International Journal of Vegetable Science, 15:3–12, 2009

ISSN: 1931-5260 print / 1931-5279 online
DOI: 10.1080/19315260802446351

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Reflective Plastic Mulch but not a Resistance-Inducing Treatment Reduced *Watermelon Mosaic Virus* Incidence and Yield Losses in Squash

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Plant viruses, especially those transmitted by aphids in a nonpersistent manner, cause significant yield losses in cucurbit crops. Studies have shown that UV-reflective mulches can reduce insects from entering a crop with coincidental reductions in virus incidence. Treatment of plants with various forms of resistance-inducing agents have also shown promise for their ability to reduce plant virus incidence and associated losses. In this article, we describe a two-trial study that evaluated the integration of UV-reflective plastic mulch and a commercially available resistance-inducing treatment, BioYield™, to reduce the incidence and disease-related yield losses in summer squash [*Cucurbita pepo* var. *melopepo* (L.) Alef.] caused by the aphid-borne plant virus, *Watermelon mosaic virus* (WMV). In the spring trial, there was significantly reduced WMV incidence and whole treatment average enzyme-linked immunosorbent assay (ELISA) values among squash plants grown on silver-on-black (UV-reflective) mulch compared with plants grown on nonreflective black mulch. Significantly greater squash fruit yields were obtained for plants grown on the silver-on-black mulch relative to the black mulch treatment. In the fall trial, highly UV-reflective silver mulch was used in addition to silver-on-black and black mulches. WMV incidence and whole treatment average ELISA values were significantly lower for squash plants in the silver mulch treatment compared with silver-on-black and black mulch treatments. The silver-on-black treatment resulted in lower WMV incidence and whole treatment average ELISA

Funding was provided by the USDA Southern Region Integrated Pest Management Program, grant no. 2003-334103-13722.

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values compared with the black mulch treatment. Squash plant yields were significantly greater for plants in the silver-on-black mulch treatment than for those in the silver or black mulch treatments. Treatment of squash plants with BioYield™ did not reduce virus incidence, or whole treatment average ELISA values, nor did it result in higher squash fruit yields compared with the nontreated control in spring or fall trials.

Keywords *Cucurbita pepo* var. *melopepo*, Potyvirus, Aphids, BioYield™.

INTRODUCTION

Aphid-borne plant viruses, transmitted in a nonpersistent manner, are particularly difficult to manage when resistant plant varieties are not available. The acquisition and subsequent inoculation of these viruses by their aphid vector tends to be too rapid for effective management using insecticides (Hull, 2002). *Papaya ringspot virus, Watermelon mosaic virus* (WMV), *Zucchini yellow mosaic virus* (genus *Potyvirus*), and *Cucumber mosaic virus* (CMV; genus *Cucumovirus*) are transmitted by aphids in a nonpersistent manner and are a constant threat to summer squash [*Cucurbita pepo* var. *melopepo* (L.) Alef.] production (Zitter, 1996). Summer squash is often grown in early summer in the southeastern United States in order to avoid abundant sources of virus and large vector populations that may occur at later times in the growing season (Chalfant et al., 1977).

One effective management tool against insect-borne plant viruses involves use of UV-reflective mulches, an approach used to reduce virus incidence in squash crops (Brown et al., 1993; Chalfant et al., 1977; Diaz-Perez et al., 2003; Summers et al., 1995; Wyman et al., 1979). The reflective properties of the mulch, specifically the UV-reflective properties, repel aphids from landing on squash plants (Csizinsky et al., 1999; Summers et al., 1995). Various forms of reflective mulch have been used and include aluminum-based paints applied to standard plastic mulch prepared to provide different degrees of reflectance (Csizinsky et al., 1999; Summers et al., 1995; Wyman et al., 1979). In most cases, use of UV-reflective mulches reduced virus incidence and associated losses in yield.

Plant growth–promoting rhizobacteria (PGPR) were used to induce resistance to infection by CMV and *Tomato mottle virus* (ToMoV; genus *Begomovirus*). A study carried out in the greenhouse involved application of single strains of PGPR to cucumber (*Cucumis sativus* L.) as seed treatments (Raupach et al., 1996). In that study, when CMV was mechanically inoculated onto cotyledons of cucumber seedlings, there was a one-week delay in onset of symptoms in some PGPR-treated plants compared with nontreated CMV-inoculated controls. Those PGPR-treated plants that did not develop symptoms by 7 days postinoculation (dpi) remained symptomless throughout the experiment (study ended at 28 dpi) and contained no detectable amounts of CMV in noninoculated leaves. The induced resistance elicited by PGPR in cucumber plants to CMV was evaluated with tomato (*Lycopersicon esculentum* Mill.) for protection against CMV and ToMoV (Murphy et al., 2000; Zehnder et al., 2000). Varying levels of protection against these viruses was observed suggesting that PGPR may be less effective as a single component in management schemes but rather may work well in an integrated management system. More recent PGPR-based formulations include combinations of bacterial strains with the carrier chitosan.

A commercially available treatment, BioYield™ (Gustafson Inc., Plano, Texas), led to enhanced tomato plant growth and expression of what may be a form of induced mature plant resistance to CMV when tested in greenhouse conditions (Murphy et al., 2003). The efficacy of this material in the field needs to be clarified.

UV-reflective and nonreflective mulches were evaluated with and without treatment of squash plants with the resistance-inducing formulation, BioYield™, for their ability to reduce incidence of watermelon mosaic disease and associated yield losses.

MATERIALS AND METHODS

Two field trials were performed at the E.V. Smith Agricultural Research and Education Center, Shorter, Ala.; one trial was in the spring 2006 (spring trial) and the second was in the fall 2006 (fall trial). In each season, the experimental design was a split-plot with mulch as the whole plot treatment and plant treatment (i.e., BioYield™ and nontreated control) as the subplot treatment. Plant treatments were randomized within each mulch treatment and each plant treatment consisted of a single row of 12 plants replicated four times. Mulch treatments were spaced 15 m apart to limit interplot interference of aphid migration.

For the spring trial, two mulch treatments were used: UV-nonreflective black mulch and UV-reflective silver-on-black mulch. For the fall trial, three mulch treatments were used: UV-nonreflective black mulch, UV-reflective silver-on-black mulch, and a highly UV-reflective silver mulch (all mulches were purchased from Irrigation Mart, Ruskin, La.). In this article, the UV-nonreflective black mulch, UV-reflective silver-on-black mulch, and highly UV-reflective silver mulch will be referred to as black, silver-on-black, and silver mulches, respectively.

BioYield™ consists of endospores (4.0×10^{10} colony forming units; $CFU\cdot L^{-1}$) of plant growth–promoting rhizobacteria strains GB03 and IN937a (*B. subtilis* and *B. amolyliquefaciens*, resepectively) mixed with the carrier chitosan. The BioYield™ was mixed with a soilless growth medium (Speedling Inc., Bushnell, Fla.) at a ratio of 1:40 (v/v), as recommended by the manufacturer, to achieve a bacterial density of 10^9 $CFU\cdot L^{-1}$ in the medium. The medium was placed into Styrofoam trays (Speedling) with 72 cavities per tray.

Squash seed, cv. Dixie, were sown in individual cavities of a Styrofoam tray in a temperature-controlled greenhouse at the Auburn University Plant Science Research Complex, Auburn, Ala. Nontreated control plants were sown and grown in a similar manner but in Styrofoam trays containing unaugmented soilless growth medium. Treatments were in separate Styrofoam trays and care was taken to avoid contamination of soilless growth medium between treatments. At transplanting, 2- to 3-week-old seedlings were transported to the field (15 May for spring trial; 6 Sept. for fall trial) and planted 30 cm apart on raised beds (20 cm high by 84 cm wide). Prior to bed preparation, P_20_5 fertilizer was applied as a broadcast at 2.5 kg per 30.5 m of row. All beds were fumigated with methyl bromide (335 $kg{\cdot}ha^{-1}$ of 67% methyl bromide+33% chloropicrin). A drip irrigation line to deliver water and fertilizer was laid before beds were manually covered with plastic mulch.

Squash seedlings were virus free when transplanted to the field. Plants were started from seed in the greenhouse, a stringently controlled environment regarding insects (vectors for the viruses), and WMV is not seed transmitted in squash. WMV symptoms in squash are easily observed, especially when plants are infected at an early stage of growth. There was no indication of infection prior to or shortly after being transplanted to the field.

Disease evaluations involved testing foliar tissues for infection by WMV. The occurrence of WMV at the E.V. Smith Agricultural Research and Education Center is highly predictable and was used as a natural plant virus system in previous projects (Murphy et al., 2008; Sikora et al., 2006). Two young leaves were collected from each plant approximately 60 days after transplanting. Each sample was wrapped in a dampened paper towel, placed in a zip-type bag and transported to the laboratory for analysis. Virus was detected using a commercial enzyme-linked immunosorbent assay (ELISA) kit (Agdia, Inc., Elkhart, Ind.) specific to WMV and performed according to manufacturer instructions. Leaf samples were processed using a motorized leaf squeezing apparatus with addition of extraction buffer according to manufacturer instructions at a ratio of 1 g tissue to 5 mL of buffer. Leaf extracts were added to microtiter plates at a final dilution of 1:25 (tissue:buffer). After adding substrate, reactions were allowed to develop at room temperature for approximately 60 min and then recorded using a Sunrise microtiter plate reader (Phenix Research Products, Hayward, Calif.). A sample was considered positive for presence of virus if the ELISA absorbance value was greater than the healthy control threshold. The healthy control threshold was determined from the average ELISA absorbance value plus three standard deviations of three negative control samples that were included on each microtiter plate. Two types of ELISA data are presented: (1) whole treatment average ELISA, referred to as ELISA(total), representing the average ELISA absorbance value for all samples within the respective treatment including samples considered positive and negative for

WMV infection, and (2) ELISA(positive), representing the average ELISA values for samples considered positive for WMV infection.

Squash fruit was harvested and marketable number and yield, according to USDA Standards (No. 1 grade), were determined. The data were subjected to analysis of variance (SAS, Cary, N.C.). The use of different treatments in spring and fall trials did not allow analyses to be pooled, and the trials were treated separately.

RESULTS

Spring Trial

Only mulch affected results in the spring trial (Table 1). WMV infection, based on occurrence of symptomatic plants, was noted by 20 days after seedlings were transplanted. Heavy rains occurred during the early part of the season, which kept infection levels relatively low, but WMV incidence increased dramatically by mid-season. The final total incidence of WMV, based on detection of virus by ELISA, was 46.3%.

For the spring trial, there was a significantly lower incidence of WMV among squash plants grown on the silver-on-black mulch treatment than for those grown on black mulch (Table 2).

Average ELISA(total) absorbance values indicated significantly lower amounts of virus among squash plants in the silver-on-black mulch treatment compared with squash plants in the black mulch treatment (Table 2). Since the ELISA(total) values include samples that are positive and negative for

Table 1: ANOVA table for affects of mulch, plant treatment and their interaction on disease incidence, virus accumulation in leaves, and yield characteristics in the spring and fall trials.

Source	Disease incidence	ELISA(total)	ELISA(positive)	Marketable Number	Marketable Yield
Spring trial					
Mulch (M)	**	*	NS	**	**
Plant treatment (T)	NS	NS	NS	NS	NS
Interaction M × T	NS	NS	NS	NS	NS
Fall trial					
Mulch (M)	**	**	NS	**	**
Plant treatment (T)	NS	NS	NS	NS	NS
Interaction M × T	NS	NS	NS	NS	NS

NS, *, **nonsignificant or significant at $P \leq 0.05$ or $P \leq 0.01$, ANOVA.

Table 2: Virus incidence and accumulation in squash plants grown with different mulches and treated or not with Bio Yield™.

	Spring trial		
	Incidence[a]	ELISA(total)[b]	ELISA(positive)[c]
Mulch			
Black	60.0 a[d]	0.374 a	0.571 a
Silver-on-black	34.4 b	0.270 b	0.637 a
Plant treatment			
BioYield™	41.8 a	0.292 a	0.588 a
Nontreated control	51.6 a	0.348 a	0.603 a
	Fall trial		
	Incidence	**ELISA(total)**	**ELISA(positive)**
Mulch			
Black	96.1 a	0.522 a	0.538 a
Silver-on-black	74.6 b	0.475 a	0.600 a
Silver	52.5 c	0.308 b	0.502 a
Plant treatment			
BioYield™	73.9 a	0.439 a	0.559 a
Nontreated control	74.5 a	0.428 a	0.542 a

[a]Incidence represents the percentage of plants in a respective treatment infected with Watermelon mosaic virus (WMV).
[b]ELISA(total) represents the average ELISA absorbance value for all plants in the respective treatment, including samples that are positive and negative for WMV infection.
[c]ELISA(positive) represents the average ELISA absorbance value for those samples considered positive for infection by WMV in the respective treatment. A sample was positive for WMV infection if the ELISA absorbance value was greater than the average plus three standard deviations of the negative control samples.
[d]Means in columns followed by the same lowercase letter are not significantly different ($P < 0.05$). The data were analyzed with analysis of variance (SAS, Cary, N.C.) and for significant ANOVA treatment means separated using LSD mean separation tests.

WMV infection, it is understandable that a lower incidence of virus in a treatment may result in a lower ELISA(total) value. For example, the silver-on-black mulch treatment had lower incidence and average ELISA(total) values compared with those for black mulch treatment. To more directly compare levels of WMV accumulation within infected plants, ELISA(positive) absorbance values were analyzed for those samples shown to be positive for virus infection. In this case, no differences in WMV accumulation were observed among mulch or plant treatments (Table 1).

Only mulch affected marketable numbers of fruit and yield (Table 1). Marketable numbers of fruit and yield were significantly greater for squash plants grown on silver-on-black mulch than for plants grown on black mulch (Table 3).

Fall Trial

Only mulch affected results in the fall trial (Table 1). Symptomatic squash plants were observed by 10 days after being transplanted to the field. The

Table 3: Marketable number and yield of squash fruit from plants grown with different mulches and treated or not with Bio Yield™.

	Spring trial	
	Marketable no.[a]	**Marketable yield (kg)**[a]
Mulch		
Black	8.49 b[b]	0.83 b
Silver-on-black	10.09 a	1.02 a
Plant treatment		
BioYield™	9.21 a	0.92 a
Nontreated control	9.39 a	0.93 a
	Fall trial	
	Marketable no.[a]	**Marketable yield (kg)**[a]
Mulch		
Black	8.85 b[b]	0.68 b
Silver-on-black	12.30 a	1.01 a
Silver	8.74 b	0.75 b
Plant treatment		
BioYield™	10.17 a	0.84 a
Nontreated control	10.05 a	0.82 a

[a]Squash fruit was harvested at fruit maturity and graded as marketable based on standardized categories.
[b]Means in columns followed by the same lowercase letter are not significantly different ($P < 0.05$). The data were analyzed with analysis of variance (SAS, Cary, N.C.) and for significant ANOVA treatment means separated using LSD mean separation tests.

WMV total incidence was 83.8%, which appeared to be higher than that observed in spring trials.

WMV incidence was significantly lower among squash plants grown on silver mulch than for plants grown on silver-on-black and black mulches (Table 2). A lower WMV incidence occurred among squash plants grown on silver-on-black mulch than on black mulch (Table 2).

Average ELISA(total) absorbance values for WMV accumulation were significantly lower among squash plants grown on silver mulch, but no differences were observed between silver-on-black and black mulches (Table 2).

Only mulch affected marketable number and yield of fruit (Table 1). Numbers and yield of marketable squash fruit was significantly greater for plants grown on silver-on-black mulch than on either silver or black mulches (Table 3).

DISCUSSION

Squash plants grown on the silver mulch and silver-on-black mulch had significantly lower WMV incidence and ELISA(total) values than squash plants grown on black mulch. Reductions in WMV incidence associated with watermelon mosaic most likely were due to the reflective properties of the silver and silver-on-black mulches compared with the nonreflective black mulch. The significant reduction in average ELISA(total) values corresponds to the lower incidence of WMV among those treatments; that is, average ELISA(total) values included squash plants that were positive and negative for WMV infection. Treatments with lower virus incidence had more samples that were negative, that is, low ELISA values, for virus. To determine whether WMV accumulation in infected squash plants differed among treatments, we analyzed those plants that had positive ELISA values. No differences in WMV were detected, indicating that, although treatments may have reduced numbers of infected plants, those that were infected accumulated similar amounts of WMV among treatments.

The ability of aluminum or silver-based mulches to reduce virus incidence among squash plants has been demonstrated (Brown et al., 1993; Chalfant et al., 1977; Diaz-Perez et al., 2003; Summers et al., 1995; Wyman et al., 1979). Reflective mulches have been used to reduce the incidence of other virus-vector systems in other crops such as the whitefly-transmitted ToMoV in tomato (Csizinsky et al., 1995, 1999), aphid-borne potyviruses in iris (*Iris brevicaulis* Raf.) and tulip (*Tulip batalinii* L.; Wilson, 1999) and the thrips-transmitted *Tomato spotted wilt virus* (TSWV) in tomato (Diaz-Perez et al., 2003; Momol et al., 2004; Riley and Pappu, 2000).

The reflective properties of various mulches may work on two levels to reduce virus incidence. First, the vector may be repelled due to reflective properties of the mulch, thereby eliminating the introduction of virus into the crop. This is likely to be more effective early in the season when vector populations are relatively low and the mulched plants are small, allowing extensive exposure to the reflected light off the mulch. Second, the introduction of virus into a crop is highly probable during the course of the growing season. If the introduction of virus into the crop is delayed long enough for plants to mature, infection may occur but with little, if any, effect on the plant. Reduced effects of virus infection in maturing plants may be associated with a form of mature plant resistance. Summers et al. (1995) observed the positive effects of delaying infection on squash yield. Diaz-Perez et al. (2003) noted a linear relationship between delay of TSWV infection in tomato and reduced disease-related effects on plant growth and fruit yields.

Despite the significant reduction in WMV incidence among squash plants grown on the highly reflective silver mulch, fruit yields (marketable numbers and weight) for these plants did not differ from plants grown on the nonreflective black mulch. Significantly greater squash fruit yields did occur for plants grown on the silver-on-black mulch (spring and fall trials). A similar observation occurred with TSWV in tomato (Riley and Pappu, 2000). The positive effects on squash fruit yield due to the silver-on-black mulch may be due to effects on air and or soil temperatures. A highly reflective mulch was shown to reduce long-wave radiation, which lowered air temperature around lower, mature leaves (Ham et al., 1991). The silver-on-black mulch may have caused an increase in soil temperature relative to the highly reflective silver mulch (Csizinsky et al., 1995, 1999). These potential above- and below-ground effects on plant growth, along with a reduced incidence of WMV, may have contributed to significantly greater squash fruit yields for plants grown on silver-on-black mulch.

The BioYield™ treatment did not afford any detectable protection of squash plants against infection by WMV. Growth-promotion effects, based on general plant size and color during the course of the season, were not observed and fruit yields did not differ from the nontreated control plants. We hypothesized that the BioYield™ treatment would induce resistance to WMV infection and enhance plant growth, thereby reducing time for plants to achieve a state of maturity and associated resistance or combinations thereof. Previous studies that focused on protection against CMV in cucumber and tomato suggested decent levels of protection, although all involved what might be considered an artificial means of virus infection (i.e., mechanical, rub inoculation; Murphy et al., 2003; Raupach et al., 1996; Zehnder et al., 2000). PGPR-induced protection was observed in tomato against ToMoV with transmission occurring under natural transmission by the whitefly vector (Murphy et al., 2000). In that study, the level of protection varied and it was suggested that occurrence of other viruses, including potyviruses, may have negatively affected protective properties of PGPR treatments. If this was the case, it may be that resistance induced by some PGPR formulations have specificity for viruses targeted for resistance. We have not observed PGPR-induced resistance against potyvirus infection in cucurbits, pepper (*Capsicum annuun* L.), or tomato (J. F. Murphy, unpublished data, 1997–2003), which may provide one explanation for the lack of protection afforded the BioYield™-treated squash plants infected with WMV. The study described in this article provided an opportunity to test the efficacy of BioYield™ to protect squash plants from WMV infection under natural conditions. Previous studies performed at this location sustained high levels of WMV incidence (Murphy et al., 2008; Sikora et al., 2006). Our current results agree with previous observations that BioYield™ does not induce protection against viruses in the genus *Potyvirus*.

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