CALIFORNIA MELON RESEARCH BOARD

2011 RESEARCH SUMMARY / REPORT

The combination of particle film applications and insecticide-based aphid management for mosaic virus incidence and potentially-associated 'brown blotch' symptoms reduction

INVESTIGATORS:

W. Douglas Gubler
Cooperative Extension Specialist
Department of Plant Pathology
University of California, Davis
One Shields Avenue
Davis, CA 95616

Andrew M. Sutherland
Postdoctoral Researcher
Department of Plant Pathology
University of California, Davis
One Shields Avenue
Davis, CA 95616

Bryce W. Falk
Professor
Department of Plant Pathology
University of California, Davis
One Shields Avenue
Davis, CA 95616

COOPERATORS:

Gary Walker; Growers Ag Service
Steve Danna; Danna Farms, Inc.
Bart Fisher; Fisher Ranch, Inc.

Laboratory Technicians: Tera Pitman, Zsofia Kiss

Field Assistants: Anna Erickson, Stacey Haack, Mason Hearn, Cody LaRue

Introduction

Worldwide, field-grown cucurbits are subject to several viral mosaic pathogens, resulting in extensive yield losses. Disease incidence has been documented as approaching 100% in some regions of California. The four most common and important of these viruses, *Cucumber mosaic cucumovirus* (CMV), *Watermelon mosaic potyvirus 2* (WMV2), *Papaya ringspot potyvirus type W* (PRSV-W), and *Zucchini yellows mosaic potyvirus* (ZYMV), are all transmitted in a nonpersistent manner by various aphid species. Transmission requires only seconds of stylet probing by a hungry aphid and can be accomplished by many (if not any) aphid species. These mosaic viruses have a wide host range and are likely to infect and be harbored by many other agricultural crops and weeds simultaneously growing in the agroecosystem. This diverse landscape likewise supports many aphid species, all of which are potential vectors, regardless of whether they establish breeding populations on melons (designated hereafter as colonizing species). In fact, it has been argued that non-colonizing (transient) species are more efficient vectors of nonpersistent viruses since virus retention and transmission ability decrease rapidly once a viruliferous aphid is allowed to feed on an uninfected plant. Pesticides are relatively ineffective at reducing virus incidence because any transient alate aphid can create new infection centers daily while passing through the crop prior to ingesting a lethal insecticide dose. For this reason it is important to decrease aphid landing rates in order to minimize new infection centers and overall virus incidence.

While in flight, aphids respond to visual stimuli from the foliage of potential hosts in order to initiate landing. Interference with the receipt of these appropriate stimuli could reduce the frequency of aphid settling rates, perhaps especially of transient species, and therefore decrease virus transmission. Hindrance of UV vision of insects, whether by absorbance or reflectance, has been associated with lower vector densities and subsequent lower incidence of disease. Reflective mulches and reflective particle film applications have been used to reduce immigration and settling of alate aphids onto various crops in the greenhouse and in commercial fields, sometimes resulting in decreases in virus incidence. These materials reflect short-wave light, including UV, and may interfere with alate aphids' in-flight selection of host plants. Some evidence suggests this interference effect may be different in transient species than in colonizing species, creating the potential to alter the aphid species composition alighting on virus hosts. If, in agricultural pathosystems including nonpersistent viruses with wide host and vector ranges,

applications of reflective particle films alter the aphid species composition, possibly by interfering with visual host cues, then virus incidence may be reduced if transient species will be dissuaded and colonizing species can be managed through integrated pest management including systemic insecticide applications aimed at aphid colonies.

'Brown blotch' is a postharvest defect known from green flesh honeydew varieties characterized by sharply-defined large brown discolored areas on the epidermis of the fruit first evident following cold storage. The defect is restricted to the epidermis and does not extend into the flesh. Melon variety, growing season, and cold storage conditions may all contribute to the prevalence of 'brown blotch'. Some researchers have suggested ultraviolet radiation as the cause of 'brown blotch'. More recently, however, 'brown blotch' has been thought to be associated with mosaic virus infection. To date, no causal organism has been identified for this costly defect, and it is often considered a physiological problem. If 'brown blotch' symptoms are indeed tied to mosaic virus infection, then inoculation of a susceptible variety at various crop growth stages, followed by appropriate cold storage, should yield symptomatic fruit. Furthermore, in that case, virus particles may be successfully isolated from symptomatic fruit.

Objectives

1. Evaluate the ability of the combination of particle film applications and systemic insecticide applications to decrease overall aphid density and to alter the species composition of alate aphids settling on melon plants.
2. Evaluate the ability of this treatment combination to reduce observed virus incidence as compared to no treatment at all.
3. Establish the existence or nonexistence of an association between specific mosaic virus infection and the occurrence of "brown blotch" symptoms.

Materials and Methods

Field sites and treatments

Two commercial honeydew fields in the Sutter Basin, northwest of Robbins, California were selected as research sites to fulfill objectives 1 and 2. Fields were preirrigated via sprinkler or furrow, beds (80" or 72" from center to center) were mechanically shaped, seeds were directly sowed in a single line per bed, and then the crop was subsequently subirrigated or furrow irrigated as needed. Site 1 was planted July 16, 2011, and was adjacent to rice and pumpkin fields as well as several large canals. Site 2 was planted July 18, 2011, and was adjacent to rice and in relatively close proximity to small walnut, pecan, almond, olive, pomegranate and citrus orchards. Site 1 was organized within a split-plot design, where half of the experimental area was regularly treated with systemic insecticides (Admire Pro at seeding, then Venom 10 days later, then two applications of Assail, at three and six weeks after seeding) and then each half divided into six treatment areas of equal size [approximately four acres (1.6 ha) each], representing experimental units. Each of these treatment areas was then assigned to receive one of three reflective particle film treatments (Table 1): no treatment (untreated control: UTC), attempted vector reduction via kaolin suspension applications (four applications of Surround® at 25 lbs. / acre, beginning two weeks after seeding, at an interval of two weeks: Surround), or attempted vector reduction via calcium carbonate applications (four applications of Purshade Ultra® at 0.5 gallons / acre, beginning two weeks after seeding, at an interval of two weeks). Site 2 was divided into three experimental blocks, with each block then divided into six treatment areas of equal size [approximately four acres (1.6 ha) each], representing experimental units. Each of these treatment areas was then assigned to receive one of six reflective particle film / systemic insecticide combination treatments (Table 1): In this manner, fields contained either two or three experimental units per treatment, representing all six reflective particle film / systemic insecticide combination treatments. Surround® (NovaSource, Tessenderlo Kerley, Inc.; Phoenix, Arizona, USA) is a wettable powder based on hydrophilic kaolin particles (95%). Purshade Ultra® (was from Purfresh, Inc.; Fremont, California, USA; now from Tessenderlo Kerley, Inc.; Phoenix, Arizona, USA) is an emulsifiable concentrate composed mainly of dissolved calcium carbonate (62.5%). Both products are widely used as sunburn protectants in many fruit and bulb crops, including commercial melons in California. The first application of materials was made August 3, 2011.

One research field at the Plant Pathology field house location on the UC Davis campus was established in order to address objective 3. The field was preirrigated to field capacity and

then cultivated for weed management. Each of four 200 foot (~ 61 m) long beds (80" from center to center) were mechanically shaped and direct seeded with 'Saturno', a green flesh honeydew variety known to exhibit 'brown blotch' symptoms, at one of four different dates: July 18, July 28, August 8, and August 18, 2011, in order to create four distinct crop ages growing simultaneously. Point-bonded polyester floating row covers (DuPont AG-06) were placed over each row after seeding in order to minimize natural mosaic virus infection. These row covers were removed for two weeks during full bloom (from five weeks after seeding until seven weeks after seeding) to allow for pollination and desirable fruit set levels and then were replaced until harvest. Each row was divided equally, by length, into 15 experimental units, each of which was approximately 12 feet (3.7m) long and contained approximately 12 plants. These units were then randomly assigned to one of three virus inoculation treatments: uninoculated, mechanically inoculated with WMV2, or mechanically inoculated with PRSV-W, so that each row contained five units representing each treatment. All virus inoculation was performed on September 2, 2011, when crop age was 15 days, 25 days, 35 days, or 45 days after seeding, depending on row, by rubbing leaves of every plant in a unit with a piece of cheesecloth periodically dipped in a viruliferous suspension made by adding 24g of symptomatic plant material to one liter of buffer stock solution containing 5g of charcoal dust and 30g of carborundum as abrasive materials.

Aphid traps

At the two commercial field sites, aphid trapping stations were set up, three to each treatment area in a linear array, immediately following the first application of materials. In this way, there were three subsamples per experimental unit, six experimental units per subplot or block, two subplots at site 1 and three blocks at site 2, for a total of 36 trapping stations at site 1 and 54 trapping stations at site 2. Stations consisted of sticky yellow double-sided insect-monitoring cards (10 cm X 16cm; Seabright Laboratories, Emeryville, CA), oriented horizontally and rigidly mounted, approximately 30-60cm from the ground, on one meter lengths of bamboo stake with a wooden clothespin coupled with a 90cm long marking flag. During each sampling period, sticky traps were positioned directly above the melon canopy level. Traps were collected and replaced weekly. Trapping began two weeks after seeding and continued until the week before the first harvest. Collected traps were brought back to the laboratory, and the number of aphids on each side was counted and recorded using a dissecting microscope. A subset of sticky

cards from the center of each unit was used for aphid species identification and species composition descriptions, facilitated by use of several regional keys to alates. Apterous aphids migrating from adjacent plants were disregarded in this study since aphid landing rates and infection center initiations were the primary foci. Harvesting began September 20 at site 1 and September 25 at site 2. In all cases, alate aphids (sensu latu) were identified using key morphological characteristics including siphunculi (cornicles), the cauda, and the unguis of the terminal antennal segment. Aphid species were assigned to one of two categories: colonizing species (commonly found in and complete life cycle on melons), or transient species (occasionally found in melons but either was not observed reproducing on or cannot complete life cycle on melons), based on sampling information and the University of California Integrated Pest Management database website (ucipm.edu). Sampling dates were also expressed as crop age (in weeks after planting), a discrete variable common to all. A mixed model approach was used for data analysis; with site and block as random variables, treatment and crop age as fixed variables, and trap stations as random subsamples nested within blocks. Treatment and crop age effects on overall aphid density, colonizing species density, transient species density, and aphid species composition (as %) were measured via fixed effect F tests. Means comparisons between treatments were through use of Tukey's honestly significant difference (HSD) test on least squared means from the mixed model. All statistical analysis was conducted using JMP Start Statistics software (SAS Institute, Cary, North Carolina, USA).

Virus incidence and identification

At the two commercial field sites, two weeks before harvest, virus incidence was assessed within each experimental unit by a group sampling approach. Field-trained scouts slowly walked the length of the center row in each unit, systematically stopping to collect from ten groups, each comprised of ten adjacent plants, at regular intervals along the row. Each group sample, consisting of ten entire, expanding leaves, was then subjected to laboratory verification and virus identification using reverse transcription-polymerase chain reaction (RT-PCR). Total RNA was extracted using the Qiagen RNeasy Mini Kit. These RNA samples were tested using several different sets of oligonucleotide primers for a selected number of viruses (primer sequences listed in Table 2). The virus screening included CMV, WMV2, ZYMV, and PRSV-W. RT-PCR was performed at 42°C for 60 min (reverse transcription), followed by 35 cycles of

94°C for 30 sec, annealing at 52°C for 45 sec, 72°C for 1 min for 35 cycles; and final extension for 7 min at 72°C. Products were analyzed by agarose gel electrophoresis and GelRed staining. A mixed model approach was used for data analysis; with location (site) and block as random variables, and treatment as a fixed variable. Treatment effect on virus incidence (as proportion of group samples testing positive for mosaic viruses) was measured via a fixed effect F test. Means comparisons between treatments were through use of the HSD test on least squared means from the mixed model. All statistical analysis was conducted using JMP Start Statistics software.

'Brown blotch' symptoms assessment

At row maturity; based on size, whitish (rather than green) ground color, and absence of pubescence; five fruit were harvested from each experimental unit, for a total of 75 fruit per row. Fruit were labeled according to row, unit and plant number and then subjected to cold storage at 43^{o}F (6.1^{o}C) for ten days. After this cold storage period, meant to parallel a typical commercial storage and shipping period, fruit were removed and individually examined for 'brown blotch' symptoms. Symptomatic fruit were photographed, and tissue samples were taken from the parent vine and the fruit itself for virus detection and identification.

Results

Aphid density

According to mixed model analysis, there was a significant treatment effect on overall aphid density recovered ($F_{5, 558} = 2.70$, $P = 0.02$). The only significant treatment difference detected was between plants treated with systemic insecticides only (6.69 ± 0.59 aphids / card / week, lowest overall mean) and plants treated with kaolin suspensions plus systemic insecticides (8.77 ± 0.76 aphids / card / week, highest mean) (Figure 1). Differences in location (site) accounted for about 53% of observed variation in the data, with overall aphid density significantly higher ($F_{1, 578} = 152.1$, $P < 0.0001$) at site 2 (10.1 ± 0.35 aphids / card / week) than at site 1 (3.92 ± 0.28 aphids / card / week). There was also a significant effect of crop age on overall aphid density ($F_{6, 558} = 59.6$, $P < 0.0001$), with aphid density generally increasing as

plants aged, highest seven weeks after seeding, and then declining sharply as fruit matured and vegetative growth decreased (Figure 2).

Aphid species composition

More than ten aphid species were encountered during the seven-week sampling period. Of these, only one was found reproducing in the crop and, together with field observations and information obtained at ucipm.edu, was therefore designated as a colonizing species: *Aphis gossypii*, the melon or cotton aphid. Aphid species composition varied substantially between sites and by date, was considerably different and much more diverse than in 2010, and was characterized by nearly equal proportions of colonizing and transient species. Prevalent transient species included *Aphis craccivora*, the cowpea aphid, *Myzus persicae*, the green peach aphid, and, especially at site 2 (near small pecan orchard), *Melanocallis caryaefoliae*, the black pecan aphid. Other species encountered, in order of prevalence, included *Tinocallis* spp., a pest of oaks, *Therioaphis trifolii*, the spotted alfalfa aphid, *Tetraneura nigiabdominalis*, the grassroot aphid, *Acyrthosiphon pisum*, the pea aphid, and *Sipha glyceriae*, a pest of rice. Many of the species recovered have been recorded to be able to transmit nonpersistent mosaic viruses. There was a significant effect of treatment detected on the aphid species composition trapped on sticky cards ($F_{5, 167} = 3.95$, $P = 0.002$), with a larger proportion of colonizing species and a smaller proportion of transient species trapped above plants treated with calcium carbonate plus systemic insecticides, kaolin plus systemic insecticides, and calcium carbonate only than above plants treated with systemic insecticides only (Figure 3). Overall, the proportion trapped of the colonizing species *A. gossypii* increased significantly with crop age, presumably since this species was increasing in density within the melon fields.

Virus incidence and identification

Out of 300 group samples, WMV2 was recovered 22 times, and CMV was recovered twice. Both ZYMV and PRSV-W were never detected. When considered overall (WMV2 + CMV), mosaic virus infection rates were highest in plants treated with calcium carbonate only (12% of group samples, n = 50) and lowest in plants treated with systemic insecticides only, kaolin only, and kaolin plus systemic insecticides (all three: 6% of group samples, each: n = 50),

but these differences were not statistically significant (Likelihood Ratio Contingency Analysis: $\chi^2 = 2.09$, df = 5, P = 0.84).

'Brown blotch' symptoms assessment

There were no 'brown blotch' symptoms observed in fruit from plants inoculated with either WMV2 or PRSV-W at 45 days after seeding. All other data was lost due to sudden wilt symptoms, followed by collapse and death of all plants, in all three remaining younger rows. These symptoms were thought to be associated with heavy rainfall received at the research site during early October. Introductions of surface-sterilized symptomatic root tissue onto water agar yielded fungal colonies identified as belonging to a *Pythium* spp., a known sudden wilt pathogen in cucurbits.

Discussion

The most noticeable effect of reflective particle film applications was on aphid species composition. When plants were left untreated, there were generally larger proportions of aphid species encountered that we considered to be casually passing through or migrating from other preferred crops during harvests or disturbances (designated as 'transient' species). In this study, aphid traps were positioned directly above treated plants, and so these comparisons address differences in aphid host selection and landing rather than actual feeding through probing, which is necessary for virus transmission. Nevertheless, since all aphids, including transient species, can be considered as potential vectors of nonpersistent viruses, reductions in landing rates due to applications of reflective particle film may in fact reduce virus transmission and incidence. Density of the sole colonizing species observed in this study, *Aphis gossypii*, the melon aphid, however, was not reduced due to reflective particle film applications. In fact, many times, melon aphids represented a significantly higher proportion of total aphids encountered above treated plants. Perhaps this species, as a colonizer, was able to utilize host cues other than those (mainly visual cues) affected by reflective applications and therefore made no distinctions. By analogy, this suggests that melon aphids are able to locate melons, a preferred host, through means other than visual cues, perhaps olfactory cues, and so were less affected by interference with visual cues. Pest species, such as melon aphid in commercial melon fields, tend to increase locally, so

that adjacent plants are utilized as populations increase, resulting in a continuous pest patch and, possibly, a large continuous virus infection center. In theory, such populations in a field can be successfully managed with insecticides. Transient species, however, can contribute to many small, discrete infection centers as they settle and probe plants during migrations through an area. Additionally, the transient nature of these aphids does not lend itself to management via insecticide applications. Therefore, in response to reflective applications, there may be a valuable decrease in new virus infection centers due to the interference with the hosts' visual cues and, subsequently, a decreased landing rate by transient species.

Regular applications of systemic insecticides (without reflective particle films) resulted in the lowest overall mean aphid density in this study. This effect was not statistically significant, however, since three of the treatments containing reflective films (calcium carbonate only, kaolin only, and calcium carbonate plus systemic insecticides) were statistically similar in terms of overall aphid density (see Figure 1). It should be noted, however, that plants treated with systemic insecticides only also harbored the highest proportion of migrant aphid species (0.67 ± 0.056). Presumably, regular insecticide applications reduced or eradicated populations of colonizing species while failing to prevent the landing of transient species.

There were no differences between treatments in terms of virus incidence as determined via PCR identification. In 2010, this was also the case, although overall incidence was much lower (0.06 – 0.11%). Also in 2010, PRSV-W was the most prevalent virus encountered, found in 66% of submitted symptomatic samples. No PRSV-W was detected in 2011. This failure to identify PRSV-W within group samples may be due to the use of inappropriate primers. No internal positive control was successfully used during PRSV-W detection due to difficulty in finding an appropriate primer sequence to correspond to sample material. For this reason, it is prudent to disregard 2011 information regarding PRSV-W detection. Some levels of PRSV-W were expected due to prevalence last year. Samples have been retained, and efforts are underway to find a more appropriate method of detection.

Although there may be differences in aphid species composition and perhaps also in subsequent virus incidence in plants treated with reflective particle films, this may not translate into an economic benefit to the commercial melon grower unless the cost of these applications is shown to offset the losses due to viruses that would occur in their absence. The virus incidence

was low in this study (0.6% minimum - 12% maximum, based on group sampling approximations). It would be valuable to conduct similar studies in regions where incidence and potential loss are higher.

Table 1. Treatment materials and application information for an investigation into the effect of the combination of reflective particle film applications and systemic insecticide applications on aphid density, aphid species composition and the resulting incidence of nonpersistently-transmitted mosaic viruses.

Treatment	**Product**	**Rate**	**# Applications (Crop Growth Stage During Applications)**
untreated	n / a	n / a	n / a
kaolin	Surround®: 95% kaolin as wettable powder	25 lbs. / acre	4 (two weeks after planting and every two weeks thereafter)
calcium carbonate	Purshade Ultra®: 62.5% calcium carbonate as emulsifiable concentrate	0.5 gal. / acre	4 (two weeks after planting and every two weeks thereafter)
systemic insecticides	Admire Pro: 42.8% imidacloprid, then Venom (site 1 only): 70% dinotefuran, then Assail: 70% acetamiprid	10.5 oz. / acre, then 6 lb. a.i. / acre (site 1 only), then 5.3 oz. / acre	1 (at seeding), then 1 (10 d after seeding, site 1 only), then 2 (three and six weeks after seeding)
kaolin **plus** systemic insecticides	combination of two treatments (kaolin and systemic insecticides) listed above	combination (as above)	combination (as above)
calcium carbonate **plus** systemic inseticides	combination of two treatments (calcium carbonate and systemic insecticides) listed above	combination (as above)	combination (as above)

Table 2. List of primers used to identify specific cucurbit mosaic viruses (see text for key to acronyms) by reverse-transcription polymerase chain reaction from melon shoot tissue.

Virus	**Sequence**	**Product size**
CMV	Fwd 5'-CATGGCTTTCCAAGGTACCAG-3' Rev 5'-CTAAAGACCGTTAACCACCTGC-3'	850 bp
ZYMV	Fwd 5'-GCCGGATCAAATTGAGTTAT-3' Rev 5'-TAACATCACGTGCAGTGTG-3'	541 bp
WMV2	Fwd 5'-AGCTTAGACCATTTGCTTGAG-3' Rev 5'-CCGAAATGCTAACTGTGACC-3'	645 bp
PRSV-W	Fwd 5'-TCGTGCCACTCAATCACAAT-3' Rev 5'-CACGAGCCCTATCAGGTGTT-3'	432 bp

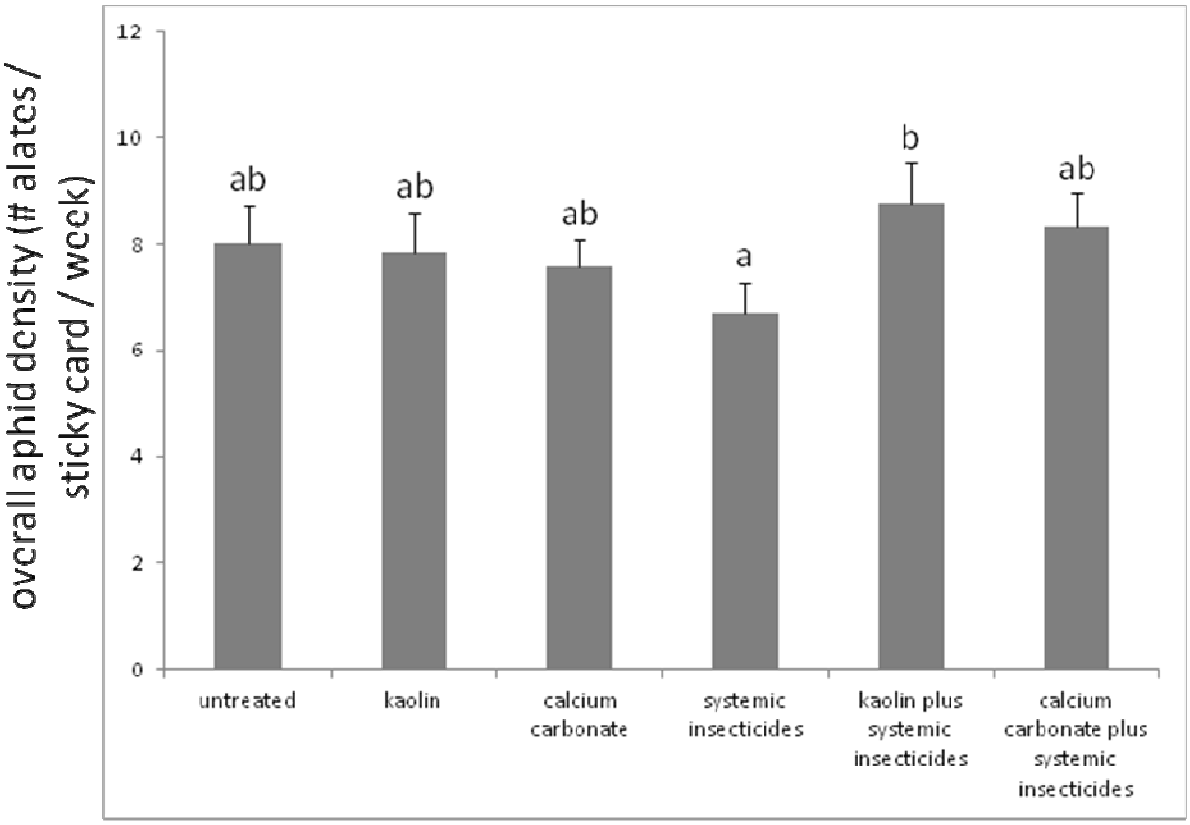

Figure 1. Mean aphid density (as number of alate individuals recovered weekly on both sides of a yellow sticky card directly above developing plants) over seven weeks of sampling within two commercial melon fields, as affected by reflective particle film applications (kaolin or calcium carbonate suspension), systemic insecticide applications (imidacloprid, dinotefuran and acetamiprid), and the combination of reflective particle film applications and systemic insecticide applications. Treatments accompanied by the same letter are not significantly different according to Tukey's Honestly Significant Difference test on least squared means from mixed model output where site and block were random variables, treatment and crop age were fixed variables, and trap stations were random subsamples nested within blocks.

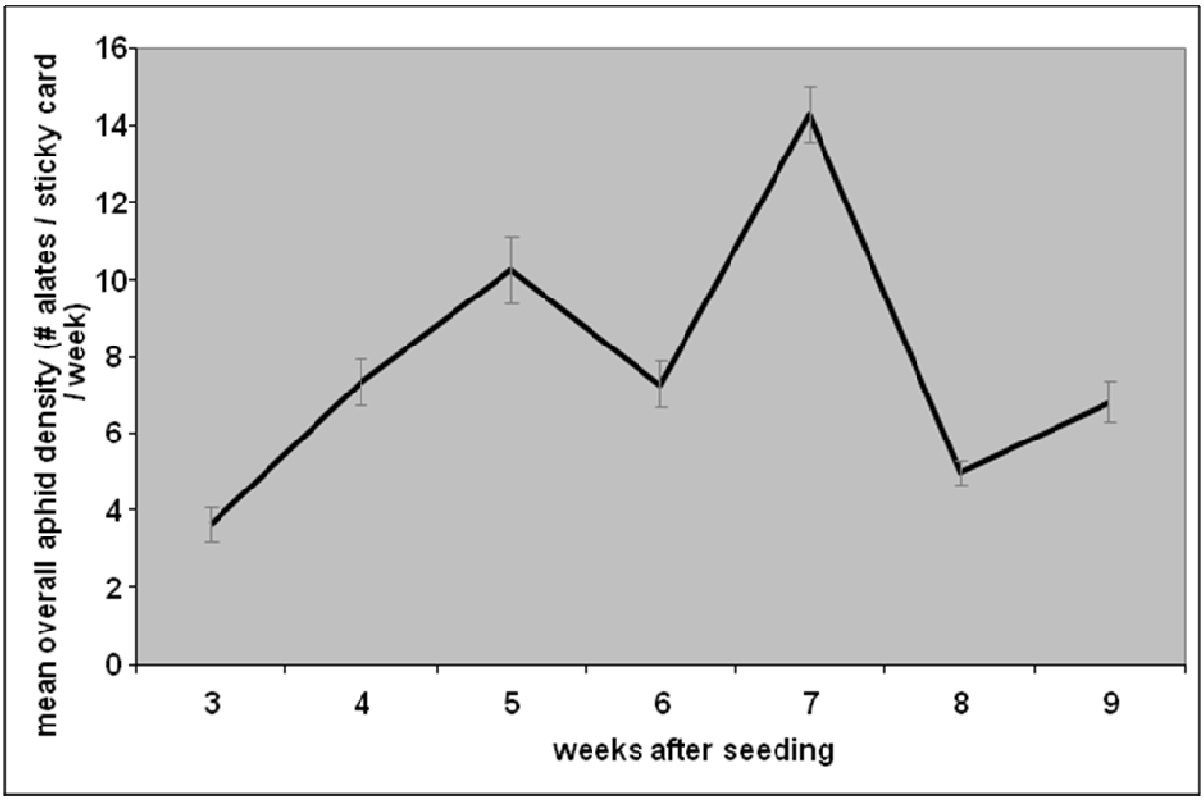

Figure 2. Overall mean aphid density at two commercial melon fields during seven week experiment investigating the effects of reflective particle film applications, systemic insecticide applications, and the combination of reflective particle film and systemic insecticide applications on aphid species composition and resulting mosaic virus incidence.

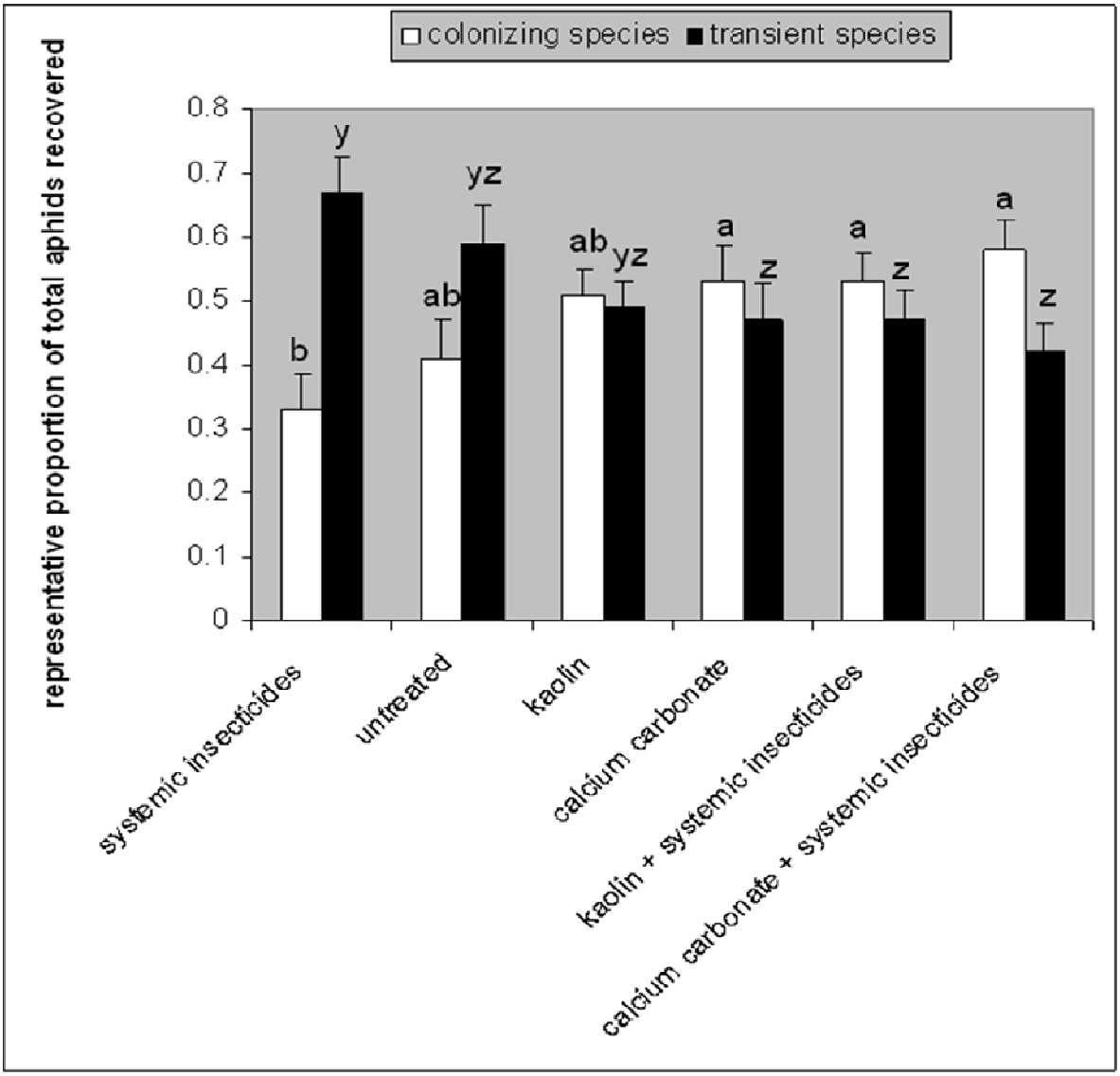

Figure 3. Aphid species composition observed on yellow sticky cards, deployed weekly, directly above melon plants subjected to various reflective particle film application applications, systemic insecticide applications, and the combination of reflective particle film and systemic insecticide applications. Colonizing species are commonly found in melons and can complete development on melons, transient species either are uncommonly found in melons but can complete development on melons or cannot complete development on melons. Treatments accompanied by the same letter are not significantly different according to Tukey's Honestly Significant Difference test on least squared means from mixed model output where site and block were random variables, and treatment and crop age were fixed variables.

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