolin 608, kaolin 611 and guar gum) and stimulants (kaolin 607, talc, chitosan and potato and cassava starches). In this case, the greatest inhibition was achieved with Surround®, ∼19 and 9 times the number of punctures with eggs and number of eggs, respectively. In a suspension at 200 g L^{-1}, kaolin and liquid limestone applied to apple and mango fruits resulted in an inhibition of *C. capitata* oviposition (Ourique *et al.*, 2017). The average number of punctures in apples and mangoes was 7 to 8 times and 3 times lower, respectively, when treated with both products.

Few ripe fruit species are white in colour and white can be considered a very neutral surface, reflecting a range of wavelengths within the visible spectrum of tephritids. According to Díaz-Fleischer *et al.* (2000), in laboratory experiments, females such as *A. fraterculus*, *A. ludens* and *C. capitata* generally show

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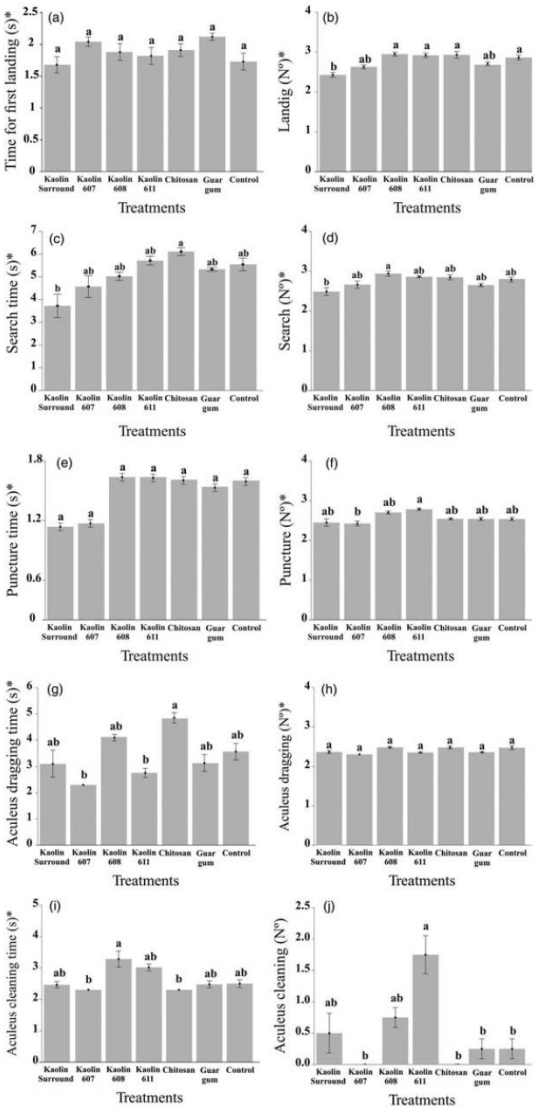

Figure 3. Oviposition behaviour (number mean ± standard deviation) of *C. capitata* in grapes, submitted the suspensions at 200 g L^{-1}. Time of first landing (a) number of landings (b) search time (c) number of search (d) puncture time (e) number of punctures (f) aculeus dragging time (g) number of aculeus dragging (i) cleaning time of the aculeus (j) (number of cleaning of the aculeus). * Data transformed into log ($x + 10$).

little or no discrimination between white spheres (substrate for oviposition) and spheres of other colours. With the use of suspension at 200 g L^{-1}, fruits from T1, T2, T3 and T4 treatments showed whitish colour, evidenced by luminosity values ≥80. Surround® and kaolin 607 reduced the oviposition of *C. capitata* and both showed high luminosity value of 94.62 ± 0.82 and 83.64 ± 0.30, respectively, which also indicates reflectance. The colour change resulting from the effects of these films probably impaired the perception of host, a fact already reported by Katsoyannos *et al.* (1986) for wild *C. capitata* flies. In the laboratory, the authors found that flies preferred to oviposit in spheres coloured in black, blue and red than in those coloured in yellow and white, which received smaller number of eggs. The preference observed for certain colours depends on both colour tone and intensity of total light reflected (brightness) and white spheres showed 100% reflectance (Katsoyannos *et al.*, 1986).

https://doi.org/10.1017/S0007485321000250

In all bioassays, when fruits were dissected for egg counting, it was observed that grapes with mineral films had punctures with eggs, but had a reduced number of eggs; however, smaller number of punctures with greater amount of eggs was observed under the fruit pedicel. Perhaps, this behaviour is owed to the perception that flies had towards the films in fruit, making them search for a more appropriate place without foreign substances for oviposition. It was observed that fruits with films had changed colour but did not prevent *C. capitata* from finding and accepting the host. However, the changed colour somehow prevented flies from having prolonged direct contact with foreign substances, causing them to look for alternative places in the fruit to oviposit.

According to Mazor and Erez (2004), kaolin-treated fruits are visually recognized by flies as host, but their colour does not match what not expect something appropriate for oviposition. Even in inappropriate hosts, in an attempt to leave offspring, fruit flies can oviposit on these substrates (Aluja and Mangan, 2008). In the absence of a primary host, *C. capitata* searches for an alternative host, such as *Opuntia ficus-indica* (L.) Mill and *Pereskia bahiensis* Gürke, to ensure offspring survival, even though they are poorly suited hosts for larval development (Leite *et al.*, 2017; Leite *et al.*, 2019).

Natural polymers have wide applicability in several study areas owing to their properties such as biocompatibility, biodegradability, high availability and non-toxicity (Azevedo *et al.*, 2007). The use of natural films at both suspension rates did not reduce Medfly ovipositions. This result was not expected, mainly owing to the colour change provided by these films. Chitosan affected the posture of *C. capitata*, with a consequent increase in the number of eggs; this result may have an application in bio-factories for massal rearing of fly, especially when aiming to sterile insect technique.

Regarding oviposition behaviour, *C. capitata* took the same time to recognize fruits with and without films (fig. 3a). It was observed that the average number of landings was lower in treatment with Surround® (2.43 ± 0.094) compared to that in control (2.92 ± 0.094). These results are in accordance with those obtained by Mazor and Erez (2004) in studies of *C. capitata* oviposition in nectarine, in which average landing was 0.05 in kaolin-treated fruits and 4.95 in untreated fruits. The authors attributed their results to the whitish colour left by the film on fruits, impairing the detection of hosts by flies (Mazor and Erez, 2004). In the present study, the number of *C. capitata* landings on fruits treated with Surround® was five times lower than that in untreated fruits (taking into account original unprocessed data). Probably, the particle films masked the volatile emission of fruits, interfering in the oviposition behaviour of fly. Studies using other films on 'Golden Delicious' apple fruits confirm that volatile compounds can be inhibited by up to 75% (Saftner, 1999) for this type of coverage. However, in the present study, the determination of volatiles by means of chromatographic analysis would be necessary to confirm this hypothesis.

Mineral films form a physical barrier over fruit, which is evidenced by the change in pulp firmness (table 3); however, this barrier did not influence the duration of aculeus insertion (puncture) (fig. 3e). Mineral films resulted in an increase in pulp firmness compared to control, which may have negatively affected oviposition at the highest suspension. *Ceratitis capitata* females prefer to oviposit on grape fruits with more advanced physiological development stage, that is, with lower firmness, lower TA and higher content of TSS (Gómez *et al.*, 2019). The same fact has already been observed by Jang and Light (1991) for *Bactrocera* (*Dacus*) *dorsalis* Hendel in papaya.

Some fruits also possess epicarps that show resistance so that some species with short aculeus, like *C. capitata*, are unable to make punctures and deposit eggs (Aluja and Mangan, 2008). According to Saour and Makee (2004), mineral particles make fruit surface rough and may make them unsuitable for oviposition. Among the variables determined or observed in this study, the number of punctures without eggs occurred in all bioassays and in all treatments, but without significant difference. This resistance, mainly provided by minerals films, may influence flies to make punctures without depositing eggs on fruits. Films should also inhibit this behaviour, since, for certain thin-skinned fruits, the injury caused by puncture also results in microorganism contamination (Engelbrecht *et al.*, 2004). It is observed that films resulted in a reduction in the number of landings of fly on fruits, but did not prevent them from recognizing and puncturing the treated grapes; this fact was also reported for blueberry fruits treated with Surround® and exposed to the fly *R. mendax* (Lemoyne *et al.*, (2008). The interference of films in colour (brightness, chroma and hue angle) and, probably, in the dispersion of volatiles, made it difficult for the females to recognize the fruits while the firmness may have acted directly in oviposition. *Ceratitis capitata* has short aculeus, smaller than other tefritids and usually selects fruits in more advanced maturation stages to oviposit.

After the puncture, flies exhibit the behaviour of circulating the fruit and occasionally dragging ovipositor to deposit marking pheromone (Díaz-Fleischer *et al.*, 2000). All treatments showed this behaviour, without significant difference. According to Díaz-Fleischer *et al.* (2000) flies clean aculeus to disperse marking pheromone and remove fruit pieces that are attached to the aculeus. It was observed that this cleaning was not mandatory, and in kaolin 607 and chitosan treatments, flies did not perform this procedure (fig. 3j). The absence of aculeus cleaning behaviour reinforces the hypothesis that flies did not recognize chitosan as an inappropriate substrate for oviposition, otherwise, an increase in oviposition regardless of suspension and type of test (in choice and non-choice) would have been observed. Such a hypothesis can be made because, in kaolin-treated blueberry fruits, *R. mendax* females made relatively short walks, followed by frequent cleaning sessions, suggesting that some fragment in the film would have hindered the perception of stimuli (chemical compounds on the surface, blocked or absorbed by the particle film) needed to assess the suitability of hosts (Lemoyne *et al.*, 2008).

The results obtained in this study are promising, given the scientific evidence that films of mineral particles such as kaolin (Surround®, 607, 608 and 611) change the firmness, luminosity, chroma and hue angle of fruits and reduce the oviposition of *C. capitata*. In addition, we also observed that natural polymers do not deter *C. capitata* females, but rather seems to stimulate oviposition.

Acknowledgements. We are grateful to the Foundation for Research Support of the State of Bahia (FAPESB) and the CAPES Foundation (Brazilian Ministry of Education; Finance Code 001) for financial support. The companies Brasilminas and NovaSource for donating the kaolin samples.

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ANNEX II

insects

MDPI

Article

Influence of Mineral Particle Films and Biomaterials on Guava Fruits and Implications for the Oviposition of *Anastrepha obliqua* (Macquart) (Diptera: Tephritidae)

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Simple Summary: Among the main phytosanitary problems that affect the production and commercialization of fresh fruits, the occurrence of fruit flies (Diptera: Tephritidae) is one of the main obstacles. The control of these tephritids is mainly performed through the use of toxic baits. The use of mineral films and biomaterials may constitute a viable alternative in relation to the traditional insecticide method, mainly because they do not contaminate the environment and do not leave toxic residues harmful to humans and animals in treated products. Therefore, by modifying the color and texture of the fruit cuticule that covers the plant tissues, kaolin affects the perception of arthropod pests, impairing the localization process and acceptance of the host plant and, consequently, its feeding and oviposition. In this study, we hypothesized that the color changes of guava fruits because of mineral particle films and biomaterials can affect the oviposition of fruit flies. The results obtained are promising and show that mineral films and biomaterials interfering with the color of guavas inhibited the oviposition of *A. obliqua*. Therefore, they can be used to protect guava fruits from the damage caused by this pest.

Abstract: *Anastrepha obliqua* (Macquart, 1835) is an important pest of tropical fruits, especially Anacardiaceae and Myrtaceae, in the Americas. The objective of this study was to evaluate the influence of mineral films and biomaterials on the coloring of guava fruits (*Psidium guajava* L.) and implications for the oviposition of *A. obliqua*. Before the bioassays, color, firmness characteristics, total soluble solids, pH, and titratable acidity were determined to characterize the maturation stage of the fruits. Pieces of guava fruit covered in aluminum foil were immersed in suspensions of mineral particles (Surround® WP kaolin; kaolins 605, 607, 608, and 611; and talc) and biomaterials (chitosan, cassava and potato starch, and guar gum) and distilled water (control). After drying, the fruits were exposed to two *A. obliqua* pairs for 48 h in choice and non-choice tests, and the numbers of eggs per fruit were counted. Mineral films (Surround® WP kaolin, and kaolins 605, 607, 608, and 611) and biomaterials (cassava and potato starch) interfered with the color of guava (luminosity, chroma, and hue angle), inhibiting the oviposition of *A. obliqua*. Talc, chitosan, and guar gum did not influence the oviposition of *A. obliqua* in guava.

Keywords: chitosan; eggs; fruit flies; kaolin; luminosity

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Citation: da Costa, D.R.; Leite, S.A.; dos Santos, M.P.; Coelho, B.S.; Moreira, A.A.; Domingues da Silva, C.A.; Joachim-Bravo, I.S.; Castellani, M.A. Influence of Mineral Particle Films and Biomaterials on Guava Fruits and Implications for the Oviposition of *Anastrepha obliqua* (Macquart) (Diptera: Tephritidae). *Insects* **2021**, *12*, 373. https://doi.org/10.3390/insects12050373

Academic Editor: Lukasz L. Stelinski

Received: 11 March 2021
Accepted: 16 April 2021
Published: 21 April 2021

1. Introduction

Brazil is the world's largest red guava (*Psidium guajava* L.) producer, reaching 578,600 tons in 2019, of which 34% was exported [1,2]. Among the most cultivated guava varieties, "Paluma and Pedro Sato" have a dual aptitude, for consumption in natura and processing industries [3].

The valorization of guava trees as raw material for the food industry and the increased consumption of in natura fruit are proportional to changes in the production system and commercialization. This is particularly true concerning the quality of the fruits produced, which can be affected by phytosanitary problems [4].

Guava is one of the fruits most affected by fruit flies (Diptera: Tephritidae) in Brazil [5]. Fruit fly larvae cause serious damage to fruit growth because they feed on the fruit pulp, making the fruit unsuitable for consumption in natura or industrialization [6]. Several factors, such as climate, altitude, geographical location, hosts, and adjacent orchards, can influence the diversity and dominance of fruit fly species in orchards [7]. Among these species, *Anastrepha obliqua* (Macquart, 1835) is an important pest of tropical fruits in the Americas, with great genetic variability among its populations and a wide geographical distribution, from northern Mexico to southeastern Brazil [8]. The most common hosts of *A. obliqua* are fruits of the family Anacardiaceae, such as the mango (*Mangifera indica* L.), the genus *Spondias* [9,10], and within the Myrtaceae family, mainly fruits of guava [11]. *Anastrepha obliqua* reach the peak of oviposition between 15 and 25 days, producing an average of 137 eggs per female, depositing one egg per oviposition [12,13].

To locate the host plant, female fruit flies can select oviposition sites based on the host plant species, size, color, odor, flavor, and maturation stage of the fruits, and avoid fruits previously oviposited [14]. Chemical stimuli, nutritional and inhibitory substances, or food stimulants also affect resource localization [15]. Fruit flies respond negatively to visual stimuli with high reflectance and wavelengths less than 520 nm, reducing oviposition and the capture of adults in traps [16–18].

The population suppression of fruit flies via behavioral manipulation using toxic baits (a mixture of attractive food and lethal agents) has become an important component of integrated pest management (IPM) programs worldwide [19–27]. However, the intensive use of toxic baits, such as the insecticide spinosad, can cause serious biological imbalances in fruit orchards by selecting resistant populations of this pest [28]. In addition, spinosad could also affect useful Arthropodofauna [29]. Thus, chemical insecticides are being used less to manage this pest, mainly because of pressure from consumers who prefer fresh fruits without residues, making it necessary to evaluate alternative strategies to manage this pest [30].

Mineral kaolin particle films and biomaterials are viable options for use in the replacement of synthetic chemical insecticides to avoid environmental contamination and the spread of toxic residues to humans and animals in the treated products [31,32].

Kaolin is an aluminosilicate mineral that is chemically inert, white, and formulated for use in plants [33]. The mechanisms of action of kaolin against insect pests include repellent, tactile, or visual interference, committed or interrupted oviposition and feeding activity, and decreased longevity and survival [34]. Therefore, by modifying the color and texture of the fruit cuticule that covers the plant tissues, kaolin affects the perception of arthropod pests, impairing the localization process and acceptance of the host plant and, consequently, its feeding and oviposition [35–37]. Unlike traditional agricultural chemicals, mineral kaolin particle films are inert and have no biochemical or physiological effects on plants or arthropod pests [38]. Thus, kaolin used in isolation does not cause fruit fly mortality [39,40], affect fruit fly attachment capacity on substrates treated with kaolin, or interfere with female oviposition behavior [41]; however, it can interfere with oviposition behavior [42]. When associated with entomopathogenic fungi, this product can cause insect pest mortality [43].

In addition to kaolin, biomaterial-based particle films have been used to protect cultivated plants because of their high availability, biodegradability and biocompatibility, and

low toxicity [44,45]. In agriculture, these biomaterials are used mainly for the coating and preservation of fruits before and after harvest [46,47]. Cellulose, agar, starch, pectin, guar gum, alginates, carrageenan, xanthan gum, chitin, and chitosan are among the most commonly used natural polymers [47]. For example, chitosan is used to treat seeds, stimulate plant growth, and control phytopathogens [46,48]. When encapsulated in nanoparticles, chitosan is released gradually [46,47,49,50]. Chitosan also delays the fruit ripening process and inhibits the development of eggs and larvae of the *Anastrepha ludens* (Loew) [51,52].

Particle films based on minerals and biomaterials have been studied as important tools for the management of fruit flies in apples [53,54], nectarines [31,53], cherries [42], blueberries [40], citrus and peaches [31], and grapes [55]. Therefore, we hypothesized that the color changes of guava fruits, because of mineral particle films and biomaterials, can affect the oviposition of fruit flies, reducing their infestation in the field.

The objective of the present study was to evaluate the influence of mineral particles and biomaterial films on the coloring of guava fruits and their implications for the oviposition of *A. obliqua* in the laboratory.

2. Material and Methods

2.1. Origin of Anastrepha Obliqua and Fruits Used in Bioassays

Adults of *A. obliqua* fruit flies were obtained from Embrapa Mandioca and Fruticultura and maintained in an air-conditioned room of the Entomology Laboratory at the State University of Southwest Bahia in acrylic cages (30 × 30 × 30 cm). They were fed daily with a Bionis-based diet®, sugar (proportion 1:3) [56] and water and maintained at 25 ± 2 °C and 70 ± 10% relative humidity. Guava fruits of the Pedro Sato variety were offered to adult *A. obliqua* every two days for oviposition, and posteriorly removed and placed in plastic trays containing vermiculite to obtain larvae and pupae. The pupae were placed in 500 mL plastic pots containing a thin layer of vermiculite covered with paper towels until adult emergence.

The guava fruits (*Psidium guajava* L.) Pedro Sato variety with red colored pulp were obtained from the local fresh fruit trade and selected at maturation stage 2, based on the description by Azzolini et al. [57]. The use of guava fruits with red pulp in the present *A. obliqua* oviposition study facilitated the visualization of eggs and minimized possible experimental errors because of the contrast of the white color of the eggs of *A. obliqua* compared to the red color of the guava pulp.

Fruits were selected based on the light green color of the epicarp (peel), color uniformity, hue angle (between 116 and 113 h), and absence of oviposition orifices of fruit flies. The guavas were washed with 1% hypochlorite and cut in the part median, in average into 2 × 2 × 1 cm pieces (length, width, and height, respectively) (6 pieces). Based on the methodology described by Joachim-Bravo et al. [58], the pieces of guava were packaged in aluminum foil, such that only the peels were exposed for oviposition, and they were subsequently used in bioassays.

Before starting the bioassays, the physicochemical characteristics of the guava fruits, including firmness, color, total soluble solids (TSS), pH, and titratable acidity (TA), were determined to characterize their ripening stage. Firmness was evaluated using a penetrometer (model WA68, Italy) with an 8 mm diameter tip. Two readings were taken per fruit on opposite sides in the equatorial region, on 20 fruits, with results expressed in Newtons. The TSS content was determined by direct readings on a digital refractometer (Reichert, model r^2 mini, Porto, Portugal); the results were expressed in °Brix, and the TA was determined by titrimetry [59], with results expressed as the % of citric acid per 100 g of pulp. The pH of 100 mL of guava juice was determined by direct readings using a digital potentiometer (Mars, model MB-10, São Paulo).

The color of the guava was determined previously and after applying the treatments on each piece of fruit, immediately after drying, using a colorimeter (CR-400, Minolta, Osaka, Japan). The apparatus was calibrated on a white ceramic plate using a D65 illuminant (z = 85.7; x = 0.3175; y = 0.3253). The luminosity values (L) were determined, which

varied from 0 to 100 (black/white) and intensities of red/green (+/- (a) and yellow/blue (+/) (b). Additionally, the color parameters were estimated as chroma C = $(a^2 + b^2)$ 1/2, which represents the color purity, and the hue angle (Hue) H = tg^{-1} (b/a), which represents the color tone [40].

2.2. Oviposition: Non-Choice Tests

Two non-choice tests were performed to evaluate the effect of fruit acceptance of treated guava pieces as oviposition substrates. A completely random design was used with 11 treatments and four repetitions, evaluated on three consecutive days (one repetition every 48 h). Each non-choice test was performed using either a 100 or 200 g L^{-1} concentration of the tested mineral particle films or biomaterials. The treatments were as follows: T1, Surround® WP kaolin; T2, kaolin 605 white; T3, kaolin 607 cream; T4, kaolin 608 white; T5, kaolin 611 grey; T6, talc 657; T7, chitosan; T8, cassava starch; T9, potato starch; T10, guar gum; and T11, control (distilled water). The particle films were dispersed in distilled water at concentrations of 100 and 200 g L^{-1} and guar gum was added to these suspensions at 5 g L^{-1}, guar gum was used because it improves the viscosity and stability of formulations [60,61] except in the treatment T11 (control). These two concentrations were used because in preliminary tests with lower concentrations there was no verified effect on oviposition by the fruit fly. In the treatment with guar gum at 200 g L^{-1}, the concentration of this substance in distilled water was also doubled (10 g L^{-1}) to verify the effects of increasing the concentration.

Chitosan was obtained from the shells of crustaceans, dissolved in distilled water, and maintained under agitation for 2 min. Surround® WP kaolin was obtained from NovaSource (Phoenix, AZ, USA), and kaolins 605, 607, 608, and 611, and talc were acquired from Brasilminas (Guarulhos, SP, Brazil). Biomaterial particle films were obtained from a natural product market (Indianópolis, SP, Brazil).

The bioassays were performed in the laboratory at 25 ± 2 °C and 70% relative humidity, with a 12 h photophase. The plot consisted of a plastic cage with a capacity of 3.5 L, containing a piece of treated guava and two pairs of 15-day-old naive *A. obliqua*, with 8 females per treatment, totaling 88 females. The pieces of guava were individually immersed for 10 s in 60 mL of each solution in a beaker. After immersion, the guava pieces were dried at 25 ± 2 °C for 1 h. Subsequently, a piece of guava was randomly selected and exposed to the fruit flies for 48 h in each cage over a disposable plastic cup with a capacity of 50 mL and subsequently removed to determine the number of eggs.

2.3. Oviposition: Choice Tests

The bioassay of choice was developed with an experimental design similar to that described in the previous section, with 10 combined treatments and 8 females per treatment, totaling 80 females/replica and 240 females in total (3 replicates). The difference was that in this bioassay, two pieces of guava were offered to the fruit flies by cage: one was treated with mineral film or biomaterial film, and the other was untreated and immersed in distilled water (control).

The methodology was the same as described in the previous bioassay, except for the control offered to the fruit flies jointly with the other treatments. The mineral particle films and biomaterials were mixed in distilled water at a concentration of 100 g L^{-1} and 200 g L^{-1}, respectively. Guar gum was added to all treatments at a concentration of 5 g L^{-1}, except for 200 g L^{-1}, in which guar gum was used at a concentration of 10 g L^{-1}. After immersion and drying, the pieces of guava (treated and untreated (control)) were separated by 10 cm and placed on plastic cups with a 50 mL capacity, in the lower part of each cage, containing one pair of fruit flies.

2.4. Statistical Analyses

The oviposition data of the non-choice test and color of the fruits (luminosity, chroma, and hue angle) were subjected to Bartlett and Shapiro–Wilk tests to evaluate the presence

of homoscedasticity of variances of the treatments and the normality of the residues, respectively. When these assumptions were violated, the hue angle data after applying 100 and 200 g L^{-1} treatments and the number of eggs were transformed by $\sqrt{x} + 1$. Then, the data were compared using general linear models in the R software package "nlme" [62] and "lsmeans" [63]. A paired t-test was used to compare the average values of luminosity, chroma, and hue angle before and after applying the suspensions of 100 and 200 g L^{-1} [64].

The oviposition data obtained in the choice tests did not fit the assumptions of the analysis of variance, making it necessary to utilize randomization-type Monte Carlo simulations, with thousands of simulations to guarantee a 95% probability. To verify differences between treatments, a priori orthogonal contrasts were performed using R version 3.6.1 [64].

3. Results

3.1. Fruit Characterization

Before immersion in the treatments, guavas presented average values of TSS, TA, and pH were 7.0 ± 0.17 °Brix, 0.52 ± 0.01, and 3.40 ± 0.52, respectively. The average firmness of guava pulp was 45 ± 0.91 N. The color of the guavas before treatments at a concentrations of 100 g L^{-1} differed only in the chroma parameter (F = 82.101; df = 10, 43; $p < 0.001$), ranging from 37.73 ± 1.82 (kaolin 607) to 40.01 ± 0.32 (Surround® WP kaolin); however, they did not differ from the control. The luminosity (F = 1.7272; df = 10, 43; $p = 0.11583$) and color angle (F = 1.2427; d f= 10, 43; $p = 0.3017$) did not differ between treatments (Table 1).

Table 1. Luminosity, chroma and hue angle (mean ± standard deviation) of the guavas before and after immersion in suspensions at 100 g L^{-1}.

Treatments	Before Immersion in Suspension at 100 g L^{-1}			After Immersion in Suspension at 100 g L^{-1}		
	Luminosity	Chroma	Hue Angle	Luminosity	Chroma	Hue Angle
T1-Kaolin Surround® WP	54.71 ± 0.12 a	40.01 ± 0.32 a	113.78 ± 1.11 a	86.55 ± 1.73 a	2.87 ± 0.07 e	123.00 ± 0.0 e
T2-Kaolin 605 white	55.94 ± 1.15 a	39.01 ± 0.63 ab	114.32 ± 1.70 a	83.39 ± 1.72 a	3.45 ± 0.38 e	138.25 ± 2.63 bc
T3-Kaolin 607 cream	53.86 ± 1.91 a	37.73 ± 1.82 b	114.17 ± 1.00 a	74.12 ± 2.36 b	20.40 ± 1.61 c	152.75 ± 0.5 a
T4- Kaolin 608 white	55.05 ± 1.01 a	38.36 ± 0.42 ab	115.61 ± 2.67 a	70.41 ± 4.80 bc	2.73 ± 0.18 e	126.75 ± 5.62 de
T5- Kaolin 611 grey	53.04 ± 1.35 a	37.96 ± 0.47 ab	114.25 ± 0.95 a	70.99 ± 3.00 bc	13.80 ± 1.03 d	143.5 ± 1.91 b
T6-Talc 657	56.14 ± 1.52 a	38.36 ± 1.32 ab	116.45 ± 1.31 a	73.42 ± 2.25 b	11.59 ± 1.41 d	137.25 ± 2.36 c
T7-Chitosan	55.44 ± 1.54 a	39.09 ± 0.60 ab	115.10 ± 1.16 a	64.69 ± 0.98 cd	28.41 ± 1.38 b	124.75 ± 4.03 de
T8-Cassava starch	56.13 ± 2.10 a	39.41 ± 0.55 ab	115.51 ± 1.68 a	68.71 ± 3.51 bcd	22.19 ± 1.37 c	129.75 ± 0.96 d
T9-Potato starch	55.70 ± 1.98 a	39.53 ± 1.27 ab	114.06 ± 1.96 a	62.73 ± 2.83 de	30.12 ± 1.85 b	136.25 ± 2.87 c
T10-Guar gum	54.08 ± 1.78 a	39.08 ± 1.44 ab	113.69 ± 1.68 a	58.01 ± 2.61 ef	40.63 ± 0.89 a	112.00 ± 0.82 f
T11-Distilled water	55.74 ± 1.77 a	39.70 ± 0.4 ab	114.94 ± 1.33 a	55.77 ± 2.06 f	40.20 ± 2.08 a	112,25 ± 1.70 f
CoefficientVariation (%)	2.86	2.5	1.37	3.89	6.54	2.05

Means followed by the same lowercase letter in the column are not different by the Tukey test ($p < 0.05$). Four repetitions per treatment were used.

Film suspensions at 100 g L^{-1} affected the luminosity (t = 11.454; df = 43; $p < 0.001$), chroma (t = 9.9953; df = 43; $p < 0.001$), and hue angle (t = −8.0453; df = 39; $p < 0.001$). A comparison of the luminosity values before and after immersion in the 100 g L^{-1} suspension showed that all films increased the luminosity and hue angle, with a decrease in the chroma of the fruits, indicating immersion in mineral films and biomaterials influenced the change of guavas color (Table 1).

Differences were observed between treatments in luminosity (F = 49.405; df = 10, 43; $p < 0.001$), chroma (F = 480.53; df = 10, 43; $p < 0.001$), and hue angle (F = 187.934; df = 10, 43; $p < 0.001$) (Table 1) after immersion in 100 g L^{-1} suspensions. The luminosity and hue angles of the guava fruits before immersion in the suspensions were consistently lower than those after immersion in all treatments. Luminosity varied from 0 (black) to 100 (white), and the guavas after treatments had values between 55.77 ± 2.06 and 86.55 ± 1.73. The highest luminosities were observed in the fruits treated with Surround® WP kaolin and kaolin 605, and the lowest was in the fruits treated with distilled water, followed by guar gum. In contrast, the largest hue angle was observed in fruits treated with kaolin

607, and the smallest was in those treated with distilled water and guar gum, with values ranging from 112 ± 0.82 to 152.75 ± 0.5.

Except for the control and guar gum, the chroma or purity of the color of the guava fruits before immersion in the suspensions was always lower than those after immersion in all treatments, with values ranging from 2.73 ± 0.18 to 40.63 ± 0.89 (Table 1). The highest chroma values were observed in fruits with treatments of guar gum and the control, and the lowest was in treatments with Surround® WP kaolin and kaolins 605 and 608.

Guavas immersed in the 200 g L^{-1} suspension differed in luminosity (t = −11.293; df = 43; $p < 0.001$), chroma (t = 13.794; df = 43; $p < 0.001$), and hue angle (t = 235.42; df = 43; $p < 0.001$) (Table 2), compared to guavas before immersion (Table 2). The color values of the guavas after immersion at 200 g L^{-1} were different from those of guavas before immersion in the suspensions, demonstrating that all films modified this parameter.

Table 2. Luminosity, chroma and hue angle (mean ± standard deviation) of the guavas before and after immersion in suspensions at 200 g L^{-1}.

Treatments	Before Immersion in Suspension at 200 g L^{-1}			After Immersion in Suspension at 200 g L^{-1}		
	Luminosity	Chroma	Hue Angle	Luminosity	Chroma	Hue Angle
T1- Kaolin Surround® WP	53.60 ± 5.3 a	40.07 ± 2.09 a	113.77 ± 2.40 a	91.08 ± 2.98 a	3.52 ± 0.21 h	98.44 ± 4.02 d
T2- Kaolin 605 white	52.96 ± 6.38 a	41.86 ± 1.87 a	114.34 ± 2.50 a	91.18 ± 0.75 a	4.57 ± 0.52 gh	106.27 ± 10.18 cd
T3- Kaolin 607 cream	54.52 ± 4.24 a	39.58 ± 1.78 a	116.95 ± 3.29 a	79.59 ± 4.26 bc	14.09 ± 0.94 d	154.84 ± 1.49 a
T4- Kaolin 608 white	55.19 ± 3.68 a	43.13 ± 1.29 a	116.44 ± 4.57 a	72.69 ± 1.75 c	6.24 ± 0.68 efg	134.09 ± 1.01 b
T5- Kaolin 611 grey	49.63 ± 3.39 a	39.14 ± 3.57 a	114.06 ± 2.41 a	75.47 ± 2.12 c	7.98 ± 0.40 e	133.04 ± 1.22 b
T6- Talc 657	49.72 ± 4.80 a	39.34 ± 3.66 a	116.26 ± 5.07 a	84.60 ± 1.68 ab	6.92 ± 0.23 ef	127.05 ± 2.25 b
T7- Chitosan	55.86 ± 2.71 a	41.95 ± 1.71 a	114.48 ± 2.14 a	58.07 ± 1.86 d	18.95 ± 0.98 c	110.94 ± 2.61 cd
T8- Cassava starch	58.62 ± 2.34 a	42.96 ± 1.10 a	116.85 ± 1.98 a	79.79 ± 1.23 bc	5.49 ± 0.30 fg	110.14 ± 4.36 cd
T9- Potato starch	57.28 ± 2.26 a	39.35 ± 1.49 a	116.76 ± 5.84 a	73.97 ± 3.82 c	7.08 ± 0.68 ef	106.36 ± 1.88 cd
T10- Guar gum	51.21 ± 2.21 a	40.90 ± 1.21 a	114.46 ± 2.59 a	57.47 ± 6.04 d	37.70 ± 1.10 b	114.14 ± 1.04 c
T11- Distilled water	51.69 ± 0.72 a	40.25 ± 0.41 a	114.86 ± 2.14 a	55.84 ± 2.84 d	39.68 ± 1.18 a	115.67 ± 2.57 c
CoefficientVariation (%)	7.18	5.09	2.97	4.09	5.34	3.26

Means followed by the same lowercase letter in the column are not different by the Tukey test ($p < 0.05$). Four repetitions per treatment were used.

There were no differences in luminosity (F = 1.4729; df = 10, 43; $p = 19.36$), chroma (F = 2.0251; df = 10, 43; $p = 0.6254$), or hue angle (F = 0.53799; df = 10, 43; $p = 0.85047$) in guava fruits before immersion in 200 g L^{-1} suspensions (Table 2). However, differences in luminosity (F = 1.4729; df = 10, 43; $p = 19.36$), chroma (F = 1.4729; df = 10, 43; $p = 19.36$), and hue angle (F = 1.4729; df = 10, 43; $p = 19.36$) (Table 2) were observed in fruits after immersion. The highest luminosities and lowest chroma of the guava fruits after immersion in the suspensions were observed in the Surround® WP kaolin and kaolin 605 treatments, respectively. However, the lowest luminosities and the highest chroma were observed in fruits treated with distilled water and guar gum, respectively. The major hue angle was observed in fruits treated with kaolin 607 and the smallest in those treated with Surround® WP kaolin, with values of 154.84 ± 1.49 (kaolin 607) and 98.44 ± 4.02 (Surround® WP kaolin).

The luminosities of the fruits immersed in the 200 g L^{-1} suspensions were always greater than those of the fruits immersed in the 100 g L^{-1} suspensions (t = 4.9029; df = 43; $p < 0.0001$), except for chitosan (Tables 1 and 2).

3.2. Oviposition: Non-Choice Tests

The number of eggs deposited by *A. obliqua* females in the pieces of guava immersed in the 100 g L^{-1} (AIC = 120.38; df = 43) and 200 g L^{-1} suspensions (AIC = 112.7; df = 43) varied between treatments in the non-choice test (Table 3). A small number of eggs were deposited by females of *A. obliqua* in the pieces of fruit treated with Surround® WP kaolin and kaolin 608 at 100 g L^{-1} concentration and the highest were in those treated with chitosan at the same concentration.

Table 3. Estimates for GLM parameters with model Gaussian for the number of eggs (mean ± SE) of *A. obliqua* in guavas, subjected to suspensions at 100 and 200 g L^{-1} no-choice tests.

Treatments	Suspension at 100 g L^{-1}					Suspension at 200 g L^{-1}				
	Estimate	Error Standard	Z-Value	*p*-Value	Eggs (N°) [1]	Estimate	Error Standard	Z-Value	*p*-Value	Eggs (N°) [1]
(Intercept)	0.707	0.4177	0.0999	0.0999	-	−4.017	0.000	0.000	1.0000	-
T1-Kaolin Surround® WP	-	-	-	-	0.70 ± 0.42 a	-	-	-	-	0.0 ± 0.38 a
T2- Kaolin 605 white	0.539	0.5907	0.3690	0.3682	1.25 ± 0.42 ab	3.231	0.000	0.597	0.5547	0.32 ± 0.38 a
T3- Kaolin 607 cream	0.323	0.5907	0.5884	0.5884	1.03 ± 0.42 ab	4.228	0.000	0.000	1.0000	0.0 ± 0.38 a
T4- Kaolin 608 white	0.161	0.5907	0.7863	0.7863	0.87 ± 0.42 a	1.436	0.000	0.265	0.7924	0.14 ± 0.38 a
T5- Kaolin 611 grey	0.730	0.5907	0.2249	0.2249	1.44 ±0.42 ab	3.677	0.000	0.000	1.0000	0.0 ± 0.38 a
T6- Talc 657	0.515	0.5907	0.3896	0.3896	1.22 ± 0.42 ab	−6.206	0.000	0.000	1.0000	0.0 ± 0.38 a
T7- Chitosan	2.109	0.5907	0.0011 **	0.0011 **	2.85 ± 0.42 b	5.590	0.000	1.033	0.3092	0.56 ± 0.38 ab
T8- Cassava starch	0.871	0.5907	0.1498	0.1499	1.58 ± 0.42 ab	5.403	0.000	0.998	0.3255	1.17 ± 0.38 ab
T9- Potato starch	1.840	0.5907	0.0038 **	0.0038 **	2.55 ± 0.42 b	2.046	0.000	0.378	0.7078	0.17 ± 0.38 a
T10- Guar gum	0.865	0.5907	0.1524	0.1524	1.57 ± 0.42 ab	1.500	0.000	2.771	0.0091 **	1.50 ± 0.38 b
T11- Distilled Water	1.677	0.5907	0.0077 **	0.0077 **	2.38 ± 0.42 b	2.175	0.000	4.017	0.0003 ***	2.17 ± 0.38 b
AIC					120.38					112.7

** $p \leq 0.01$, *** $p \leq 0.001$; [1] Data transformed in $\sqrt{x+1}$. Mean ± SD values in the same column followed by the same letter do not differ significantly at $p < 0.01$.

However, in the 200 g L^{-1} concentration, a small number of eggs was deposited by *A. obliqua* females into pieces of fruit treated with Surround® WP kaolin; kaolins 605, 607, 608, and 611; potato starch; and talc. The largest was for that treated with distilled water.

3.3. Oviposition: Choice Tests

In the choice bioassays, the number of eggs deposited by *A. obliqua* females in pieces of guava immersed in concentrations of 100 g L^{-1} (F = 6.424; df = 10; $p < 0.0001$) and 200 g L^{-1} (F = 2.006; df = 10; $p = 0.048$) varied between treatments (Figure 1).

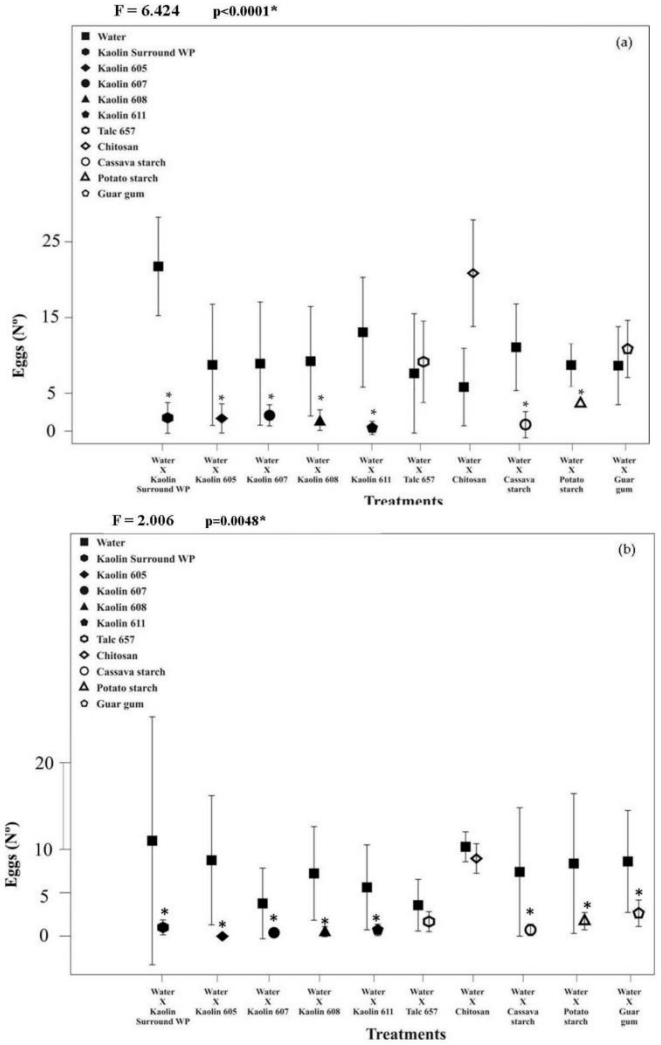

Figure 1. Number (N°) of *A. obliqua* eggs (mean ± standard deviation) in guavas, submitted the suspensions mineral and biomaterials at 100 g L^{-1} (**a**) and 200 g L^{-1} (**b**). Four repetitions per treatment were used.

Except for fruits treated with talc and chitosan at 100 g L^{-1}, guar gum at 5 g L^{-1} (Figure 1a), and those treated with chitosan at 200 g L^{-1} (Figure 1b), a small number of postures of *A. obliqua* occurred in the other treatments with films of mineral particles of kaolin and biomaterials based on potato and cassava starch (Figure 1a). Talc applied at a 200 g L^{-1} concentration decreased the number of eggs deposited by *A. obliqua* females in the guava pieces. However, the observed variations in the standard deviation of the means were consistent with the small numbers of eggs deposited by *A. obliqua* in fruits treated with Surround® WP kaolin, kaolin 611, cassava, and potato starch at 100 g L^{-1} concentration and only those treated with kaolins 605 and 608 at a concentration of 200 g L^{-1} of.

4. Discussion

The similarity in luminosity and hue angle of the peel between the guava fruits used in the bioassays before applying the suspensions of mineral particle films and biomaterials confirmed that they were in a similar stage of maturation, with small variations in chroma (Table 1). These results corroborate those obtained by Azzolini et al. [57], who characterized maturity stage 2. This is important because the insertion of the aculeus of the flies in the fruits depends on several factors, including the type of host (primary or secondary), evidence of previous use by conspecifics (presence of pheromone marking), and quality of the fruit (i.e., degree maturation) [15]. Visual and tactile stimuli influence the recognition and acceptance of fruit as places of oviposition, making it difficult to location of oviposition sites and/ or the fixation of females on coated fruits [41]. In present study, the reduction in the oviposition of *A. obliqua* may not have been caused by the difficulty in locating the fruit due to the color change (visual stimulus) and the change in the texture of the skin due to the presence of the films (tactile stimulus).

The small number of eggs deposited by *A. obliqua* females in the pieces of fruit treated with Surround® WP kaolin and kaolin 608 at a 100 g L^{-1} concentration and in those treated with Surround® WP kaolin; kaolins 605, 607, 608, and 611; and potato starch and talc at 200 g L^{-1} in the non-choice test indicated that the mineral particle films used at the minor concentration were more suitable for protecting guava fruits than those of biomaterials. These results corroborate those of studies on kaolin applications that inhibited the oviposition of *C. capitata* in apples [54] and citrus fruits [31] at a concentration of 30 g L^{-1} in the laboratory and with those conducted in citrus orchards [32,65] and apples [66] sprayed with 50 g L^{-1} Surround® kaolin. However, the increase in the number of treatments with fewer postures of *A. obliqua*, both for mineral particles and for biomaterials in the fruits treated at a concentration of 200 g L^{-1} can be attributed to the uniform coating of the fruits provided by the higher concentration of these products [67].

In the non-choice test, when the treated and untreated fruits were offered simultaneously to laying *A. obliqua* females, an effect of the mineral particles and biomaterial films was observed regardless of concentration (100 g L^{-1} or 200 g L^{-1}). All mineral films and biomaterials based on potato and cassava starch and guar gum reduced *A. obliqua* oviposition. The preference of some tefrithids for certain colors depends on both color tone (chroma) and the intensity of the total reflected light (luminosity) [68]. For example, *A. obliqua* is attracted by wavelengths ranging from 340 nm to 670 nm, with a peak of attraction between 380 and 570 nm, corresponding to the electromagnetic spectrum where ultraviolet and visible light occur [18]. Therefore, the change of the natural green color of the guava fruit peel to the white color of the films of mineral particles or biomaterials probably impaired the perception of the *A. obliqua* females. Studies have shown that fruits or spheres covered with white coating reduce the oviposition of fruit flies [16,18,68]. The white color has a high reflectance and is less visually attractive to fruit flies, as demonstrated for *C. capitata* [68,69], *Bactrocera dorsalis* (Hendel) [70], and *A. obliqua* [18].

In general, it was verified that the 200 g L^{-1} suspension inhibited oviposition in choice and non-choice tests. Inhibition of oviposition of *C. capitata* was also obtained with the use of kaolin (Inducal®) and calcareous liquid, applied at the same concentration, in

apple and mango fruits [71]. However, it was observed that 50% of the particle film-based biomaterials in the choice and non-choice tests did not protect the fruits from oviposition by *A. obliqua*. The exceptions were for potato starch, applied at a concentration of 200 g L^{-1}, which reduced the oviposition of flies in the bioassays of choice and non-choice, and cassava starch in the choice bioassay at the two concentrations tested. Several studies have been conducted with particle films based on edible biomaterials, such as starches, for post-harvest protection of fruits [72–75].

In the present study, potato and cassava starches were demonstrated to be promising for the protection of guava fruits because, in addition to preserving the color of the peel, they protected the fruit pulp from *A. obliqua* oviposition after 48 h of exposure to the insects. However, further studies in the laboratory and field should be conducted because with increased concentrations, the starch base films became brittle, exposing the fruit to flies. This is a common result, particularly in treatments with higher concentrations of this product [74,75].

The chitosan base film did not differ from the control in both bioassays for the number of eggs deposited by *A. obliqua*. This was because the product formed a semitransparent film, which delayed the ripening of the guava fruits and maintained them at the same color as the maturation stage 2 peel, similar to that of the control fruits. The maintenance of peel integrity and delaying the ripening of guava fruits are effects of chitosan, as observed by Hong et al. [76]. When applied to grapes, chitosan did not inhibit *C. capitata* but stimulated oviposition by this fruit fly [54]. Studies conducted after oviposition revealed that chitosan inhibited the development of eggs and larvae of *A. ludens* and *A. obliqua* in mangos [52,77].

Guar gum added to all suspensions of mineral particles and biomaterial films did not affect the oviposition of *A. obliqua*, except in the choice bioassay, when it was used at 10 g L^{-1}. Guar gum acts as a thickener, improving the viscosity and stability of formulations, and is commonly used in chemical and biological insecticide formulations [60,61] and as a diet for the mass production of the fruit flies and parasitoids [78]. In a similar study, guar gum, when used as a thickener in suspensions of mineral films and biomaterials, did not affect the inhibition of oviposition by *C. capitata* [55].

5. Conclusions

The results obtained in the present study are promising and show that mineral films (Surround® kaolin, and kaolins 605, 607, 608, and 611) and biomaterials (cassava and potato starch) changed the color of guavas (luminosity, chroma, and hue angle), inhibiting the oviposition of *A. obliqua*. Therefore, they can be used to protect guava fruits from the damage caused by this pest. Additionally, different species of fruit flies vary their oviposition behavior in fruits treated with the studied particles. New studies should test films of mineral particles and biomaterials in other hosts for females of species of economic importance, since the oviposition behavior of fruit flies is probably regulated by an interaction of factors. Finally, it demonstrates the potential of biomaterials to protect fruits against attack by fruit flies, mainly because they are edible and rapidly degrade.

Author Contributions: Conceptualization, D.R.d.C., M.A.C., C.A.D.d.S. and I.S.J.-B.; methodology, D.R.d.C. and M.A.C.; software, D.R.d.C. and M.P.d.S.; formal analysis, D.R.d.C., M.P.d.S. and M.A.C.; investigation, D.R.d.C. and M.A.C.; resources, D.R.d.C. and M.A.C.; writing—original draft preparation, D.R.d.C. and M.A.C.; writing—review and editing, D.R.d.C., S.A.L., M.P.d.S., B.S.C., A.A.M., C.A.D.d.S., I.S.J.-B. and M.A.C. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Foundation for Research Support of the State of Bahia (FAPESB) and CAPES Foundation (Brazilian Ministry of Education) Finance Code 001.

Institutional Review Board Statement: Not applicable.

Data Availability Statement: Data is contained within the article.

Acknowledgments: We are grateful to the Foundation for Research Support of the State of Bahia (FAPESB) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) (Brazilian Ministry of Education; Finance Code 001) for scholarships and financial support. The companies Brasilminas and NovaSource for donating the kaolin samples.

Conflicts of Interest: The authors declare no conflict of interest.

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