

Horticultural Entomology

Effect of Plastic Mulch Colors on *Anasa tristis* (Hemiptera: Coreidae) Population Dynamics in Summer Squash, *Cucurbita pepo* (Cucurbitales: Cucurbitaceae)

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Abstract

The squash bug, *Anasa tristis* (De Geer), is a serious pest of cucurbit crops across the United States, especially within summer squash (*Cucurbita pepo* L.) systems. Using their piercing sucking mouthparts, squash bugs feed on both leaf tissue and fruits, often leading to leaf necrosis, marketable fruit loss, and even plant death. To date, the relationship between squash bug presence and plasticulture has not been adequately investigated. This 2-yr study evaluated the effects of white, black, and reflective plastic mulch colors on the occurrence of all squash bug life stages and marketable zucchini yield in Virginia. In both years, *A. tristis* adults and egg masses were more numerous on zucchini plants grown in white and reflective plastic mulch compared to bare ground plants. Greater nymphal densities and marketable fruit yield were observed in certain plastic mulch treatments versus the bare ground treatment, yet these differences were not consistent in both years. Contrary to the repellency effects reflective mulches have on other cucurbit insect pests, our research suggests that reflective and other plastic mulch colors can negatively impact squash bug management, especially in regions with high *A. tristis* pressure. Our study offers new insights for cucurbit growers to use when considering whether they should implement plasticulture in their growing systems.

Keywords: squash bug, cucurbits, plastic mulch, integrated pest management, cultural control

The squash bug, *Anasa tristis* De Geer, is a formidable pest of cucurbit crops across much of the continental United States, threatening an estimated \$1.37 billion in cucurbit production each year (USDA-NASS 2018). *Anasa tristis* can complete its life cycle on a variety of cucurbit species, yet it prefers those in the *Cucurbita* genus, specifically *C. pepo* cultivars (e.g., summer squash and zucchini; Bonjour et al. 1991, Bonjour et al. 1993). Using its piercing-sucking mouthparts, squash bug feeds on the xylem, phloem, and plant cell tissues of the leaves, stems, and fruit (Neal 1993). This feeding can have a variety of negative effects on the host plant, leading to localized leaf necrosis, vascular disruption, fruit rot, marketable yield loss, and even plant death (Woodson and Fargo 1991, Neal 1993, Palumbo et al. 1993). Squash bug feeding can create opportunistic entry sites for cucurbit fruit pathogens such as anthracnose, choanephora fruit rot, and grey mold rot (Doughty et al. 2016). *Anasa tristis* adults are

also vectors of *Serratia marcescens* Bizio, a phloem-colonizing bacterium that causes cucurbit yellow vine disease (CYVD) in summer squash, pumpkin, watermelons, and cantaloupe (Bruton et al. 2003). Current squash bug management strategies focus primarily on the application of broad-spectrum insecticides; however, most insecticides labeled for squash bug control (e.g., organophosphates, carbamates, pyrethroids, and neonicotinoids) are harmful to natural enemy and pollinator species (Elzen 2001, Doughty et al. 2016, D'Avila et al. 2018). Additionally, repetitive use of these insecticides may result in outbreaks of secondary pests such as aphids, whiteflies, and thrips (Kuhar et al. 2005). For these reasons, alternative, non-chemical controls are needed to improve the sustainability and effectiveness of squash bug integrated pest management.

Plasticulture, or the use of plastic mulch, is commonly used in vegetable production to suppress weeds, retain soil moisture,

regulate soil temperature, improve irrigation efficacy, and enhance organic matter retention (Jabran 2019). Plasticulture has also been shown to influence insect pest behavior, acting as a repellent or visual disrupter (Vincent et al. 2003, Diaz and Fereres 2007). For example, Nottingham and Kuhar (2016) observed reduced pest densities in snap beans grown on reflective plastic mulch, potentially through the augmentation of light reflectance and concomitant temperature around vegetable plants. Within cucurbit systems, reflective mulch can significantly decrease the presence of aphids, thrips, whiteflies, and cucumber beetles, and as a result, minimize the prevalence of insect-vectored plant diseases (Henshaw et al. 1991, Summers et al. 1995, Caldwell and Clark 1999, Frank and Liburd 2005). Given these benefits, investigating the impact mulch can have on incidence of squash bug seems a logical next step; however, to date, only one study (Cartwright et al. 1990) has investigated the effects of different plastic mulches on squash bug presence.

In their 2-yr study, Cartwright et al. (1990) observed greater numbers of squash bugs but no difference in yield (i.e., mean number of fruit/plant) in reflective plastic mulch versus bare ground. Yet, these findings were inconsistent between years, planting dates, or between specific mulch colors and bare ground treatments. To clarify these varied results, the influence of various plastic mulch colors on squash bug warrants additional consideration. The findings of this single study may also not be indicative of all regions of the United States, as this experiment was conducted in Stillwater, Oklahoma (Doughty et al. 2016). Here, we seek to further investigate the effects of plastic mulches on squash bug abundance and damage, using a different geographic location and increased experimental replication in Virginia summer squash systems.

Materials and Methods

Experimental Design

We conducted field experiments to test the effects of mulch color on *A. tristis* during the summers of 2019 and 2020 at Kentland and Homefield Farms in Whitethorne, VA. To do so, we constructed four treatments of plastic mulch beds (1.2 × 9.1 m/block) as a Latin square design in early June. Treatments included black, white, and reflective mulch (Berry Hill Irrigation, Buffalo Junction, VA) and bare ground. Zucchini seeds (*var.* Dunja, Harris Seeds) were sown directly in mid-June at 0.76 m spacing within each treatment bed. Seven to 10 d after sowing, stand counts were taken for each germinated treatment bed. Plots with sparse germination (<50%) were replanted with greenhouse grown seedlings (sown on the same date as in the field) so there were at least ten plants per plot. Tiffany teff grass (Hancock Seed Company, Dade City, FL) was sown between plastic mulch rows at a rate of 11 kg seed/ha, acting as a living mulch and weed suppressant throughout the experiment. A 9% concentration (total fatty acid) solution commercial blend of caprylic and capric acid (Home Plate non-selective herbicide; Certis USA, Columbia, MD) was applied to bare ground plots to manage weeds on two dates: before squash germination and immediately following germination. Subsequent weed management was accomplished through weekly manual removal of weeds.

Insect Sampling

Approximately 3 wk after zucchini germination, we began sampling for squash bug at weekly intervals. Five randomly selected plants within each plot were surveyed for adults, nymphs, and egg masses. Surveys included visual checks of the entire plant and the plastic mulch directly below the plant, specifically focusing on the spaces

where the plant stem meets the mulch and along the mulch teff grass margin. Additional consideration was given to these areas because previous research suggested that *A. tristis* adults and nymphs commonly reside in inconspicuous or hidden areas adjacent to the plants (Palumbo et al. 1991a, Doughty et al. 2016). In total, insect sampling was performed for six consecutive weeks, starting the first week of July and ending the second week in August.

Marketable Fruit Sampling

Once plants began to develop fruit, we harvested all undamaged, marketable zucchini from each plot three times per week from the fourth week of July through the second week of August by manually picking all market sized fruit (220–250 g). Damaged fruit was picked during each harvest date but was discarded and not included in our marketable fruit yield counts. Numerous factors, such as improper pollination, microbial pathogens, and feeding damage from other cucurbit insect species, can influence summer squash fruit quality. Since we did not specifically account for these potential predictors of fruit quality, the number of unmarketable fruit was not recorded as an additional response variable.

Statistical Analysis

Insect count and marketable yield data were separated by year. The number of marketable fruit collected per plot was summed for each sampling week and divided by the number of plants within each plot. Weekly *A. tristis* counts and marketable fruit yield/plant were normalized using a square-root transformation. To test the fixed effects of mulch treatment, sampling week, and their interaction on insect counts and yield, we used a generalized linear mixed model (GLMM) with block and site location as random factors. Although we were not interested in the effect of sampling week alone, it was included as a fixed factor in order to test its interaction with mulch treatment on *A. tristis* counts and yield. This interaction allowed us to distinguish whether the effect of mulch treatment was consistent across our *A. tristis* and yield sampling periods. Multiple comparisons between mulch treatments were conducted using a Tukey's honestly significant difference (HSD) test ($\alpha = 0.05$). All statistical analyses were completed using JMP 15.0.0 software.

Results

Adults

For both years, mulch treatment and sampling week were significant predictors of *A. tristis* adult counts, whereas the mulch*sampling week interaction was not significant (GLMM; Table 1). In 2019 and 2020, a nearly five-fold difference was observed in reflective and white mulch versus bare ground plots (Tukey HSD; Figs. 1a and 2a). In 2020, significantly more adults were also found in black mulch compared to bare ground treatments.

Egg Masses

Similar to the adult counts, mulch treatment and sampling week had significant effects on observed egg masses in both years (GLMM, Table 1). In 2020, mulch*sampling week interaction was a significant predictor of *A. tristis* egg masses, meaning the relative differences among mulch treatments were influenced by the week the egg mass counts were performed. In 2019 and 2020, roughly twice as many egg masses were found in black, white, and reflective plots than in bare ground plots (Tukey HSD; Figs. 1b and 2b).

Table 1. Estimated degrees of freedom, *F* statistics, and *P* values for the generalized linear mixed model describing the effects of mulch treatment, sampling week, and their interaction on *A. tristis* counts and marketable fruit/plant in Whitethorne, VA in 2019 and 2020

Year	Response variables	Fixed factors	df	F statistic	<i>P</i> value
2019	Adults	Mulch	3, 141.5	7.12	0.0002
		Sampling week	5, 139.5	8.23	< 0.0001
		Mulch*Sampling week	15, 139.5	1.16	0.312
	Egg masses	Mulch	3, 141.5	10.95	< 0.0001
		Sampling week	5, 139.5	21.03	< 0.0001
		Mulch*Sampling week	15, 139.5	0.88	0.586
	Nymphs	Mulch	3, 141.5	1.27	0.287
		Sampling week	5, 139.5	17.06	< 0.0001
		Mulch*Sampling week	15, 139.5	0.820	0.654
	Marketable fruit/plant	Mulch	3, 64.9	6.178	0.0009
		Sampling week	2, 66.2	4.893	0.010
		Mulch*Sampling week	6, 66.2	0.568	0.755
2020	Adults	Mulch	3, 164	8.65	< 0.0001
		Sampling week	5, 164	2.48	0.034
		Mulch*Sampling week	15, 164	1.23	0.254
	Egg masses	Mulch	3, 164	12.58	< 0.0001
		Sampling week	5, 164	19.64	< 0.0001
		Mulch*Sampling week	15, 164	2.04	0.015
	Nymphs	Mulch	3, 164	5.90	0.0008
		Sampling week	5, 164	31.89	< 0.0001
		Mulch*Sampling week	15, 164	1.47	0.122
	Marketable fruit/plant	Mulch	3, 80	4.66	0.0047
		Sampling week	2, 80	9.91	< 0.0001
		Mulch*Sampling week	6, 80	3.00	0.011

Bold *P* values indicate significant effect ($P < 0.05$) of fixed factors on response variables.

Nymphs

In 2019, mulch treatment had no significant effect on nymph counts, and sampling week was our only significant predictor (GLMM; Table 1; Fig. 1c). In 2020, sampling week and mulch treatment significantly affected observed nymphs. Specifically, we counted over three times as many nymphs on zucchini plants in reflective and black mulch than on bare ground plants (Tukey HSD; Fig. 2c).

Marketable Yield

Sampling week and mulch treatment predictors had significant effects on marketable fruit/plant produced in 2019 and 2020 (GLMM; Table 1). The mulch*sampling week interaction was only a significant predictor of marketable fruit/plant in 2020. In 2019, plants in black and reflective mulches produced about twice as many marketable zucchini as bare ground plants (Tukey HSD; Fig. 1d). However, in 2020, plants in bare ground treatments produced the same marketable yield as plants in the three mulch color treatments (Fig. 2d).

Discussion

In our study, we observed significantly more *A. tristis* adults and egg masses on summer squash grown with white, black, or reflective mulch compared to bare ground, yet no differences in adult or egg mass numbers among the three mulch color treatments (Figs. 1 and 2). The observed similarity in population dynamics for adults and egg masses was expected, as the two life stages are highly correlated throughout much of the growing season (Harmon et al. 2003). More specifically, once adults reach peak density, there is a subsequent peak in egg mass density (Fargo et al. 1988, Palumbo et al. 1991b). Our findings also support a preference by *A. tristis* adults for squash grown in plastic mulched systems, which appears to be unaffected by mulch color. Preference for plastic mulch systems may be a result

of *A. tristis* adults' propensity to congregate near the crown and lower, older leaves of their host plant (Palumbo et al. 1991a). During the initial weeks of the summer squash vegetative growth phase, the plants are small and offer inadequate refuge for the colonizing adults. Holes in the plastic mulch where squash is planted provide shelter for adults to feed on the young plant's main stem and mate relatively protected from natural enemies. One of the most prominent natural enemies of *A. tristis* adults, parasitoid fly *Trichopoda pennipes* Fabr. (Diptera: Tachnidae), actively searches for the bugs in the canopy of host plants (Beard 1940). However, it is unknown whether *T. pennipes* will forage for host bugs underneath the plastic mulch. Squash bug copulation can last >20 h for a single adult pair, leaving the bugs vulnerable for extended periods of time (Sears et al. 2020). The protective cover of plastic mulch may then allow *A. tristis* to optimize its fitness and fecundity early in the season when shelter near host plants is sparse. Future research is needed to completely discern if squash bug adults experience decreased rates of predation and parasitism when using plastic mulch as refuge.

Nymphal densities were highly variable and did not follow the pattern of the adult and egg mass numbers. Mulch treatment was only a significant predictor of nymph counts in 2020 (Table 1; Fig. 2c). Previous research suggests that although all *A. tristis* life stages exhibit an aggregated distribution, nymphs show the greatest degree of aggregation (Palumbo et al. 1991c). For this reason, nymph distribution may be patchier and require additional sampling to accurately determine nymphal abundance (Palumbo et al. 1991c). Therefore, a potentially patchy distribution of nymphs in conjunction with identical numbers of plants surveyed for all life stages may explain inconsistent results among nymphs between and within treatment groups.

Even with significant differences in *A. tristis* adult and egg mass numbers between mulched and bare ground plots, differences among mulch treatments observed in marketable fruit yield varied between years, as plants in plastic mulch produced more marketable fruit

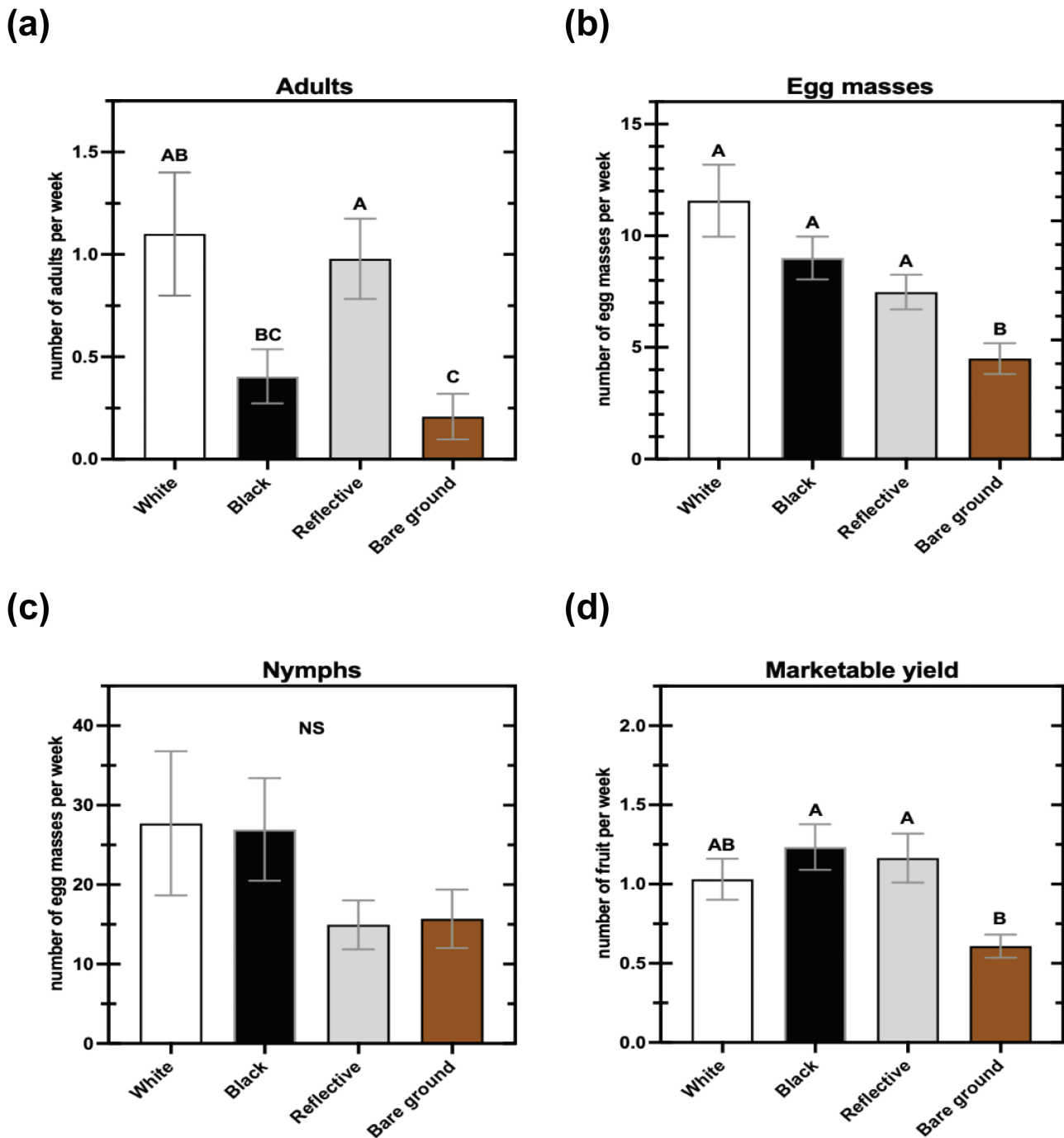


Fig. 1. Mean counts of *A. tristis* (a) adults, (b) egg masses, and (c) nymphs per 6-wk sample period, and (d) mean marketable zucchini fruit produced per plant during the 3-wk harvest period, for different mulch treatments in Whitethorne, VA in 2019. Letters display significant differences between treatment groups (Tukey HSD, $P < 0.05$). 'NS' indicates no significant difference among mulch treatments, and bars show standard error.

than bare ground plants only in 2019 (Table 1; Figs. 1d and 2d). As mentioned earlier, the use of plastic mulch in vegetable systems provides numerous horticultural advantages (Jabran 2019). In cucurbit crops, mulch increases yield and plant biomass, lowers soil moisture evaporation, and improves irrigation efficiency (Conway et al. 1989, Kirnak and Demirtas 2006, Torres-Oliver et al. 2018). The benefits of plastic mulch may have had a compensatory effect in 2019, increasing yield to even exceed reductions resultant from increased squash bug pressure. Contrarily, even with reduced *A. tristis* pressure, bare ground zucchini produced similar marketable

fruit yield to mulched plants in 2020, further suggesting a compensatory/beneficial effect of plastic mulch on yield. Disentangling relationships among insect pressure, plastic mulch, and yield has the added difficulty of research lacking a well-established relationship between squash bug pressure and yield. To date, research-based threshold values for adults, egg masses, and nymphs have not been firmly established. Although we did find some differences in insect pressure, mulch, and marketable yield between treatments, our study did not include numbers of fruit discarded due to *A. tristis* damage. It is possible that mulched plants with large densities of squash bugs

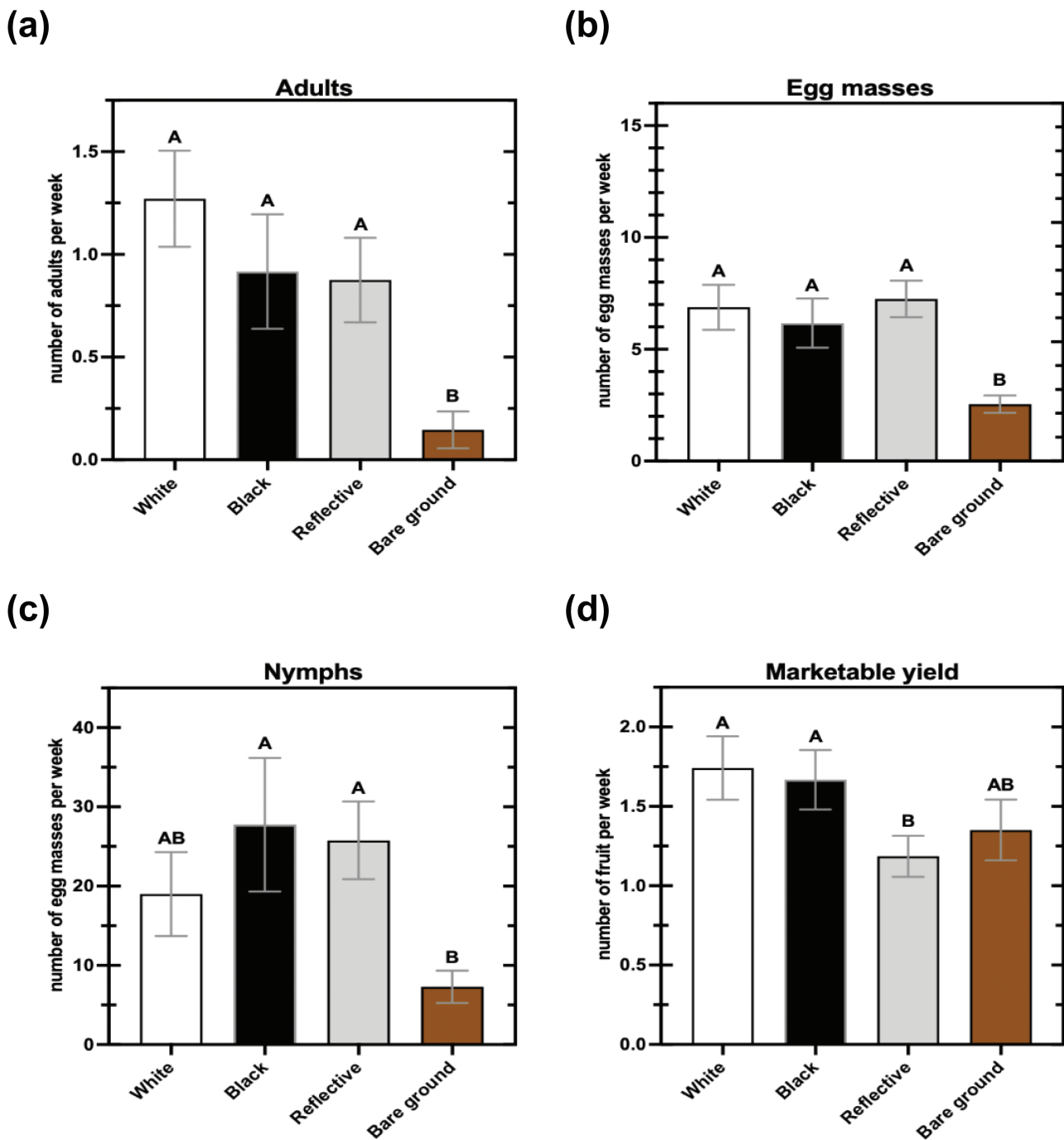


Fig. 2. Mean counts of *A. tristis* (a) adults, (b) egg masses, and (c) nymphs per 6-wk sample period, and (d) mean marketable zucchini fruit produced per plant during the 3-wk harvest period, for different mulch treatments in Whitethorne, VA in 2020. Letters display significant differences between treatment groups (Tukey HSD, $P < 0.05$), and bars show standard error.

incurred greater fruit damage than those grown in bare ground with lower bug densities. In order to correctly assess yield loss from squash bug damage, additional fruit quality predictor variables like pollination success, presence/absence of microbial pathogens, and counts of other cucurbit insect pests are required. Future experimentation attempting to connect squash bug and its influence on damaged fruit numbers should include the aforementioned predictors to accurately elucidate how *A. tristis* specifically influences overall marketable yield in summer squash.

Despite many differences in methodology and experimental design, our study and Cartwright et al. (1990) identified similar patterns in

squash bug presence and yield across different mulch treatments. Both studies observed more adults and egg masses on squash in plastic mulch versus bare soil plots, and inconsistent nymph presence among treatments between years. Although we demonstrated differences in yield between mulch treatments, methodological ambiguity in Cartwright et al. (1990) makes comparing yield between the two studies difficult. For example, they only recorded *total* yield in the second year of their study with no known differentiation between marketable and damaged fruit. Additionally, they offered no explanation of how or for what duration they conducted their yield collections and varied their squash planting dates and subsequent *A. tristis* sampling time frames dramatically

between years. Overall, our study clarifies and expands the results described in Cartwright et al. (1990) by using uniform planting dates, increasing mulch treatment and sequential sampling replication, and including 2 yr of marketable yield data.

When considering the implementation of plasticulture in summer squash production, growers should be mindful of the entire cucurbit pest complex, particularly which species are routinely economically destructive in their region. Despite the possible horticultural attributes provided by plastic mulch, our research conducted in southwest Virginia suggests that plastic mulch can negatively impact squash bug management, especially in regions with high *A. tristic* pressure. Growers in such areas looking to minimize *A. tristic* presence may benefit from forgoing the use of plastic mulch, and instead, utilize a system that uses herbicide applications, cover crops, or other weed suppression tactics. On the other hand, growers in locations that experience significant pressure from cucumber beetles, aphids, or whiteflies can help protect their cucurbits from pest colonization with the repellency effects offered by reflective plastic mulch (Summers et al. 1995, Caldwell and Clark 1999, Vincent et al. 2003, Frank and Liburd 2005). Availability of essential farm equipment (e.g., plastic mulch layer) and added costs of specific plastic mulch colors (e.g., reflective, red, blue, etc.) will also inevitably impact whether a certain plasticulture system is implemented. In most cases, cucurbit growers will face a mixed assemblage of insect pests throughout the growing season, requiring them to conduct cost-benefit analyses of all possible cultural, biological, and chemical control strategies. Using the newly acquired knowledge put forth by this study, growers can more confidently decide if plasticulture fits within their squash bug management plans.

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