

Settlement rate of *Aphis gossypii* (Hemiptera, Aphididae) and transmission efficiency of *Cucumber mosaic virus* in melons protected with kaolin-particle films

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Abstract

The objective of our study was to evaluate the effect of Surround® (95% kaolin) on the settlement of *Aphis gossypii* Glover on melon plants and on the transmission of a non-persistent virus, *Cucumber mosaic virus* (CMV). The first step was to conduct a transmission experiment using apterae aphids under laboratory conditions. Results of these tests showed that Surround® is unable to avoid the transmission of non-persistent viruses, although a significant reduction in the transmission rate of CMV in the Surround®-treated plants was observed in some cases. Also, the efficacy of Surround® was tested using alatae aphids that were released in large cages under greenhouse conditions. The results obtained indicated that there was a significant difference in one assay at 24 h, and in two assays at 48 h, when the mean number of aphids settled on test plants treated and untreated with Surround® was compared. However, Surround® was able to reduce the transmission rate of CMV by only 10% and there were no statistically significant differences between treated and untreated control plants under our experimental conditions.

Key words: hydrophilic kaolin particle film, control, non-persistent transmission, CMV.

Resumen

Grado de asentamiento de *Aphis gossypii* (Hemiptera, Aphididae) y tasa de transmisión del virus del mosaico del pepino en melón protegido con películas de partículas de caolín

El objetivo de este trabajo fue evaluar el efecto de Surround® (95% caolín) en el asentamiento de *Aphis gossypii* Glover en plantas de melón, y consecuentemente, en la transmisión de virus no persistentes, como es el caso del virus del mosaico del pepino (CMV). Inicialmente, se llevaron a cabo ensayos de transmisión en condiciones de laboratorio con pulgones ápteros. Los resultados indican que el Surround® no evita la transmisión de virus no persistentes, aunque en algún caso se produjo una disminución significativa del porcentaje de transmisión en las plantas tratadas con el producto. Por otro lado, se realizaron ensayos para evaluar la eficacia del producto en condiciones de invernadero con pulgones alados confinados en jaulones. Los resultados obtenidos, al comparar el número medio de pulgones asentados en las plantas tratadas con Surround® frente a las no tratadas, indicaron que hay diferencias significativas en uno de los ensayos a las 24 h, y en dos de los tres ensayos a las 48 h. Sin embargo, en esos mismos ensayos se observó que Surround® redujo la tasa de transmisión de CMV en torno a un 10%, aunque las diferencias obtenidas frente al testigo no fueron estadísticamente significativas.

Palabras clave: película de partículas hidrofílicas de caolín, control, transmisión no persistente, CMV.

Introduction

The study of mineral particles has been taking place over several decades with the aim of identifying those with insecticide properties and to determine their mechanism of action. Kaolin is a white aluminosilica-

te comprised of fine non-abrasive particles which, in modified form, have been used in a number of industrial applications including paints, cosmetics and pharmaceutical products. Recently, USDA-ARS and Engelhard Corporation (Iselin, NJ, USA) collaborated to develop kaolin-based films for use in agriculture (Glenn *et al.*, 1999). With new technologies, kaolin particles have been produced with a specific size and shape and with reflecting properties. Initially, the particles

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were created with hydrophobic properties and were coated with complexes of chromium, stearic acid and organic zirconate. Later, the product was formulated with hydrophilic properties to facilitate its dispersion in water and to make it easier to apply using conventional sprays, also obtaining good results in insect control and diseases affecting pear crops (Knight *et al.*, 2000; Puterka *et al.*, 2000). As well as its efficacy in pest and disease control, it also had a series of agronomical advantages such as increased photosynthetic activity favoring plant growth (Glenn and Puterka, 2000; Tworowski *et al.*, 2002), protection of the fruit from sunburns and heat stress and also a potential to enhance the color (Glenn *et al.*, 2001). It also acts as a fungicide and insecticide against different arthropod species interfering with their feeding, oviposition, selection of plant host, movement and causing dehydration due to adherence of the kaolin particles to its cuticle (Glenn *et al.*, 1999; Knight *et al.*, 2000, 2001; Lapointe, 2000; Puterka *et al.*, 2000, 2003; Unruh *et al.*, 2000; Díaz *et al.*, 2002; Gemei and Tong-Xian, 2002).

Of the 30 virus species that affect Cucurbitaceae worldwide (Lovisolo, 1980) the *Cucumber mosaic virus* (CMV, genus *Cucumovirus*) is the one with the highest incidence in Spanish field crops (Luis-Arteaga *et al.*, 1998; Alonso-Prados *et al.*, 2003). This virus is transmitted in a non-persistent manner by aphids (Pirone and Harris, 1970), of which *Aphis gossypii* is the most effective vector (Labonne *et al.*, 1982). CMV causes appreciable losses in yield if the plants are infected in the early growth stages (Alonso-Prados *et al.*, 1997). The application of conventional insecticides is ineffective in the control of CMV since transmission occurs very quickly in the aphids' surface tests after landing on the plant (Raccah, 1986). It is, therefore, very important to develop preventive methods that eliminate or reduce the landing rate of aphids. The possibility of controlling, with variable efficacy, the incidence of non-persistent virus using chemical methods such as mineral oils, repellants and pyrethroids has been demonstrated (Perring *et al.*, 1999) and also with physical methods such as agro-textile coats (Avilla *et al.*, 1994), barrier crops (Jones, 1993) or surface reflectance which interfere with the localization of the plant by the vector. This is the case of kaolinite or bentonite-based cements supplemented with an adherent compound (Nawrocka *et al.*, 1975; Marco, 1993) or plastic sheets painted different colors (Brown *et al.*, 1993; Farias-Larios and Orozco-Santos, 1997).

The objective of the present work was to assess the degree of efficacy of Surround® as a possible preven-

tive method capable of reducing the landing rate and the degree of settlement of *A. gossypii* and the transmission of CMV to melon plants.

Material and Methods

Surround® WP is a wettable powder based on hydrophilic kaolin particles (95% active ingredient), registered by the company Engelhard Corporation (Iselin, NJ, USA). The product is diluted in water at a dose of 60 g l⁻¹ and shaken vigorously to achieve a homogeneous mixture. It is applied in two applications using a manual spray gun (Hozelock Polyspray 2, model 4075, Haddenham, Buckinghamshire, UK) with a 30 min interval between applications to achieve a better coverage of the leaf surface.

To assess the degree of efficacy against CMV transmission two different experiments were performed: one in laboratory and another in greenhouse conditions. Both experiments used melon (*Cucumis melo* L.) seedlings (cv. Regal) and the M6 isolate of CMV (kindly provided by Dr. E. Moriones, CSIC, Malaga, Spain). Plants were grown in pots using a mixture of equal parts of vermiculite and soil substrate. They were watered three times a week and a nutritional complex 20-20-20 (Nutrichem 60, Miller Chemical, Hannover, PE, USA) was added to the irrigation water in a proportion of 0.25 g l⁻¹ of water. Plants were kept in a test chamber at a temperature of 26:20°C (day:night) and a photoperiod of 16:8 h (light:dark).

Experiments were performed with a clone of *Aphis gossypii* Glover that was obtained from a virginopara collected in 1998 on a melon plant in El Ejido, Almería, Spain. This colony was kept on Regal melon in a test chamber at a temperature of 23:18°C (D:N) and a photoperiod of 16:8 h (L:D).

The source plants for the virus were inoculated mechanically using desiccated tissues approximately one month before the experiment and kept in the same conditions as those described above. Infection by CMV was assessed at 30 days after finishing the transmission assay by DAS-ELISA using commercial antibodies from the laboratory Agdia Inc. (Elkhart, IN, USA).

CMV transmission assays in laboratory conditions

This assay was done by following the transmission protocol described by Fereres *et al.* (1992). The plants

used as indicators of infection (receptor plants) were melon seedlings in the one true leaf stage arranged in 28 alveoli trays. Before starting the experiment, the colony of the *A. gossypii* clone was synchronized to obtain apterae adults of the same age between 7 and 9 days old. These adults were introduced into plastic boxes ($3.5 \times 5.5 \times 1$ cm) with approximately 25 individuals/box to be subjected to a 1 h pre-acquisition starving period. In order to evaluate the degree of efficacy of Surround® in the process of viral acquisition the aphids were then exposed to a 5 min acquisition access period on a melon plant previously infected with CMV (virus source plant) that had been treated with Surround® 1 h before starting the experiment. After the acquisition access period, aphids were placed on the test Regal melon plants (receptors) in groups of 5 aphids/plant for a 2 h inoculation period. The same protocol was followed for the control, but the CMV source plant was used as a virus source before being treated with Surround®. In parallel, test plants (receptors) were treated with Surround® and the same previously described virus source plant was used (treated and untreated) for transmission tests to assess the effect of Surround® on CMV inoculation by *A. gossypii*. Two hours after inoculation, the test plants were treated with Confidor (10 ml of insecticide solution that was added at a dose of 0.5 ml of commercial product l^{-1}). Plants were then placed in a plant growth chamber. Three experiments were carried out on 28 plants for each treatment. Data were analyzed by the Chi-squared test and Fisher's exact test when the expected values were less than 5 (Statview, Abacus Concepts, 1987).

Experiments of aphid settlement and CMV transmission in greenhouse conditions

For this experiment, melon seedlings were used in the one true leaf stage and were transplanted one day before the experiment into 10 cm diameter pots. These seedlings were treated with Surround® as described previously and another 28 seedlings were left untreated as a control. For these transmission experiments under greenhouse conditions an arena test, similar to the one described by Summer *et al.* (1990) was used. The plants were placed inside the cages ($100 \times 100 \times 50$ cm) equipped with antiaphid mesh and arranged in a circle (Fig. 1). A cage was used for each treatment, one with 28 untreated melon plants and one with 28 treated plants. These cages were placed in a greenhouse

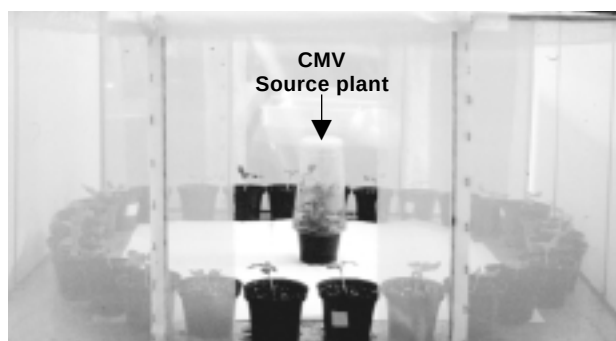


Figure 1. Arena test designed to assess the effects of Surround®, on aphid settlement and virus transmission by *A. gossypii*. The CMV-infected source plant was placed in the middle of the cage ($100 \times 100 \times 50$ cm). Melon test plants surrounded the virus source plant.

adjusted to a temperature of $25 \pm 2^\circ\text{C}$ and a relative humidity of $75 \pm 5\%$. A pepper (*Capsicum annuum* L. cv. Yolo Wonder) plant was placed in the middle of the circle, which had been inoculated 30 days before with CMV and presented clear symptoms. Two hundred alate aphids of between 7 and 9 days old from the *A. gossypii* colony that had been fasting for 1 h were released on the pepper plant. The viral source plant was covered with a methacrylate cylinder adapted to the pot so the aphids would be in contact with the virus source for 10 min. The cylinder was then taken away and the aphids were allowed to disperse freely. The *A. gossypii* clone used in this study did not survive on the pepper plant since it is adapted to melon and the aphids rapidly dispersed throughout the cage. The number of individuals per melon seedling was counted 24 and 48 h after the release. After 48 h the virus source plant was removed from each of the cages and the melon plants were treated with Confidor 20 SL (adding 10 ml/pot of insecticide solution directly to the substrate) to eliminate the risk of secondary dispersion of the virus.

Three experiments were performed (28 plants/treatment) in the same greenhouse conditions as those previously described, the first two at the beginning and middle of June, respectively, and the third at the end of September.

For the statistical analysis of the mean number of aphids settling on the plants tested the Mann-Whitney U test was used (Statview, Siegel, 1956) and Chi-squared to compare the percentage transmission between treated and non-treated plants and the Fisher Exact test when the expected values were less than 5 (Statview, Abacus Concepts, 1987).

Results

CMV transmission experiments in laboratory conditions

The results obtained (Table 1) indicate that Surround® does not prevent non-persistent viral transmission in laboratory conditions. However, in one of the three experiments a significant reduction was observed ($P < 0.05$) in the percentage CMV transmission when the receptor plants were treated with Surround®. In the two other experiments carried out there was also a drop in the CMV transmission rate in plants treated with Surround®, although the differences compared with the control were not statistically significant. Treatment of the receptor plants seemed to be more effective (126/168 plants infected in the whole experiment, *versus* 145/168 in untreated receptor plants) than treatment of the source plants (138/168 infections in treated plants compared to 133/168 in untreated plants) although differences did not reach statistical significance.

Experiments of aphid settlement and CMV transmission in greenhouse conditions

When the mean number of aphids settling on plants treated with Surround® were compared to those not tre-

ated (Table 2) it was observed that at 24 h there were no significant differences in two assays but there were in one assay and at 48 h there were significant differences in one assay and not in the other two. The reduction in the number of aphids settling on the melon plants treated with Surround® was more evident at 48 h than at 24 h. The percentages of CMV transmission in the three assays (Table 3) indicated that Surround® did not significantly reduce ($P > 0.05$) CMV transmission by alate aphids in greenhouse conditions in a confined space such as that used in our experimental design. However, in the three assays carried out there was a lower transmission rate in plants treated with Surround® compared to the control but the difference was not greater than 14% in any case.

Discussion

Transmission experiments by aphids in greenhouse conditions in a confined space have been used over the years to assess propensity of the vector in viral transmission (Irwin and Ruesink, 1986). This type of transmission experiment has been carried out by other researchers both with apterae and alate aphids and in most cases with viruses of the genus *Potyvirus* (Feres *et al.*, 1993; Webb and Kok-Yokomi, 1993; Yuan and Ullman, 1996). In our case, to obtain a high trans-

Table 1. CMV transmission rate (M-6) in melon plants treated with Surround® in laboratory conditions

Treatment	Transmission efficacy (%) ^a			
	Assay 1	Assay 2	Assay 3	Total
Treated source-untreated receptor (TS-UTR)	92.86 (26/28)	89.29 (25/28)	67.86 (19/28)	83.33 (70/84)
Treated source-treated receptor (TS-TR)	82.14 (23/28)	89.29 (25/28)	71.43 (20/28)	80.95 (68/84)
Untreated source-treated receptor (UTS-TR)	78.57 (22/28)	85.71 (24/28)	42.86 (12/28)*	69.05 (58/84)*
<i>Control</i>				
Untreated source-untreated receptor	92.86 (26/28)	100 (28/28)	75 (21/28)	89.29 (75/84)
<i>Assay 1</i>		<i>Assay 2</i>		
(TS-UTR) vs Control	$\chi^2 = 0$; $P > 0.99$	(TS-UTR) vs Control	$\chi^2 = 0.08$; $P = 0.77$	
(TS-TR) vs Control	$\chi^2 = 1.47$; $P = 0.26$	(TS-TR) vs Control	$\chi^2 = 0$; $P > 0.99$	
(UTS-TR) vs Control	$\chi^2 = 2.33$; $P = 0.15$	(UTS-TR) vs Control	$\chi^2 = 4.67$; $P = 0.11$	
<i>Assay 3</i>		<i>Total</i>		
(TS-UTR) vs Control	$\chi^2 = 0.35$; $P = 0.55$	(TS-UTR) vs Control	$\chi^2 = 1.26$; $P = 0.26$	
(TS-TR) vs Control	$\chi^2 = 0.09$; $P = 0.76$	(TS-TR) vs Control	$\chi^2 = 2.30$; $P = 0.13$	
(UTS-TR) vs Control	$\chi^2 = 5.98$; $P = 0.01$	(UTS-TR) vs Control	$\chi^2 = 10.43$; $P = 0.001$	

^a Number of plants infected per total number of plants tested is in parentheses. * Significant differences ($P \leq 0.05$) compared to control according to Chi-squared test and when the expected values were lower than 5 using a Fisher exact test (Statview, Abacus Concepts, 1987).

Table 2. Mean $\pm$ SE of the total number of aphids settled on each melon plant treated with Surround®

Assay	Time of exposure	No. aphids on source plants		Untreated (control)	Treated with Surround®	U	P
		UT	TS	Media $\pm$ SE	Mean $\pm$ SE		
E1	24 h	30	30	1.57 $\pm$ 0.3 a	0.82 $\pm$ 0.2 a	483.0	0.1170
	48 h	5	18	2.68 $\pm$ 0.4 a	1.36 $\pm$ 0.2 b	546.0	0.0101
E2	24 h	20	20	2.0 $\pm$ 0.3 a	2.07 $\pm$ 0.4 a	386.0	0.9202
	48 h	4	14	1.93 $\pm$ 0.3 a	2.04 $\pm$ 0.4 a	405.0	0.8270
E3	24 h	23	41	1.71 $\pm$ 0.4 a	0.5 $\pm$ 0.2 b	527.0	0.0138
	48 h	4	22	2.46 $\pm$ 0.5 a	0.68 $\pm$ 0.2 b	594.5	0.0005

UT: untreated receptor plants (control). TS: receptor plants treated with Surround®. Means $\pm$ SE followed by different letters in rows indicate significant differences ($P \leq 0.05$) according to Mann-Whitney U test (Statview, Siegel, 1956).

mission rate we used a virus of the genus *Cucumovirus*, since *A. gossypii* is cited as the most effective CMV vector (Labonne *et al.*, 1982).

It has been shown that Surround® can interfere with the feeding and settling process of several arthropod pest species of pear, apple and grape crops such as pear psylla, *Cacopsylla pyri* (L.), the acarid *Tetranychus urticae* Koch, the aphid *Aphis spiraecola* Patch (Glenn *et al.*, 1999) and the cercopid *Homalodisca coagulata* (Say) (Puterka *et al.*, 2003). This repellent effect could interfere with the settling and test phase of the aphids in receptor plants treated with Surround®, reducing the intracellular penetrations required for the aphids to inoculate non-persistent viruses such as CMV. This repellent property seems to be confirmed by analyzing the data obtained in the experiment carried out in cages under greenhouse conditions (Table 2). The number of aphids settling in the treated

plants was significantly less than in those not treated with Surround®, in one of the experiments at 24 h and in two of the three experiments at 48 h. These results largely coincide with assays carried out with reflecting plastic sheets (Kennedy *et al.*, 1961; Kring, 1962; Antignus *et al.*, 1996) or with plants bleached either with kaolinite or bentonite (Nawrocka *et al.*, 1975; Marco, 1993) that impedes recognition and settling of the aphid and, therefore, protects the crop from the transmission of non-persistent viruses. We have also observed in our greenhouse experiments that the number of aphids in the virus source plant (pepper infected with CMV), after 48h, was greater in the case of the cage containing receptor plants treated with Surround® than in the control cage with non-treated plants (Table 2). This suggests that the product interferes with processes of localization and settling of *A. gossypii* on treated melon plants, forcing the insect to return to the virus source plant in spite of the fact that in the case of the clone used here this is not a host plant that it usually feeds on. The effect of Surround® has been observed in other insect pests such as *H. coagulata* (Puterka *et al.*, 2003) and *Bemisia argentifolii* Bellows (Gemei and Tong-Xian, 2002).

Although Surround® interferes in the settling of *A. gossypii* on the melon plants studied, our transmission results both in laboratory and in greenhouse conditions indicate that the product does not significantly reduce CMV transmission. This supports the idea that, in non-persistent viruses, colonization is not necessary for effective transmission (Racchah *et al.*, 1985; Perez *et al.*, 1995). However, a tendency is observed towards a lower transmission percentage in plants treated with the product. In our no-choice experimental conditions the aphids cannot choose between treated and non-tre-

Table 3. Percentage of plants infected with CMV when the melon plants were treated with Surround®

Assay	Transmission efficacy (%) ^a		χ^2	P
	Untreated (control)	Treated with Surround®		
E1	28.57 (8/28) a	17.86 (5/28) a	0.900	0.3406
E2	78.57 (22/28) a	64.29 (18/28) a	0.237	0.2348
E3	17.86 (5/28) a	14.29 (4/28) a	0.716	0.7344
Total	41.67 (35/84) a	32.14 (27/84) a	1.64	0.2009

^a Number of plants infected per total number of plants tested is in parentheses. The significant differences ($P \leq 0.05$) were analysed by the Chi-squared test and when the expected values were lower than 5 using a Fisher exact test (Statview, Abacus Concepts, 1987). There were no significant differences between treatments.

ated plants. Our transmission results could have varied if the experiment had been carried out in an open and larger space giving the vector the possibility of choosing between plants treated and those not treated with Surround®.

From the results obtained here we can conclude that a reduced number of aphids settle on plants treated with Surround®. However, to confirm this finding field experiments must be carried out in which the aphids can choose freely between treated and untreated plants. The mechanism of action of Surround® seems to be based on interference in the insect's vision as also occurs with reflecting surfaces (Kennedy *et al.*, 1961). This also seems to impede the movement and settling of the insect on the plant (Glenn *et al.*, 1999, Puterka *et al.*, 2003), and, therefore, reduces the probability that the insects carry out superficial tests on the leaf thus reducing the inoculation potential of non-persistent viruses.

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