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Use of Living and Dying Mulches as Barriers To Protect Zucchini from Insect-Caused Viruses and Phytotoxemias

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Summary

A field study was conducted to examine the influence of interplanting zucchini (*Cucurbita pepo* L.) with the cover crops white clover (*Trifolium repens* L.) and buckwheat (*Fagopyrum esculentum*) on densities of aphids and whiteflies, occurrences of aphid-caused viruses and whitefly-induced phytotoxemia, and crop yield. The white clover and buckwheat cover crops were used as a living and dying mulch, respectively. Zucchini plants grown in bare-ground plots had greater aphid numbers and higher incidences of virus-infected plants than those in the white clover and buckwheat treatment plots. Whitefly nymph numbers were similar among treatment types. However, the severity of squash silverleaf disorder was significantly lower on zucchini plants in white clover treatments than in buckwheat treatments on each sampling date. Marketable fruit yields were also significantly greater in the mulch treatments than in bare-ground plots. The feasibility of using white clover and buckwheat as barrier plants is discussed.

Introduction

Cucurbitaceous crops, such as squash and cucumber, are susceptible to several insect-caused viruses and physiological plant disorders. Non-persistent viruses (NPVs), such as zucchini yellow mosaic virus (ZYMV), transmitted by several aphid species, are regarded as the most destructive pathogens affecting cucurbits throughout the world. Among aphids, the melon aphid (*Aphis gossypii* Glover), a worldwide pest on field crops, is one of the most efficient transmitters of plant viruses. Other important aphid-transmitted viruses of cucurbits include cucumber mosaic virus (CMV), watermelon mosaic virus (WMV), and papaya ringspot virus, watermelon strain (PRSV-w). Infected plants are stunted, yield fewer fruits than healthy plants, and the fruits are frequently distorted, rendering them unmarketable. In Hawai'i, cucurbit crop losses due to aphid-transmitted viruses sometimes approach 100 percent.

In addition to aphids, whiteflies can be severe pests of cucurbit crops. Some can induce phytotoxemias in several plant species. Phytotoxemia is an adverse, often delayed reaction of plants to toxins introduced during insect feeding. The silverleaf whitefly (*Bemisia argentifolii* Bellows and Perring) is a severe pest of several agricultural crops throughout the world. Silverleaf whiteflies are responsible for a phytotoxemia know as squash silverleaf disorder (SSL). Feeding by immature silverleaf whiteflies causes this physiological disorder, and symptom severity is dependant on the number of immature whiteflies per unit of leaf area. If populations become high enough to cause significant leaf silvering, there will be a reduction in photosynthesis, resulting in smaller plants and severe yield losses in squash fields. In addition to causing SSL, whitefly damage generally includes a decline in plant vigor, irregular fruit ripening (such as is seen in tomatoes), and transmission of plant viruses.

Current control strategies for aphids and whiteflies rely mostly on insecticide inputs aimed at killing them before they damage a crop. However, the use of insecticides may not reliably control the spread of an insect-transmitted virus, especially non-persistent viruses. Non-persistent viruses are transmitted non-specifically by a large number of aphid species after very brief feeding probes (1–2 minutes or less). Integrating cultural-control techniques, such as mixed cropping or the growing of two or more plant species within the same field, with other pest-suppression methods has the potential to reduce insect pest

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numbers and the resulting transmission of viruses and phytotoxemias.

Using zucchini as a model cucurbit system, this study investigated the value of mixed-cropping systems (specifically, use of living and dying mulch) for reducing aphid and whitefly populations and their associated plant impairments (specifically, non-persistent viruses and squash silverleaf disorder). *Dying mulches* are cover crops, grown within a main crop, that will began to senesce and eventually die prior to completion of the main crop harvest period. The dying mulch's purpose is to serve some benefit prior to dying, such as increasing the numbers of beneficial organisms, improving soil fertility, or suppressing nematodes. *Living mulches* are cover crops interplanted or undersown with a main crop that will continue to grow and mature along with the main crop; they are intended to serve a function such as weed or insect suppression. *Barrier plants* are secondary plants grown within or bordering a primary cash crop for the purpose of controlling insect-caused plant diseases. This paper describes a field experiment designed to use both living and dying mulches as barrier plants to reduce occurrence of NPVs and SSL disorder.

Materials and methods

Experiment layout

A field experiment was conducted at the University of Hawai'i at Mānoa Poamoho Research Station on the island of O'ahu in 2003. The three cropping habitats examined during this study were zucchini plants undersown either in (1) a living mulch, white clover var. New Zealand (*Trifolium repens* L.), seeded at ~54 g per row; (2) a dying mulch, buckwheat (*Fagopyrum esculentum* Moench, Peaceful Valley Farm Supply, Grass Valley, Calif.), broadcast-seeded at a rate of ~1.81 kg (4 lb) per plot; or (3) grown in bare-ground monoculture (the "control" treatment). Experimental plots were 13.7 m x 13.7 m (45 ft x 45 ft), with each treatment replicated four times and arranged in a randomized complete block design.

White clover was sown on March 31, 2003 and buckwheat on September 18, 2003. On September 24, a weed string-trimmer was used to clear eight 76-cm (2.5-ft) rows in the white clover plots. Afterward, a motorized hand tiller was used to cultivate the rows. On October 13, 2-week-old greenhouse-grown zucchini plants ('Spineless Beauty', Syngenta Seed, Boise, Idaho) were transplanted into each treatment plot. Each plot contained eight rows of zucchini 1.5 m (5 ft) apart with plants spaced 1.2 m (4 ft) apart within them. Alleyways between plots were a minimum of 7 m wide and kept free of weeds. Bordering the study sites were rows of Sudax (a sorghum–sudan grass hybrid), which were sprayed weekly with GF 120 Naturalyte fruit fly bait to help manage populations of the melon fly, *Dacus cucurbitae* Coquillett.

Insect counts

Counts of adult whiteflies and aphids on zucchini leaves were taken at weekly intervals beginning 7 days after planting (DAP) and continuing until 49 DAP. A total of 16 plants were randomly selected from each plot during a sampling period by gently turning over one randomly selected leaf from each plant and recording the number of insects found. Because the alate (winged) aphids are responsible for virus spread, only this aphid morph was counted. In addition, aphids (winged and wingless) and immature stages (eggs, nymphs, and pupae) of whiteflies were sampled by taking a 3.14 cm^2 disc sample from the foliage of 12 randomly selected zucchini plants per plot using a cork bore sampler. The disc samples were then taken to a laboratory and examined under a microscope. The numbers of immature whiteflies and aphids found on these discs were recorded.

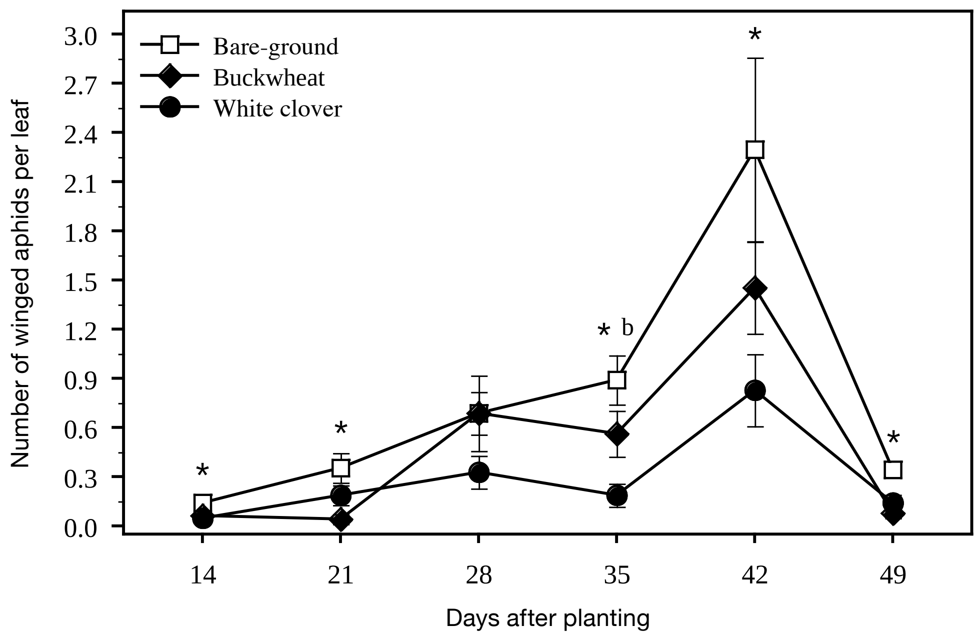
Disease and phytotoxemia rating

The percentage of zucchini plants with a non-persistent virus was determined by visually inspecting all zucchini plants in each plot. The percentage of plants showing viral symptoms (e.g., mosaic leaves, distorted and/or mottled fruit) was recorded weekly. Silverleaf disorder was rated on the new leaf growth of all plants in three randomly selected interior rows of each plot. A scale of 0 to 5 was used during each inspection date, with 0 indicating no symptoms and 5 indicating that the entire leaf contained a silver coloring. Plants were evaluated for viral symptoms every five days beginning 30 DAP, and silverleaf severity symptoms were assessed every 10 days beginning 16 DAP.

Statistical analysis

The effects of mulch type on each experimental factor were analyzed using analysis of variance (Proc GLM, SAS Institute) and predetermined orthogonal comparisons to separate mean differences. Within the model, the following predetermined statistical contrasts were conducted: zucchini monoculture vs. mulches (buck-

Figure 1. Mean population densities of winged aphids in bare-ground (zucchini monoculture), buckwheat (zucchini-buckwheat), and white clover (zucchini-white clover).



* indicates average numbers in bare-ground were significantly greater than in buckwheat + white clover.
[b] indicates average numbers in buckwheat were significantly greater than in white clover ($P < 0.05$).

wheat and white clover), and buckwheat vs. white clover. Treatment comparisons were considered significantly different if $P < 0.05$*.

Results

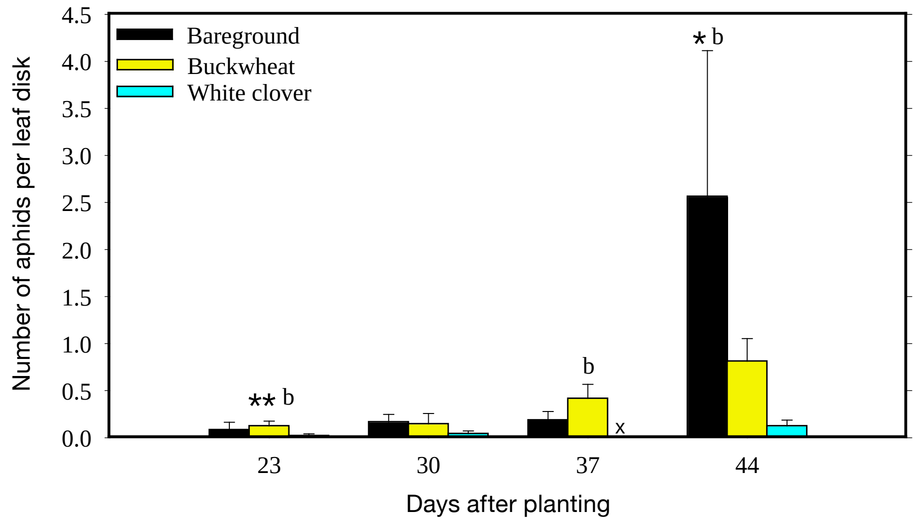
Insect counts

The number of winged aphids found on zucchini plants from whole-leaf counts was significantly greater in bare-ground plots than in either mulch treatment on all sample dates (Fig. 1, $P < 0.05$). However, aphid counts were similar on zucchini plants in buckwheat and white clover plots on most dates. Only at 35 DAP were aphid counts significantly higher in buckwheat than in white clover plots. Aphid counts from leaf disc samples differed from whole-leaf counts. Significantly fewer aphids were found on disc samples collected from bare-ground plots compared with the two mulch treatments on the initial sample date, 23 DAP (Fig. 2, $P < 0.05$). However, by the final sample period (44 DAP), aphid counts from disc samples were significantly higher in bare-ground plots than in the two mulch treatments. Significantly more aphids were found on zucchini leaf disc samples collected from buckwheat than in white clover plots on three of the four sample dates ($P < 0.05$).

Adult whitefly numbers from whole-leaf counts were similar among treatments on most sample dates (Fig. 3, $P > 0.05$). However, counts were significantly lower in bare-ground zucchini compared with the two mulch treatments at 21 DAP. On the final two sampling dates (35 and 42 DAP), whitefly counts were also significantly lower on zucchini plants in white clover compared with buckwheat plots. Whitefly egg counts taken from leaf disc samples were similar among treatments on three of the four sampling dates (Fig. 4, $P > 0.05$). Only on the initial sampling date (23 DAP) were there significantly

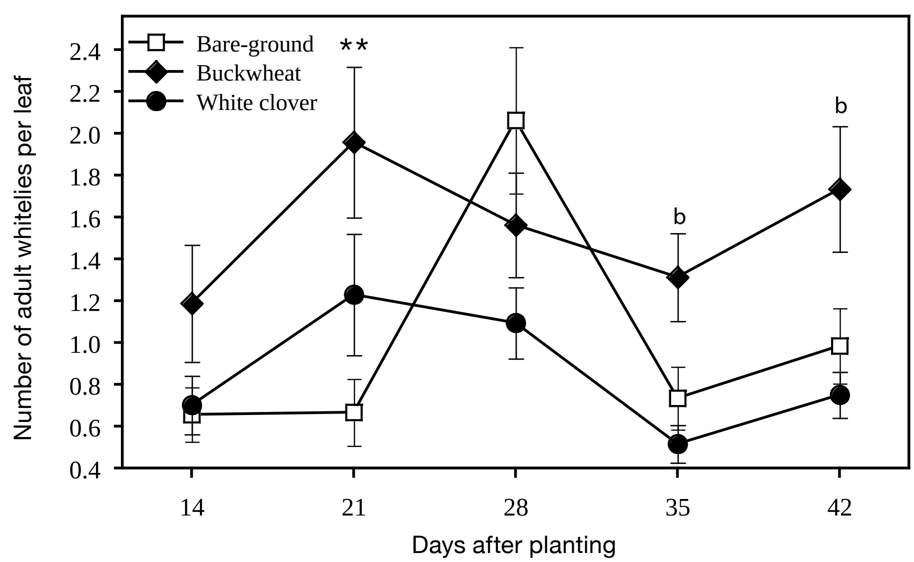
*The P value is a statistical estimate of the probability that a difference between treatments found during an experiment happened by chance. For example, a P value of 0.05 (P = 0.05) means there is a 5-in-100 chance that the result occurred by chance, and thus a 95 percent probability that the result occurred because of the effect of treatments. The lower the P value, the more likely it is that any difference between treatment data means was caused by treatment effect. Thus P < 0.05 means that the result has a better than 95 percent chance of being a valid result, while P > 0.05 indicates that less confidence can be placed in the result.

Figure 2. Mean population densities of aphids collected from zucchini leaf disc samples (3.14 cm²) in bare-ground (zucchini monoculture), buckwheat (zucchini-buckwheat), and white clover (zucchini-white clover) treatments.



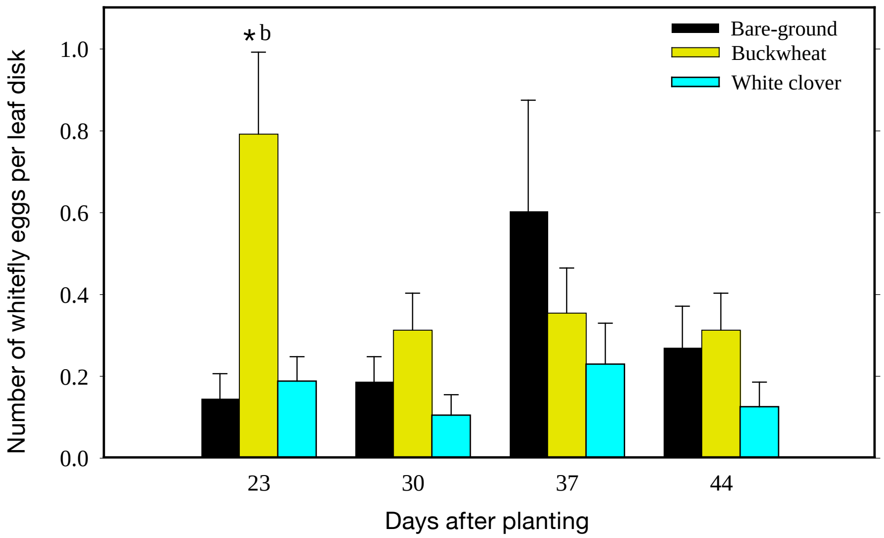
** indicates average numbers in (buckwheat + white clover) were significantly greater than in the bare-ground treatment.
[b] indicates numbers in buckwheat were significantly greater than white clover; x signifies no aphid found.
* indicates average numbers in bare-ground were significantly greater than in buckwheat + white clover ($P < 0.05$).

Figure 3. Mean population densities of adult whiteflies in bare-ground (zucchini monoculture), buckwheat (zucchini-buckwheat), and white clover (zucchini-white clover) treatments.



** indicates average numbers in buckwheat + white clover were significantly greater than in the bare-ground treatment.
[b] indicates numbers in buckwheat were significantly greater than in the white clover treatment ($P < 0.05$).

Figure 4. Mean population densities of whitefly eggs collected from zucchini leaf disc samples (3.14 cm²) in bare-ground (zucchini monoculture), buckwheat (zucchini-buckwheat), and white clover (zucchini-white clover).



* indicates average numbers in bare-ground significantly greater than buckwheat + white clover.
[b] indicates numbers in buckwheat were significantly greater than white clover ($P < 0.05$).

Table 1. Mean squash silverleaf disorder severity symptoms (± SE) on plants in three zucchini crop habitats.

	Days after planting			
Treatment	16 [*,b]	26 [b]	36	46 [b]
Bare-ground	3.51 ± 0.13	1.28 ± 0.09	0.87 ± 0.03	x
Buckwheat	2.20 ± 0.15	1.53 ± 0.09	1.05 ± 0.03	1.07 ± 0.04
White clover	0.65 ± 0.09	0.99 ± 0.02	0.85 ± 0.03	0.95 ± 0.02

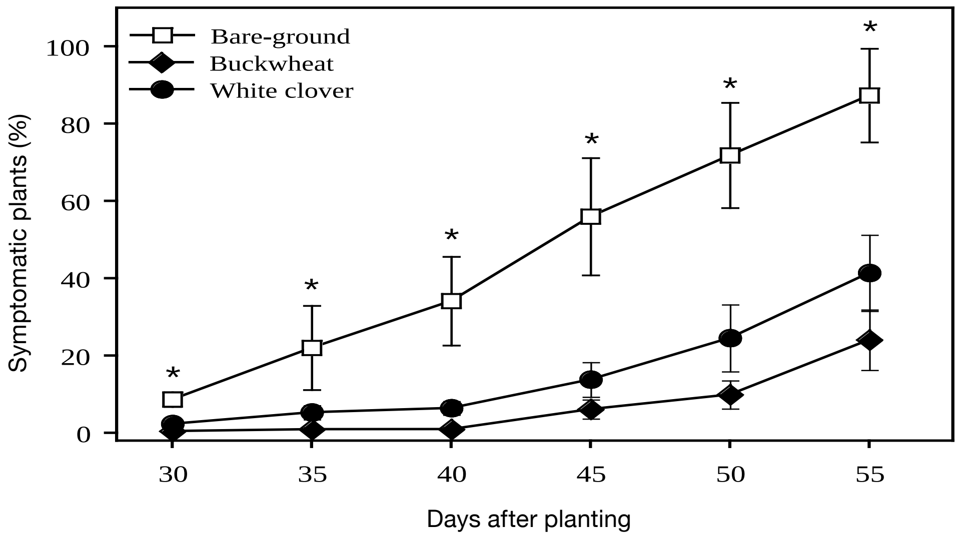
* indicates average numbers in bare-ground treatment were significantly greater than in buckwheat + white clover.
[b] indicates numbers in buckwheat were significantly greater than in white clover ($P < 0.05$).
x indicates plants in treatments were not inspected for silverleaf symptoms.

different whitefly egg counts among treatments. On this date, fewer whitefly eggs were counted on leaf disc samples collected in bare-ground than in mulch treatments and in white clover than in buckwheat plots ($P < 0.05$). At 30 DAP, whitefly egg counts were suggestively higher (nearly significant different) in buckwheat than in white clover ($P = 0.053$). No differences were detected among treatment comparisons in the number of immature whiteflies (nymphs and pupae) over the samplings.

Virus and phytotoxemia

On each sampling date, a significantly higher percentage of zucchini plants displayed symptoms of a non-persistent viral disease in bare-ground plots compared with the two mulch treatments (Fig. 5, $P < 0.05$). However, there were no significant differences in the proportion of virus-infected plants between the buckwheat and white clover treatment plots. SSL disorder severity symptoms caused by immature whitefly feeding were significantly greater in bare-ground plots compared to the two mulch treatments at the initial inspection (16 DAP, Table 1). But symptom severity was significantly lower in the white clover than in the buckwheat treatment on each inspection date ($P < 0.05$). Zucchini plants in bare-ground treatment

Figure 5. Mean percentage of plants displaying symptoms of a non-persistent virus in bare-ground (zucchini monoculture), buckwheat (zucchini-buckwheat), and white clover (zucchini-white clover).



* indicates average numbers in bare-ground were significantly greater than buckwheat + white clover ($P < 0.05$).

plots could not be rated for SSL disorder symptoms at the final inspection (46 DAP) because of the high occurrence and severe display of NPV infection symptoms.

Crop yield

Marketable crop yields were significantly higher in the mulch plots than in the bare-ground plots **(**Fig. 6, $P < 0.05$). Marketable yields were 2.3 and 2.8 times greater in buckwheat and white clover plots, respectively, than in bare-ground treatments. A significantly greater weight of fruits had viral symptoms in bare-ground plots compared with the mulch treatments ($P < 0.05$). A percentage of fruits in all treatments were unmarketable due to melon fly and pickle worm damage. The melon fly and pickle worm damaged zucchini fruits by ovipositing eggs and boring inside the fruit, respectively.

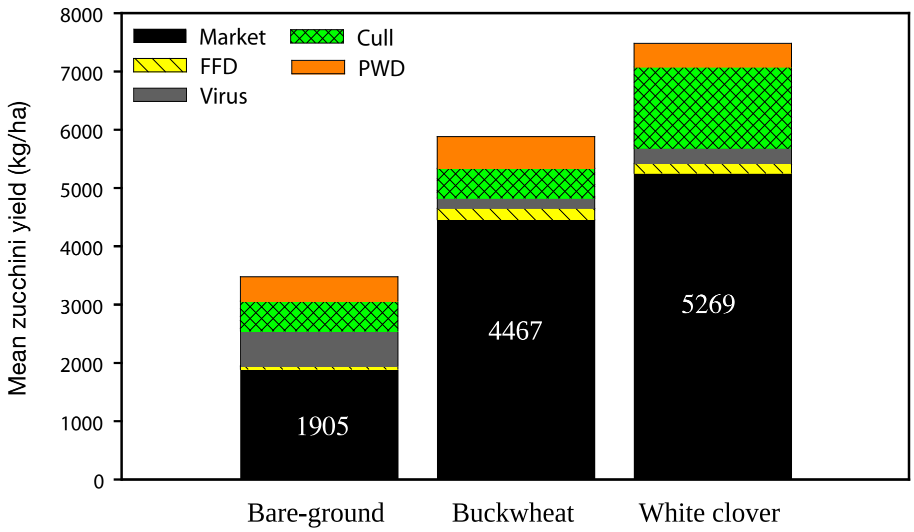
Discussion

The purpose of this study was to determine whether a living mulch (white clover) or a dying mulch (buckwheat) could reduce the incidence of aphid-caused non-persistent viruses and whitefly-caused squash silverleaf disorder. We found that both cover cropping strategies effectively reduced the occurrence of non-persistent viruses compared with bare-ground zucchini and contributed to significantly higher marketable yields. There were also fewer winged aphids found on zucchini plants in the mulch plots than in bare-ground zucchini. Although densities of immature whiteflies were similar on zucchini plants in white clover and buckwheat plots, squash silverleaf disorder severity symptoms in zucchini plants were higher in buckwheat than in white clover plots throughout the zucchini crop cycle. It is known that under severe silvering, zucchini fruit yields are reduced; thus, we believe that greater SSL severity symptoms in buckwheat plots contributed to yields being slightly lower than in white clover plots. Also, the severity of symptoms depended on the number of whitefly nymphs per leaf area. Zucchini plants and associated leaves in the white clover plots were distinctly larger than those in buckwheat and bare-ground plots. Thus, the plants in the white clover plots could sustain a higher number of immature whiteflies before being effected by SSL disorder.

Conclusion

Using cover crops as dying or living mulches seems to be a promising, non-chemical management tactic for reducing populations of aphids and the occurrence of aphid-caused NPVs. Since white clover is a low-growing living mulch, its potential to compete with the main crop is minimal. The advantage of using a dying mulch, such as buckwheat in this experiment, is that if the plant-

Figure 6. Mean zucchini fruit yield in bare-ground (zucchini monoculture), buckwheat (zucchini-buckwheat), and white clover (zucchini-white clover).



"Market" indicates marketable fruit, FFD indicates fruit fly damaged fruits, "virus" indicates fruits displaying viral symptoms, "cull" indicates naturally deformed fruit, and PWD indicates pickle worm damaged fruits. Marketable yields were significantly higher in buckwheat + white clover than in bare-ground plots ($P < 0.05$).

ing date is appropriately scheduled with regards to the main crop's growth cycle, the dying mulch will began to senesce before the main crop is planted and therefore will less likely compete with the main crop for nutrients and other resources. Another potential benefit of a dying mulch is that if it is harboring beneficial arthropods, they may readily move to the cash crop as it senesces.

In this study, white clover appeared to be more suitable as a barrier crop for zucchini plantings. The occurrence of NPVs and SSL disorder were reduced in white clover–zucchini plots. SSL severity symptoms were sometime greatest in buckwheat plots. Further, whitefly densities were sometime highest in buckwheat plots. Similar findings were recorded in some ongoing studies aimed at determining the mechanisms responsible for lower virus incidence in zucchini planted with barrier plants. Thus, in areas were whitefly densities are high, buckwheat may not be a feasible barrier plant. However, if the main concern is NPVs, both buckwheat and white clover may be favorable barrier plants. For those farmers looking to create more sustainable cropping practices or protect their crops from viruses and SSL disorder, using cover crops as living or dying mulches may be viable management strategies.

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Zucchini interplanted into a buckwheat dying mulch

Zucchini interplanted into a white clover living mulch