Kaolin-based Foliar Reflectant Affects Physiology and Incidence of Beet Curly Top Virus but not Yield of Chile Pepper

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Abstract. Kaolin reflectant treatments have been shown to reduce stress due to the environment, pests, and pathogens in many plants. We tested the effect of kaolin on yield, beet curly top virus (BCTV) incidence, and physiological parameters (measured as hyperspectral reflectance) of field-grown chile pepper (*Capsicum annuum* L.) in southern New Mexico. Curly top incidence was significantly lower in kaolin-treated chile blocks than untreated blocks. Peppers treated with the kaolin-reflectant showed significantly less water stress and higher photochemical reflectance than untreated plants during active growth periods. Treated plants had significantly higher levels of chlorophyll a and higher reflectance than untreated plants. Yield from treated plants was not significantly different from that from untreated plants. We did not detect any deleterious effects on peppers due to application of kaolin. Kaolin treatments suppressed beet curly top virus on chile and reduced water stress parameters during the hottest months of the growing season, suggesting that it could be useful in New Mexico chile production in years with moderate disease pressure.

Chile pepper (*Capsicum annuum* L.) production in the southwestern U.S. is limited by problems from the environment, diseases, and pests. Environmental constraints such as water stress, high solar irradiance, and soil moisture fluctuations are common in the arid and semi-arid chile producing areas of the southwestern U.S. Exposure to these various stresses influences physiological processes such as photosynthesis, and transpiration, which in turn affects plant growth and yield.

One significant pest problem for chile peppers in New Mexico is beet curly top virus (BCTV), a curtovirus, which is transmitted by the beet leafhopper *Circulifer tenellus* (Baker). BCTV infects a broad range of hosts that includes other crops and weeds from many plant families (Bennett, 1971). Disease symptoms on chile include severe stunting and chlorosis and death of young plants. BCTV-infected plants produce either no fruit or highly misshapen fruit, resulting in no marketable yield. High temperatures, low humidity, and high light intensity increase the severity and the rate of development of curly top disease in plants (Douglass and Cook, 1952).

Chile is not a primary host for the leafhopper, and the insect does not carry out its life cycle on the plant, but the vector can still efficiently transmit the virus to the plant with feeding

times as short as 15 min (Bennett, 1971). The leafhopper vector prefers arid to semi-arid conditions, bright sunny areas, and stressed plants; conditions that are frequently found in the southwestern U.S. (Douglass and Cook, 1952). Since *C. tenellus* is less likely to enter a shaded closed plant canopy, young plants are more susceptible to curly top than older plants (Romney 1943). Unfortunately, no effective measures are available to control curly top or the leafhopper vector on chile.

Reducing plant stress is important in ensuring optimum crop growth, yield, and quality. Studies conducted on a variety of crops including soybean, dryland cotton, artichoke, melons, and peach have shown that foliar applications of white kaolin mineral reduce leaf and fruit tissue temperature, and plant transpiration and water use, as a result of increased foliage reflection of infrared radiation (Baradas et al., 1976; Basnizki and Evenari, 1975; Glenn et al., 1999, 2002; Jifon and Syvertsen, 2003; Lipton and Matoba, 1971; Moreschet et al., 1979). In addition, leaf coating with white kaolin mineral reduces foliar diseases (Frederiksen, 1986; Glenn et al., 1999; Kamp, 1985; Schon, 1993; Ziv and Han, 1990) and suppresses insect pests including vectors of plant viruses (Cottrell et al., 2002; Liang and Lui, 2002; Marco, 1993; Puterka, et al., 2000; Showler 2002).

The objective of this work was to evaluate the ability of a kaolin-based foliar reflectant to improve yield, reduce plant stress, and reduce beet curly top virus incidence in chile.

Materials and Methods

This study was conducted during 2002 and

2003 at New Mexico State University's Leyendecker Plant Research Station at Las Cruces. Seed of chile pepper (*Capsicum annuum* 'B18') was planted in plots on 106 cm beds in mid-April 2002 and in early April 2003. In 2002, mefenoxam (175.4 mL·ha⁻¹), and carbofuran (2.3 L·ha⁻¹) were applied at planting. Seedlings were thinned to 25.5 to 30.5 cm spacing. No fungicide or insecticides were used in the 2003 trials. During both years, plants were furrow irrigated at 7- to 14-d intervals.

There were two treatments for this work, kaolin-treated and untreated. Beginning at the six- to eight-leaf stage, plants were treated with a 3% kaolin suspension (Surround WP, Engelhard, Iselin, N.J.) until thoroughly wetted using hand-held pump action sprayers that were constantly agitated. Plants were treated from late May through early July for a total of four applications in 2002, and from June through early September for a total of nine applications in 2003. Plants were treated at about 10-d intervals. Kaolin treatments began at the same plant size, but on different dates in 2002 and 2003 due to differences in plant growth rates during the 2 years. Treatments were terminated earlier in 2002 than 2003 because of the frequent rains in July and August 2002, which washed the kaolin from the plants. Treatments were arranged in a randomized complete block design with four replications in 2002 and three replications in 2003. Plots were 100 m in length and 8 rows in width in 2002, and 300 m in length and 24 rows in width in 2003.

During 2003, chile single leaf reflectance over the wavelength range 350 to 2500 nm was measured for treated and untreated plants using a spectroradiometer (FieldSpec Pro; Analytical Spectral Devices ASD, Boulder, Colo.). Individual leaves were scanned on the upper (adaxial) surface using an external integrating sphere (1800-12; LI-COR, Lincoln, Neb.) specifically designed for measurement of leaf surface reflectance and transmittance. The integrating sphere was connected to the spectroradiometer by a fiberoptic cable.

Leaf reflectance was measured from two randomly chosen plants per plots at three height levels of the chile plant (upper, middle, and lower), one leaf per height level. Fifty readings were taken for each single leaf and averaged to reduce noise. Leaves from treated leaves were cleaned and the white particles were removed by gently rubbing with a cotton pad before scanning. Leaf reflectance was measured on two randomly chosen plants from each treated and untreated plots. Hyperspectral measurements (those made from 350 to 2500 nm) were recorded weekly from early July until harvest. The spectra were stored on a hard disk for subsequent viewing and data analysis using ASD ViewSpecPro version 4.2 software.

The collected hyperspectral data was used to calculate several vegetation physiological parameters such as water stress, chlorophyll content, photosynthesis, and nitrogen content. These parameters were used to assess differences in the spectral properties between the treated and untreated chile plants. Moisture stress index (MSI) was calculated as the ratio between reflectance (R) at 1600 nm and reflectance (R)

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Table 1. Effect of kaolin treatments on yield of chile.

Treatment	Avg total yield (kg $\pm$ SE)		Avg marketable yield (kg $\pm$ SE)	
	2002	2003	2002	2003
Kaolin treated	3.94 $\pm$ 0.27	4.15 $\pm$ 0.98	2.10 $\pm$ 0.26	3.28 $\pm$ 0.83
Untreated	3.26 $\pm$ 0.71	3.77 $\pm$ 0.94	2.30 $\pm$ 0.5	3.02 $\pm$ 0.76

Table 2. Effect of kaolin treatments on moisture stress, photochemical reflectance, and *Beet curly top virus* (BCTV) incidence in 2003; MSI = moisture stress index, WBI = water band index, PRI = photochemical reflectance index.

Treatment	MSI $\pm$ SE (1600/820 nm)	WBI $\pm$ SE (900/970 nm)	PRI $\pm$ SE (531–570 nm)/ (531 + 570 nm)	Mean BCTV incidence (no. infected plants $\pm$ SE)
Kaolin treated	0.525 $\pm$ 0.011 a ^c	1.049 $\pm$ 0.002 a	0.14 $\pm$ 0.001 a	143 $\pm$ 23.2 a
Untreated	0.542 $\pm$ 0.014 b	1.045 $\pm$ 0.002 b	0.009 $\pm$ 0.001 b	171 $\pm$ 26.7 b
P value	0.013	0.015	0.003	0.015

^cMean separation within column for MSI and WBI from 3 July through 3 Aug., PRI from 3 July through 3 Sept. Means within columns followed by the same letter are not significantly different.

measured at 820 nm (Hunt and Rock, 1989). Water band index (WBI), which is correlated with the plant water content, was calculated as the ratio between reflectance at 900 nm and reflectance value measured at 970 nm (Gamon and Qiu, 1999; Penuelas et al., 1997a).

Chlorophyll-based difference index or Chlorophyll normalized difference index (Chl NDI) was calculated as $(R_{750} - R_{705}) / (R_{750} + R_{705})$ (Datt, 1998; Gitelson and Merzlyak, 1994; Gitelson et al., 1996). The chlorophyll-a concentration (RARS-a) was calculated using the reflectance ratio (R_{675} / R_{700}) , while the

Chlorophyll-b concentration (RARS-b) was calculated using $[(R_{675} / R_{650}) \times R_{700}]$ (Datt, 1998). The photochemical reflectance index (PRI) was calculated as $(R_{531} - R_{570}) / (R_{531} + R_{570})$ (Gamon et al., 1990; Penuelas et al., 1997b). Nitrogen index was calculated as $(R_{800} - R_{600}) / (R_{800} + R_{600})$ (Ma et al., 1996). The red-edge, which shows the vitality of the plant, was calculated by the ratio of reflectance at 750 and 700 nm, (Gitelson and Merzlyak, 1996).

Chile pods were harvested on 24 Sept. 2002, and 25 Sept. 2003. Within each plot, four sampling areas were arbitrarily selected for assessing

chile yield. Each sampling area consisted of five adjacent plants without curly top symptoms. All pods from the five plants were hand-harvested and weighed, and designated as total yield. The harvested pods were then screened to exclude all nonmarketable pods. Pods were considered nonmarketable if they were noticeably blemished from factors such as sunburn, blossom-end rot, or fungal disease. The remaining pods were weighed, and the resulting weight was designated as marketable yield. The average total yield and average marketable yield were computed from the four sampling areas within a plot.

Curly top incidence was assessed in each plot by counting the number of plants showing virus symptoms out of about 600 plants. Only plants taller than 25 cm were included because we wanted to assess curly top incidence that occurred after the kaolin treatments were initiated. The plants were about 25 cm height at the first kaolin treatment, and since infected plants stop increasing in height, and virus-infected plants <25 cm would have been inoculated before the onset of the kaolin treatments; only those taller than 25 cm would have been inoculated after the onset of treatments. Symptoms used for assessment of virus infection included chlorosis, stunting, and stiff plant architecture. BCTV infection was confirmed in selected samples by PCR analysis (Creamer et al., 2003).

ANOVA procedures for a randomized complete block design were used to test for

Fig. 1. Moisture stress index (MSI) (a), water band index (WBI) (b), photochemical reflectance index (PRI) (c), and chlorophyll a (RARS a) (d) of kaolin-treated and untreated chile in 2003. *Significant differences between treatments $\alpha = 0.05$.

Table 3. Effect of kaolin treatments on chlorophyll and nitrogen stress in 2003; RARS-a = chlorophyll-a, RARS-b = chlorophyll-b, CDBI = chlorophyll based difference index..

Treatment	RARS-a ± SE (675/700 nm)	RARS-b ± SE (675/650 nm) × (700 nm)	CDBI ± SE (750–705 nm)/ (750 + 705 nm)	Nitrogen index ± SE (800–600 nm)/ (800 + 600 nm)
Kaolin treated	0.552 ± 0.010 a ²	0.169 ± 0.011 a	0.485 ± 0.024 a	0.711 ± 0.018 a
Untreated	0.488 ± 0.008b	0.167 ± 0.010 a	0.476 ± 0.022 a	0.719 ± 0.023 a
P value	0.0001			

²Means within columns followed by the same letter are not significantly different.

experimental factors (block and treatment). Treatment differences in yield, curly top virus infection, and physiological variables were gauged at a 5% significance level.

Results and Discussion

In both years of the experiment, kaolin treatments did not visibly harm chile. Average total yield and average marketable yield in kaolin-treated plants were not statistically different ($P > 0.4$) from untreated plants in 2002 and 2003 (Table 1). While yield was not significantly improved in treated plants, yield taken was only from plants without BCTV-infection. Likely total yield from all plants in a treatment would have been significantly better in treated blocks compared to untreated, since curly top infected plants produce no marketable pods, leading to complete yield loss in infected plants. Thus, yield would be higher in treated blocks if all plants were harvested.

In 2003, the average incidence of beet curly top virus in the chile test field was 26%. The average number of beet curly top virus-infected plants per sprayed block was 143, which was significantly different ($P = 0.015$) from the average number of infected plants per untreated block, 171 (Table 2). The trials in 2002 showed no difference in curly top incidence in kaolin-treated chile compared to untreated, and the field incidence of disease was very low ($<0.5\%$).

These results suggest that kaolin treatment can significantly reduce curly top incidence in chile seasons with moderate virus incidence (26% in 2003). This level of virus infection was higher than levels in 2002 (0.5% to 1%), but lower than average field infection levels compared to 2001 (30% to 50%) (Creamer et al., 2003). It is not surprising that the 2002 results showed no difference in curly top incidence in kaolin-treated chile compared to untreated, given the very low incidence of virus in the field.

The spectral analysis showed that the mean water band index (WBI), which is indicative of plant water content, was significantly ($P = 0.015$) higher in treated plants than untreated plants during July and August sampling dates (Table 2, Fig. 1). Similarly, the moisture stress index (MSI) mean values increased during this high temperature period (Table 2, Fig. 1). MSI mean values significantly ($P = 0.013$) differed between treated and untreated plants during July and August. The lowest value for WBI and highest for MIS were recorded on 29 July in the untreated plants. Similar findings were reported by Jifon and Syvertsen (2003) who found that physiological effects of kaolin applications on grapefruit trees were most prominent midday on warm sunny days.

We found significant differences in the photochemical reflectance index (PRI) between the overall means of treated and untreated plants ($P = 0.003$) (Table 2, Fig. 1). Chlorophyll a was also significantly ($P = 0.0001$) higher in treated than untreated plants as measured by the chlorophyll-a index (RARS-a) (Table 3, Fig. 1). Kaolin was also shown to increase the chlorophyll ratio and leaf area for beans (Tworowski et al., 2002).

Indices of chlorophyll-b (RARS-b), total chlorophyll (CDBI), nitrogen index, (Table 3), and red edge (data not shown) were not substantially affected by application of particle film. No significant differences were found in the overall means between treated and untreated chile plants for the four indices at all sampling dates.

Kaolin treatments provided suppression of beet curly top virus on chile and reduced water stress parameters during the hottest months of the growing season. These treatments were not begun until after virus had already entered the field. Early season kaolin treatments would likely decrease virus infection by suppressing the leafhopper vector, but not improve the water status of the plant since the weather would be cooler. Applications of kaolin at the onset of hot temperatures in New Mexico, which usually occurs in late May to early June, might help plants from being subjected to severe water stress for more of the growing season. Kaolin treatments did not reduce water stress in plants at the end of the growing season in September (Fig. 1a). At this point, the plants have already set fruit, which continue to ripen and the temperatures are often cooler than the preceding three months. This suggests that these late applications may not be as important for decreasing plant stress. Additional research should help answer these questions about timing of applications of kaolin.

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