EVALUATION OF LIVING AND SYNTHETIC MULCHES WITH AND WITHOUT IMIDACLOPRID FOR SUPPRESSION OF WHITEFLIES AND APHIDS, AND INSECT-TRANSMITTED VIRAL DISEASES IN ZUCCHINI SQUASH

By

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To my Mom and Dad
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Field studies were conducted in north-central Florida to evaluate the effect of living and synthetic mulches with and without imidacloprid (Admire 2F) on the density of silverleaf whiteflies, \textit{(Bemisia tabaci} Genn. B-biotype) and aphids (Hemiptera: Aphididae) and their transmission of plant viruses in zucchini squash, \textit{(Cucurbita pepo} L). Living mulch, buckwheat \textit{(Fagopyrum esculentum} Moench) and two synthetic mulches (reflective and white) were evaluated in the fall of 2005 and 2006. Five treatments were evaluated including reflective mulch with imidacloprid, reflective mulch without imidacloprid, living mulch with imidacloprid, living mulch without imidacloprid and a standard white mulch (control). The experimental design was a randomized complete block with four replicates. Whiteflies and aphids were evaluated through foliar counts, yellow sticky traps, and pan traps. Natural enemies were established through foliar counts and the marketable yields of squash were evaluated by weight.

Data from yellow sticky traps indicated that the addition of imidacloprid enhanced the control of whiteflies in squash growing with buckwheat and reflective mulch. Similarly, foliar counts indicated that these two treatments had fewer numbers of whiteflies than the white
synthetic mulch (control). The suppression of immature whiteflies resulted in fewer silverleaf symptoms on the leaves.

The addition of imidacloprid to reflective mulch significantly affected apterous aphids in both years. However, counts of alate aphids from pan traps were inconsistent for both field seasons. These counts indicated that there were no significant ($P < 0.05$) differences between treatments with and without imidacloprid. In 2006, the control treatment had significantly ($P < 0.05$) higher numbers of alate aphids per trap compared with all other treatments.

In 2005, buckwheat treatments had significantly more natural enemies per leaf than reflective mulch treatments. Imidacloprid did not appear to affect natural enemy populations because there were no significant differences in their numbers between treatments with and without Admire 2F.

In 2005, there was no incidence of insect-transmitted viruses; whereas in 2006, *Cucurbit leaf crumple virus* (CuLCrV), a virus new to Florida was found. *Cucurbit leaf crumple virus* is transmitted by whiteflies in a persistent manner. Higher incidences of plants infected with CuLCrV were recorded on the white mulch compared with all other treatments.

Buckwheat mulch had a significant negative effect on the size of squash plants and marketable yields. Yield results were consistent between both years. The treatment with reflective mulch and Admire 2F resulted in significantly higher yields than the living mulch and control treatments. No additional benefits resulted from combining reflective mulch with imidacloprid in terms of yield in both years. The living mulch treatment resulted in consistently lower yields than all the other treatments evaluated. The research indicates that enhanced reduction in the abundance of whiteflies and aphids can be achieved when mulches are combined with imidacloprid but this does not necessarily mean an increase in marketable yields.
CHAPTER 1
INTRODUCTION

Cucurbits are a major vegetable crop grown in the USA, Caribbean and other parts of the world. Their production is important worldwide and these crops are commercially available throughout the year. Cucurbit production competes in importance with other fruit and vegetable crops worldwide (Gaba et al. 2004).

The family Cucurbitaceae consists of three genera: Cucumis L., (melon and cucumber), Citrullus Schrader (watermelon), and Cucurbita L. (summer and winter squashes). Of the squashes Florida produces primarily summer squashes, (Cucurbita pepo L.) including crookneck squash, straightneck squash, scallop squash, and zucchini squash (Mossler and Nesheim 2001). Winter squash such as acorn, butternut squash, and spaghetti squash can also be produced.

Florida is ranked second nationally after Georgia in the production of fresh market squash. In the 2006 field-seasons, a total of 4249 ha of cucurbits was planted in Florida and 4131 ha were harvested with a value of approximately 38.76 million USD (NASS-2006). In Florida, almost all of the squash are produced for fresh market. Summer production is mainly for local markets while fall production is exported. Squash is one of the crops in the state that is shipped every month of the year thus providing a continuous income to the state of Florida (Mossler and Nesheim 2001).

Although squash is produced throughout the state of Florida, the southeast part of the state (Miami-Dade County) is the principal squash-producing region. Other important producing regions include the southwest counties (Lee, Hendry, and Collier), west-central counties (Hillsborough, Hardee, and Manatee) and north Florida counties (Alachua, Columbia, and Gilchrist). Squashes can tolerate temperatures of 18 to 27 °C but grows best between 24 and 29 °C. In regions where frost damage is not a threat, squash is planted anytime between August and
March. In north Florida where frost damage is likely, fall planting occurs in August and September and in spring season between February and March.

Squash is one of the vegetables that are grown on synthetic mulches in Florida. Various colors of synthetic mulches such as clear, white, black, yellow, and silver are available (Olson et al. 2005). Growers use white, or white on black in the fall and black in the winter and spring (Zitter 1977, Csizinszky et al. 1997, Frank and Liburd 2005). Black mulches keep the soil warm during the cool season while white mulches are used during the warm season. Alternatively, reflective silver mulches have the benefit of reducing pests and the occurrence of viral diseases when used in the fall (Olson et al. 2005).

Squash is planted mainly through direct seeding or transplanting seedlings. Squash matures within 40 to 65 days. Squash plants have both male and female flowers on the same plant and therefore require a pollination agent for fertilization to take place (Mossler and Nesheim 2001). Usually, one hive of honeybees per 0.8 ha is maintained to attain sufficient pollination (Olson et al. 2005).

Several factors affect the squash industry in Florida resulting in losses to growers. Some of these factors are the results of increasing costs associated with controlling insects and their related problems, and outbreaks of new pests and diseases (Akad et al. submitted). Like other squashes, zucchini squash is attacked by a broad array of insect pests and plant pathogens such as viruses, bacteria, fungi, and nematodes.

Some of the major foliar diseases affecting cucurbits include powdery mildew caused by *Sphaerotheca fuliginea* (Schlechtend.:Fr.) Pollacci and *Erysiphe cichoracearum* DC. and downy mildew caused by *Pseudoperonospora cubensis* Berk & M. A. Curtis (Zitter et al. 1996). The occurrence of these diseases is more common than viral diseases. While preventative sprays are
applied against downy and powdery mildew, control may not be necessary if the disease occurs in the last 3 weeks of the growing period. Other foliar diseases affecting squash include wet rot and phytophthora foliar blight, which can cause fruits to rot. Both diseases can be managed effectively by soil drench of metalaxyl/chlorothalonil (Mossler and Nesheim 2001). Post harvest diseases of squash include bacterial soft rot, alternaria rot, fusarium rot, rhizopus storage rot and pythium fruit rot. These diseases which, cause the fruit to rot, may begin in the field and cause fruit to deteriorate under storage. Pythium fruit rot is the most common disease causing the crown of older squash to rot. Post harvest diseases can however be controlled through proper handling during and after harvest (Mossler and Nesheim 2001).

Insect pests of squash include pickleworm, *Diaphania nitidalis* Stoll, and melonworm, *Diaphania hyalinata* L. Both pests are tropical moths and are able to overwinter in Florida causing damage to squash among other cucurbits. Their young larvae are known to cause damage on the blossoms, foliage of young vegetative buds and the apical growth of vines. Other pests that damage the foliage and occasionally the fruits are squash bug, *Anasa tristis* DeGeer; melon thrips, *Thrips palmi* Karny; fall armyworm, *Spodoptera frugiperda* (J. E. Smith); beet armyworm, *Spodoptera exigua* Hübner; and leafminers, *Liriomyza trifolii* Burgess and *L. sativae* Blanchard. To manage these pests, Florida growers use biopesticides such as *Bacillus thuringiensis* (B.t.) against melonworm, fall and beet armyworm and other caterpillars, and malathion and SpinTor against sucking pests (such as aphids, mites), beetles, squash vine borer and leaf miners (Mossler and Nesheim 2001).

In Florida, the silverleaf whitefly, *Bemisia tabaci* (Gennadius), biotype B (also known as *Bemisia argentifolii* Bellows and Perring), is a serious economic pest of squash. Both adults and immatures feed on phloem sap (Brown et al. 1995). In addition, the B biotype whiteflies cause
silverleaf disorder of squash (SSL) an important physiological disorder of some cucurbits. Squash silverleaf is associated with the feeding of immature whiteflies of B biotype and is characterized by silvering of the adaxial leaf surface and blanching of the fruit (Schuster et al. 1991, Yokomi et al. 1990, Costa et al. 1993). Substantial economic losses in Florida have now been associated with SSL due to poor fruit quality and yield reduction (Yokomi et al. 1990, Liburd and Frank 2007).

Whiteflies also transmit geminiviruses to squash. Recently, two whitefly-transmitted viruses were recorded in squash in Florida. The \textit{Squash vein yellowing virus} (SqVYV) an Ipomovirus, affects squash, pumpkin and watermelon and \textit{Cucurbit leaf crumple virus} (CuLCrV); a begomovirus, affects all cucurbits except honeydew melon (Brown et al. 2002, Adkins et al. 2007, Akad et al. submitted). \textit{Cucurbit leaf crumple virus} is a relatively new virus to Florida and first appeared in north-central Florida during the fall 2006 (Webb et al. 2007). The emergence of newly introduced whitefly-borne viruses to areas where they never existed before has been attributed to high whitefly populations and possibly movement of infected plant material from other states (Webb 1991, Polston and Anderson 1997). \textit{Cucurbit leaf crumple virus} is transmitted by whiteflies in a persistent, circulative manner. Typical symptoms of CuLCrV on leaves include curling, yellowing, and crumpling. Symptoms can also develop on the fruits causing abrasions or even green streaks like on the yellow squash causing the fruits to be unmarketable (Webb et al. 2007).

Aphids are also important pests of squash, causing damage through sucking plant sap and, more importantly, through transmission of viral diseases. They are the most important vectors of plant viruses and are known to transmit 275 different viral disorders (Nault 1997). In Florida, the most damaging viruses affecting squash are \textit{Papaya ring spot virus-Watermelon strain} (PRSV-
W), *Watermelon mosaic virus* (WMV), *Zucchini yellow mosaic virus* (ZYMV) and *Cucumber mosaic virus* (CMV). Symptoms of these viruses on leaves include: leaves curling downward and wrinkling, vein banding, mosaic patterns, mottling, blistering and distorted (strapleaf) leaves which are reduced in size. Fruit symptoms include discoloration with mosaic patterns and reduced misshapen and warty texture (Alderz et al. 1983, Schubert and McRitchie 1984, Purcifull et al. 1988, Mossler and Nesheim 2001, Gianessi et al. 2002).

Mosaic viruses are transmitted in a non-persistent manner by several aphid species. Of the known species associated with virus spread, the melon aphid *Aphis gossypii* Glover, is considered a primary pest of cucurbits (Damicone et al. 2007). Alate aphids are able to acquire/transmit the viruses as they are probing to test the plant for host suitability. Once the virus is acquired aphid vectors remain viruliferous for a short period of time (minute to hours). Damage to host plants by whiteflies and aphids can also result in reduced plant growth and quality due to excessive removal of plant assimilates from phloem. Moreover, excretion of honeydew, a sugar-rich compound, by whiteflies and aphids on the leaves and fruits, promote growth of sooty mold (*Capnodium* spp.) further reducing their quality (Palumbo et al. 2001).

Pesticides have been the most popular pest management tactic in high value crops (Hummel et al. 2002) including cucurbits (Frank and Liburd 2005). However, awareness has increased concerning the use of pesticides due to their effects on human, environment and other non-target organisms (Antignus 2000). In addition, high pesticide usage increases the cost of production and potential for resistance development. Subsequently, there has been increased emphasis on sustainable cultural pest management tactics.

Cultural control strategies entail manipulating components of the agro-ecosystem to reduce pest numbers to below economically damaging levels (Hilje et al. 2001). Living mulches and
inert ground covers such as synthetic, sawdust, straw and husk mulches have been evaluated as a means of managing key pests in vegetable crops. UV-reflective mulches have been demonstrated to reduce whitefly and aphids and/or the incidence of the viruses they transmit in various vegetable crops (Wolfenbarger and Moore, 1968, Brown et al. 1993, Csizinszky et al. 1995, Smith et al. 2000, Stapleton and Summers 2002, Reitz et al. 2003, Summers et al. 2004, Frank and Liburd 2005). UV-reflective mulches reflect short-wave light, which repels incoming herbivores, and reduces their incidence of alighting on plants. This, consequently, affects the rate of virus transmission and spread by these pests.

Synthetic mulches have also been shown to increase yields (Summer et al. 2004, Frank and Liburd 2005). Their use has been adopted in many parts of the world including Florida in production of vegetables. UV-reflective mulches however, lose their efficacy once the crop canopy covers 70% of the mulch (Smith et al. 2000). In addition, they are expensive and pose a problem of disposal, which may have an undesirable effect on the environment.

Living mulches are a low-cost alternative to synthetic mulches (Hilje et al. 2001) and are safe for the environment. The main idea of using these mulches is to provide resources for natural enemies and manipulate host seeking behavior of insect herbivores (Roots 1973, Hilje et al. 2001). For instance, whiteflies and aphids locate their host through indiscriminate visual attraction to yellow (green) (Kring 1972, van Lenteren 1990). Alate aphids locate their host by contrasting the soil background with the green color of the foliage. Thus, reducing the contrast between the plant and the bare ground as with living mulches will affect the number of insects alighting on the host plant (Hilje et al. 2001, Cradock et al. 2002, Hilje and Stansly 2007).

Living mulches in zucchini squash have been demonstrated to reduce the number of whiteflies and aphids and delay the onset of insect-borne viruses in squash in Florida and Hawaii.
In addition, they have been shown to increase yields in squash and other vegetables when compared with the bare ground (Hooks et al. 1998, Frank and Liburd 2005, Hilje and Stansly 2007).

**Justification**

Living mulches are a cheaper and more sustainable alternative to reflective mulches. Zucchini squash interplanted with living mulch, buckwheat *Fagopyrum esculentum* Moench had lower populations of whiteflies and aphids and delayed the first appearance of virus incidence in the crop (Hooks et al. 1998, Frank and Liburd 2005). Reflective mulches have been shown to reduce whitefly and aphid populations and subsequently delay the onset of the viral transmitted diseases (Stapleton and Summers 2002, Summers et al. 2004, Frank and Liburd 2005). However, the reliance on living or reflective mulch as a single tactic is inadequate to control virus spread (Polston and Anderson 1997). Furthermore, reflective mulches lose its insect repelling ability once the plant growth covers the reflecting surfaces (Smith et al. 2000). It is, however, not yet known if aphid and whitefly populations can be further reduced by the addition of a reduced-risk insecticide that could lead to more economic gains. Further reduction in whitefly and aphid populations will increase the number of marketable fruits and subsequently enhance growers’ profits. Imidacloprid (Admire 2F) is an effective reduced-risk insecticide with systemic activity and can be used to manage whiteflies and aphids. It is however not known if imidacloprid will affect the natural enemy populations associated with living mulch in zucchini.

There is a need to integrate other tactics with reduced-risk insecticide to prolong their use in managing whiteflies and aphids and other problems in cucurbits. Since isolated cases of resistance to whiteflies have already been reported (Osborne 2006 personal comm.). In our study, Admire 2F will be used once at the beginning of the season when control is most needed.
Hypothesis

It is hypothesized that the use of synthetic (reflective) and living mulches (buckwheat) in combination with a reduced-risk insecticide (Admire 2F) will further suppress whitefly and aphid populations and subsequently delay the onset of viral diseases.

The goal of my study is to determine if mulches in combination with Admire 2F (imidacloprid) could offer added advantage in suppressing key pests as well as insect-transmitted viruses and consequently increase yields. In addition, I seek to demonstrate an economic viable management program for aphid and whiteflies and their related viral diseases of squash that can be used in Florida and elsewhere.

Specific Objectives

• To investigate the use of reflective and a living mulch, buckwheat (*Fagopyrum esculentum* Moench) with and without imidacloprid for controlling aphids and whiteflies.

• To study the effects of reflective and living mulch (buckwheat) with and without imidacloprid on natural enemies (beneficial insects) that suppress aphid and whiteflies.

• To evaluate the effectiveness of reflective and a living mulch with and without imidacloprid to viral diseases of zucchini.

• To study the impact of reflective and a living mulch with and without imidacloprid to plant size and consequently the marketable yields of zucchini.
CHAPTER 2
LITERATURE REVIEW

Whiteflies

Whiteflies, *Bemisia tabaci* Gennadius 1889 (Hemiptera: Aleyrodidae) are a serious pests in many cropping systems worldwide (Brown et al. 1995, Smith et al. 2000, Rauch and Nauen 2003, Denholm and Nauen 2005). *Bemisia tabaci* is a highly polyphagous insect attacking approximately 540 different plant species in 77 families (Basu 1995). Both the adults and nymphs cause direct damage through sucking the plant sap (Brown et al. 1995). Symptoms associated with direct feeding include chlorotic halos associated with reduced chlorophyll levels within 1-2 mm of the feeding site of the nymph (Basu 1995, McAuslane et al. 2004). Plant damage can also occur indirectly by inducing systemic plant disorders (Yokomi et al. 1990) and transmitting viruses (Polston and Anderson 1997). In addition, whiteflies excrete honeydew, a sugar-rich substrate that promotes the growth of sooty mold (*Capnodium* spp.) on harvestable plant parts and leaves, lowering their quality (Basu 1995, Brown et al. 1995).

Squash silver leaf disorder (SSL) is a systemic plant disorder associated with the feeding of immature whiteflies (*B. biotype of B. tabaci*) and characterized by silvering of the upper leaf surface (Schuster et al. 1991, Yokomi et al. 1990, Hooks et al. 1998, Frank and Liburd 2005). The initial stage of SSL includes yellowing of veins in expanding leaves and as symptoms progress, the entire upper leaf surface becomes silvered. This silver appearance is due to the formation of air space within the mesophyll and palisade cell layers and separation of the upper epidermis from the underlying palisade cells (Paris et al. 1987, McAuslane et al. 2004).

Furthermore, infested leaves of *Cucurbita* plants have reduced levels of chlorophyll and higher reflectance than normal leaves (McAuslane et al. 2000, McAuslane et al. 2004). The
silvering on the leaves is irreversible and symptoms severity increases as the number of immature whiteflies per plant increases. As few as two immature whiteflies per plant, feeding for about 14 days are able to induce silverleaf symptoms on zucchini (Costa et al. 1993). Symptoms are also manifested on the fruits where green-fruited squashes appear lighter in color or the color is streaked longitudinally while the yellow-fruited squash appear pale or white (Schuster et al. 1991, Yokomi et al. 1990).

_Bemisia tabaci_ was not a pest in Florida cucurbits production until 1987 when an outbreak occurred on poinsettia _Euphorbia pulcherrima_ (Poinsettia), which was associated with a new biotype, B. This biotype later spread to other crops including squash and tomatoes (Mossler and Nesheim 2001). The B biotype was thought to have been introduced into Florida in the early 1980s possibly through the movement of ornamentals (Brown et al. 1995, Polston and Anderson, 1997), but economic losses were not observed until an outbreak of the disorder occurred during the 1987-88 vegetable season (Schuster et al. 1991). Economic losses to many crops as a result of direct feeding damage and whitefly-transmitted geminiviruses led to the rise of _B. tabaci_ as a pest and virus vector in the tropics and subtropics in the late 1980s (Yokomi et al. 1990, Schuster et al. 1991, Brown et al. 1995, Polston and Anderson 1997).

**Taxonomy**

Infestation on poinsettias in Florida indicated a new strain, since the strain previously, strain A was not affecting poinsettia. The new strain was named strain B, or biotype B to distinguish it from the existing strain A or biotype A. These two biotypes have similar morphological appearance but different biological characteristics (Brown et al. 1995). It is known that B-biotype has a wider host range and lays more eggs than A-biotype. Also, based on the amount of honey dew produced, B-biotype ingests more phloem sap than biotype-A (Brown et al. 1995). The feeding of immatures of the B-biotype can cause plant disorders, which is not
the case with biotype A. In addition, B-biotype has greater efficiency in transmitting geminivirus
diseases than the A strain (Brown et al. 1995) but not clostroviruses in which strain A is better.

According to DNA differentiation tests and mating incompatibilities as evidenced by the
absence of females in the progeny, the B-biotype was described as a distinct species and a
common name ‘silverleaf whitefly’ was suggested due to the damage they cause on squash
(Perring et al. 1993). Concurring with the earlier described differences of strain B with the other
existing strains, Bellows et al. (1994) added the morphological differences based on the fourth
instar pupal case. These differences alongside with allozymic diagnoses allowed them to classify
the strain as a new species, *Bemisia argentifolii* (Bellows & Perring) (Bellows et al. 1994).

This naming has undergone some debates with some colleagues arguing that if B-biotype
is a new species then all the other biotypes should be elevated to species level. In this regard,
Brown et al. (1995) proposed that *B. tabaci* is in fact a ‘species complex’. At the time of writing,
‘B-biotype’ is the most commonly used name.

**Biology and Behavior of B Biotype**

The B biotype of *B. tabaci* has six life stages: egg, four instars, and the adult. The eggs are
usually elongate-oval but occasionally may be reniform. The apex distal end of the egg is acute
and the basal portion is usually broad with a pedicel or stalk of varying length by which the
female attaches the egg to the host (Gill 1990, Byrne and Bellows 1991). Eggs may be laid
singly in small groups or in circular or semicircular pattern on the underside of the leaf due to the
rotation of the female as she oviposits (McAuslane et al. 2000). Freshly laid eggs are translucent,
creamy white but turn pale brown just before hatching. Each female can lay a minimum of 50
eggs and up to 300 eggs have been recorded (Basu 1995).

The crawler is the first instar, and the only mobile stage of the immature stages. Crawlers
are pale, translucent white, oval with a convex dorsum and flat ventral side (Basu 1995). The
stage has well developed legs and antenna. After emerging, the crawler moves and settles a few millimeters from the egg to find a feeding site but they are also capable of moving within the leaves (Triplehorn and Johnson 2005). Once settled and feeding the crawler excretes a small amount of wax (Gill 1990). Subsequent instars (second, third and fourth) are sedentary and only have limited movement during molts when they reinsert their stylets. They both have a scale-like covering, which is a waxy secretion that helps the instars adhere themselves to the leaf surface (McAuslane 2000, Triplehorn and Johnson 2005).

The fourth instar has three distinct developmental stages even though there is no molting between them: substage one is a feeding, flattened and opaque nymphal phase, substage two is a nonfeeding nymphal phase, which is thickened and covered with wax, and a pharate adult phase. The early stage (substage one) is feeding and hence only the last nymphal phases are referred to as a pupa. The major difference between the substages two and three is the red eyes which are more visible in the pharate stage due to the developing adult inside them (Gill 1990, Byrne and Bellows 1991, Osborne and Landa 1992, Basu 1995).

Adults emerge through a T-shaped slit in the pupal case of the last nymphal instar. A characteristic round-shaped hole is left on the case if an adult parasitoid emerged from the pupa (McAuslane 2000). The adult head is broader than long, with seven-segmented filiform antennae. Mating can occur within hours of emergence once the wings are hardened and expanded. Mated females produce both females and males whereas unmated ones only males (Byrne and Bellows 1991, McAuslane 2000).

Females of *B. tabaci* usually oviposit on the abaxial side of young leaves and the same leaf is used for oviposition and feeding. They usually prefer a moderate degree of pubescence to either glabrous or extremely hairy leaf surfaces for oviposition. *Bemisia tabaci* refrains from
ovipositing on very young leaves (van Lenteren 1990). Vertical distribution patterns along the plant stratum have been described for *B. tabaci* life stages. Eggs are usually found in the upper plant strata where they are laid while the mature nymphs are on the lower strata. Other factors that seem to affect oviposition and feeding site selection by whiteflies include phototropism, leaf shape, and nitrogen content (Bentz et al. 1995, McAuslane et al. 2000).

Whiteflies have been described as weak fliers and move with the wind current, as they probe host plants. *Bemisia tabaci* is known to orient toward either yellow or blue/ultraviolet light but not both at the same time, a behavior that is related to migration and plant colonization (Basu 1995, Antignus 2000). Ultraviolet light is related to migratory behavior whereas yellow radiation induces vegetative behavior, which could be a part of host selection mechanism. This is important as whiteflies do not appear to have olfactory cues in host-selection (van Lenteren 1990, Byrne and Bellows 1991, Antignus 2000). Once within the host location, whiteflies use color as cue to select landing sites for feeding and oviposition (Byrne and Bellows 1991).

**Monitoring**

Adult whiteflies can be monitored by use of yellow sticky traps and leaf turn method in-situ. In-situ counts are done to estimate the absolute population in the field. This should be done in the morning when the whiteflies are least mobile. Yellow sticky traps have been reported to give a good correlation between catches and actual whitefly numbers in the field in some systems. For this reason, these traps have been proposed for monitoring whiteflies in integrated pest management systems (Basu 1995). Nymph age tends to correlate with leaf age since they complete their development on the leaf they were oviposited. Estimates of egg density are usually taken from upper stratum leaves, while nymphaal densities are taken from the middle and lower canopies depending on the host crop (Basu 1995). Quantification of immature whiteflies
involves cutting of leaf discs of a designated area from selected leaves. The leaf disks are then examined under a microscope for identification of nymphal stages.

**Whitefly-Transmitted Geminiviruses**

*Bemisia tabaci* biotype B has been described as a notorious pest due to its ability to transmit viruses to various crops. Whitefly-transmitted viruses cause yield losses worldwide on important food and industrial crops (Polston and Anderson, 1997). Yield losses due to whitefly-transmitted viruses have been described to be in the range of 20-100% depending on the crop, season, and vector prevalence (Basu 1995). Before 1990, geminiviruses transmitted by whiteflies (*Bemisia tabaci* Genn.) were primarily a problem for legume production in the Western Hemisphere. Emergence and spread of new geminivirus diseases have been directly linked to evolution of variants of the viruses (Varma and Malathi, 2003), the appearance of biotype B and the increase of the vector population (Polston and Anderson, 1997, Varma and Malathi, 2003) in the Americas and the Caribbean Basin.

The geminivirus genome is made up of circular, single-stranded DNA, which is encapsidated in twinned subunits of a single capsid protein. They form the second largest family, Geminiviridae of plant viruses. In terms of genome structures, most of them are bipartite, having two equal components, A and B, while the monopartite have one larger DNA component. Geminiviruses have been classified into four genera: *Mastrevirus*, *Curtovirus*, *Topocuvirus*, and *Begomovirus*, depending on their vector and genomic characteristics (Varma and Malathi, 2003). The majority of the known geminiviruses (80%) is transmitted by whiteflies: Begomoviruses, which have mostly bipartite genomes and infect dicotyledonous plants.

Geminiviruses are transmitted in a persistent, circulative way (Polston and Anderson, 1997). They have a minimum acquisition period of 30-60 min, with inoculation periods that are less than 30 min, and infectivity is retained for at least few days. Typical symptoms include vein

**Aphids**

Aphids are one of the most varied and most studied groups of the hemipterans (Kring 1972, Powell et al. 2005). Their reproductive potential, salivary secretions, and ability to transmit viral diseases make them the most potent and world-wide enemies of many crops (Brown et al. 1993, Kucharek et al 2001, Stapleton and Summers 2002, Soria et al. 2003, Liburd and Frank 2007, Ng and Perry 2004). Viral diseases cause damage to both yield and fruit quality resulting in significant losses (Kucharek et al. 2001). More than 50 viruses have been listed that affect cucurbits. Until 2001, there were only five reported aphid-transmitted viruses affecting cucurbits in Florida. These included *Cucumber mosaic virus* (CMV), *Zucchini yellow mosaic virus* (ZYMV), *Watermelon mosaic virus strain 2* (WMV-2) now called WMV, *Papaya ring spot virus type W* (PRS-V-W) and *Watermelon leaf mottle virus* (Purcifull et al. 1998). PRSV-W and WMV are the most commonly occurring viruses in Florida. Another aphid-transmitted virus that has emerged recently in Florida is PRSV-Tigre (Webb et al. 2003). Typical symptoms of these viruses on the leaves include: leaf curling downward and wrinkling, vein banding, mosaic patterns, mottling, blistering, leaf distortions and reduction of leaf lamina (strapleaf). Fruit symptoms include discoloration with mosaic patterns, reduced size, misshapen, with warty texture (Alderz et al. 1983, Schubert and McRitchie 1984, Purcifull et al. 1988, Mossler and Nesheim 2001, Kucharek et al. 2001, Gianessi et al. 2002).

**Biology and Behavior of Aphids**

Variations in aphid life cycles are well known. They alternate primary hosts with secondary hosts, sexual with parthenogenetic forms, wingless with winged forms, and migrant with
nonmigrant forms. All these variations may sometimes occur within the annual cycle of a single species as it does in the melon aphid, *Aphis gossypii* Glover (Kring 1972). Holocyclic species have a sexual generation in the autumn and during the remainder of the year, they are asexual bearing females (viviparae). Anholocyclic aphids have no sexual generation, and only viviparae are produced throughout the year. Heteroeccious or host-alternating aphids use different species for winter (primary) and summer (secondary) hosts while monoecious species utilize the same plants throughout the year (Halbert and Voegtlin 1995).

The evolution of parthenogenesis, enabling reproduction without males for part or all the lifecycle, confers a reproductive advantage by doubling the intrinsic rate of population increase (*r*<sub>m</sub>) relative to sexually reproducing individuals (Powell et al. 2005). When conditions are favorable (low aphid density, high host quality), most aphid species continue to maximize investment in reproduction by producing apterous progeny. As density increases, and plant nutritional quality declines, production of alate offspring is stimulated.

Host selection by aphids is not a random process (Powell et al. 2005, Nault 1997). They employ a variety of sensory and behavioral mechanisms to locate and recognize their host plants (Powell et al. 2005). Landing by migrating alate aphids involves a phototactic response to plant-reflected wavelengths (Nault 1997). After landing, a variety of cues may be detected as the aphid walks across the leaf surface. Stylet penetration is attempted as a reflex following tarsal contact with any solid surface even in the presence of repellent cues (Powell et al. 2005). The first few stylet penetrations initiated after the plant contact are usually brief (<1 min) and are limited to the epidermis when the aphid tries to distinguish host from non host. Aphid stylets and labium apparently lack external contact chemoreceptors and therefore plant sap may have to be ingested to contact a gustatory organ located in the epipharyngeal area. If the sap is desirable it is sucked
into the cibarium (sucking pump). Aphids make one or more probes before taking flight or moving to the lower leaf surface where they establish contact with phloem (Nault 1997, Powell et al. 2005). This behavior is especially important since transmission of stylet-borne viruses can occur from the test probes (Nault 1997). For this reason, non colonizing aphids can be more important in virus transmission than those that colonize the crop.

Several factors, such as effects of light, sound, and odor are responsible for active selection of alighting surfaces by aphids (Kring 1972). Alighting may be a response stimulus to a particular site (colored object, trap, or plant) or it may be the result of a lack of interfering stimuli. For this reason, takeoff and flight activity are increased in the presence of shortwave light. Certain white surfaces and aluminum surfaces that reflect shortwave, long wave and varying amount of infrared energy do reduce the number of aphids alighting on plants or in traps they surround (Kring 1972). Host-seeking aphids are attracted to the relatively long wave radiation reflected from the soil and vegetation (Cradock et al. 2002). Yellow and green surfaces (wavelength 500-580nm) are particularly attractive.

**Monitoring**

Several trapping techniques can be used to monitor alate aphids. These aphids have poor visual acuity but they are known to be responsive to yellow and green frequencies (wavelength 500-580 nm). For this reason commercial yellow sticky traps are available to monitor aphid populations in the field and greenhouse. Pan traps are small containers that are filled with a preservative for collecting aphids. They have functions similar to the sticky traps, but the aphids are collected in the liquid preservatives. Suction traps have also been used to monitor aphids in small grain fields.
Aphid-Transmitted Viruses

Aphid-borne viruses can kill infected plants and significantly affect the crop yield (Eastop 1977). Aphid-borne non-persistently transmitted viruses are of economic importance for many cucurbit growers. They form the bulk of aphid transmitted viruses (Hull 2002, Hooks and Fereres, 2006). Non-persistent viruses are transmitted by many aphid species including *Aphis gossypii*, *Myzus persicae*, *Acyrthosiphon (Aulacorthum) solani*, *Aphis craccivora*, and *Macrosiphum euphorbiae* and many others as they search for suitable hosts to invade. *Aphis gossypii*, the melon aphid is considered as one of the most important vector because it is able to colonize and reproduce on cucurbit crops (Damicone et al. 2007). In other cases this aphid has not been considered important because it tends to remain on the plant (Mora-Aguilera 1995).

Non-persistent viruses are defined as those that have a very short acquisition and inoculation times (seconds to minutes) (Nault 1997, Ng and Perry 2004). The viruliferous aphids retain their ability to infect for a very short period. These viruses have been termed as ‘stylet-borne’ viruses due to the fact that the virions are restricted to the stylets. These viruses are transmitted to healthy plants within very brief probing period (Nault 1997). It is known that during acquisition and inoculation stylets of aphids do not penetrate beyond epidermal cells (Nault 1997). Conversely, semi-persistent viruses are vectored by a few aphid species that will be able to feed on and colonize the host plant. Acquisition of the virions occurs within minutes, but the ability to transmit can be increased with prolonged feeding (Ng and Perry, 2004). In addition, the virions are retained for hours to days within the body of the vector. Semi-persistently transmitted viruses have also been termed as ‘foregut borne’ and like the non-persistently transmitted viruses they cannot be recovered from the hemolymph (Nault 1997). Furthermore, the ability of viruliferous immatures to infect other plants is lost during molting in both types of transmission.
On the other hand persistent transmitted viruses require longer periods to be acquired or transmitted (hours to days) (Ng and Perry, 2004). They also require more than one week latent period (Nault 1997). Furthermore, infected aphids remain viruliferous for extended periods.

**Management of Whiteflies and Aphids and their Related Problems**

In the zucchini crop, no single control method has been used to control whiteflies and aphids and their associated problems (Hooks et al 1998, Frank 2004). Whiteflies and aphids are notorious pests in zucchini and greatly known for the multi-component damage they cause to plants. Traditionally, pesticides have been the main management tool for whiteflies and aphids. However, due to the problems associated with pesticidal tactics, other alternative methods have been investigated. It is known that non-persistently transmitted viruses have not been managed effectively by the use of insecticides since the viruses transmitted before the vectors are killed (Brown et al. 1993, Soria et al. 2003). Similarly, geminiviruses have not been controlled by use of insecticides (Polston and Anderson 1997). Furthermore, the use of insecticides increases cost of production and many virus vectors have developed resistance to various chemical classes (Dittrich 1990, Wang et al. 2002).

Use of systemic insecticides, however, can reduce secondary virus spread in the field by preventing the build up of vectors on primary sources if the major vector is a species that colonizes that crop. Virus control can also be achieved using plant’s genetic resistance. This however, can be of limited use since it is not always available (Cradock et al. 2002). Furthermore, resistance can be overcome under high pressure of viruliferous populations. Tomato varieties with resistance genes of Tomato yellow leaf curl virus (TYLCV) were overcome when moderate to high populations of viruliferous whiteflies were inoculated early in the growing season (Polston and Anderson, 1997). With this in mind, vector management is very important in the control of insect-transmitted viruses.
Cultural control techniques have been proposed as means of managing whiteflies and aphids and their related problems (Antignus 2000). These techniques are tailored toward manipulating the insects’ behavior in host recognition, resulting in arrestment of the vectors before they land on the host plant.

**Living Mulches**

Living mulches are cover crops that are intentionally maintained as living ground cover throughout the growing season of the main crop. They are distinguished from other cover crops in that they are not removed before the main crop is planted (Nakamoto and Tsukamoto 2006). There has been increased interest in the use of living mulches for pest suppression as a means of responding to environmental concerns regarding the use of pesticides. These cover crops suppress weeds, reduce soil erosion, enhance soil fertility and quality, and reduce damage imposed by herbivores.

It has been suggested that diverse habitats contain lower populations of herbivorous insects as compared to simple habitats. Root (1973) found that insect pests were less dense in *Brassica oleracea* grown in diverse habitats than in pure stands. To explain his findings, he proposed two hypotheses. The natural enemy hypothesis predicts that predator and parasitoid populations will occur in greater diversity and at greater densities in diverse plantings because these systems provide additional resources (e.g. food, shelter) for the enemies. The other hypothesis, resource concentration, predicts that herbivores are more likely to find and remain in pure stands of their host plants because of availability of necessary resources in these areas.

Use of living mulch as a means of vegetative diversification can also affect transmission of insect-vectored viruses depending on the mode of transmission of the particular virus. Inclusion of living mulch provides additional feeding sites for infectious aphids around the crop. This has been termed as a ‘protection crop’ and hence reducing the incidence and the spread of aphid-
borne non-persistently transmitted virus (Toba et al. 1977). In this case the living mulch will be serving as a virus-sink where the ‘infected’ aphid is likely to loose its virus during probing. The protection crop must be immune from the target virus or viruses and suitable for probing by the aphid and should not serve as a host for aphid reproduction (Toba et al. 1977).

Diversified habitats can also affect herbivore damage through their influence on factors such as immigration and emigration of pest or enemies searching behavior of both generalists versus specialist insects. These habitats will not only affect host-finding for host-specific pests but also for species-specific enemies (Costello and Altieri 1995). Furthermore, *Bemisia tabaci* spent less time on host plants and appeared restless in a multiple plant species environment (Frank 2004). Therefore, it is possible that damage associated with the pest will be reduced in such a scenario.

Among the agronomic benefits attributed to living mulches, reduction of damage (direct and indirect) has received attention in crop production (Toba et al. 1977, Andow et al. 1986, Hooks et al 1998, Frank and Liburd 2005, Hilje and Stansly 2007). Use of living mulch has been shown to result in fewer whiteflies, aphids and/or delayed occurrence of insect-transmitted viruses in various crops (Kloen and Altieri 1990, Costello and Altieri 1995, Hooks et al. 1998, Frank and Liburd 2005). However, in cases where excessive competition leads to poor plant quality in mixed cropping systems, the advantages of reduced pest densities may be lost because of poor crop yields (Frank and Liburd 2005).

**Synthetic Mulches**

The synthetic mulches are primarily polyethylene (colored or clear plastic), but may include treated paper, wax coated papers, and aluminum and steel foils. Silver or gray reflective mulches have been used successfully to delay and reduce the incidence of aphid-borne virus diseases in squash and other crops (Brown et al. 1993, Stapleton and Summers 2002, Summers et

Reflective and colored mulches have been reported to modify soil moisture and temperature (Csizinszky et al. 1997), which results in increased crop earliness, growth, and yield (Stapleton and Summers 2002, Greer et al. 2003) and providing greater benefit and cost effectiveness to the grower. Colored or reflective mulches are most effective early in the crop cycle, before the developing plant canopy covers the mulch. They reduce attack by soil pathogens and consequently reduce fruit rot, and fertilizer leaching (Cradock et al. 2002). Plants grown over reflective mulch produced significantly higher yields than those grown on bare ground (Stapleton and Summers 2002, Frank and Liburd 2005). However, disposal of synthetic mulches following crop termination can be problematic and may interfere with farming practices such as cultivation. Furthermore, the effectiveness of the mulches is lost over time through plant growth either by shading or covering the reflective surface (Zitter 1977).

**Reduced Risk-Insecticides**

Reduced-risk pesticides are newer classes of compounds that pose a lower health risk to humans and environment. A reduced-risk insecticide is defined as one that may reasonably be expected to accomplish one or more of the following: 1) reduce pesticide risks to human health, 2) reduce pesticides risks to non-target organisms, 3) reduce the potential for contamination of valued, environmental resources, or 4) broaden adoption of IPM or make it more effective. Imidacloprid is a reduced-risk insecticide that belongs to the neonicotinoids group of insecticides.

Neonicotinoids act on the insect central nervous system as antagonists of the postsynaptic nicotinic acetylcholine receptors (Denholm and Nauen 2005). They show marked selectivity
within the Class Insecta and present no hazard to mammals. They exhibit excellent contact and systemic activity and have become widely used for controlling sucking insect pests including whiteflies, aphids, planthoppers, and leafhoppers (Palumbo et al. 2001, Liu 2004, Denholm and Nauen 2005).

Soil-applied imidacloprid (Natwick et al. 1996) has been the primary insecticide treatment used for B. tabaci control in vegetable, melon, and greenhouse production systems (Palumbo et al. 2001). In treated plants, the compound and its metabolites are initially toxic to feeding adults, and act as anti-feedants. Establishment by immature whiteflies is significantly reduced because of suppressed egg deposition. Residual control of B. tabaci populations can vary from 1 to 10 weeks, depending on formulation, rate, depth of placement, soil type, application method, and cropping system (Palumbo et al. 2001). Due to its rapid uptake and systematic translocation within newly emerging plants, prophylactic applications of imidacloprid have been reported to reduce incidences of aphid-borne viruses (Palumbo et al. 2001). However, unlike most other systemic insecticides, imidacloprid is relatively immobile in the soil and requires precise placement where root uptake can occur (Natwick et al. 1996). Imidacloprid can cause phytotoxicity to foliage on young plants depending on the application rates and timing.
CHAPTER 3
TO INVESTIGATE THE EFFECT OF LIVING (BUCKWHEAT) AND REFLECTIVE MULCHES WITH AND WITHOUT A REDUCED-RISK INSECTICIDE ON WHITEFLIES, APHIDS AND NATURAL ENEMIES IN ZUCCHINI SQUASH

Whiteflies and aphids (Hemiptera: Aphididae) are economic pests of fresh market zucchini squash (Cucurbita pepo L.) affecting production in Florida. These pests cause damage to squash plants directly by sucking on their sap or indirectly by transmitting viruses. Aphids vector non-persistently transmitted viruses, which are considered a major limiting factor to cucurbit production in Florida (Purcifull et al. 1988, Wolfenbarger and Moore 1967). Before 1988, when the first economic damage associated with the pest was recorded on cucurbits the sweetpotato whitefly (Bemisia tabaci Genn.) was not a major pest in Florida, (Schuster et al. 1991). Squash silverleaf disorder (SSL) is a physiological disorder associated with the feeding of immatures of B biotype whiteflies that causes the upper surface of the leaf to be silvered. The symptoms can be present on the fruit causing the color to look paler as in the case of zucchini and yellow squash. Recently, whiteflies have been implicated in the transmission of new whitefly-borne virus diseases in squash (Adkins et al. 2007, Akad et al. submitted). This multi-faceted damage of whiteflies and aphids make them probably the most economic pests of squash.

Control of whiteflies and aphids on squash has been based on the use of insecticides. However, this control cannot be relied on alone as the pests are prone to develop resistance. Additionally, the control of insect-transmitted viruses cannot rely on insecticidal tactics (Kring and Schuster 1992, Brown et al. 1993) especially in the case where the virus is transmitted non-persistently. These viruses are transmitted before the aphid can obtain a lethal dosage to kill them (Stapleton and Summers 2002). Nevertheless, insecticides can be used to reduce the proliferation of viruses by preventing build-up on the host.
Cultural control tactics are safer alternatives and their use has shown some promising results in managing whiteflies and aphids in various crops. In particular, UV-reflective mulch repels aphids (Alderz and Everett 1968, Wolfenbarger and Moore 1968), whiteflies (Csizinszky et al. 1997, Summers and Stapleton 2002, Summers et al. 2004, Frank and Liburd 2005). Alate aphids and adult whiteflies are repelled by UV wavelengths reflected by the silver and aluminum pigment of the synthetic mulch. Conversely, reflective mulches are known to lose their reflectivity to dust or shading as the crop canopy grows and hence they may not be able to provide sufficient protection against insect pest alone (Zitter and Simons 1980, Smith et al. 2000). The addition of a reduced-risk insecticide may maintain or enhance their effectiveness against pests of zucchini squash.

Living mulches are cheaper alternative to reflective mulches. Living mulches have been shown to be effective in distracting whiteflies and other pests from locating their host (Hooks et al. 1998, Frank and Liburd 2005, Hilje and Stansly 2007). These mulches are used to diversify habitats. Such diversified habitats have lower populations of herbivores when compared with monocultures (Root 1973, Andow et al. 1986, Kloen and Altieri 1990, Hooks et al. 1998, Costello and Altieri 1995), which ultimately lead to the delay of the onset of insect-transmitted viral diseases. In recent studies, living mulches have been shown to reduce the damage inflicted by aphids and whiteflies when interplanted with zucchini squash (Hooks et al. 1998, Frank and Liburd 2005).

Despite being shown to have some negative effects on whiteflies and aphids, living mulches have not been tested in combination with a reduced-risk insecticide. It is not known therefore, if the addition of a reduced-risk insecticide will negatively affect the natural enemies associated with the living mulches or produce synergistic effects in reducing pest populations.
The study was conducted with the aim of integrating cultural and reduced-risk insecticide tactics for pest management of whiteflies and aphids in zucchini squash. The hypothesis of the study was that use of the insecticide with the mulches could result in further reduction in pest populations and thus lead to economic gains. Specific objectives were to investigate the effect of living mulch buckwheat, (*Fagopyrum esculentum* Moench), and reflective mulch in combination with a reduced-risk insecticide (imidacloprid) to manage whiteflies and aphids in zucchini squash. The effects of these treatments on the natural enemies associated with squash were also evaluated.

**Materials and Methods**

**Plot Preparation, Irrigation system, and Experimental Design**

The experiment was conducted at the Plant Science Research and Education Unit in Citra, Florida. Field plots measuring 10.36 m x 10.36 m were prepared, each containing four beds. Each plot was separated from the adjacent plots by 7.6 meters of bare soil on all sides and was kept weed free throughout the experiment. Planting beds were prepared with a 6-foot center wheel spacing tractor (2-wheel drive Model 6615, John Deere) and were fumigated with methyl bromide 80/20 formulation (80 % methyl bromide, 20% chloropicrin) at the rate of 283.5 kg/ha. Fumigation was done 2 weeks before planting squash. The fumigant was injected into the soil and immediately the planting rows were covered with the respective synthetic mulch treatments. Drip irrigation lines (5/8 inch, 10 mm thickness) were placed in the center of the beds prior to covering with plastic mulch. Living mulch rows were also fumigated and temporarily covered (2 wk) with synthetic mulch, to allow the methyl bromide to properly fumigate those beds. The plastic mulch was removed before planting buckwheat seeds. Before planting squash, the living mulch; (buckwheat) was hand-seeded between the rows 21 and 18 days in 2005 and 2006 respectively. At the time of planting squash plants the buckwheat mulch was 20 cm high. There
were two rows of living mulch on each side of the squash plants. Living mulch was planted approximately 23 cm adjacent to squash plants (Fig. 3a). In the case of synthetic mulches the entire 76 cm on top bed surfaces were covered with the mulch (Fig. 3b).

Treatments were arranged in a randomized complete block design with four replications. The treatments evaluated consisted of: 1) reflective mulch (1.33 mm thick 66 inch wide) with the reduced-risk insecticide (Admire® 2F Bayer, Kansas City, MO); 2) reflective mulch without Admire® 2F; 3) living mulch with Admire® 2F 4) living mulch without Admire® 2F; and 5) standard synthetic white mulch (1.33 mm, thick, 66 inch wide), which served as the control. The reduced risk-insecticide Admire® 2F was applied 2 weeks after squash planting through the drip lines at the rate of 1.684 L/ha on the appropriate treatments. Planting holes were cut in the center of each plastic mulch strip or on ground unmulched beds, and two squash seeds, cv ‘Wild Cat®’ (Harris Moran Seed Company) were hand seeded per hole. Plant spacing was maintained at approximately 92 cm in the two years of the study with planting dates on 29 September in 2005 and 3 October in 2006. After germination, the missing plants were replaced using already established seedlings from the greenhouse. In 2006, the missing plants were not only from germination failure, but also from crow damage.

Agronomic practices for squash followed the standard production guide for squash in North Florida (Olson et al. 2005). However, no insecticides were applied during the growing season except Admire 2F on the specific treatments. A fungicide, azoxystrobin (Amistar; 80 WP); was sprayed as required against powdery mildew during early stages of the crop. Base fertilizer-dressing N-P-K (10-10-10) at the rate 624 kg per hectare was applied to the soil at planting. For the first four weeks the squash crop was top-dressed with nitrogen, potassium and phosphorous each at 0.7 kg weekly and thereafter increased to 0.9 kg at blossom. Weed
management was done mechanically as required. In 2006, reflective tapes and propane canister were used to keep the crows away during the day time.

**Foliar Sampling**

Adult whiteflies and aphids were counted from the leaves *in-situ* by leaf-turn method. This was initiated 3 weeks after planting and carried out weekly until final harvest. Nine plants located on the outside rows of each plot were randomly selected. Insect pest counts were established by gently turning a leaf and counting the number whiteflies and aphids encountered. A total of nine leaves were counted in each plot giving a total of 36 leaves per treatment.

Nymphal whitefly population was determined from the nine selected leaves (discussed above), three from each stratum (upper, mid, lower). The leaves were excised and placed in a 1-gallon self-sealing Ziploc bags and returned to the Small Fruit and Vegetable IPM Laboratory, UF, Gainesville, FL. In the laboratory, a 3.14-cm² leaf disc was taken from each leaf using a cork borer and examined for whitefly immature stages under a 40X-dissecting microscope (MEIJI EMZ, Meiji techno co. Ltd Tokyo Japan) (as adapted from Frank and Liburd 2005). The distribution of the whitefly immatures along the plant strata was also established.

**Trap Sampling**

Adult whiteflies were monitored with yellow sticky, Pherocon® AM unbaited traps (YST), (Great Lakes IPM, Vestaburg, MI). Three traps were placed in each plot, one in the middle and the other two on a diagonal line at the two opposite sides of the plot. The traps were left in the field for a period of 24 h and collected and taken back to the Small Fruit and Vegetable IPM laboratory to establish counts. The first set of traps were placed into the field one week after germination and there after once every week for eight and six weeks in 2005 and 2006 respectively.
Alate aphids were monitored using blue (PackerWare®) and clear water pan-traps (Pioneer/ Tri-State Plastics Inc., Dickson, KY). Four pan traps were used per plot, two of each color, placed at the four corners of each plot within the interior rows. Each pan trap contained approximately 250 cm$^3$ of 5% detergent solution (Colgate-Palmolive Co. New York, NY). Each trap was supported on a tomato cage and trap height was adjusted accordingly to plant height. The traps were left in the field for one week and sampling was conducted for seven and six weeks in 2005 and 2006 respectively. In 2005, the number of alate aphids trapped were established in the field, while in 2006 bowl contents were emptied into individual vials and labeled accordingly. Vials were then transported to the laboratory for counting.

**Physiological Disorder Evaluation**

In 2006, symptoms of silverleaf on the squash were assessed on 10 plants within the interior rows. Squash with silverleaf symptoms were scored using an arbitrary scale (Yokomi et al. 1990) where index 0 signified a ‘healthy’ leaf with no symptoms and index 5 leaves were completely silvered.

**Natural Enemy Counts**

Natural enemies were sampled using *in-situ* counts from the same zucchini plants as described above. Six leaves from six plants located on the outside row of each plot were randomly selected. The leaves were gently turned and the numbers of natural enemies encountered were recorded from them. Sampling was initiated 3 weeks after planting and conducted every other week until the final harvest.

**Statistical Analysis**

Data from whitefly and aphid counts (foliar, whitefly immatures, and trap counts) were analyzed using repeated measures analysis (PROC MIXED, SAS Institute 2003) to examine the interaction effect between treatment and time (sampling weeks). Overall, least square means
values were computed and means were compared to determine the effects of mulch treatments. The standard errors of means (S.E.M) were also calculated (PROC MEANS, SAS Institute 2003).

Data from natural enemy counts, silverleaf score index were subjected to an analysis of variance (ANOVA) using SAS GLM (SAS 9.1 version) and treatment means separated by least significant differences (LSD) test (SAS Institute 2003). Where necessary, the data were log-transformed to meet assumptions for ANOVA. Comparisons of immature counts from the treatments were made based on the average upper, middle and lower leaf disc counts.

Results

Foliar Counts

In 2005, buckwheat with Admire 2F was equally effective as reflective mulch with Admire 2F in controlling adult whiteflies per leaf ($F = 43.29; \text{df} = 4, 72; P < 0.0001$) [Table 3-1]. Similarly, buckwheat alone was not significantly different from reflective mulch alone. Addition of Admire 2F to buckwheat and reflective mulches resulted in enhanced control of whiteflies per leaf compared with the other treatments. Conversely, plants grown with white mulch (control) had significantly higher number of whiteflies per leaf than all the other treatments. There were significant ($F = 7.86; \text{df} = 20, 72; P < 0.0001$) interaction effects between treatments and sampling weeks. Treatment differences were observed in 5 out of the 6 weeks sampled.

Apterous aphid counts per leaf indicated that buckwheat with Admire 2F had similar numbers as those recorded in reflective mulch with Admire 2F. Both treatments resulted in significantly fewer numbers of aphids than all the other mulches tested including white mulch (control) ($F = 10.42; \text{df} = 4, 72; P < 0.0001$) [Table 3-1]. The addition of Admire 2F to the mulches reduced the number of apterous aphids per leaf but not alate aphids. There were significant ($F = 3.37; \text{df} = 20, 72; P < 0.0001$) interaction effects between treatment and
sampling week. Of the six weeks sampled, treatment differences were observed in 3 out of the 6 weeks. Similarly, there were significant ($F = 2.40; \text{df} = 20, 72; P < 0.0036$) interaction effects between treatment and time in alate aphids counted per leaf. Among the weeks sampled, treatment differences were only observed in 2 weeks of sampling, and during both dates reflective mulch with Admire 2F had the highest alate aphids counted per leaf. Overall, reflective mulch with Admire 2F recorded significantly higher number of alate aphids per leaf compared with all the other treatments except reflective mulch alone ($F = 5.13; \text{df} = 4, 72; P < 0.0011$) [Table 3-1].

In 2006, fewer adult whiteflies per leaf were recorded compared with 2005, though a similar trend was observed among the treatments (Table 3-2). Plant growing with living and reflective mulch treatments continued significantly fewer of adult whiteflies per leaf than those growing on white mulch ($F = 49.71; \text{df} = 4, 72; P < 0.0001$) (Table 3-2). Buckwheat with Admire 2F had the fewest whiteflies counted per leaf, but it was not significantly different from reflective with Admire 2F. The addition of Admire 2F to the buckwheat treatment resulted in further reduction of whiteflies per leaf. During the study there were significant ($F = 3.63; \text{df} = 20, 72; P < 0.0001$) interaction effects between treatment and sampling week, with significant treatment differences observed in all weeks.

In the case of aphids per leaf, although there were significant ($F = 2.01; \text{df} = 20, 72; P < 0.0166$) interaction effects between treatments and time the difference was only observed in one week of sampling. Both buckwheat and reflective mulches with Admire 2F had significantly fewer apterous aphids than white mulch (control) ($F = 3.14; \text{df} = 4, 72; P < 0.0193$) [Table 3-2]. Buckwheat with and without Admire 2F had significantly fewer of alate aphids than the white mulch which was similar to the reflective mulches. The addition of Admire 2F did not affect the
number of alates per leaf when used with any of the mulches (buckwheat or reflective). Although there were no significant \( F = 0.86; \text{df} = 4, 72; P < 0.6330 \) interaction effects between treatments and time, differences among the treatments were observed on the fifth week of sampling. On this date, white synthetic mulch resulted in significantly higher number of alate aphids than all the other treatments.

**Whitefly Immature Counts**

Results of whitefly immature counts in the laboratory were consistent across the two years. Most of the whitefly nymphs were concentrated in the lower plant stratum with the least on the upper stratum (Figs. 3-2 & 3-3). In 2005, white mulch had significantly higher number of immatures per \( 3.14\text{-cm}^2 \) leaf disc compared with all the other treatments \( (F = 13.91; \text{df} = 4, 72; P = 0.0001) \) [Table 3-3]. Treatments with Admire 2F contained the fewest number of whitefly immatures per leaf disc. Buckwheat alone was not significantly different from reflective mulch alone. There were significant \( F = 2.91; \text{df} = 4, 72; P = 0.0005 \) interaction effects between treatment and time.

In 2006, the number of whitefly immatures varied significantly among treatments \( (F = 11.90; \text{df} = 4, 72; P < 0.0001) \) [Table 3-3]. No significant difference occurred among reflective mulch, reflective mulch with Admire 2F and buckwheat mulch with Admire 2F. The addition of Admire 2F to reflective mulch did not result in further significant reduction of immature whiteflies. Whitefly adult populations on plants growing within buckwheat in combination with Admire 2F resulted in lower whitefly immatures than those in buckwheat mulch alone. Buckwheat mulch with Admire 2F was not significantly different from reflective with Admire 2F and reflective alone. White mulch resulted in significantly higher numbers of immatures per leaf disc compared with all the other treatments. The interaction effects between treatment and
time was significant ($F = 1.90; \text{df} = 20, 72; P < 0.0255$), treatment differences were observed in 4 out of the 6 weeks sampled.

**Trap Counts**

**Whiteflies**

Significantly ($P < 0.05$) fewer adult whiteflies were captured on yellow sticky traps (YST) within reflective and buckwheat treatments with Admire 2F than those within the buckwheat alone and standard white (control) in 2005 ($F = 22.21; \text{df} = 4, 96; P < .0001$) [Table 3-4]. Buckwheat alone was not significantly different from the white mulch (control). Traps set within reflective with Admire 2F caught the least number of whiteflies. There were significant ($F = 4.26; \text{df} = 28, 72; P < 0.0001$) interaction effects between treatment and sampling week. Treatment differences were observed in 6 out of the 8 weeks sampled. In most of the sampling dates, white (control) resulted in the highest number of whiteflies counted per trap.

In 2006 however, YST within the buckwheat alone mulch contained significantly ($P < 0.05$) fewer whiteflies than white mulch, which was higher than all the other treatments ($F = 27.66; \text{df} = 4, 72; P < .0001$) [Table 3-4]. The addition of Admire 2F to buckwheat and reflective mulches did affect the number of whiteflies per trap, and these mulches resulted in significantly fewer whiteflies compared with the mulches tested alone. There were significant ($F = 3.79; \text{df} = 20, 72; P < 0.0001$) interaction effects between treatment and sampling week. Of the six weeks sampled treatment differences were observed in weeks 1, 4, 5, and 6.

**Aphids**

During the 2-yr study, the color of the pan traps (clear versus blue) did not have a significant ($t = -0.86, \text{Pr} > |t| = 0.3908$) in 2005 and $t = 0.03, \text{Pr} > |t| = 0.9736$ in 2006 effect on trap catches and hence means of pooled alate aphid counts are reported here. In 2005, pan traps within reflective mulch with Admire 2F caught significantly higher numbers of alate aphids than
those in the control, which did not differ significantly with those in reflective mulch and buckwheat treatments ($F = 2.51; \text{df} = 4, 268; P = 0.0392$) [Table 3-5].

In 2006, pan traps within the white synthetic mulch caught significantly more alate aphids than all other treatments ($F = 9.54; \text{df} = 4, 84; P < 0.0001$) [Table 3-5]. The addition of Admire 2F did not affect the number of alate aphids captured. Surprisingly, reflective alone had the fewest number of alate aphids trapped in the pan traps of all the treatments evaluated including reflective with Admire 2F. Significant ($F = 3.63; \text{df} = 4, 84; P < 0.0001$) interaction effects between treatments and sampling dates were observed. Among the seven weeks of sampling, treatment differences were observed only in week 2 and 4.

**Physiological Disorders**

Silverleaf symptoms differed significantly among the treatments ($F = 44.60; \text{df} = 4, 34; P > 0.001$). Plants growing within white mulch had almost the entire upper leaf surface silvered as indicated by the high index score (Fig. 3. 4). Treatments with Admire 2F (reflective and buckwheat) had significantly lower index scores than all other treatments. There was no difference between reflective mulch and buckwheat treatment alone.

**Natural Enemies**

The major families recorded in the study were Syrphiridae, Coccinelidae, Chrysopidae, and Apidae and spiders. In 2005, significantly more natural enemies were recorded on squash in the buckwheat treatment with Admire 2F than any other treatment except buckwheat alone ($F = 3.43; \text{df} = 4,353; P = 0.009$) [Table 3-6]. The treatment reflective mulch with Admire 2F had the fewest natural enemies but it was not significantly different from reflective mulch alone. In 2006, there were no significant differences in the number of natural enemies among the treatments ($F = 0.69; \text{df} = 4, 347; P < .6006$) [Table 3-6].
Discussion

The response of whiteflies on zucchini interplanted with living mulch was more consistent than that of alate aphids in the two-yr study. My study shows that living and reflective mulches with and without Admire 2F were able to provide significant protection against whiteflies as compared to the control. Crops grown within these mulches were likely protected from pest infestation due to the negative effect that these mulches have on the orientation of the insect to the plant. Living mulch, (buckwheat) increases the vegetation diversity that is known to decrease the host apparency and hence fewer numbers of whiteflies and aphids landed on squash. On the other hand, UV reflective mulch is known to repel whiteflies and hence prevent them from landing on the host plants (Antignus 2000). This was the case in my study where significantly fewer whiteflies were recorded in reflective mulch. The current study provides another instance where living mulch was demonstrated to control whiteflies. Previous researches have shown that the inclusion of living mulches with zucchini plants (Frank and Liburd 2005), and tomatoes (Hilje and Stansly 2007) successfully reduced whiteflies populations in the main crop.

Whitefly populations were higher in 2005 than in 2006. In both years, foliar counts revealed that buckwheat was equally as effective as reflective mulch when tested alone or in combination with the reduced-risk insecticide Admire 2F. In 2005, however YST indicated that reflective mulch alone was superior to living mulch in controlling whiteflies. The situation was reversed in 2006 when living mulch alone was superior to reflective mulch alone. The reason for these differences are not clear but could be related to stage of maturity of living mulch or other external factors.

Although previous studies have reported reduction of whiteflies when using living mulch, none of the studies evaluated the mulch in combination with a reduced-risk insecticide. I found that addition of Admire 2F to the mulches further reduced whitefly populations. More research is
needed to fully investigate how these two strategies (living mulch with Admire 2F or reflective mulch with Admire 2F can be integrated in zucchini production.

Likewise, the number of nymphs per leaf disc was higher in 2005 than in 2006. Our results indicated that treatments with Admire 2F provided significantly greater protection from whitefly immatures than treatments without. The reduction in number of immature whiteflies per leaf is important since they are responsible for inducing silverleaf symptoms. It was evident that most of the whitefly immatures were concentrated in the lower strata of the zucchini plants. This area provides more nitrogen and fewer trichomes (Bentz et al. 1995). The immature whiteflies of B biotype are associated with a physiological disorder called squash silverleaf symptom (SSL). The occurrence of SSL was recorded in 2006. Silverleaf symptoms were more severe on the white mulch, which had the highest whitefly population compared with all the other treatments. A positive correlation between the number of whitefly immatures and SSL symptoms has been reported before by Costa et al. (1993).

In this study, the mulch treatments had varying effects on alate aphids. These aphids are important in the transmission of non-persistent viruses such as the mosaic viruses. Previous studies have reported that the UV-reflective mulch was able to confuse and repel alate aphids from landing on the plants (Wolfenbarger & Moore 1968, Brown et al. 1993, Summer et al. 2004). Our results in 2006 were in agreement with previous research where the reflective mulch treatments afforded the best protection against alate aphids. In this study it is a reported that addition of Admire 2F did not enhance reduction of alate aphids as revealed by the pan traps and foliar counts. In contrast, Admire 2F provided a significant amount of control of apterous aphids in 2005, but in 2006 the reduction was not as dramatic, perhaps because the populations were not as high. Furthermore, Admire 2F is a systemic insecticide and could be more lethal to apterous
aphids that are sedentary as opposed to the migrating alatae. It is not surprising that mulches without the reduced-risk insecticide, including the white mulch (control), did not differ significantly ($P > 0.05$) from each other in both years. The color of pan traps did not affect the number of aphids caught. This is in harmony with the findings of Frank and Liburd (2005).

In Florida, white mulch is used alone or on top of black in zucchini production during the warm seasons to keep the soil cool (Liburd and Frank 2007, Liburd et al. *in press*). In our study, we report that white polyethylene mulch resulted in consistently high numbers of whiteflies (adults and nymphs) and alate aphids in at least one season. Earlier studies have also reported that white mulch is associated with high numbers of immatures and adult whiteflies (Csizinszky et al. 1997). In addition, a high number of aphids have been trapped out plants with white polyethylene mulch compared with bare ground or / and other mulches (Alderz and Everett 1968, Zitter 1977, Zitter and Simons 1980, Frank and Liburd 2005).

The counts of natural enemies associated with whiteflies and aphids were inconsistent throughout the two field seasons. In 2005, more natural enemies were recorded in plants in plots treated with living mulch (buckwheat) than those with reflective mulch with Admire 2F. However, in 2006 no significant differences among the treatments were recorded. Admire 2F did not have adverse effects on natural enemies associated with the mulches as indicated by non-significant differences between treatments with and without Admire 2F. The natural enemies counted on the leaves were mainly predators and consisted of Syrphiridae, Coccinelidae, Chrysopidae and Apidae and spiders. Spiders made up the bulk (39%) of the recordings followed by coleopterans (29%). Buckwheat is an annual plant whose flowers produce nectar that attracts a high percentage of beneficial insects including pollinators. When used as a living mulch, buckwheat supports a high number of natural enemies, which could contribute to the reduction of
whitefly and aphid populations. It is known that certain species of natural enemies can play a role in regulating whitefly populations (Gerling 1990). In the current study, the natural enemy populations were very low as revealed by the in-situ counts. Therefore other factors contributed to the reduction in the whitefly population.

There are two theories that can be used to explain the reduction of pests in diversified plantings (Root 1973). The first one is ‘resource concentration’ that theorizes that pests will opt to remain and establish in pure stands as opposed to mixed plantings, which may be limited in preferred resources (food, shelter). The second, is the ‘natural enemy’ hypothesis that assumes that diverse habitats have diverse insects and hence predators and parasitoids could contribute to pest reduction. Because no consistent effects on natural enemies were observed I can conclude that resource concentration may have contributed to pest reduction in my study. It can also be speculated that population reduction of whiteflies in the squash planted with the living mulch was related to the host plant quality. In the study, zucchini plants growing with buckwheat were noticeably smaller as indicated by the plant sizes (CHAPTER 5). Quality reduction could be as a result of increased competition for resources such as nutrients, light, and water leading to a higher rate of herbivore emigration and/or lower population growth. Additionally, the differential in immigration and reproduction of herbivores could also be used to explain the differences in herbivore response in crop monocultures and crop mixtures (Andow et al. 1986).

In the current study, it can be concluded that addition of Admire 2F enhanced control of whiteflies and to some extent apterous aphids and not alate aphids. Living mulch with Admire 2F and reflective mulch with Admire 2F gave protection equal against whiteflies and aphids. Admire 2F did not affect beneficial insects associated with the mulches. The economics of
incorporating a reduced-risk insecticide with a living or reflective mulch will have to be studied before recommendations can be made.
Table 3-1. Effect of living and reflective mulches alone or in combination with Admire 2F on the number of adult whiteflies and aphids per zucchini squash leaf, Citra, FL (2005) [Foliar counts]

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Whiteflies (adult)</th>
<th>Aphids (apterous)</th>
<th>Aphid (alate)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reflective + Admire 2F</td>
<td>10.38 ± 2.02 c</td>
<td>0.56 ± 0.21 b</td>
<td>0.92 ± 0.29 a</td>
</tr>
<tr>
<td>Reflective</td>
<td>15.22 ± 2.22 b</td>
<td>3.48 ± 0.80 a</td>
<td>0.69 ± 0.21 ba</td>
</tr>
<tr>
<td>Buckwheat</td>
<td>17.09 ± 2.32 b</td>
<td>3.19 ± 1.02 a</td>
<td>0.31 ± 0.06 c</td>
</tr>
<tr>
<td>Buckwheat + Admire 2F</td>
<td>10.40 ± 1.73 c</td>
<td>0.44 ± 0.12 b</td>
<td>0.37 ± 0.06 bc</td>
</tr>
<tr>
<td>Control</td>
<td>38.27 ± 6.20 a</td>
<td>3.07 ± 0.69 a</td>
<td>0.40 ± 0.10 bc</td>
</tr>
</tbody>
</table>

Means followed by the same letters are not significantly different ($P = 0.05$ according to least square means test following repeated measures analysis, LS).

- $F = 43.29; \ df = 4, 72; \ P < 0.0001$
- $F = 10.42; \ df = 4, 72; \ P < 0.0001$
- $F = 5.13; \ df = 4, 72; \ P < 0.0011$
Table 3-2. Effect of living and reflective mulches alone or in combination with Admire 2F on the number of adult whiteflies and aphids per zucchini squash leaf, Citra, FL (2006) [Foliar counts]

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Whiteflies (adult)</th>
<th>Aphids (apterous)</th>
<th>Aphid (alate)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reflective + Admire 2F</td>
<td>5.13 ± 0.71 bc</td>
<td>0.04 ± 0.02 b</td>
<td>0.25 ± 0.06 ab</td>
</tr>
<tr>
<td>Reflective</td>
<td>7.75 ± 0.96 b</td>
<td>0.28 ± 0.12 a</td>
<td>0.17 ± 0.05 ab</td>
</tr>
<tr>
<td>Buckwheat</td>
<td>8.27 ± 0.97 b</td>
<td>0.21 ± 0.09 ab</td>
<td>0.16 ± 0.04 b</td>
</tr>
<tr>
<td>Buckwheat + Admire 2F</td>
<td>3.23 ± 0.42 c</td>
<td>0.01 ± 0.01 b</td>
<td>0.14 ± 0.04 b</td>
</tr>
<tr>
<td>Control</td>
<td>16.03 ± 2.04 a</td>
<td>0.31 ± 0.11 a</td>
<td>0.29 ± 0.08 a</td>
</tr>
</tbody>
</table>

Whitefly data transformed (log₁₀) before analysis, means are presented in the original counts. Means followed by the same letter are not significantly different ($P = 0.05$ according to least square means test (LS) following repeated measures analysis).

- $^a F = 26.67; \text{df} = 4, 72; P < 0.0001$
- $^b F = 3.14; \text{df} = 4, 72; P < 0.0193$
- $^c F = 0.86; \text{df} = 4, 72; P < 0.6330$
Table 3-3. Effect of living and reflective mulches alone or in combination with Admire 2F on the number of whitefly immatures per treatment in zucchini Citra, FL

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean ± SEM whitefly immature per leaf disc (3.14 cm²)</th>
<th>2005a</th>
<th>2006b</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reflective + Admire 2F</td>
<td>0.33 ± 0.10 c</td>
<td>0.30 ± 0.09 c</td>
<td></td>
</tr>
<tr>
<td>Reflective</td>
<td>1.86 ± 0.32 b</td>
<td>0.98 ± 0.26 bc</td>
<td></td>
</tr>
<tr>
<td>Buckwheat</td>
<td>2.11 ± 0.46 b</td>
<td>1.77 ± 0.45 b</td>
<td></td>
</tr>
<tr>
<td>Buckwheat + Admire 2F</td>
<td>0.42 ± 0.15 c</td>
<td>0.44 ± 0.20 c</td>
<td></td>
</tr>
<tr>
<td>White (control)</td>
<td>3.49 ± 0.77 a</td>
<td>2.83 ± 0.64 a</td>
<td></td>
</tr>
</tbody>
</table>

Means followed by the same letter are not significantly different (\( P = 0.05 \) according to least square means test following repeated measures analysis, LS).

\( a \) \( F = 13.91; \) df = 4, 72; \( P < 0.0001 \)

\( b \) \( F = 11.90; \) df = 4, 72; \( P < 0.0001 \)
Table 3-4. Effect of living and reflective mulches alone or in combination with Admire 2F on the number of whiteflies caught per yellow sticky trap in Citra, FL

<table>
<thead>
<tr>
<th></th>
<th>Mean ± SEM counts per YST</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2005&lt;sup&gt;a&lt;/sup&gt;</td>
<td>2006&lt;sup&gt;b&lt;/sup&gt;</td>
<td></td>
</tr>
<tr>
<td>Reflective + Admire 2F</td>
<td>10.74 ± 1.67 c</td>
<td>13.22 ± 1.54 d</td>
<td></td>
</tr>
<tr>
<td>Reflective</td>
<td>20.80 ± 3.81 b</td>
<td>27.82 ± 4.41 b</td>
<td></td>
</tr>
<tr>
<td>Buckwheat</td>
<td>31.92 ± 6.36 a</td>
<td>18.90 ± 3.30 c</td>
<td></td>
</tr>
<tr>
<td>Buckwheat + Admire 2F</td>
<td>17.33 ± 3.11 b</td>
<td>12.01 ± 1.29 d</td>
<td></td>
</tr>
<tr>
<td>White (control)</td>
<td>51.33 ± 11.32 a</td>
<td>37.72 ± 4.71 a</td>
<td></td>
</tr>
</tbody>
</table>

YST data (2005) whitefly data transformed (log<sub>10</sub>) before analysis, means are presented in the original counts. Means followed by the same letter are not significantly different (P = 0.05 according to least square means test following repeated measures analysis, LS).

<sup>a</sup> F = 22.21; df = 4, 96; P < 0.0001
<sup>b</sup> F = 27.66; df = 4, 72; P < 0.0001
Table 3-5. Effect of living and reflective mulches alone or in combination with Admire 2F on the number of alate aphids trapped per pan-trap in Citra, FL

<table>
<thead>
<tr>
<th></th>
<th>Mean ± SEM counts per pan trap</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2005(^a)</td>
</tr>
<tr>
<td>Reflective + Admire 2F</td>
<td>3.07 ± 0.43 a</td>
</tr>
<tr>
<td>Reflective</td>
<td>2.36 ± 0.36 ab</td>
</tr>
<tr>
<td>Buckwheat</td>
<td>2.14 ± 0.27 ab</td>
</tr>
<tr>
<td>Buckwheat + Admire 2F</td>
<td>2.21 ± 0.28 ab</td>
</tr>
<tr>
<td>White (control)</td>
<td>1.97 ± 0.28 b</td>
</tr>
</tbody>
</table>

Means followed by the same letter are not significantly different ($P = 0.05$ according to least square means test following repeated measures analysis, LS).

\(^a\) $F = 2.51$; df = 4, 268; $P < 0.0392$

\(^b\) $F = 9.54$; df = 4, 84; $P < 0.0001$
Table 3-6. Effect of living and reflective mulch alone or in combination with Admire 2F on number of natural enemies per treatment in zucchini Citra, FL

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Mean natural enemies per treatment ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2005&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Reflective + Admire 2F</td>
<td>0.18 ± 0.06 c</td>
</tr>
<tr>
<td>Reflective</td>
<td>0.26 ± 0.06 bc</td>
</tr>
<tr>
<td>Buckwheat</td>
<td>0.40 ± 0.08 ab</td>
</tr>
<tr>
<td>Buckwheat + Admire 2F</td>
<td>0.49 ± 0.08 a</td>
</tr>
<tr>
<td>White (control)</td>
<td>0.28 ± 0.06 bc</td>
</tr>
</tbody>
</table>

Means followed by the same letter are not significantly different $P = 0.05$ (LSD)

<sup>a</sup> $F = 3.43$; df = 4, 353; $P < 0.009$

<sup>b</sup> $F = 0.69$; df = 4, 347; $P < 0.6006$
Figure 3-1. (A) Zucchini squash growing with living mulch (B) Zucchini squash growing on UV-reflective mulch
Figure 3-2. Distribution of immature whiteflies on different plant strata of zucchini in Citra, FL (2005)
Figure 3-3. Distribution of immature whiteflies on different plant strata of zucchini in Citra, FL (2006)
Figure 3-4. Effect of living and reflective mulches alone or in combination with Admire 2F on silverleaf symptoms presented by the score indices per treatment in zucchini Citra, FL (2006).
Virus diseases have a great impact on cucurbit vegetable production in the United States including Florida (Purcifull et al. 1998, Gaba et al. 2004, Damicone et al. 2007). Unlike most of the other diseases in squash virus rely on insect vectors for their spread from one plant to another. Aphids (Hemiptera: Aphididae) and whiteflies are among the most important vectors transmitting viruses to zucchini squash, *Cucurbita pepo* L. (Nault 1997, Ng and Perry 2004). Aphids are known to transmit potyviruses and cucumoviruses including *Papaya ring spot virus-watermelon strain* (PRSV-W), *Watermelon mosaic virus* (WMV), *Zucchini yellow mosaic virus* (ZYMV) and *Cucumber mosaic virus* (CMV). These viruses are transmitted in a non-persistent manner, which is characterized by the short time needed for virus acquisition and transmission to a healthy plant. Similarly, the viruses have a short retention time in the aphids. Whiteflies (B-biotype, *Bemisia tabaci* Genn.) also cause damage through the transmission of plant viruses.

There are several whitefly-transmitted viruses reported to infect squash, including *Cucurbit leaf crumple virus* (CuLCrV) also known as *Cucurbit leaf curl virus* (CuLCV), *Squash leaf curl virus* (Brown et al. 2002), *Squash vein yellowing virus* (SqVYV) (Adkins et al. 2007) and *Cucurbit yellow stunting disorder virus* (CYSDV) (Kao et al. 2000). Among these diseases, only CuLCrV and SqVYV have been recorded in Florida. *Cucurbit leaf crumple virus* is a begomovirus, which is transmitted in a persistent, circulative manner by whiteflies.

Pesticides have been the primary tactic for managing whiteflies and aphids. This method has limitations in that insecticides have not sufficiently controlled virus transmission, especially when the whitefly population is high. It is even more problematic in case of aphid vectors where the virus is transmitted before the insecticide kills them (Brown et al. 1993, Kring and Schuster...
In addition, insecticide resistance of whiteflies (Dittrich et al. 1990) and aphids (Wang et al. 2002) to selected chemical classes has further restricted their use. Nonetheless, insecticides can be used to decrease the spread of the virus by reducing the vector population at the primary and secondary source (Brown et al. 1993). In addition, several non-pesticidal methods, including cultural control practices that are intended for reducing the vector population and hence interrupting their epidemiological cycle. In this light, several control methods have been evaluated for the control of viral diseases in cucurbits including: border crops, intercrops, mineral oils (Zitter and Simons 1980, Damicone et al. 2007, Fereres 2000), living mulches (Hooks et al. 1998, Frank and Liburd 2005), floating row covers (Webb and Linda 1992), and reflective mulches (Alderz and Everett 1968, Wolfenbarger and Moore 1968, Greenough et al. 1990, Kring and Schuster 1992, Stapleton and Summer 2002, Summers et al. 2004, Frank and Liburd 2005).

Synthetic UV-reflective mulch has been reported to successfully protect various vegetable crops against insect pests and hence reduce the incidence of viral diseases they transmit (Csizinszky et al. 1997, Reitz et al. 2003, Stapleton and Summers 2002, Summers et al. 2004). Reflective mulches reduce whitefly and alate aphid populations because they reflect short-wave UV light, which repels incoming insects, thus preventing them from alighting on plants (Zitter and Simons 1980). The crop is thus protected against aphids and whiteflies during the early growing period and consequently could delay the onset of insect-vectored viruses.

Living mulch is a cost effective alternative to reflective mulches (Hilje et al. 2001). These mulches have been shown to be effective in hindering whiteflies and other pests from locating their hosts and consequently transmit viruses (Hooks et al. 1998, Frank and Liburd 2005, Hilje and Stansly 2007). Hilje and Stansly (2007) reduced the number of adult whiteflies and the
incidence of *Tomato yellow mottle virus* (ToYMoV) in tomatoes in Costa Rica. The mulches have also been used to diversify habitats in zucchini plantings and hence influence the ability of herbivores to recognize their host, resulting in reduced spread of the viruses they vector (Hooks et al. 1998, Liburd and Frank 2007). Furthermore, the mulches provide food resources (honey, pollen) and shelter for natural enemies that contribute to pest reduction (Root 1973).

Neonicotinoid insecticides, such as imidacloprid (Admire 2F) [Bayer Cropscience US], are systemic in plants when applied as soil drenches and can assist in managing whiteflies (Palumbo et al. 2001). However, frequent application of neonicotinoids and high populations of whiteflies can increase selection pressure, which may eventually reduce their efficacy (Liburd and Frank 2007).

Previous studies have shown that buckwheat (*Fagopyrum esculentum* Moench) used as a living mulch has potential to reduce insect pests and spread of viruses in zucchini crop (Hooks et al. 1998, Frank and Liburd 2005). The goal of my research was to develop sustainable integrated pest management strategies for whiteflies and aphids and the viruses they vector by integrating cultural control and reduced-risk pesticides tactics. Specifically, I wanted to determine if reduced pest populations in mulches in combination with Admire 2F (imidacloprid) could offer the added advantage of suppression of insect-transmitted viruses. This study evaluated the effect of silver reflective mulch and living mulch alone or in combination with Admire 2F (imidacloprid) to reduce whitefly and aphid incidence and spread of viral diseases on zucchini squash in Florida.

**Materials and Methods**

**Field Plot Preparation and Experimental Design**

The experiment was conducted at the University of Florida Plant Science Research and Education Unit in Citra, Florida. Field plots measured 10.4 m x 10.4 m. Zucchini squash, seeds variety Wild Cat® (Harris Moran), were planted on the four beds of each plot. Treatments
evaluated in this experiment included 1) reflective mulch with the reduced-risk insecticide; imidacloprid (Admire® 2F Bayer, Kansas City, MO), 2) reflective mulch without Admire® 2F, 3) living mulch with Admire® 2F, 4) living mulch without Admire 2F, and 5) standard synthetic white mulch (control). Before planting squash the living mulch, buckwheat was hand seeded between the rows 21 and 18 days in 2005 and 2006, respectively. The reduced risk-insecticide imidacloprid [Admire® 2F] was applied 2 weeks after squash planting through the drip lines at the rate of 1.684 liters per hectare. The treatments were arranged in randomized complete block design with four replications. Agronomic practices followed the standard production guide for squash in North Florida (Olson et al. 2005). However, no insecticides were applied during the growing season except Admire 2F on the specified treatments. The fungicide azoxystrobin (Amistar; 80 WP), was sprayed as required against powdery mildew in the early stages of the crop.

**Virus Screening**

In 2005, plants were visually observed for virus symptoms weekly starting from the mid-growing season until the last week of the experiment. Two weeks before the end of the experiment, I excised a young leaf (third leaf from the tip) from six plants per plot, regardless of whether or not they had symptoms and transported the leaf to the laboratory. Leaves were stored at 4 °C overnight and assayed using an enzyme-linked immunosorbent assay (ELISA) [Clark and Adams 1977] for *Papaya ring spot virus-Watermelon strain* (PRSV-W), *Watermelon mosaic virus* (WMV), *Zucchini yellow mosaic virus* (ZYMV) and *Cucumber mosaic virus* (CMV). Approximately 0.2 g of each leaf sample was grounded in phosphate buffered saline (PBST) at a 1:20 dilution using a hand-held macerator to release the virus particles into the suspension. After 24 h at 3-4 °C, 100 µL of each sample was added to duplicate wells in a 96-well plate, (Nunc, Denmark) that had previously been coated with specific antibodies at 1 µg/ml. Each plate
included a positive control and five negative controls (healthy squash). The plates were incubated overnight at 4 °C. After 24 h the plates were emptied and quickly rinsed and blotted once before washing with an ELX 50 Auto Strip Washer (Bio-Tek, Winooski, VT) using 8 washes of PBST without soaking. Enzyme conjugates (ECB) were prepared by diluting specific antibodies in buffer (Agdia Inc.) using predetermined dilution factors. Plates were covered with Parafilm before being incubated at 30 ºC for 4 h in a moist chamber. After incubation and washing (as above), 100 µL of substrate (10% diethanolamine adjusted to pH 9.8 + phosphatase substrate) (Sigma, St. Louis, MO) was added to each well. The color change was visually assessed (yellow, positive and clear, negative) before measuring the absorbance of the samples at 450 nm using an EL 800 Universal Microplate reader (Bio-Tek, Winooski, VT).

In 2006, visual observations of viral symptoms and incidences were recorded and monitored in the field. Viral symptoms including mosaic, mottling, leaf crumpling, and distortion (fig. 4-1) were noted on squash plants growing within white synthetic mulch treatments. The symptomatic leaves were excised from the plants and transported back to the laboratory. Each sample was assayed using ELISA for eight viral diseases including ZYMV, WMV, PRSV-W CMV, Tobacco streak virus (TSV), Watermelon leaf mottle virus (WLMV), Papaya ring spot virus-type T (PRSV-Tigre), Squash mosaic virus (SqMV) and Tomato spotted wilt virus (TSWV). Procedures followed were as described above except that TSV and TSWV were tested with reagent kits (Agdia Inc.) and the manufacturer’s directions were followed. All other antisera were obtained from D. Purcifull, University of Florida.

**PCR Analysis**

Twenty leaf samples were collected from the field (as earlier described) from symptomatic squash plants and tested using polymerase chain reaction (PCR) for the presence of begomoviruses. Primers PAR1c496/ PAL1v1978, which amplifies a region of the begomoviruses
A component, and PBL1v2040/PCRc154, which amplifies a B component of the bipartite genomes were used (Akad et al. submitted). The amplified sequences were then compared with the known genome of begomoviruses. The analysis was conducted by the Plant Pathology Laboratory of J. E. Polston, Department of Plant Pathology, University of Florida, Gainesville.

**Greenhouse Screening**

In order to determine if whiteflies were capable of transmitting symptoms noted in the field to healthy squash plants, a greenhouse study was initiated. Symptomatic leaves were collected from the field, transported back to the Vegetable Entomology Laboratory. Leaves were cut using a razor blade under water before placing each leaf in a 400-ml beaker with water. Each leaf in the beaker was placed each cage. Whiteflies were aspirated from a ‘virus free’ colony which was maintained at the Plant pathology laboratory and introduced into the cages. The whiteflies were left to feed on the leaves for 24 hr before introducing approximately two-week old squash plants (variety Prelude II) in each cage. The whiteflies were left to feed on the leaves and the visual symptoms of the virus recorded.

**Data Analysis**

Data from virus incidence were subjected to an analysis of variance (ANOVA) using SAS GLM (SAS 9.1 version) and treatment means separated by least significant differences (LSD) test (SAS Institute 2003).

**Results**

In 2005, there were no symptomatic plants observed in the field. Similarly, the plants tested negative for ZYMV, WMV, PRSV-W, and CMV viruses. In 2006, however, symptomatic plants were observed in November. Zucchini leaves were observed showing mottling, curling and crumpling symptoms (Figure 5-1). These viral symptoms were significantly more severe on squash growing on white synthetic mulch (control), where they were first observed, than other
treatments evaluated ($F = 9.96; \text{df} = 4, 15; P < 0.0004$) [Figure 5-2]. The symptoms later spread to other plots with time.

Cumulative disease incidence among the plants growing under buckwheat and reflective mulch treatments was significantly lower than those under the white mulch treatment (Figure 5-2). Addition of Admire 2F to reflective mulch treatments resulted in a significant reduction in the number of plants showing virus symptoms, which was not significantly different from buckwheat mulch with Admire 2F.

**Greenhouse Results**

Our greenhouse test indicated that whiteflies were able to transmit the virus from infected field samples to healthy plants. Plants began to show virus symptoms after approximately 10 days. After 14 days all the plants in the cages showed the viral symptoms and PCR analysis was positive for a begomovirus.

**PCR Analysis Results**

The samples submitted for the PCR assays produced PCR products using the degenerate primers PAR1c496/ PAL1v1978 and PBL1v2040/PCRC154. When sequences were submitted for a Basic Local Alignment Search Tool (BLAST) search the results revealed over 95% sequence identity with *Cucurbit leaf crumple virus* (*CuLCrV*). (Akad et al. submitted).

**Discussion**

In this study, there was no incidence of aphid-transmitted virus diseases. Alate aphid populations were also low during the two field studies (CHAPTER 3) that are significant in transmission of the viruses. However, this low population could not be directly related to the absence of the viruses. Furthermore, it is reported that even a small population of alate aphids can be sufficient to spread viruses especially non-persistently transmitted viruses (Fereres 2000).
The occurrence of viral diseases may vary from year to year in Florida (Mossler and Nesheim 2001).

The use of reflective or living mulches reduced the incidence of CuLCrV, which is persistently transmitted by whiteflies. The lowest incidence of virus-infected plants was observed in the treatments that combined reflective or living mulch with Admire 2F. This positively correlated ($r = 0.96$) with the numbers of whiteflies recorded in those treatment plots. It was noted that mulches (reflective and living mulch with Admire 2F) had fewer numbers of whiteflies (CHAPTER 3) and consequently resulted in low incidence of CuLCrV. The standard white mulch treatment (control) had the highest incidence of CuLCrV. This mulch had earlier reported the highest numbers of whiteflies (adults and immatures) throughout the study. *Cucurbit leaf crumple virus* is transmitted by whiteflies only, and a reduction in whitefly numbers in the two treatments (reflective and living mulch) (CHAPTER 3) lead to reduction of infected plants. There is therefore an added advantage of combining reflective or buckwheat mulch with Admire 2F as seen in our field trial. It has been suggested that management of viruses is achieved better with a combination of two or more control strategies. Jones (2001) reported some benefits of combining different tactics including cultural, chemical, and biological. These tactics all have different modes of action that act together resulting in enhanced disease suppression than any method used alone.

Although living and reflective mulches are known to interfere with the insect’s host recognition activity, their actual modes of actions are different. Reflective mulch repels whiteflies, interfering with their orientation (Zitter and Simons 1980, Csizinszky et al. 1997). The effectiveness of UV reflective mulch in reducing the incidence of CuLCrV is attributed to its ability to repel whiteflies, which would be finding their way to the host plant. Alternatively,
living mulches are known to reduce the contrast between the ground and the host plants which apparently guide the whiteflies and other insects to the host (Cradock et al. 2002).

*Cucurbit leaf crumple virus* (CuLCrV) has been reported in Arizona, Texas, California and Northern Mexico (Wiebe 2003), but had not been found in Florida before winter 2006. This spring (2007), CuLCrV was recorded in southwest Florida in watermelon (S.E. Webb, Pers. Comm.). *Cucurbit leaf crumple virus* is transmitted by whiteflies in a persistent manner and has a wide host range within the family Cucurbitaceae, infecting most of the domestically grown cucurbits. It has also been reported to infect beans (Brown et al. 2002). Symptoms on the leaves are very noticeable. The virus can also affect the fruit of some cucurbits. The disease may have been accidentally introduced into Florida through infected watermelon seedlings from another state (Akad et al. submitted). It is known that appearance and distribution of the new geminivirus diseases are associated with the coming of biotype B and its population increase in the state (Polston and Anderson, 1997), as well as evolution of variants of the viruses (Varma and Malathi, 2003). The probable geographic origin of CuLCrV is southwestern USA and northern Mexico (Brown et al. 2002).

Our study suggests that the use of living or reflective mulch alone or in combination with imidacloprid (Admire 2F) can be used to reduce whitefly populations and reduce the incidence of *Cucurbit leaf crumple virus*. There are benefits to be carried by combining a living or reflective mulches with a reduced-risk insecticide.
Figure 4-1. Effects of mulches on *Cucurbit leaf crumple virus* on zucchini squash plants grown with either living mulch or reflective ground covers as compared with white synthetic mulch in Citra, FL (2006). Virus incidence = number of plants per plot with virus present. Values with the same letter do not differ ($P < 0.05$) according to LSD test.
Figure 4-2. *Cucurbit leaf crumple virus* symptoms recorded on zucchini squash plants in Citra, Florida (2006)
Consumption of zucchini squash, *Cucurbita pepo* L. has increased due to preferential use of the squash as a salad and a cooked vegetable compared to other summer squashes (Stephen 2003). Zucchini squash, is a high value vegetable crop in Florida and the state is ranked second nationally after Georgia in the production of fresh market squash. In the 2006 field-seasons, a total of 4249 ha of cucurbits was planted in Florida and 98% of the squash produced were harvested with an exceeding value of approximately 38.76 million USD (NASS-2006). Squash is one of the crops in Florida that is exported every month of the year (Mossler and Nesheim 2001), thus providing a continuous income to the state. Almost all the squash is produced for fresh market with summer production mainly for local markets and fall production for export. In Florida, damage due to pest infestation is probably one of the major problems affecting the squash industry. In this regard, it is important to use appropriate and effective control strategies that are sustainable and will improve plant productivity.

The use of mulches on raised beds is now a common practice in some vegetable production systems in Florida. Mulches have several advantages including controlling weeds, regulating soil temperatures, retaining moisture, and increasing crop yields (Olson 2005). In addition, some of the mulches have been shown to have negative effects on pests, thus reducing their incidences. In particular, reflective mulch has received increased attention in the production of various crops. Previous research has shown increased yields of various crops grown on reflective mulch i.e. strawberries (Rhainds et al. 2001), cantaloupe and cucumber (Summers and Stapleton 2002), and zucchini squash (Summers et al. 2004, Frank and Liburd 2005). Reflective mulch reflects
sunlight back to the crop canopy increasing the amount of photosynthesis available to contribute to plant growth and vigor.

Living mulches are a cheaper alternative to reflective mulch. These mulches are interplanted with the main crop for various benefits such as improving soil fertility and pest suppressing (Liburd et al. *in press*). Previous research investigating the use of living mulches in various crops has reported mixed results with respect to marketable yields. Hooks et al. (1998) recorded increased yield in zucchini squash in Hawaii. Similarly, Hilje and Stansly (2007) reported increased yield in tomatoes when interplanted with living mulches. Alternatively, Andow et al. (1986) saw decrease in yield in cabbage interplanted with living mulches as compared with bare ground between plants.

Several factors must be considered when growing living mulches with a main crop. For instance, it is important to select a living mulch whose growth pattern has least effect on the main crop. Frank (2004) reported that buckwheat, *Fagopyrum esculentum* Moench was superior to white clover; *Trifolium repens* L., when interplanted with zucchini squash. Secondly, living mulch must be planted at the correct spacing to reduce competition between the main crop and the mulch.

Earlier, we reported that living and reflective mulch in combination with the insecticide Admire 2 F were effective in controlling whiteflies and aphids associated with zucchini squash (CHAPTER 3). It will be interesting to see if this reduction will translate to increased plant size and hence better yields.

My hypothesis was that since Admire 2F would contribute to further reduction in pest pressure (whiteflies and aphids) during the early season, the treated plants would be able to compensate for growth and hence produce higher marketable yields.
The specific objective was to evaluate the impact of buckwheat and reflective mulch with and without a reduced-risk insecticide on plant growth (size) and marketable yields of zucchini squash. It will be my intention to make recommendations if any of the combinations could result in economic gains for squash growers in Florida.

**Materials and Methods**

Field experiments were set up at the University of Florida Plant Science Research and Education Unit in Citra, Florida. Field plots measuring 10.36 m x 10.36 m were prepared, each containing four beds. The beds were 76 cm top width and 30 cm high and the distance between them was 1.06 meters. Each plot was separated from the adjacent plots by 7.6 m of bare soil on all sides that was kept weed free throughout the experiment. Living mulch, (buckwheat, *Fagopyrum esculentum* Moench) was hand seeded between the rows 21 days and 18 days in 2005 and 2006 respectively, before planting squash. The four treatments evaluated were: 1) reflective mulch with the reduced-risk insecticide (Admire 2F, Bayer, Kansas City, MO), 2) reflective mulch without Admire 2F, 3) living mulch with Admire 2F, 4) living mulch without Admire 2F and standard synthetic white mulch which served as the control. The reduced risk-insecticide Admire 2F was applied 2 weeks after squash planting through the drip lines at the rate of 1.684 liters per hectare on the appropriate treatments.

Planting holes were cut in the center of each plastic mulch strip and in the center of the non-mulched beds and two squash seeds, variety Wild Cat®, were hand seeded per hole. Planting spacing was maintained at 45 cm between the plants in the row during the 2005 and 2006 field seasons. After germination the missing plants were replaced using seedlings raised in the greenhouse for that purpose. The agronomic practices for squash followed the standard production guide for squash in North Florida (Olson et al. 2005). The same plants were used for sampling for the insect pests (CHAPTER 3).
Plant Size Sampling

Ten plants were randomly selected from the inner rows that had not been damaged during pest sampling to estimate plant size. The plant size measurements were taken using a technique adopted from Frank and Liburd (2005). Using a tape measure, the plant height was taken as the length of the squash from the ground to the terminal bud. The plant width data was taken by measuring the length between the two widest opposing lateral shoots growing from the same plant.

 Marketable Yields Sampling

Yield data were collected from the three inner rows of each plot that had not been damaged during sampling. Zucchini squash was harvested at immature stage (soft, thin, edible rind shells) with edible seeds at approximately 20-25 cm long. Fruits were harvested and weighed in the field every other day for three weeks.

Data Analysis

Plant size and yield data were analyzed using analysis of variance (ANOVA) SAS GLM (SAS 9.1 version [2001]). Treatment means were separated using LSD means separation procedures and were considered significant when $P < 0.05$.

Results

Plant Size

Plant widths for reflective mulch treatments with and without Admire 2F were significantly larger than those for buckwheat and the white mulch (control) treatments ($F = 11.64; \text{df} = 4, 34; P < .0001$) [Table 5-1]. Buckwheat treatments resulted in the smallest width and there was no significant difference between treatments with and without Admire 2F.

The heights of zucchini plants grown in reflective mulch with and without Admire 2F were not significantly different. However, these plants were significantly taller than all other
treatments, including the control. Zucchini plants grown within living mulch with Admire 2F were significantly taller than plants grown in living mulch alone, a treatment that resulted in the least height when compared with all the other treatments ($F = 41.92; \text{df} = 4, 34; P < .0001$) [Table 5-1].

** Marketable Yields**

Zucchini squash yields differed significantly among the treatments ($F = 37.56; \text{df} = 4,167; P < 0.0001$) [Table 5-2]. In 2005, zucchini plants grown with reflective mulch with Admire 2F produced significantly higher yields than those from buckwheat and white mulch (control) treatments. Overall, plants growing within reflective and white mulch treatments had 58 and 54% respectively, more zucchini than those growing in the living mulch. Reflective mulch alone and white mulch provided similar yields.

In 2006, reflective mulch plots produced significantly higher yields than the white mulch (control) ($F = 53.40; \text{df} = 4, 133; P < .0001$) [Table 5-2]. Overall, reflective mulch treatment resulted in the highest yields compared with all the other mulches. As in 2005, buckwheat plots produced the least yields when compared with all the other treatments. Actually plants growing within the living mulch alone produced 74 and 64% fewer marketable squash than reflective and white mulches.

**Discussion**

Zucchini plants interplanted with the living mulch, buckwheat, were smaller in size and eventually yielded less than those growing on the synthetic mulches. My results were consistent with the findings of Frank (2004). Plants growing with buckwheat mulch were smaller and had lower yields when compared with plants grown with synthetic mulches. When living mulches are interplanted with a main crop (zucchini) they share the same scarce natural resources (light, nutrients), which could lead to competition and can negatively affect the productivity of the main
crop. In our study, the competition between zucchini plants and buckwheat was observed to be greatest during the early stages of growth. Although squash plants were able to regain some level of vigorous growth after buckwheat senesced, they were not able to compensate for yield. Furthermore, by the time the buckwheat senesced, the fall (cool season) temperatures were already present, which may have been unfavorable for the future growth of squash. The squash plant is a warm-season crop and grows best between 24 and 29 °C (Mossler and Nesheim 2001). It is possible that early-season competition is a critical factor that could affect fall zucchini production when interplanted with living mulches. The addition of Admire 2F was able to enhance zucchini yields growing within buckwheat in at least one of the seasons (2005).

However, in spite of the larger plant size there were no differences in yields.

In our study, buckwheat considerably delayed the flowering of zucchini plants and hence the time of harvesting. Previous studies have also reported similar effects of a living mulch when interplanted with a cash crop. For instance, growth of cantaloupe was delayed by wheat, *Triticum aestivum* L. and fruits failed to mature by the time harvest was completed in other treatments (Toba et al. 1977). In another study, maturation of cabbage heads was delayed and was smaller in living mulch treatments compared to bare ground (Andow et al. 1986).

During the 2-yr study, the reflective mulch treatment resulted in the largest plants and the highest yields. Previous studies have reported similar findings (Brown et al. 1993, Summers et al. 1995, Csizinszky et al. 1997, Stapleton and Summers 2002, Summers et al. 2004, Frank and Liburd 2005). It is known that UV-reflective mulch has high photosynthetically active radiation, which contributes to both plant growth and crop earliness (Stapleton and Summers 2002, Olson et al. 2005). In our study, the addition of Admire 2F did not increase yields significantly in reflective mulch treatments.
Synthetic white mulch treatment yielded more zucchini fruits than living mulch plots despite having a high whitefly population in both years. In 2006, white mulch had the highest incidences of whitefly-transmitted virus (CHAPTER 4). It is known that zucchini yield can be affected by the occurrence of insect-transmitted diseases. In 2006, a whitefly-transmitted disease was present; however, this disease did not affect the yields of zucchini possibly because it appeared late in the growing season. Yield losses due to whitefly-transmitted viruses have been described to be within a range of 20-100% depending on the crop, season, and vector prevalence (Basu 1995).

Indeed, living mulches were able to reduce pest problems (CHAPTER 3 & 4) but resulted in a huge negative impact on the yields. This adverse effect of living mulches is a big factor that may limit their adoption in pest management programs. Additionally, the living mulch-insecticide combination treatment did not increase yield sufficiently to justify the cost of Admire 2F. For the growers to adopt this tactic the net gains associated with mulches plus Admire 2F have to be large enough to justify their use this was not the case in this study. Further research needs to be done before the living mulch is recommended to growers. In cases where reflective mulches cannot be used, living mulch is a cheap alternative, since it has been shown to result in higher yields than bare ground in other studies (Frank 2004, Hilje and Stansly 2007). It is worth mentioning that other costs (extra drip lines, natural resources like water, nutrients) need to be considered before using living mulches.
Table 5-1. Effect of living and reflective mulches alone or in combination with Admire 2F on plant size of zucchini squash Citra, FL (2006)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Plant Width</th>
<th>Plant Height</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reflective + Admire 2F</td>
<td>42.08 ± 2.00</td>
<td>23.71 ± 0.39</td>
</tr>
<tr>
<td>Reflective</td>
<td>40.09 ± 1.94</td>
<td>22.15 ± 0.69</td>
</tr>
<tr>
<td>Buckwheat</td>
<td>31.62 ± 1.54</td>
<td>13.75 ± 0.58</td>
</tr>
<tr>
<td>Buckwheat + Admire 2F</td>
<td>32.64 ± 1.81</td>
<td>16.10 ± 0.73</td>
</tr>
<tr>
<td>Control (white mulch)</td>
<td>38.18 ± 2.00</td>
<td>18.39 ± 0.70</td>
</tr>
</tbody>
</table>

Means followed by the same letter are not significantly different $P = 0.05$ (LSD)

\[ a \quad F = 11.64; \text{df} = 4, 34; \quad P < 0.0001 \]

\[ b \quad F = 41.92; \text{df} = 4, 34; \quad P < 0.0001 \]
Table 5-2. Effect of living and reflective mulches alone or in combination with Admire 2F on marketable yields of zucchini in squash Citra, FL

<table>
<thead>
<tr>
<th>Treatment</th>
<th>2005 Mean ± SEM</th>
<th>2006 Mean ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reflective + Admire 2F</td>
<td>39.47 ± 4.07 a</td>
<td>32.97 ± 3.42 a</td>
</tr>
<tr>
<td>Reflective</td>
<td>36.26 ± 3.87 ab</td>
<td>32.11 ± 3.67 a</td>
</tr>
<tr>
<td>Buckwheat</td>
<td>15.1 ± 2.70 d</td>
<td>8.29 ± 1.21 c</td>
</tr>
<tr>
<td>Buckwheat + Admire 2F</td>
<td>20.39 ± 3.59 c</td>
<td>8.54 ± 1.47 c</td>
</tr>
<tr>
<td>White (control)</td>
<td>33.45 ± 3.62 b</td>
<td>23.37 ± 2.35 b</td>
</tr>
</tbody>
</table>

Means ± SEM followed by the same letter are not significantly different $P = 0.05$ (LSD)

\[ F = 37.56; \text{df} = 4,167; P < 0.0001 \]

\[ F = 53.40; \text{df} = 4, 133; P < 0.0001 \]
The field experiments demonstrated that the addition of imidacloprid to living and reflective mulches enhanced the control of adult whiteflies in zucchini squash. Specifically, the addition of imidacloprid to buckwheat further reduced whitefly densities (adults and immatures) in both years as revealed by foliar and yellow sticky trap samples. The addition of imidacloprid to reflective mulch enhanced the control of aphids (wingless) in both years. However a lower population was always recorded with the addition of imidacloprid. In almost all cases buckwheat in combination with imidacloprid gave protection to squash against whiteflies equal to that provided by reflective mulch with imidacloprid. Only in 2005, did counts from the yellow sticky traps indicate that reflective mulch with imidacloprid was superior to buckwheat in combination with the reduced-risk insecticide.

The addition of imidacloprid did not affect the alate aphid population densities in zucchini as indicated by foliar and pan trap samples. Surprisingly, pan traps set within squash growing on reflective mulch with imidacloprid caught more alate aphids in 2006 than those within reflective mulch alone. This could not be explained as it is known that reflective mulch interferes with the insect’s host locating ability and hence reduces their landings on squash plants. The addition of imidacloprid to reflective mulch reduced apterous aphids per leaf in both years. However, buckwheat combined with imidacloprid reduced the populations only in 2005. Such differential effects of treatment combinations on the pests indicates that development of an integrated pest management program for the zucchini crop can be a complicated process, since the crop is affected by many pest species.
The study also suggests that the use of reflective and buckwheat mulches with imidacloprid can be used to effectively reduce the incidence of whitefly-transmitted virus. This further supports the argument that mulches can be deployed against various virus diseases. Only in 2005, that buckwheat with imidacloprid treatment produced higher yields than the buckwheat mulch alone.

The yields of squash planted with buckwheat were greatly reduced. Living mulches such as buckwheat are cheaper alternatives to synthetic (reflective) mulches. However, crop spacing and competition for resources need to be evaluated further before any recommendation can be made.

A cost analysis of cost per hectare for using these mulches is as follows. The cost of applying reflective mulch/labor is 1374 USD/ha. The cost of drip tapes is 150 USD/1829 meter roll. The average cost of planting buckwheat is 178 USD/ha. The cost of adding imidacloprid (Admire 2F) to the mulches at the recommended rate imidacloprid is 257 USD per ha. Buckwheat mulch with imidacloprid is more cost effective than reflective with imidacloprid. However, it is worth mentioning that buckwheat has some other additional costs which include extra drip lines to support plant growth and management practices such thinning after planting.

Although reflective mulch is more expensive, the cost of the mulch installation can be justified because significantly higher yields were obtained. The reflective mulch treatment with imidacloprid was much more expensive than reflective mulch alone and yet no additional gains resulted in terms of yields. Therefore, it may not be necessary to combine the two tactics together because mulch alone was sufficient to give satisfactory yields. Since there were no benefits from using this combination, it may not be recommended to growers as an option. However, it may worth mentioning that the benefits of reflective mulches are short lived since they diminish once
the crop canopy covers them. It is possible in cases of high pest pressure that the use of reflective mulch and imidacloprid may be warranted since early virus infection can significantly reduce yield. Imidacloprid can be used to reduce the spread of whitefly-transmitted viruses by reducing the vector population on the primary and secondary host.

Currently, there is a rising demand for sustainable agriculture and to increase organic farm holdings in the United States and the rest of the world. This means that the use of cultural based tactics for pests and diseases in various crops will be the first line of defense for most growers. This study supports the importance of buckwheat for managing whiteflies and aphids in zucchini as a cheaper alternative to reflective mulches. Therefore, buckwheat is likely to become more popular in organic farming as a means of responding to pests and disease management needs provided that adverse competition effects on crop yield can be worked out.

An important aspect of buckwheat is that it grows erect and therefore does not pose problems with shading or growing into squash. Therefore, different planting spacing can be evaluated to determine the optimal spacing in the relation to the cash crop to reduce the competition for natural resources. Zucchini crop may be planted in double or triple rows between wider strips of living mulch to lessen competition between the crop and the mulch. If the mulch is planted before the vegetable crop, like in our case, strips of the mulch must be prepared for the crop by tilling, mowing, or applying herbicides. Another study can be conducted to establish the optimal time of planting squash into the living mulch so that maximum usage of beneficial insects can be achieved. A comprehensive survey for CuLCrV, diagnosing the severity of the disease and potential risks in Florida would be an important problem to address.
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BIOGRAPHICAL SKETCH

Teresia Nyoike was born in the Thika District of Central, Kenya, Africa. She graduated with a Bachelor of Science in Agriculture (Crop Protection) on August 2001 from the University of Nairobi, Kenya. In August 2001, she joined Dudutech Kenya Ltd., an integrated pest management company that produced bio-control agents for local and export markets. In 2002, she had laboratory training on biological control agents for root knot nematodes- *Pasteuria penetrans* at Reading University (UK). During her four years in the company, she worked as a nematologist with specific responsibilities of developing on-farm control systems for root knot nematodes using biological control agents. In August 2005, she joined the Small Fruit and Vegetable IPM Laboratory, University of Florida, in Gainesville to pursue her MS in Entomology. She is a member of the Gamma Sigma Delta honors society in agriculture.