DEVELOPING INTEGRATED PEST MANAGEMENT STRATEGIES FOR GREENHOUSE GERBERA DAISIES

by

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ABSTRACT

The serpentine leafminer, *Liriomyza trifolii* (Burgess) (Diptera: Agromyzidae) is a key pest in protected cultivation of ornamentals and vegetables. *L. trifolii* is the primary pest of greenhouse gerberas. Secondary pests including mites (*Tetranychus urticae* Koch), thrips (*Frankliniella occidentalis* (Pergande)), whiteflies (*Trialeurodes vaporarioum* (Westwood), and *Bemisia tabaci* (Gennadius)), aphids (*Myzus persicae (Sulzer)*), and powdery mildew-causing fungal pathogens (from the genera *Podosphaera, Erysiphe, Leveillula, Golovinomyces*, and *Oidium*) also require management. *L. trifolii* is resistant to many commercially available pesticides, while secondary pests are susceptible. Natural enemies can effectively control leafminer populations where pesticide use has been avoided. Pesticides when used often disrupt leafminer biocontrol resulting in over-use of pesticides yet ineffective control of pests. We investigated the compatibility of pesticides, commonly used against leafminers, mites, thrips, whiteflies, and fungal pathogens, with natural enemies of *L. trifolii* (*Diglyphus isaea* (Walker) (Hymenoptera: Eulophidae)) and *T. urticae* (*Neoseiulus californicus* (McGregor)

(Arachnida: Acari: Phytoseiidae)). While commonly used pesticides, e.g., abamectin and spinosad were found to cause severe mortality in the natural enemies, a few others like bifenazate, pyriproxyfen, spiromesifen, and spirotetramat were found to be compatible with a biologically-based control program.

Sixty cultivars of *Gerbera jamesonii* Bolus varied in leafminer preference and damage in a greenhouse choice test. Leaf toughness measured using a penetrometer varied among cultivars, but did not correlate with resistance. A biologically-based Integrated Pest Management (IPM) program was compared with a traditional chemical control regime in a grower greenhouse under realistic growing conditions. Not only was the biologically-based method possible, but also proved cost-effective. Traditional management using insecticides was more expensive and failed to control leafminers, resulting in low quality plants and flowers compared with the biologically-based IPM program. Implementation of a biologically-based IPM program can increase the competitiveness of our local cut flower industry by providing cost effective pest control for a sustainable production system.

INDEX WORDS: *Liriomyza trifolii;* integrated pest management; greenhouse pest management; greenhouse IPM; biologically-based IPM; *Diglyphus isaea; Neoseiulus californicus*

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To

M. C. Abraham and Elizabeth Abraham, my parents

Tomci M. Abraham, my brother

and

Tina P. Thomas, my wife

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Summary

The cut flower industry is one of many businesses that have moved from the United States to other countries where production costs are lower. Once among the largest producers of cut flowers in the world, US producers are no longer able to compete with overseas growers. Revival of this industry in the US now depends in part on the development of effective and cost- efficient control mechanisms for various pests and diseases. Such control measures would increase product quality and provide a reasonable profit for the grower. While some flowers have considerable shelf life when shipped dry, other flowers do not. Such a situation exists in the case of gerbera (*Gerbera jamesonii* Bolus)

While overseas competitors are shipping gerberas dry, our local growers can deliver them in water, thereby increasing the shelf life and providing a better quality produce at competitive prices. Competitiveness of this industry depends on how effectively we control their pests. Major pests vary depending on the flower system in cultivation. In the case of greenhouse gerbera production, the primary pests are leafminers. Leafminers have effective and established biological control agents and are resistant to insecticides (Minkenberg and van Lenteren 1986). The influx of other secondary pests like aphids, mites, whiteflies and thrips during the growing

seasonwarrant insecticide applications which kill the biocontrol agents thereby disrupting leafminer control.

This project sought compatible alternative strategies involving biocontrol agents and pesticides (if and when needed), so that effective control of leafminers and secondary pests were made possible. Using the gerbera system as a model, results can later be applied to other cut flower systems to increase their competitiveness in the US cut flower market.

Cut flower industry

The United States was the largest producer of cut flowers in the world in 1981 with an excess of $2 billion in revenue. The majority of production came from Florida and California and cultivation was done in greenhouses consisting of roses, carnations and chrysanthemums (Parrella and Jones 1987). Currently, cut flowers comprise half of the United States imports of floriculture and nursery products worth over $1.3 B (Jerardo 2005). Colombia, Ecuador, and the Netherlands are among the countries from which the vast majority of our cut flowers are imported (Bader 2006).

Domestic growers have been competing with cut flower growers outside the continental US (mainly South America) and profits have been dwindling (Parrella and Jones 1987) to an extent that the US cut flower industry is on the brink of being eliminated. During the period from 1981-1984, when the demand for cut flowers domestically had increased by a rate of 7% annually, domestic sales increased only slightly, while those of imported flowers grew at 16.6% and reached a market share of 48% by 1984 (Parrella and Jones 1987). While personal expenditures on ornamental and

cut flowers increased by 3.8% from 2003 to 2004, an increase of 16% in imports was seen (Jerardo 2005). The import share of US cut flower consumption, which increased to 64%, was responsible for a 21% growth in exports from Colombia alone. The increasing share of household income spent on cut flowers does not support the domestic industry but is shipped abroad. On average, an American household spends $10 on cut flowers annually, of which $6.40 goes overseas mainly to South American competitors (Jerardo 2005). To increase the competitiveness of the US cut flower industry, the insect control costs should be brought to the lowest possible level, because most of the other variables like soil, nematodes, and weeds can be controlled better before the crop is even planted. That leaves arthropods and pathogens to be controlled, the costs of which accrue due to expensive and ineffective pesticides, frequent applications, and insecticide resistance problems (Parrella and Jones 1987).

These problems explain why through the years, the area under protected cultivation and cut flowers have decreased, not just in the state of Georgia, but also nationwide (Vilsack and Clark 2007). The Georgia floriculture industry employs 9000 individuals with revenue of more than $152.5 M and is a distant second to Florida in the Southeastern region of the United states (Vilsack and Clark 2007).

Why Gerbera?

Gerbera was one of the insignificant cut flowers in the American market until 2000. Sales alone increased from $20 M in 2000 to more than $31 M (> 1 B stems) in 2004 (Jerardo 2005). While the unit cost for gerbera and the average number of growers have remained almost constant through these years of growth, the average sale per

grower has been steadily increasing with a value of more than $100, 000 every year. The specific share of imports of gerbera though were not available for comparison (Jerardo 2005). A market analysis by the UGA Center for Agribusiness and Economic Development (Wolfe et al. 2007) found that Roses, Daisies, and Carnations topped the list of most requested locally grown cut flowers, and that gladiolus and gerbera daisies received the highest price per stem in South Georgia. While cut flowers in general have a short life time in the greenhouses where they are maintained and harvested for their produce and removed, gerberas remain for around 3 years yielding their highest when they are a year old and tapering significantly after the 3 year mark.

Story of Gerbera

Gerbera (*Gerbera jamesonii* Bolus) is a native of Transvaal, South Africa. It was in 1737 that Jan Frederik Gronovius, an influential botanist in the Netherlands and England, named this genus after a contemporary plant lover, Traugott Gerber, the director of the oldest botanical gardens in Moscow, Russia. In 1878 Anton Rehmann, an Austrian botanist in Poland discovered this new species, but the credit of the species name went to Robert Jameson, a merchant in Durban South Africa who rediscovered the plant in Barberton and later introduced this species to England. Harry Bolus, the English botanist in South Africa, credited Jameson with the discovery of this species which today has become the most popular gerbera, known also as Barberton Daisy. With all the advances in science and the hybridization done thus far, *Gerbera jamesonii* Bolus has been bred into the third most popular cut flower in the world (Seifert 2003).

Major Pest

Leafminers: Over 10,000 species of holometabolous insects from across the four orders: Diptera, Coleoptera, Hymenoptera and Lepidoptera have developed the leaf mining habit (Connor and Taverner 1997). Larvae of leafminers live and feed within the leaves, damaging the mesophyll tissue. Their feeding tracks, called mines, are whitish or grey and externally visible in leaves with varying shapes from narrow linear galleries to wide chambers (Hering and Martin 1951). The damage caused by gallery formation of the leafminers also reduces the photosynthetic capacity of the leaves, causing premature leaf abscission and entry of other pathogens into the plant (Salvo and Valladares 2007).

In the Order Diptera, members of the family Agromyzidae are generally known as leafminers, even though only 75% of the total 1800 species actually mine leaves (Bader 2006). Leafminers from the genera *Agromyza*, *Liriomyza* and *Phytomyza* (Diptera: Agromyzidae) are worldwide pests on many agricultural crops (Spencer 1973) and affect the aesthetic value of ornamental plants and edible leaves (Spencer 1973, Minkenberg and van Lenteren 1986, Maier 2001).

The genus *Liriomyza* contains more than 330 species (Liu et al. 2009) and is distributed widely in temperate and tropic regions (Parrella 1987, EPPO 2006, Malipatil and Ridland 2008). Within this genus there are 23 economically important species (Spencer 1973) with a few of them being polyphagous. While the family Agromyzidae has at least ten polyphagous members (Spencer 1977), six of them occur in the genus *Liriomyza* (Liu et al. 2009). Even though there are differences among the types of mines made by various species in this genus, generally all are known as "serpentine leafminers"

(Steyskal 1973). Many *Liriomyza* larvae do create very narrow serpentine mines initially which gradually enlarge to twist the leaf (Needham et al. 1928). It is to be noted that members in other genera also produce serpentine mines (Spencer 1973), however *Liriomyza* mines differ with locations on the leaves and shape (Parrella et al. 1985) and are influenced by the developmental stage of the leaf and host (Parrella 1987). The leaf puncturing behavior of female leafminers precedes the feeding behavior and may lead to oviposition. All punctures are used for feeding but not necessarily for oviposition. Leaf puncture size varies with the size of the adult female and puncturing damage often leads to reduced photosynthesis and death in young plants (Parrella et al. 1985).

Economically important *Lyriomyza* species: Historically *Liriomyza* leafminers have been minor pests because of the hymenopteran parasitoids that kept their populations in check (Parrella and Keil 1984). The use of broad spectrum pesticides to control pests also affected the biocontrol agents, spurring an increase in leafminer populations. This facilitated their transition to economically important pests during the 1980s (Parrella and Keil 1984). Until Spencer (1965) cleared the confusion regarding the taxonomy of *L. trifolii* (Burgess), *L. sativae* Blanchard was considered the major pest in chrysanthemums in California and celery in Florida. Partly due to the availability of taxonomic information to correctly identify *L. trifolii* and other information regarding its polyphagous nature and host associations, and partly due to the natural taking over, *L. trifolii* was recognized as the dominant leafminer species later (Schuster and Beck 1981, Poe and Knodel-Montz 1982). The fact that *L. trifolii* was historically an economic pest in horticultural crops gives credence to misidentification probably being the problem (Price and Stanley 1982). *L. bryoniae* (Kaltenbach) was another polyphagous leafminer

that has been found on ornamentals (Gerberas) in Taiwan. After the arrival of *L. trifolii* in 1988 the leafminer population did not remain low, as it had prior to that date (Wang and Lin 1988). In chrysanthemum, *L. huidobrensis* (Blanchard) is another leafminer whose mines are broad and characteristic of being on both upper and lower sides of the leaves while remaining close to the principal veins and midrib. It has caused minor damage in Colombia and California (Price 1982) but is the most economically important pest in greenhouses in the Netherlands (Lanzoni et al. 2002).

In crops where leaves are not the marketable product, there is tolerance for more leafminer attack before pesticides need to be applied. That is the condition in solanaceous crops like tomatoes (Liu et al. 2009), but not in ornamentals where leaves are also important. As early as the 1980s, *L. trifolii* was acknowledged as the primary pest on chrysanthemums and numerous other bedding plants that included verbena, calendula, etc. (Parrella et al. 1984). With a much lower reproductive potential and greater susceptibility to insecticides, *L. huidobrensis* was not expected to be a serious pest in ornamentals (Parrella and Bethke 1984). Earlier when *L. sativae* was the major pest in California, squash growers actually benefitted from the leafminers, which promoted the senescence of older leaves and thereby helping the harvest process. This was not the case when *L. sativae* was replaced by a more damaging *L. trifolii* (Trumble 1982). There are other instances where low infestations of *Liriomyza* have actually benefitted famers with a higher yield (Kotze and Dennill 1996).

***Liriomyza trifolii* (Burgess)**

This was the first serpentine leafminer in North America and was originally described as *Oscinis trifolii* (Burgess 1880) and collected from white clover (*Trifolium repens* L.). *L. trifolii* is known by several common names like serpentine leafminer, American serpentine leafminer, broad bean leafminer, California leafminer, celery leafminer, chrysanthemum leafminer (Malipatil and Ridland 2008). After a lot of confusion owing to the loss of the holotype, Spencer (1965) designated a neotype. Apart from the morphological differences, identification of *L. trifolii* adults was definitively made by gel electrophoresis (Menken and Ulenberg 1983, Zehnder et al. 1983), and morphological characteristics of the female genitalia (Knodel-Montz and Poe 1982). The synonyms *Agromyza phaseolunulata* Frost (1943), *Oscinis trifolii* Burgess (1880), *L. trifolii* de Meijere (1925), and *L. alliovora* Frick (1955) have been documented (Spencer 1973). Even though not a synonym, *L. sativae* could have been credited with damages caused by *L. trifolii* due to confusion in identifying the two species until 1965 (Parrella and Keil 1984) unless *L. trifolii* supplanted *L. sativae* in its host preferences like what happened in tomato and celery (Zehnder and Trumble 1984a).

Origin and distribution: Originally a nearctic and neotropical species, *L. trifolii* is now considered cosmopolitan, with Florida being its endemic focus (Spencer 1965), though original collections were made from Washington D.C. and Iowa (Charlton and Allen 1981). Importations by the flower industry within the country and to various countries from Florida aided the spread of *L.trifolii* into California (Parrella 1982), Colombia (Price 1982) , Turkey (Uygun et al. 1995), Kenya, England, France and the Netherlands (Spencer 1981) and others. *L. trifolii* currently has spread to over 5 continents and 92

countries (EPPO 2006, Malipatil and Ridland 2008). The distribution has followed the pattern of importation of ornamental flowers like chrysanthemum from Florida except for the instances in the Canary Islands where they were found in 1976 without such a flower importation (Minkenberg and van Lenteren 1986).

Host plants: *L. trifolii* is one among the six polyphagous species out of more than 330 species in the genus *Liriomyza* (Spencer 1973, Liu et al. 2009) that attack a wide range of host plants from ornamentals, vegetable and other crops, and weeds. Stegmaier (1966) listed 47 plant genera in 10 families as hosts, while 120 species in 21 families were reported by Spencer (1981), 169 species from 31 families (Pitkin 2009) and 400 species (Reitz and Trumble 2002). Ornamental plants are significant because their importation may have contributed to the spread of leafminers and on hosts such as chrysanthemums, gerbera and marigold (*Tagetes)* (Dempewolf 2004). Compositae comprise 40% of the total known host plants, followed by Leguminosae (Minkenberg and van Lenteren 1986).

Studies on the host plant preference of *L. trifolii* differed in their findings. While Charlton and Allen (1981) found that legumes were favored over chrysanthemums, Parrella et al (1983) found these leafminers to be more fecund on chrysanthemums when compared to tomatoes and celery. In an earlier study, Parrella et al. (1981b) mentioned that chrysanthemums were not the preferred host among many other ornamentals and vegetable crops. Fagoonee and Toory (1983) saw a preference for pink bean plants over potatoes. While *L. trifolii* are serious pests in each of these systems, preference for host plants seems to vary according to the availability of plants in an area and the plants to which that particular population of leafminers are accustomed (Parrella et al. 1983, Reitz and Trumble 2002).

Life history and biology: *L. trifolii* is about 2 mm long, with a yellow head and plum red eyes. A yellow patch is noticeable at the hind end of the mesonotum, leaving the rest of the thorax and abdomen grayish black. The legs and underside have a pale yellow shade (Minkenberg and van Lenteren 1986).

Males usually emerge before females, with mating occurring in the morning hours (Dimetry 1971) within the first 24h (Parrella 1987). Even though a single mating is sufficient to fertilize all eggs laid (Minkenberg and van Lenteren 1986), males and females engage in multiple matings to maximize egg production (Charlton and Allen 1981). *L. trifolii* has a reproductive potential that is three times other economically important *Liriomyza* (Parrella et al. 1981a)

Host plant feeding seems to benefit *L. trifolii* in three ways,

1. to confirm the host plant
2. to ingest specific proteins that will aid egg maturation
3. to feed on carbohydrates (Minkenberg and van Lenteren 1986).

Regardless of the host plant, the first event observed when a female initiates a leaf puncturing sequence is a bending of the abdomen to position the ovipositor perpendicular to the leaf. A series of rapid thrusts follows until the ovipositor has penetrated the leaf surface. Once that is accomplished, the thrusts become slower and more deliberate. Creating one out of two possible types of leaf punctures, the female then damages mesophyll cells. By twisting the abdomen from side to side, a large fan-shaped leaf puncture is created, or without twisting the abdomen after puncturing it, a tubular leaf

puncture is produced. Eggs are then deposited in tubular leaf punctures. The subtle difference between oviposition behavior and the creation of a tubular leaf puncture without an egg is that oviposition entails a pause in slow thrusting followed by a final thrust to deposit an egg. The female is often seen to host feed from the wound after a leaf puncture is made (Bethke and Parrella 1985). Even though males do not create punctures, they are observed to feed from those punctures made by females, the size of which are about 0.15-0.3 mm (Minkenberg and van Lenteren 1986, Parrella 1987). The ratio of punctured holes to oviposition varies with conditions (Minkenberg and van Lenteren 1986, Parrella 1987). Even though there was speculation for feeding puncture/egg ratio being an index of host preference (Hussey and Gurney 1962), evidence was lacking (Ibrahim and Madge 1977). Eggs are 0.2 x 0.1 mm, translucent initially and turn creamy later. They are laid just below the epidermis (Minkenberg 1988).

Initial studies could not determine if *L. trifolii* preferentially fed from the top tier of the plant (Schuster and Beck 1981). Zehnder and Trumble (1984b) saw that in presence of *L. sativae*, *L. trifolii* inhabited the lower tier while *L. sativae* fed from the mid-tier. When *L. sativae* was absent though, *L. trifolii* was seen to inhabit and feed from both lower and middle tier of the plant (Zoebisch and Schuster 1990). The larvae prefer the palisade mesophyll in chrysanthemum (Parrella et al. 1985). Initially colorless, the larva gains a yellow color as it matures through three larval stages. The third instar cuts an opening at the end of the mine to exit (Minkenberg and van Lenteren 1986). While leafminers feed throughout the day, they oviposit during midday, and both larval and adult emergence is between 0900-1200 h (Charlton and Allen 1981).

Development from egg to adult, which depends on temperature, took the least time at 32.5°C, with an average of 12.2 d in pink bean plants and 14.3 d in chrysanthemums (Charlton and Allen 1981). Adult longevity and fecundity were found to increase 2-3 fold when adults fed on honey rather than host plant leaf punctures. Hence it could be assumed that aphid populations producing honey dew or even flower nectar could boost leafminer populations (Charlton and Allen 1981). However speculations are that such laboratory studies could have overestimated the field longevity and fecundity of these leafminers, which have not been measured in natural conditions (Parrella 1987). Most larvae that emerge from the leaves drop down to pupate in the soil while some do so in exposed places (Charlton and Allen 1981).

Effects of abiotic factors on biology and development: Mortality of *L. trifolii* decreases with increase in temperature from 11.5°C, to reach minimum at about 25°C and then increases again (Parrella et al. 1981a, Miller and Isger 1985). The same temperature (25°C) was optimum for emergence of *L. trifolii*, while after 30°C a sharp increase in immature mortality was recorded (Minkenberg and van Lenteren 1986). The development time though, was seen to decrease from 15°C to 35°C (64d to 14d) (Leibee 1982, 1984). With decrease in temperature from 35°C, female longevity increased and peaked at around 20°C and then dipped slightly (Leibee 1982, 1984). Humidity was not found to play a major role, except in extreme cases of drought or moisture. Pupal emergence increased with an increase in moisture, and after submerging pupae for 4h, 24h and 75h survival rates were 96%, 50% and 0% respectively (Charlton and Allen 1981). Anecdotally, *L. trifolii* has been noted to show a positive phototactic response

with more damage along paths and borders where there is more incident light (Minkenberg and van Lenteren 1986).

Studies indicate that relative humidity has a positive relation with development to adult from the pupae, hence making greenhouses a preferred environment (Charlton and Allen 1981). *L. trifolii* pupae are hardy and probably cannot be affected by either drying or flooding the soil, without harming the plants (Charlton and Allen 1981). A linear relationship was found to exist between fertilization rate and density of *L. trifolii* on chrysanthemums (Price and Harbaugh 1981, Harbaugh et al. 1983), and larval mortality was lower with increased fertilization (Poe et al. 1976). Physical barriers on leaf surfaces could influence survivability of leafminers. Hooked trichomes on pink beans caused premature death of *L. trifolii* (Charlton and Allen 1981).

Control Measures

Monitoring is an important step required in any control program. For leafminers in general, and *L. trifolii* in particular, yellow sticky cards have worked well (Broadbent 1982).

Chemical controls and insecticide resistance: After rigorous use of pesticides for a long time, leafminers have evolved to be resistant to almost all chemistries (Keil and Parrella 1982). While none of them work effectively against adults, few are effective against larval stages (Civelek and Weintraub 2003). To be effective against the larvae, the chemicals need to be translaminar. Two such chemicals used successfully against larval *L. trifolii* are cyromazine (N-cyclopropyl-1, 3, 5-triazine-2, 4, 6-triamine), which is an insect growth regulator and labeled for use in vegetables (Trigard) and ornamentals

(Citation), and abamectin, a GABA agonist, also registered for use on vegetable and ornamental crops (Civelek and Weintraub 2003, Ferguson 2004). Both of these pesticides are larvicides and have limited (sublethal in abamectin) to no effect on adults (cyromazine) (Schuster and Everett 1983). They seemed to have met the expectation of being long-lived insecticides against *L. trifolii* (Leibee 1988) until recently.

With *L. trifolii*'s long history of exposure to pesticides, it is difficult to employ the usual concept of rotating pesticides to prevent development of resistance because the pesticides used in a rotation program should still be effective on the target (Parrella and Trumble 1989). *L. trifolii* has presented more challenges in greenhouses because of the intense pressure with pesticides when compared to leafminer problems in field grown crops (Parrella and Trumble 1989). Pesticide resistance is a serious worldwide problem in agriculture. In leafminers, *Liriomyza* spp. have developed resistance to most, if not all, of the different chemistries that have been developed. Genung (1957) was the first to suggest the possibility of insecticide resistance by *Liriomyza* leafminers.

L. trifolii developed resistance to insecticides first in Florida and California (Parrella and Keil 1984, Parrella et al. 1984), and has posed problems for leafminer control elsewhere. Having been introduced from the primary focus (Florida), *L. trifolii* has even shown resistance in other localities without similar insecticide pressure (Poe and Knodel-Montz 1982). Among the reasons for the rapid development of resistance by *L. trifolii* compared to other *Liriomyza* leafminers is the high reproductive potential they have (Parrella et al. 1981a). *L. trifolii* is a more serious pest in ornamentals that are cultivated in greenhouses, where pesticides are heavily sprayed, and immigration of susceptible leafminers rarely occurs when compared to other field grown crops (Parrella

et al. 1984). However studies by Mason et al. (1989) did not agree entirely. They said that even though the fecundity was considerably higher than other *Liriomyza* spp., and that adequate immigration was not occurring, these were not causes sufficient to bring the rapid development of resistance. They credited the development of resistance mainly to the rigorous use of insecticides within the confines of glasshouses and a combination of the other aforesaid reasons.

The average effective field life of an insecticide on *L. trifolii* has been less than three years in Florida (Table 1.1) (Parrella et al. 1984). The failure to control these leafminers with available insecticides in California, prompted a special local needs registration ("24c") for permethrin (Pounce) in 1979, and microencapsulated methyl parathion (Penncap-M) in 1980 for use in greenhouses growing chrysanthemums. Resistance to these chemistries that were being sprayed as many as 70 times a year was noticed in some populations by 1981 and all over California in 1982 (Parrella et al. 1984), making the situation similar to that of Florida.

When tested with four leafminer species in 1985 against methamidophos, *L. trifolii* collected from celery in Florida showed the highest resistance. This was attributed to the high usage of the said chemical for control in Florida. However, the *L. trifolii* in California chrysanthemums came next even though they had not been exposed to this chemical, but acquired resistance since all the population initially came from Florida (Parrella and Keil 1985). *L. sativae* was the next that showed resistance due to high doses of chemicals applied towards its control earlier (Parrella and Keil 1985).

Table 1.1 History of insecticide use on *Liriomyza* spp. in vegetables in Florida

(Leibee 1981, Ferguson 2004)

Insecticide	Date first used	Effective field life (yr)
Nicotine sulfate	<1945	?
Chlordane	1947	11
Toxaphene	1947	5
Parathion	1948	10
Diazinon	1958	3
Azinphosmethyl	1961	13
Dimethoate	1961	13
Naled	1961	13
Oxamyl	1975	2
Methamidophos	1977	4
Permethrin	1978	2
Cyromazine	1983	21
Abamectin	1990	14

When three different strains of *L. trifolii* were compared for resistance against abamectin, cyromazine, and spinosad on chrysanthemums, the resistance seemed to be unstable (Ferguson 2004). The only other reports of resistance to cyromazine, one from Florida in celery plantings, and the other in a laboratory test with ornamentals where resistance was seen against cyromazine, abamectin and spinosad, was also highly unstable. Once they started the rotation of abamectin and cyromazine regimen, another

instance was not cited in the celery plantings. Similar results were found in a later study where resistance did revert easily (in this case, in less than eight generations) whenever resistance had not reached high levels (Ferguson 2004). In California, where *L. trifolii* is a major problem in ornamentals, it has been advised to rotate with a new class of insecticide every two months whereas in vegetables an abamectin, and cyromazine rotation is advised (Ferguson 2004) because the different modes of action make them unlikely candidates for developing cross resistance (Leibee 1981)

Cartap, bensultap, and thiocyclam, which are derivatives of nereis toxin (secretions of marine annelids), were found to be successful in varied levels against larval and adult forms of *L. trifolii* (Civelek and Weintraub 2003). Thiocyclam and cartap provided 100% mortality against day-old larvae, while bensultap gave 71% mortality 8 days after the application. When adult females were allowed to feed on leaves treated with the same chemistries, thiocyclam gave 100% mortality and cartap 96% while cyromazine had no effect (Civelek and Weintraub 2003).

In other agricultural crops, it has been found that the withdrawal of a resistant chemistry for a significant time allows resistance reversion in the species. However, this practice tested in *L. trifolii* did not yield favorable results after a 10 month (15 generations) study involving permethrin and chlorpyrifos. Even though reductions in resistance were seen in a consistent fashion, it was not enough to resume use of those insecticides (Parrella and Trumble 1989). *L. trifolii* larval resistance to permethrin was documented in 1983 (Parrella) and remained the same in this study (Parrella and Trumble 1989).

Generally, leafminers are at an advantage of getting partial protection from chemicals by embedding themselves within the leaves. This protection changes form when the larva progresses from the third instar to a pupa. Combining this with the rapid rate at which *L. trifolii* develops resistance to the chemicals, it becomes impossible to control these leafminers exclusively using a chemical or two. Reducing the number of applications is an effective way to slow resistance development in *L. trifolii*, but the issue arises in ornamentals where tolerance for leafminer attack is close to zero. *L. trifolii* has the capacity to greatly reduce the marketability or render entire crops unmarketable in ornamentals (Parrella et al. 1982). The slightest damage will render the produce worthless, and hence farmers will spray insecticides at the slightest presence of leafminers (Hara 1986). In such situations, it would be challenging to control the overuse of pesticides and control leafminers (Mason et al. 1989). The option here would be to use all the available natural enemies to enable maximum control. In every field/greenhouse population of *L. trifolii*, there may be some that escape the insecticide, and to target them there needs to be parasites or parasitoids. These, when working complementary to pesticides (if any), would give us the best shot at controlling *L. trifolii* (Leibee 1981). The scenario changes when the number of effective chemicals in our hand decreases. Such a situation would increase the role played by parasitoids and predators. With chemicals affecting the natural enemy complex and generating pest outbreaks, Integrated Pest Management (IPM) would be an alternative for *L. trifolii* control (Trumble 1985).

Biocontrol: Biological control, whenever viable, has been a pest management strategy and is gaining increasing interest in Japan, Europe, North and South America (Bader et

al. 2006). Using insecticides that cause minimal harm to natural enemies is the best mechanism to effect conservation biocontrol (Liu et al. 2009). Habitat diversification, by including weed stands to harbor parasitoid populations, may also work effectively (Schuster et al. 1982). Studies showed that while eulophids were seen to be more populous in natural ecosystems, braconids were seen in higher numbers in cultivated areas (Liu et al. 2009). Since crop monocultures may disrupt biocontrol (Liu et al. 2009), the success of natural control will depend on matching *L. trifolii* in the host system that is needed, together with the parasitoid that works best (Johnson and Hara 1987).

Successful introductions of natural enemies have facilitated classical biocontrol in some of the Pacific Ocean islands─ Hawaii, Tonga and Guam as early as 1970s-1980s (Liu et al. 2009). However, as stated before, parasitoids would differ in effectiveness as the system, host plant and area changes, so finding the best parasitoid that works in the system of interest would be important in determining the success of any biocontrol program to suppress *Liriomyza* leafminers.

Augmentative biocontrol has been implemented by inoculative releases of parasitoids early in the season to help build up populations later and provide control. This has been effective in areas where disruptive use of chemicals has been avoided (Liu et al. 2009). The success of such an effort though would depend on the ability of the natural enemy to establish a maximum population, and be released in synchrony with the host, and integrated with other natural enemies or existing control measures (Liu et al. 2009). However, more information about successful release rates, their economic analyses, availability of cost-effective natural enemies, and augmentation of native natural enemies would be the key to such a control mechanism (Liu et al. 2009).

Natural enemies: Natural enemy communities are rich in the areas of origin of *Liriomyza* leafminers (Spencer 1973, Minkenberg and van Lenteren 1986, Murphy and LaSalle 1999). Increasing numbers of parasitoids of *L. trifolii* have been reported through various studies. Originally, 36 species in 19 genera worldwide were reported (Minkenberg and van Lenteren 1986, Johnson and Hara 1987), and now around 140 species of parasitoids and a few species of predators (which include nematodes) and entomopathogens (Liu et al. 2009) are known.

Parasitoids have been used effectively in two major agricultural areas: ornamental crops in greenhouse and commercial vegetable production (Liu et al. 2009) through different strategies. Inoculation and augmentation are the two methods employed in protected culture, while conservation biocontrol has been successful in field crops (Liu et al. 2009). Parasitoids in field crops/open systems tend to be more species rich but also depend on many abiotic factors which are not controlled in such scenarios (Salvo et al. 2005).

Among parasitoids, those from the family Eulophidae have not been specific to any single kind of leafminer, and hence could effect control of leafminers in general (Liu et al. 2009). The most common among the parasitoids of *L. trifolii* are from the genera *Diglyphus, Chrysocharis* (Minkenberg and van Lenteren 1986) and *Dacnusa*. *Diglyphus* and *Dacnusa* among others are commercially available for leafminer control and used in many countries (van Lenteren 1995, Ozawa et al. 1999, Abd-Rabou 2006). As for Georgia, there is a native larval-pupal parasitoid (Wharton 1984) *Oenonogastra microrhopalae* (Ashmead) which was also found in Ohio and Ontario (Minkenberg and van Lenteren 1986).

A successful biocontrol program depends on knowledge of the biology of the biocontrol agents and the target pest. With a lot of potential biocontrol agents, biological information is lacking (Minkenberg and van Lenteren 1986). *Diglyphus* spp. were among the most common parasitoids found and used in many studies (Price and Stanley 1982, Zehnder and Trumble 1984a, Johnson and Hara 1987, Sher et al. 2000, Ozawa et al. 2001, Patel et al. 2003, Burgio et al. 2007). Simulation models to predict release rates of this parasitoid have also been successful to an extent (Heinz et al. 1993).

Diglyphus begini (Ashmead): *Diglyphus begini* (Hymenoptera: Eulophidae) an important larval ectoparasitoid of *L. trifolii* has a recorded host range including 13 species of leafminers from two Dipteran families in 25 host plants from 12 families (Heinz and Parrella 1990b). An initial description of its development was given by Webster and Parks (1913). *D. begini* (Ashmead 1904) is a facultative gregarious parasitoid of nearctic and neotropical distribution (Minkenberg and van Lenteren 1986). *Diglyphus* is a synovigenic, and idiobiont wasp (Abd-Rabou 2006).

Cushman (1926) suggested that while looking for hosts that are concealed within plant tissue, parasitoids would narrow their search to locations or microhabitats. Similar observation was made by Doutt (1957) where parasitoids zeroed in on mines and not necessarily where there were potential hosts. They then searched by probing into the mines with their ovipositor until contacting an immature leafminer. Any such larvae would be stung and this could eventually kill them irrespective of a decision to oviposit or not (Allen and Charlton 1981). Even though larger and mature hosts are preferred for oviposition, smaller ones may be used for host feeding and later oviposition near the mine or rejected after extensive probing of host viscera by the ovipositor, accounting for

additional mortality (Allen and Charlton 1981, Heinz and Parrella 1989). After the egg hatches, the larva attaches itself to the leafminer and consumes the body fluids and completes its development mostly from a single leafminer larva. The parasitoid then pupates within pillar like structures from the larval meconium, and later the adult emerges through a hole it cuts in the leaf.

For the first 2d after emergence, *D. begini* does not seem to oviposit into any of the hosts that are attacked, and after the second day, 1.3 hosts are killed for every host that they oviposit into. Fecundity or oviposition does not depend on the presence of males; nevertheless unmated females produce only males. In order to produce females, they need to mate with males (Heinz and Parrella 1990b). Even though there has been a study that indicated a 1:1 sex ratio (Coote and Ellis 1986), populations often tend to be heavily male biased (~ 72%), however there has been no documentation for sex specific differential mortality in the field. It was also observed in multiple situations that females mostly emerged out of the larger hosts while males emerged from the smaller ones (Heinz and Parrella 1990b).

Allen and Charlton (1981) showed that at 25°C, the development time for *D. begini* was approximately 10.4 d (1.2d as egg, 5.4 d as larva, and 3.8 days as pupa). It was different at 24°C, where females had a slightly longer development time of almost 2 weeks and males took 1.6 d less (Heinz and Parrella 1990b). At emergence the life expectancy for females is 6.3 d and 3.4 d for males and life span had a positive correlation with their body size (Heinz and Parrella 1990b). Oviposition was highest on the third day after emergence and peaked for most of the population during 2-5 d after emergence, producing approximately 6 offspring in their lifetime. Fecundity was a factor

that again correlated directly with the body size of the female (Heinz and Parrella 1990b). Longevity of *D. begini* differed in studies conducted by Allen and Charlton (1981) where they lived for 3.5 d when fed water alone, and lived for 17 d while provided with *L. trifolii* larvae only and 29.8 d when provided water and honey. Individual *D. begini* on average killed a total of 268 *L. trifolii* for oviposition and 448 by stinging and not ovipositing for a grand total of 716. These values were incredibly higher than the projected values from the Heinz and Parrella study (1990b) .

Several studies proved the effectiveness of this parasitoid against *L. trifolii* in ornamentals (Heinz et al. 1988, Heinz and Parrella 1990a, 1992). Within 8 weeks of first release, populations of *L. trifolii* were brought down to zero in marigolds (Heinz and Parrella 1990a). Successful releases have been made in greenhouse chrysanthemums also (Parrella et al. 1992). Studies reported the recurring costs (not including worker benefits, facilities lease, profit margins, utility costs or costs of shipping) to produce 1000 *D. begini* per day to be US $ 19.40 (Parrella et al. 1989) and US $19.20 (Rathman et al. 1991).

<u>*Other important Parasitoids:*</u> *Dacnusa* is a braconid wasp and an endoparasitic, proovigenic and koinobiont wasp that attacks all instars of the host larvae (Abd-Rabou 2006). Other species in the genus *Diglyphus* that controls various *Liriomyza* leafminers have similar biology. *Diglyphus isaea* (Walker) is one of the few commercially available parasitoids for *L. trifolii,* prices of which varied from US$86-112 for 250 adults in early 2008 (Liu et al. 2009) and have gone down since. *D. isaea* is a complex of cryptic species, four of which are seen in China (Sha et al. 2007). Three releases of *D. isaea* at a rate of 0.15 females/plant resulted in 100% increase in leafminer larval mortality, and

95% parasitism from sampled *L. trifolii* (Ozawa et al. 1999, 2001, Liu et al. 2009). When released at a rate of 100 adults/100m^2, larval populations of *L. trifolii* were seen to decrease to <1 larva /leaf (Ulubilir and Sekeroglu 1997, Liu et al. 2009); the economic viability of such an inundative release however needs to be calculated.

No studies on the biology of *Oenonogastra microrhopalae* have been done except for some toxicology studies (Oetting 1985). We do know that they are larval-pupal parasitoids on *L. trifolii* (Wharton 1984).

Insecticide effects on natural enemies: It has been shown that fields where insecticides are applied have low densities of parasitoids (Price and Stanley 1982). However, like the varied efficacy of different chemicals against *L. trifolii*, their effects against natural enemies also varied (Trumble 1985). Avermectin did not adversely affect the biocontrol agents in a study that compared cyromazine, avermectin and methomyl, while cyromazine facilitated the highest reduction in parasitism during this study. Methomyl acted on adult parasitoids, but cyromazine was detrimental to immatures. This could be useful information to control irregular outbreaks of leafminers or secondary pests (Trumble 1985). Compared to methamidophos, methomyl decreased parasitoid populations by more than 50% (Trumble and Toscano 1983), however Poe et al. (1978) did not find methomyl to be toxic to leafminer parasitoids, when studies were done in tomato fields with *L. sativae* in the presence of six hymenopteran parasitoids including *Diglyphus intermedius* (Girault). Whether methomyl can be used sparingly needs to be researched (Trumble and Toscano 1983). Parasitoid populations were not detrimentally affected in a study that compared methamidophos, methomyl and cryolite, though

methomyl effected a slight but insignificant decrease in parasitoid counts when compared to the control plots (Trumble 1982).

In a study where potted chrysanthemums were treated with permethrin and diazinon, *D. begini* were unaffected after sprays of permethrin and continued to give good control of *L. trifolii*, while their populations plummeted after sprays of diazinon resulting in eight-fold increases in leafminer populations (Allen and Charlton 1981). Literature is in agreement that parasitoid populations increase during reduced pesticide applications (Johnson et al. 1980). When pesticides do not interfere, natural enemies often control leafminer populations to 90-100% (Liu et al. 2009). Special attention is required towards native natural enemies, which are often unnoticed under heavy pesticide pressure. Chances are that such native parasitoids would be more successful against the leafminers than exotic ones (Liu et al. 2009).

Entomopathogens—Microbial control: Fungus: Efficacies of fungal pathogens have not been promising when 11 entomopathogenic fungal strains were tested against *L. trifolii* and *L. sativae.* Puparia of the leafminers were placed in peat inoculated with suspensions of *Beauveria bassiana* (Bals.) Vuill., *Metarhizium anisopliae* (Metsch.) Sorokin, *Paecilomyces farinosus* (Hotmskiold), and *P. fumosoroseus* (Wize) Brown and Smith. *L. trifolii* was susceptible to the fungus while *L. sativae* was not. One strain of *M. anisopliae, P. farinosus* and 2 strains of *P. fumosoroseus* caused around 75-80% mortality (Bordat et al. 1988, Liu et al. 2009). Studies conducted on *L. bryoniae* showed *M. anisopliae* and *Paecilomyces lilacinus* to effect 60-88% and 70-94% control respectively (Liu et al. 2009). Research remains open as for fungus and parasitoid interactions, maximizing performance (selection of strain timing of application and other

strategies), and application technologies that minimize adverse effects to the environment (Liu et al. 2009).

Bacteria: Strains of *Bacillus thuringiensis*, a microbial insecticide, are known to produce crystalline cytoplasmic protein inclusions and control certain species of Lepidoptera and Diptera (van Frankenhuyzen 1993). Success of a certain strain on the house fly *Musca domestica* L. prompted this study on *L. trifolii*. While treating in beans with an economic threshold of 4-5 larvae per leaf, sprays were made with *B. thuringiensis* Berliner (60×10^6/mg) at a recommended rate of 75 g/100 L water. A spray every 2-3 weeks effected best control of *L. trifolii* and resulted in higher yields than those not treated with *B. thuringiensis* (*Bt*). The reduction in leafminer parasitoids in the treated plots was speculated to be caused due to the reduction in leafminer populations and not because *Bt* was toxic to the parasitoids per se (Cikman and Nuray 2006). GCSC-BtA (Germany-China Scientific Cooperation-*B. thuringiensis*-Abamectin) was tested on several leafminer species including *L. sativae* and their parasitoids and found to be effective with very low reductions in parasitoid populations (Sengonca and Liu 2003, Cikman and Nuray 2006). However, whether *Bt* would be a viable control in cut flowers needs to be investigated because of the reduced tolerance to mines or larvae lower than 4-5 larvae per leaf indicated in the above study. It was interesting to see that *Bt* treated plots showed significantly higher yields than the control plot that had no leafminers.

Nematodes: In greenhouses or laboratory conditions with high humidity, *Liriomyza* control by nematodes could be as high as 85-97% (Harris et al. 1990, Broadbent and Olthof 1995, Liu et al. 2009). Efficacy depended on pest species and humidity (Hara et al. 1993) but not as much on temperature (Williams and Macdonald 1995, Liu et al.

2009). Glycerin was an effective adjuvant that increased mortality of *L. trifolii* by *Steinernema carpocapsae* (Weiser) (Broadbent and Olthof 1995). At times, low host mortality and sensitivity demonstrated these nematodes unreliable (Liu et al. 2009). *S. carpocapsae*, depending on various factors, could successfully be used together with the parasitoid *Diglyphus begini* to control *L. trifolii* (Sher et al. 2000). High costs and variable effectiveness have prevented the widespread use of entomopathogenic nematodes (Liu et al. 2009). Further research can undo this anomaly.

Botanicals: Neem seed extract (from *Azadirachta indica* A.Juss) as a broad spectrum foliar spray has been effective against many insects by inhibiting feeding or regulating their growth and sometimes both (Rembold et al. 1982). It has worked successfully against *L. sativae* and *L. trifolii* (Webb et al. 1983, Fagoonee and Toory 1984). A 0.4% concentration of neem extract was found to cause significant mortality of late instars and pupae of *L. trifolii* in research and commercial greenhouses with chrysanthemums and lasted for 3 weeks. Though repeated applications of neem did not show any signs of phytotoxicity, the neem application did not save the foliage from leafminer damage when compared to the chemical control. It was also found that 0.1% sprays did not provide significant control compared to 0.4% (Larew et al. 1985). While applying on beans, it was found that sprays with 0.1 and 0.5% concentrations gave 91 and 100% control against eggs and larvae. Residual control of larvae, even though on a declining rate (86% - 44% mortality) was achieved until 7 d (Webb et al. 1983). Even though *L. trifolii* and *L. sativae* differed in their inhibition from ovipositing on leaves sprayed with neem, development of both larvae failed. It was also found that the repellent action of neem was largely lost after 22 h, but neem continued to account for mortality of larvae up to 10

d. It was speculated that higher concentrations of neem extract might repel *L. trifolii* better (Webb et al. 1983).

Host plant resistance: Several host plant resistance studies have been conducted in celery, one of the worst affected vegetables by *L. trifolii* damage. After losses spread over all vegetable producing areas of the US, California Celery Research Advisory conducted trials with 159 accessions from throughout the world to find out the role of host plant resistance in combating the menace of *L. trifolii* (Trumble and Quiros 1988). The narrow leaf architecture of filiform leaves seemed to have helped *A. leptophyllum* (Pers.) F. Muell., an accession from Australia, to resist (no mines) *L. trifolii* attack. Among the tolerant (consistently fewer mines) accessions were one each from Taiwan and Australia, which showed profound variation during peak leafminer populations (Trumble and Quiros 1988). *A. leptophyllum* and its antibiosis-based resistance mechanism though could not be used for immediate breeding studies because the plant does not hybridize sexually. The accessions with fewer leafminer attacks were not of great help either because it would not make a great difference in monoculture settings where there was no choice for *L. trifolii* and so would cause substantial damage anyway (Trumble and Quiros 1988). *A. prostratum* (A230) showed promising results in the lab, showing antixenosis even though there was limited oviposition in the field.

With 30% of all work in breeding companies focusing on resistance breeding (van Lenteren 2007), finding a gerbera accession that is resistant to leafminer attack could be the key for breeding programs to come up with a solution. Antibiosis and antixenosis mechanisms could be effective tools of resistance to control leafminer attacks, but need to be investigated before anything promising can be found. Partial resistance could

supplement biological control and maybe work synergistically to control leafminer pests (van Lenteren 2007).

Cultural and other methods practiced: Insects in greenhouse crops originate in probably three ways; carried over from previous crop, blown into greenhouses, already present in the seedlings/cuttings during transplant. While sterilization takes care of pests being carried over, it is not always completely successful. Dividing large areas and having physical barriers for the smaller areas where the planting and harvesting are done at the same time would prevent movement of adults from a finished crop to another that has just been planted. Fine mesh screenings would prevent flying or blown insects from entering the greenhouses. Very fine screening of 20 * 20 per 2.54 cm^2 is required to exclude *L. trifolii*. A successful IPM program that included different components of control for chrysanthemums in greenhouses was detailed by Parrella and Jones (1987).

Use of a flame thrower to burn down harvested plants made sure that no leafminer from a harvested crop would move to another bed that was not harvested or harbor immature stages that would damage later crops. Other practices included manual monitoring and collection, swabbing diesel oil or 90-weight oil on polyethylene curtains that were installed in green houses, and a sweep net version of the oily polyethylene curtain technique (Price 1982).

Using weeds to attract *L. trifolii* to avoid economic damage on the ornamentals was a suggested mode of control, however studies did not find any potential weed that could impact leafminer control because they were not preferred over the crop of interest (Schuster et al. 1982, Zoebisch et al. 1984).

Integrated Pest Management (IPM):

In areas like turf, cut flowers, backyards of homes, landscape plantings, and ornamentals, the concept of economic thresholds (Stern et al. 1959) is not applicable in the strict sense. What is more plausible in such a scenario is Aesthetic Injury Level (AIL) which was first elucidated by Olkowski (1974) by considering the aesthetic and economic value of a crop. However, AIL in its strict sense would again not fit the aforementioned scenarios because of the lack or variable level of tolerance for insect damage on respective products or belongings. A florist would reject a flower with insect damage, whereas a homeowner would tolerate to a greater extent before calling the pesticide applicator. The modified version of AIL (Parrella and Jones 1987) takes into consideration the fact that there are non–marketable portions in products that have very low tolerance for insect damage, especially in floriculture. It would involve the use of a few selective and effective pesticides to keep the produce marketable, the pesticide being safe enough/less detrimental to the natural enemies, and a strict pest population monitoring program. The tolerance level is mostly fixed through the monitoring and sampling efforts, and a combined effort of all mechanisms available is used to keep the produce marketable and within the AIL (Parrella and Jones 1987) .

Shifting from a conventional pesticide program to IPM resulted in significant reduction in leafminers and damage in tomatoes in Mexico (Trumble and Alvarado-Rodriguez 1993), celery in California (Trumble et al. 1997, Reitz et al. 1999), and tomatoes in California (Trumble et al. 1993) together with reduced pesticide use, leafminer populations, potential environmental problems, and increased worker health and safety (Liu et al. 2009). For the success of IPM in monocultures like those in

ornamental crops, matching the best natural enemy with the *Liriomyza* species that causes the most problems would be the best solution (Johnson and Hara 1987).

Current Problems:

Currently the gerbera production system has established biocontrol agents that work successfully. However, the influx of secondary pests like mites, thrips, whiteflies, and aphids warrants the use of pesticides and disrupts the existing biocontrol mechanism. The solution to this disadvantageous situation is to devise a balanced control strategy which would take care of the leafminer, *L. trifolii*, and also control the secondary pests without disrupting the biocontrols that are in place. There are many questions requiring answers, a few of which were addressed herein.

Research Objectives

Project 1. Compatibility of commonly used insecticides and fungicides to natural enemies

Natural enemies have been shown to successfully suppress leafminer populations in the absence of chemicals (Liu et al. 2009). Current grower practices and suite of pests frequenting the greenhouses makes it difficult to exclude chemicals from the system. While pesticides are not effective against leafminers, they are efficient and effective against secondary pests. Though an “all pesticide”-chemical control regime for pest control doesn’t meet the criteria of cost effective pest control, growers are reluctant and/or lack the information regarding how to integrate control practices. Effects of some commonly used pesticides on various individual natural enemies have been evaluated previously. However, with the amount of commonly used pesticides and potential natural

enemies against pests in this system, there is a lacuna that needs immediate remediation. This study was carried out to investigate the compatibility of commonly used pesticides with the leafminer parasitoid, *Diglyphus isaea*, and the predatory mite *Neoseiulus californicus* (McGregor). This project provided information concerning which of these components (chemical or biological) if any, can be integrated into a pest management program for the suite of primary and secondary pests in greenhouse gerberas.

Project 2. Resistance mechanisms in gerbera cultivars to *Liriomyza trifolii*

Since chemicals are an ineffective control option for serpentine leafminers *L. trifolii,* other options need to be explored. Host plant resistance is a "no chemical method" which if successful, could be a major component of an integrated pest management plan in greenhouse gerberas. While such mechanisms have been explored in depth in vegetables (Trumble and Quiros 1988, Trumble et al. 1990, Black et al. 2003) and some ornamentals (Nair 2011), little to no work has been done in cut flowers. This could be because methods like host plant resistance are not expected to totally remove or control damage by the pest. The aesthetic tolerance for damage in this system being practically zero, it is understandable that no investigation into host plant resistance has been conducted. However, if there is a successful mechanism of resistance in these plants, they could synergistically work with other control mechanisms effected in the system. This study was hence conducted to evaluate gerbera cultivars for their resistance to the leafminer species *Liriomyza trifolii* in 1) a choice field test, and 2) a lab test investigating the physical characteristic of leaf toughness as a potential mechanism. The results showed that there was no overt resistance mechanism that was giving any cultivar impunity from leafminers or their damage. While some cultivars showed significantly

less leafminer damage than others, they more often than not, did not either correspond with a physical attribute that would provide resistance or provide resistance for an entire cultivar group (color variants within a certain cultivar).

Project 3. Case Study- Is a biologically- based control program cost effective for greenhouse gerbera daisies?

Integrating biological and chemical control methods is a concept that was initially called for in 1959 (Stern et al.) to control spotted alfalfa aphid in a field crop where economic injury levels existed. However, pest control in the cut flower industry operates on an aesthetic injury level where the tolerance for damage by pests is practically zero. Grower adoption requires that the feasibility of such a program be demonstrated. Currently, pesticides are the primary control option. Confidence in natural enemy reduction in pest-induced damage, if not the pest itself is lacking. In this study, we compared a chemically- based control program with a biologically- based program in a realistic greenhouse situation. The chemically- based control followed current practices, and sprayed chemicals, while the biological control program utilized the leafminer natural enemy- *Diglyphus isaea* to control leafminers, and chemicals (only when needed) that were less toxic to the natural enemy to control secondary/occasional pests like mites, thrips, whiteflies, aphids, and fungal pathogens. A grower greenhouse was selected for this study and showed that biological control was not only biologically possible but also cost effective. The grower ended up spending more dollars per 100 sq ft for ineffective control of pests in the chemical control program when compared to a biologically- based program. This should make a successful case for adoption of an integrated pest management program.

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CHAPTER 2

PESTICIDE COMPATIBILITY WITH NATURAL ENEMIES FOR LEAFMINER MANAGEMENT IN GREENHOUSE GERBERA DAISIES

To be submitted to the *Journal of Economic Entomology*

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Abstract

We studied the compatibility of various pesticides used in commercial greenhouse management with two biological control agents; a leafminer parasitoid (*Diglyphus isaea* (Walker)), and a predatory mite (*Neoseiulus californicus* (McGregor)). These natural enemies were exposed to miticides, fungicides, and insecticides used against leafminers, thrips and whiteflies according to label directions in laboratory vial assays, after which mortality at 12, 24, and 48 hours (h) was recorded. Greater mortality of predatory mites than leafminer parasitoids was observed overall, illustrating that fewer pesticides were compatible with predatory mites compared with the parasitoid. However, some commonly used pesticides were found to cause high mortality to both the leafminer parasitoid and predatory mites. Twospotted spider mite (*Tetranychus urticae* Koch) infestations often disrupt leafminer (*Liriomyza trifolii* (Burgess)) biocontrol programs. Therefore, potentially compatible miticides (bifenazate, hexythiazox, spiromesifen, acequinocyl, etoxazole, and clofentezine) identified in laboratory trials were also evaluated in a greenhouse study to determine if they were compatible with leafminer parasitism during a 4 week period. All six of them were compatible with leafminer biocontrol and did not affect parasitoid survivability in the long run.

KEYWORDS *Liriomyza trifolii; Diglyphus isaea;* Greenhouse pest management; Greenhouse biocontrol; leafminer biocontrol; Safe pesticides.

Introduction

The primary pests affecting greenhouse gerberas are serpentine leafminers, *Liriomyza trifolii* (Diptera: Agromyzidae), which have a wide distribution and attack more than 400 species (Reitz and Trumble 2002) of plants including vegetables and ornamentals. The larvae feed on the palisade mesophyll (Parrella et al. 1985) and decrease photosynthesis and yield, directly affecting the marketable produce. Rigorous and extended use of pesticides has rendered leafminers resistant to almost all chemistries (Keil and Parrella 1982). Leafminers are also protected from pesticides by being concealed within the leaves in their larval stages. Successful biocontrol has been implemented by augmentative releases of parasitoids. This has however been effective in areas only where disruptive use of chemical controls has been avoided (Liu et al. 2009).

The influx of secondary pests like mites, thrips, whiteflies, and aphids, and pathogens causing powdery mildew through the season necessitates pesticide sprays that in turn kill the leafminer parasitoids and disrupt biocontrol. The unique situation in greenhouse gerbera production suggests the potential for integrated pest management (IPM) as an effective solution. While pesticides work against secondary pests, they also disrupt biological control of the primary pest. Knowing which chemicals can be used against secondary pests without harming the natural enemies of primary or secondary pests would facilitate implementation of an integrated pest management (IPM) program for greenhouse gerberas. While there is information about compatibility of pesticides to several parasitoids in several production systems (Biobest , Koppert) gaps exist in the greenhouse gerbera system regarding commonly used pesticides and the natural enemies that have potential.

Materials and Methods

We evaluated the compatibility of commonly used pesticides in greenhouse gerberas with 2 natural enemies: a leafminer parasitoid (*Diglyphus isaea*), a wasp that feeds on the immature leafminer as part of its life cycle, and a predatory mite (*Neoseieulus californicus*), a mite that is predaceous on other pest mite species (Rincon Vitova Insectaries, Ventura, CA). There are at least 6 major pests that are targeted in greenhouse gerbera management: leafminers (*Liriomyza trifolii*), mites (*Tetranychus urticae*), thrips (*Frankliniella occidentalis*), whiteflies (*Trialeurodes vaporariorum*, and *Bemisia tabaci*), aphids (*Myzus persicae*), and pathogens causing powdery mildew (from the genera *Podosphaera*, *Erysiphe, Leveillula, Golovinomyces*, and *Oidium*). Hence at least 5 groups of pesticides (Table 2.1) need to be evaluated, because aphids are often targeted by the same insecticides but at a lower rate than when used against pests like whiteflies or leafminers. Following a laboratory study in which the toxicity of these chemicals within 48 h was documented, pesticides that caused the least mortality from among the treatments in the miticide group were used in a greenhouse study to investigate the toxicity post 48 h.

Laboratory Study

Experimental Protocol: Pesticides (Table 2.1) selected for the lab assays are commonly used in greenhouse management. Nine pesticides and a water control were evaluated. Since pesticides recommended against aphids are also used against other pests but at a higher rate, they were not evaluated as a separate group. Previously documented vial assay methods (Bjorksten and Robinson 2005, Wu and Miyata 2005) were modified and

employed as leaf dip assays for the parasitoid wasps, and as pesticide swirl assays for predatory mites.

Leafminer parasitoid *(D. isaea)*: Gerbera plugs that had not previously been treated were obtained from Speedling Inc., Blairsville, GA. A single leaf was removed from the plug and covered with cotton around the petiole and inserted into one end of a 1.5 cm long section of Tygon® tubing and hydrated when necessary. The leaf was then completely dipped in the respective treatments (aqueous pesticide solutions at label rates or water control) for 10 seconds each and allowed to dry for at least 3 h. After the inside of the vial was streaked with honey (as a food source for the parasitoids), 10 *D. isaea* parasitoids were introduced. The tubing with the leaf inside was then inserted at the neck region of the vial and sealed using parafilm™.

Predatory Mites (*N. californicus)*: A solution (10-15 ml) of the designated treatment was poured into each glass vial and swirled for even coverage over the surface of the glass. After allowing at least 3 h for drying, a drop of honey was streaked inside each vial, and then 10 adult *N. californicus* mites were inserted and the vial capped.

Design and Data Collection: Five experiments where an experimental unit was a vial were conducted, and the experiment consisted of 10 replicates for each of the 10 treatments all of which were placed on a lab counter with a 14 h light: 10 h dark period and held at 22-25°C. Each experiment was repeated on 2 other days for a total of 15 trials. Live adult parasitoids and adult mites (viewed through a microscope) were counted 12, 24, and 48 h after the treatment. Any movement by the natural enemy

designated them as alive while the lack of movement when disturbed resulted in counting them as dead.

Greenhouse Miticide Study

Location and Experimental design: The study was conducted at the UGA-Griffin campus. After selecting and housing 170 potted gerbera plants of the Gerbera 'Festival Mini Yellow Shade' cultivar in similar growth stages, an excess of 500 adult *L. trifolii* collected from grower and research greenhouses were released into the greenhouse. Treatments included 6 miticides (bifenazate, hexythiazox, spiromesifen, acequinocyl, etoxazole, and clofentezine) and a (water) control and were applied a week after the flies were introduced. Each cage (Bug dorm rearing cage, # 1452, BioQuip Products, Rancho Dominguez, CA) was an experimental unit and housed 4 potted plants for a total of 168 plants in 42 cages. Twenty four hours later, 10-12 parasitoids (*D. isaea*) purchased from Rincon Vitova Insectaries Inc., Ventura, CA. were released into each cage. During the test period, the greenhouse was maintained at 25-32°C and 85% humidity.

Data Collection and Evaluation: Seven days after the parasitoids were released, 3 leaves were sampled from each experimental unit and inspected under a microscope for parasitoid and leafminer activity. After the first sampling date, cages were removed so that the leafminer pressure and the parasitoid availability would be equal for all the plants, while residual toxicity would determine the actual activity of leafminer and *D. isaea*. The greenhouse was flooded with an excess of 600 adult leafminers and 72 h later, 250 parasitoids. Sampling was then repeated every seventh day thereafter for three weeks spanning 14 June through 5 July, 2011.

Statistical Analyses

The experiments were analyzed as randomized complete block designs. Replications were considered as the block factor. Data were subjected to analysis of variance (ANOVA) using the general linear model procedure (PROC GLM, SAS Institute 2003) and means were separated using Tukey's HSD test.

Laboratory Study: Treatment means were analyzed separately for each study. When initial analysis determined that date was significant ($P< 0.05$), trials for each experiment were subsequently analyzed separately. The tiered method advocated by IOBC (International Organization of Biological Control) considers pesticides from lab studies causing mortality rates of 30- 79% to be slightly harmful and < 30% mortality harmless (Stark et al. 2007), and chemicals falling in both these categories to qualifyto be part of IPM programs. Pesticides in this study that caused mortalities within these values at least twice out of the three trials were considered at least "less harmful".

Greenhouse Study: Data were analyzed as above, first to find the difference in parasitism rate (average number of parasitoids/ total number of leafminers in the experimental unit) between the treatments. Additional analyses investigated the differences based on average number of leafminers, average number of parasitoids, number of live leafminers, and total (sum of live and dead) leafminers.

Results

Laboratory study

Following the criteria accepted by IOBC (Stark et al. 2007), chemicals tested in laboratories are divided into four categories based on their toxicity. Those causing < 30% mortality are considered harmless, 30-79% slightly harmful, 80-98% moderately harmful, and > 99% considered harmful. The same criteria were used to elucidate our lab experiment results.

Leafminer chemicals (D. isaea at 48 h): Novaluron, and petroleum oil were harmless (<30% mortality within 48 h in at least 2 out of the 3 trials) (Table 2.7). Azadirachtin, cyromazine, and acetamiprid were slightly harmful, causing mortality in the range of 30-79%. Lambda cyhalothrin was found to be moderately harmful with a mortality of 80-98%. Dinotefuran and bifenthrin were harmful and caused mortality > 99% within 48 h (*f* range= 27.04- 47.96; *df*= 9, 99; p value= <0.0001) (Table 2.2). Though spiromesifen was tested together with leafminer chemicals, it actually is not labeled for use against leafminers. It was tested at the whitefly rate as an additional whitefly chemical.

Leafminer chemicals (N. californicus at 48 h): At the 48 h mark, none of the pesticides were harmless to the predatory mites (Table 2.7). Cyromazine, novaluron and petroleum oil were found to be slightly harmful (30-79% mortality). Azadirachtin was moderately harmful, with 80-98% mortality; dinotefuran, bifenthrin, lambda cyhalothrin, and acetamiprid were harmful and caused > 99% mortality in the predatory mites (*f* range= 16.84- 46.24; *df*= 9, 99; p value= <0.0001) (Table 2.2). The low mortality in the cyromazine treatment, and novaluron at the 48 h mark does not ensure their harmlessness

though because of their being insect growth regulators (IGRs) with effects not showing up until later.

***Miticides (D. isaea at 48 h)*:** Clofentazine and acequinocyl were harmless and caused < 30% moratlity within 48 h (Table 2.7). Bifenazate, hexythiazox, spiromesifen, etoxazole, and milbemectin were slightly harmful and caused 30-79% mortality. Abamectin caused 80-98% mortality and spinosad > 99% and were moderately harmful and harmful to *D. isaea* respectively (*f* range= 17.46- 84.97; *df*= 9, 99; p value= <0.0001) (Table 2.3). However most of the miticides that demonstrated lower mortality at the 48 h mark were IGRs and only a prolonged study (Greenhouse Study detailed below) could confirm if they are actually safe to *D. isaea* for a longer period.

Miticides (N. californicus at 48 h): Etoxazole, bifenazate, hexythiazox, clofentazine, and spiromesifen were slightly harmful and caused 30-79% mortality *f* range= 12.85-43.56; *df*= 9, 99; p value= <0.0001) (Tables 2.3, 2.7). However, a majority of them being IGRs and specifically miticides would not neccesarily make them compatible with a biological control program involving predatory mites unless selective toxicity to pest mite species is proven. While acequinocyl caused 80-98% mortality, abamectin, spinosad and milbemectin caused > 99% mortality even at the 48 h mark and hence were harmful.

Whitefly chemicals (D. isaea at 48 h): Pyriproxifen, and spiromesifen caused < 30% mortality at the 48 h mark (*f* range= 20.07- 24.71; *df*= 9, 99; p value= <0.0001) (Tables 2.4, 2.7) and hence considered harmless to *D. isaea*. Spirotetramat, flonicamid, Pyridaben, and chlorpyrifos at their respective median label rates (Table 2.1) were found

to cause 30-79% mortality. Pyriproxyfen is an IGR and caused low mortality, while spirotetramat and spiromesifen are not IGRs and can be components in an IPM program. Kinoprene, thiamethoxam, imidacloprid, and lambda cyhalothrin caused 80-98% mortality and are probably best not used in a biological based IPM program.

Whitefly chemicals (N. californicus at 48 h): Flonicamid, spirotetramat, thiamethoxam, and spiromesifen were slightly harmful, causing 30-79% mortality within 48 h (*f* range= 21.7- 24.94; *df*= 9, 99; p value= <0.0001) (Tables 2.4, 2.7). Pyriproxifen, and chlorpyrifos, caused 80-98% mortality (moderately harmful), while kinoprene, imidacloprid, pyridaben and lambda cyhalothrin caused > 99% mortality (harmful) in the predatory mites.

Thripicides (D. isaea at 48 h): Flonicamid, cyfluthrin, insecticidal soap, *B. bassiana*, and acetamiprid were found to be slightly harmful because they inflicted mortality within the range of 30-79% in 48 h (*f* range= 31.2- 40.96; *df*= 9, 99; p value= <0.0001) (Tables 2.5, 2.7). While abamectin, fluvalinate, and chlorfenapyr caused 80-98% mortality (moderately harmful) in *D.isaea*, spinosad was responsible for >99% (harmful).

Thripicides (N. californicus at 48 h): Flonicamid and insecticidal soap caused 30-79% mortality (slightly harmful), while *B. bassiana*, and acetamiprid were moderately harmful and caused 80-98% mortality (*f* range= 15.04- 32.61; *df*= 9, 99; p value= <0.0001) (Tables 2.5, 2.7). Abamectin, spinosad, cyfluthrin, fluvalinate, and chlorfenapyr, caused > 99% mortality in the mites (harmful).

Fungicides (D. isaea at 48 h): All tested fungicides showed lower than 79% mortality in *D. isaea* within 48 h and hence qualify to be used in IPM programs. Butanone, fosetyl-

aluminum, azoxystrobin, potassium bicarbonate, pyraclostrobin, copper sulfate, and piperalin caused < 30% and hence are considered harmless (*f* range= 1.53- 4.92; *df*= 9, 99; p value range= <0.0001- 0.15) (Tables 2.6, 2.7). Rosemary oil (EcoSmart), and sulfur were the only ones that caused higher mortality but still remained within 30-79% and hence are considered only slightly harmful.

Fungicides (N. californicus at 48 h): Butanone and copper sulfate caused 30-79% mortality in mites (*f* range= 16.11- 70.13; *df*= 9, 99; p value= <0.0001) (Tables 2.6, 2.7), hence slightly harmful. Sulfur was moderately harmful and caused 80-98% mortality while fosetyl-alumium, rosemary oil, azoxystrobin, potassium bicarbonate, pyraclostrobin, and peperalin caused >99% mortality (harmful) in *N. californicus*.

While there were slight differences in individual mortality values attributed to specific pesticides, the ones consistently inflicting high mortality on natural enemies were clearly identified. In general, more pesticides were compatible with the parasitoids (*D. isaea*) than the predatory mites (*N. californicus*) (Table 2.7). Salient points distilled from the results above are given below where (*df*= 9, 99; *f values* ranged from 12- 119; *p* values <0.0001).

1. Six miticides cause less mortality than the industry standard, abamectin in the parasitoid *D. isaea* even at 48 h.
2. Spinosad, a good control for thrips, caused high mortality in the parasitoid.
3. Mortality of *D. isaea* parasitoids due to the fungicides did not vary significantly from the water control (*df*= 9, 99; *f* ranged from= 1.53- 5.5; *p* value ranged from <0.0001 -

0.1511), but they inflicted high mortality on the predatory mites *N. californicus* (Table 2.6).

Greenhouse Study

Treatments did not differ from the control in parasitism rates over 4 weeks, confirming compatibility observed in laboratory studies (*f* range= 0.22- 1.38; *df*= 6, 41; P values range= 0.2615- 0.9673) (Appendix Table 2). The fluctuation in parasitism level was not restricted to the treatments but the control also followed the same trend. There was no significant difference between the treatments and control in any of the parameters that were additionally tested: average number of leafminers (*f* range= 0.95- 1.27; *df*= 6, 41; P values range= 0.3016 - 0.4774) (Appendix Table 3), average number of parasitoids (*f* range= 0.18- 1.54; *df*= 6, 41; P values range= 0.1985 - 0.9800) (Appendix Table 4), number of live leafminers (*f* range= 0.95- 1.27; *df*= 6, 41; P values range= 0.3016 - 0.4774) (Appendix Table 5), and total (sum of live and dead) leafminers (*f* range= 0.31- 1.51; *df*= 6, 41; P values range= 0.1964 - 0.9276) (Appendix Table 6). Parasitism, which started high in the first week fell in the second week and returned to its highest level by the 4th week.

Discussion

Laboratory study

For each of the groups of chemicals that were tested, a majority were found to be toxic to the leafminer parasitoid *Diglyphus isaea* at the 48 h mark, and even more so for the predatory mite *Neoseiulus californicus*. Some of those that were found to be less toxic, were “insect growth regulators” (IGRs) and hence would not be expected to show negative effects until later. Several studies have looked at effects of fewer pesticides on

leafminer parasitoids in either field (Poe et al. 1978, Johnson et al. 1980, Oetting 1985, Hara 1986, Weintraub and Horowitz 1998, Civelek and Weintraub 2003) or lab studies (Bjorksten and Robinson 2005, Wu and Miyata 2005) and demonstrated toxic effects or the lack thereof on natural enemies. This study however looked at a large number of pesticides commonly applied against at least 6 major pests in the greenhouse gerbera system and investigated their compatibility with natural enemies that have the potential of controlling the two most important pests. Most other studies looked at fewer chemicals targeting a single important pest in their respective systems.

Effects on D. isaea: Since *L. trifolii* are often chemically resistant, most of the chemicals labelled for use against them rarely control populations to a significant level. However, that rarely serves as an incentive to not spray pesticides in the greenhouses. Growers often rely on pesticides as the only solution to pest problems as they (when effective) allow for tangible and observable effects immediately, as opposed to biological control methods which take more time and do not eliminate a pest completely. The knowledge that novaluron, petroleum oil, azadirachtin, cyromazin and acetamiprid are at most slightly harmful to the leafminer parasitoid could encourage the use of such chemicals for leafminer control when inevitable. Mites are the most commonly encountered among the secondary pests in this system and chemicals are effective in controlling them. Within 48h though, there were more miticides that were potentially harmless to the leafminer parasitoid than harmful. That abamectin is toxic to parasitoids has been shown previously (Hara 1986, Bjorksten and Robinson 2005). Our results on the effect of spinosad coroborates similar findings in protected cultivation (Jones et al. 2005) and field situations where high mortality was observed in hymenopterans in spite of its being

accepted by many as a biorational pesticide (Williams et al. 2003). This also cautions and emphasizes the importance of individual componenets of an integrated management program in cut flowers. Spinosad as a miticide has a recommended rate of 22 oz/100 gal and as a thrips material 6 oz / 100 gal. Even though less toxic at the lower rate, spinosad caused severe mortality to the leafminer parasitoid at both rates. Abamectin is the industry standard for mite control and spinosad is an effective thrips control material. Their both being harmful to natural enemies removes significant control options from a grower's pesticide armory.

Apart from the IGRs, only spirotetramat and spiromesifen demonstrated potential as whitefly insecticides that could integrate with biological control of the leafminer. However, both are in the insecticide class 23 which inhibits acetyl CoA carboxylase (IRAC 2011). This provides few options for rotation of pesticides. As a thrips control material, flonicamid, cyfluthrin, acetamiprid, insecticidal soap, and *Beauveria bassiana* were seemingly safe to the leafminer parasitoid, but from a grower's perspective, the natural products are not first choice options because they do not immediately show effects. Flonicamid comes under the chemical class 9c and is a feeding blocker (IRAC 2011), while the natural products effect control in other ways. Cyfluthirin, which comes in the pyrethroid class, and acetamiprid, which is a neonicotinoid, could be effective components though. Spinosad is effective for thrips control (Jones et al. 2005), but demonstrate negative effects on parasitoid populations. Fungicides in general were found to cause low mortality in the parasitoid wasp *D. isaea*. EcoSmart, a ready–to-use rosemary oil concoction and sulfur were the only fungicides among those tested (Tables 2.6, 2.7) that caused > 30% mortality in *D. isaea*, but still less than 79% and hence

usable in IPM programs. Our data suggest that fungicides do not cause immediate negative effects on leafminer parasitoids.

Effects on the Predatory mite: Mites are the most frequently encountered secondary pests in greenhouse gerberas. Unless a miticide specifically toxic to pest mite species is available, integration of miticides and predatory mites would not be possible in an IPM program. Cyromazine is accepted as being safe for natural enemies in general (Biobest , Koppert), and our study noted the same. However, we observed heightened activity by the surviving mites in the vial closer to the lid. Whether the phenomenon is a synergistic effect or a repellent effect needs a closer investigation

From among the whitefly chemicals, flonicamid, thiomethoxam, spiromesifen, and spirotetramat were only slightly harmful to predatory mites. Spiromesifen and spirotetramat were safe options also to the leafminer parasitoids and hence add to the number of options to rotate. Among commonly used thrips control materials, only flonicamid and insecticidal soap showed potential to integrate with pest mite biocontrol. While miticides in general were not completely toxic to the insect natural enemy (leafminer parasitoid), insecticides in general seemed to harm the non-insect natural enemy (predatory mite).

The salient inference from the lab assays is identfication of pesticides that can be safely integrated with a biological control regime. Focusing on safety of the leafminer parasitoid, *D. isaea,* primarily, there are slightly more pesticides that are potentially compatible than with predatory mites (Table 2.7). Reevaluating our control options from

the available compatible chemistries to effectively rotate, and convincing growers to adopt only those options in an IPM program would be the challenge going forward.

Greenhouse Miticide Study

Mites being the most frequently encountered among the secondary pests makes their control an important component in any IPM program in this system. Our prolonged greenhouse study showed that the residual effect of miticides was not detrimental to *D. isaea* in the long run. Even though the parasitism rate dropped below 30% in the second week, the fact that the fluctuation occurred in all treatments, including the control, and that there were no differences in other parameters that were analyzed, indicates that the effect was due to life history traits. After one week of high parasitism (> 70%), there were very few leafminers for the parasitoids to parasitize the following week. All the treatments followed a similar pattern and reached a peak parasitism by the fourth week, which also meant that the miticides did not detrimentally affect *D. isaea* development in the weeks prior (2^{nd} or 3^{rd} week) when the parasitoids were in younger and more vulnerable stages. Results indicated that bifenazate, hexythiazox, spiromesifen, acequinocyl, etoxazole, and clofentazine are not injurious at least in the long run for the development and population buildup of *D. isaea*. This gives us valuable information for integrating biological and chemical control to keep the most important pests in this system in check. The primary pest can be controlled using its natural enemy, and the major secondary pest can be controlled by rotating safe chemicals that do not harm the leafminer parasitoid, *D. isaea*.

Additionally, from these results (Table 2.7), we would be able to integrate options to control the primary pest in this system (leafminer) using its natural enemies and use less disruptive options from among the chemicals to control the secondary pests. The benefits from such a strategy are multifold, 1) lower pesticide footprint in the premises and environment, 2) enhanced safety to the workers and producers alike, 3) better management of the pest and diseases leading to a better crop, and 4) overall a sustainable production system. With the increase of insecticide resistant pests, the possibility of insecticide resistant natural enemies (Rosenheim and Hoy 1988) will need to be strongly explored.

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Table 2.1. List of chemicals with trade name, active ingredients, formulation, median label rates (per 100 gallon of water unless otherwise mentioned) for respective target pests.

Trade name	Active Ingredient	Target Pests					
		Spider mites	Leaf miners	Thrips	White flies	Aphids	Fungal Pathogens
Avid 0.15 EC	Abamectin	4 oz			8 oz		--
Ultiflora	Milbemectin	12 fl oz	--	--	--	--	--
TetraSan 5WDG	Etoxazole	12 oz	--	--	--	--	--
Floramite WSP	Bifenazate	3 fl oz	--	--	--	--	--
Hexygon DF	Hexythiazox	1.5 fl oz	--	--	--	--	--
Judo	Spiromesifen	2.5 fl oz	--	--	3 fl oz	--	--
Ovation SC	Clofentezine	2 fl oz	--	--	--	--	--
Pylon	Chlorfenapyr	3.9 fl oz	--	15 fl oz	--	--	--
Sanmite WP	Pyridaben	4 fl oz	--	--	5 fl oz	--	--
Shuttle O	Acequinocyl	9.6 fl oz	--	--	--	--	--
Conserve SC	Spinosad	22 fl oz		6 fl oz	--	--	--
Duraguard ME	Chlorpyrifos			37.5 fl oz			--
Kontos	Spirotetramat	--	--	--	1.7 fl oz		--
Pedestal SC	Novaluron	--		7 fl oz		--	--
Citation WP	Cyromazine		2.66 oz			2.66 oz	--

Safari 20 SG	Dinotefuran	--	0.375 lb				--
Azatin XL	Azadirachtin	--	13 fl oz	14 fl oz	13 fl oz	14 fl oz	--
Scimitar GC	Lambda Cyhalothrin	4 oz	3.25oz		4 oz	3.25 oz	--
TriStar 30 SG	Acetamiprid	--	7.35 fl oz	6 fl oz	4 fl oz	1.3 fl oz	--
Flagship 25 WG	Thiamethoxam	--	--	--	3 oz		--
Aria	Flonicamid	--	--	2.5 oz	3.6 oz	0.9 oz	--
Talstar One	Bifenthrin	16.25 fl oz	32.6 fl oz		16.25 fl oz		--
Naturalis L	*B. bassiana*	65 fl oz	--		65 fl oz		--
Mavrik Aquaflow	Fluvalinate	7 fl oz	--		7 fl oz		--
Marathon 1 G	Imidacloprid	--	15 oz/ 1000 sq. ft.				--
Decathlon 20 WP	Cyfluthrin	--	--		1.9 oz		--
Distance	Pyriproxyfen	--	--	--	7 fl oz	--	--
PureSpray Oil	Petroleum Oil	2-5 tbsp/ gal		--		2-5 tbsp/ gal	
Enstar	Kinoprene	7.5 fl oz					
MPede	Insecticidal Soap	2 gal					
Pipron LC	Piperalin	--	--	--	--	--	6 fl oz
Milstop	Potassium bicarbonate	--	--	--	--	--	2.5 lbs
Pageant	Pyraclostrobin	--	--	--	--	--	9 oz

EcoSmart RTU	Rosemary Oil	--	--	--	--	--	
Sulfur 6L	Sulfur	--	--	--	--	--	6 fl oz
Aliette WDG	Fosetyl-aluminum	--	--	--	--	--	64 oz
Strike 50 WDG	Butanone	--	--	--	--	--	2 oz
Phyton 27	Copper Sulfate	--	--	--	--	--	2 fl oz
Heritage	Azoxystrobin	--	--	--	--	--	20 oz

Table 2.2. Means (±SE) of number of live natural enemies (*D. isaea* and *N. californicus*) at each observation time of 12, 24, and 48 h in each of three trials (Tr 1, Tr 2, Tr 3) after exposure to leafminer-targeted materials at median label rates (Table 2.1) out of a total of 10 natural enemies in each experimental unit.

12 h	*D. isaea*			*N. californicus*		
Treatment	Tr 1	Tr 2	Tr 3	Tr 1	Tr 2	Tr 3
Control	9.2±0.25a	9.8±0.13a	9.7±0.15a	8.6±0.54a	7.9±0.72a	9.0±0.42a
Spiromesifen	8.1±0.6ab	9.0±0.39a	9.4±0.27a	8±0.52ab	3.5±1.14bc	5.0±1.11bc
Cyromazine	9.0±0.52ab	9.0±0.26a	8.9±0.31a	8.7±0.42a	7.0±0.67a	7.2±1.19ab
Novaluron	6.6±0.85bc	10±0a	9.1±0.31a	6.4±0.83abc	2.6±1.09bcd	2.3±0.6cd
Petroleum Oil	5.0±0.77cd	9.9±0.1a	9.4±0.22a	4.4±0.69c	5.0±0.93ab	6.1±1.12ab
Azadirachtin	8.6±0.3ab	9.3±0.26a	9.1±0.23a	5.8±0.59bc	5.2±0.55ab	2.4±0.52cd
Acetamiprid	7.3±0.56abc	8.3±0.42a	6.2±0.47b	1.4±0.48d	1.3±0.42cd	1.7±0.4cd
Dinotefuran	1.3±0.45e	4.8±0.65b	3.8±0.51c	0.3±0.21d	1.2±0.39cd	0.0±0d
Bifenthrin	1.1±0.38e	2.6±0.27c	2.0±0.58c	0.0±0d	0.0±0d	0.0±0d
Lambda cyhalothrin	3.1±0.55de	5.0±0.76b	2.8±0.61c	0.0±0d	0.2±0.13d	0.0±0d

df	9, 99	9, 99	9, 99	9, 99	9, 99	9, 99
f	30.90	46.00	40.11	49.46	18.13	22.43
P value	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
24 h						
Control	8.4±0.45ab	9.8±0.13a	9.7±0.15a	8.4±0.54ab	7.6±0.73a	9.0±0.42a
Spiromesifen	6.0±0.7bcd	8.7±0.52ab	8.9±0.43a	6.6±0.37abc	3.5±1.14bc	4.8±1.05bc
Cyromazine	8.9±0.5a	7.7±0.58b	8.8±0.29a	8.5±0.43a	6.8±0.61a	7.2±1.19ab
Novaluron	5.6±0.88cd	9.5±0.40ab	9.1±0.31a	5.9±1.0bc	2.6±1.09bcd	2.3±0.6cd
Petroleum Oil	4.0±0.84de	9.7±0.15ab	8.9±0.31a	4.2±0.63c	5.0±0.93ab	5.9±1.05ab
Azadirachtin	8.0±0.37abc	8.2±0.42ab	8.6±0.31a	4.7±0.87c	4.8±0.63ab	2.0±0.47cd
Acetamiprid	6.7±0.56abc	7.8±0.51ab	6.2±0.47b	0.9±0.41d	0.9±0.31cd	1.7±0.4cd
Dinotefuran	1.1±0.38f	2.9±0.57cd	3.3±0.58c	0.1±0.1d	0.8±0.33cd	0.0±0d
Bifenthrin	0.7±0.3f	1.0±0.15d	1.9±0.59c	0.0±0d	0.0±0d	0.0±0d
Lambda cyhalothrin	1.8±0.39ef	3.5±0.56c	1.8±0.44c	0.0±0d	0.0±0d	0.0±0d
df	9, 99	9, 99	9, 99	9, 99	9, 99	9, 99

f	28.67	53.82	66.47	38.24	18.18	22.02
P value	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
48 h						
Control	7.7±0.52a	9.6±0.22a	9.0±0.42a	8.0±0.6a	7.0±0.92a	8.4±0.97a
Spiromesifen	5.3±0.68ab	7.8±0.63abc	7.9±0.53ab	6.4±0.4a	3.5±1.14bc	3.9±0.91bc
Cyromazine	7.6±0.62a	6.6±0.62c	6.4±0.58bc	8.1±0.53a	6.8±0.61a	6.4±1.38ab
Novaluron	4.2±0.7b	8.9±0.57ab	7.7±0.5ab	5.8±1.05ab	2.6±1.09bcd	0.8±0.7cd
Petroleum Oil	2.7±0.83bc	8.7±0.56abc	7.4±0.4abc	3.9±0.72b	4.2±0.89ab	5.9±1.05ab
Azadirachtin	7.4±0.58a	6.6±0.6c	6.6±0.52bc	0.9±0.28c	0.6±0.43cd	0.0±0d
Acetamiprid	5.3±0.68ab	7.1±0.48bc	5.4±0.37c	0.2±0.13c	0.1±0.1d	0.7±.03cd
Dinotefuran	0.4±0.16c	1.1±0.35d	2.4±0.45d	0.0±0c	0.0±0d	0.0±0d
Bifenthrin	0.2±0.13c	0.8±0.2d	1.5±0.56d	0.0±0c	0.0±0d	0.0±0d
Lambda cyhalothrin	1.0±0.33c	1.8±0.55d	0.6±0.22d	0.0±0c	0.0±0d	0.0±0d
df	9, 99	9, 99	9, 99	9, 99	9, 99	9, 99
f	27.04	47.96	39.45	46.24	16.84	18.32

P value	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001

Table 2.3. Means (±SE) of number of live natural enemies (*D. isaea* and *N. californicus*) at each observation time of 12, 24, and 48 h in each of three trials (Tr 1, Tr 2, Tr 3) after exposure to miticides at median label rates (Table 2.1) out of a total of 10 natural enemies in each experimental unit.

12 h	*D. isaea*			*N. californicus*		
Treatment	Tr 1	Tr 2	Tr 3	Tr 1	Tr 2	Tr 3
Control	4.5±0.75b	9.0±0.21a	9.1±0.43a	5.8±0.95a	9.0±0.3a	5.4±0.43b
Hexythiazox	8.7±0.58a	8.9±0.38a	8.9±0.46a	4.0±0.7ab	7.7±0.47a	6.0±0.56ab
Milbemectin	8.6±0.33a	9.0±0.37a	8.6±0.37a	1.2±0.33c	0.4±0.22d	0.5±0.22d
Clofentezine	8.6±0.5a	9.2±0.25a	8.5±0.54a	4.6±0.69a	7.9±0.62a	7.7±0.52a
Spiromesifen	7.9±0.53a	9.2±0.33a	8.7±0.54a	6.6±0.87a	7.0±0.79a	7.6±0.4a
Bifenazate	7.6±0.72a	8.9±0.41a	8.8±0.39a	5.0±0.49a	8.3±0.3a	5.9±0.50ab
Etoxazole	2.6±0.62bc	9.5±0.34a	8.4±0.48a	5.9±0.55a	7.67±0.62a	7.0±0.56ab
Acequinocyl	2.875±0.6b	8.89±0.39a	8.8±0.66a	1.7±0.47bc	4.3±0.83b	3.1±0.55c
Abamectin	3.2±0.42b	2.0±0.42b	4.7±0.65b	1.8±0.33bc	3.6±0.69bc	1.7±0.45cd
Spinosad	0.2±0.13c	0.0±0c	0.1±0.1c	1.0±0.33c	1.0±0.33cd	0.6±0.22d

df	9, 99	9, 99	9, 99	9, 99	9, 99	9, 99
f	31.62	119.51	34.49	13.43	30.21	39.37
P value	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
24 h						
Control	3.4±0.79b	8.3±0.3a	9.0±0.42a	5.0±0.94abc	7.7±0.47a	5.1±0.41b
Hexythiazox	7.6±0.78a	8.6±0.43a	7.4±0.52a	2.5±0.72cdef	6.8±0.57a	5.7±0.63ab
Milbemectin	7.7±0.42a	8.1±0.48a	7.9±0.59a	0.2±0.13f	0.1±0.1d	0.0±0.0c
Clofentezine	7.1±0.53a	8.2±0.44a	7.6±0.64a	3.3±0.63bcde	6.67±0.57a	7.3±0.62a
Spiromesifen	7.0±0.67a	8.9±0.38a	6.9±0.78a	6.3±0.86a	5.8±0.88ab	7.4±0.34a
Bifenazate	7.0±0.79a	8.6±0.43a	7.7±0.45a	3.8±0.77abcd	7.0±0.54a	5.1±0.64b
Etoxazole	2.4±0.6bc	9.2±0.33a	7.6±0.72a	5.3±0.7ab	6.67±0.52a	6.9±0.59ab
Acequinocyl	2.25±0.6bc	8.75±0.52a	8.5±0.65a	1.2±0.42def	3.6±0.86bc	1.9±0.50c
Abamectin	2.6±0.43bc	1.0±0.42b	3.2±0.55b	0.9±0.31ef	2.2±0.39cd	1.2±0.42c
Spinosad	0.2±0.13c	0.0±0b	0.0±0c	0.7±0.26ef	0.4±0.22d	0.1±0.1c
df	9, 99	9, 99	9, 99	9, 99	9, 99	9, 99

f	20.50	85.14	24.88	13.23	23.34	38.70
P value	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
48 h						
Control	2.3±0.6b	8.2±0.29ab	9.0±0.42a	4.4±0.97abc	6.7±0.56a	4.8±0.33b
Hexythiazox	6.6±0.62a	8.3±0.40ab	6.3±0.68ab	1.9±0.8cde	5.3±0.75a	5.5.65ab
Milbemectin	7.2±0.25a	6.8±0.39b	6.7±0.7ab	0.0±0e	0.0±0c	0.0±0.0c
Clofentezine	5.6±0.69a	7.8±0.55ab	7.1±0.74ab	3.0±0.71bcd	6.3±0.56a	7.3±0.62a
Spiromesifen	6.0±0.63a	8.5±0.40ab	5.8±0.77b	5.8±0.99a	3.9±1.07ab	7.2±0.39a
Bifenazate	5.6±0.72a	8.4±0.45ab	6.1±0.71ab	3.4±0.78abcd	5.8±0.81a	5.0±0.65b
Etoxazole	1.9±0.5b	8.8±0.36a	6.9±0.94ab	4.7±0.7ab	6.2a±0.61a	6.6±0.64ab
Acequinocyl	1.5±0.6b	7.89±0.60ab	7.1±0.81ab	0.8±0.39de	2.1±0.85bc	0.9±0.43c
Abamectin	1.3±0.26b	0.0±0c	1.7±0.50c	0.0±0e	1.3±0.26bc	0.5±0.31c
Spinosad	0.0±0b	0.0±0c	0.0±0c	0.0±0e	0.1±0.1c	0.1±0.1c
df	9, 99	9, 99	9, 99	9, 99	9, 99	9, 99
f	23.53	84.97	17.46	12.85	16.68	43.56

P value	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001

Table 2.4. Means (±SE) of number of live natural enemies (*D. isaea* and *N. californicus*) at each observation time of 12, 24, and 48 h in each of three trials (Tr 1, Tr 2, Tr 3) after exposure to whitefly-targeted materials at median label rates (Table 2.1) out of a total of 10 natural enemies in each experimental unit.

12 h	*D. isaea*			*N. californicus*		
Treatment	Tr 1	Tr 2	Tr 3	Tr 1	Tr 2	Tr 3
Control	8.6±0.4a	9.2±0.36a	9.1±0.38a	3.1±0.7bc	8.6±0.43a	8.0±0.65a
Spirotetramat	8.0±0.49ab	8.1±0.38a	9.3±0.26a	4.8±0.84ab	3.6±0.87cde	5.4±0.65bc
Pyriproxyfen	8.5±0.37a	8.3±0.37a	8.7±0.26a	2.6±0.62bcd	3.2±0.51cde	2.2±0.39ef
Flonicamid	6.4±0.59abc	7.4±0.45ab	7.9±0.66a	6.8±0.7a	7.6±0.86ab	7.3±0.68ab
Kinoprene	8.4±0.34ab	7.7±0.56a	9.1±0.23a	2.2±0.47bcd	2.8±0.69cdef	2.9±0.46de
Chlorpyrifos	4.5±0.7cde	5.7±0.3bc	8.8a	4.2±0.57ab	4.0±0.63cd	3.8±0.61cde
Pyridaben	4.9±0.35cd	3.8±0.39cd	8.5±0.5a	0.7±0.47cd	0.4±0.22f	1.4±0.3ef
Lambda Cyhalothrin	2.5±0.43e	4.0±0.61cd	5.1±0.53b	0.2±0.13d	0.9±0.46ef	0.2±0.13f
Imidacloprid	6.1±0.67bc	3.0±0.43d	4.33±0.59b	1.5±0.64cd	1.63±0.48def	2.5±0.43ef
Thiamethoxam	3.0±0.49de	2.3±0.40d	3.3±0.84b	4.2±0.47ab	5.3±0.56bc	5.2±0.81bcd

df	9, 99	9, 99	9, 99	9, 99	9, 99	9, 99
f	20.35	34.07	20.24	12.19	20.19	23.92
P value	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
24 h						
Control	8.4±0.48a	9.1±0.41a	8.9±0.43a	2.9±0.64bcd	8.2±0.51a	7.7±0.73a
Spirotetramat	6.3±0.75abc	6.9±0.6ab	9.2±0.33a	4.3±0.76ab	3.2±0.95b	5.2±0.69abc
Pyriproxyfen	7.5±0.56ab	6.9±0.55ab	8.1±0.41a	1.4±0.52cde	2.1±0.6bcd	1.8±0.33de
Flonicamid	5.6±0.4bcd	6.0±0.73b	7.6±0.7a	6.6±0.78a	7.3±0.96a	7.0±0.76ab
Kinoprene	5.8±0.66abc	5.7±0.45b	7.6±0.27a	0.7±0.3de	1.4±0.3bcd	2.3±0.45de
Chlorpyrifos	3.0±0.7de	4.7±0.37bc	8.1a	3.6±0.62bc	2.9±0.64bc	3.3±0.67cd
Pyridaben	4.4±0.37cde	3.0±0.36cd	7.1±0.84a	0.5±0.34de	0.0±0d	0.1±0.1e
Lambda Cyhalothrin	2.1±0.38e	3.0±0.54cd	4.2±0.59b	0.0±0e	0.3±0.21cd	0.0±0e
Imidacloprid	5.3±0.82bcd	2.63±0.42cd	3.1±0.58b	1.2±0.59cde	0.13±0.1cd	1.25±0.33de
Thiamethoxam	1.7±0.47e	1.6±0.43d	3.0±0.9b	2.9±0.59bcd	3.3±0.67b	4.9±0.95bc
df	9, 99	9, 99	9, 99	9, 99	9, 99	9, 99

f	14.13	21.45	15.23	14.11	22.19	24.55
P value	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
48 h						
Control	7.6±0.43a	9.0±0.47a	8.1±0.67a	2.3±0.42bcd	8.0±0.5a	7.4±0.85a
Spirotetramat	5.3±0.87ab	6.3±0.72b	9.1±0.35a	3.8±0.77b	2.9±1.01bc	5.0±0.75ab
Pyriproxyfen	7.1±0.5a	6.2±0.57b	7.6±0.45a	0.7±0.3de	0.1±0.1d	0.8±0.25d
Flonicamid	4.4±0.43b	5.5±0.73bc	6.6±0.9a	6.0±0.86a	6.8±1.06a	4.9±0.77ab
Kinoprene	1.5±0.37c	3.2±0.39cde	2.5±0.48b	0.0±0e	0.0±0d	0.0±0d
Chlorpyrifos	1.7±0.3c	3.8±0.36cd	7.7a	1.4±0.34cde	1.1±0.74bcd	2.2±0.42cd
Pyridaben	3.2±0.44bc	2.5±0.27de	6.6±0.9a	0.2±0.2e	0.0±0d	0.0±0d
Lambda Cyhalothrin	1.0±0.26c	2.1±0.62de	2.4±0.43b	0.0±0e	0.3±0.21cd	0.0±0d
Imidacloprid	4.4±0.95b	1.75±0.37de	1.63±0.43b	0.0±0e	0.0±0d	0.13±0.1d
Thiamethoxam	1.1±0.38c	1.0±0.37e	1.9±0.77b	2.7±0.5bc	3.2±0.68b	4.5±1.07bc
df	9, 99	9, 99	9, 99	9, 99	9, 99	9, 99
f	20.07	24.71	20.39	21.70	24.94	24.88

P value	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001

Table 2.5. Means (±SE) of number of live natural enemies (*D. isaea* and *N. californicus*) at each observation time of 12, 24, and 48 h in each of three trials (Tr 1, Tr 2, Tr 3) after exposure to thrips materials (thripicides) at median label rates (Table 2.1) out of a total of 10 natural enemies in each experimental unit.

12 h	*D. isaea*			*N. californicus*		
Treatment	Tr 1	Tr 2	Tr 3	Tr 1	Tr 2	Tr 3
Control	9.3±0.5a	8.9±0.28a	9.9±0.1a	7.6±0.67a	7.3±0.76a	8.9±0.5a
Acetamiprid	7.4±0.64ab	7.5±0.43ab	7.9±0.43b	1.6±0.65de	6.5±0.62ab	4.5±1.13c
Flonicamid	8.2±0.55a	9.0±0.39a	8.0±0.26b	5.9±0.8ab	7.6±0.7a	5.7±0.75bc
Insecticidal soap	9.8±0.2a	8.2±0.49ab	8.6±0.4ab	4.2±1.11bcd	5.7±0.52ab	7.6±0.6ab
B. bassiana	8.1±0.69a	8.2±0.13ab	7.7±0.3b	5.0±0.45abc	5.5±0.54ab	5.4±0.52bc
Cyfluthrin	4.6±0.76c	6.3±0.45bc	5.5±0.56c	0.4±0.4e	2.1±0.43cd	1.2±0.33d
Fluvalinate	5.4±0.75bc	4.5±0.62cd	4.7±0.52c	0.2±0.2e	0.3±0.15d	0.6±0.22d
Abamectin	4.6±0.56c	4.3±0.5cd	8.7±0.3ab	2.4±0.52cde	4.5±0.62bc	5.6±0.58bc
Carbonitrile	5.0±0.49bc	4.1±0.84d	7.7±0.54b	0.4±0.22e	1.9±0.35d	4.5±0.86c
Spinosad	1.7±0.37c	0.4±0.16e	1.3±0.37c	1.4±0.72de	6.9±0.55ab	6.9±0.57ab

df	9, 99	9, 99	9, 99	9, 99	9, 99	9, 99
f	19.38	34.95	40.68	17.21	20.64	15.05
P value	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
24 h						
Control	8.9±0.6a	8.1±0.31a	9.3±0.26a	7.4±0.72a	7.1±0.71a	8.7±0.52a
Acetamiprid	6.9±0.74ab	7.0±0.4a	7.1±0.53b	1.6±0.65cde	5.9±0.5ab	3.0±0.77cde
Flonicamid	7.2±0.59ab	8.2±0.49a	7.1±0.41b	5.8±0.84ab	5.0±0.76abc	5.2±0.8bc
Insecticidal soap	9.1±0.43a	7.4±0.4a	8.4±0.45ab	4.1±1.14bcd	3.3±0.45cd	7.5±0.58ab
B. bassiana	6.9±0.82ab	7.4±0.34a	7.6±0.3ab	4.4±0.58bc	4.7±0.52bc	3.6±0.27c
Cyfluthrin	3.3±0.73c	4.2±0.76b	4.6±0.56cd	0.4±0.4e	0.2±0.2e	0.8±0.33ef
Fluvalinate	4.7±0.63bc	3.0±0.67b	3.9±0.59d	0.1±0.1e	0.0±0e	0.2±0.2f
Abamectin	2.2±0.63cd	2.9±0.57b	6.7±0.62bc	2.4±0.52cde	2.3±0.52de	3.5±0.58cd
Carbonitrile	2.6±0.4cd	2.2±0.8bc	3.5±0.56d	0.4±0.22e	0.1±0.1e	1.1±0.31def
Spinosad	0.5±0.17d	0.3±0.15c	1.0±0.3e	1.4±0.72de	6.3±0.67ab	4.6±0.58c
df	9, 99	9, 99	9, 99	9, 99	9, 99	9, 99

f	24.89	29.06	29.03	15.12	27.40	28.48
P value	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
48 h						
Control	7.6±0.58ab	6.9±0.38a	7.2±0.25a	7.0±0.68a	6.0±0.68a	8.6±0.56a
Acetamiprid	4.5±0.58cd	6.9±0.41a	6.7±0.54a	0.9±0.38cd	2.4±0.62b	2.1±0.71bcd
Flonicamid	6.0±0.75bc	6.8±0.44a	6.5±0.40a	4.4±0.99ab	0.7±0.15c	4.6±0.82b
Insecticidal Soap	8.5±0.37a	6.7±0.3a	6.9±0.4a	3.1±1.31bc	1.2±0.25bc	2.8±1.18bc
B. bassiana	5.9±0.87bc	6.1±0.5a	6.7±0.56a	3.7±0.65bc	1.2±0.39bc	0.0±0d
Cyfluthrin	1.6±0.45e	2.5±0.86b	3.6±0.5bc	0.1±0.1d	0.0±0c	0.0±0d
Fluvalinate	2.7±0.63de	2.0±0.63bc	2.2±0.47cd	0.0±0d	0.0±0c	0.0±0d
Abamectin	1.0±0.33e	2.0±0.45bc	4.2±0.61b	0.0±0d	0.0±0c	0.0±0d
Chlorfenapyr	0.8±0.25e	1.0±0.47bc	0.5±0.17de	0.0±0d	0.0±0c	0.0±0d
Spinosad	0.3±0.15e	0.1±0.1c	0.2±0.13e	1.2±0.59cd	0.0±0c	0.7±0.33cd
df	9, 99	9, 99	9, 99	9, 99	9, 99	9, 99
f	32.47	31.20	40.96	15.04	32.61	27.01

P value	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001

Table 2.6. Means (±SE) of number of live natural enemies (*D. isaea* and *N. californicus*) at each observation time of 12, 24, and 48 h in each of three trials (Tr 1, Tr 2, Tr 3) after exposure to fungicides at median label rates (Table 2.1) out of a total of 10 natural enemies in each experimental unit.

12 h	*D.isaea*			*N. californicus*		
Treatment	Tr 1	Tr 2	Tr 3	Tr 1	Tr 2	Tr 3
Control	8.9±0.43a	6.9±0.43b	8.8±0.63a	8.4±0.45a	9.0±0.39a	9.3±0.39a
Sulfur	8.4±0.34a	8.9±0.28a	8.4±0.33a	5.7±0.94abc	7.5±0.34abc	5.1±1.12bc
Piperalin	9.0±0.3a	8.7±0.42ab	9.3±0.26a	0.0±0e	0.0±0e	1.5±0.5c
Pyraclostrobin	9.4±0.22a	9.3±0.26a	9.2±0.42a	3.1±0.59cd	5.5±0.78cd	7.8±1.05ab
Fosetyl-aluminum	9.5±0.22a	9.4±0.22a	8.9±0.41a	2.7±0.68de	7.4±0.48abcd	4.8±1.25bc
Copper sulfate	9.3±0.21a	8.1±0.43ab	8.3±0.65a	5.7±0.80abc	6.7±0.6bcd	4.2±0.93bc
Butanone	8.9±0.41a	7.8±0.47ab	8.7±0.56a	6.6±0.93ab	8.6±0.58ab	9.2±0.25a
Pot. bicarbonate	9.7±0.15a	8.8±0.39a	9.2±0.33a	4.4±0.64bcd	5.2±0.55d	7.5±0.91ab
Azoxystrobin	8.9±0.41a	8.4±0.3ab	9.4±0.5a	4.1±0.67bcd	8.0±0.47ab	8.8±0.49a
Rosemary Oil	9.0±0.3a	7.7±0.56ab	8.4±0.52a	0.0±0e	0.1±0.1e	3.4±0.69c

df	9, 99	9, 99	9, 99	9, 99	9, 99	9, 99
f	1.14	3.7	0.69	17.44	46.29	11.8
P value	0.3480	.00006	0.7172	< 0.0001	< 0.0001	< 0.0001
24 h						
Control	8.8±0.42ab	6.7±0.52b	8.1±0.66a	7.3±0.56a	9.0±0.39a	8.8±0.59a
Sulfur	7.8±0.53 ab	8.5±0.34ab	7.8±0.53a	3.9±1.24b	3.3±0.62c	3.4±0.96bcd
Piperalin	8.8±0.33ab	8.4±0.37ab	8.5±0.52a	0.0±0c	0.0±0d	0.4±0.22d
Pyraclostrobin	8.5±0.37ab	9.1±0.28a	8.8±0.44a	0.5±0.17c	1.3±0.5d	4.2±0.98bc
Fosetyl-aluminum	8.8±0.33ab	9.1±0.28a	8.6±0.45a	0.4±0.22c	0.3±0.16d	2.2±0.69cd
Copper sulfate	8.8±0.36ab	7.9±0.43ab	7.4±0.78a	4.1±1.14b	6.4±0.62b	1.8±0.51cd
Butanone	8.8±0.42ab	7.4±0.56ab	7.9±0.59a	6.5±0.99ab	8.4±0.54a	8.5±0.52a
Pot. bicarbonate	8.9±0.23a	8.5±0.4ab	9.0±0.33a	0.0±0c	0.5±0.22d	4.4±0.78bc
Azoxystrobin	8.3±0.33ab	7.5±0.45ab	8.8±0.49a	0.4±0.16c	0.9±0.23d	5.9±0.86ab
Rosemary Oil	7.3±0.21b	6.7±0.72b	7.2±0.65a	0.0±0c	0.0±0d	0.8±0.47d
df	9, 99	9, 99	9, 99	9, 99	9, 99	9, 99

f	2.27	3.74	1.29	18.55	79.09	18.19
P value	0.0255	0.0006	0.2557	< 0.0001	< 0.0001	< 0.0001
48 h						
Control	8.8±0.42a	5.9±0.38bc	7.0±0.68a	7.3±0.59a	8.2±0.33a	8.6±0.6a
Sulfur	6.9±0.59	6.9±0.5ab	6.9±0.59a	3.4±1.28b	1.5±0.65c	1.4±0.52b
Piperalin	8.0±0.33a	7.5±0.48ab	7.4±0.58a	0.0±0c	0.0±0c	0.0±0b
Pyraclostrobin	8.0±0.49a	8.7±0.3a	8.3±0.47a	0.0±0c	0.0±0c	1.2±0.77b
Fosetyl-aluminum	8.0±0.37a	7.8±0.55ab	7.1±0.82a	0.0±0c	0.1±0.1c	0.4±0.3b
Copper sulfate	7.8±0.55a	7.3±0.37ab	6.5±0.81a	3.5±1.27b	6.0±0.71b	0.7±0.26b
Butanone	7.8±0.57a	6.3±0.6abc	7.3±0.62a	6.1±1.1ab	7.6±0.80ab	7.0±0.92a
Pot. bicarbonate	7.5±0.5a	7.6±0.3ab	7.9±0.41a	0.0±0c	0.0±0c	0.0±0b
Azoxystrobin	7.1±0.55ab	7.2±0.53ab	8.0±0.65a	0.0±0c	0.1±0.1c	1.0±0.39b
Rosemary Oil	5.1±0.38b	4.1±0.99c	5.6±0.88a	0.0±0c	0.0±0c	0.0±0b
df	9, 99	9, 99	9, 99	9, 99	9, 99	9, 99
f	4.92	5.50	1.53	16.11	70.13	40.97

P value	< 0.0001	< 0.0001	0.1511	< 0.0001	< 0.0001	< 0.0001

Table 2.7. Summary of compatibility of pesticides with natural enemies following IOBC guidelines (Stark et al 2007)

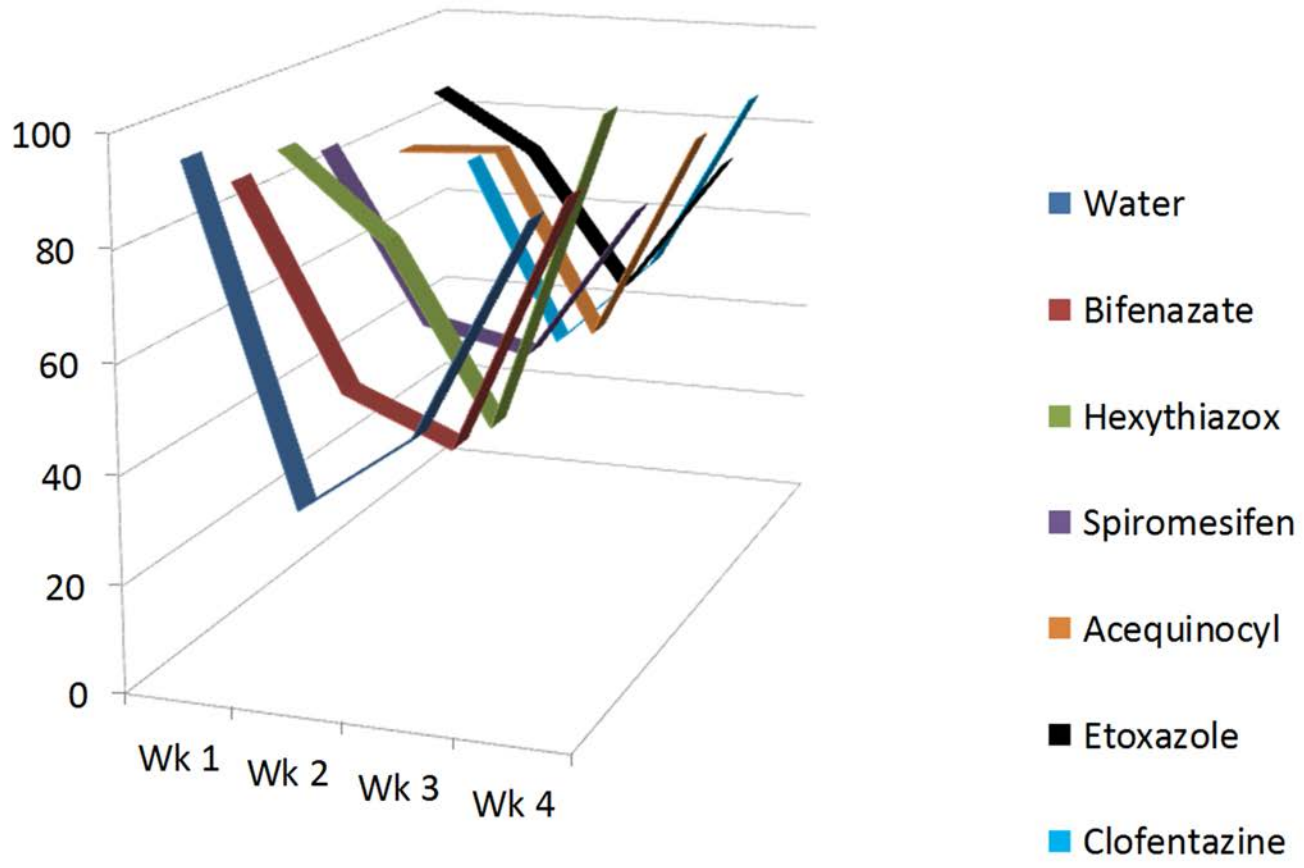
*Safety to natural enemies denoted by following legends: D. isaea- #, and N. californicus- **

Leafminer Materials	Miticides	Thripicides	Whitefly chemicals	Fungicides
Harmless (< 30% mortality within 48 h)				
Novaluron #	Clofentezine #		Pyriproxyfen #	Butanone #
Petroleum Oil #	Acequinocyl #		Spiromesifen #	Fosetyl-aluminum #
				Azoxystrobin #
				Potassium bicarbonate #
				Pyraclostrobin #
				Copper Sulfate #
				Piperalin #
Slightly Harmful (30-79% mortality within 48 h)				
Azadirachtin #	Bifenazate # *	Flonicamid # *	Flonicamid # *	Sulfur #
Cyromazine # *	Hexythiazox # *	Cyfluthrin #	Chlorpyrifos #	Rosemary Oil #
Petroleum Oil *	Spiromesifen # *	Insecticidal Soap # *	Spirotetramat # *	Butanone *
Acetamiprid #	Milbemectin #	*B. bassiana* #	Pyridaben #	Copper Sulfate *
Novaluron *	Etoxazole # *	Acetamiprid #	Thiamethoxam *	
	Clofentezine *		Spiromesifen *	
Moderately Harmful (80-98% mortality within 48 h)				

Lambda Cyhalothrin #	Abamectin #	Abamectin #	Kinoprene #	Sulfur *
Azadirachtin *	Acequinocyl *	Fluvalinate #	Thiamethoxam #	
		Chlorfenapyr #	Imidacloprid #	
		B. bassiana *	Lambda Cyhalothrin #	
		Acetamiprid *	Pyriproxyfen *	
			Chlorpyrifos *	
Harmful (>99% mortality within 48 h)				
Dinotefuran # *	Spinosad # *	Spinosad # *	Kinoprene *	Fosetyl-aluminum *
Bifenthrin # *	Milbemectin *	Abamectin *	Imidacloprid *	Rosemary Oil *
Lambda Cyhalothrin *	Abamectin *	Cyfluthrin *	Pyridaben *	Azoxystrobin *
Acetamiprid *		Fluvalinate *	Lambda Cyhalothrin *	Potassium bicarbonate *
		Chlorfenapyr *		Pyraclostrobin *
				Piperalin *

Figure Captions

Fig.2.1. Average parasitism in 6 miticide treatments and a water control ove a four week period


100
80
60
40
20
0
Wk 1
Wk 2
Wk 3
Wk 4
Water
Bifenazate
Hexythiazox
Spiromesifen
Acequinocyl
Etoxazole
Clofentazine

CHAPTER 3

NONPREFERENCE AMONG GERBERA CULTIVARS BY THE LEAFMINER *LIRIOMYZA TRIFOLII* (AGROMYZIDAE: DIPTERA)

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Abstract

The leafminer, *Liriomyza trifolii* (Burgess) is a key pest of gerbera daisies (*Gerbera jamesonii* Bolus), which are among the most preferred cut flowers in the world. While insecticides often fail to control this pest, parasitoids have proven to be effective. To maintain the parasitoids in the system, pesticide applications should be avoided. However, the influx of secondary pests like mites, thrips, whiteflies, and aphids during the growing season necessitates chemical sprays, which are effective in controlling the secondary pests, but are often toxic to the natural enemy and hence disrupt biological control. Since chemicals are not easily avoided in this system, an alternative method to avoid leafminers was sought, using host plant resistance, which can be an important component of integrated pest management (IPM) programs. Sixty gerbera cultivars were evaluated for potential resistance to *L. trifolii*. A range in susceptibility measured as leaf punctures and developing mines was evident for the first five weeks of a six-week exposure period. However, consistent exposure to high numbers of leafminers resulted in similar expression of damage among all cultivars after five weeks. Differences among cultivars in force required to puncture leaves could not be consistently associated with damage due to leafminers.

Index words: host plant resistance; Gerbera, leafminers,

Species used in this study: 60 cultivars of *Gerbera jamesonii*,

Significance to the cut flower industry

Gerbera daisies are the third most preferred cut flowers in the world, and increasing in demand in the United States. The lack of cost-effective options to control the complex of primary and secondary pests however impedes development of a sustainable production system. Anecdotal evidence indicated variable infestation among gerbera cultivars by the primary pest leafminer, *Liriomyza trifolii*. A range in susceptibility among 60 cultivars was observed, suggesting that early and heavily infested plants could serve as early indicator plants, while those that were initially less preferred may provide some benefit in an IPM program. All cultivars evaluated, however, eventually became equally damaged when in the presence of high populations of leafminers for five weeks.

Introduction

The primary pests affecting greenhouse gerberas are serpentine leafminers, *Liriomyza trifolii* (Burgess) (Diptera: Agromyzidae), which have a wide distribution and attack more than 400 species (Reitz and Trumble 2002) of plants including vegetables and ornamentals. The larvae feed on the palisade mesophyll (Parrella et al. 1985) and decrease photosynthesis and yield, directly affecting the marketable produce. Rigorous and extended use of pesticides has rendered leafminers resistant to almost all chemistries (Keil and Parrella 1982). Leafminers are also protected from chemicals by being concealed within the leaves in their larval stages. Successful biocontrol has been achieved using augmentative releases of parasitoids. This has however been effective in areas only where disruptive use of chemicals have been avoided (Liu et al. 2009).

The influx of secondary pests like mites, thrips, whiteflies, aphids, and pathogens causing powdery mildew through the season necessitate pesticide sprays, which in turn kill the leafminer parasitoids. Insecticide toxicology assays demonstrated that many of the commonly used pesticides (against secondary pests) cause high mortality in beneficial arthropods (leafminer parasitoid *Diglyphus isaea* (Walker) and the predatory mite *Neoseiulus californicus* (McGregor)) and hence disrupt effective pest management (Chapter 2). While pesticides are not effective against leafminers, they certainly are detrimental to the effective buildup of natural enemy populations. Host plant resistance could avoid the pest, reducing the need for chemical intervention.

Host plant resistance studies in vegetables have identified effective mechanisms against leafminers. The narrow leaf architecture in celery (Trumble and Quiros 1988), trichomes and acyl sugars (within the trichomes) in wild tomatoes (Hawthorne et al. 1992), and jasmonic acid sprays in celery (Black et al. 2003) have all successfully reduced leafminer damage/ host feeding. However, similar studies in ornamentals or cut flowers are lacking. Resistance could be an innate function of the plant through the chemical contents within the leaf (Black et al. 2003), or a function of the toughness of leaf as found to deter lace bugs (Nair 2011). Even partial resistance could supplement biological control and work synergistically to control leafminer pests (van Lenteren 2007).

In this unique system, while pesticides work against secondary pests, they disrupt biological control of the primary pest. IPM can provide an effective solution for gerberas. Finding a successful host plant resistance mechanism would assist in designing an IPM protocol. A successful IPM program would control leafminers, the primary pest,

through host plant resistance, natural enemies, or a combination of both, and utilize pesticides compatible with biological control to manage the secondary pests and pathogens.

Materials & Methods

Plant Material: Seeds of 60 gerbera cultivars (*Gerbera jamesonii*, Ball® horticultural company, West Chicago, IL, Table 3.1) were germinated in a commercial facility (Speedling® Inc., Blairsville, GA). Seeds were planted in cell packs (128 cells/ tray) filled with Fafard super fine germinating mix (Agawam, MA) and, after being watered lightly, kept in the germination chamber at 75° F and 80-100% relative humidity until complete germination was achieved a week later. When plants were well rooted after 7 wk, they were transplanted into larger cell packs (36 cells/ tray) and housed in a greenhouse on the UGA-Griffin campus.

Greenhouse Choice Study: Sixty gerbera cultivars were evaluated for leafminer feeding or oviposition, and subsequent development in a greenhouse choice study. A randomized complete block design with 10 replications for each of the 60 cultivars was employed in the experiment. Each plant was an experimental unit. All the plants were exposed to high leafminer pressure for 72 h at a commercial greenhouse and then returned to the UGA-Griffin campus facility. High leafminer pressure was maintained by 2 biweekly introductions, each an excess of 500 *L. trifolii* captured from other greenhouses on the UGA-Griffin Campus and grower greenhouses in Thomaston, GA. Data collection began 48 h after relocation to UGA-Griffin campus. Data included the numbers of stings— (puncture marks caused by egg laying or feeding by the leafminer) and the number of mines— (silver patterns characterized by the lack of chlorophyll due to the feeding and

development of the leafminer larva within the leaf) found in a 15 cm^2 area of each of 3 upper leaves on the plant (non-destructive sampling). Data were collected weekly on each of the 600 experimental units from 11 May to 15 June 2011. Age of larvae was not assessed during the study.

Penetrometer Study: From the results of the greenhouse experiments, 15 cultivars were selected from among 4 categories: High number of stings and mines (cultivar # 2, 53, 28, 39), medium number of stings and high number of mines (cultivar # 40, 49, 35), high number of stings and low number of mines (cultivar # 16, 56), low number of stings and mines (cultivar # 5, 7, 30, 50, 55, 57). *L. trifolii* oviposition is exclusively through the dorsal side of the leaf while other leafminer species use a combination of dorsal and ventral side or exclusively one side also (Parrella and Bethke 1984, Bethke and Parrella 1985, Parrella 1987). Using a penetrometer force gauge (Chatillon DFX-010-NIST Digital Force Gauge), the force required (in newtons) to penetrate the dorsal side of the leaf was assessed. Ten observations each from 3 similarly aged leaves for each cultivar were taken, equaling 30 observations for each cultivar. Each leaf was placed on a stage attached to the force gauge and the pointed portion of the instrument was lowered according to prescribed operating procedures between leaf veins and observations recorded.

Statistical Analyses: Data were subjected to analysis of variance (ANOVA) using the general linear model procedure (SAS Institute 2003, Cary, NC). Means in both studies were separated using Tukey's HSD test at $\alpha = 0.05$. Data from the penetrometer study were further subjected to a correlation analysis using PROC CORR (SAS Institute 2003,

Cary, NC) to determine if leaf damage had a correlation with leaf toughness or lack thereof.

Results and Discussion

Greenhouse Choice Study: Even though mines in several plants were absent during the first week, all plants in the study sustained oviposition or feeding punctures at that point (Tables 3.2, 3.3). Numbers of punctures and mines varied by cultivar, but were not always consistent from week to week. Trends were identified indicating differential susceptibility among cultivars. While there were no cultivars that were immune to *L. trifolii,* 'Gerbera Jaguar Pink'(Cultivar # 5), 'Gerbera Jaguar Rose Deep'(Cultivar # 7), 'Gerbera Jaguar Salmon Pastel'(Cultivar # 9) and 'Gerbera Revolution Spring Pastels'(Cultivar # 57), consistently showed less damage (Table 3.5). Sustained exposure to high populations of leafminers rendered plants equally damaged by the sixth week when there were no more significant differences in cultivar damage (F values range= 1.33-3.75, df= 59, 599, p values range= < 0.0001 – 0.059, Tables 3.2, 3.3).

While cultivar groups of 'Gerbera Jaguar' and 'Gerbera Revolution' showed potential for lower leafminer preferences, the non-preference did not extend to all color variants in the group. 'Gerbera Jaguar Rose Deep' (Cultivar # 7) was among cultivars that had least damage while 'Gerbera Jaguar Fire Dark Eye' (Cultivar # 2) sustained consistently high damage. While 'Gerbera Revolution Spring Pastel' (Cultivar # 57) showed lower damage, 'Gerbera Revolution Yellow' (Cultivar # 59) and 'Gerbera Mega Revolution Yellow' (Cultivar # 49) sustained heavier leafminer damage.

The mine damage values for the cultivar lines averaged across the six observation dates (Table 3.6) (f= 4.21; df= 5, 169; p value = 0.0010) identified the cultivar lines 'Gerbera Revolution' and 'Gerbera Festival Mini' as having significantly less mine damage overall. Cultivar lines 'Gerbera Jaguar', 'Gerbera Royal', 'Gerbera Festival', and 'Gerbera Mega Revolution' had significantly higher damages and were not significantly different amongst them.

Penetrometer Study: Cultivars showed significant differences in the force required to penetrate the dorsal surface of the leaves ((*f*= 13.68*; df*= 14, 449*; p* value < 0.0001), Fig 3.1). However, the force required to penetrate the surface was not consistent with the preference or non-preference of leafminer damage from the correlation analysis (R=0.0032; P=0.4948). Data from leafminer non-preferred cultivars like 'Gerbera Revolution Scarlet Dark Eye' (cultivar # 56), 'Gerbera Festival White Shade' (cultivar # 30), and leafminer preferred 'Gerbera Festival Spider Salmon Eye' (cultivar # 40) corresponded with the force required to penetrate the surface. A higher force to penetrate the surface in non-preferred cultivars and, less force required to penetrate the surface in highly preferred ones.

However, leafminer preferred cultivars like 'Gerbera Festival Semi DB Yellow' (cultivar # 39), 'Gerbera Festival Mini Yellow' (cultivar # 35), and non-preferred cultivars like 'Gerbera Royal Semi DB Pink Dark Eye' (cultivar # 16) and 'Gerbera Jaguar Rose Deep' (cultivar # 7) inversely corresponded with the force required to penetrate the leaf surface. Preferred cultivars in this situation required higher force to penetrate, while non-preferred cultivars required less force to penetrate. Hence in

general, the preference or non-preference of leafminer attack did not align with the force required to penetrate the dorsal surface of leaf.

Anecdotal evidence that yellow cultivars attract more leafminers than other colors is consistent with the fact that yellow sticky cards are best in attracting leafminers (Tryon Jr. et al. 1980) and an effective tool in sampling (Musgrave et al. 1975, Jones and Parrella 1986). Our experiment was conducted on plants without flowers to assess foliar-based potential for avoidance or antibiosis. Observations of the different cultivars in our study showed very little variation within the spectrum of being pubescent or glabrous. The texture though seemed to have some difference, and hence the investigation into leaf toughness as a factor to deter leafminer oviposition and resultant damage.

Punctures and mines did not correspond in this study. Punctures are a function of either feeding behavior or oviposition, and feeding frequency has been shown to 'not predict' leafminer preference or damage (Fagoonee and Toory 1983). Punctures can hence only be an indicator of leafminer preference while the best measure of resistance is a lower number of mines. There was no consistent preference by leafminers for yellow cultivars. While there were some yellow cultivars that were among the most damaged, all yellow cultivars were not heavily damaged. There were pink and orange cultivars that sustained heavier damage than certain yellow cultivars, but no yellow cultivars ranked very low in number of punctures and mines (Tables 3.4, 3.5). Also, innate mechanisms might be expected to be a trait of a certain cultivar group. With 10-12 cultivars coming under the same general cultivar group, we expected that they would be armed with the same defense mechanism and hence remain together in being preferred by leafminers or sustaining damage. The results however didn't agree with that. For example, while

‘Gerbera Jaguar Rose Deep’ (Cultivar # 7) and ‘Gerbera Jaguar Pink’ (Cultivar # 5) showed low levels of damage, ‘Gerbera Jaguar Fire Dark Eye’ (Cultivar # 2) sustained heavy leafminer damage. ‘Gerbera Jaguar’ (Cultivar # 5, 7, and 9) and ‘Gerbera Revolution’ (Cultivar # 57) were two cultivar groups where at least a few of them showed reduced leafminer damage (Table 3.4).

While more cultivars in the Gerbera Jaguar line consistently showed low mine damage on individual observation dates (Table 3.4), cultivar lines (including all cultivars in the cultivar group) ‘Gerbera Revolution’ and ‘Gerbera Festival Mini’ showed less mine damage across the duration of the experiment (Table 3.6). There might be some innate quality in these lines that could help in resistance breeding in gerberas.

Leaf toughness was not a predictor of leafminer preference. Color variants of the cultivar group ‘Gerbera Revolution’ varied in the force required to penetrate the dorsal leaf surface and did not correspond with leafminer preferences from the greenhouse choice study. ‘Gerbera Revolution Scarlet Dark Eye’ (Cultivar # 56) required high force to penetrate the surface, and was one of the cultivars with lower number of mines developing in spite of high number of stings. ‘Gerbera Revolution Red Shade Dark Eye’ (Cultivar # 53) was among the cultivars that sustained high leafminer damage, but the force required to penetrate the surface was just intermediate but higher than that required to penetrate ‘Gerbera Revolution Pastel range Dark Eye’ (Cultivar # 55), which sustained low leafminer damage.

Related species like *G. ambigua* (Cass.) Schultz. Bip., *G. crocea* (L.) Kuntze, *G. linnaei* Cass., *G. serrata* (Thunb.) Druce, *G. tomentosa* DC, *G. viridifolia* (DC.) Sch.

Bip., and *G. wrightii* Harv. might provide sources of resistant germplasm. Alternatively jasmonic acid sprays that were successful in celery (Black et al. 2003) could be explored. The search for an effective host plant resistance mechanism will have to continue for the reason that it can tremendously help the IPM program that will then result. The leafminer *L. trifolii* has been a successful cosmopolitan pest and will continue to drive pest management in gerbera production for years to come. Our answer to that would depend on finding successful components that could be weaved into an integrated program to control the suite of pests in this system.

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Table 3.1. Gerbera cultivars evaluated for leafminer *L. trifolii* preference

#	Cultivar name
1	Jaguar Fire
2	Jaguar Fire Dark Eye
3	Jaguar Orange Deep
4	Jaguar Orange Picotee
5	Jaguar Pink
6	Jaguar Red
7	Jaguar Rose Deep
8	Jaguar Rose Picotee
9	Jaguar Salmon Pastel
10	Jaguar Scar Shade Dark Eye
11	Jaguar tangerine
12	Jaguar White
13	Jaguar Yellow
14	Jaguar Yellow Dark Eye
15	Royal Mix
16	Royal Semi-Double Pink Dark Eye
17	Royal Semi-Double Vanilla Dark Eye
18	Royal Semi-Double Watermelon Dark Eye
19	Durora Mini-Double Mix
20	Festival Apricot
21	Festival Apricot Dark Eye
22	Festival Cream
23	Festival Mix Dark Eye
24	Festival Peach Dark Eye
25	Festival Pink Shade Dark Eye
26	Festival Red Dark Eye
27	Festival Salmon
28	Festival Salmon Orange Shade
29	Festival Scarlet Dark Eye
30	Festival White Shade
31	Festival Yellow Lemon
32	Festival Mini Orange Shade
33	Festival Mini Pink Soft
34	Festival Mini Pastel Deep Shade
35	Festival Mini Yellow Shade
36	Mini Revolution Mix
37	Festival Semi-Double Orange Shade
38	Festival Semi-Double Rose Shade
39	Festival Semi-Double Yellow
40	Festival Spider Salmon Eye
41	Festival Spider Yellow
42	Kameleo Micro Mix

43	Mega Revolution Champagne
44	Mega Revolution Golden Yellow Dark Eye
45	Mega Revolution Orange Dark Eye
46	Mega Revolution Purple Shade
47	Mega Revolution Scarlet Dark Eye
48	Mega Revolution White
49	Mega Revolution Yellow Shade
50	Revolution Pink
51	Revolution Pink Baby
52	Revolution Pink Pastel Dark Eye
53	Revolution Red Shade Dark Eye
54	Revolution Rose Shade
55	Revolution Pastel Orange Dark Eye
56	Revolution Scarlet Dark Eye
57	Revolution Spring Pastels
58	Revolution White
59	Revolution Yellow
60	Yellow Dark Eye

Table 3.2. Mean ±SE number of *L. trifolii* oviposition and feeding leaf punctures per gerbera plant by cultivar

Cultivar	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Jaguar Fire	28.5±5.41af	14.8±4.07a	25.3±6.31ac	19.6±4.24ab	20.7±7.23ad	19.4±4.07ab
Jaguar Fire Dark Eye	47.8±8.32a	32.0±3.42a	36.7±9.66ac	46.0±8.29ab	38.5±13.17ad	26.5±3.43ab
Jaguar Orange Deep	13.6±4.08cf	12.5±3.62a	22.2±4.10ac	27.4±5.83ab	29.7±7.61ad	18.9±5.35ab
Jaguar Orange Picotee	24.4±5.46af	24.6±4.22a	15.2±4.53ac	16.7±5.88b	17.5±4.89ad	16.6±6.29ab
Jaguar Pink	13.7±3.58cf	10.4±3.62a	12.5±1.15ac	20.2±4.78ab	22.8±3.46ad	2.9±1.16b
Jaguar Red	38.6±6.64ae	18.0±4.42a	33.7±6.04ac	28.2±6.06ab	37.9±4.60ad	29.1±4.53ab
Jaguar Rose Deep	9.4±2.38ef	7.0±2.96a	9.6±4.37bc	15.8±7.31b	4.852±1.60d	16.8±8.02ab
Jaguar Rose Picotee	6.6±2.34f	9.3±2.69a	13.4±3.19ac	15.8±3.53b	9.0±3.21cd	7.6±3.18ab
Jaguar Salmon Pastel	16.1±3.07bf	12.9±3.76a	13.5±3.61ac	12.3±2.91b	15.8±5.00bd	15.8±3.37ab
Jaguar Scar Shade Dark Eye	15.1±3.70cf	19.0±3.79a	31.3±8.14ac	30.6±8.06ab	33.9±10.51ad	22.1±4.83ab
Jaguar tangerine	29.0±6.15af	16.1±2.76a	31.5±6.53ac	32.6±6.06ab	23.1±4.36ad	15.9±3.54ab
Jaguar White	19.7±7.19af	18.4±6.05a	18.0±5.43ac	28.5±7.73ab	21.6±4.85ad	13.4±3.99ab
Jaguar Yellow	37.7±8.60ae	24.7±4.21a	29.2±3.98ac	26.3±4.77ab	16.5±2.83bd	14.8±2.98ab
Jaguar Yellow Dark Eye	15.5±3.88cf	12.1±3.84a	23.8±5.59ac	14.7±3.96b	17.8±5.34ad	11.8±2.59ab
Royal Mix	18.4±2.13af	18.4±3.59a	21.3±6.27ac	14.5±4.44b	20.6±4.48ad	16.1±3.56ab
Royal Semi-Double Pink Dark	13.0±2.63cf	22.7±3.54a	24.6±3.22ac	41.7±10.03ab	51.1±10.14a	27.3±11.04ab

Eye						
Royal Semi-Double Vanilla Dark Eye	25.7±5.74af	20.7±3.34a	31.6±6.92ac	29.9±5.63ab	21.4±4.32ad	22.9±7.32ab
Royal Semi-Double Watermelon Dark Eye	27.1±3.41af	29.0±7.39a	19.3±4.20ac	33.8±7.80ab	20.9±3.46ad	31.7±6.88ab
Durora Mini-Double Mix	32.6±10.02af	19.0±4.31a	13.0±2.85ac	22.4±4.56ab	16.4±3.41bd	15.1±4.16ab
Festival Apricot	22.4±5.60af	33.2±10.61a	31.6±4.38ac	27.7±5.75ab	25.6±9.11ad	36.3±11.60a
Festival Apricot Dark Eye	10.7±2.54df	12.2±3.75a	7.8±1.73c	15.4±2.08b	19.9±3.92ad	13.1±3.83ab
Festival Cream	40.3±8.20ad	30.9±5.55a	26.7±8.81ac	42.2±8.59ab	25.2±7.53ad	16.6±3.83ab
Festival Mix Dark Eye	23.5±5.95af	17.2±5.44a	23.2±4.54ac	28.6±6.42ab	26.7±4.23ad	28.8±6.45ab
Festival Peach Dark Eye	12.4±2.81cf	12.7±3.47a	15.8±3.95ac	17.0±3.68b	29.2±5.07ad	14.3±3.44ab
Festival Pink Shade Dark Eye	26.0±6.76af	21.5±4.64a	31.5±8.22ac	49.3±12.01ab	29.1±8.12ad	25.0±5.21ab
Festival Red Dark Eye	14.4±1.75cf	10.2±2.49a	24.3±7.63ac	35.1±6.88ab	26.6±4.54ad	15.0±3.63ab
Festival Salmon	22.0±3.63af	22.4±5.66a	16.9±5.71ac	29.4±6.56ab	26.3±6.46ad	12.5±2.42ab
Festival Salmon Orange Shade	23.5±4.51af	8.8±2.61a	19.7±5.66ac	57.5±16.57a	44.3±9.40ab	16.8±3.21ab
Festival Scarlet Dark Eye	23.7±5.06af	22.5±6.23a	17.3±3.74ac	28.8±7.89ab	17.6±3.83ad	19.2±4.15ab
Festival White Shade	46.5±7.23ab	33.1±2.55a	23.8±2.12ac	24.4±5.00ab	21.2±7.07ad	17.7±4.58ab
Festival Yellow Lemon	31.5±4.81af	18.1±3.77a	27.7±4.64ac	30.5±6.62ab	26.4±6.65ad	30.3±5.40ab
Festival Mini Orange Shade	28.0±4.50af	30.2±6.25a	31.3±10.09ac	44.5±7.45ab	33.4±5.26ad	15.5±4.09ab
Festival Mini Pink Soft	22.8±3.70af	20.1±3.70a	24.0±5.80ac	22.6±4.97ab	31.0±6.20ad	20.1±2.70ab

Festival Mini Pastel Deep Shade	35.2±6.48af	23.6±5.41a	24.2±5.31ac	29.7±6.05ab	16.6±3.90bd	11.7±2.61ab
Festival Mini Yellow Shade	42.7±8.57ac	19.2±4.79a	16.7±2.66ac	19.0±3.07ab	23.6±7.37ad	10.6±2.13ab
Mini Revolution Mix	23.6±5.41af	23.3±5.11a	30.5±6.16ac	26.4±5.32ab	28.8±7.99ad	23.0±5.21ab
Festival Semi-Double Orange Shade	38.5±6.68ae	28.5±4.17a	43.2±4.77a	37.1±8.16ab	27.0±4.67ad	22.1±2.57ab
Festival Semi-Double Rose Shade	26.9±5.60af	21.4±4.19a	41.0±9.56ab	35.2±6.70ab	33.4±3.97ad	30.3±5.31ab
Festival Semi-Double Yellow	35.1±5.83af	26.0±5.10a	40.8±8.79ab	50.4±8.74ab	41.8±7.29ac	30.9±6.05ab
Festival Spider Salmon Eye	29.8±5.01af	24.0±4.63a	25.0±5.63ac	25.9±6.61ab	26.2±5.26ad	7.1±2.03ab
Festival Spider Yellow	38.8±5.34ae	20.1±6.56a	31.3±5.93ac	45.6±11.30ab	31.5±5.67ad	26.2±5.31ab
Kameleo Micro Mix	21.2±4.08af	15.6±5.22a	16.1±5.14ac	19.4±4.06ab	9.9±1.29cd	19.0±4.50ab
Mega Revolution Champagne	30.0±6.28af	31.3±6.51a	27.7±5.75ac	26.2±3.89ab	29.0±5.69ad	30.3±8.41ab
Mega Revolution Golden Yellow Dark Eye	12.4±3.66cf	13.3±3.66a	19.5±5.48ac	25.1±5.35ab	17.5±2.42ad	14.6±5.14ab
Mega Revolution Orange Dark Eye	22.5±6.65af	14.2±2.68a	15.8±3.58ac	27.4±4.37ab	28.5±4.97ad	18.1±4.66ab
Mega Revolution Purple Shade	26.9±8.42af	16.6±4.44a	20.4±4.78ac	19.2±5.74ab	17.9±6.62ad	22.7±7.90ab
Mega Revolution Scarlet Dark Eye	35.2±10.86af	27.2±9.27a	17.5±4.87ac	25.4±10.00ab	25.0±5.41ad	13.7±4.18ab
Mega Revolution White	11.0±3.77df	10.0±1.42a	13.1±4.52ac	14.8±4.42b	17.8±5.06ad	16.8±5.20ab
Mega Revolution Yellow Shade	27.0±4.57af	23.5±4.39a	22.1±7.26ac	26.2±5.56ab	25.0±7.20ad	25.9±5.35ab

Revolution Pink	15.6±4.19cf	13.8±2.45a	21.3±3.83ac	26.0±4.83ab	13.7±4.66bd	14.1±5.41ab
Revolution Pink Baby	23.1±5.81af	26.3±4.75a	22.9±6.10ac	41.8±12.37ab	26.0±7.99ad	24.4±5.98ab
Revolution Pink Pastel Dark Eye	38.2±5.77ae	31.2±4.69a	32.8±5.87ac	37.4±10.05ab	25.1±5.53ad	19.1±4.27ab
Revolution Red Shade Dark Eye	14.2±1.67cf	21.7±5.34a	16.7±3.53ac	40.7±10.70ab	37.8±7.93ad	14.7±4.05ab
Revolution Rose Shade	42.7±6.46ac	25.7±3.02a	25.1±4.26ac	29.6±6.22ab	33.0±7.36ad	24.6±8.54ab
Revolution Pastel Orange Dark Eye	8.4±3.41ef	10.0±2.10a	10.2±3.05bc	16.6±3.32b	11.9±2.64bd	18.2±5.07ab
Revolution Scarlet Dark Eye	29.7±6.16af	28.1±6.44a	34.5±6.34abc	47.2±6.09ab	38.1±7.38ad	17.9±6.13ab
Revolution Spring Pastels	13.5±3.13cf	13.2±3.10a	12.6±1.79ac	17.1±3.69b	27.3±4.95ad	13.4±8.62ab
Revolution White	23.8±6.83af	32.3±4.90a	27.8±6.16ac	27.4±6.38ab	23.6±6.30ad	23.3±7.42ab
Revolution Yellow	27.5±3.55af	26.2±4.94a	28.0±4.56ac	29.6±6.08ab	20.5±4.32ad	25.6±5.84ab
Yellow Dark Eye	16.3±3.76bf	14.1±4.09a	12.6±3.34ac	33.3±9.28ab	23.5±7.04ad	23.1±5.79ab
F	3.75	2.35	2.32	2.35	2.18	1.52
df	59, 599	59, 599	59, 599	59, 599	59, 599	59, 599
P	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0102

Table 3.3. Mean ±SE number of *L. trifolii* mines per gerbera plant by cultivar (n= 10)

CV	Week 1	Week 2	Week 3	Week 4	Week 5	Week 6
Jaguar Fire	2.1±0.58ad	4.3±2.30ab	3.8±0.79bc	8.6±0.54ab	7.3±0.80ab	11.4±1.53a
Jaguar Fire Dark Eye	3.3±1.06ad	4.8±1.30ab	6.6±1.14ac	17.3±1.46a	16.2±1.51ab	19.8±1.92a
Jaguar Orange Deep	2.1±0.42ad	2.5±0.72ab	4.3±0.68bc	6.3±0.97ab	8.9±1.48ab	9.0±2.45a
Jaguar Orange Picotee	1.7±0.55ad	3.3±0.53ab	4.7±0.96bc	7.3±0.97ab	9.6±1.10ab	12.4±1.64a
Jaguar Pink	1.7±0.40ad	2.2±0.62ab	1.9±0.50c	3.2±1.07b	6.2±1.23ab	8.7±2.15a
Jaguar Red	3.4±0.74ad	4.4±1.27ab	3.3±0.41bc	8.3±1.48ab	13.9±0.97ab	15.9±1.58a
Jaguar Rose Deep	0.6±0.21d	1.4±0.30ab	2.1±0.65c	3.3±0.94b	3.7±0.49b	10.4±1.02a
Jaguar Rose Picotee	2.9±0.80ad	3.5±1.96ab	4.3±0.73bc	5.8±0.80b	8.0±1.03ab	8.3±2.14a
Jaguar Salmon Pastel	1.3±0.36bd	2.2±0.43ab	2.0±0.37c	3.3±0.64b	6.2±1.14ab	7.2±1.33a
Jaguar Scar Shade Dark Eye	2.6±0.94ad	2.3±0.53ab	3.5±1.07bc	11.4±0.83ab	8.3±1.49ab	7.3±1.28a
Jaguar tangerine	3.5±0.84ad	5.6±1.13ab	3.0±0.58c	4.5±0.80b	6.6±0.84ab	9.2±1.64a
Jaguar White	2.1±0.60ad	3.2±0.91ab	5.0±1.57bc	7.7±0.76ab	8.4±1.26ab	8.1±1.47a
Jaguar Yellow	4.6±0.91a	6.1±1.07a	6.0±0.64ac	7.8±1.02ab	10.3±1.55ab	16.8±3.36a
Jaguar Yellow Dark Eye	1.2±0.20bd	2.8±0.73ab	3.5±0.59bc	6.6±1.32ab	8.2±1.29ab	11.4±1.57a

Royal Mix	1.8±0.48ad	2.8±0.76ab	4.1±0.84bc	6.7±1.25ab	10.2±2.20ab	11.1±1.22a
Royal Semi-Double Pink Dark Eye	1.7±0.42ad	1.6±0.32ab	4.9±0.84bc	6.4±0.89ab	5.0±0.55b	7.6±1.15a
Royal Semi-Double Vanilla Dark Eye	2.1±0.54ad	2.9±0.66ab	3.7±0.41bc	6.0±0.75b	5.7±0.95ab	7.3±0.60a
Royal Semi-Double Watermelon Dark Eye	3.3±0.76ad	5.5±0.97ab	9.3±1.11ab	7.9±1.30ab	5.3±0.50b	9.8±2.16a
Durora Mini-Double Mix	1.7±0.29ad	2.5±1.02ab	3.5±1.01bc	3.7±1.02b	5.8±0.94ab	5.7±0.96a
Festival Apricot	1.2±0.31bd	2.4±0.44ab	3.2±0.64bc	5.1±0.53b	8.2±0.98ab	7.9±1.59a
Festival Apricot Dark Eye	2.1±0.50ad	3.1±0.81ab	5.3±0.95ac	6.5±1.31ab	8.3±2.47ab	9.6±3.48a
Festival Cream	4.4±0.77ab	4.8±1.34ab	6.7±0.91ac	7.0±1.13ab	7.1±1.33ab	7.1±1.48a
Festival Mix Dark Eye	2.9±0.86ad	2.6±0.59ab	4.6±0.56bc	6.6±1.27ab	7.5±1.37ab	9.3±2.18a
Festival Peach Dark Eye	4.2±0.69ac	6.0±1.24ab	3.3±0.68bc	8.9±0.74ab	9.1±0.86ab	9.0±1.57a
Festival Pink Shade Dark Eye	2.5±0.93ad	2.5±0.72ab	4.5±1.04bc	6.9±0.57ab	7.0±0.80ab	9.4±1.31a
Festival Red Dark Eye	3.3±0.81ad	4.4±1.21ab	5.0±1.37bc	6.1±0.56b	6.5±0.82ab	12.3±2.02a
Festival Salmon	2.1±0.39ad	2.3±0.46ab	4.0±0.52bc	5.4±0.91b	9.7±0.75ab	14.1±2.21a
Festival Salmon Orange Shade	2.8±0.63ad	5.8±0.90ab	4.8±0.80bc	5.6±0.92b	9.0±0.64ab	9.2±1.93a
Festival Scarlet Dark Eye	1.6±0.33ad	1.6±0.42ab	4.0±1.05bc	9.2±0.82ab	6.9±1.07ab	6.1±1.22a
Festival White Shade	2.5±0.48ad	1.8±0.55ab	2.5±0.71c	4.5±0.81b	4.9±1.21b	14.9±2.30a

Festival Yellow Lemon	2.7±0.35ad	2.4±0.39ab	5.2±1.00ac	12.6±0.93ab	14.4±1.62ab	14.0±1.13a
Festival Mini Orange Shade	1.7±0.67ad	1.5±0.46ab	4.2±0.90bc	5.6±0.75b	5.5±0.82ab	7.0±1.29a
Festival Mini Pink Soft	0.7±0.28d	1.7±0.56ab	2.5±0.61c	5.1±0.70b	18.6±0.43a	18.2±0.79a
Festival Mini Pastel Deep Shade	1.8±0.47ad	1.3±0.70ab	2.4±0.54c	4.4±1.02b	6.3±0.67ab	12.9±1.42a
Festival Mini Yellow Shade	3.7±1.05ad	5.9±1.04ab	7.0±1.41ac	8.5±1.53ab	7.0±1.15ab	11.6±2.53a
Mini Revolution Mix	1.0±0.35d	1.4±0.70ab	2.2±0.39c	5.5±0.82b	6.0±1.15ab	7.1±1.52a
Festival Semi-Double Orange Shade	2.8±0.81ad	5.5±1.83ab	4.0±0.52bc	9.1±1.18ab	7.7±0.88ab	9.7±1.22a
Festival Semi-Double Rose Shade	3.6±0.78ad	4.1±1.00ab	6.0±1.20ac	8.6±1.48ab	7.7±0.87ab	8.0±0.74a
Festival Semi-Double Yellow	2.9±0.66ad	2.7±0.54ab	4.5±0.62bc	7.4±1.09ab	7.2±0.73ab	9.5±1.61a
Festival Spider Salmon Eye	1.9±0.30ad	2.4±0.57ab	4.9±0.75bc	8.4±0.62ab	11.0±1.48ab	11.5±1.81a
Festival Spider Yellow	2.9±a0.65d	4.4±1.11ab	5.5±1.51ac	9.0±1.56ab	8.2±1.05ab	8.3±1.37a
Kameleo Micro Mix	1.7±0.41ad	2.5±0.71ab	6.2±0.82ac	7.5±0.55ab	7.8±0.94ab	12.1±0.58a
Mega Revolution Champagne	1.1±0.29cd	2.8±0.37ab	3.3±0.72bc	5.9±0.83b	9.8±2.93ab	10.1±1.67a
Mega Revolution Golden Yellow Dark Eye	2.1±0.34ad	3.5±0.78ab	4.0±0.54bc	11.9±1.23ab	9.1±1.72ab	10.8±2.09a
Mega Revolution Orange Dark Eye	2.7±0.63ad	5.3±1.33ab	5.2±0.91ac	8.4±0.53ab	9.5±1.02ab	9.8±1.07a
Mega Revolution Purple Shade	1.2±0.24bd	2.7±0.52ab	2.8±0.56c	4.0±0.45b	6.1±0.93ab	6.0±1.63a

Mega Revolution Scarlet Dark Eye	1.4±0.30bd	4.5±1.01ab	6.1±1.31ac	6.0±1.34b	6.1±0.66ab	8.6±1.31a
Mega Revolution White	0.7±0.25d	2.0±0.43ab	4.1±1.03bc	4.7±0.79b	5.4±0.82ab	8.2±1.74a
Mega Revolution Yellow Shade	1.7±0.36ad	4.6±0.94ab	11.3±1.16a	8.1±1.91ab	13.7±0.66ab	16.3±1.07a
Revolution Pink	1.9±0.43ad	0.9±0.36b	3.6±0.89bc	4.6±0.67b	7.2±1.49ab	5.5±1.29a
Revolution Pink Baby	1.6±0.39ad	3.5±0.99ab	3.0±0.58c	5.3±0.64b	6.7±1.29ab	6.7±1.44a
Revolution Pink Pastel Dark Eye	3.6±0.64ad	2.6±0.64ab	1.9±0.38c	4.1±0.36b	5.9±1.09ab	5.3±1.31a
Revolution Red Shade Dark Eye	1.5±0.23ad	2.4±0.97ab	3.6±0.83bc	9.7±0.89ab	10.4±1.28ab	9.9±0.88a
Revolution Rose Shade	1.7±0.41ad	4.1±0.95ab	3.6±0.79bc	5.0±0.71b	7.6±0.76ab	8.1±0.95a
Revolution Pastel Orange Dark Eye	0.8±0.22d	1.3±0.33ab	2.3±0.48c	3.7±0.36b	6.7±0.98ab	7.9±1.24a
Revolution Scarlet Dark Eye	1.9±0.50ad	3.5±0.47ab	3.4±0.55bc	3.7±0.50b	5.8±0.76ab	7.3±2.31a
Revolution Spring Pastels	0.7±0.20d	1.5±0.47ab	2.8±0.92c	3.7±0.73b	3.7±0.45b	5.4±0.65a
Revolution White	1.3±0.26bd	1.5±0.30ab	3.4±0.54bc	4.5±0.81b	7.5±1.33ab	6.2±1.34a
Revolution Yellow	3.7±0.60ad	5.3±1.01ab	6.1±1.02ac	9.1±1.31ab	9.4±1.87ab	10.7±1.81a
Yellow Dark Eye	1.0±0.43d	2.2±0.60ab	3.0±0.45c	4.9±0.72b	8.6±1.39ab	11.31±1.48a
F	3.27	2.61	2.54	1.84	1.52	1.33
df	59, 599	59, 599	59, 599	59, 599	59, 599	59, 599
P	< 0.0001	< 0.0001	< 0.0001	0.0003	0.0103	0.0590

Table 3.4. Gerbera cultivars least preferred by the leafminer *L. trifolii* with < 20% of highest damage on at least 2 observation dates

Cultivar #	Cultivar name
5	Gerbera Jaguar Pink
7	Gerbera Jaguar Rose Deep
9	Gerbera Jaguar Salmon Pastel
57	Gerbera Revolution Spring Pastel

Table 3.5. Gerbera cultivars highly preferred by the leafminer *L. trifolii* with > 80% of highest damage on at least 2 observation dates

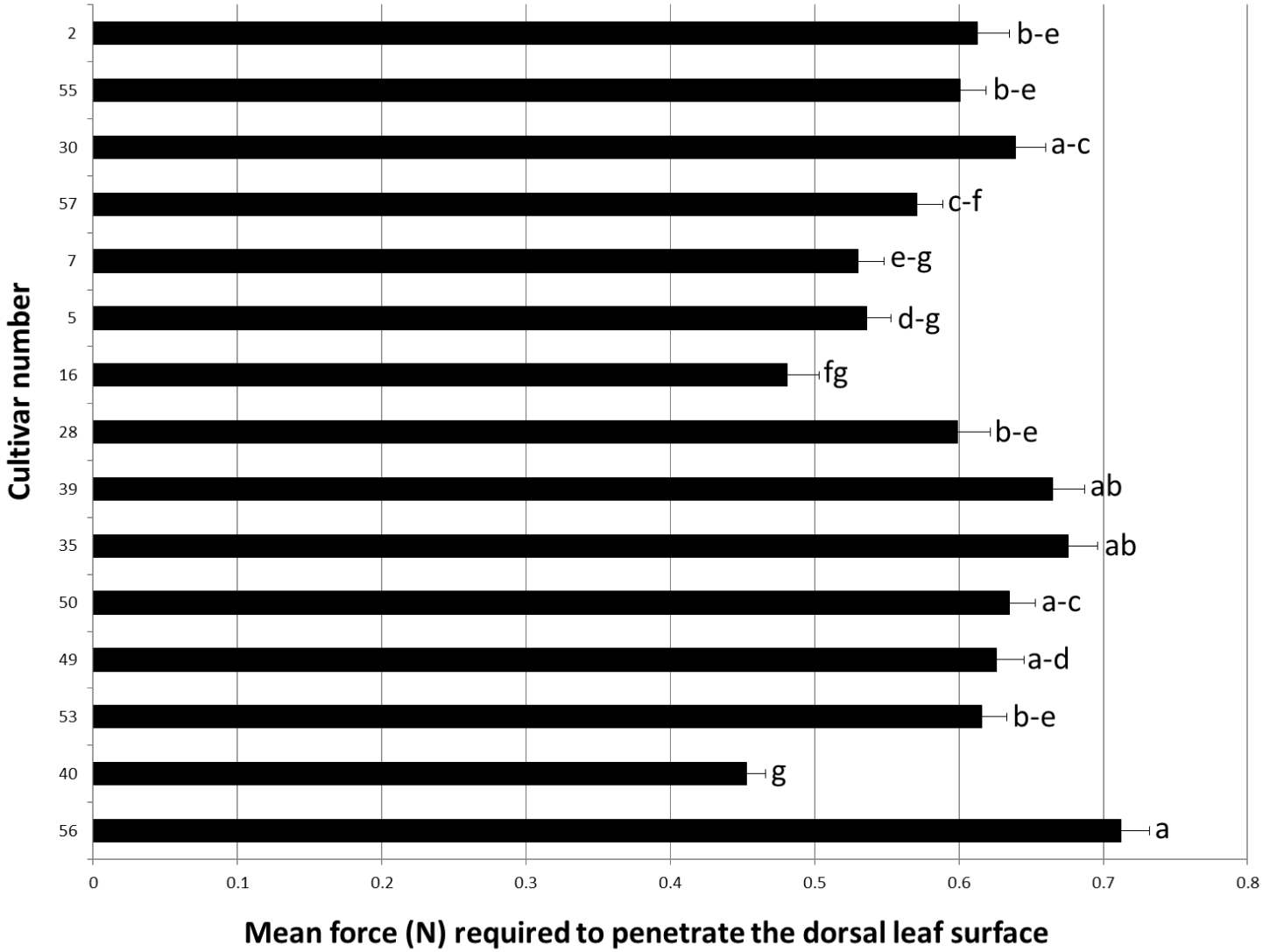
Cultivar #	Cultivar name
2	Gerbera Jaguar Fire Dark Eye
13	Gerbera Jaguar Yellow
18	Gerbera Royal Semi-Double Watermelon Dark Eye
22	Gerbera Festival Cream
24	Gerbera Festival Peach Dark Eye
33	Gerbera Festival Mini Pink Soft
35	Gerbera Festival Mini Yellow Shade
49	Gerbera Mega Revolution Yellow Shade
59	Gerbera Revolution Yellow

Table 3.6. Mean± SE of L. *trifolii* mines on cultivar lines across six observation dates

Cultivar Lines	Damage ± SE
Gerbera Jaguar	5.2895± 0.19 ab
Gerbera Royal	5.3535±0.28 ab
Gerbera Festival	5.4614±0.14 a
Gerbera Festival Mini	3.9872±0.32 c
Gerbera Mega Revolution	5.1286±0.23 ab
Gerbera Revolution	4.4353±0.18 bc

Figure Captions

Fig. 3.1. Force (means ± SE) in newtons required to penetrate the dorsal surface of gerbera cultivars (cultivar names appear in Table 1). N = 30 for each cultivar number shown. Bars with same case letters are not significantly different (Tukey's HSD, α=0.05, p value < 0.0001).


Cultivar number
2
55
30
57
7
5
16
28
39
35
50
49
53
40
56
b-e
b-e
a-c
c-f
e-g
d-g
fg
b-e
ab
ab
a-c
a-d
b-e
g
a
0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.8
Mean force (N) required to penetrate the dorsal leaf surface

CHAPTER 4

CASE STUDY- COMPARISON OF TRADITIONAL AND BIOLOGICALLY-BASED LEAFMINER CONTROL IN GREENHOUSE GERBERA DAISIES

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Abstract

The leafminer *Liriomyza trifolii* (Burgess) is a key pest of gerbera daisies (*Gerbera jamesonii* Bolus), which are among the most preferred cut flowers in the world. While insecticides often fail to control this pest, parasitoids have proven to be effective. To maintain the parasitoids in the system, pesticide applications should be avoided. However, the influx of secondary pests like mites, thrips, whiteflies, and aphids during the growing season necessitates chemical sprays, which are effective in controlling the secondary pests, but are often toxic to the natural enemy and hence disrupt biological control. Since natural enemies provide effective control of *L. trifolii* and chemicals are not easily avoided in this system, an alternative strategy combining both of these would ideally be the solution. In this study we compared a traditional chemically-based control regime with a biologically-based control program, and found that a biologically-based control program reduced overall leafminer populations and provided insect control at a lower cost than the chemically-based regime. Our data suggest that growers would benefit from adopting a biologically-based control program in terms of amount spent on insect control and better looking plants and flowers.

Index words: integrated pest management; cut flower pest management; biologically-based control.

Significance to the cut flower industry:

Gerbera daisies (*Gerbera jamesonii* Bolus) are the third most preferred cut flowers in the world and increasing in demand in the United States (Seifert 2003). Chemically resistant leafminers, *Liriomyza trifolii* Burgess are the primary pest in this

system and can be controlled effectively by natural enemies in the absence of toxic chemicals (Liu et al. 2009). Secondary pests like mites, thrips, whiteflies, and aphids often require intervention with pesticides that are toxic to the natural enemy and disrupt leafminer control. Current strategies to control the suite of pests in this system are ineffective and hence an impediment to sustainable production. While natural enemies can effectively control leafminers, pesticides are required in order to effectively control the secondary pests. A biologically-based control program that combines these two control methods without harming leafminer biocontrol would be an ideal solution. Through our project, we compared a traditional chemically-based control program with a biologically-based control program and found that the biologically-based program not only resulted in better looking plants and flowers, but also spent less in insect control costs during the 81 weeks we monitored.

Introduction

The primary pests affecting greenhouse gerberas are serpentine leafminers, *Liriomyza trifolii* (Burgess) (Diptera: Agromyzidae), which have a wide distribution and attack more than 400 species (Reitz and Trumble 2002) of plants including vegetables and ornamentals. The larvae feed on the palisade mesophyll (Parrella et al. 1985) and decrease photosynthesis and yield, directly affecting the marketable produce. Rigorous and extended use of pesticides has rendered leafminers resistant to almost all chemistries (Keil and Parrella 1982). Leafminers are also protected from chemicals by being embedded within the leaves in their larval stages. Successful biocontrol has been implemented by augmentative releases of parasitoids. This has however been effective in areas only where disruptive use of chemicals has been avoided (Liu et al. 2009).

The influx of secondary pests like mites, thrips, whiteflies, and aphids, and pathogens causing powdery mildew through the season necessitates pesticide sprays which in turn kill the leafminer parasitoids. Insecticide toxicology assays in our lab revealed that many of the commonly used chemicals (against secondary pests) cause high mortality in beneficial arthropods (leafminer parasitoid *Diglyphus isaea* (Walker) and the predatory mite *Neoseiulus californicus* (McGregor)) and hence will disrupt effective pest management (Chapter 2). While pesticides are not effective against leafminers, they certainly are detrimental to the effective buildup of natural enemy populations. Pesticides however are not an easily eliminated component in the pest management program of this system. Any pest management program in the cut flower system would inevitably utilize some pesticides as long as other cost effective options are not available.

What is a defining characteristic of this system though is that the primary pest cannot be effectively controlled by chemicals, while secondary pests can. Additionally, the primary pest, leafminer can be controlled by natural enemies, but will be successful only when toxic sprays are avoided in the system. Integrated pest management (IPM) programs vary according to systems and pests involved. What might work in one system might not be optimal for another. There have been programs in other systems that were developed and successful (Parrella and Jones 1987). Following the same line, unless we find cost-effective biological solutions to control the whole suite of primary and secondary pests in this system, the practical solution would be to integrate natural enemies and safe chemicals for controlling the pest complex. *Diglyphus isaea,* a parasitoid of the leafminer *Liriomyza trifolii,* has been demonstrated to be successful (Liu et al. 2009) and is available commercially. Commonly used pesticides that are relatively

harmless to the natural enemy have been identified in toxicology studies in our lab (Chapter 2). Using the information, we wanted to see if a biological based control program would be possible and viable compared with a chemical based approach.

Materials and Methods

Liu et al (Liu et al. 2009) had previously documented that biological control in the absence of chemicals could effectively control leafminers. Whether such an approach was possible from a business perspective was however not known. Our objective was to investigate if a biologically-based control program would be cost effective for a grower to undertake. Only an actual grower greenhouse could simulate field conditions and such a study, if eventually successful, would claim merit. In actual field conditions, we wanted to compare a chemically-based control regime, where growers more often than not spray excessively for any pest problem, with a biologically-based control program where *L. trifolii*, the primary pest was controlled by natural enemies and the secondary pests were controlled by pesticides that did not disrupt (Chapter 2) the biological control.

Location of Study

A grower greenhouse located in Thomaston, GA, about 35 miles south of the UGA-Griffin campus was selected. Two out of several greenhouses were selected to implement one of either chemically-based control or biologically-based control, which were the treatments. In the greenhouse selected for chemically-based control, 3 benches that comprised of 5 pots in width and 50 pots in length for a total of 250 potted Gerbera plants on a bench was selected as our experimental area. In the biologically- based control greenhouse, 3 benches in the front (denoted as A in figures and tables) and 3

benches in the back (denoted as B in figures and tables) were selected as experimental areas for the test. The project spanned from the 4th week in 2010, to week 32 in 2011for a total of 81 weeks. Monitoring was done weekly in 2010 and biweekly in 2011.

Chemical control house

This house was maintained by the grower and received regular pesticide application as deemed necessary to control insects, mites and pathogens. During the project period 2010-2011, 132 instances of chemicals/sprays (Fig. 1-3) were applied to control various pests out of which 93 were exclusively for insect/ mite control, and the rest for fungal pathogens.

Biological control house

This house was selected due to the availability of new plants that were not sprayed with any pesticide prior to monitoring. Any pesticide application in this house was conducted only after consultation with the authors. Leafminer parasitoids *Diglyphus isaea* (Rincon Vitova Insectaries, Ventura, CA.), were introduced in late April and early May for populations to build up. Due to seasonal preferences for flowers, there was one instance when an entire section of plants in the house was changed (disrupting the system that prevailed until then). The introduction of chemically treated plants into the biocontrol house on February 22nd warranted a release of *D. isaea* (Rincon Vitova Insectaries, Ventura, CA.) in the 2nd week of March. During the project period, there were 57 instances of chemicals/sprays applied to control various secondary pests out of which only 19 were exclusively for insect/mite control and the rest for fungal pathogens.

Yellow sticky card monitoring

Three yellow sticky cards were placed on stakes in each of the benches in the experimental area slightly above foliage height to monitor the pest and natural enemy population through the season. Each card was an experimental unit. Cards were removed and brought back to the lab and new cards replaced every week in 2010 and bi-weekly in the 2011 growing season. Once in the lab, counts were made from the yellow sticky card for numbers of leafminers, fungus gnats, *D. isaea*, and other wasps. Counting was done from the central nine squares in the sticky card, omitting the peripheral incomplete squares on the shorter sides of the card.

Leaf counts

The number of mines— (silver patterns characterized by the lack of chlorophyll due to the feeding and development of the leafminer larva within the leaf) in 50 random leaves on either sides of the middle bench, and one side of the outer benches in the 3 experimental areas mentioned above were counted weekly in 2010 and biweekly in 2011. Each sampled leaf was an experimental unit.

Statistical Analyses

The experiment was analyzed as randomized complete block design with replications (rows) considered as blocking factor. Data were subjected to analysis of variance (ANOVA) using the general linear model procedure (SAS Institute 2003, Cary, NC). Means were separated with Fisher's protected least significant difference (LSD) test.

Results and Discussions

Initial analysis was done to see if there was difference between the two treatments, chemical based control (denoted by C in the tables and figures) and biological based control (denoted by A and B in the tables and figures). While there was significant difference between the two methods, individual dates were shown to be a significant contributing factor for differences. Subsequent analyses hence looked at each individual observation date.

Yellow sticky card monitoring

At the start of monitoring in the greenhouses in early 2010, there was a higher leafminer population in the biocontrol house than the chemical control house (Figs. 4.1, 4.2). Based on data from sticky cards, the populations of leafminers varied during the season, with significant differences between the chemical control house and biological control house. During the peak growing season in 2010, when leafminer populations are historically high, the biological control plants demonstrated considerably fewer leafminers (Table 4.1, Figs 4.2, 4.3) and subsequent damage (Figs. 4.8, 4.9). During 2011, the same pattern followed, but the number of leafminers had decreased from the previous year (Table 4.1). For the 19 observation dates spanning June 24th 2010 to October 18th 2010, leafminers were significantly higher in the chemical control house than in the biocontrol house for 9 dates and lower or not significantly different in the rest. There were a couple of occasions when the leafminer population was higher in one section of the biocontrol house than in the chemical control house, but the population generally remained low. In general, during the active growing season, leafminer

populations in the chemical control house were significantly higher on most observation dates than in the biocontrol house.

D. isaea, slowly increased in numbers over a 45 day period after the initial release. While *D. isaea* populations increased in the biocontrol house, they were understandably low in the chemical control house. Over time though, the number of *D. isaea* in the chemical control house also increased in spite of the harmful pesticide sprays. Their numbers remained lower than those from the biocontrol house until the first week of August at which point higher numbers were found in the chemical control house until the third week of September and then reverted the other way.

In the third week of February, the grower replaced a whole section of plants in the biocontrol house with plants that were treated with organophosphates, chlorpyrifos (DuraGuard) and imidacloprid (Marathon) due to business reasons. The number of leafminers at this point significantly increased, while parasitoid numbers remained low. After a re-introduction of parasitoids, in about 2 months' time, the parasitoid populations were back to keeping the leafminer populations in check once again. It remained so until the first week of August when the study was terminated.

Leaf counts

Leaf counts were another measurement that provided us an indication as to how much leafminer pressure the plants were being exposed to. Higher leafminer numbers would mean higher number of mines on each leaf which would in turn mean more leafminers developing within them. After a cold winter, leafminer populations were very low when monitoring started. Nevertheless, mines on leaves in the biocontrol house were

slightly higher than in the chemical control house initially. Even after the initial introduction of parasitoid wasps in the biocontrol house, mine numbers remained slightly higher than in the chemical control house. This continued until a steady population was established by the second week of June. Numbers of mines on leaves in the biocontrol house began to fall significantly below the numbers from the chemical control house. Mine counts in leaves in the chemical control house were increasing at a high rate, while those in the biocontrol house remained significantly low. The week of July 7th through August 5th was when the number of mines reached its highest. Three tank mixes at weekly intervals could only bring the number of mines from around 250 to around 175 on these occasions, while the average number of mines in the biocontrol house was around 50.

The introduction of chemically treated plants into the biocontrol house in February 2011 disrupted biocontrol, and resulted in a higher number of mines for about 7 weeks. Parasitoid populations after the release in March established by that time and were able to keep the leafminer population in check from then on. *D. isaea* populations were effective in keeping the leafminer populations from increasing in a similar manner as it did in 2010.

Over the period of the study, visual data (Figs. 4.8, 4.9) clearly showed biologically-based control to provide a superior control for leafminer than a traditional chemically-based control. From the fluctuations in populations of both leafminers and *D. isaea* in both greenhouses over time, it could be inferred that the location of the two observation houses being across from each other allowed for movement of both leafminers and parasitoid wasps to and fro. With regards to controlling leafminer

populations, the biocontrol house must have functioned similar to a banker system to the chemical control house. While a banker plant system has been effective in greenhouse vegetable cultivation (Avilla et al. 2004, Blümel 2004), efficacy in floral systems is still being evaluated (Van Driesche et al. 2008, Frank 2010). Another option could be the maintenance of a centrally located greenhouse in grower greenhouse situations that which follows biologically-based control and hence can harbor natural enemies or act as a refuge.

This case study shows that pesticides were not an effective control option for chemically-resistant leafminer *Liriomyza trifolii*. There were 71 sprays/chemicals applied for leafminer control alone (in the chemical control house), in spite of which high numbers of leafminers continuously inflicted damages. While highly toxic pesticides do kill a few leafminers, it does not result in a significant decline in leafminer populations or damage. While the biocontrol house received fewer sprays, which were specific to the secondary pests, significant control was also achieved of the leafminer. The result was apparent in better kept plants and flowers. The data support the fact that leafminer control is possible with its natural enemy *D. isaea* in the absence of harmful chemicals. In this particular situation where the primary pest, *L. trifolii*, is chemically resistant and cannot be controlled by pesticides, while secondary pests can effectively be controlled by chemicals, the practical way is an integrated pest management (IPM) approach. By integrating chemical and biological control and following a biologically-based control method, the primary pest would be controlled by its natural enemy, for example *Diglyphus isaea*, and the secondary pests would be controlled using specific chemicals

that are less harmful to these natural enemies but will still effectively control the pests (Chapter 2).

Is a biologically-based integrated management program cost effective?

Using the spray schedule data and records of natural enemy releases (Table 3), the cost of insect control in a 100 sq ft area was calculated. The retail costs of chemicals were ascertained from Griffin Greenhouse and Nursery Supplies. Inc, Tewksbury, MA. The cost of plant protection chemicals (at median label rates), which included insecticides and fungicides for 2010-2011 (Table 4) in the chemical control house was $76.30, while that in the biological control house was $24.09. Looking at just the insect control costs for the same period, the chemical control in 100 sq ft was $62.10, while biologically-based control was $10.04, excluding labor costs. Factoring in labor costs for spraying chemicals (at the rate of $15/h for spraying about 3500 sq. ft of greenhouse), an additional $31.20 in the chemical control house, and $17.20 in the biological control house were spent. The total amount spent per 100 sq ft hence was $107.5 in the chemical control house and $42.49 in the biologically-based IPM house. This was assuming that the cost of labor in releasing parasitoids was the same as application of chemicals in a given area, and that the labor involved in scouting for pests would remain the same irrespective of the control regime.

The prices of newer specific pesticides seem to be driving the total cost of control. Older broad spectrum pesticides have lower application costs and are also detrimental to natural enemies. Newer chemistries are costlier for the claim that they are specific and effective against the target pests. As the number of sprays of such chemicals increases,

the actual gap between costs of chemical control versus a biological based control would also increase. While considering the cost of insect control and the fact that the only natural enemy (*D. isaea*) in the system targets the leafminer, it can be inferred that the difference in costs is the amount spent on ineffective control of leafminers alone. While biocontrol in itself might not be as cheap as other broad spectrum insecticides, it surely is comparable to the costs of newer pesticides. The difference would however remain in how many more times insecticide sprays would be required against leafminers even though pesticides do not render effective control. Any other pest problem in this system would have required a pesticide application in both the chemical and biological control houses.

There have been studies that looked into the financial feasibility of biological control programs in the past (Trumble and Morse 1993, Trumble et al. 1997, Wright et al. 2002) with results varying along the spectrum of cost effectiveness. Additionally, such analyses usually investigated the control of a single pest. In contrast, this study examined the cost effectiveness of a biologically-based control program for a suite of pests. Implementation of IPM or biological control programs needs to be examined on a case by case basis. Only in situations where such programs are biologically and financially viable can they be adopted successfully. Through this study, it is evident that a biologically-based IPM program not only results in plants and flowers with less leafminer damage but also requires lower expenses towards overall insect control. With the primary pest being chemically resistant, pesticides are certainly not an effective option. If a biologicall control for the primary pest is to be successfully incorporated with the chemical control for secondary pests, pesticides that are toxic to the leafminer natural

enemy need be avoided. While that is possible, it may require a change in the grower's pest control paradigm. A holistic understanding that parasitoid-pest interactions ultimately benefit the system needs to happen. The idea behind this model can be transferred to systems with similar pest complexes. Their adoption in the field will, however, depend on demonstrations of both biological and financial viability of such an insect control regime like what has been shown above.

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Table 4.1. Mean value of Leafminers ± SE on yellow sticky cards for each observation dates.

Week & Date	A- Biocontrol	B- Biocontrol	C- Chemical
wk13 Mar 31 2010	1.3±0.2 a	0.2±0.07 a	0.5±0.13 a
wk14 Apr 8 2010*	4±0.96 a	1.2±0.49 b	0.7±0.33 b
wk15 Apr 15 2010	0.3±0.21 a	0.9±0.45 a	0.7±0.27 a
wk16 Apr 22 2010*	1.3±0.53 a	0.4±0.18 b	0.2±0.17 b
wk17 Apr 28 2010**	6.7±1.88 a	1.7±0.5 b	0.8±0.4 b
wk18 May 6 2010***	26.6±2.62 a	14.8±2.01 b	5.2±0.87 c
wk19 May 12 2010*	4.3±0.95 b	9.8±1.85 a	5.7±0.21 b
wk20 May 19 2010	10.8±2.86 a	5.9±0.91 a	11.2±1.7 a
wk21 May 26 2010***	33.2±5.25 a	33.9±3.17 a	3.2±1.07 b
wk22 Jun 3 2010***	46±4.56 b	71.1±8.9 a	15±2.81 c
wk23 Jun 9 2010*	11.3±1.39 b	17.3±3.05 ab	26.3±4.85 a
wk24 Jun 16 2010*	22.7±2.19 b	45.1±9.3 a	25.3±3.02 b
wk25 Jun 24 2010	85.6±13.29 a	111.2±15.0 a	116.7±12.82 a
wk26 Jun 30 2010***	20.3±4.95 b	26.9±3.44 b	207.7±26.02 a
wk27 Jul 7 2010***	16.2±2.1 b	24.6±3.86 b	182.3±36.8 a
wk28 Jul 14 2010***	43.7±5.02 b	42.6±6.33 b	510.3±48.6 a
wk29 Jul 21 2010***	74.3±10.1 b	112.2±10.44 b	687.2±121.38 a
wk30 Jul 28 2010***	28.9±5.98 b	41.9±6.01 b	220.5±35.82 a
wk31 Aug 5 2010***	96.7±9.45 b	87±9.19 b	265.8±31.91 a
wk32 Aug 11 2010***	40.8±5.43 b	44.6±5.32 b	78±3.57 a
wk33 Aug 18 2010***	9.3±2.47 b	22.1±4.77 b	135±13.23 a
wk34 Aug 24 2010***	18.6±2.26 b	36.8±6.58 b	124.7±9.93 a
wk35 Aug 30 2010**	24.4±3.5 b	35.2±3.49 a	45.2±3.03 a
wk36 Sep 7 2010**	17.4±2.25 b	30.3±4.5 a	32.7±2.38 a
wk37 Sep 14 2010**	25.4±4.03 b	37.8±3.54 a	17±3.08 b
wk38 Sep 20 2010*	46.8±6.18 ab	66.5±5.39 a	27.5±3.39 b
wk39 Sep 27 2010	35.9±4.74 b	51.9±3.92a	48±7.14 ab
wk40 Oct 5 2010	12.9±3.52 a	10.3±1.85 a	17.3±2.55 a

wk41 Oct 12 2010**	4.6±0.89 b	7.2±1.22 b	12.7±1.51 a
wk42 Oct 18 2010	11.4±4.79 a	14.4±1.58 a	18±3.44 a
wk43 Oct 26 2010***	6.4±1.45 b	7.1±1.31 b	81.5±7.48 a
wk44 Nov 2 2010***	9.2±1.59 b	3.9±1.14 b	143.3±18.01 a
wk45 Nov 9 2010***	3.4±0.8 b	2.4±0.6 b	55.3±3.74 a
wk46 Nov 16 2010***	2.1±0.87 b	1.9±0.35 b	21.3±3.51 a
wk47 Nov 23 2010***	5.1±1.22 b	4±0.79 b	60±5.45 a
wk48 Nov 30 2010***	8.5±1.57 b	5.2±1.63 b	54.9±8.02 a
wk49 Dec 7 2010***	12.7±2.48 b	3±1.02 b	94.2±9.81 a
wk50 Dec 14 2010***	4±0.89 b	0.9±0.35 b	66.5±3.14 a
wk51 Dec 21 2010***	1.8±0.59 b	1.4±0.5 b	53.2±5.86 a
wk52 Dec 29 2010***	2±0.62 b	1±0.33 b	70.2±5.12 a
wk1 Jan 5 2011***	1.4±0.44 b	0.3±0.17 b	55.8±6.97 a
wk2 Jan 18 2011***	0.6±0.33 b	0.3±0.16 b	59.2±6.06 a
wk4 Jan 25 2011***	0.6±0.24 b	0.4±0.17 b	50.8±4.38 a
wk6 Feb 8 2011***	9.7±2.7 b	2±0.68 b	54±7.13 a
wk8 Feb 22 2011*	36.3±4.82 b	33.6±5.2 b	64.5±8.09 a
wk10 Mar 8 2011***	83.1±12.22 b	77.6±6.93 b	159.3±16.87 a
wk12 Mar 23 2011	217.6±17.78 a	206.1±32.9 a	250.5±19.3 a
wk14 Apr 5 2011	357.7±31.72 a	388±26.52 a	445±56.82 a
wk16 Apr 19 2011	582.3±56.46 ab	453.2±41.0 b	763±132.34 a
wk18 May 4 2011**	144.5±16.1 b	106.5±16.7 b	223.2±27.84 a
wk20 May 17 2011***	28.3±4.51 b	19.9±2.3 b	71.5±5.59 a
wk22 May 31 2011	159.2±29.47 a	134.2±19.38 a	171.8±13.16 a
wk24 Jun 14 2011***	40.6±10.46 b	40.1±5.63 b	126.8±14.02 a
wk26 Jun 28 2011	53.7±7.08 a	58.1±5.04 a	44.7±5.33 a
wk28 Jul 12 2011	97.9±10.95 a	61.9±7.32 b	80.7±10.9 ab
wk30 Jul 26 2011	33.3±4.13 a	25.9±2.9 a	32.3±3.79 a
wk32 Aug 9 2011*	32±4.97 b	58.3±6.0 a	34.1±5.53 b

***A** denotes front benches in the biocontrol house, **B** denotes back benches in the biocontrol house, and **C** denotes the chemical control house. Significance level indicated near the dates. Analysis compared A, B, and C on each date for significant differences.*

Table 4.2. Mean value of *Diglyphus isaea* (leafminer parasitoid) ± SE on yellow sticky cards for each observation date.

Week & Date	A- Biocontrol	B- Biocontrol	C- Chemical
wk13 Mar 31 2010***	0.2±0.07 b	1.3±0.42 a	0±0 c
wk14 Apr 8 2010	0.8±0.22 a	0.6±0.24 a	0.3±0.21 a
wk15 Apr 15 2010	2.5±1.05 a	2.8±0.52 a	1.2±0.40 a
wk16 Apr 22 2010	2±0.38 a	1.8±0.72 a	1.7±0.67 a
wk17 Apr 28 2010**	4.7±1.08 a	2.4±0.73 b	1.3±0.49 b
wk18 May 6 2010*	8.7±0.92 a	6±0.81 ab	4.8±0.87b
wk19 May 12 2010	4.9±1.28 a	5.2±0.64 a	5.7±1.2 a
wk20 May 19 2010	6.1±1.96 a	5.8±0.77 a	8±1.46 a
wk21 May 26 2010**	22.3±2.99 a	21.9±2.84 a	5.7±1.3 b
wk22 Jun 3 2010	17.4±1.0 a	17.3±2.36 a	16.3±2.17 a
wk23 Jun 9 2010	7.8±1.25 a	11.6±1.51 a	11.2±2.13 a
wk24 Jun 16 2010***	49±7.27 b	74.7±8.0 a	4.7±1.83 c
wk25 Jun 24 2010***	121.2±13.07 a	108.6±10.96 a	7.8±2.38 b
wk26 Jun 30 2010**	20.1±4.14 a	30.9±5.69 a	2.3±0.91 b
wk27 Jul 7 2010**	17.4±2.28 a	20.7±3.07 a	3.5±0.95 b
wk28 Jul 14 2010**	17.6±2.92 b	35.6±5.34 a	8.3±2.15 b
wk29 Jul 21 2010	38.9±5.36 ab	56.4±8.33 a	22.7±8.84 b
wk30 Jul 28 2010*	51.2±10.4 a	42.3±6.24 b	20.8±2.91 c
wk31 Aug 5 2010	115.9±13.77 ab	144.2±23.75 a	70.3±11 b
wk32 Aug 11 2010	49.2±5.26 a	36±3.96 a	37.2±4.96 a
wk33 Aug 18 2010***	8.6±1.81 b	11.6±2.52 b	55.5±8.20 a
wk34 Aug 24 2010***	8.6±1.96 b	9.3±1.47 b	34.5±5.55 a
wk35 Aug 30 2010***	7.7±1.53 b	8.4±1.38 b	29.3±4.56 a
wk36 Sep 7 2010***	7.4±1.33 b	9.1±1.11 b	25±4.14 a
wk37 Sep 14 2010*	15.7±3.73 b	17.6±2.8 b	38±5.01 a
wk38 Sep 20 2010	30±5.44 a	43.9±10.0 a	43.3±3.28 a
wk39 Sep 27 2010	37.8±8.75 a	38.4±6.7 a	15.2±3.47 b
wk40 Oct 5 2010*	33.4±6.39 a	34.7±4.3 a	13.7±2.41 b

wk41 Oct 12 2010**	40.6±9.93 a	57.4±3.37 a	2±0.25 b
wk42 Oct 18 2010***	19.3±2.73 b	46.7±5.33 a	6.3±1.42 b
wk43 Oct 26 2010**	20.3±2.95 b	41.6±7.48 a	7±1.43 b
wk44 Nov 2 2010*	5.9±1.21 a	4.7±0.98 ab	2.3±0.98 b
wk45 Nov 9 2010**	3.6±0.76 a	2.1±0.48 a	0±0 b
wk46 Nov 16 2010**	3.9±0.67 a	2.8±0.54 a	0.2±0.16 b
wk47 Nov 23 2010*	7.8±1.67 a	6±2.16 a	0.7±0.42 b
wk48 Nov 30 2010**	15±3.66 a	6.8±1.23 b	0.2±0.16 b
wk49 Dec 7 2010**	14.3±2.67 a	8.4±1.66 a	0±0 b
wk50 Dec 14 2010*	8±2.35 a	4.3±1.19 ab	0±0 b
wk51 Dec 21 2010*	5±1.38 a	1.3±0.33 b	0±0 b
wk52 Dec 29 2010	0.3±0.23 a	0.3±0.16 a	0±0 a
wk1 Jan 5 2011	0.1±0.1 a	0±0 a	0±0 a
wk2 Jan 18 2011	0±0 a	0.1±0.1 a	0±0 a
wk4 Jan 25 2011	0±0 a	0±0 a	0±0 a
wk6 Feb 8 2011	0.3±0.23 a	0.6±0.24 a	0±0 a
wk8 Feb 22 2011	0.5±0.34 a	0±0 a	0±0 a
wk10 Mar 8 2011	0.9±0.45 a	0.4±0.17 a	0±0 a
wk12 Mar 23 2011	0.2±0.13 a	0.3±0.23 a	0±0 a
wk14 Apr 5 2011	0.2±0.14 a	0.9±0.42 a	0±0 a
wk16 Apr 19 2011**	45±11.35 a	44.6±14.74 a	0.8±0.65 b
wk18 May 4 2011***	164.8±19.72 a	175.9±21.67 a	11.2±2.93 b
wk20 May 17 2011	59.2±15.64 a	40±6.38 a	34.7±6.51 a
wk22 May 31 2011	78.1±20.8 a	30.7±4.52 b	51.5±3.65 ab
wk24 Jun 14 2011***	42.6±10.58 b	30.9±4.98 b	118.5±10.81 a
wk26 Jun 28 2011	21.4±4.36 ab	13±2.75 b	38.2±8.82 a
wk28 Jul 12 2011***	43.6±11.43 b	46.8±9.89 b	162±24.65 a
wk30 Jul 26 2011	39.6±7.61 a	36.4±7.34 a	46.5±10.46 a
wk32 Aug 9 2011	14.4±2.81 a	21.6±8.83 a	16.5±2.82 a

***A** denotes front rows in the biocontrol house, **B** denotes back rows in the biocontrol house, and **C** denotes the chemical control house. Significance level indicated beside the dates. Analysis compared A, B, and C on each dates for significant differences.*

Table 4.3. Costs of pesticides and natural enemies.

As per quote from "Griffin Greenhouse and Nursery Supplies. Inc."

Active ingredient	Container vol.	cost	High rate/ 100 gallon	Median rate/ 100 gallon	Cost per 100 sqft (high)	Cost per 100 sqft (median)
Spiromesifen	8 oz	423.76	4 oz	3 oz	$1.06	$0.79
Pyriproxyfen	32 oz	289.90	8 oz	7 oz	$0.36	$0.32
Acetamiprid	8 oz	31.00	8 oz	7.25 oz	$0.16	$0.14
Spirotetramat	250 ml	193.20	50 ml	50 ml	$0.19	$0.19
Pyridalyl	16 oz	118.92	8 oz	8 oz	$0.30	$0.30
Pymetrozine	15 oz	182.57	5 oz	3.75 oz	$0.30	$0.23
Dinotefuran	3 lb	452.47	0.5 lb	0.4 lb	$0.38	$0.30
Etoxazole	16 oz	110.85	16 oz	12 oz	$0.55	$0.42
Abamectin	8 oz	110.77	8 oz	4 oz	$0.55	$0.28
Bifenazate	32 oz	342.94	4 oz	3 oz	$0.21	$0.16
Spinosad	32 oz	178.31	22 oz	22 oz	$0.61	$0.61
Cyromazine	16 oz	402.42	2.66 oz	2.66 oz	$0.33	$0.33
Chlorpyrifos	128 oz	294.53	50 oz	50 oz	$0.58	$0.58
Chlorfenapyr	16 oz	513.19	5.2 oz	5.2	$0.84	$0.84
Imidacloprid	80 oz	85.73	1.5 oz	1.5 oz	$1.60	$1.60
Cyfluthrin	8 oz	86.42	1.9 oz	1.9 oz	$0.10	$0.10
Acephate	16 oz	13.69	1.4 lb/107639 sqft	1.4	$0.02	$0.02
Lambda Cyhalothrin	32 oz	194.07	5 oz	4 oz	$0.15	$0.12
Azadirachtin	32 oz	225.40	16 oz	14 oz	$0.56	$0.49
Fenpropathrin	32 oz	166.35	16 oz	16	$0.42	$0.42
Fenpyroximate	32 oz	164.08	24 oz	20	$0.61	$0.51
Pyraclostrobin	16 oz	76.00	12 oz		$0.29	
Fludioxonil	8 oz	208.91	4 oz		$0.52	
Copper Sulfate	8 oz	55.13	2.5 oz		$0.09	
Fenhexamid	2.5 lb	311.13	2.5 lb/ 107639 sqft		$0.29	
Potassium bicarbonate	5 lb	55.14	2.5lbs		$0.14	
Thiophenate-methyl	16 oz	70.57	24 oz		$0.53	
D.isaea (1 b)	250		77.5		$0.62	
5+ bottles			59.8		$0.48	

Table 4.4. Calculation of cost of control for 100 sq.ft. area in chemical control and biologically-based control house at median label rates.

	Chemical control house	Biological control house
2010		
Insecticides	36.57	6.68
Natural enemies		0.96
Fungicides	12.42	10.03
2011		
Insecticides	25.55	3.36
Natural enemies		0.48
Fungicides	1.78	2.58
Total plant protection cost	76.3	24.09
Insect control costs	62.1	10.04
Total costs including labor	107.5	42.49
Leafminer control for 81 wk	~50.00	1.44

Figure 4.1. Average number of leaf mines from 50 random leaves on each observation date -week 4-12 2010 and the spray and natural enemy release data for the enclosed period.

A *denotes front rows in biocontrol house,* ***B*** *denotes back rows in biocontrol house, and* ***C*** *denotes rows in chemical control house.*

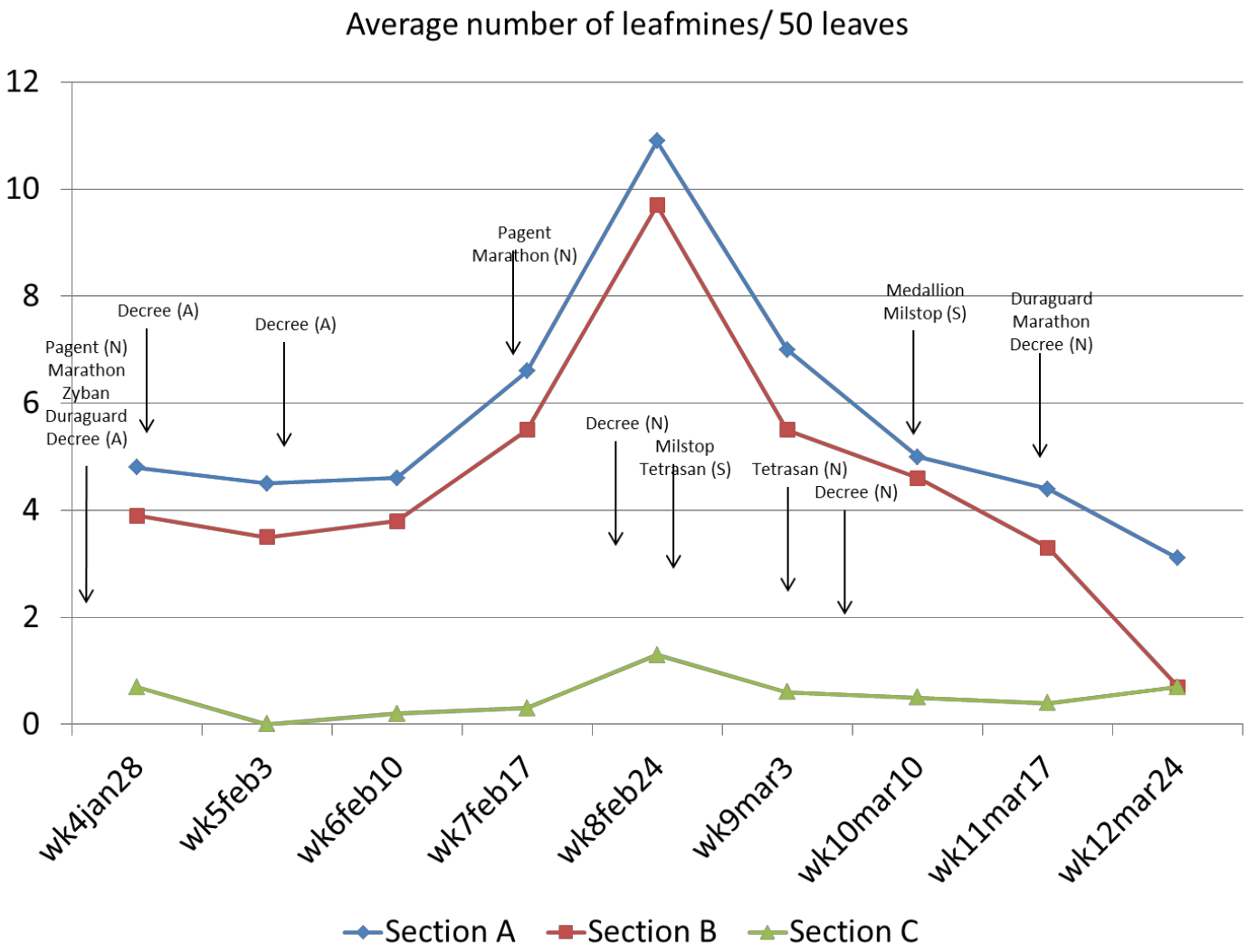

Average number of leafmines/ 50 leaves
12
10
8
6
4
2
0
Pagent (N)
Marathon
Zyban
Duraguard
Decree (A)
Decree (A)
Decree (A)
Pagent
Marathon (N)
Decree (N)
Milstop
Tetrasan (S)
Tetrasan (N)
Decree (N)
Medallion
Milstop (S)
Duraguard
Marathon
Decree (N)
wk4jan28
wk5feb3
wk6feb10
wk7feb17
wk8feb24
wk9mar3
wk10mar10
wk11mar17
wk12mar24
Section A
Section B
Section C

Figure 4.2. Average number of leaf mines from 50 random leaves on each observation date -week 13-32 2010 and the spray and natural enemy release data for the enclosed period.

A *denotes front rows in biocontrol house,* ***B*** *denotes back rows in biocontrol house, and* ***C*** *denotes rows in chemical control house.*

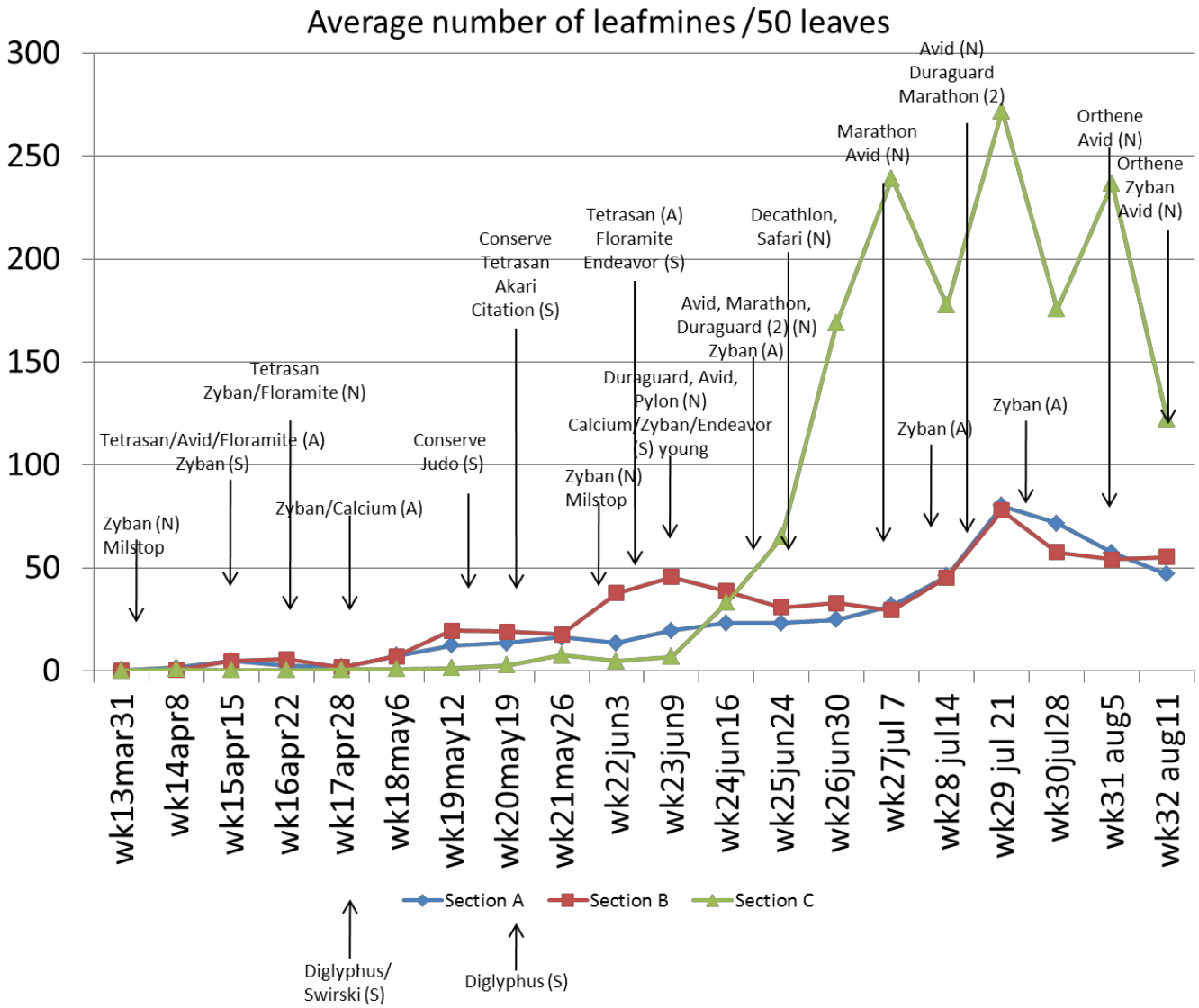

Average number of leafmines /50 leaves
300
250
200
150
100
50
0
wk13mar31
wk14apr8
wk15apr15
wk16apr22
wk17apr28
wk18may6
wk19may12
wk20may19
wk21may26
wk22jun3
wk23jun9
wk24jun16
wk25jun24
wk26jun30
wk27jul 7
wk28 jul14
wk29 jul 21
wk30jul28
wk31 aug5
wk32 aug11
Zyban (N) Milstop
Tetrasan/Avid/Floramite (A) Zyban (S)
Tetrasan Zyban/Floramite (N)
Zyban/Calcium (A)
Conserve Judo (S)
Conserve Tetrasan Akari Citation (S)
Zyban (N) Milstop
Tetrasan (A) Floramite Endeavor (S)
Duraguard, Avid, Pylon (N) Calcium/Zyban/Endeavor (S) young
Avid, Marathon, Duraguard (2) (N) Zyban (A)
Decathlon, Safari (N)
Marathon Avid (N)
Zyban (A)
Avid (N) Duraguard Marathon (2)
Zyban (A)
Orthene Avid (N)
Orthene Zyban Avid (N)
Section A
Section B
Section C
Diglyphus/ Swirski (S)
Diglyphus (S)

Figure 4.3. Average number of leaf mines from 50 random leaves on each observation date -week 33 2010- Wk 2 2011 and the spray and natural enemy release data for the enclosed period.

A *denotes front rows in biocontrol house,* ***B*** *denotes back rows in biocontrol house, and* ***C*** *denotes rows in chemical control house.*

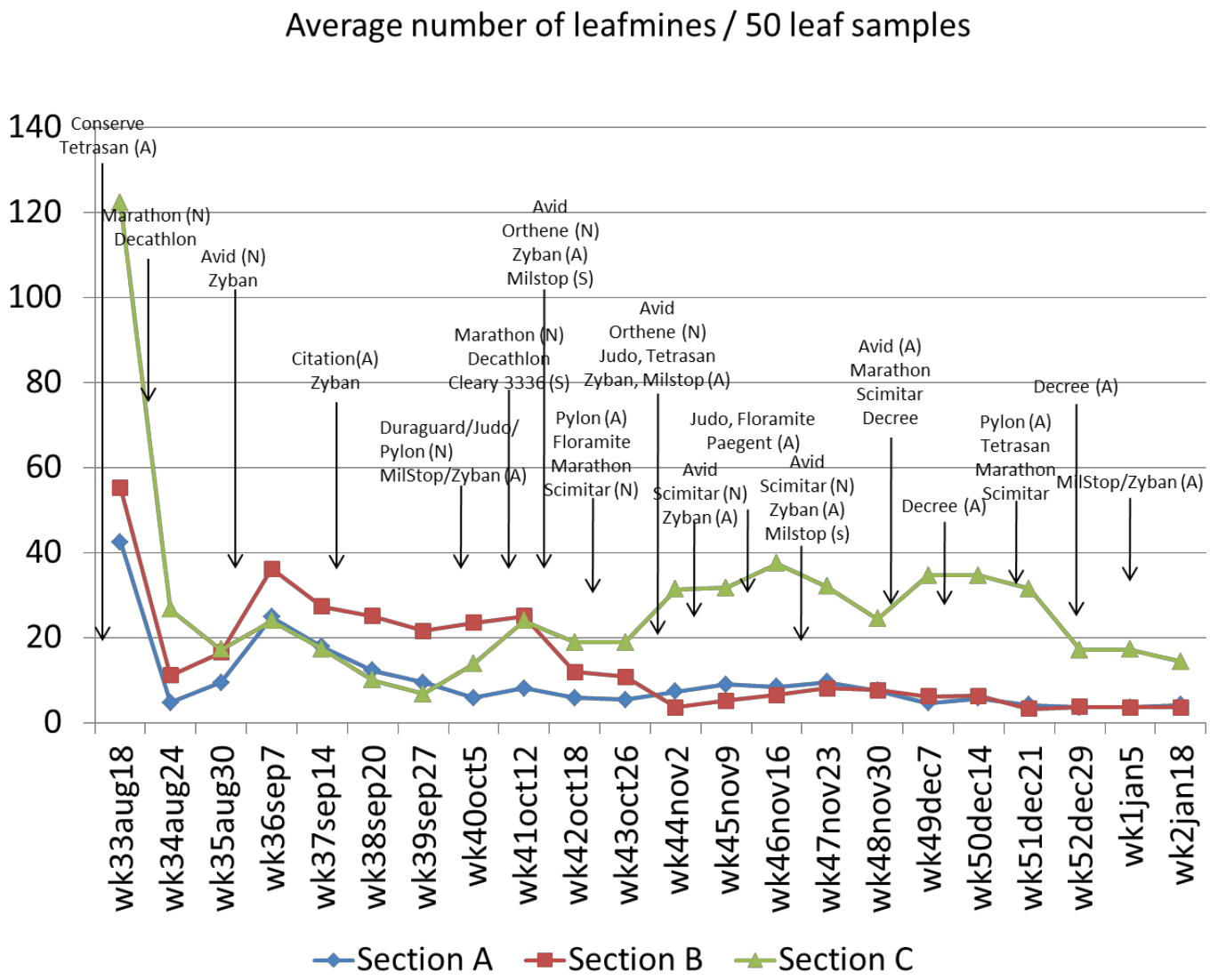
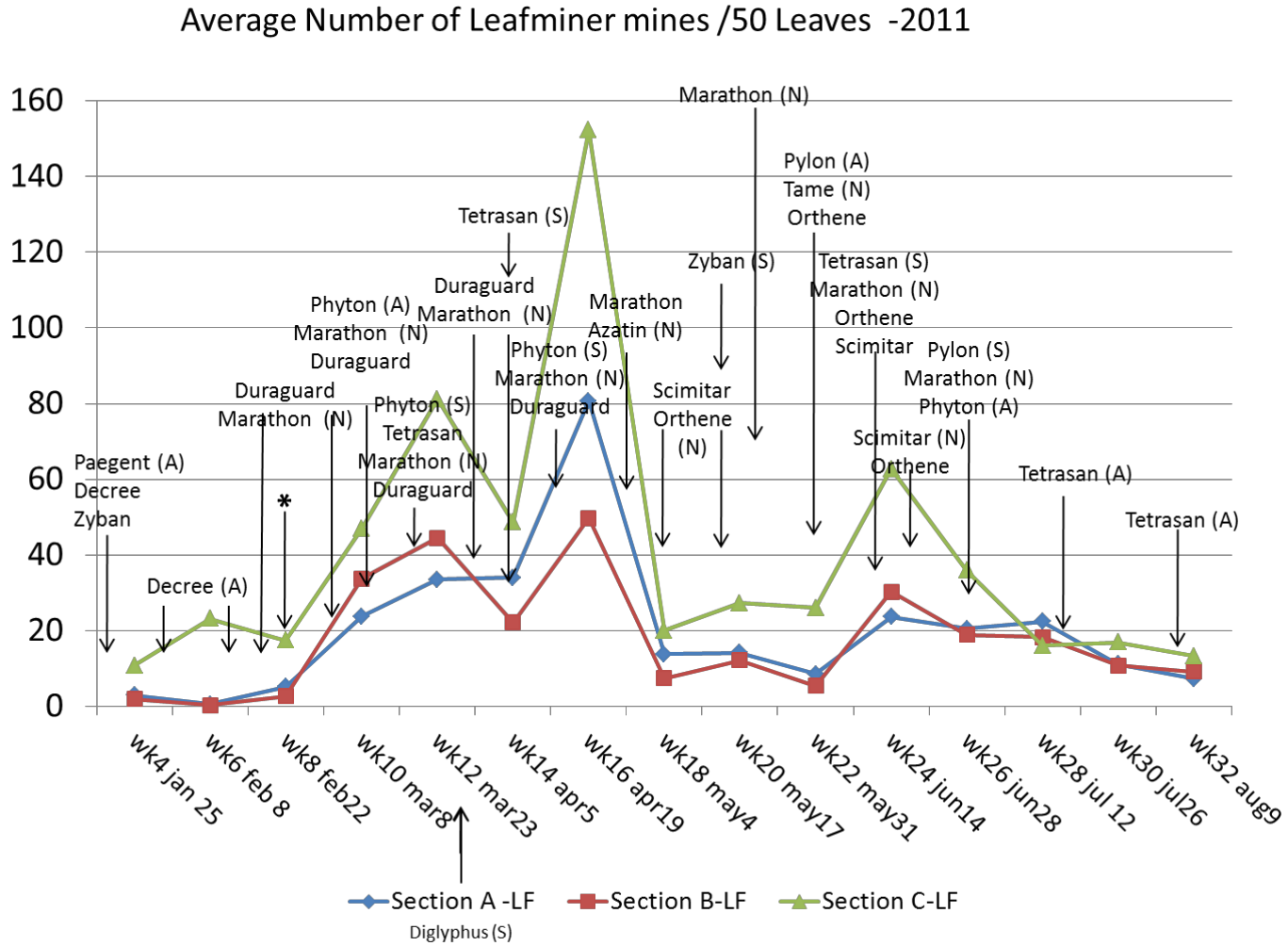

Average number of leafmines / 50 leaf samples
140
120
100
80
60
40
20
0
Conserve
Tetrasan (A)
Marathon (N)
Decathlon
Avid (N)
Zyban
Citation(A)
Zyban
Duraguard/Judo/
Pylon (N)
MilStop/Zyban (A)
Marathon (N)
Decathlon
Cleary 3336 (S)
Avid
Orthene (N)
Zyban (A)
Milstop (S)
Pylon (A)
Floramite
Marathon
Scimitar (N)
Avid
Orthene (N)
Judo, Tetrasan
Zyban, Milstop (A)
Avid
Scimitar (N)
Zyban (A)
Judo, Floramite
Paegent (A)
Avid
Scimitar (N)
Zyban (A)
Milstop (s)
Avid (A)
Marathon
Scimitar
Decree
Decree (A)
Pylon (A)
Tetrasan
Marathon
Scimitar
Decree (A)
MilStop/Zyban (A)
wk33aug18
wk34aug24
wk35aug30
wk36sep7
wk37sep14
wk38sep20
wk39sep27
wk40oct5
wk41oct12
wk42oct18
wk43oct26
wk44nov2
wk45nov9
wk46nov16
wk47nov23
wk48nov30
wk49dec7
wk50dec14
wk51dec21
wk52dec29
wk1jan5
wk2jan18
Section A
Section B
Section C

Figure 4.4. Average number of leaf mines from 50 random leaves on each observation date - week 4- 32 2011 and the spray and natural enemy release data for the enclosed period.

A *denotes front rows in biocontrol house,* ***B*** *denotes back rows in biocontrol house, and* ***C*** *denotes rows in chemical control house.*



**Feb 22 Wk 9 – moved plants into South house – plants had been treated w/ Duraguard & Merit in the previous wks

Figure 4.5. Average number of leafminers (lines) and *Diglyphus* (bars) caught on Yellow Sticky cards on each observation date- week 13- 32

A *denotes front rows in biocontrol house,* ***B*** *denotes back rows in biocontrol house, and* ***C*** *denotes rows in chemical control house.*

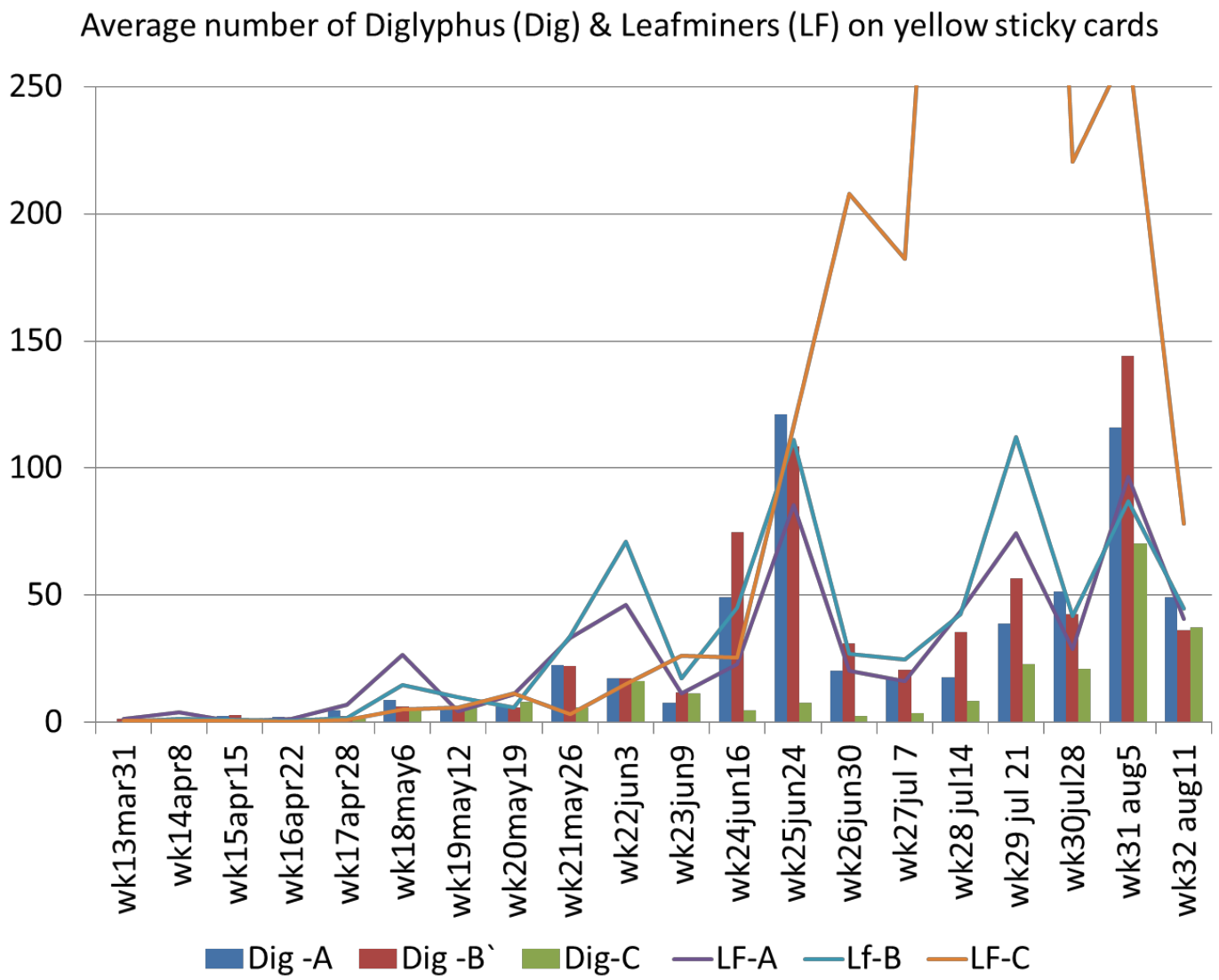

Average number of Diglyphus (Dig) & Leafminers (LF) on yellow sticky cards
250
200
150
100
50
0
wk13mar31
wk14apr8
wk15apr15
wk16apr22
wk17apr28
wk18may6
wk19may12
wk20may19
wk21may26
wk22jun3
wk23jun9
wk24jun16
wk25jun24
wk26jun30
wk27jul 7
wk28 jul14
wk29 jul 21
wk30jul28
wk31 aug5
wk32 aug11
Dig -A
Dig -B`
Dig-C
LF-A
Lf-B
LF-C

Figure 4.6. Average number of leafminers (lines) and *Diglyphus* (bars) caught on Yellow Sticky cards on each observation date- week 33 2010 – Week 2 2011

A *denotes front rows in biocontrol house,* ***B*** *denotes back rows in biocontrol house, and* ***C*** *denotes rows in chemical control house.*

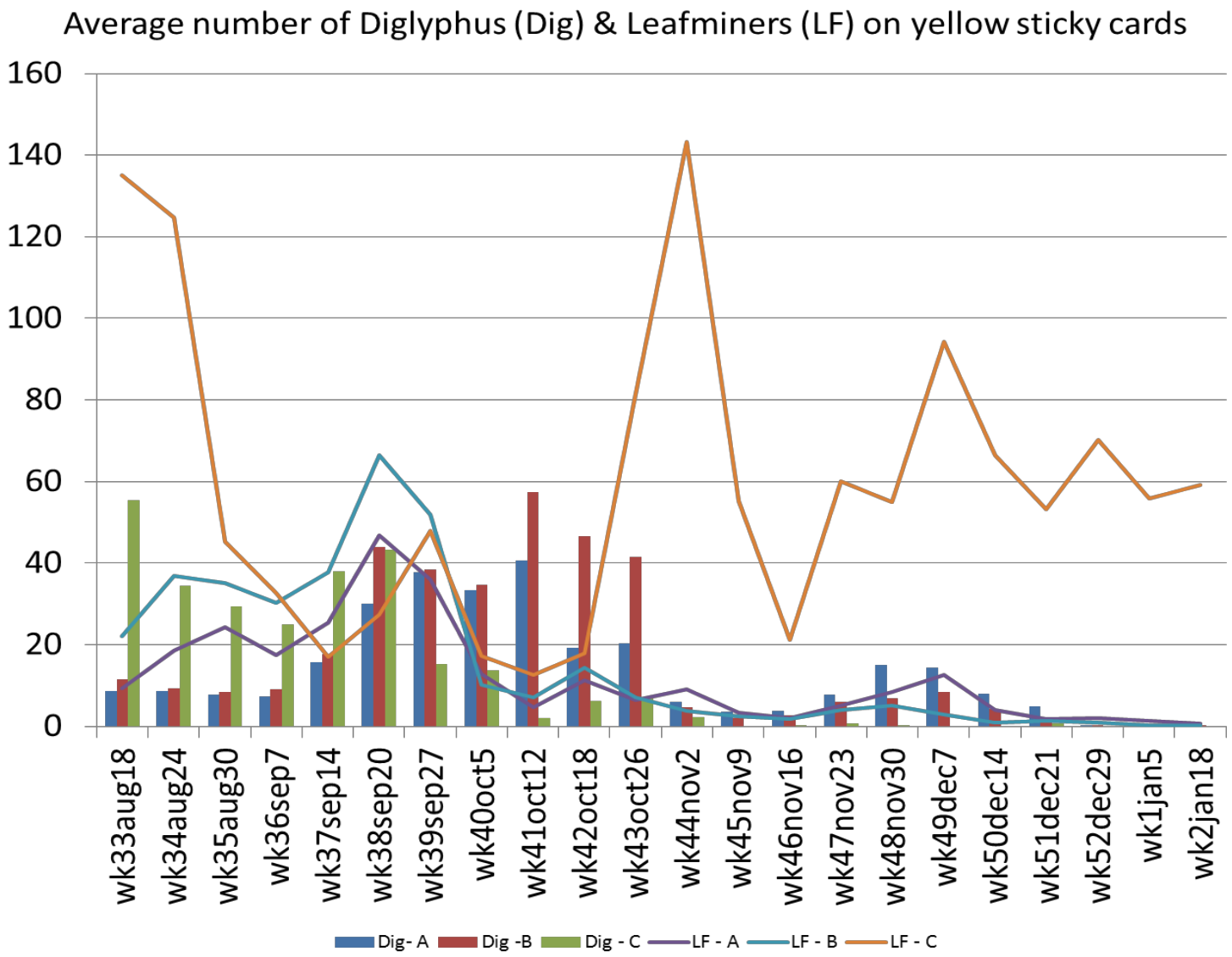

Average number of Diglyphus (Dig) & Leafminers (LF) on yellow sticky cards
160
140
120
100
80
60
40
20
0
wk33aug18
wk34aug24
wk35aug30
wk36sep7
wk37sep14
wk38sep20
wk39sep27
wk40oct5
wk41oct12
wk42oct18
wk43oct26
wk44nov2
wk45nov9
wk46nov16
wk47nov23
wk48nov30
wk49dec7
wk50dec14
wk51dec21
wk52dec29
wk1jan5
wk2jan18
Dig- A
Dig -B
Dig - C
LF - A
LF - B
LF - C

Figure 4.7. Average number of leafminers (lines) and *Diglyphus* (bars) caught on Yellow Sticky cards on each observation date- week 4 – 32 2 2011

A *denotes front rows in biocontrol house,* ***B*** *denotes back rows in biocontrol house, and* ***C*** *denotes rows in chemical control house.*

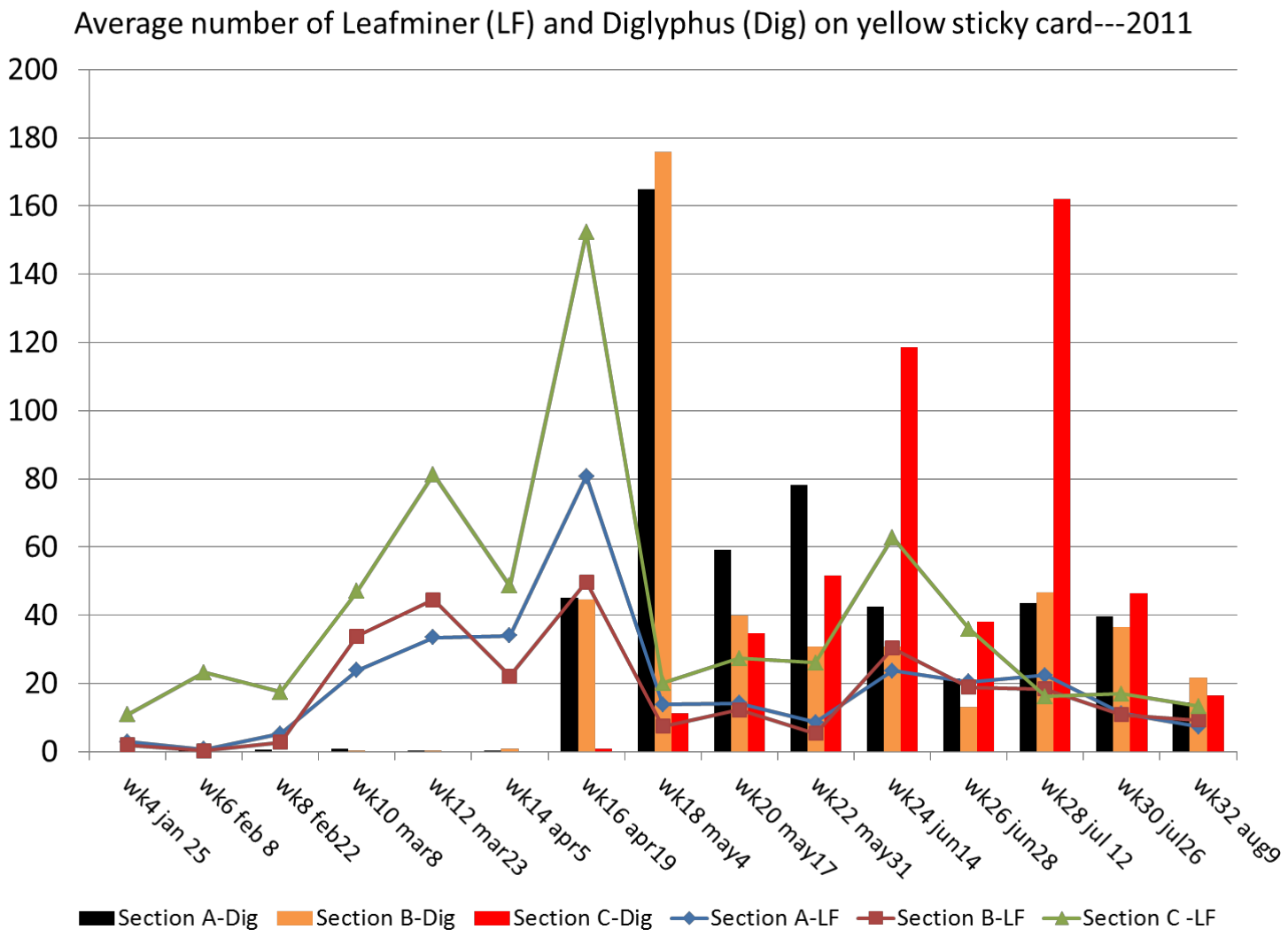

Average number of Leafminer (LF) and Diglyphus (Dig) on yellow sticky card---2011
200
180
160
140
120
100
80
60
40
20
0
wk4 jan 25
wk6 feb 8
wk8 feb22
wk10 mar8
wk12 mar23
wk14 apr5
wk16 apr19
wk18 may4
wk20 may17
wk22 may31
wk24 jun14
wk26 jun28
wk28 jul 12
wk30 jul26
wk32 aug9
Section A-Dig
Section B-Dig
Section C-Dig
Section A-LF
Section B-LF
Section C -LF

Figure 4.8. Gerbera plants and flower with leafminer damage in the chemical control house in August 2010

Figure 4.9. Clean gerbera plants and flowers in the biocontrol house in August 2010

CHAPTER 5

SUMMARY

The United States was once the largest producer of cut flowers in the world. Lack of effective pest control strategies and dwindling profits, among other reasons, have made our growers less competitive in the global market to an extent that we now import cut flowers worth at least $1 B. Cost-effective insect and pathogen controls need to be developed for the cut flower industry to be competitive again. Gerbera daisies offer a unique competitive advantage for local growers. While shipped from overseas, gerbera daisies are often not accompanied by a water source. This limits the shelf life and income earning potential for the growers. Adding a water source increases the shelf life, hence also the income and profit potential thereby. In Georgia, gerberas are among the top three sought after cut flowers, and among the top two that garner the highest price per stem. However, current local production does not always meet existing demand.

The primary pests affecting greenhouse gerberas are serpentine leafminers, *Liriomyza trifolii* (Diptera: Agromyzidae), which have a wide distribution and attack more than 400 species (Reitz and Trumble 2002) of plants including vegetables and ornamentals. The larvae feed on the palisade mesophyll (Parrella et al. 1985) and decrease photosynthesis and yield, directly affecting the marketable produce. Rigorous and extended use of pesticides has rendered leafminers resistant to almost all chemistries (Keil and Parrella 1982). Leafminers are also protected from chemicals by being concealed within the leaves in their larval stages. Successful biocontrol has been

implemented by augmentative releases of parasitoids. This has, however, been effective in areas only where disruptive use of chemicals has been avoided (Liu et al. 2009).

The influx of secondary pests like mites, thrips, whiteflies, and aphids, and pathogens causing powdery mildew through the season necessitates pesticide sprays which in turn kill the leafminer parasitoids. While chemicals are not effective against leafminers, commonly used pesticides are detrimental to the effective buildup of natural enemy populations. Pesticides however are not an easily eliminated component in the pest management program of this system. Any pest management program in the cut flower system would inevitably utilize pesticides because they are considered cost effective. In greenhouse gerberas, pesticides are effective and efficient in controlling secondary/occasional pests. The solution would be to integrate biological and chemical tactics to achieve control of the whole suite of pests.

Knowing the compatibility of commonly used pesticides will equip the grower with decision-making criteria regarding which chemical could be used without disrupting natural control of the leafminer, *L. trifolii*. Also, the availability of even partial host plant resistance mechanisms would synergistically help in not just controlling leafminer but potentially also secondary pests in this system. Once that information is available, it needs to be demonstrated that such an integration of tactics would actually benefit the grower economically. Aesthetic injury levels in cut flowers are practically zero; hence it is important to establish the biological and financial feasibility of such a plan in a business setting.

From our pesticide compatibility studies, we understood that many commonly used pesticides are detrimental to effective buildup of natural enemy populations. Abamectin (Avid®), which is an industry standard for mite control, and spinosad (Conserve®), the industry standard for thrips, were found to cause severe mortality in the leafminer parasitoid *D. isaea*. We also identified some that are compatible with natural enemies like spirotetramat (Kontos®) for whiteflies, and flonicamid (Aria®) for thrips. More chemicals were toxic to the predatory mite than to the leafminer natural enemy. Also there were at least 6 miticides (bifenazate, etoxazole, hexythiazox, spiromesifen, acequinocyl, and clofentezine) that were safe to the leafminer parasitoid not just within 48 h period but also in the long run. Most commonly used fungicides did not affect the leafminer parasitoids, while they were harmful to the predatory mites. With leafminers being the primary pest, mites being the major among the secondary pests, and powdery mildew an inevitable pathogen in this system, we now have the information to integrate chemical and biological control to effectively impact pest control. While biological control would effectively keep the primary pest in check, safe pesticides could be used to control the secondary pests, including the fungal pathogens causing powdery mildew.

With respect to host plant resistance, we found significant differences among susceptibility of 60 cultivars to leafminer damage in our studies. However, they could not be traced back to an effective resistance mechanism. Resistance was either not present throughout the various color variants in a cultivar group, hence precluding the possibility of an innate cultivar specific mechanism, or not consistent on a physical attribute such as leaf toughness. At least one cultivar among the Gerbera Jaguar and Gerbera Revolution groups showed low leafminer damage consistently. We did not

however find an effective mechanism of resistance in any of the 60 cultivars that were assessed in our study. However, it does not mean the absence of a resistance mechanism in this system. Additional investigation into related species of Gerbera like *G. ambigua, G. crocea, G. linnaei, G. serrate, G. tomentosa, G. viridifolia, G. wrightii* from their area of origin, South Africa, or other cultivars, if available, might be directions to pursue in the future.

With the information about compatibility of pesticides with natural enemies in hand, we investigated if an integrated approach was possible and financially feasible in a business setting. The study which took place in a grower greenhouse spanned over a year and a half with one of two greenhouses monitored under a chemical control regime, and the other under a biological based control regime. Not only did the biologically-based control provide for better looking plants and flowers, lower leafminer populations and damage over the peak growing season in 2010 and 2011, it was also found to be cost-effective. While the grower spent at least $62.10 for ineffective pest control in 100 sq ft. area of the chemical control house, insect control in the biological based control house cost only $10.04 excluding labor costs. Including labor costs, these figures were $107.5 in the chemical control house and $42.49 in the biocontrol house. Our study proved that a biologically-based IPM is not only biologically feasible but also cost effective in the greenhouse gerbera system. A future step would include evaluating this program in multiple greenhouses and conducting workshops for growers so that they are prepared for a change in paradigm in pest control. This idea can then be executed in other cut flower systems where pest complexes are similar.

Through our study, we added to the already existing larger knowledgebase about compatibility of pesticides to natural enemies compiled by Koppert and Biobest. More importantly, it gave us the decision-making criteria to integrate relevant components into a management strategy to control the primary and secondary pests in greenhouse gerberas in a cost-effective and environmentally friendly manner. We have been able to provide research verified recommendations of biologically-based solutions to production impediments in the cut flower system that will benefit our local growers. By equipping growers with this information, a sustainable production system with effective pest management, and less negative environmental impact can be achieved.

APPENDIX

Table A.1. Analysis of variance of number of natural enemies (*D. isaea*, and *N. californicus*) alive 12, 24, and 48 h after chemical treatments.

Treatments	Non-target	Run	Time	f	df	p value	Trt	Rep
Miticides	*D. isaea*	1	12h	31.62	9, 99	< 0.0001	10	10
			24h	20.50	9, 99	< 0.0001	10	10
			48h	23.53	9, 99	< 0.0001	10	10
		2	12h	119.51	9, 99	< 0.0001	10	10
			24h	85.14	9, 99	< 0.0001	10	10
			48h	84.97	9, 99	< 0.0001	10	10
		3	12h	34.49	9, 99	< 0.0001	10	10
			24h	24.88	9, 99	< 0.0001	10	10
			48h	17.46	9, 99	< 0.0001	10	10
	N. californicus	1	12h	13.43	9, 99	< 0.0001	10	10
			24h	13.23	9, 99	< 0.0001	10	10
			48h	12.85	9, 99	< 0.0001	10	10
		2	12h	30.21	9, 99	< 0.0001	10	10

			24h	23.34	9, 99	< 0.0001	10	10
			48h	16.68	9, 99	< 0.0001	10	10
		3	12h	39.37	9, 99	< 0.0001	10	10
			24h	38.70	9, 99	< 0.0001	10	10
			48h	43.56	9, 99	< 0.0001	10	10
Leafminer materials	*D. isaea*	1	12h	30.90	9, 99	< 0.0001	10	10
			24h	28.67	9, 99	< 0.0001	10	10
			48h	27.04	9, 99	< 0.0001	10	10
		2	12h	46.00	9, 99	< 0.0001	10	10
			24h	53.82	9, 99	< 0.0001	10	10
			48h	47.96	9, 99	< 0.0001	10	10
		3	12h	40.11	9, 99	< 0.0001	10	10
			24h	66.47	9, 99	< 0.0001	10	10
			48h	39.45	9, 99	< 0.0001	10	10
	N. californicus	1	12h	49.46	9, 99	< 0.0001	10	10
			24h	38.24	9, 99	< 0.0001	10	10
			48h	46.24	9, 99	< 0.0001	10	10

		2	12h	18.13	9, 99	< 0.0001	10	10
			24h	18.18	9, 99	< 0.0001	10	10
			48h	16.84	9, 99	< 0.0001	10	10
		3	12h	22.43	9, 99	< 0.0001	10	10
			24h	22.02	9, 99	< 0.0001	10	10
			48h	18.32	9, 99	< 0.0001	10	10
Thripicides	*D. isaea*	1	12h	19.38	9, 99	< 0.0001	10	10
			24h	24.89	9, 99	< 0.0001	10	10
			48h	32.47	9, 99	< 0.0001	10	10
		2	12h	34.95	9, 99	< 0.0001	10	10
			24h	29.06	9, 99	< 0.0001	10	10
			48h	31.20	9, 99	< 0.0001	10	10
		3	12h	40.68	9, 99	< 0.0001	10	10
			24h	29.03	9, 99	< 0.0001	10	10
			48h	40.96	9, 99	< 0.0001	10	10
	N. californicus	1	12h	17.21	9, 99	< 0.0001	10	10
			24h	15.12	9, 99	< 0.0001	10	10

			48h	15.04	9, 99	< 0.0001	10	10
		2	12h	20.64	9, 99	< 0.0001	10	10
			24h	27.40	9, 99	< 0.0001	10	10
			48h	32.61	9, 99	< 0.0001	10	10
		3	12h	15.05	9, 99	< 0.0001	10	10
			24h	28.48	9, 99	< 0.0001	10	10
			48h	27.01	9, 99	< 0.0001	10	10
Whitefly materials	*D. isaea*	1	12h	20.35	9, 99	< 0.0001	10	10
			24h	14.13	9, 99	< 0.0001	10	10
			48h	20.07	9, 99	< 0.0001	10	10
		2	12h	34.07	9, 99	< 0.0001	10	10
			24h	21.45	9, 99	< 0.0001	10	10
			48h	24.71	9, 99	< 0.0001	10	10
		3	12h	20.24	9, 99	< 0.0001	10	10
			24h	15.23	9, 99	< 0.0001	10	10
			48h	20.39	9, 99	< 0.0001	10	10
	N. californicus	1	12h	12.19	9, 99	< 0.0001	10	10

			24h	14.11	9, 99	< 0.0001	10	10
			48h	21.70	9, 99	< 0.0001	10	10
		2	12h	20.19	9, 99	< 0.0001	10	10
			24h	22.19	9, 99	< 0.0001	10	10
			48h	24.94	9, 99	< 0.0001	10	10
		3	12h	23.92	9, 99	< 0.0001	10	10
			24h	24.55	9, 99	< 0.0001	10	10
			48h	24.88	9, 99	< 0.0001	10	10
Fungicides	*D. isaea*	1	12h	1.14	9, 99	0.3480	10	10
			24h	2.27	9, 99	0.0255	10	10
			48h	4.92	9, 99	< 0.0001	10	10
		2	12h	3.7	9, 99	0.0006	10	10
			24h	3.74	9, 99	0.0006	10	10
			48h	5.50	9, 99	< 0.0001	10	10
		3	12h	0.69	9, 99	0.7172	10	10
			24h	1.29	9, 99	0.2557	10	10
			48h	1.53	9, 99	0.1511	10	10

N. californicus	1	12h	17.44	9, 99	< 0.0001	10	10
		24h	18.55	9, 99	< 0.0001	10	10
		48h	16.11	9, 99	< 0.0001	10	10
	2	12h	46.29	9, 99	< 0.0001	10	10
		24h	79.09	9, 99	< 0.0001	10	10
		48h	70.13	9, 99	< 0.0001	10	10
	3	12h	11.8	9, 99	< 0.0001	10	10
		24h	18.19	9, 99	< 0.0001	10	10
		48h	40.97	9, 99	< 0.0001	10	10

Table A.2. Analysis of variance and means (±SE) for the residual toxicity of miticides, over a four week period, on average percent of parasitism by *D. isaea.*

Trt (a.i.)	Week 1	Week 2	Week 3	Week 4
Control	95.4±3.1 a	35.0±1.2 a	49.7±9.4 a	89.1±4.4 a
Hexythiazox	89.8±5.2 a	73.6±2.7 a	41.2±7.0 a	100±0 a
Bifenazate	87.7±8.5 a	50.4±1.8 a	42.6±8.3 a	89.2±7.4 a
Etoxazole	87.6±6.5 a	74.6±3.2 a	49.0±3.0 a	76.2±10.4 a
Spiromesifen	86.1±7.3 a	53.0±1.3 a	49.8±14.5 a	78.7±16 a
Acequinocyl	78.7±6.9 a	79.7±9.7 a	44.3±8.7 a	85.1±5.1 a
Clofentezine	69.5±16.3 a	28.5±8.9 a	49.8±5.8 a	86.2±7.4 a
df	6, 41	6, 41	6, 41	6, 41
F value	0.98	1.38	0.22	0.75
P value	0.4557	0.2615	0.9673	0.6145

Table A.3. Analysis of variance and means (±SE) for the residual toxicity of miticides, over a four week period, on average *L. trifolii* population.

Trt (ai)	Week 1	Week 2	Week 3	Week 4
Control	0.28±0.21 a	0.8±0.4 a	5.44±1.6 a	0.56±0.25 a
Hexythiazox	0.28±0.1 a	0.33±0.23 a	5.16±1.15 a	0.0±0 a
Bifenazate	0.22±0.22 a	0.6±0.28 a	7.56±1.3 a	0.39±0.22 a
Etoxazole	0.61±0.25 a	0.2±0.11 a	6.05±1.39 a	0.61±0.22 a
Spiromesifen	0.5±0.37 a	0.4±0.27 a	4.77±1.49 a	0.22±0.11 a
Acequinocyl	0.89±0.25 a	1.44±0.75 a	8.44±2.03 a	0.44±0.14 a
Clofentezine	0.22±0.11 a	1.67±0.67 a	4.72±0.87 a	0.56±0.31 a
df	6, 41	6, 41	6, 41	6, 41
F value	1.27	1.23	0.95	1.15
P value	0.3016	0.3265	0.4774	0.3568

Table A.4. Analysis of variance and means (±SE) for the residual toxicity of miticides, over a four week period, on average population of parasitoid (*D.isaea*).

Trt (ai)	Week 1	Week 2	Week 3	Week 4
Control	2.9±1.1 a	0.7±0.32 a	5.0±1.32 a	3.8±1.33 a
Hexythiazox	3.5±0.78 a	0.7±0.16 a	4.4±1.16 a	3.1±0.58 a
Bifenazate	2.5±0.55 a	0.9±0.22 a	7.3±2.29 a	3.2±0.92 a
Etoxazole	1.9±0.66 a	1.3±0.7 a	5.8±1.89 a	3.1±0.86 a
Spiromesifen	2.6±0.62 a	1.5±0.51 a	3.0±0.98 a	2.6±1.22 a
Acequinocyl	4.3±1.32 a	1.8±0.68 a	6.5±1.48 a	3.1±0.81 a
Clofentezine	1.0±0.48 a	0.7±0.27 a	5.80.74 a	3.6±0.59 a
df	6, 41	6, 41	6, 41	6, 41
F value	1.54	0.78	0.86	0.18
P value	0.1985	0.5938	0.5369	0.9800

Table A.5. Analysis of variance and means (±SE) for the residual toxicity of miticides, over a four week period, on sum of live leafminers in the three sampled leaves from each experimental unit.

Trt (ai)	Week 1	Week 2	Week 3	Week 4
Control	0.833±0.65 a	2.4±1.19 a	16.33±4.82 a	1.67±0.76 a
Hexythiazox	0.833±0.31 a	1.0±0.68 a	15.5±3.46 a	0.0±0 a
Bifenazate	0.667±0.67 a	1.8±0.85 a	22.66±3.95 a	1.16±0.65 a
Etoxazole	1.83±0.75 a	0.6±0.34 a	18.16±4.17 a	1.83±0.65 a
Spiromesifen	1.5±1.12 a	1.2±0.80 a	14.33±4.48 a	0.67±0.33 a
Acequinocyl	2.667±0.76 a	4.3±2.26 a	25.33±6.1 a	1.33±0.42 a
Clofentezine	0.667±0.33 a	5.0±2.0 a	14.16±2.6 a	1.67±0.92 a
df	6, 41	6, 41	6, 41	6, 41
F value	1.27	1.23	0.95	1.15
P value	0.3016	0.3265	0.4774	0.3568

Table A.6. Analysis of variance and means (±SE) for the residual toxicity of miticides, over a four week period, on sum of leafminers live and dead (= # of parasitoids)

Trt (ai)	Week 1	Week 2	Week 3	Week 4
Control	9.67±3.96a	4.8±1.43a	31.33±5.92a	13.0±4.73a
Hexythiazox	11.33±2.6a	3.16±0.70a	28.67±6.41a	9.167±1.74a
Bifenazate	8.167±2.13a	5.0±1.22a	44.67±9.64a	10.67±2.93a
Etoxazole	7.667±2.5a	5.8±2.43a	35.67±9.81a	11.0±2.31a
Spiromesifen	9.33±2.28a	6.4±1.48a	23.33±6.21a	8.33±3.56a
Acequinocyl	15.667±3.86a	9.833±3.32a	44..83±6.71a	10.67±2.23a
Clofentezine	3.667±1.33a	9.5±2.75a	31.67±3.64a	12.5±2.14a
df	6, 41	6, 41	6, 41	6, 41
F value	1.55	1.31	1.17	0.31
P value	0.1964	0.2908	0.3458	0.9276