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SUPPRESSION OF WHITEFLIES, *BEMISIA TABACI* (HEMIPTERA: ALEYRODIDAE) AND INCIDENCE OF *CUCURBIT LEAF CRUMPLE VIRUS*, A WHITEFLY-TRANSMITTED VIRUS OF ZUCCHINI SQUASH NEW TO FLORIDA, WITH MULCHES AND IMIDACLOPRID

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ABSTRACT

The sweetpotato whitefly, *Bemisia tabaci B* biotype (Gennadius) (Homoptera: Aleyrodidae) also known as *Bemisia argentifolii* (Bellows and Perring) is a key pest in zucchini squash (*Cucurbita pepo* L.) causing both direct and indirect damage. A field experiment was conducted in the fall of 2005 and repeated in the fall of 2006 to investigate the effect of mulches alone or in combination with a reduced-risk insecticide, imidacloprid (Admire® 2F) on whiteflies and related problems in zucchini squash. UV-reflective mulch and the living mulch, buckwheat (*Fagopyrum esculentum* Moench), with and without imidacloprid, were evaluated in a randomized complete block design with 4 replications. White mulch (standard) was included as a control in the experiment. Mulches in combination with imidacloprid resulted in fewer whiteflies per leaf than those without imidacloprid in 2005 and 2006. More whiteflies were recorded on zucchini plants growing on white synthetic mulch than all other treatments in both years. In the 2006 growing season, these plants also showed the first symptoms of viral infection caused by *Cucurbit leaf crumple virus* (CuLCrV), a whitefly-transmitted geminivirus. Both living and reflective mulches were more effective than white mulch in reducing the densities of whiteflies and the incidence of CuLCrV on zucchini plants.

Key Words: *Bemisia tabaci*, *Cucurbit leaf crumple virus*, mulches, imidacloprid, zucchini squash

RESUMEN

Bemisia tabaci biotipo B (Gennadius) (Homoptera: Aleyrodidae) también conocida como *Bemisia argentifolii* (Bellows and Perring) es una plaga principal del cultivo de calabacín (*Cucurbita pepo* L.) causando daños directos e indirectos. En un experimento llevado a cabo en el otoño del 2005 y repetido en la misma época en el 2006 investigo el efecto de coberturas solas o en combinación con el insecticida de riesgo reducido imidacloprid (Admire® 2F) para el control de mosca blanca y otros problemas relacionados en calabacín. Coberturas UV-reflectivas, y coberturas naturales (*Fagopyrum esculentum* Moench), solas o en combinación con imidacloprid fueron evaluadas usando el diseño de bloques completos al azar con cuatro repeticiones. También una cobertura blanca fue incluida como control del experimento. Coberturas combinadas con imidacloprid en el 2005 y 2006 tuvieron significativamente menos mosca blanca. La cobertura de plástico blanco mostro el mayor número de mosca blanca en ambos años. En la temporada del 2006, las plantas mostraron los primeros síntomas de infecciones virales causadas por el virus del plegamiento de las hojas de calabazas (CuLCrV), el cual es un geminivirus transmitido por la mosca blanca. Coberturas vivas y reflectivas fueron más efectivas que las coberturas blancas en la reducción del número de mosca blanca y la incidencia de CuLCrV en las plantas de calabacín.

Translation provided by the authors.

Sweetpotato whitefly, *Bemisia tabaci B* biotype (Gennadius), also known as *Bemisia argentifolii* (Bellows and Perring), is a major pest of zucchini squash (*Cucurbita pepo* L.) and other cucurbits in Florida. Both the adult and immature stages cause direct damage through sucking plant sap (Brown et al. 1995). Nymphs in particular cause a physiological disorder known as "squash silverleaf" (SSL), which is characterized by silvering of the leaves and blanching of the fruits rendering them unmarketable (Yokomi et al. 1990; Schuster et al. 1991). Whiteflies excrete honeydew, which promotes the growth of sooty mold (*Capnodium* spp.) on leaves and harvestable plant parts, lowering their quality (Basu 1995; Brown et al. 1995). Whiteflies also transmit geminiviruses in many crops, including squash, causing significant yield losses (Polston & Anderson 1997).

Aphids (Homoptera: Aphididae) are also key pests of squash causing direct damage through feeding and transmitting viruses. Important aphid-transmitted viruses affecting squash in

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Florida include *Papaya ringspot virus* type W (PRSV-W), *Watermelon mosaic virus* (WMV), *Zucchini yellow mosaic virus* (ZYMV) and *Cucumber mosaic virus* (CMV) (Frank & Liburd 2005).

In Florida, pesticides have been the primary tactic for managing whiteflies and the viral diseases they transmit in tomatoes (Schuster et al. 2007). Similarly, insecticides play a major role in the pest management of squash (Frank & Liburd 2005). However, this method has not sufficiently controlled the spread of the non-persistently transmitted aphid-borne viruses in cucurbits (Webb et al. 1993) and may not be effective for managing whitefly-transmitted viruses. In addition, heavy reliance on insecticides increases the selection pressure and potential for resistance in whitefly populations (Dittrich et al. 1990).

Several control methods have been reviewed for the control of viral diseases in cucurbits including border crops (Damicone et al. 2007), intercrops (Liburd et al. *in press*), living mulches (Hooks et al. 1998; Frank & Liburd 2005), floating row covers (Webb & Linda 1992), and reflective mulches (Alderz & Everett 1968; Wolfenbarger & Moore 1968; Greenough et al. 1990; Kring & Schuster 1992; Stapleton & Summers 2002; Summers et al. 2004; Frank & Liburd 2005). An integrated approach involving the use of a living or reflective mulch with a reduced-risk insecticide may provide a more sustainable approach for whitefly and disease management (Liburd & Nyoike 2008).

Synthetic UV-reflective mulch has been reported to successfully protect various vegetable crops against insect pests and to reduce the incidence of viral diseases (Csizinszky et al. 1997; Smith et al. 2000; Stapleton & Summers 2002; Reitz et al. 2003; Summers et al. 2004). These mulches protect the crop during the early growing period from insect herbivores and delay the onset of insect-vectored viruses. Alternatively, living mulches have also been shown to reduce the possibility of whiteflies from locating their hosts and subsequently reduce transmission of viruses (Hooks et al. 1998; Frank & Liburd 2005). Living mulches provide food resources (honey and pollen) and shelter for natural enemies that could contribute to the reduction of pest populations (Root 1973). Hilje & Stansly (2008) reported a reduction in the number of adult whiteflies and the incidence of *Tomato yellow mottle virus* (ToYMoV) in tomatoes in Costa Rica when tomatoes were grown with living ground covers. Buckwheat (*Fagopyrum esculentum* Moench) is a living mulch shown to reduce insect pests and the spread of aphid-transmitted viruses in zucchini crops (Hooks et al. 1998; Frank & Liburd 2005).

Imidacloprid (Admire® 2F) [Bayer CropScience US], is a neonicotinoid that is systemic in plants and can be applied as soil drench to manage whiteflies (Palumbo et al. 2001). Neonicotinoid insecticides, act on the insect central nervous system as agonists of the postsynaptic nicotinic acetylcholine receptors (nAChRs) (Denholm & Nauen 2005), causing the nerve to be over stimulated, which leads to death. The U.S Environmental Protection Agency (EPA) has defined pesticides that poses reduce risks to human health, environment, and non-target organisms as "reduced-risk insecticides" (Myers et al. 2006). Imidacloprid has low environmental risk (Palumbo 2004) and can be used in conjunction with mulches to enhance their effects on the target pests.

Our hypothesis was that the addition of imidacloprid would further reduce whitefly numbers in reflective and living mulches and reduce the incidence of virus transmission.

Materials and Methods

Field Plot Preparation and Experimental Design

A field experiment was conducted at the University of Florida Plant Science Research and Education Unit in Citra, Florida in the fall of 2005 and repeated in 2006. In both years field plots measured 10.4 m × 10.4 m and were separated from adjacent plots by 7.6 m of bare soil on all sides. This soil was kept weed-free mechanically throughout the experiments. Each plot consisted of 4 beds. Planting beds were prepared with a 6-foot center wheel spacing tractor (2-wheel drive Model 6615, John Deere, Moline, IL) and were fumigated with methyl bromide 80/20 formulation (80% methyl bromide, 20% chloropicrin) at the rate of 283.5 kg/ha. Fumigation was done as a standard procedure for planting squash to kill soil pathogens, weeds, and nematodes. Two weeks before planting squash, the fumigant was injected into the soil and immediately the planting rows were covered with the respective synthetic mulch treatments. Drip irrigation lines (5/8 inch, 10 mm thickness) were placed in the center of the beds prior to covering with plastic mulch. Living mulch rows were also fumigated and temporarily covered (2 weeks) with synthetic mulch, to allow the methyl bromide to properly fumigate the beds. The plastic mulch was removed before planting buckwheat seeds. Living mulch, buckwheat, was hand seeded between the rows 18 d before planting squash. The same planting procedures and treatments were evaluated in 2005 and 2006 growing seasons.

Treatments were arranged in randomized complete block design with 4 replications. The treatments evaluated were as follows: (1) reflective mulch (Intergro, Lake Alfred, FL) with the reduced-risk insecticide imidacloprid (Admire® 2F, Bayer, Kansas City, MO); (2) reflective mulch without Admire® 2F; (3) living mulch with imidacloprid; (4) living mulch without imidacloprid and (5) standard synthetic white mulch (control). Imidacloprid was applied 2 weeks after squash planting through the drip lines at the rate of 1.684 L/ha.

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Planting holes were cut in the center of each plastic mulch strip and in the center of the non-mulched beds and 2 squash seeds, variety Wild Cat® (Harris Moran, Modesto, CA) were hand seeded per hole.

Agronomic practices followed the standard production guide for squash in North Florida (Olson et al. 2005). However, no insecticides were applied during the growing season except Admire® 2F on the specified treatments. A fungicide, azoxystrobin (Amistar 80 WP), was sprayed as required against powdery mildew in the early stages of the crop. Base dressing fertilizer N-P-K (10-10-10) was applied to the soil at the rate of 560 kg/ha. For the first 4 weeks, the squash plants were top-dressed with nitrogen, potassium, and phosphorous, each at 13 kg/ha weekly until blossom when the rates were increased to 20 kg/ha.

Whitefly Sampling

In-situ counts of adult whiteflies per leaf were obtained by randomly selecting 9 plants, picking 1 leaf per plant located in the outer rows of each plot. Counts were done on the selected leaves by gently turning a leaf and counting the number of adult whiteflies on the leaf. *In-situ* counts began 3 weeks after planting zucchini and continued for 6 weeks during each year of the study. A total of 36 leaves per treatment were examined each week.

Monitoring Virus Incidence

Visual observations of viral symptoms and incidence were recorded and monitored in the field. This was done by walking every row in each plot and recording the number of plants showing virus symptoms. Plants showing viral symptoms (mosaic, mottling, leaf crumpling and distortion) had 1 young leaf excised (third leaf from the tip) and transported to the laboratory in a cooler. Leaves were assayed for the most commonly occurring aphid-transmitted cucurbit viruses including *Papaya ringspot virus* type W, *Watermelon mosaic virus* (WMV), *Zucchini yellow mosaic virus* (ZYMV) and *Cucumber mosaic virus* (CMV) by a double antibody sandwich enzyme-linked immunosorbent assay (DAS-ELISA) with specific polyclonal antisera (provided by D. E. Purcifull (University of Florida). Alkaline phosphatase-antibody conjugates were prepared by the method of Clark & Adams (1977).

In addition, 20 leaf samples were collected from the field and submitted to the Plant Pathology Laboratory, University of Florida, Gainesville, FL for identification of a suspected whitefly-transmitted virus (Akad et al. 2008).

Data Analysis

Whitefly counts from the leaves were analyzed by repeated measures, PROC MIXED, and least significant differences (LSD) procedure was used to test for treatment mean differences at $\alpha = 0.05$ (SAS Institute 2003). Where necessary, the data were log transformed to meet the assumptions for analysis of variance (ANOVA). Virus incidence data were subjected to ANOVA using PROC GLM (SAS Institute 2003) and treatment means separated by LSD test. Finally, a regression analysis PROC REG (SAS Institute 2003) was conducted to correlate whitefly population with virus incidence.

Results

Foliar Counts

In 2005, buckwheat with imidacloprid was equally effective as reflective mulch with imidacloprid in controlling adult whiteflies per leaf ($F = 43.29$; $df = 4, 72$; $P < 0.0001$) (Table 1). Similarly, buckwheat alone was not significantly different from reflective mulch alone. Addition of imidacloprid to buckwheat and reflective mulches resulted in enhanced control of whiteflies per leaf compared with the other treatments. Conversely, plants grown with white mulch (control) had significantly higher number of whiteflies per leaf than all the other treatments. There were significant ($F = 7.86$; $df = 20, 72$; $P < 0.0001$) interaction effects between treatments and sampling weeks. Treatment differences were observed in 5 out of the 6 weeks sampled.

In 2006, fewer adult whiteflies per leaf were recorded compared with 2005, though a similar trend was observed among the treatments (Table 1). Plants growing with living and reflective mulch treatments had significantly fewer adult whiteflies per leaf than those growing on white mulch (control) ($F = 26.67$; $df = 4, 72$; $P < 0.0001$) (Table 1). Plants in the buckwheat with imidacloprid treatment had the fewest whiteflies per leaf, but counts were not significantly different from those within the reflective with imidacloprid treatment. The addition of imidacloprid to the buckwheat treatment resulted in a further reduction of whiteflies per leaf. During the study there were significant ($F = 3.63$; $df = 20, 72$; $P < 0.0001$) interaction effects between treatments and sampling weeks, with significant treatment differences observed in all weeks.

Monitoring Virus Incidence

In Nov 2006, zucchini leaves were observed showing mottling, curling, and crumpling symptoms (Fig. 1). These symptoms, typical of viral infection, were significantly more severe on squash growing on white synthetic mulch (control), where they were first observed, than other treatments evaluated ($F = 9.96$; $df = 4, 15$; $P < 0.0004$) (Fig. 2). The symptoms later spread to other plots. Cumulative disease incidence among the plants

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TABLE 1. MEAN NUMBERS OF ADULT WHITEFLIES PER LEAF ON ZUCCHINI SQUASH PLANT GROWN WITH EITHER LIVING MULCH OR REFLECTIVE MULCH AS COMPARED WITH A STANDARD CONTROL WHITE SYNTHETIC MULCH FOR ALL SAMPLING DATES IN CITRA, FL.

	Mean ± SEM whitefly numbers per leaf	
	2005[a]	2006[b]
Reflective + Imidacloprid	10.38 ± 2.02 c	5.13 ± 0.71 bc
Reflective	15.22 ± 2.22 b	7.75 ± 0.96 b
Buckwheat	17.09 ± 2.32 b	8.27 ± 0.97 b
Buckwheat + Imidacloprid	10.40 ± 1.73 c	3.23 ± 0.42 c
Control	38.27 ± 6.20 a	16.03 ± 2.04 a

Means followed by the same letter are not significantly different ($P = 0.05$ according to least square means test following repeated measures analysis, LS).
[a]$F = 43.29$; $df = 4, 72$; $P < 0.0001$.
[b]$F = 26.67$; $df = 4, 72$; $P < 0.0001$.

growing under buckwheat and reflective mulch treatments was lower than those under the white mulch treatment (Fig. 2). Addition of imidacloprid to reflective and buckwheat treatments resulted in a decrease in the number of plants showing virus symptoms, which was not significantly different from reflective mulch alone.

Virus Identification

ELISA results were negative for all the common aphid-transmitted cucurbit viruses. Field samples submitted to the Plant Pathology tested positive for a whitefly-transmitted geminivirus after PCR analysis. This virus was identified as *Cucurbit leaf crumple virus* (CuLCrV). Whiteflies were able to transmit the virus from infected field samples to healthy plants in the greenhouse (Akad et al. 2008).

DISCUSSION

The use of reflective and living mulches reduced the incidence of CuLCrV, which is persistently transmitted by whiteflies. The lowest number of whiteflies and incidence of virus-infected plants were observed in plots that had been treated with imidacloprid at planting; consequently our hypothesis was supported. This was evident given a strong positive correlation ($r = 0.96$) between the number of whiteflies per leaf and virus incidence. The standard white mulch treatment (control) had the highest incidence of CuLCrV. Plants in the white mulch treatment also had the highest numbers of whiteflies throughout the study. *Cucurbit leaf crumple virus* is transmitted by whiteflies only, and a reduction in whitefly numbers in the 2 treatments (reflective and living mulch) led to a reduction in the number of infected plants. It has been suggested that management of viruses is better achieved with a combination of 2 or more control strategies. Jones (2001) reported some benefits of combining different tactics including cultural, chemical, and biological. These tactics all have different modes of action that act together resulting in greater disease suppression than any method used alone. However, in our study reflective mulch alone gave equal protection to zucchini plants from the virus to that afforded by reflective mulch with imidacloprid. Therefore, no additional benefits were derived from imidacloprid with reflective mulch, which may imply that UV reflective mulch can be used alone.

In related studies reflective mulch alone has successfully delayed and decreased the spread of viruses transmitted by various insect pests (Reitz et al. 2003; Stapleton & Summers 2002; Summers et al. 2004). Alternatively, living mulch has also been reported to reduce whitefly and aphid numbers and the incidence of insect-borne viruses in zucchini squash and tomatoes, respectively (Hooks et al. 1998; Hilje & Stansly 2008). When



Fig. 1. *Cucurbit leaf crumple virus* symptoms recorded on zucchini squash plants in Citra, FL (2006), with severe Silverleaf symptoms in the background. Photo by Teresia Nyoike.

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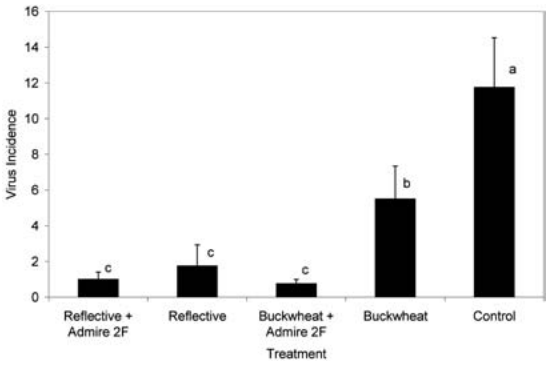


Fig. 2. Effects of mulches on the incidence of *Cucurbit leaf crumple virus* in zucchini squash plants grown with either living mulch or reflective ground covers as compared with white synthetic mulch (control) in Citra, FL, in 2006. Virus incidence = number of plants per plot with virus symptoms. Values with the same letter do not differ ($P < 0.05$) according to LSD test.

considering the number of adult whiteflies per leaf in our study, living mulch alone compared well with reflective mulch alone. The actual modes of action through which living and reflective mulches interfere with the insect's host recognition are different (Hilje & Stansly 2008). Reflective mulch reflects shortwave light, which repels whiteflies, interfering with their orientation (Zitter & Simons 1980; Csizinszky et al. 1997). The effectiveness of UV reflective mulch in reducing the incidence of CuLCrV is attributed to its ability to repel whiteflies, preventing them from alighting on host plants. Alternatively, living mulches are known to reduce the contrast between the ground and the host plants, which prevents the insects from alighting on its host (Cradock et al. 2002).

Cucurbit leaf crumple virus has been reported in Arizona, Texas, California, and Northern Mexico (Wiebe 2003), but had not been found in Florida before fall 2006 when it was found in our field plots and in Hastings, FL in commercial field squash (Akad et al. 2008). The virus is transmitted by whiteflies in a persistent manner and has a host-range limited mostly to the family Cucurbitaceae, infecting most of the domestically grown cucurbits. It also infects snap beans, *Phaseolus vulgaris* L. (Brown et al. 2002). Symptoms on the leaves are very noticeable (Fig. 1). The virus can also affect the fruit of squash (Hagen et al. 2008). *Cucurbit leaf crumple virus* has now been reported in several counties in southwest and west central Florida affecting squash and watermelon (*Citrullus lanatus* L.) (Akad et al. 2008). It is known that the appearance and distribution of new geminivirus diseases is associated with the coming of biotype *B* and its population increase in the state (Polston & Anderson 1997), as well as evolution of variants of the viruses (Varma & Malathi 2003).

In our study, living mulch alone was comparable to reflective mulch alone in suppressing whitefly activity. Our study suggests that the use of living or reflective mulch alone or in combination with imidacloprid can be used to reduce whitefly populations and reduce the incidence of CuLCrV-infected squash plants. There are additional benefits to be derived by combining a living mulches with a reduced-risk insecticide such as Admire® 2F. Whether this reduction can translate to higher yields will be examined in future studies.

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