AN ABSTRACT OF THE THESIS OF

Joel A. Reich for the degree of Master of Science in Horticulture presented on March 2, 2006.

Title: Field and Greenhouse Studies with *Acalymma* and *Diabrotica*: Protection of Cucurbits with a Kaolin-based Particle Film; Feeding Damage to Cucumbers with and without Cucurbitacin.

Abstract approved:

Glenn C. Fisher

Research was conducted in the field and greenhouse to determine if a kaolinite-based particle film, Surround®, would reduce colonization and early season feeding damage in cucurbits by the Western Striped Cucumber Beetle, *Acalymma trivitattum* (Mannerheim). Greenhouse studies were designed to determine effect of particle film on cucumber dry weight production and (2) relationship between cucurbitacin content and adult feeding damage to two cucumber varieties,

Experiments were conducted in 2004 – 05 on commercial farms in western Oregon during 2004 and 2005. Greenhouse enclosure trials were also conducted in 2004 and 2005. Different rates of solution and manners of application were evaluated. Young plantings of cucurbit crops which received label-rate applications of the kaolinite-based particle film (KBPF) had fewer *A. trivitattum* adults and less feeding damage than untreated plants. Weight of marketable fruit harvested from plants dipped in the particle film solution prior to transplanting or sprayed with the particle film immediately after transplanting were significantly greater than weight of marketable fruit harvested from untreated plants.

In the greenhouse, a 1x rate applied to the top sides of leaves significantly increased dry weight over the UTC. Plant damage to cucumber seedlings from both Acalymma and Diabrotica adults was significantly reduced by the kaolin based particle film. However, in the absence of insect pests, the kaolin-based particle film significantly reduced plant dry weights of plants compared to the UTC when applied to both underside and topside of leaves at 1x rate as well as when applied at the 2x rate to the topside of leaves.

KBPF treated cucurbit seedlings attract fewer WSCB, sustain less feeding damage as seedlings and yield better than untreated plants.

Field and Greenhouse Studies with *Acalymma* and *Diabrotica*: Protection of Cucurbits with a Kaolin-based Particle Film; Feeding Damage to Cucumbers with and without Cucurbitacin

by
Joel A. Reich

A THESIS

submitted to

Oregon State University

in partial fulfillment of
the requirements for the
degree of

Master of Science

Presented March 2, 2006

Commencement June 2006

Master of Science thesis of Joel A. Reich
presented on March 2, 2006.

APPROVED:

Major Professor, representing Horticulture

Head of the Department of Horticulture

Dean of the Graduate School

I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request

---

Joel A. Reich, Author

ACKNOWLEDGEMENTS

I wish to thank first of all my major advisor, Dr. Glenn Fisher, for his assistance and guidance throughout the thesis writing process. He not only helped with the academic side of this undertaking but with the frustration-management side as well. I especially want to thank him for accepting me as his student under rather unusual circumstances.

I also wish to thank the other members of my committee, Dr. Anita Azarenko and Dr. Paul Jepson, who took time to offer me guidance even though they are incredibly busy with their other responsibilities.

Deep gratitude is what I feel for Jack Giovannini of the Statistics Department, a fellow graduate student who offered indispensable and significant aid in the preparation and execution of my statistical analyses. Without his help I would still be swimming in a morass of data and spreadsheets.

To my family I wish to express my deepest gratitude. Without their encouragement and support, from day one through the present, I could not have made it this far. Especially during the sometimes rocky experience of my masters education, their counsel was invaluable.

I wish to express thanks to the growers who allowed me to conduct trials on their farms and often assisted me at critical moments: John Eveland at Gathering Together farm, Steve Haley at McDowell Creek Family Farm, Jeff and Eleanor at Persephone Farm and Andy Bennett and Scott Polschneider at Stahlbush Island Farms.

I also owe thanks to all the O.S.U. staff members who assisted me, from the Horticulture Dept. staff to the greenhouse staff to the Veg. Farm crew and everyone in between.

Finally I wish to thank all of my friends and acquaintances in the Corvallis area who have shared their lives with me. You all have made my time in Corvallis so much more than just a degree program, it has been a great time in my life. Thank you.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF FIGURES (cont.)

LIST OF TABLES

LIST OF TABLES (cont.)

Field and Greenhouse Studies with *Acalymma* and *Diabrotica*: Protection of Cucurbits with a Kaolin-based Particle Film; Feeding Damage to Cucumbers with and without Cucurbitacin

Introduction

Cucumber beetles

The Western Striped Cucumber Beetle (*Acalymma trivittatum* (Mannerheim)) (*A. trivittatum*) and the Striped Cucumber Beetle (*Acalymma vittatum* (Fabricius)) (SCB) are common pests of Cucurbit crops throughout large areas of North America, especially cucumbers, pumpkins and winter and summer squash (Ferguson and Metcalf, 1985, Chambliss and Jones, 1966). The most noticeable damage is from adult feeding on foliage, flowers and fruits. Often the adults kill seedlings before emergence of the third true leaf (Hoffman et al., 1996). The larvae, which live in the soil, feed on root tissue as well as portions of fruits in contact with the soil. The larval root feeding, while not as visually apparent as adult feeding, can cause significant loss of vigor in affected plants (Ellers-Kirk et al., 2000). In addition to the direct damage done by beetle feeding, *A. vittatum* is a known vector of a bacterial disease *Erwinia tracheifolia*, the causal agent of bacterial wilt in cucurbits and several other crops (Bradbury, 1970).

For many years the western spotted cucumber beetle subspecies, *Diabrottica undecimpunctata undecimpunctata* (Mannerheim) (*D. uu*), has been an occasional pest of seedling cucurbits when adults of the over-wintering generation invade seedling stands, defoliating and often killing plants with less than four true leaves (Fisher, 2005). *A. trivittatum*, although known as a serious pest in California for over a century (Michelbacher et al,), has only recently become an economic pest in western Oregon (McGrath, Luna, 2005). Farmers in the region grow a number of *A. trivittatum*'s preferred hosts, which belong to the family Cucurbitaceae, including pumpkins (*Cucurbita maxima*) (for seeds, pie filling and jack-o-lanterns), a variety of winter squashes (*Cucurbita spp.*) (such as butternut, delicata and acorn), cucumbers (*Cucumis sativa*) (both for the fresh market and for commercial pickling operations) and summer squashes (*Cucurbita pepo)* (such as zucchini and crookneck).

Acalymma **and** ***Diabrotica***

Acalymma trivittatum (Mannerheim) has been a pest in the western United States and many parts of Mexico since its original description by Mannerheim in 1843. At that time this beetle was known as *Diabrotica trivittata*. In 1947 the genus was revised by Barber who placed the species under *Acalymma* as part of a major taxonomic reorganization. Details regarding this reorganization, as well as the current description of, and key for, *A. trivittatum* can be found in Munroe and Smith (1980).

The most prevalent species of cucumber beetles causing damage during the two years of this study was *A. trivittatum*. Research on *A. trivittatum* has been sparse. In 1953 Michelbacher et al. described the damage done to melon crops in northern California by this pest and suggested control with DDT. The only other definitive work on this species as a pest is reported by Hoffman et al. (1996). He describes research using kairomone-baited traps to manage various members of the cucumber beetle complex in New York and California. Rodriguez-del-Bosque and Magallanes-Estala (1994) discussed the seasonal abundance of *A. trivittatum* in northeastern Mexico, but it is only mentioned in an accessory fashion.

Most scientific entomological literature on the *Acalymma* concerns a well-studied relative, *Acalymma vittatum* Fabricius, the Striped Cucumber Beetle, found throughout North America east of the Rocky Mountains. These two insects are nearly identical in regards to behavior, ecology, host range and appearance. The only significant differences are in geographic range and several small morphological distinctions (Munroe and Smith, 1980). Unfortunately there is also indication that some research results for and species identifications of *A. trivittatum* have been reported as *A. vittatum* (LaBonte, 2005). This may explain why there are relatively few scientific publications addressing *A. trivitattum.* I have provided in the 'Literature Review' details regarding *A. vittatum* pertinent to an understanding of *A. trivittatum*.

Controlling Cucumber beetles

Control of *Acalymma* beetles damaging cucurbit seedlings in conventional farming systems of western Oregon is usually by the application of one of many synthetic carbamate, organophosphosphate or pyrethroid insecticides. They are applied as broadcast or band sprays at seedling emergence or immediately after transplanting. Subsequent preventative or curative foliar sprays are usually applied after heavy rainfall or irrigation (McGrath, 2005). Well-timed applications of these chemicals generally provide reliable, cost-effective control (Fisher, 2005). For growers operating under organic label restrictions, application of synthetic pesticides is not allowable. Oregon Tilth reports that in 2005 there were approximately 200 acres of cucurbits (excluding melons) certified as organic in western Oregon, (Oregon Tilth, 2005). There are many non-organic farmers also interested in using pesticides and practices that fall under organic product standards and/or believed to have minimal environmental impact.

These two groups of growers have had difficulty protecting seedling cucurbits from *A. trivittatum* that has caused substantial losses the past three years. There are few options available that are effective and practical. Many organic producers have experience with floating row covers but few use them extensively because of their initial expense, susceptibility to strong winds, and cost of labor to install, maintain and store them (Fisher, 2005). Initially, this project was undertaken to determine if kaolinite, formulated as a particle film and applied to seedling cucurbits, would reduce seedling damage and if so, which use patterns would be most effective against *A. trivitattum*. During the course of the study, I was also able to develop information on the *D. uu* using greenhouse studies to evaluate efficacy of the kaolinite-based product as well as to investigate the hypothesis that a cucurbitacin-free cultivar of cucumber would be effective in reducing seedling feeding damage compared to a cucurbutacin containing cultivar.

Commercially available kaolin, sold under the trade name Surround WP®, has been approved for use on certified organic crops by the Organic Materials Review Institute (OMRI) (http://www.omri.org) and the Washington State Department of Agriculture (WSDA) (http://agr.wa.gov/foodanimal/organic/materialslist.htm) among others. Further information regarding its status as an organically-approved material can be found at the manufacturer website (http://www.engelhard.com/).

Literature Review

The story of *A. trivittatum* and its affinity for Cucurbit crops is an interesting one, beginning well before the existence of modern humans, in central Mexico. Mexico and Central America have long been recognized as centers of genetic diversity that have given rise to many modern crops. Corn, avocados, amaranth, chiles and many dry beans all have their origins in the region. Many types of squash were also developed by indigenous peoples in the region using the genetic resources of the various wild members of the family Cucurbitaceae (Cucurbits) found there (Foster and Cordell, 1992).

The family Cucurbitaceae is an extremely widespread taxonomic unit, with distinct genera having evolved in Africa (22 genera including *Citrullus, Cucumis, Telfairia*), Asia (20 genera including *Benincasa, Luffa, Momordica, Trichosanthes*), and the Americas (55 genera including *Cucurbita, Sechium, Sicana*). Two economically unimportant genera also arose in Europe. Generally, melons come from Africa, cucumbers from south Asia and squash from tropical and sub-tropical America. (Pitrat et al. 1999).

One thing that all wild Cucurbits have in common is an intense bitterness, provided by a group of approximately 20 oxygenated tetracyclic triterpenes known as Cucurbitacins (Metcalf et al., 1980, Ferguson and Metcalf, 1985). These compounds have been found to be among the most bitter in the world, with human sensory lab panelists able to detect Cucurbitacin B in water at concentrations as low as 1 ppb. At higher concentrations, Cucurbitacins are extremely toxic, with Cucurbitacin B having an LD_{50} for mice of 1.0 mg./Kg. of body weight. (David and Vallance, 1955).

The extremely bitter Cucurbitacins (Cucs) serve as highly effective allomones for the Cucurbitaceae, strongly discouraging herbivory among nearly all potential consumers. The exception are beetles (order: Coleoptera) of the family Chrysomelidae, subfamily Galerucinae, tribe Luperini. The tribe Luperini is further divided into the Aulacophorina (Old World genera) and the Diabroticina (New World genera). Among the Diabroticina, the genera *Acalymma* and

Diabrotica both contain species which feed on both wild and cultivated Cucurbits (Chambliss and Jones, 1966, Metcalf et al. 1980).

The most obvious advantage that accrued to the beetles that developed the ability to consume Cucurbitacin without negative effects is that they secured for themselves a monopoly on a widespread group of host plants that was unavailable to other herbivores (potential competitors). A second evolutionary advantage resulting from this food source was the ability of these species to sequester Cucurbitacin within their bodies (principally in the haemolymph), resulting in a highly effective defensive mechanism against predation (Gould and Massey, 1984). While Gould and Massey (1984) reported inconclusive results when measuring predator interactions with diabroticites fed on cucurbitacin-rich and cucurbitacin-free diets, most other research has shown a predator preference for cucurbitacin-free beetles (Howe et al. 1976, Ferguson and Metcalf, 1984).

While the wild ancestors of today's modern squashes radiated out from southern Mexico, so did the ancestors of much of the Luperini tribe of Chrysomelid beetles, including the focus of this project, *Acalymma vittatum*. During the coevolutionary process that occurred between these two groups (Cucurbits and Luperine beetles) most Luperine beetles developed the ability to consume high levels of cucurbitacins, for the reasons stated above. In some instances species, such as *Acalymma vittatum*, became obligate feeders on Cucurbits, with larvae unable to complete development unless they had fed on cucurbitacins. With the cucurbitacins now serving as kairomones for many Luperine beetles, highly specific chemical responses developed within the beetles to help them locate Cucurbits (Chambliss and Jones, 1966. Metcalf et al. 1980).

Over time, other species developed behaviors that left behind the historical attachment to the Cucurbitaceae. Specifically, many Luperine beetles became obligate feeders on members of the Poaceae (most notably corn). Several of these species are the beetles that today make up the corn rootworm complex that is so well known to corn farmers of the American mid-west (*Diabrotica virgifera* LeConte, *D. undecimpunctata howardi* Barber, etc.) While the behaviors of these corn root worm species no longer call for feeding on Cucurbits, laboratory studies have shown that they have retained the chemically-based feeding response to the presence of cucurbitacins (Howe et al., 1976).

Acalymma vittatum, more than any other member of its tribe, has retained its strong historical association with the Cucurbitaceae. Not only are larvae unable to complete development without a diet of cucurbitacins, but cucurbitacins act as a phagostimulant on adult *A. vittatum*, causing the beetles to feed compulsively until the source of cucurbitacins has been completely exploited (Metcalf, Metcalf, Rhodes, 1980).

The obligate nature of the aforementioned relationship would be enough, by itself, to make *A. vittatum* a pest on crops in the Cucurbitaceae family. Unfortunately for growers of Cucurbits, there is one more biochemical relationship working against them.

Smyth and Hoffman (2003) described the effects of a male-produced aggregation pheromone that appears to be largely responsible for the long-noted ability of *A. vittatum* to reach high levels of infestation in Cucurbit plantings in a remarkably short time (Webster, 1895). Spring emergence of Cucurbits often coincides with the post-diapause activities of adult *A. vittatum*. The first *A. vittatum* to arrive in Cucurbit plantings are invariably males, known as 'pioneer males'. Smyth and Hoffman (2003) determined that these 'pioneer males', once they have begun feeding on the cucurbitacin-rich cotyledons, begin to release an aggregation pheromone which attracts other males from the surrounding area (the precise radius has not been determined). They observed that within 24 hours of placing 10 male *A. vittatum* on a young squash plant, up to 150 additional males had arrived and begun to feed on the same plant. Females, which also respond to this aggregation pheromone, do not arrive in large numbers until a significant number of males have begun feeding and emitting the aggregation pheromone.

This aggregation behavior, occurring at an early and susceptible growth stage of Cucurbit crops, combined with the phagostimulant and arrestant effects of cucurbitacins on *A. vittatum*, often results in the serious injury or death of young plants. Destruction of a plant at an early stage obviously results in a 100% loss of that plant's yield, while Hoffman et al. (2000) showed that ≥20% defoliation during early growth of winter squash can lead to significant decreases in both yield of marketable fruit and total-plant dry matter at harvest. Since beetle feeding damage to foliage at later stages of growth appears to have a significantly smaller effect on eventual yield, the goal of this project has been to find ways to prevent or reduce massive feeding damage to Cucurbit crops during the early (first 4-6 weeks) part of the season.

History of Alternative Management Strategies

Due to the potentially devastating damage caused by *A. vittatum*, as well as the desire among many growers to avoid using synthetic pesticides except when unavoidable, it is not surprising that a number of alternative approaches have been pioneered in recent years. Some of these approaches have focused on controlling the beetles themselves, while others have attempted to make the crop less attractive to the beetles.

Ellers-Kirk et al. (2000) evaluated the potential of several entomopathogenic nematodes (including *Steinernema feltiae* Filipjev, *S. riobravis, S. carpocapsae* Weiser and *Heterorhabditis bacteriophora* Poinar) for reducing populations of larval (root-feeding) *A. vittatum*. They found that the introduction of *S. riobravis* was responsible for a ≈50% decrease in larval survivorship among *A. vittatum* under both conventional and organic soil management systems. A previous study by Reed et al. (1986) demonstrated that trickle irrigation systems are an effective and economical way to introduce entomophagous nematodes into farm soils for control of *A. vittatum* larvae. A complementary paper by Necibi et al (1992) found that the use of black plastic mulch can increase the effectiveness of entomophagous nematodes against diabroticite beetle larvae by fostering conditions favorable to the nematodes and unfavorable for diabroticite larvae. These combined techniques appear to have potential for managing the larvae, but not for newly arriving adults.

Using a different approach, Radin and Drummond (1994) studied the effects of using a highly-attractive trap crop (*Cucurbita maxima* Duchesne cv. 'NK530') to keep *A. vittatum* from colonizing a cash crop of cucumber, *Cucumis* *sativa*. They found that the trap crop was highly effective at attracting *A. vittatum* away from the cucumbers, at least for the first three days of colonization. This three day window would, theoretically, allow a grower to treat the trap crop with an insecticide to kill the beetles before they were able to colonize the cash crop of cucumbers. Furthermore, Radin and Drummond found that the effectiveness of the trap crop was greatest during cooler (early-season) conditions. The trap cropping approach appears to be well suited to the production of cucumbers. Unfortunately, the highly attractive trap crop used in their study (*Cucurbita maxima*) is one of the cash crops that this project hopes to find ways to protect. There is no indication that the particularly attractive cultivar used in the Radin and Drummond study could be used successfully in a planting of pumpkins, winter squashes or summer squashes.

In a different approach to trap cropping, Pair (1997) used highly attractive squash plants (*Cucurbita pepo* cv. 'lemondrop') that had been systemically treated with either imidocloprid or carbofuran to attract and kill early-colonizing *A. vittatum* in cantaloupe, squash and watermelon plantings. While the treated trap plants accounted for roughly 1% of the plantings, they succeeded in attracting and killing >30% of the *A vittatum* in the plots. This approach holds some promise for conventional growers, but offers no solutions to organic farmers or those who would rather avoid the use of synthetic pesticides.

A novel approach to *A. vittatum* control, resulting from induced systemic resistance (ISR) in host plants, was discovered largely by accident and reported by Zehnder et al (1997). Zehnder et al. were investigating the potential of plant growth-promoting rhizobacteria (PGPR) to induce systemic resistance against bacterial wilt (*Erwinia tracaeohila*) in cucumbers. They found that PGPR were able to significantly reduce the incidence of bacterial wilt, but they also noticed significantly lower numbers of the disease vector, *A. vittatum*, on treated cucumber plants. This effect was not expected, and the explanation provided by Zehnder et al., while reasonable, has still not been proved. According to their 1997 paper: "PGPR induce physiological changes in the plant leading to changes in the production or accumulation of plant allelochemicals acting as beetle attractants".

This explanation holds that the amount of beetle-attracting cucurbitacins is significantly reduced in PGPR treated plants. This approach to cucumber beetle control holds great potential, but the hypothesis must be tested in further studies of the metabolic pathways leading to cucurbitacin synthesis in both untreated and PGPR-treated plants.

Another method for managing *A. vittatum* involves the use of aluminum-coated plastic mulch. Many growers of cucurbits and other crops use black (and sometimes other colored) plastic mulches for a variety of reasons, including increased soil temperature and decreased emergence of weeds. Caldwell and Clarke (1999) conducted research into the effectiveness of aluminum-coated plastic mulch at discouraging *A. vittatum* from feeding in cucumber and squash plantings. They compared the effects of aluminum-coated plastic mulch with the standard black plastic mulch as well as with black plastic mulch that had two strips of aluminum affixed to it running parallel to the rows. In both squash and cucumbers they found that beetle numbers were between two and six times higher in black plastic plots as compared to the aluminum-coated plots. The reduced numbers of beetles in the aluminum-coated plots stayed below the action threshold designated by the researchers for pesticide application, and the experimental plots yielded the same amount of marketable produce as the black plastic plots. Unfortunately, the significantly higher cost of the aluminum-coated mulch greatly reduced the profitability of the crop, even when the savings on pesticide applications were taken into account. Furthermore, very few of the cucurbit growers in western Oregon use any kind of plastic mulch in their production systems. Adopting this strategy would call for a major shift in practices. Unless more economical aluminum-coated plastic mulch becomes available, it is unlikely that this approach to *A. vittatum* management will be widely adopted.

Particle Films as Crop Protectants

Mineral-based particle films have long been considered as useful crop protectants. From the 1920s through the 1960s many growers and researchers attempted to apply mineral-based particle films to a variety of agricultural production challenges. Principal among these challenges were fungal infections of foliage and arthropod pest damage to all above-ground plant parts. While a variety of positive results were described, interest in the use of these materials dwindled in the era of cheap and effective (as well as environmentally deleterious) synthetic chemical pesticides. In recent years, due to increasing concerns about environmental degradation on the part of both agricultural producers and consumers, interest in the use of mineral-based particle films has begun to rise (Glenn et al, 1999).

Beginning in 2000, a variety of scientific papers have reported on the effectiveness of a commercially available, organically approved formulation of kaolinite clay. This product is manufactured by the Englehard Corporation (Iselin, N.J.) and sold under the name Surround WP™. The mineral particle found in this product is M96 (M-96-018 Kaolin) that has been sized to ≤2 µm and made hydrophobic by treating it with a proprietary synthetic hydrocarbon (Englehard, Iselin, N.J.). The formulation of the product was the result of collaboration between USDA-ARS and the Englehard Corporation which sought to improve the effectiveness of kaolin-based sprays, which historically had not been highly effective (Knight et al, 2000).

Initial testing and marketing focused on tree fruits, particularly apple, pear and citrus. Unruh et al (2000) found that applications of three formulations of kaolin-based sprays (including the formulation described above) significantly reduced larval walking rate, fruit discovery rate and fruit penetration rate by codling moth, *Cydia pomonella* (L.) on apples in the laboratory. In apple and pear orchards they found that oviposition by codling moths was significantly reduced in both the first and second annual generations of adults.

Also in 2000, Knight et al reported similar results when measuring the effects of kaolin-based particle films on female oviposition and larval development and feeding of the obliquebanded leafroller, *Choristoneura rosaceana* (Harris) on apples. The authors of both articles suggested that kaolin particle films alone could provide adequate crop protection in orchards with low to moderate lepidopteran pest pressure.

Research in Florida citrus crops, reported by LaPointe (2000), measured the effects of a hydrophilic formulation of a kaolin-based particle film on the root weevil *Diaprepes abbreviatus* (L.), a common pest of citrus in Florida and the Caribbean basin. Foliar feeding by adults in greenhouse trials was reduced by 68-84% on treated as opposed to untreated plant material. In addition, oviposition by females was also greatly affected. During the study, which employed both choice and no-choice enclosures, females oviposited >19,000 eggs on untreated foliage and no eggs on treated plant material.

By 2002, articles began appearing which demonstrated that kaolin-based particle films could be used effectively as protection from arthropod pests in a wide variety of crops, not just the genera *Malus, Pyrus and Citrus*. Research was conducted on the effects of kaolin-based sprays on several agricultural pest-host interactions: Black pecan aphid, *Melanocallis caryaefoliae* (Davis), on pecan trees, *Carya illinoensis* (Wang) (Cottrell et al, 2002); Silverleaf whitefly, *Bemisis argentifolii* (Bellows and Perring), on cantaloupe (Liang and Liu, 2002); Boll weevil, *Anthonomis grandis grandis* (Boheman), on cotton, *Gossypium hirsutum* (L.) (Showler, 2002[1]); Beet armyworm, *Spodoptera exigua* (Hübner), on cotton, *Gossypium hirsutum* (L.) (Showler, 2002[2]); Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), on apple, nectarine and persimmon (Mazor and Erez, 2003); and olive fruit fly, *Bactrocera oleae* (Gmelin), on olive (Saour and Makee, 2003). All of these articles reported similar results to those discussed above: Application of kaolin-based particle films resulted in reduced oviposition, increased juvenile or larval mortality, decreased damage to fruits and/or flowers and an overall decrease in feeding behavior on the part of the pest organism. The relative uniformity of these results, spread over a wide array of host/pest relationships, suggests that there is great potential for kaolin-based particle films to provide considerable protection of cucurbit crops from damage by adult *A. vittatum*.

Materials and Methods

Research was conducted during the 2004 and 2005 growing seasons. Sites included on-farm locations in the central Willamette Valley as well as greenhouses located on the Oregon State University campus, Corvallis, Oregon. Reference specimens of the insect under study, *A. trivittatum*, were collected during September, 2005 and subsequently pinned and labeled. Mr. Jim LaBonte, systematist for the Plant Division of the Oregon Department of Agriculture, identified the beetles to species as *Acalymma trivattatum* (Mannerheim).

In 2004, on-farm sites included: Stahlbusch Island Farms Incorporated's 'Highway 34 field' (SIFI 34), located two miles east of Corvallis; Gathering Together Farms' main farm (GTF 2004), located one mile south of Philomath, Oregon; McDowell Creek Family Farm (McDowell 2004), located seven miles southeast of Lebanon, Oregon; and Persephone Farm (Persephone), located eight miles southeast of Lebanon, Oregon. Greenhouse trials were conducted in the West Greenhouses, wing 15-3, on the Oregon State University campus.

In 2005, on-farm sites included: Stahlbusch Island Farms Incorporated's 'Riverside field' (SIFI Riverside), located two miles east of Corvallis; Gathering Together Farms' main farm (GTF 2005), located one mile south of Philomath, Oregon; McDowell Creek Family Farm (McDowell 2005), located seven miles southeast of Lebanon, Oregon; and adjacent to Kiger Island Nursery (Kiger), located four miles south of Corvallis. Greenhouse trials were conducted in the same location as in 2004.

At all trial locations the growers provided for the overall culture and care of the crops on which research was conducted. The only exception was that I applied the kaolin-based particle film at all sites but one. I collected all data germane to the experiments conducted.

The kaolin-based particle film (KBPF) treatments applied in this study utilized Surround® WP, provided by the Englehard Corporation (Iselin, NJ). Unless otherwise noted, all applications were made with a mixture consisting of 177 grams Surround per liter of water (equivalent to three cups per gallon, as recommended on product packaging). Applications were made using a 15.14 liter, 45 p.s.i. backpack sprayer (Solo, model 425, Newport News, VA) with a single nozzle wand. Applications were made such that all upper plant surfaces above ground were covered with the KBPF. Studies where both upper and lower plant surfaces were coated are indicated.

Efficacy of the KBPF was evaluated by different methods as time and knowledge about the interactions among crop, beetle, weather and irrigation events developed. In the field I had two sites where I was able to collect information on numbers of beetles present on treated and untreated plants through time. A leaf damage rating scale was soon adopted when it became apparent that I would be physically unable to take the time necessary to count beetle numbers at the other sites. The damage rating scale allowed me to quantify damage from the beetles in a rapid, replicable and efficient manner using a rating scale with a range of 0-4 that could be used as the plants grew and developed increasing, and differing, numbers of leaves.

The scale used is similar to those used by Pena et al. (1984), Peterson et al. (1989), White (1990) and Hallet et al. (2004) to measure damage caused to a variety of crops by several different arthropod pests. The visual damage scale is also similar to the one used by Baker and Robinson (1985) to measure damage to cucurbit seedlings by various *Acalymma* species in New York.

The damage scoring system used was as follows: '0'= no beetle feeding damage apparent on any leaves; '1'= beetle feeding damage present on 1%-25% of leaves; '2'= beetle feeding damage present on 26%-50% of leaves; '3'= beetle feeding damage present on 51%-75% of leaves; '4'= beetle feeding damage present on 76%-100% of leaves, or the plant had been killed by beetle feeding. Beetle damage was typically in the form of holes through leaves or the removal of the lower epidermis of the cotyledons. A leaf was considered damaged if there were one or more holes present. A cotyledon was considered damaged if it was desiccated due to feeding on the underside (*A. trivittatum* feeding on cotyledons almost never creates holes). For purposes of this study, cotyledons were considered to be ‘leaves’. Data was always collected between 1-2 pm in order to maintain uniform sampling intervals.

One trial compares treatments using leaf damage scale values by treatment, numbers of dead seedlings by treatment and the corresponding yields of *Delicata* var. winter squash by treatment.

Because *A. trivittatum* damage in cucurbits grown in western Oregon is most severe during the seedling stage, my experiments with KBPF were initially constructed to provide data on reducing colonizing beetles as well as seedling damage reduction. This was to be done on both transplanted and direct-seeded cucurbits for the three to four week the adults cause damage to seedlings by recording beetles and/or damage to seedlings at 5 to 7 day intervals while beetles were attacking the plantings.

The treatments I evaluated were methods of application: (1) dipping transplants into a prepared KBPF solution and allowing them to dry immediately prior to transplanting (2) applying the KBPF once at transplanting time or at seedling emergence for seeded crops (3) applying KBPF at transplanting (or emergence of seedlings) and again ten days later. Some of the trials were modified because overhead irrigation applied at planting and usually within four or five days later removed most of the visual residue of the KBPF on treated plants. These trials were designed to determine the value of maintaining a KBPF coating on leaves to protect plants from *A. trivittatum* feeding for the first 10 days after transplanting versus 20 days after transplanting and usually necessitated from 1 to 3 additional sprays depending on the duration (10 or 20 days) of protection desired.

In the greenhouse I evaluated feeding preferences of both *A. trivittatum* and Duu when exposed to KBPF-treated and untreated cucurbit seedlings. Finally, a cucurbitacin-free variety of cucumbers was evaluated against a cucurbitacin-containing variety for feeding preference by *D. uu*.

2004 Field Trials

<u>SIFI 34</u>:

This trial was designed to determine whether: 1) a single application of KBPF would reduce the number of *A. trivittatum* colonizing seedling pumpkins and 2) if two applications, spaced ten days apart, would be more effective than one. Plants in plots receiving treatment ‘0’ (a.k.a. “control”) were sprayed with water only on 6/2 and 6/12. Plants in plots receiving treatment ‘1’ (a.k.a. “1 spray”) were sprayed on all upper surfaces with a uniform coating of KBPF on 6/2, followed by a water spray on 6/12. Plants in plots receiving treatment ‘2’ (a.k.a. “2 spray”) were sprayed on all upper surfaces with a uniform coating of KBPF on 6/2 and 6/12.

Initiated on 6/2, this trial was placed in a seedling field of pumpkins (variety ‘Golden Delicious’) planted on 5/22. The trial consisted of three treatments with four replications, arranged in a randomized complete block design. Each plot was 40’ x 14’ (14’ width accommodated five rows, while 40’ length accommodated 6 to 7 plants per row), for a total trial area of 160’ x 42’ containing approximately 372 plants. The trial was located at one edge of a larger field that was under ‘conventional’ management. The field was chosen because of reported damage by an *Acalymma* species in prior years. Since insecticidal sprays were used on the rest of the field, a buffer zone of 12’ surrounded the trial on two sides, while the other two sides were bordered by woodland. Spray applications on the rest of the field occurred during periods without measurable wind, in order to reduce the possibility of spray drift.

Plot comparisons were made by counting and recording total numbers of beetles on all plants within a plot for each sample date and statistically analyzing the number of beetles present on each plant within plots. Counts were conducted shortly after dawn, when temperatures were relatively cool and beetle movement was therefore minimal. Beetles were counted if they were found anywhere on the plant or in the crevices adjacent to the stem where it emerged from the soil. Counts were made at five day intervals, starting on 6/2, prior to the first application of KBPF, and continuing through 6/22, by which time beetle numbers had decreased sharply. By 6/2 it was determined that seedling emergence was complete and >75% of the plants had begun to expand the first true leaf. The remaining ≈25% of plants were in the cotyledon stage. Actual beetle numbers were only measured for one trial in each of the two study years (SIFI 34 in 2004, GTF 2005) because it would have been too time intensive to count beetles at all trials.

GTF 2004:

A significant change in experimental design occurred between the start of the SIFI 34 trial and the start of the GTF 2004 trial and should be noted. As mentioned earlier, irrigation events tend to wash off much of the KBPF. Due to the realities of on-farm research, it is not always possible to know exactly when an irrigation event will take place. As a result, the '1 spray' vs. '2 spray' approach led to inconsistencies in the amount of time that plants in a particular plot were *actually* covered with KBPF. This led to the development of the '10 day' vs. '20 day' approach. Under this system, KBPF would be applied on a given day and *maintained* for either 10 or 20 days. In practice, this meant keeping track of when irrigation events occurred so that KBPF could be reapplied immediately afterwards. In this fashion, coverage could be maintained for exactly 10 or 20 days. At the end of the designated coverage period the KBPF was washed off, either by fortuitously-timed irrigation or by a high pressure water spray applied with the use of a backpack sprayer.

This trial was designed to compare three KBPF treatments for reduction of damage to seedling winter squash (var. delicata). The treatments were 1) one application only to field transplants made the day of transplanting, 2) two applications made to field transplants, the second applied ten days after the first, and 3) seedlings dipped into the standard rate of KBPF immediately prior to transplanting. An untreated check plot was included for comparison.

The four treatments were: 'Dip', '10 day', '20 day' and 'no spray'. Plants in plots receiving treatment 'no spray' (a.k.a. "control") were sprayed with water whenever other plots received applications of KBPF. Plants in plots receiving treatment 'Dip' were dipped in a label-rate solution of KBPF immediately prior to planting and then had their coating of KBPF maintained for ten days (overhead irrigation for three hours or more tends to remove most of the KBPF). Plants in plots receiving treatment ‘1 Spray’ (a.k.a. “10 day coverage”) were sprayed on all upper surfaces with a uniform coating of KBPF immediately after planting on 6/12 and this coating was maintained for ten days. Plants in plots receiving treatment ‘2 Spray’ (a.k.a. “20 day coverage”) were sprayed on all upper surfaces with a uniform coating of KBPF immediately after planting on 6/12 and this coating was maintained for twenty days. A maintenance application was required for treatments ‘Dip’, ‘1 spray’ and ‘2 spray’ on 6/17. A second maintenance application was required for ‘2 spray’ plots on 6/22. The ‘Dip’ treatment was only used in the trials conducted at Gathering Together Farm and Persephone Farm. This was because only those farms establish cucurbit crops using greenhouse-raised transplants. The practice of planting greenhouse-raised seedlings is common for organic farms (which both GTF and Persephone are), but almost unheard of on conventional farms, which tend to direct seed much larger acreages. The practice of dipping seedlings prior to planting offers the opportunity to provide more complete coverage, because both upper and lower leaf surfaces are covered, thus it seemed reasonable to evaluate this manner of applying the KBPF. In addition, this trial involved counting the number of dead plants in each plot on 6/29. Since most yield losses caused by *A. trivittatum* are caused by plant mortality prior to fruit set, this seemed like a valuable data set to collect.

Initiated on 6/12, the ‘GTF 2004’ trial was located in a field of transplanted winter squash seedlings (var. ‘Delicata’). The trial consisted of four treatments with four replications, arranged in a ‘randomized block’ pattern. Each plot was 25’ x 6’ (this 6’ width accommodated two rows and the 25’ length accommodated 11-12 plants), for a total trial area of 200’ x 12’, containing approximately 352 plants. The trial was located in the middle of a ≈2 acre field that was being managed according to both USDA and Oregon Tilth organic regulations. Other crops being grown in the vicinity included chard, lettuce and onions. This site was selected because it had a history of *A. trivittatum* infestations.

Data was collected from 6/12 through 7/7 at five day intervals. Data regarding beetle feeding damage to plants was recorded and compared using a visual damage rating scale with a range of 0-4. The scale used is similar to those used by Pena et al. (1984), Peterson et al. (1989), White (1990) and Hallet et al. (2004) to measure damage caused to a variety of crops by several different arthropod pests. The visual damage scale is also similar to the one used by Baker and Robinson (1985) to measure damage to cucurbit seedlings by various *Acalymma* species in New York.

The damage scoring system used was as follows: '0'= no beetle feeding damage apparent on any leaves; '1'= beetle feeding damage present on 1%-25% of leaves; '2'= beetle feeding damage present on 26%-50% of leaves; '3'= beetle feeding damage present on 51%-75% of leaves; '4'= beetle feeding damage present on 76%-100% of leaves, or the plant had been killed by beetle feeding. Beetle damage was typically in the form of holes through leaves or the removal of the lower epidermis of the cotyledons. A leaf was considered damaged if there were one or more holes present. A cotyledon was considered damaged if it was desiccated due to feeding on the underside (*A. trivittatum* feeding on cotyledons almost never creates holes). For purposes of this study, cotyledons were considered to be 'leaves'. Data was always collected between 1-2 pm in order to maintain uniform sampling intervals. This particular rating system, using percentages of leaves damaged by beetle feeding, was chosen because it allowed use of the same scale even as the plants grew and developed increasing, and differing, numbers of leaves.

McDowell 2004:

This trial was designed to determine two things: 1) Does application of KBPF reduce *A. trivittatum* damage to young pumpkin plants, and 2) Is there a difference in damage to plants protected with KBPF for ten days compared to those protected with KBPF for 20 days. This second question is important because, if there is no difference, growers could save themselves the expense of extended and unnecessary applications. Plants in plots receiving treatment '1' (a.k.a. "control") were sprayed with water whenever other plots received applications of KBPF. Plants in plots receiving treatment '2' (a.k.a. "10 day coverage") were sprayed on all upper surfaces with a uniform coating of KBPF shortly after seedling emergence, on 6/24, and this coating was maintained for ten days. Plants in plots receiving treatment '3' (a.k.a. "20 day coverage") were sprayed on all upper surfaces with a uniform coating of KBPF shortly after seedling emergence, on 6/24, and this coating was maintained for twenty days. A maintenance application was required for treatments '2' and '3' on 6/29. Further maintenance applications were required for '3' plots on 7/5 and 7/10.

Initiated on 6/24, this trial was located in a field of direct seeded pumpkins *var*. 'jack o' lantern'. The trial consisted of three treatments with four replications, arranged in a 'randomized block' pattern. Each plot was 40' x 14' (the 14' width accommodated four rows and the 40' length allowed for 17 to 20 plants), for a total trial area of 160' x 42', containing approximately 840 plants. The trial was located near the southeastern corner of a ≈6 acre field under conventional management. Other crops being grown in the vicinity included corn, wheat, winter squash and cut flowers. This site was selected because an adjacent field of winter squash seedlings had been nearly destroyed between 6/3/04 and 6/22/04, indicating that beetle pressure in the area was high. Notably, this grower had never seen *A. trivittatum* prior to the 2004 growing season, indicating that all adult *A. trivittatum* present had migrated in during the 2004 season.

Data was collected from 6/24 through 7/20 at five day intervals. Data regarding beetle feeding damage to plants was recorded and compared using the rating scale as previously described for GTF 2004.

Persephone Farms 2004:

This trial was designed to answer the same questions as the GTF 2004 trial and therefore used four similar treatments: 'Control', 'one spray', 'two sprays' and 'dip'. This trial was designed before the "10 day coverage vs. 20 day coverage' model was devised, so KBPF coatings were washed off by irrigation events and not replaced. As in the other trials, 'control' received a water spray whenever KBPF was applied to other plots. 'One spray' received an overhead application immediately after planting on 6/3. 'Two sprays' also received an overhead application on 6/3, as well as a second application one week later on 6/10. Transplants destined for ‘dip’ plots were dipped in a bucket containing a label rate mixture of KBPF and water and allowed to dry before being planted on 6/3. These plots received no further applications of KBPF. As opposed to all other trials reported in this study, the managers of Persephone Farm chose to lay out the perimeters of the trial and make all required applications. Partly as a result of this difference, reliable data for this trial was only collected on 6/29. Nonetheless, this data does demonstrate a clear trend and is therefore reported. In addition, this trial involved counting the number of dead plants in each plot on 6/29. Since most yield losses caused by *A. trivittatum* are caused by plant mortality prior to fruit set, this seemed like a valuable data set to collect.

Initiated on 6/3, this trial was located in a field of transplanted pumpkin seedlings (var. ‘Sugar Pie’). The trial consisted of four treatments with four replications, arranged in a non-randomized complete block design. Each plot was 18’ x 12’ (this 12’ width accommodated three rows and the 18’ length allowed for six plants), for a total trial area of 288’ x 12’, containing approximately 288 plants. The trial was located near the middle of a ≈4 acre field that was being managed according to Oregon Tilth organic regulations. Other crops being grown in the vicinity included broccoli and various winter squashes. This site was selected for two reasons. First, it had a history of severe *A. trivittatum* damage. Second, the mangers of the farm were, to my knowledge, the first to use KBPF to protect their cucurbits from *A. trivittatum.*

2005 Field Trials

SIFI Riverside

This trial was designed to determine two things: 1) Does application of KBPF reduce *A. trivittatum* damage to young pumpkin plants, and 2) Is there a difference in damage to plants protected with KBPF for ten days compared to those protected with KBPF for 20 days. While the experimental plots were laid out on 6/3, substantial seedling emergence, and therefore the beginning of the trial, did not occur until 6/6. Furthermore, due to cool, rainy weather, *A. trivittatum* did not colonize the field until 6/9. Due to this delay, coverage periods were changed from 10 day/20 day to 14 day/21 day. Plants in plots receiving treatment '1' (a.k.a. "control") were sprayed with water on all dates when other treatment blocks received KBPF applications. Plants in plots receiving treatment '2' (a.k.a. "14 day coverage")were sprayed on all upper surfaces with a uniform coating of KBPF on 6/6 and 6/13, as well as water spray on 6/20. Plants in plots receiving treatment '3' (a.k.a. "21 day coverage") were sprayed on all upper surfaces with a uniform coating of KBPF on 6/6, 6/13 and 6/20. The trial was designed to determine whether: 1) application of KBPF would have an effect on the number of *A. trivittatum* colonizing young plantings of pumpkin and 2) if one of two coverage periods, 14-day or 21-day, would be more effective than the other. In addition, measurements were taken on a single date, late in the trial, to compare the number of plants in each plot that had been killed by beetle feeding damage.

Initiated on 6/3, this trial was laid out in a seedling field of pumpkins (variety 'Golden Delicious') planted on 5/25. The trial consisted of three treatments with four replications, arranged in a 'randomized block' pattern. Each plot was 25' x 15' (this 15' width accommodated five rows and the 25' length allowed for approximately 10 plants), for a total trial area of 100' x 45' containing approximately 600 plants. The trial was located near the western edge of a larger field that was under 'conventional' management. The field was chosen because *A. trivittatum* had been observed there in prior years. Since insecticidal sprays were used on the rest of the field, a buffer zone of 24' surrounded the trial on three sides, while the other side was bordered by four unsprayed rows and a 25' wide gravel road. Spray applications on the rest of the field occurred during periods without measurable wind in order to reduce the possibility of spray drift.

Comparisons were made by measuring the beetle feeding damage to young plants using the 0-4 damage scale described above. Measurements at this site were always taken between 10 am and 11 am in order to maintain uniform sampling intervals. Measurements were taken at seven day intervals starting on 6/6 and continuing through 7/3

GTF 2005

This trial was designed to determine two things: 1) Does application of KBPF reduce *A. trivittatum* damage to young winter squash plants, and 2) Is there a difference in damage to plants protected with KBPF for ten days compared to those protected with KBPF for 20 days? Plants in plots receiving treatment '0' (a.k.a. "control") were sprayed with water whenever other plots received applications of KBPF. Plants in plots receiving treatment '1' (a.k.a. "10 day coverage") were sprayed on all upper surfaces with a uniform coating of KBPF immediately after planting on 6/15 and this coating was maintained for ten days. Plants in plots receiving treatment '2' (a.k.a. "20 day coverage") were sprayed on all upper surfaces with a uniform coating of KBPF immediately after planting on 6/15 and this coating was maintained for twenty days. A maintenance application was required for treatments '1' and '2' on 6/21. A second maintenance application was required for '2' plots on 6/28.

Initiated on 6/15, this trial was located in a field of transplanted winter squash seedlings (var. 'Delicata'). The trial consisted of three treatments with four replications, arranged in a 'randomized block' pattern. Each plot was 20' x 4' (this 4' width accommodated one row and the 20' length allowed for 9-10 plants), for a total trial area of 80' x 12' containing exactly 112 plants. The trial was located near the southern margin of a ≈4 acre field that was being managed according to both USDA and Oregon Tilth organic regulations. Other crops being grown in the vicinity included chard, onions, butternut squash and fava beans. This site was selected because it had a history of *A. trivittatum* infestations.

Data was collected from 6/15 through 7/10 at five day intervals. Comparisons were made both by counting the number of beetles present on each plant in the entire trial area as well as by measuring beetle feeding damage to each plant. Beetle counts were conducted shortly after dawn (7 am to 8 am), when temperatures were relatively cool and beetle movement was therefore minimal. Beetles were counted if they were found anywhere on the plant or in the crevices adjacent to the stem where it emerged from the soil. Data regarding beetle feeding damage to plants was recorded and compared using the same artificial scale (0-4) described above. Data regarding beetle feeding damage to plants was collected between 8 am and 9 am.

McDowell Creek 2005

This trial was designed to determine two things: 1) Does application of KBPF reduce *A. trivittatum* damage to young pumpkin plants, and 2) Is there a difference in damage to plants protected with KBPF for ten days compared to those protected with KBPF for 20 days. Plants in plots receiving treatment '0' were sprayed with water whenever other plots received applications of KBPF. Plants in plots receiving treatment '1' were sprayed on all upper surfaces with a uniform coating of KBPF shortly after seedling emergence on 7/13, and this coating was maintained for ten days. Plants in plots receiving treatment '2' were sprayed on all upper surfaces with a uniform coating of KBPF shortly after seedling emergence, on 7/13, and this coating was maintained for twenty days. A maintenance application was required for treatments '1' and '2' on 7/19. Further maintenance applications were required for '3' plots on 7/23 and 7/28.

Initiated on 7/13, this trial was located in a field of direct seeded pumpkins (var. 'Jack o' lantern'). The trial consisted of three treatments with four replications, arranged in a 'randomized block' pattern. Each plot was 50' x 15' (this 15' width accommodated four rows and the 50' length would allow for approximately 20 plants), for a total trial area of 150' x 60' containing approximately 960 plants. The trial was located near the western margin of a ≈8 acre field under conventional management. Other crops being grown in the vicinity included corn, wheat, winter squash and cut flowers. This site was selected because several adjacent fields had experienced heavy *A. trivittatum* pressure during the preceding year

No data was collected for this trial because no *A. trivittatum* ever arrived in the field. In an unusual twist, large numbers of imported cabbage loopers, *Trichoplusia ni*, did appear in the field and caused considerable damage to the seedling pumpkins. While specific data was not collected, casual observation indicated less looper damage in plots treated with KBPF.

Kiger Island 2005

This trial was designed, as in the case of the 'greenhouse trials', to determine if *A. trivittatum* feeding response was significantly different on standard variety cucumbers (i.e. Marketmore 76) and cucurbitacin-free cucumber varieties (i.e. Marketmore 80 or 97).

Cucumber seeds of the three varieties were planted, with the intention of growing the resulting plants until the maturation of the first fruits. During that span of time, weekly measurements of *A. trivittatum* feeding damage would be taken, using the 0-4 scale described above. The resulting data would then be compared in order to determine if there had been a variety effect on beetle feeding damage.

To this end, seeds were planted in a research plot on Kiger Island on 7/6. The trial was laid out in a randomized block design containing three treatments (Marketmore 76, 80 and 97) and four replications. Each plot measured 4' x 5' and was surrounded by an ≈1' wide path, resulting in a ≈20' x 17' total trial area. Prior to planting, beds received 0.2 g/ft^2 application of bifenthrin to protect against known plant pathogenic nematode populations and were then rototilled to provide adequate tilth. After planting, a top-dressing of Osmocote fertilizer was applied.

By 7/18 all seedlings had emerged but no *A. trivittatum* were present. Weekly visits to the site were made through 9/5 but no *A. trivittatum* were ever found. Approximately one dozen *D. uu* were encountered in the trial plots on 8/1 and 8/8, but they were found feeding exclusively on the weed *Chenopodium album* (Lambs quarters). As a result, no feeding damage data was collected. This outcome was quite unexpected, since adjacent growers had reported seeing *A. trivittatum* during 2004. This result serves to underline the rather mysterious nature of the movements of *A. trivittatum* throughout local and regional landscapes.

2004 Greenhouse Trials

KBPF Enclosure Trials

This set of trials was designed to determine, in a more controlled setting than on-farm research can provide, whether *A. trivittatum* are less likely to feed on cucumber seedlings if they have been treated with KBPF. Trials were conducted during July and August, 2004 in space 15-3 in the Oregon State University West Greenhouses.

In addition, some of the trials replaced *Acalymma trivittatum* with *Diabrotica undecimpunctata undecimpunctata*. Both species are pests in the Willamette Valley, both are members of the tribe Luperini, and both have been demonstrated to feed compulsively when presented with cucurbitacin-containing plant material. While not central to this project, testing the response of *D. uu* to KBPF-treated seedlings provided an opportunity to show that *A. trivittatum* response to KBPF was not a fluke. In addition, if KBPF is shown to discourage feeding by *D. uu* on cucumber seedlings, it may lead to its use in other crops, such as green beans, on which *D. uu* is a serious economic pest.

The design of these greenhouse trials was relatively simple. Each trial consisted of three enclosures. Each enclosure contained four cucumber seedlings. Seedlings were either treated with KBPF or not treated with KBPF. Once the enclosures had been prepared in this fashion, ten beetles were introduced to each enclosure for 48 hours. At the end of the 48 hours the beetles were removed and the seedlings were rated, using the artificial scale (0-4) described earlier.

Specifically, the enclosures were made of overturned four-gallon, white plastic buckets. Two opposing sides of each bucket were removed. One was replaced with nylon mesh in such a way as to allow access by hand when untied, as well as ventilation. The other was replaced with clear plastic to allow observation. Seedlings were cucumber, *Cucumis sativus* 'Marketmore 76', and were grown in 2''x 2'' nursery containers. Two seedlings were grown in each container. Treated seedlings were sprayed from above with a label rate solution of KBPF using a backpack sprayer. At the end of each 48 hour trial beetles were removed, counted (to verify that none had escaped) and frozen.

Each enclosure contained one of three treatments: ‘KBPF/KBPF’ where both containers (each containing two seedlings) had been treated with KBPF, ‘No KBPF/No KBPF’ where neither of the two containers had been treated with KBPF, and ‘KBPF/No KBPF’ where one container (two seedlings) had been treated with KBPF and one had not. The enclosures were arranged on a flat table top as follows: Left-‘KBPF/KBPF’, Middle-‘No KBPF/No KBPF’, Right-‘KBPF/no KBPF’.

‘Marketmore 76/80’ Enclosure Trials

While selecting a cultivar to use in the trials described above, it was discovered that several seed companies were selling a “cucurbitacin-free” cultivar. The stated purpose of this cultivar was to allow growers to avoid cucumbers with bitter blossom-ends, a condition that occasionally occurs when cucurbitacins develop in the distal end of the fruit. This “bitter-free” cultivar, called ‘Marketmore 80’ was described as a near-isogenic variant of the very popular ‘Marketmore 76’ cultivar, with the only difference being in the production of cucurbitacin. If in fact this cultivar was free of cucurbitacins, it would stand to reason that it would not elicit the cucurbitacin-induced compulsive feeding behavior on the part of *A. trivittatum*. If this turned out to be the case, breeding cucurbit crops to be cucurbitacin-free could emerge as a new paradigm in the management of *A. trivittatum*.

In order to test this possibility, a second set of trials was devised, based on the KBPF Enclosure Trials described above. All aspects were as described above, with two exceptions. First, the variable of interest became cultivar, not whether KBPF had been applied or not. Accordingly, the enclosures contained: Left-‘Marketmore 80/Marketmore 80’, Middle-‘Marketmore 76/Marketmore 76’, Right-‘Marketmore 80/Marketmore 76’. Second, only Duu were used, due to availability. Whereas the Duu are present in high numbers throughout the growing season, *Acalymma trivittatum* has distinct generations. Unfortunately, during 8/05 very few *A. trivittatum* were available.

2005 Greenhouse Trials

KBPF Enclosure Trials

The greenhouse trials in 2005 were largely the same as the greenhouse trials in 2004, with several important differences. First, only *A. trivittatum*, were used, since they are the focus of this project. Second, the arrangement of the three enclosures was modified. Rather than always have the 'KBPF/KBPF' enclosure on the left, the 'No KBPF/ No KBPF' enclosure always in the center and the 'KBPF/ No KBPF' enclosure always on the right, the position of each treatment was randomized, as well as the positioning of the two different treatments within the enclosure in the case of the 'KBPF/ No KBPF' enclosure. Third, no 'Marketmore 76/80' trials were conducted due to the results in 2004 and preliminary testing in early 2005.

With those three exceptions, the trial was conducted as it had been in 2004: 10 beetles were introduced to each of the three enclosures for 48 hours. Each enclosure contained four seedlings of *Cucumis sativus* (var. Marketmore 76) which had received one of three treatments. The treatments were again 'KBPF/KBPF', 'No KBPF/ No KBPF' and 'KBPF/ No KBPF'. Six replications of this trial were conducted between 8/12 and 8/18. At the end of each 48 hour test period the beetles were removed and damage was assessed using the 0-4 damage scale. A further set of trials was scheduled for early September but was cancelled due to a lack of live *A. trivittatum* specimens.

Statistical Analysis

SIFI-34, GTF 2004, GTF 2005 (Beetle counts)

The beetle count data were analyzed by a repeated measure ANOVA in SAS proc mixed. The Tukey adjustment was used to make treatment comparisons.

SIFI-34 (# Plants with > 5 beetles per plant)

Since the life cycle of the beetle tapers off during the end of the summer, only the days before 6/17 were analyzed by a repeated measure ANOVA in SAS proc mixed. The residual plots showed 1 large outlier. The Tukey adjustment was used to make treatment comparisons.

G.T.F 2004 (Yields)

The yield data were analyzed by a one-way ANOVA in SAS proc mixed. The residuals were examined to confirm model assumptions of normality and homogeneity of variance. The Tukey adjustment was used to make treatment comparisons.

McDowell Creek, GTF 2004, GTF 2005, SIFI Riverside (Damage ratings)

The damage rating was analyzed by taking the base 10 log to account for the larger amount of variablitiy in the control plots. Then the log damage rating data were analyzed by a repeated measure ANOVA in SAS proc mixed. The residuals were examined to confirm model assumptions of normality and homogeneity of variance. The Tukey adjustment was used to make treatment comparisons.

Persephone Farm (Damage ratings, Number of dead plants, Number of plants with >50 beetles)

The data were analyzed by an ANOVA in SAS proc mixed. Only one day of data was available, so no repeated measures techniques were necessary. The residuals were examined to confirm model assumptions of normality and homogeneity of variance. The Tukey adjustment was used to make treatment comparisons.

Greenhouse 2004 and 2005 (KBPF damage ratings)

The damage rating data was analyzed by a paired t-test separately for both beetle types, since the plants within an enclosure are not independent. The residuals were examined to confirm model assumptions of normality and homogeneity of variance.

Greenhouse 2004 (Marketmore variety damage ratings)

The damage rating data was analyzed by a paired t-test since the plants within an enclosure are not independent. The residuals were examined to confirm model assumptions of normality and homogeneity of variance.

SIFI-Riverside, GTF 2004, (Number of dead plants)

The number of dead plants on the last day was analyzed by an ANOVA in SAS proc mixed. Only the last day was used because there was no evidence of a time effect in the treated plots. The residuals were examined to confirm model assumptions of normality and homogeneity of variance. The Tukey adjustment was used to make treatment comparisons.

Results

Field Trials

SIFI 34 2004:

Analysis of the 'SIFI 34' trial indicates that plants receiving either one or two applications of KBPF had statistically fewer beetles per plant than the control plants through day 15 ($t = 10.09$, $df = 30$, $p = <.0001$, and $t = 9.93$, $df = 30$, $p = <.0001$, respectively) (Table 1.). Further, there was no significant difference between the numbers of beetles recorded in the '1 spray' and '2 spray' treatments ($t = -0.15$, $df = 30$, $p = 0.9871$) through day 20. On day 20 numbers of beetles in all plots dropped dramatically and there was no statistical difference between numbers of *A. trivittatum* per plant over the three treatments. (see figure 1 and table 1)

GTF 2004:

The 'GTF 2004' trial compared actual beetle feeding damage (rather than numbers of beetles per plant) as well as the number of dead plants by treatment. I was also able to take data on the eventual yield of marketable squash by treatment. ANOVA tests verify that 'Dip' ($t = -14.31$, $df = 45$, $p = <.0001$), '1-spray' ($t = -14.89$, $df = 45$, $p = <.0001$) and '2-spray ($t = -16.40$, $df = 45$, $p = <.0001$) resulted in significantly less beetle damage than that of the control. Of interest is the fact that analysis showed no significant difference among the treatments using the leaf damage rating scale. 'Dip' and '1-spray' treatments ($t = -0.58$, $df = 45$, $p = 0.5676$). Furthermore, while the graph below appears to show a difference in damage between '2-spray' and '1-spray' and '2-spray' and 'Dip', ANOVA tests indicate no significant difference between '2-spray' and '1-spray'($t = 1.51$, $df = 45$, $p = 0.1371$) and only little evidence of a difference between '2-spray' and 'Dip' ($t = -2.09$, $df = 45$, $p = 0.0423$).

The number of dead plants in each treatment, as measured on the last sampling date (7/7) 25 days after transplanting, indicates that all treatments were effective in reducing the number of plants killed as a result of *A. trivittatum* feeding during the trial. There is evidence that the number of dead plants differs for ‘1-spray’ versus ‘no-spray’ (t = -3.55, df = 6, p = 0.0121). There is strong evidence that the number of dead plants differs for ‘dip’ vs. ‘no spray’ (t = -4.03, df = 6, p = 0.0069). Analysis was not conducted on ‘no spray’ versus ‘2-spray’ because ‘2-spray’ had no variability (all values were zero) and this causes problems in the model. Nonetheless, the fact that all values were zero for ‘2-spray’ indicates strong evidence for a difference.

Marketable yields were lowest in the untreated control plots. All treatments had yields that were significantly greater than the control (‘no spray’). However, there were no differences noted among the three KBPF treatments. There is evidence that the yield is different for ‘1 spray’ vs. ‘no spray’ (t = 3.46, df = 9, p = 0.0301), there is evidence that the yield is different for ‘2 spray’ vs. ‘no spray’ (t = 3.74, df = 9, p = 0.0199) and there is strong evidence that the yield is different for ‘dip’ vs. ‘no spray’ (t = 3.92, df = 9, p = 0.0152). There were no significant differences between the yields for ‘dip’, ‘1-spray’ and ‘2-spray’. (see figure 2 and tables 2,3,4)

McDowell 2004:

The ‘McDowell 2004’ trial compared *A. trivittatum* leaf feeding damage among three different treatments: plants protected with two sprays for ‘10-days’, plants protected with four sprays for ‘20-days’ and untreated plants. Both of the KBPF treatments had significantly less *A. trivittatum* leaf feeding damage than the untreated plants. There is strong evidence that ‘no spray’ has different damage than ‘10-day’ (t = 14.57, df = 30, p = <.0001) and there is strong evidence that ‘no spray’ has different damage than ‘20-day’ (t = 14.75, df = 30, p = <.0001), however, there is no evidence that ‘10-day’ has different damage than ‘20-day’ (t = 0.18, df = 30, p = 0.9828). (see figure 3 and table 5)

Persephone 2004:

Twenty six days after transplanting, plants that were either dipped in KBPF solution prior to transplanting or received one spray at transplanting had significantly fewer damaged leaves than the UTC plants. The plants with the least amount of leaf feeding damage were those protected with a spray at transplanting followed by a spray 7 days later. The 'Persephone' trial, as represented by damage comparisons among four treatments on 6/29, shows a treatment effect for only one treatment, '2-spray' ($t = 4.36$, $df = 9$, $p = 0.0081$). This result differs from all other field trials where all treatments resulted in less beetle damage than the control. This may be a reflection of the fact that this trial experienced the highest beetle pressure of any trial during the two year project. When the number of dead plants per treatment is compared for 'Persephone', only the '2-spray' treatment shows any significant difference from the other treatments. (see tables 6 and 7)

SIFI Riverside:

The results from the 'SIFI-Riverside' trial continue to show a clear trend. Both '14-day' and '21-day' treatments provide strong evidence that there is a significant difference in beetle damage between themselves and the 'no spray' control ($t = 11.65$, $df = 24$, $p = <.0001$ and $t = 12.99$, $df = 24$, $p = <.0001$ respectively). As in previous trials, there is no evidence of a significant difference between '14-day' and '21-day' treatments ($t = 1.34$, $df = 24$, $p = 0.3874$).

'SIFI-Riverside' also matches previous results in terms of number of dead plants per treatment. Once again, both '14-day' and '21-day' treatments provide strong evidence that there is a significant difference in the number of plants killed due to beetle feeding between themselves and the 'no spray' control ($t = 12.66$, $df = 6$, $p = <.0001$ and $t = 13.06$, $df = 6$, $p = <.0001$ respectively). As in previous trials, there is no evidence of a significant difference between '14-day' and '21-day' treatments ($t = 0.41$, $df = 6$, $p = 0.9135$). (see figure 4 and tables 8,9)

GTF 2005:

The results from the 'GTF 2005' trial also continue to show the same clear trend as previous results. Both '10-day' and '20-day' treatments provide strong evidence that there is a significant difference in beetle damage between themselves and the 'no spray' control ($t = 7.08$, $df = 30$, $p = <.0001$ and $t = 8.35$, $df = 30$, $p =$

<.0001 respectively). As in previous trials, there is no evidence of a significant difference between '10-day' and '20-day' treatments (t = 1.27, df = 30, p = 0.4213).

The beetle count results from the 'GTF 2005' trial also support the now well-established trend. Both '10-day' and '20-day' treatments provide strong evidence that there is a significant difference in the number of beetles per plant between themselves and the 'no spray' control (t = 13.31, df = 30, p = <.0001 and t = 14.44, df = 30, p = <.0001 respectively). As in previous trials, there is no evidence of a significant difference between '14-day' and '21-day' treatments (t = 1.13, df = 30, p = 0.5014). (see figures 5, 6 and tables 10, 11)

Greenhouse trials

The KBPF greenhouse trials conducted in both 2004 and 2005 indicate that adults of both *A. trivittatum* and *Duu* display a preference for seedlings not treated with KBPF.

In 2004, choice tests were conducted on both *D. uu* and *A. trivittatum*, as described earlier. There is strong evidence that the no KBPF – KBPF difference for *D. uu* is not 0 (p-value = 0.001). It is estimated that for *D. uu* the difference in damage ratings for no KBPF - KBPF is 3.125 (95% confidence interval is 2.36 to 3.89). (see figure 7 and table 12). There is also strong evidence that *A. trivittatum* causes more feeding damage to untreated seedlings than to treated seedlings (p-value = 0.0016). It is estimated that for the *A. trivittatum* the difference in damage ratings for untreated vs. treated is 2.625 (95% confidence interval is 1.86 to 3.39). (see figure 8 and table 13)

In the 2005 KBPF greenhouse trials only *A. trivittatum* were used and more replications were conducted than in 2004. As expected, results for the 2005 trials were more or less the same as in 2004 (with the exception that no *D.uu* were used). A one-sample t-test indicated that there is strong evidence that *A. trivittatum* cause more feeding damage to untreated seedlings than to treated seedlings (p-value = 0.0013). It is estimated that the difference in damage ratings for untreated vs.

treated is 2.375 (95% confidence interval is 1.44 to 3.31). (see figures 9,10,11 and table 14)

The results of the Marketmore variety choice test trials conducted in 2004 were quite the opposite, in that they showed no clear preference at all on the part of the beetles for one cultivar over the other. As discussed earlier, only *D. uu* were used in this trial. T-test analysis indicated that there is no evidence that Marketmore 80 – Marketmore 76 is not 0 (p-value = 0.4512). It is estimated that the difference between damage scores for Marketmore 80 vs. Marketmore 76 is .125 (95% confidence interval is -.25 to .5). (see table 15)

The results of the growth rate trial in 2004 were also conclusive. One-way ANOVA tests indicated that all pairwise comparisons between treatments were significant. Plants sprayed with the label rate solution of KBPF on upper surfaces only (1x top) had higher average dry weight than the control (adjusted p-value < 0.0001. It is estimated that 1x top weighs .32 grams more than control. 95% adjusted confidence interval is 0.26 to 0.39 grams more). Control plants, in turn, had higher average dry weights than plants sprayed with a label rate solution of KBPF on both upper and lower surfaces (1x top/bot) (adjusted p-value < 0.0001. It is estimated that control weighs 0.63 grams more than top/bottom. 95% adjusted confidence interval is 0.57 to 0.70 grams more). Finally, 1x top/bot. plants had higher average dry weights than plants sprayed with a twice label rate (2x top) solution of KBPF (adjusted p-value < 0.0001. It is estimated that top/bot. weighs 0.14 grams more than 2x top. 95% adjusted confidence interval is 0.07 to 0.20 grams more). (see figure 12 and table 16)

Figure 1. Average number of *A. trivittatum* per plant by treatment and date at SIFI-34 trial.

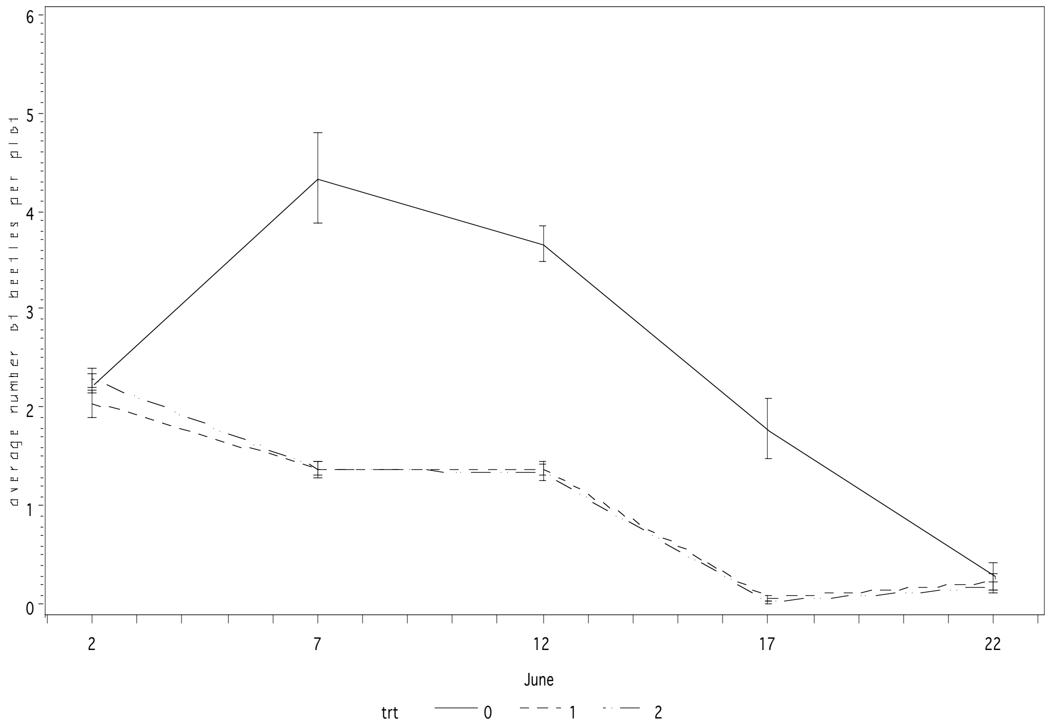

Table 1. SIFI 34, 2004. Pumpkin *Golden delicious.* Means ± SE of numbers of *A. trivittatum* per plant by treatment and date for 1 and 2 Applications of KBPF. Numbers include beetles found in soil immediately adjacent to stems.

		Average (Mean ± SE) Number of Beetles Per Plant				
Treatment	Duration	6/2/04	6/7/04	6/12/04	6/17/04	6/22/04
Control	--	2.25 ± .10a	4.33 ± .46a	3.68 ± .18a	1.79 ± .31a	.29 ± .13a
KBPF	1-spray	2.05 ± .14a	1.38 ± .06b	1.39 ± .07b	.07 ± .03b	.23 ± .08a
KBPF	2-spray	2.29 ± .10a	1.38 ± .08b	1.34 ± .08b	.03 ± .02b	.18 ± .06a
	F=	1.08	35.54	135.97	29.94	.28
	P=	.3990	.0005	<.0001	.0008	.7633

[a] Columns are original means ± SEM. Column means with different letters are significantly different with means separated using the Tukey test (P=0.05) (SAS Institute Inc. 9.1, 2002-2003).

Figure 2. Average leaf damage to delicate squash seedlings by *A. trivittatum* by treatment by date at GTF 2004.

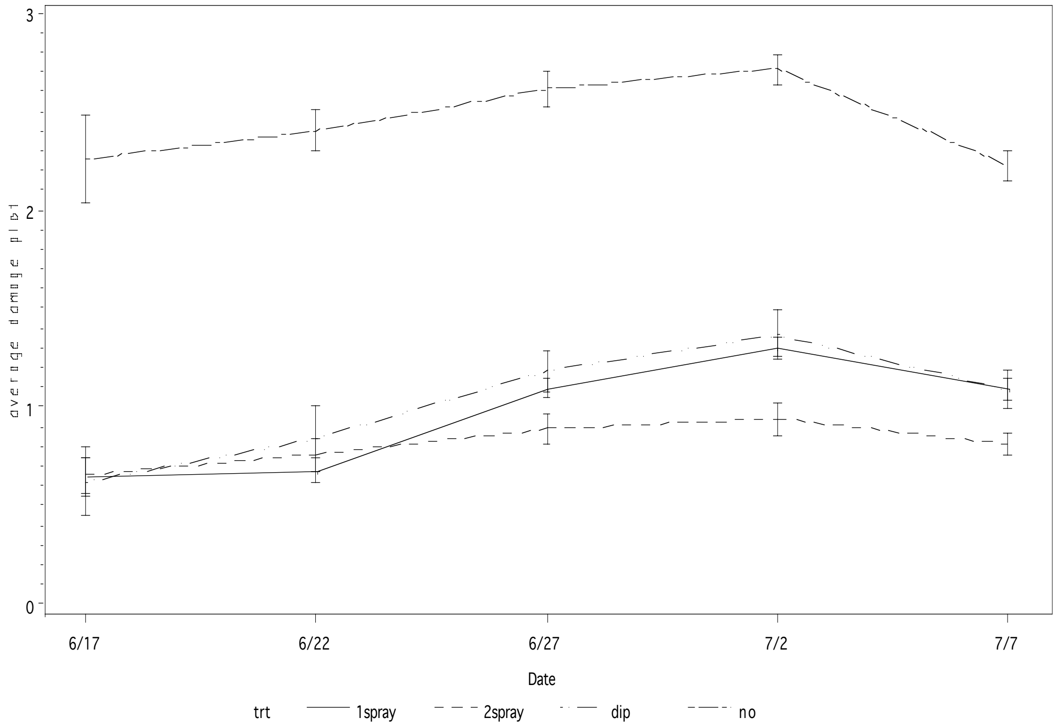

Table 2. *A. trivittatum* damage to delicata squash seedlings at Gathering Together Farm, 2004. Damage means ± SE are based on 0-4 visual damage rating scale.

		A. trivittatum Damage Rating by Date and Days after first Spray or Dip[a]				
Treatment	Duration	6/17/04[b]	6/22/04	6/27/04	7/2/04	7/7/04
Control	--	2.26 ± .22a	2.40 ± .10a	2.61 ± .08a	2.72 ± .08a	2.22 ± .07a
KBPF	Dip	.62 ± .17b	.84 ± .16b	1.18 ± .10b	1.37 ± .13b	1.09 ± .10b
KBPF	10-day	.65 ± .10b	.67 ± .06b	1.10 ± .05b	1.30 ± .05b	1.09 ± .06b
KBPF	20-day	.66 ± .09b	.76 ± .08b	.89 ± .08b	.94 ± .08b	.81 ± .05b
	F=	32.11	70.51	102.77	88.13	84.76
	P=	<.0001	<.0001	<.0001	<.0001	<.0001

[a] Beetle damage based on a rating of 0 to 4; 0 = no damage, 1 = 1% - 25% damage, 2 = 26% - 50% damage, 3 = 51% - 75% damage and 4 = >75% damage.
[b] Columns are original means ± SEM. Column means with different letters are significantly different with means separated using the Tukey test (*P*=0.05) (SAS Institute Inc. 9.1, 2002-2003).

Table 3. GTF 2004: Mean number of plants killed by *A. trivittatum* feeding on delicata squash plants at Gathering Together Farm, 2004. Plots contained 24 plants on average.

		Accumulated Number of Plants Killed *by A.* trivittatum Feeding				
Treatment	Duration	6/17/04 [a]	6/22/04	6/27/04	7/2/04	7/7/04
Control	--	3.25 ± .48a	4.50 ± .87a	6.5 ± 1.04a	7.50 ± 1.26a	8.50 ± 1.26a
KBPF	Dip	0.0 ± 0.0b	.25 ± .25b	.50 ± .29b	.75 ± .25b	.75 ± .25b
KBPF	10-day	0.0 ± 0.0b	0.0 ± 0.0c	1.00 ± .41b	1.75 ± .63c	2.25 ± .85c
KBPF	20-day	0.0 ± 0.0b	0.0 ± 0.0c	0.0 ± 0.0c	0.0 ± 0.0d	0.0 ± 0.0d
	Asymptotic Pr > X^2	.0021	.0048	.0089	.0073	.0074

[a] Columns are original means ± SEM. Column means with different letters are significantly different with means separated using the Kruskal-Wallis test (*P*=0.05) (SAS Institute Inc. 9.1, 2002-2003).

Table 4. Effect of KBPF on mean yield of marketable delicata squash at GTF 2004 trial.

		Avg. yield (in lbs.) by treatment
Treatment	Duration	10/7/04
Control	--	72.5 ± 8.68a
KBPF	Dip	104.25 ± 9.17b
KBPF	10-day	100.5 ± 5.85b
KBPF	20-day	102.75 ± 10.49b
	F=	6.94
	P=	.0102

[a] Columns are original means ± SEM. Column means with different letters are significantly different with means separated using the Tukey test (*P*=0.05) (SAS Institute Inc. 9.1, 2002-2003).

Figure 3. Average leaf damage to pumpkin seedlings by *A.* trivittatum by treatment by date during McDowell 2004 trial (log transformed).

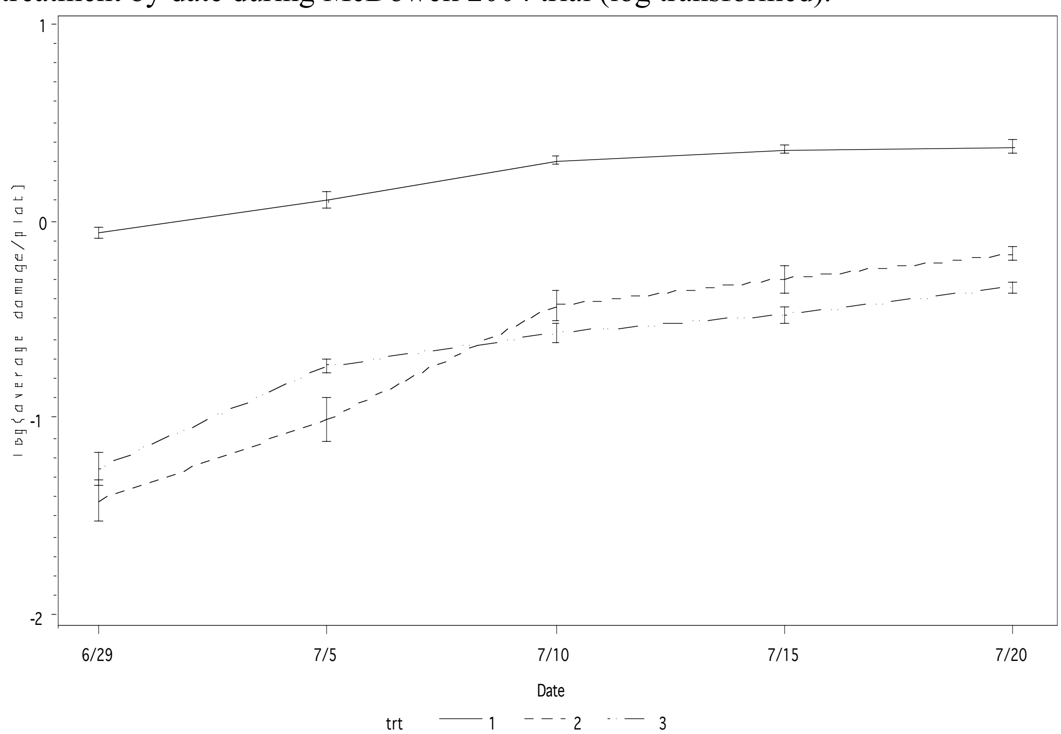

Table 5. *A. trivittatum* damage on pumpkin seedlings at McDowell Creek Farm, 2004. Damage means ± SE are based on 0-4 visual damage rating scale.

		Cucumber Beetle Damage Rating[a]				
Treatment	Duration	6/29/04[b]	7/5/04	7/10/04	7/15/04	7/20/04
Control	--	.88 ± .06a	1.30 ± .12a	2.03 ± .08a	2.28 ± .12a	2.39 ± .16a
KBPF	10-day	.03 ± .01b	.11 ± .03b	.39 ± .07b	.53 ± .08b	.68 ± .06b
KBPF	20-day	.05 ± .01b	.19 ± .01b	.28 ± .03b	.34 ± .03b	.46 ± .03b
	F=	272.5	117.88	181.74	115.52	95.40
	P=	<.0001	<.0001	<.0001	<.0001	<.0001

[a] Beetle damage based on a rating of 0 to 4; 0 = no damage, 1 = 1% - 25% damage, 2 = 26% - 50% damage, 3 = 51% - 75% damage and 4 = >75% damage.
[b] Columns are original means ± SEM. Column means with different letters are significantly different with means separated using the Tukey test (*P*=0.05) (SAS Institute Inc. 9.1, 2002-2003).

Table 6. Persephone Farm 2004. *A. trivittatum* damage on pumpkin seedlings, June 29, 2004. Damage means ± SE are based on 0-4 visual damage rating scale.

		26 Days Post transplanting, *A.* trivittatum Damage Rating[a]
Treatment	Duration	6/29/04[b]
Control	--	2.16 ± .21a
KBPF	Dip	1.86 ± .16b
KBPF	1-spray	1.78 ± .14b
KBPF	2-spray	.95 ± .26c
	F=	6.98
	P=	.0100

[a] Beetle damage based on a rating of 0 to 4; 0 = no damage, 1 = 1% - 25% damage, 2 = 26% - 50% damage, 3 = 51% - 75% damage and 4 = >75% damage.
[b] Columns are original means ± SEM. Column means with different letters are significantly different with means separated using the Tukey test (*P*=0.05) (SAS Institute Inc. 9.1, 2002-2003). Plants transplanted on 6/3/2004

Table 7. Mean ± SE of number of dead plants per plot caused by *A. trivittatum* feeding at Persephone Farm as of June 29, 2005. Plots contained 18 plants each at the beginning of the experiment.

		Dead Plants Per Plot
Treatment	Duration	6/29/04 [a]
Control	--	6.0 ± 1.22a
KBPF	Dip	6.0 ± 1.29a
KBPF	1-spray	5.0 ± 1.08a
KBPF	2-spray	2.25 ± 1.11b
	F=	2.79
	P=	.1018

[a] Columns are original means ± SEM. Column means with different letters are significantly different with means separated using the Tukey test (*P*=0.05) (SAS Institute Inc. 9.1, 2002-2003).

Figure 4. Average leaf damage to pumpkin seedlings by *A. trivittatum* by treatment by date at SIFI-Riverside trial.

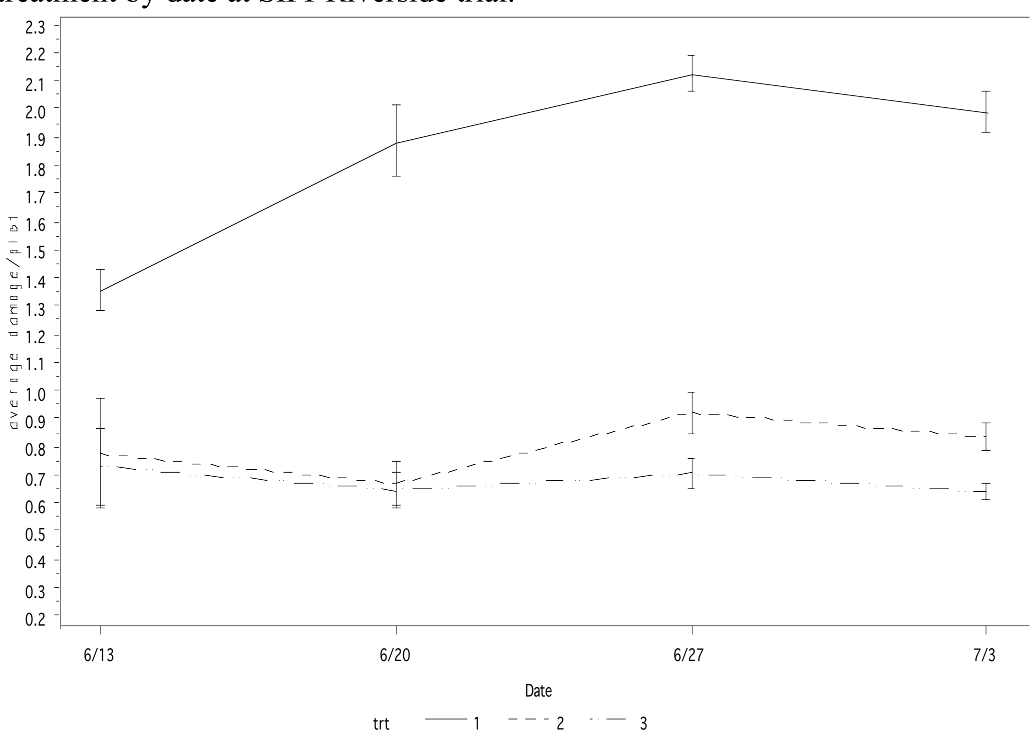

Table 8. *A. trivittatum* damage to 'golden delicious' pumpkin plants at SIFI-Riverside trial, 2005. Damage means ± SE are based on 0-4 visual damage rating scale.

		Cucumber Beetle Damage Rating[a]			
Treatment	Duration	6/13/05[b]	6/20/05	6/27/05	7/3/05
Control	--	1.36 ± .07a	1.89 ± .13a	2.13 ± .06a	1.99 ± .07a
KBPF	14-day	.77 ± .19b	.67 ± .08b	.92 ± .07b	.83 ± .05b
KBPF	21-day	.73 ± .14b	.64 ± .06b	.71 ± .05c	.64 ± .03c
	F=	5.51	50.78	111.82	147.42
	P=	.0438	.0002	<.0001	<.0001

[a] Beetle damage based on a rating of 0 to 4; 0 = no damage, 1 = 1% - 25% damage, 2 = 26% - 50% damage, 3 = 51% - 75% damage and 4 = >75% damage.
[b] Columns are original means ± SEM. Column means with different letters are significantly different with means separated using the Tukey test (*P*=0.05) (SAS Institute Inc. 9.1, 2002-2003).

Table 9. Mean number of plants killed by *A. trivittatum* feeding on 'golden delicious' pumpkin plants at SIFI-Riverside trial, 2005. Plots contained 50 plants on average.

		Plants Killed by Beetle Feeding			
Treatment	Duration	6/13/05[a]	6/20/05	6/27/05	7/3/05
Control	--	2.0 ± .71a	1.75 ± .85a	4.75 ± .85a	8.25 ± .75a
KBPF	14-day	0.5 ± .29b	0.5 ± .29b	.50 ± .29b	.50 ± .29b
KBPF	21-day	.25 ± .25b	.25 ± .25b	.25 ± .25b	.25 ± .25b
Asymptotic Pr > X^2		.1406	.2167	.0163	.0153

[a] Columns are original means ± SEM. Column means with different letters are significantly different with means separated using the Kruskal-Wallis test (*P*=0.05) (SAS Institute Inc. 9.1, 2002-2003).

Figure 5. Average leaf damage to delicate squash seedlings by *A.* trivittatum by treatment by date during GTF 2005 trial (log transformed).

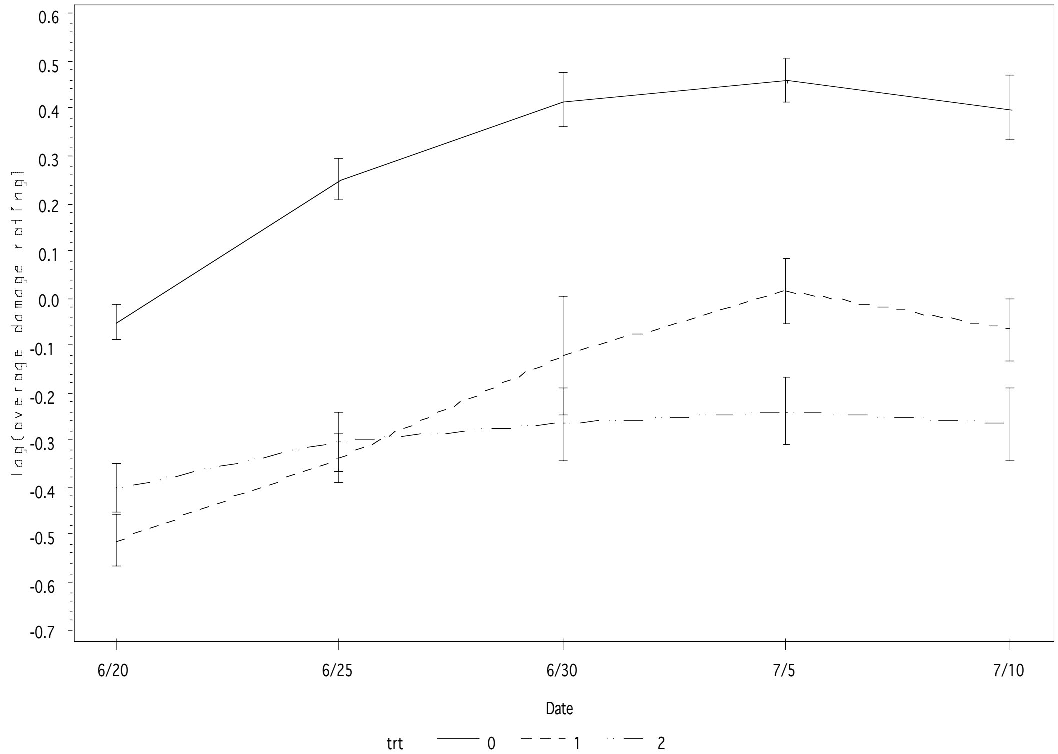

Table 10. *A. trivittatum* damage on delicate squash seedlings at Gathering Together Farm, 2005. Damage means ± SE are based on 0-4 visual damage rating scale.

		Cucumber Beetle Damage Rating[a]				
Treatment	Duration	6/20/05[b]	6/25/05	6/30/05	7/5/05	7/10/05
Control	--	.89 ± .11a	1.81 ± .21a	2.68 ± .23a	2.92 ± .18a	2.59 ± .22a
KBPF	10-day	.32 ± .08b	.47 ± .11b	.84 ± .14b	1.08 ± .13b	.89 ± .12b
KBPF	20-day	.41 ± .08b	.51 ± .11b	.57 ± .13b	.59 ± .13c	.57 ± .13b
	F=	30.88	35.46	22.91	31.25	19.08
	P=	.0007	.0005	.0016	.0007	.0025

[a] Beetle damage based on a rating of 0 to 4; 0 = no damage, 1 = 1% - 25% damage, 2 = 26% - 50% damage, 3 = 51% - 75% damage and 4 = >75% damage.
[b] Columns are original means ± SEM. Column means with different letters are significantly different with means separated using the Tukey test (*P*=0.05) (SAS Institute Inc. 9.1, 2002-2003).

Figure 6. Average number of *A. trivittatum* per plant, by treatment and date, at GTF 2005 (log transformed).

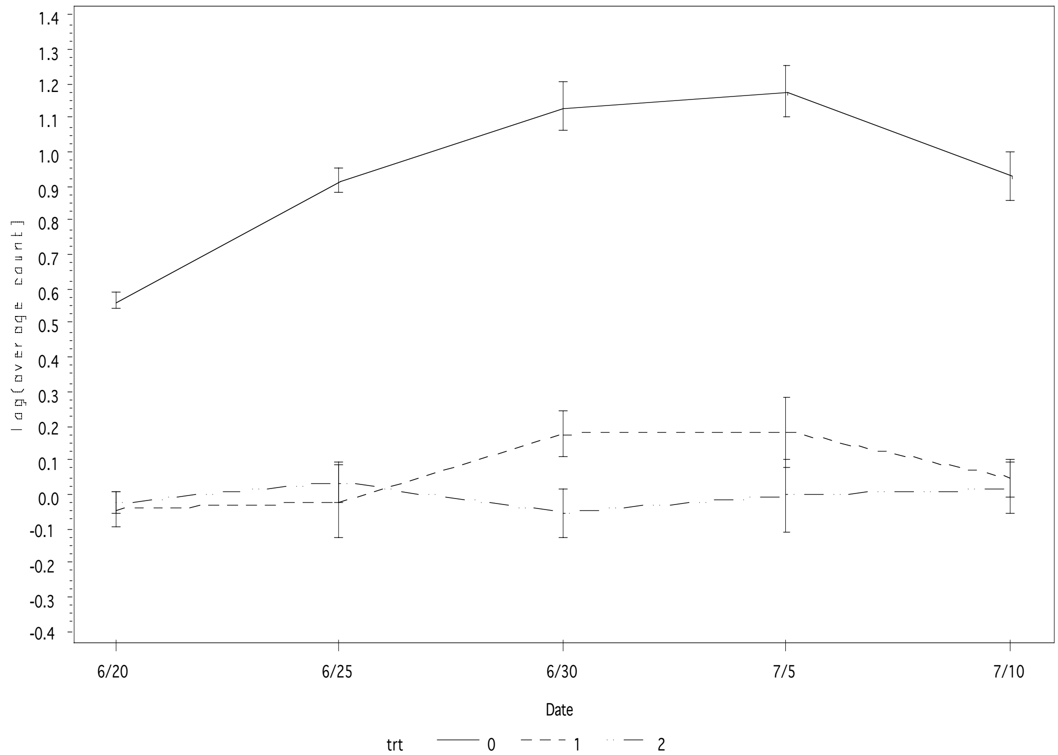

Table 11. Means ± SE of number of beetles (*A. trivittatum*) per plant by treatment and date at GTF-Delicata trial, 2005. Values include beetles found in soil immediately adjacent to stems.

		Average (Mean ± SE) Number of Beetles Per Plant				
Treatment	Duration	6/20/05	6/25/05	6/30/05	7/5/05	7/10/05
Control	--	3.70 ± .59a	8.39 ± ..92a	13.47 ± 1.76a	14.23 ± 2.15a	8.09 ± 1.32a
KBPF	10-day	.92 ± .17b	1.03 ± .20b	1.55 ± .23b	1.62 ± .25b	1.14 ± .21b
KBPF	20-day	.95 ± .15b	1.11 ± .22b	.92 ± .18c	1.08 ± .18b	1.08 ± .19b
	Asymptotic Pr > X^2	.0246	.0245	.0123	.0164	.0228

[a] Columns are original means ± SEM. Column means with different letters are significantly different with means separated using the Kruskal-Wallis test (*P*=0.05) (SAS Institute Inc. 9.1, 2002-2003).

Figure 7. Damage ratings (0-4) measured on treated and untreated cucumber seedlings after 48 hour exposure to *D. uu* during 2004 KBPF Enclosure Choice Tests.

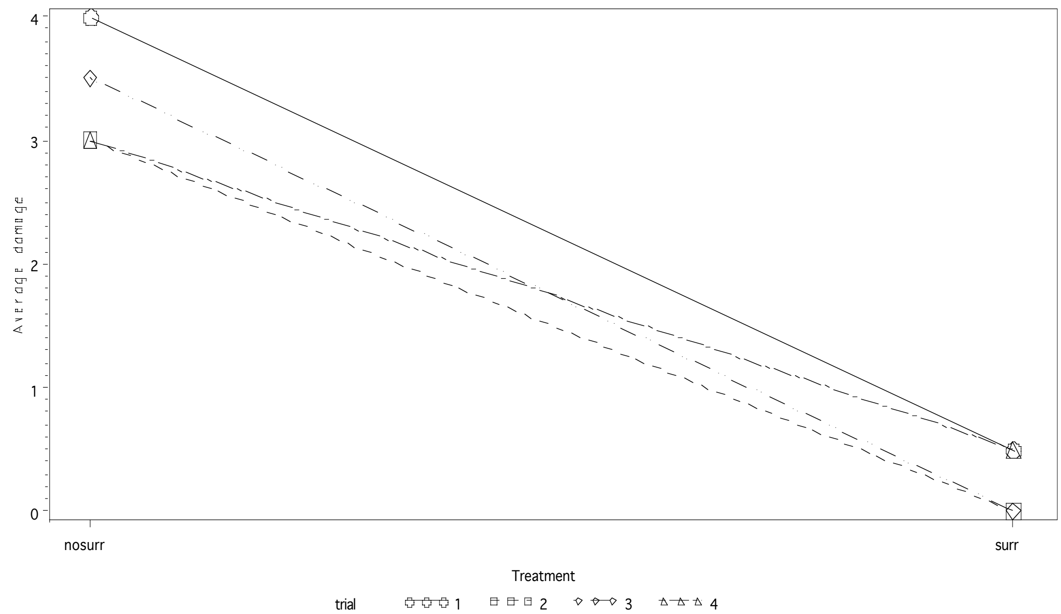

Table 12. *D. undecimpunctata* damage on cucumber seedlings in greenhouse choice test, 2004. Damage means ± SE are based on 0-4 visual damage rating scale. Means are calculated using data from four replications, conducted on 7/7, 7/13, 7/18 and 7/24.

	Greenhouse Trial Cucumber Beetle Damage Rating[a]
Treatment	
Control	3.38 ± 0.24a
KBPF	.25 ± .14b
T=	13.06
P=	0.001

[a] Beetle damage based on a rating of 0 to 4; 0 = no damage, 1 = 1% - 25% damage, 2 = 26% - 50% damage, 3 = 51% - 75% damage and 4 = >75% damage.
[b] Columns are original means ± SEM. Column means with different letters are significantly different with means separated using the Tukey test (*P*=0.05) (SAS Institute Inc. 9.1, 2002-2003).

Figure 8. Damage ratings (0-4) measured on treated and untreated cucumber seedlings after 48 hour exposure to *A. trivittatum* during 2004 KBPF Enclosure Choice Tests.

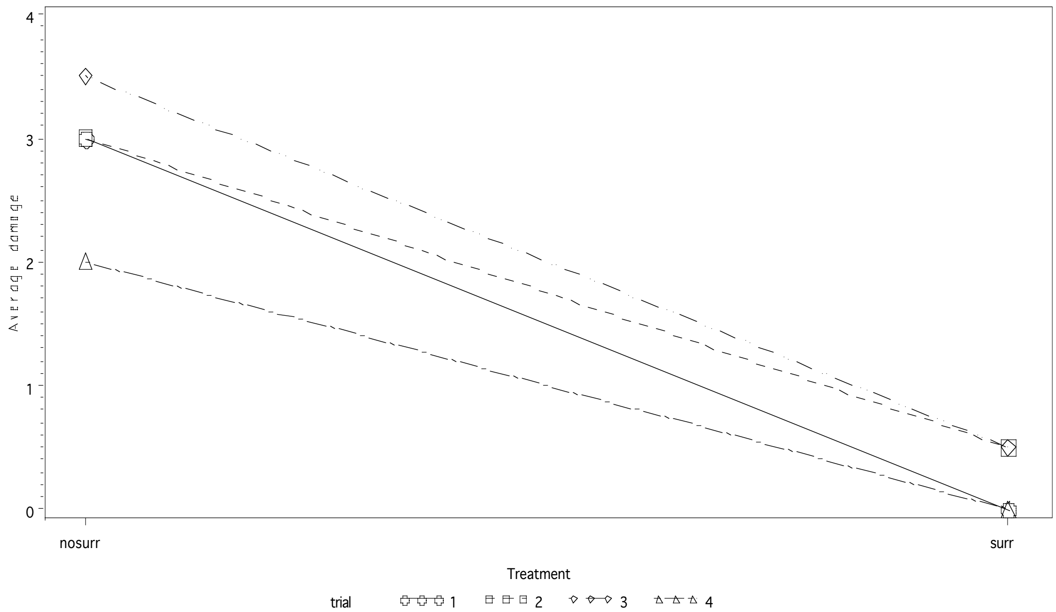

Table 13. *A. trivittatum* damage on cucumber seedlings in greenhouse choice test, 2004. Damage means ± SE are based on 0-4 visual damage rating scale. Means are calculated using data from four replications, conducted on 7/7, 7/13, 7/18 and 7/24.

	Greenhouse Trial Cucumber Beetle Damage Rating[a]
Treatment	
Control	2.88 ± 0.31a
KBPF	.25 ± .14b
T=	10.97
P=	0.002

[a] Beetle damage based on a rating of 0 to 4; 0 = no damage, 1 = 1% - 25% damage, 2 = 26% - 50% damage, 3 = 51% - 75% damage and 4 = >75% damage.
[b] Columns are original means ± SEM. Column means with different letters are significantly different with means separated using the Tukey test (*P*=0.05) (SAS Institute Inc. 9.1, 2002-2003).

Figure 9. Damage ratings (0-4) measured on treated and untreated cucumber seedlings after 48 hour exposure to *A. trivittatum* during 2005 KBPF Enclosure Choice Tests. (8/12)

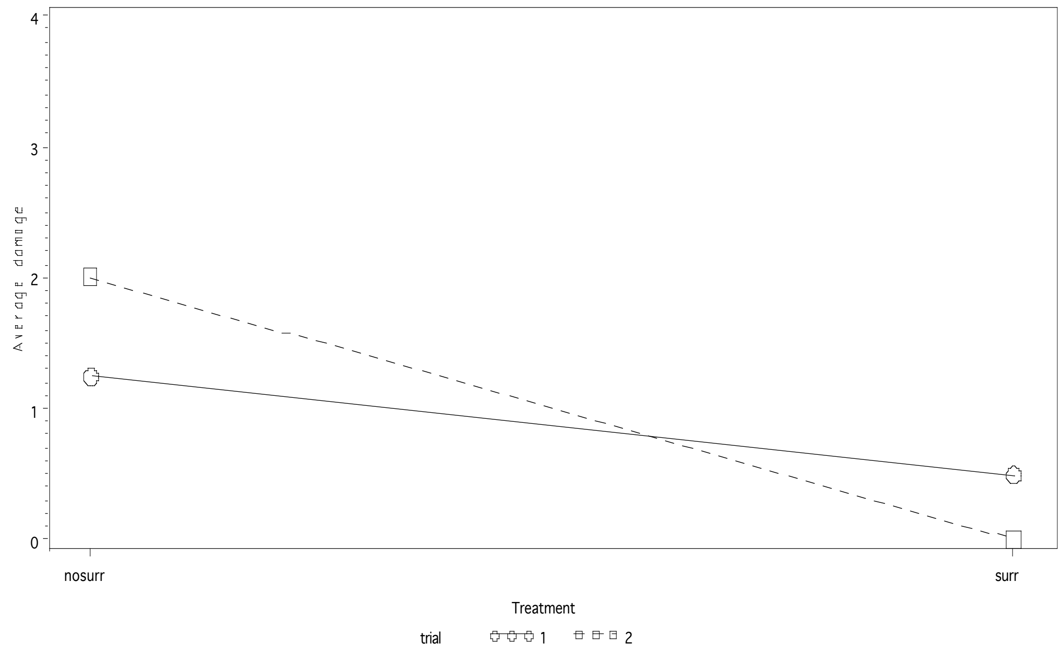

Figure 10. Damage ratings (0-4) measured on treated and untreated cucumber seedlings after 48 hour exposure to *A. trivittatum* during 2005 KBPF Enclosure Choice Tests. (8/14)

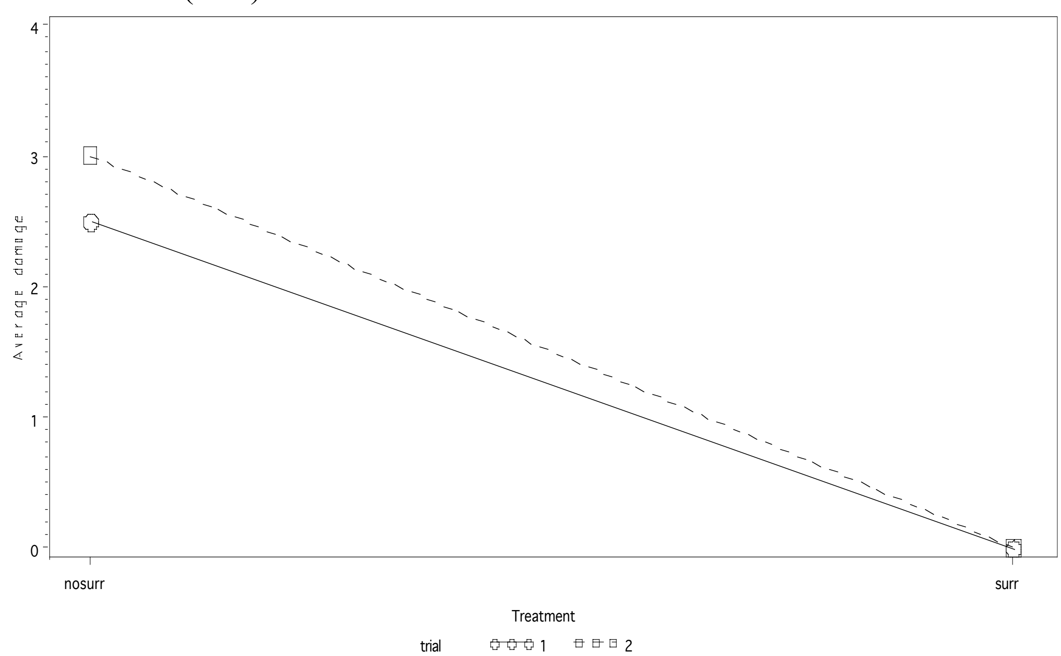

Figure 11. 2005 KBPF Enclosure Choice Test Using *A. trivittatum* (8/16)

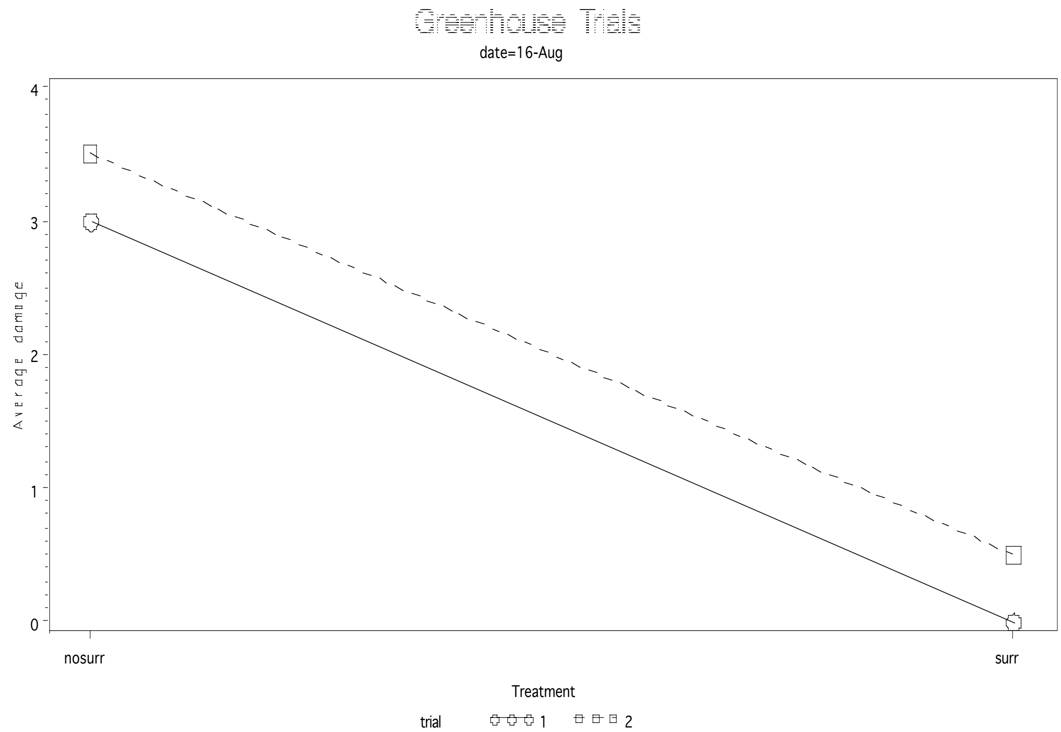

Table 14. *A. trivittatum* damage on cucumber seedlings in greenhouse choice test, 2005. Damage means ± SE are based on 0-4 visual damage rating scale.

	Greenhouse Trial Cucumber Beetle Damage Rating[a]		
Treatment	8/12/05[b]	8/14/05	8/16/05
Control	1.63 ± .38a	2.75 ± .25a	3.25 ± .25a
KBPF	.25 ± .25a	0.0 ± 0.0a	.25 ± .25b
T=	2.20	11.0	∞
P=	0.2716	0.0577	<.0001

[a] Beetle damage based on a rating of 0 to 4; 0 = no damage, 1 = 1% - 25% damage, 2 = 26% - 50% damage, 3 = 51% - 75% damage and 4 = >75% damage.
[b] Columns are original means ± SEM. Column means with different letters are significantly different with means separated using the Tukey test (*P*=0.05) (SAS Institute Inc. 9.1, 2002-2003).

Table 15. *D. undecimpunctata* damage on cucumber seedlings in variety ('Marketmore 76/80') choice test, 2004. Damage means ± SE are based on 0-4 visual damage rating scale.

		Greenhouse Variety Trial Cucumber Beetle Damage Rating[a]			
Treatment		8/1/04[b]	8/7/04	8/13/04	8/19/04
Marketmore 76'		1.00 ± 0.0a	1.00 ± .50a	0.75 ± .25a	1.25 ± .25a
'Marketmore 80'		1.25 ± .25a	1.00 ± .00a	1.0 ± 0.0a	1.25 ± .25a
	T=	1.0	0.0	1.0	0.0
	P=	0.500	1.000	0.500	1.000

[a] Beetle damage based on a rating of 0 to 4; 0 = no damage, 1 = 1% - 25% damage, 2 = 26% - 50% damage, 3 = 51% - 75% damage and 4 = >75% damage.
[b] Columns are original means ± SEM. Column means with different letters are significantly different with means separated using the Tukey test (P=0.05) (SAS Institute Inc. 9.1, 2002-2003).

Figure 12. 2004 growth rate comparison of *C. sativa* grown with different KBPF application rates and application methods.

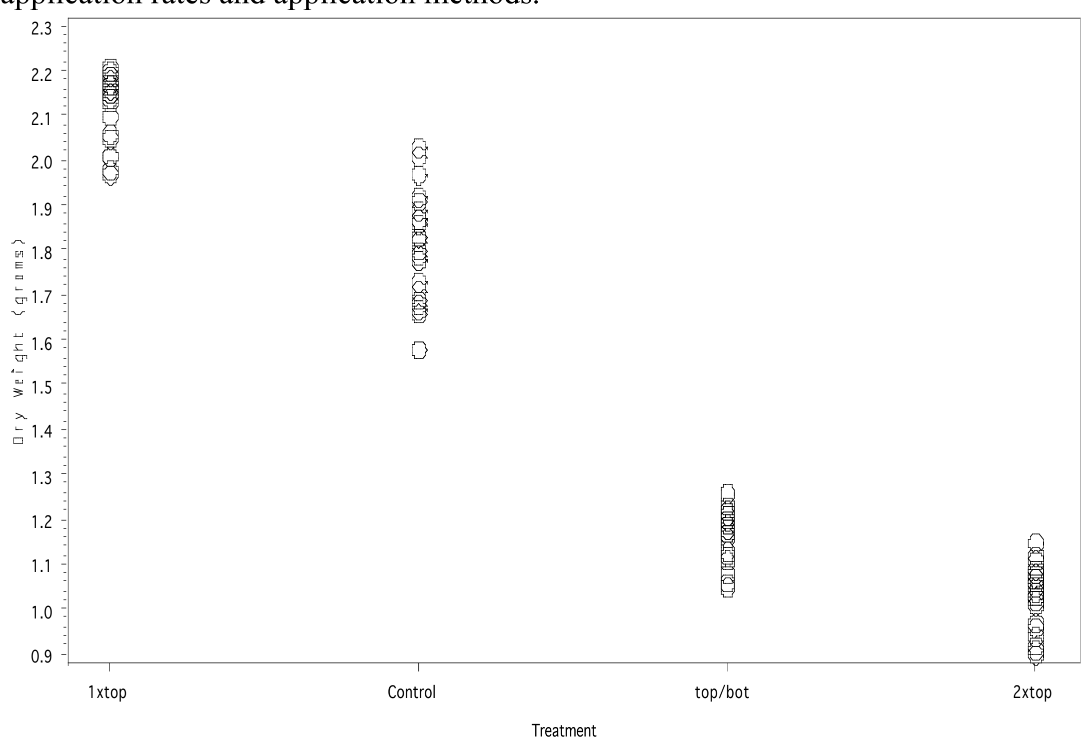

Table 16. Dry weight averages ± SE of cucumber plants grown with different rates and application types of KBPF.

	Greenhouse Trial Cucumber Plant Dry Weights (grams)
Treatment	Weight [a]
Control	1.80 ± .03a
1x top	2.13 ± .01b
2x top	1.03 ± .01c
1x top/bot.	1.17 ± .01d
F=	924.82
P=	<.0001

[a] Columns are original means ± SEM. Means with different letters are significantly different with means separated using the Tukey test (*P*=0.05) (SAS Institute Inc. 9.1, 2002-2003).

Discussion

Effects of Kaolin (KBPF) on *A. trivittatum* damage

As was expected, due to the results of previous research on other insect pests (Unruh et al., 2000, Knight et al., 2000, LaPointe, 2000, Davis, 2002, Wang, 2002, Cottrell et al, 2002, Bellows and Perring, 2002, Liang and Liu, 2002, Boheman, 2002), the application of a kaolinite-based particle film was effective in significantly reducing damage to cucurbit seedlings by *Acalymma trivittatum*.

When employed prophylactically, prior to colonization of a planting by *A. trivittatum*, the particle film succeeded in reducing both numbers of beetles and the resulting feeding damage, as compared to untreated controls, in all field trials conducted during the course of this project. Enclosure tests conducted in greenhouses indicate that *A. trivittatum* show a strong preference for untreated seedlings over seedlings which have been treated with KBPF.

The duration of the coverage period (10 days from germination or 20 days from germination) appears to have had an effect on total beetle damage, with longer coverage leading to slightly lower damage. However, this difference was only sometimes significant and was always dwarfed by the difference between either coverage treatment versus no coverage.

Response of *A. trivittatum* to kaolin particle film

The behavioral response of *A. trivittatum* upon encountering a plant surface that has been treated with a kaolin-based particle film is similar to the responses of a variety of other insects, as documented by Glenn et al. (1994). Upon coming into contact with the kaolin particles, numerous particles adhere to the exterior of the beetle, principally the tarsi. This adherence appears to irritate the beetle, as they promptly stop foraging behavior, including the characteristic waving of antennae, and attempt to remove the irritating particles. Typically, the rear tarsi are rubbed against the side of the abdomen whereas the beetle tries to use its mouthparts to remove the particles from the front tarsi. Unfortunately for the beetle, this behavior only succeeds in spreading the particles to other parts of its body, most noticeably the mouthparts, antennae and elytra. At this point the beetle continues trying to remove the particles from its body, to no effect. Many times during the course of this project beetles were observed engaging in this cleaning behavior, which often involves an elaborate 'dance' involving a ruffling of the wings. Not once was a beetle observed to have satisfactorily cleaned itself and returned to foraging and/or feeding. Frequently, the beetle would attempt to clean itself for several minutes then fly away.

Plant growth with kaolin particle film application

Of serious concern is any effect the particle film treatments might have on seedling growth. Based on the results of growth rate trials conducted for this project it appears that kaolin-based particle films do not hinder growth if: 1) Plants are treated only on upper leaf surfaces and 2) plants are treated with a label-rate (3c./gal.) solution of the kaolin powder in water. The growth trial conducted indicated that seedlings treated on upper and lower leaf surfaces, as well as seedlings treated with a 2X solution (6c./gal.) grew significantly slower.

Economic Considerations

If any new pest management strategy is to be adopted, it must be both effective and economically feasible. The results reported earlier show that KBPF is effective at reducing damage to cucurbit crops due to feeding by the *A. trivittatum*. It must now be determined if this approach will be economically appealing.

The two synthetic pyrethroids commonly used by conventional growers to control the *A. trivittatum* are Carbaryl and Esfenvalerate. Sevin® brand Carbaryl, which is applied at a rate of 1.25 lbs./acre, can be purchased for $5.60 per pound, resulting in a per acre cost of $7.00. Asana XL® brand Esfenvalerate, which is applied at a 6 oz./acre rate, can be purchased for $94/gal., resulting in a cost of $4.41 per acre.

The most common and effective control method used by organic growers is floating row cover. Agribon® AG-15 floating row cover is most commonly used.

In order to cover one acre with floating row cover, the material cost would be $656.88. Most growers use the same row cover for two seasons, so the annual cost would be approximately $328.44.

In comparison, a kaolinite-based particle film, sold as Surround WP®, is applied at a rate of 25 lbs./acre and can be purchased for $1 per pound. Assuming two applications during the early growth of a cucurbit crop, this results in a per acre cost of $50.

When these numbers are compared it is clear that conventional farmers will not switch to KBPF for economic reasons, although some may be enticed by the non-toxic nature of KBPF to overlook the associated increase in capital expenditure. Organic growers, however, will see KBPF as a significant savings over floating row covers. At a savings of ≈$275 per acre of cucurbit crop, KBPF should allow for significantly increased profitability.

Use as part of an IPM program

Growers will likely find that the use of kaolin-based particle films is an effective element of IPM programs for protection of cucurbit crops. Non-toxic and environmentally benign, kaolin adds a powerful prophylactic tool to the list of natural and synthetic products already being used.

The prophylactic nature of this product does pose one problem for IPM practitioners. Typically, applications of crop protectants are not made until an action threshold has been met, based on a monitoring program of some sort. Due to the rapidly aggregating nature of this pest, as discussed earlier, a reasonable action threshold for *A. trivittatum* in a planting of young cucurbits is one beetle. As reported earlier, the time between the arrival of one 'scout male' and a full-blown infestation can be as short as 48 hours. With this in mind, a different type of action threshold is called for. I believe that if a grower has had large numbers of *A. trivittatum* in their cucurbit crops in a given year (particularly if they have had high numbers in the latter part of the season), then *it makes sense to apply kaolin to emerging seedling cucurbits the following spring before any A. trivittatum have been spotted and identified*.

A further consideration has to do with the results reported from the 'Persephone' trial. While the majority of field trials in this project indicated there was no significant improvement in plant protection gained by extending kaolin coverage to 20 days from seedling emergence, there may be an exception when beetle numbers are particularly high. *A. trivittatum* numbers were extremely high at Persephone Farms in 2004 and they did see a statistically significant benefit in plots that received extended kaolin coverage. This result suggests that extended coverage would indeed be beneficial in situations with extremely high beetle counts.

Recommendations for future research

As with any question, the answer to the question posed in this project leads to several new questions. The most obvious question produced by my research comes from the Persephone trial in 2004, where unusually high numbers of beetles led to the only results where the extended coverage treatment proved significantly better than other treatments. This suggests that we need to develop an action threshold, based on beetle numbers, for when to maintain KBPF coverage beyond 10-14 days from germination.

Additionally, research should be conducted to determine whether a KBPF solution rate less than that on the product label would still provide significant crop protection. If so, this would represent an opportunity for financial savings on the part of growers adopting the use of KBPF.

Thirdly, I would like to see research conducted around the issue of specific cucurbit cultivars that do not produce cucurbitacins. I attempted to investigate this facet of the relationship between cucumber beetles and cucumbers during the course of my research but got inconclusive results. Based on the well-documented connection between cucurbitacins and the destructive behavior of cucumber beetles, I still believe that cucurbitacin-free cultivars can play a significant role in decreasing both crop damage and pesticide application to cucurbit crops.

References Cited

Baker, P.B. and R.W. Robinson. 1985. Evaluations of selected cucurbita accessions for cucumber beetle complex resistance. *Search: Agriculture*. 31:1-6.

Bradbury, J.F. 1970. *Erwinia tracheiphila*: CMI description of pathogenic bacteria. No. 233. Commonwealth Mycological Institute, Kew, Surrey, England.

Brewer, M.J., Story, R.N. and V.L. Wright. 1987. Development of summer squash seedlings damaged by striped and spotted cucumber beetles (Coleoptera: Chrysomelidae). *J. Econ. Entomol.* 80(5):1004-1009.

Burkness, E.C., and W.D. Hutchison. 1998. Action thresholds for striped cucumber beetle (Coleoptera: Chrysomelidae) on 'Carolina' cucumber. *Crop Protection.* 17(4):331-336.

Caldwell, J.S. and P. Clarke. 1999. Repulsion of cucumber beetles in cucumber and squash using aluminum-coated plastic mulch. *HortTechnology*. 9(2):247-250.

Chambliss, O.L. and C.M. Jones. 1966. Cucurbitacins: specific insect attractants in Cucurbitaceae. *Science.* 153:1392-1393.

Cottrell, T.E., Wood, B.W. and C.C. Reilly. 2002. Particle film affects black pecan aphid (Homoptera: Aphididae) on pecan. *J. Econ. Entomol.* 95(4):782-788.

David, A. and D.K. Vallance. 1955. *J. Pharm. Pharmacol.* 7:295-296.

Ellers-Kirk, C.D., Fleischer, S.J., Snyder, R.H. and J.P. Lynch. 2000. Potential of entomopathogenic nematodes for biological control of *Acalymma vittatum* (Coleoptera: Chrysomelidae) in cucumbers grown in conventional and organic soil management systems. *J. Econ. Entomol.* 93(3):605-612.

Ferguson, J.E. and R.L. Metcalf. 1985. Cucurbitacins: plant-derived defense compounds for diabroticites (Coleoptera: Chrysomelidae). *Jour. Chem. Ecol.* 11(3):311-318.

Fisher, G. 2005. Personal communication. Various dates throughout 2005. Oregon State University Extension specialist for entomology.

Foster, N. and L.S. Cordell (eds.). 1992. Chiles to chocolate: foods the Americas gave the world. Tucson. University of Arizona Press.

Glenn, D.M., Puterka, G.J., Vanderzwet, T., Byers, R.E. and C. Feldhake. 1999. Hydrophobic particle films: a new paradigm for suppression of arthropod pests and plant diseases. *J. Econ Entomol.* 92(4):759-771.

Gould, F. and A. Massey. 1984. Cucurbitacins and predation of the spotted cucumber beetle, *Diabrotica undecimpunctata howardi. Entomol. Exp. Appl.* 36:273-278.

Hallett, R.H., Ray, H., Holowachuk, J., Soroka, J.J. and M.Y. Gruber. 2004. Bioassay for assessing resistance of *Arabidopsis thaliana* L. (Heynh.) to the adult crucifer flea beetle, *Phyllotreta cruciferae* (Goeze) (Coleoptera: Chrysomelidae). *Can. J. of Plant Sci.* 85: 225-235.

Hoffman, M.P., Kirkwyland, J.J., Smith, R.F. and R.F. Long. 1996. Field tests with kairomone-baited traps for cucumber beetles and corn rootworms in cucurbits. *Environ. Entomol.* 25(5):1173-1181.

Hoffman, M.P., Robinson, R.W., Kyle, M.M. and J.J. Kirkwyland. 1996. Defoliation and infestation of *Cucurbita pepo* genotypes by diabroticite beetles. *HortScience* 31(3):439-442.

Hoffman, M.P., Ayyappath, R. anf J.J. Kirkwyland. 2000. Yield response of pumpkin and winter squash to simulated cucumber beetle (Coleoptera: Chrysomelidae) feeding injury. *J. Econ. Entomol.* 93(1):136-140.

Howe, W.L., J.R. Sanborn and A.M. Rhodes. 1976. Western Corn Rootworm adult and spotted cucumber beetle associations with *Cucurbita* and cucurbitacins. *Environ. Entomol*. 5:1043-1048.

Jifon, J.L. and J.P. Syvertsen. 2003. Kaolin particle film applications can increase photosynthesis and water use efficiency of 'Ruby Red' grapefruit leaves. *J. Amer. Soc. Hort. Sci.* 128(1):107-112.

Knight, A.L., Unruh, T.R., Christianson, B.A., Puterka, G.J. and D.M. Glenn. 2000. Effects of a kaolin-based particle film on obliquebanded leafroller (Lepidoptera: Tortricidae). *J. Econ. Entomol.* 93(3):744-749.

LaBonte, J. 2005. Personal communication regarding history of identification and misidentification of *A. vittatum* and *A. trivittatum* in Oregon. 12/8/05.

Lapointe, S.L. 2000. Particle film deters oviposition by *Diaprepes abbreviatus* (Coleoptera: Curculionidae). *J. Econ. Entomol.* 93(5):1459-1463.

Liang, G. and T.X. Liu. 2002. Repellancy of a kaolin particle film, Surround, and a mineral oil, Sunspray Oil, to silverleaf whitefly (Homoptera: Aleyrodidae) on melon in the laboratory. *J. Econ. Entomol.* 95(2):317-324.

Mazor, M. and A. Erez. 2003. Processed kaolin protects fruits from Mediterranean fruit fly infestations. *Crop Protection.* 23:47-51.

McGrath, D (Ed.). 2005. Pacific Northwest Insect Management Handbook. Oregon State University, Extension Service. http://pnwpest.org/pnw/insects?01VGTB17.dat

Metcalf, R.L., Metcalf, R.A. and A.M. Rhodes. 1980. Cucurbitacins as kairomones for diabroticite beetles. *Proc. Natl. Acad. Sci. USA.* 77:3769-3772.

Michelbacher, A.E., Middlekauff, W.W. and O.G. Bacon. 1953. Cucumber beetles attacking melons in northern California. *J. Econ. Entomol.* 46(3):489-494.

Munroe, D.D. and R.F. Smith. 1980. A revision of the systematics of acalymma sensu stricto barber (Coleoptera: Chrysomelidae) from North America including Mexico. *Memoirs of the Entomol. Soc. Of Canada*. No. 112.

Necibi, S., Barrett, B.A. and J.W. Johnson. 1992. Effects of a black plastic mulch on the soil and plant dispersal of cucumber beetles, *Acalymma vittatum* (F.) and *Diabrotica undecimpunctata howardi* Barber (Coleoptera: Chrysomelidae), on melons. *J. Agric. Entomol.* 9:129-135.

Oregon Tilth. 2005. Personal communication on 10/21/05. 470 Lancaster Dr. NE. Salem, Oregon 97301

Pair, S.D. 1997. Evaluation of systemically treated squash trap plants and attracticidal baits for early-season control of striped and spotted cucumber beetles (Coleoptera: Chrysomelidae) and squash bug (Hemiptera: Coreidae) in cucurbit crops. *J. Econ. Entomol.* 90(5):1307-1314.

Pena, J.E., Wadill, V.H. and S.K. O'Hair. 1984. Mites attacking cassava in southern Florida: damage descriptions and density estimate methods. *Florida Entomologist.* 67(1):141-146.

Peterson, G.C., Ali, A.E.B., Teetes, G.L., Jones, J.W. and K. Schaefer. 1989. Grain sorghum resistance to midge by yield loss and visual scores. *Crop Sci.* 29:1136-1140.

Pitrat, M., Chauvet, M. and C. Foury. 1999. Diversity, history and production of cultivated cucurbits. *Proc. 1st Int. Symp. on Cucurbits. Acta Hort.* (Eds. K. Abak and S. Büyükalaca). 21-28

Radin, A.M. and F.A. Drummond. 1994. An evaluation of the potential for the use of trap cropping for control of the striped cucumber beetle, *Acalymma vittata* (F.) (Coleoptera: Chrysomelidae). *J. Agric. Entomol.* 11(2):95-113.

Reed, D.K., Reed, G.L. and C.S. Creighton. 1986. Introduction of entomogenous nematodes into trickle irrigation systems to control striped cucumber beetle (Coleoptera: Chrysomelidae). *J. Econ. Entomol.* 79(5):1330-1333.

Rodriguez-del-Bosque, L.A. and A. Magallanes-Estala. 1994. Seasonal abundance of *Diabrotica balteata* and other diabroticina beetles (Coleoptera: Chrysomelidae) in northeastern Mexico. *Environ. Entomol.* 23(6):1409-1415.

Saour, G. and H. Makee. 2004. A kaolin-based particle film for suppression of the olive fruit fly *Bactrocera oleae* Gmelin (Diptera: Tephritidae) in olive groves. *J. Appl. Ent.* 128:28-31.

Schupp, J.R., Fallahi, E. and I.J. Chun. 2002. Effect of particle film on fruit sunburn, maturity and quality of ‘Fuji’ and ‘Honeycrisp’ apples. *HortTechnology.* 12(1):87-90.

Showler, A.T. 2002. Effects of kaolin-based particle film application on Boll Weevil (Coleoptera: Curculionidae) injury to cotton. *J. Econ. Entomol.* 95(4):754-762.

Showler, A.T. 2002. Effects of kaolin particle film on beet armyworm, *Spodoptera exigua* (Hubner) (Lepidoptera: Noctuidae), oviposition, larval feeding and development on cotton, *Gossypium hirsutum* L. *Agric., Ecosys. And Environ.* 95:265-271.

Smyth, R.R. and M.P. Hoffman. 2003. A male-produced aggregation pheromone facilitating *Acalymma vittatum* early season host plant colonization. *Jour. of Insect Behavior.* 16(3):347-359.

Tallamy, D.W. and Piotr M. Gorski. 1997. Long- and short-term effect of cucurbitacin consumption on *Acalymma vittatum* (Coleoptera: Chrysomelidae) fitness. *Environ. Entomol.* 26(3):672-677.

Thomas, A.L., Muller, M.E., Dodson, B.R., Ellersieck, M.R. and M. Kaps. 2004. A kaolin-based particle film suppresses certain insect and fungal pests while reducing heat stress in apples. *J. Amer. Pomological Soc.* 58(1):42-51.

Unruh, T.R., Knight, A.L., Upton, J., Glenn, D.M. and G.J. Puterka. 2000. Particle films for the suppression of the codling moth (Lepidoptera: Tortricidae) in apple and pear orchards. *J. Econ. Entomol.* 93(3):737-743.

Webster, F.M. 1895. North American species of diabroticites. *J. N.Y. Entomol. Soc.* 3:158-165.

Westcott, Cynthia. 1973. *The Gardeners Bug Book (4^{th} ed.).* Doubleday and Co. Garden City, N.Y. pp. 146-147,153.

White, W.H. 1990. Yellow sugarcane aphid (Homoptera: Aphididae) resistance mechanisms in selected sugarcane cultivars. *J. Econ. Entomol.* 83(5):2111-2114.

Zehnder, G., Kloepper, J., Yao, C. and G. Wei. 1997. Induction of systemic resistance in cucumber against cucumber beetles (Coleoptera: Chrysomelidae) by plant growth-promoting rhizobacteria. *J. Econ. Entomol.* 90(2):391-396.