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Impact of Kaolin Particle Film and Synthetic Insecticide Applications on Whitefly Populations *Trialeurodes vaporariorum* (Hemiptera: Aleyrodidae) and Physiological Attributes in Bean (*Phaseolus vulgaris*) Crop

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Abstract. **The bean crop is of great importance for human consumption as a source of protein. One of the most limiting insect pests of this crop in Colombia is the whitefly, *Trialeurodes vaporariorum* (Westwood). Currently, various nonchemical pest control alternatives for cleaner production are being sought. This study aimed to determine the influence of kaolin on the development of populations of whitefly in greenhouses, and its effect on the physiological characteristics of the bean crop [*Phaseolus vulgaris* (L.)]. This work was conducted in the greenhouses of the Universidad Nacional de Colombia, in Bogotá. Three experiments were carried out and four treatments were evaluated: 1) control (without any insecticide), 2) synthetic chemical insecticides, and foliar applications of kaolin at 3) 2.5%, and 4) 5% (W/V). Generally, the results showed a high percentage of efficacy (≈91%) on whitefly control in plants treated with 5% kaolin, compared with the plants not treated with insecticides in the three different experiments. In addition, foliar applications of kaolin decreased transpiration by 40% and enhanced by 43% the contents of leaf chlorophyll without affecting bean yield. In conclusion, the use of kaolin particle can be considered as an alternative tool in a program of agricultural management on the bean crop since it can control a high percentage of whitefly and it may help the plant physiology, especially under conditions of abiotic stress such as drought stress.**

The bean (*P. vulgaris*) is considered a principal component of the diet in many countries; it is a source of protein and some essential minerals. It is arguably the most important grain legume for direct human consumption (Islam et al., 2002). In Colombia, bean crops occupied 39,822 ha with an average yield of 2 tons/ha in 2012 (DANE, 2013).

Bean crops can have a high incidence of pests and diseases, causing extensive use of products of chemical synthesis for crop protection to control them. This can lead to an increase in production costs, decreasing the profitability and competitiveness of this crop (Pastor-Corrales and Schwartz, 1994; Singh, 1990). One of the most important pests in this crop is the greenhouse whitefly, *T. vaporariorum* (Westwood) (Hemiptera: Aleyrodidae) that can cause yield losses up to 50% and is one of the most prevalent pests in the Andean region in the bean crop (Manzano and van Lenteren, 2009; Rendón et al., 2001).The damage this pest generates can be caused by both nymphal instars and adults alike, as they feed on the phloem of plants. Furthermore, *T. vaporariorum* can also act as a viral diseases vector (Jones, 2003).

At the moment, crop protection is focused on decreasing the use of conventional pesticides and the development of new strategies that can be included in programs of integrated pest management (IPM). From this viewpoint, studies with inert particles such as kaolin have been developed (aluminosilicate clay) to become an alternative for the control of arthropods (Glenn et al., 1999). It has been found that foliar applications of kaolin have a positive effect on Hemiptera control such as *Agonoscena targionii* (Psyllidae) in pistachio (Saour, 2005), *Diaphorina citri* (Liviidae) in citrus (Hall et al., 2007) and *Cacopsylla pyri* (Psyllidae) in pears (Saour et al., 2010).

In addition, use of kaolin can have positive effects on the plant physiology (Glenn and Puterka, 2002). It has also been stated that leaf temperature and/or fruit decreased (Wünsche et al., 2004) and the chlorophyll content increased with kaolin particle film (Segura-Monroy et al., 2015). Furthermore, kaolin helps to regulate plant water relations and promotes water use efficiency (Glenn et al., 2010; Jifon and Syvertsen, 2003).

Studies in temperate regions have shown that kaolin foliar sprays can exert an insecticidal activity. However, this type of research in tropical areas, specifically in Andean regions is virtually nonexistent. This study may provide alternatives for IPM programs in horticultural crops. The aim was to compare the effect of using kaolin and synthetic chemical insecticides on the population dynamics of whitefly *T. vaporariorum* (Westwood) in beans and the influence of the particle film on leaf transpiration, chlorophyll content, and yield components.

Materials and Methods

Growing conditions

Three experiments were carried out at greenhouses of the Faculty of Agricultural Sciences at Universidad Nacional de Colombia, Bogotá (with geographical coordinates 4°38′17.59′′ N, 74°5′3.65′′ O) with an average altitude of 2640 m above sea level. In general, the three experiments lasted ≈120 d. Cultivar used was ICA-Cerinza, widely planted by bean farmers in the Colombian Andes. The planting pattern was 1 m between rows and 0.33 m between plants by placing one seed per site. Guard rows were established between treatments to avoid drifting. Total planted area was of 120 m^2 and plot size was 15 plants.

Each plant was watered with 750 mL of water per week throughout the crop cycle using a watering can. The irrigation frequency was three times per week. The volume of irrigation in each frequency was 250 mL. The soil characteristics were texture sandy loam soil, pH 5.5, cation exchange capacity = 17.5 meq/100 g, N = 0.45%, Ca = 14.8 meq/100 g, K = 0.79 meq/100 g, Mg = 1.26 meq/100 g, Na = 1.48 meq/100 g, P = 81.3 mg/kg, Cu = 1.40 mg/kg, Fe = 88.5 mg/kg, Mn = 6.69 mg/kg, Zn = 9.93 mg/kg, B = 1.11 mg/kg. In all experiments, plants were fertilized with 25 g of N–P–K compound fertilizer (15–15–15; Precisagro, Colombia) at 21 and 84 d after sowing (DAS). In addition, bean plants were treated with a foliar fertilizer (Wuxal®; Bayer, Colombia) at 1 mL/L (v/v) to supply microelements at 42 and 105 DAS between 800 HR and 1000 HR in all cases. Weed control was manual and disease control was carried out as necessary following recommendations by Pastor-Corrales and Schwartz (1994).

In all experiments, greenhouse was at 20 ± 3 °C, 60% to 90% relative humidity, and a natural photoperiod of 12 h. Experiments were conducted on the following dates: 1) first experiment (E1) between 30 Sept. 2011 and 30 Jan. 2012, 2) second experiment (E2) between 23 Feb. and 28 June 2012, and 3) third experiment (E3) between 24 Aug. and 21 Dec. 2012.

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Whitefly control

The treatments were 1) control (plants without application of any insecticide product), 2) bean plants treated with synthetic chemical insecticides mainly from the groups of neonicotinoids, chitin and adenosine triphosphate synthesis inhibitors, and kaolin foliar sprays (Surround® WP; Tkinet) at 3) 2.5%, and 4) 5% (w/v). The criteria used to perform treatments for the control of *T. vaporariorum* were the use of the action threshold defined for whitefly in green bean crops in Colombia, which is the presence of 12 nymphs per leaf in the lower third of the canopy. A random leaf was assessed per plant and five plants were analyzed per treatment (five leaves were analyzed per experimental plot) (Bueno et al., 2005; Cardona et al., 1993).

Regarding insecticide treatments, the active ingredients used in the three experiments were: imidacloprid (Confidor® 350 SC; Bayer) at 0.10 L a.i. per ha, buprofezin (Oportune® 25 SC; Bayer) at 0.15 L a.i. per ha, and diafenthiuron (Polo® 250 SC; Syngenta, Colombia) at 0.25 L a.i. per ha. Applications of synthetic chemical insecticides were performed as follows: in Expt. 1 (E1), plants were treated with buprofezin at 35 DAS and subsequently with imidacloprid at 77 DAS. In Expt. 2 (E2), foliar insecticide sprays were carried out at 42 and 77 DAS with imidacloprid and diafenthiuron, respectively. In the third experiment (E3), one application was made at 91 DAS with imidacloprid. Kaolin foliar applications were performed on the same dates as the insecticides in the three tests. In general, foliar applications of all products were carried out early in the morning.

Entomological measurements

Population growth of T. vaporariorum. The methodology described by Bueno et al. (2005) was used to assess the population variability of *T. vaporariorum*. Generally, samples were collected between 14 and 112 DAS at intervals of 7 d. In each sample point, five leaves of the lower third of the canopy were observed directly in each treatment using a magnifying glass to count the number of eggs, nymphs, and adults.

The percentage efficacy. The procedure described by Henderson and Tilton (1955) was used to assess the efficacy of the different treatments on the development of populations of *T. vaporariorum.* In general, the percentage efficacy in each treatment was determined by the following formula:

$$\%\,\text{Efficacy} = 100 \times \left(1 - \frac{(T_\text{a} \times C_b)}{(T_\text{b} \times C_a)}\right)$$

where T is the treated population, C is the control population, a is the population after the treatment, and b is the population before the treatment.

Physiological measurements

Leaf transpiration rate. Leaf transpiration was estimated using a steady-state porometer (Model 1600; LI-COR Biosciences, Lincoln, NE) on two fully mature leaves from the upper third of the canopy between 100 and 103 DAS at noon in the third experiment in all treatments.

Leaf chlorophyll content. The determination of this pigment was made only at 103 DAS in the third experiment on the same leaves used to assess leaf transpiration, according to the protocol described by Lichtenthaler (1987). Five grams of green leaves was taken from each treatment, and 80% acetone (v/v) was added. About 24 h elapsed before taking the reading in the spectrophotometer (BioMate TM 3; Thermo Electron Corporation, Waltham, MA, 2004) at a wavelength of 645 and 663 nm using acetone as blank. Chlorophylls were calculated using the following equations:

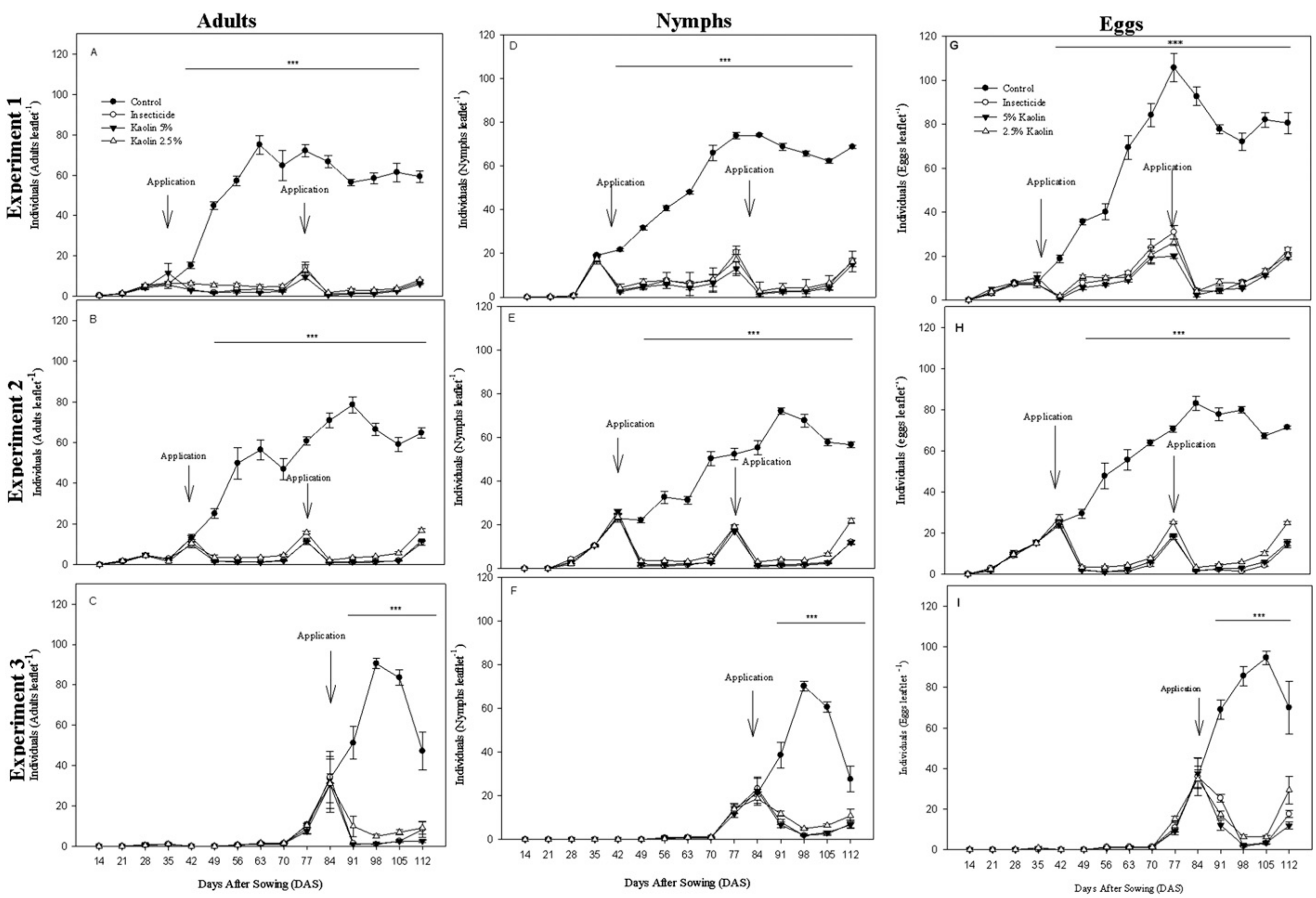

Fig. 1. Summary of population growth of *Trialeurodes vaporariorum* for adults, nymphs, and eggs per leaflet in 'ICA-Cerinza' plants for Expt. 1 (**A**, **D**, and **G**), Expt. 2 (**B**, **E**, and **H**), and Expt. 3 (**C**, **F**, and **I**), respectively. NSNo significance; ***significant difference ($P \leq 0.001$); **significant difference ($P \leq 0.01$); *significant difference ($P \leq 0.05$) compared with the control according to the Tukey test. Values are means of six replications ± standard error.

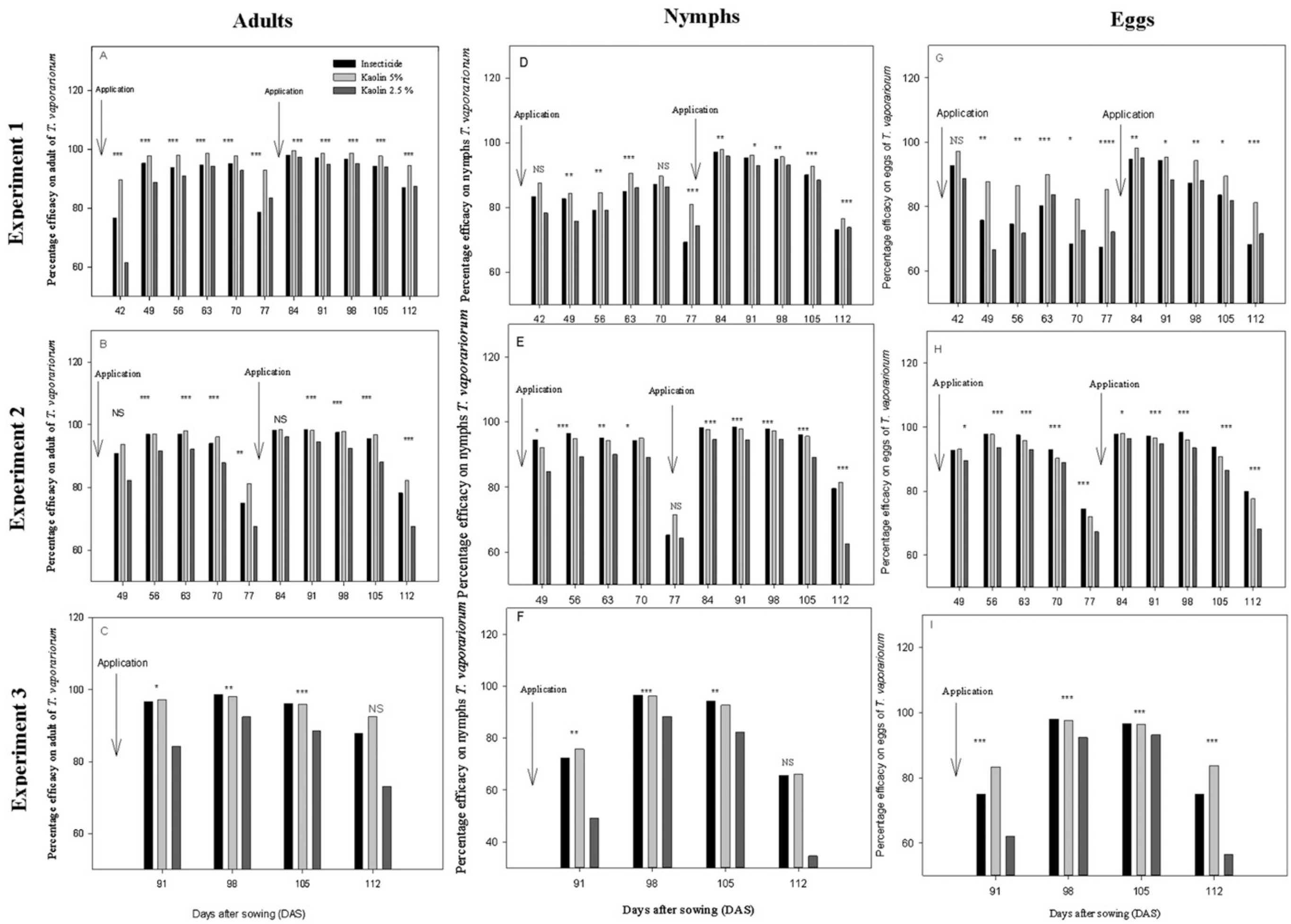

Fig. 2. Summary of percentage of treatment efficacy for adults, nymphs, and eggs per leaflet in 'ICA-Cerinza' plants for Expt. 1 (**A**, **D**, and **G**), Expt. 2 (**B**, **E**, and **H**), and Expt 3 (**C**, **F**, and **I**), respectively. [NS]No significance; ***significant difference ($P \leq 0.001$); **significant difference ($P \leq 0.01$); *significant difference ($P \leq 0.05$) compared with the control according to the Tukey test. Values are means of six replications ± standard error.

Chlorophyll a

$$= \frac{(12.7 \times D663) - (2.69 \times D645) \times V}{1000 \times W}$$

Chlorophyll b

$$= \frac{(22.8 \times D645) - (4.48 \times D663) \times V}{1000 \times W}$$

Total chlorophyll

$$= \frac{(20.2 \times D645) - (8.02 \times D663) \times V}{1000 \times W}$$

where D is the optical density, V is the volume of extract used to determine the optical density, and W is the starting material mass.

Yield components. Number of pods per plant (PN), number of seeds per pod (NSP), and mass of 1000 seeds (SW) (g) were recorded at the end of each experiment (120 DAS). Seed yield was determined by using the equation described by Önder et al. (2013).

$$\frac{\left(\mathrm{PN} \times \mathrm{Number\ plants/m^2}\right) \times (\mathrm{NSP}) \times (\mathrm{SW}\ 1,000)}{100,000}$$

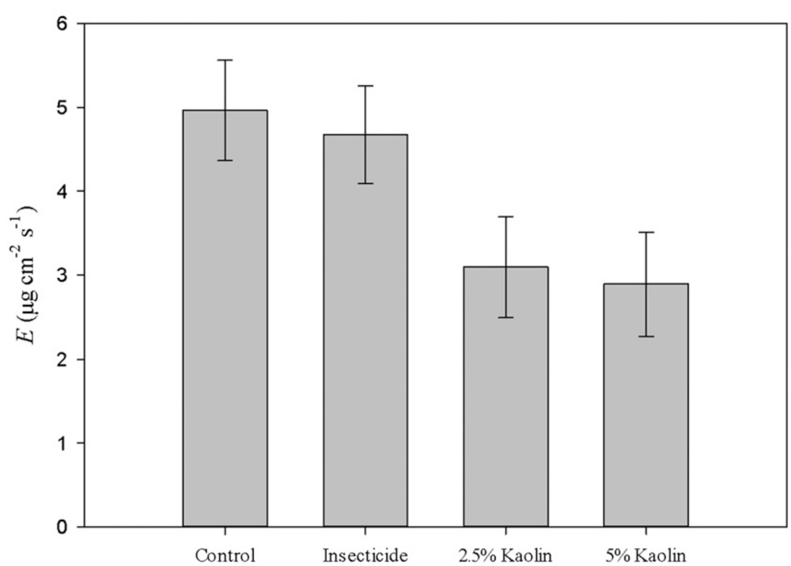

Fig. 3. Effect of foliar sprays treatments (control, chemical insecticide, 2.5% kaolin, and 5% kaolin) on leaf transpiration of 'ICA-Cerinza' beans. Values are means of six replications ± standard error.

Statistical analysis

Data were analyzed using an experimental design of completely randomized blocks, which consisted of six blocks where the different treatments were arranged. Each treatment consisted of 15 plants per experimental plot. To analyze the whitefly population, logarithmic transformation of the data were used because the data did not show a normal distribution. To analyze the percentage of efficacy of each treatment on the population of *T. vaporariorum*, percentage data were transformed using the arcsine formula. Mean comparison tests of Tukey were performed for the variables that showed significant differences in the analysis of variance. Data were analyzed using the Statistix statistical program (Version 8, Tallahassee, FL).

Results

Population variability of T. vaporariorum. Significant differences were observed in the number of eggs, nymphs, and adults of *T. vaporariorum* in bean plants due to the treatments in the three different experiments (Fig. 1). Foliar applications of agrochemicals started to be carried out at 35, 42, and 91 DAS for E1, E2, and E3, respectively at the threshold, when 12 or more nymphs were found per leaf in the lower third of the canopy. From this date, a greater population growth of *T. vaporariorum* (eggs, nymphs, and adults) in control plants (no foliar applications of insecticides) was observed. In general, control plants in E1 (Fig. 1A, D, and G) had an earlier population peak in comparison with E2 (Fig. 1B, E, and H) and E3 (Fig. 1C, F, and I). In addition, E3 showed a trend breakdown whereas E1 and E2 established a plateau. At 112 DAS, bean plants were treated with synthetic chemical insecticides and kaolin in the three different experiments. In general, plants with kaolin and/or insecticides showed on average 70% less eggs, 75% less nymphs, and 80% less adults than control plants. In addition, no differences were observed between bean plants treated with the two doses of kaolin and the ones treated with insecticides along the experiment.

Percentage efficacy on T. vaporariorum. The percentage of efficacy of the treatments (2.5% kaolin, 5% kaolin, and chemical management) was evaluated starting after the first application (at 35, 42, and 84 DAS in Expts. 1, 2, and 3, respectively) until 112 DAS using the methodology of Henderson and Tilton (1955) on whitefly populations in egg, nymph, and adult stages in the bean crop (Fig. 2). In all cases, bean plants treated with 5% kaolin showed a percentage of efficacy of ≈95% on adult population compared with 90% and 85% in plants treated with synthetic chemical insecticides and 2.5% kaolin, respectively (Fig. 2A–C). Foliar applications of 5% kaolin also showed a similar efficacy (≈90%) on populations of whitefly nymphs in comparison with the application of insecticides (≈88%) and 2.5% kaolin (≈83%) (Fig. 2D–F). Similar trends were obtained for the number of eggs (Fig. 2G–I). The efficacy of kaolin sprays at 5% was ≈90%, with synthetic chemical insecticides was ≈85% and for 2.5% kaolin was ≈80%, respectively. Thus, the treatment that exerted greatest control over the various stages of *T. vaporariorum* was 5% kaolin, followed by the chemical insecticides and finally 2.5% kaolin.

Leaf transpiration rate and leaf chlorophyll content. Leaf transpiration decreased around 38% and 42% in plants treated with 2.5% and 5% kaolin, respectively, compared with plants that did not receive kaolin applications (Fig. 3). On the other hand, bean plants treated with 5% kaolin had the highest concentration of leaf chlorophyll (a, b, and total) ($P \leq 0.05$) (Fig. 4). These

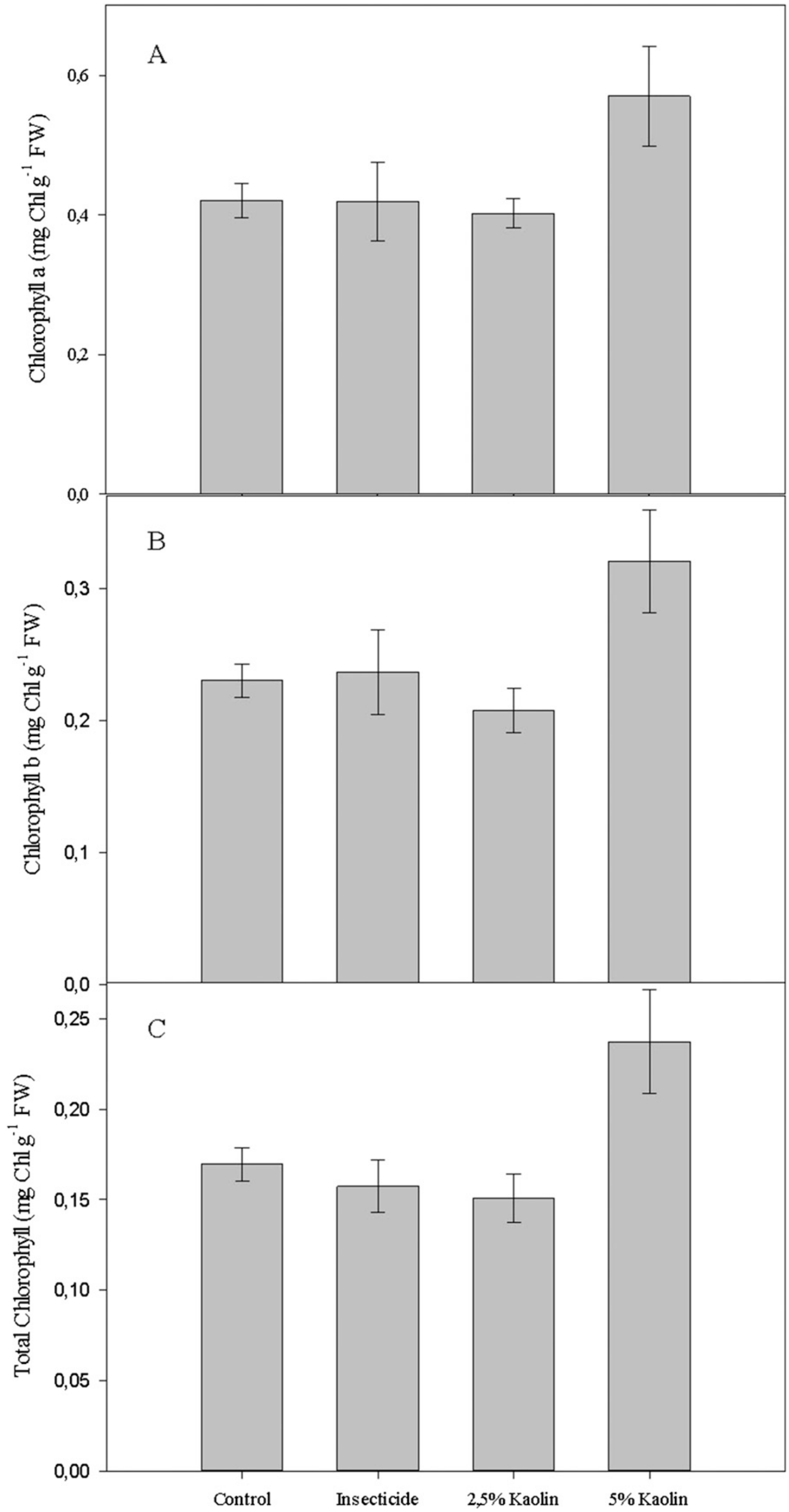

Fig. 4. Effect of foliar sprays treatments (control, chemical insecticide, 2.5% kaolin, and 5% kaolin) on the content of (**A**) chlorophyll a, (**B**) chlorophyll b, and (**C**) total chlorophyll in leaves of bean plants from the ICA-Cerinza cultivar. Each point represents the mean of six values. Values are means of six replications ± standard error.

plants had 43% more total chlorophyll than the control.

Yield components. Number of pods, number of grains, weight of pods with beans, grain weight, number of pods per plant, and number of grains per pod were not different among treatments in the three experiments (Table 1).

Discussion

Foliar applications of kaolin at both doses (2.5% and 5% w/v) controlled ≈80% of the population of *T. vaporariorum* in the different stages (eggs, nymphs, and adults) in all three experiments. Also, the percentage of efficacy of the two doses of kaolin was similar to that obtained in bean plants treated with synthetic chemical insecticides (≈90%).

Previous studies found that foliar kaolin sprays to control *A. targionii* (Hemiptera: Psyllidae) in pistachio, reduced by ≈80% the population of adults and nymphs compared with untreated plants (Saour, 2005). In addition, a reduction on the number of eggs and nymphs of ≈85% and80%, respectively, has been found due to the use of kaolin in citrus for the management of *D. citri* (Hemiptera: Liviidae) (Hall et al., 2007). Use of kaolin also reduced nymphal populations of *C. pyri* (Psyllidae) by 85% in pear trees (Saour et al., 2010). It can be concluded that use of kaolin may be considered as a useful tool in a program of IPM.

Kaolin applications exercise control in different types of arthropods, especially in the nymphal stage, probably because nymphs are vulnerable to desiccation. Kaolin is an inert dust that affects the cuticle by removal or sorption of cuticular waxes, causing a loss of water from the body, and subsequently death through desiccation (Ebeling, 1971; Hall et al., 2007). Kaolin generates a barrier that prevents the insect *Diaprepes abbreviatus* (Coleoptera: Curculionidae) from feeding, causing starvation, and preventing its oviposition in citrus plants (Lapointe, 2000). Also, Larentzaki et al. (2008) stated that an interference with the feeding capacity was the most probable cause of the higher mortality rates observed in kaolin spray treatments to control *Thrips tabaci* (Thysanoptera: Thripidae). On the other hand, kaolin may also interfere with the adhesion of *D. abbreviatus* eggs, which can help to explain the high percentage of effectiveness of Surround WP® applications on the number of eggs of *T. vaporariorum.*

Furthermore, this study also showed the reduced transpiration of kaolin in plants. Plants treated with 5% kaolin had a 40% reduction in their transpiration compared with untreated plants. Similar observations regarding the effect on leaf transpiration have been reported (Glenn et al., 2010; Moftah and Al-Humaid, 2005; Segura-Monroy et al., 2015). Use of kaolin can lower leaf temperature, causing a reduction in the vapor pressure deficit (VPD) between the leaf tissues and the atmosphere (Glenn and Puterka, 2005). A low VPD generates a lower leaf transpiration and a lesser water loss compared with untreated plants. Similarly, treatment of 5% kaolin enhanced the chlorophyll content in bean leaves. Lombardini et al. (2005) also reported increased chlorophyll content in pecan leaves after foliar applications of kaolin. Wünsche et al. (2004) found that leaves and fruit treated with kaolin absorb 20% less light because this particle increases leaf reflectance compared with untreated leaves. Therefore, a lower leaf chlorophyll content observed in plant without foliar kaolin sprays can be because these leaves absorbed more sunlight, causing an increase of the degradation of photosynthetic pigments as a photoprotection mechanism for high light conditions (Segura-Monroy et al., 2015).

To sum up, this study showed that foliar applications of kaolin, especially at 5%, presented 90% efficacy on the control of *T. vaporariorum,* values similar to those observed with synthetic chemical insecticides. Likewise, kaolin at 5% produced positive effects on the bean plant physiology, causing a 40% reduction in transpiration and an increase of 43% in leaf chlorophyll content. In consequence, we can suggest that the use of kaolin can be considered as an alternative to control *T. vaporariorum* without any negative effect on seed yield, despite this experiment was mainly performed under greenhouse conditions. In that sense, other studies carried out with kaolin under laboratory, growth chamber and/or greenhouse conditions have also contributed to design strategies to handle or understand arthropod dynamics in field conditions (Knight et al., 2000; Porcel et al., 2011). In addition, this research also allows us to conclude that kaolin sprays can show a beneficial effect on plant physiological behavior and help to handle environmental stresses.

Table 1. Foliar sprays treatments (control, chemical insecticide, 2.5% kaolin, and 5% kaolin) on yield components in 'ICA-Cerinza' beans.

Treatment	Pods/plant	Number grains/pods	Mass of 1000 grains (g)	Yield (ton/ha)
Expt. 1				
Control	9.68 B[z]	4.51	910.96	1.15
Insecticide	13.43 AB	4.34	970.32	1.65
2.5% Kaolin	15.48 A	3.66	963.56	1.66
5% Kaolin	10.79 AB	4.85	874.46	1.35
Significance	*	NS	NS	NS
Expt. 2				
Control	12.26	3.28	1,400	1.40
Insecticide	16.97	3.20	1,185.10	1.51
2.5% Kaolin	15.09	3.90	1,081	1.77
5% Kaolin	11.82	3.30	1,381.20	1.47
Significance	NS	NS	NS	NS
Expt. 3				
Control	27.27	3.55	839.34	2.39
Insecticide	27.67	3.72	741.54	2.31
2.5% Kaolin	30.43	3.70	940.19	3.11
5% Kaolin	27.60	3.27	786.44	2.07
Significance	NS	NS	NS	NS

[z]Means with different letters represent statistically significant differences according to Tukey's test ($P \leq 0.05$).

*Significantly different at $P = 0.05$ level and [NS]nonsignificant at $\alpha = 0.05$.

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