N O T E

Control of *Liriomyza trifolii* (Diptera: Agromyzidae) in Cut Flowers using *Isaria fumosorosea* (Hypocreales: Cordycipitaceae) Alone and in Combination with Insecticides[1]

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Liriomyza trifolii (Burgess), sometimes referred to as the American serpentine leafminer, readily infests greenhouses, and their larvae feed on numerous crops worldwide including ornamentals and vegetable crops (Parella 1987, Annu. Rev. Entomol. 32: 201 - 224). Damage caused by larval mining significantly reduces photosynthetic capacity of the crops and causes leaf fall during heavy infestations (Johnson et al. 1983, J. Econ. Entomol. 76: 1061 - 1063). Apart from direct damage caused by larval feeding on leaf tissues, females may act as vectors for diseases during oviposition (Matteoni and Broadbent 1988, Can. J. Plant Pathol. 10: 47 - 52).

Control of leafminers is generally by the use of parasitoids (Greathead and Greathead 1992, Biocontr. News Info. 13: 61 - 68; Murphy and LaSalle 1999, Biocontr. News Info. 20: 91N-104N) or chemical insecticides, especially in ornamental plants (Garthwaite and Thomas 2001, *In* Pesticide Usage Survey Report 164: Protected Crops (Edible and Ornamental) of Great Britain in 1999. Department of Environment, Food and Rural Affairs, London, UK). Lack of effective insecticides or the limitations on their use for the control of leafminers together with the repetitive release of large numbers of parasitoids have proven to be economically nonviable to most growers. Therefore, new approaches are necessary to reliably achieve control. The use of entomopathogens is promising because of their potential compatibility with other control measures (Rovesti and Deseo 1990, Nematol. 36: 237 - 245).

Entomopathogenic fungi hold great potential for use in greenhouse ecosystems; however, studies using fungal entomopathogens for control of leafminers in these crop systems are rare (Villacorta 1983, Entomophaga 28: 179 - 184; Samek et al.

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2006, J. Forest Sci. 52: 136 - 140). However, the potential use of entomopathogenic fungi for controlling leafminers has been demonstrated in other cropping systems. For instance, *Lecanicillium lecanii* (=*Verticillium lecanii*) (Zimmerman) Viegas was found to parasitize and cause up to 40% mortality of overwintering pupae of the horse-chestnut leafminer, *Cameraria ohridella* Deschka et Dimić (Samek et al. 2006). In addition, *Metarhizium anisopliae* (Metsch.) Sorokin was considered a potential bio-control agent for ground nut leafminer, *Aproaerema modicella* Deventer (Rao and Reddy 1997, Intl. Arachis Newsletter 17: 48 - 49) and the coffee leafminer, *Perileucoptera coffeella* Guérin-Méneville (Villacorta 1983). Some reports document fungal endophytes that act as natural toxins against leafminers (Faeth and Hammon 1996, Oecologia 108: 728 - 736; Gaylord et al. 1996, Oecologia 105: 336 - 342; Preszler and Gaylord 1996, Oecologia 108: 159 - 166), but they are yet to be exploited for use in leafminer control on a commercial scale. Most recently, *Isaria fumosorosea* Wize was isolated from infected hibernating pupae of the horse-chestnut leafminer (Zemek et al. 2007, Entomol. Res. 37: A135 - 136). However, no study has been conducted using fungal pathogens for control of *L. trifolii* in cut flowers. Therefore, this study compared the relative effectiveness of the fungus, *I. fumosorosea* strain Apopka-97 alone and in combination with pesticides to control *L. trifolii* on gerbera daisies and sunflowers.

The fungus *I. fumosorosea* used in this study is licensed to Certis USA, LLC (Columbia, MD) and has been developed as a biopesticide under the trade name *PFR* 97™. The treatments for the trial were *PFR* 97™ alone and in combination with pesticides, or pesticides only, followed by an assessment of the number of adult insects captured on yellow traps and the number of leaf mines after application in gerbera daisies, *Gerbera jamesonii* Bolus ex Hooker f. and sunflowers, *Helianthus annuus* L. The experiments were conducted during the fall of 2008 at Sunshine State Carnations, Inc. (Hobe Sound, FL) on gerbera and sunflower plants. The treatments were replicated 3 times and conducted in individual open-air greenhouses designated as spray / control (where only pesticides were applied), *PFR* 97™ (fungus alone), and *PFR* 97™ / spray (both fungus and pesticides). In the control houses, a grower standard spray program was followed in the application of pesticides. The active ingredients of pesticides used by the grower on an alternating spray schedule included 4 fungicides (triflumizole, pyraclostrobin, chlorothanil and azoxystrobin), 4 insecticides (acephate, abamectin, thiamethoxam and pymetrozine), 2 insect growth regulators (novaluron and pyriproxyfen), 1 bioinsecticide (Dipel™ – *Bacillus thuringiensis kurstaki*) and 1 fertilizer / repellent (sincocin). The houses designated *PFR* 97™ alone had a single application of the fungus, whereas the houses with *PFR* 97™ / spray followed the normal pesticide spray schedule after a single application of *PFR* 97™. Treatments were applied using a completely randomized block design with each house (block) having 4 rows and each treatment per crop had 3 blocks. For gerbera, 1 row consisted of 150 plants whereas sunflower had 900 per row. The pesticides were applied sequentially at intervals in quantities following label recommendations from the manufacturer, and *PFR* 97™ was applied at the rate of 2.0×10^7 blastospores / mL. All pesticides and *PFR* 97™ were applied using a Hahn® handgun sprayer.

Yellow sticky traps (3 × 5 Scout Sticky Trap™, OHP, Mainland, PA) stuck in the soil at a similar height (12 cm) were used to evaluate treatment effects by determining the number of adult flies that were present 7, 14, 21 and 28 d post treatment. The total number of flies captured on 12 sticky traps per treatment (2 placed at the end of a row) in the gerbera daisies and sunflowers during each evaluation period served to indicate their relative abundance (Musgrave et al. 1975, Proc. Florida State Hortic. Soc.

88: 156 - 160; Zoebish and Schuster 1990, Florida Entomol. 73: 505 - 507). Baseline data of flies captured on the sticky traps were used to determine the abundance of adults before application of treatments. Trap fly data were recorded every 7 d for 4 wks to determine treatment effects on the number of adults. The numbers of leaf mines were counted after 4 wks from 60 randomly selected excised leaves for each treatment.

The mean number of flies captured from the sticky traps and leaf mines as a result of damage from the leafminer larvae were normalized by log transformation and analyzed by one-way ANOVA (PROC GLM, SAS 1998, Cary, NC) with treatments and crop type as factors. The Duncan Multiple Range Test (DMRT) was used to determine if differences existed between treatment means ($P \leq 0.05$).

Results showed that a lower number of adult flies were captured in sunflower using the sticky traps as compared with gerbera (Tables 1, 2). It was not established why the leafminer fly population was higher in gerbera, but the difference in the phenology and morphology of the 2 crops could be an important factor. Gerbera leaves are arranged in a rosette pattern close to the ground and are succulent, sometimes producing a closed canopy that may provide favorable conditions for the flies to reproduce as well as escape from spray application.

None of the spray treatment applications were significantly different ($F = 0.75$; df = 4, 25; $P = 0.5674$) throughout the 4-wk evaluation period in sunflower (Table 1). In gerbera, the number of adult flies significantly decreased with time for the pesticides ($F = 4.51$; df = 4, 25; $P = 0.0070$); *PFR* 97™ alone ($F = 6.90$; df = 4, 25; $P = 0.0007$) and *PFR* 97™ in combination with the pesticides ($F = 9.85$; df = 4, 25; $P = 0.0001$), an indication that the population may have declined as a result of treatment application in this crop (Table 2). However, there were no differences in the abundance of adult flies from 7 - 28 d post treatment between pesticides, *PFR* 97™ alone and the combination treatments; suggesting that the use of pesticides or a combination of pesticides and *PFR* 97™ should be reconsidered because the fungus alone offers equal control and subsequently may reduce production costs.

Overall, the population of adult flies in gerbera declined with time reaching very low levels that were comparable with sunflower, 28 d after application of *PFR* 97™ (Table 2). This reduction in the number of adult flies captured on the sticky cards could be associated with a pathogenic effect of the fungus on the larval stages resulting in very few leafminers emerging as adults. Given that at maturity, the larvae penetrate the leaf surface and drop to plant debris or to the soil to pupate, this behavior might facilitate

Table 1. The mean number of *Liriomyza trifolii* leafminer adult flies captured on sticky traps in sunflower before and after pesticide applications, *PFR* 97™ alone and a combination of *PFR* 97™ and pesticides.

	Mean number of adult leafminers ± SEM days post treatment*			
Treatment	7	14	21	28
Pesticides	0.17 ± 0.00aA	0.17 ± 0.17aA	0.17 ± 0.17aA	0.17 ± 0.17aA
PFR 97 alone	0.17 ± 0.00aA	0.17 ± 0.17aA	0.17 ± 0.17aA	0.00 ± 0.00aA
PFR 97 + pesticides	0.00 ± 0.17aA	0.50 ± 0.34aA	0.17 ± 0.17aA	0.00 ± 0.00aA

*Mean values within columns bearing the same lowercase letter and across rows bearing the same uppercase letter are not significantly different (Duncan Multiple Range test, $P \leq 0.05$).

Table 2. The mean number of adult *Liriomyza trifolii* leafminer flies captured on sticky cards in gerbera before and after pesticide applications, *PFR* 97™ alone and a combination of *PFR* 97™ and pesticides.

	Mean number of adult leafminers ± SEM days post treatment*			
Treatment	7	14	21	28
Pesticides	3.00 ± 0.89aB	5.17 ± 0.08aA	0.33 ± 0.21aB	0.17 ± 0.17aB
PFR 97 alone	5.00 ± 1.6aA	6.00 ± 0.51aA	0.33 ± 0.21aB	0.33 ± 0.21aB
PFR 97 + Pesticides	7.00 ± 1.3aA	5.00 ± 0.93aA	0.17 ± 0.17aB	0.17 ± 0.17aB

*Mean values within columns bearing the same lowercase letter and across rows bearing the same uppercase letter are not significantly different (Duncan Multiple Range test, $P \leq 0.05$).

their infection with the fungus which may lead to low emergence and could explain why low numbers of adult *L. trifolii* flies were captured on the sticky traps over time. In support of this hypothesis, horse-chesnut leafminer hibernating pupae in the soil were observed to be mycosed with *I. fumosorosea* (Zemek et al. 2007).

On ornamentals, the unsightly appearance of mines always reduces the value of these crops substantially, which has led to rigorous pesticide spray schedules. As a result of the substantial economic losses caused by *Liriomyza* leafminers, as well as their ability to rapidly develop resistance to insecticides, the results obtained in this study are very promising. The fungus *I. fumosorosea* could be a suitable intervention under greenhouse conditions which are ideal for implementation of biological control programs.

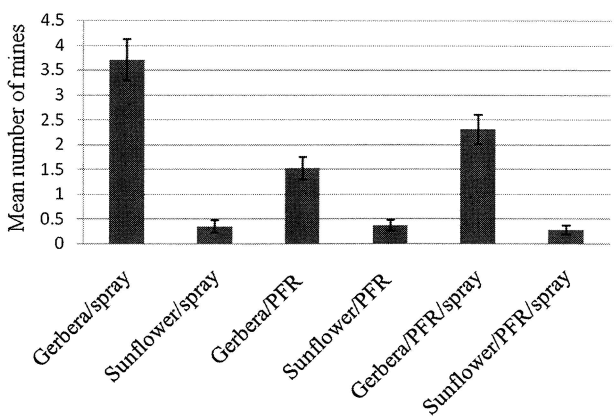

Fig. 1. Mean number of mines caused by *Liriomyza trifolii* counted in sunflower and gerbera leaves for each treatment 28 days post spray. Bars represent the mean number of mines ± SEM per treatment of each flower type (n = 60; DMRT, $P < 0.05$). Spray = pesticides.

Results show that the number of leaf mines in gerbera where *PFR* 97™ was applied were 2 times less than where pesticides were used (Fig. 1). Unexpectedly, a combination of *PFR* 97™ and pesticides application also had a higher number of mines probably indicating incompatibility with the pesticides used. Some of the fungicides on the spray schedule that were applied after application of *PFR* 97™ such as azoxystrobin, had previously been reported to slightly inhibit its fungal growth (Sterk et al. 2002, *In* Intl. Symp. Biol. Contr. Arthropods, Honolulu, HI. pp. 306 - 313; Aerts et al. 1997, Med. Fac. Agric. Sci. Univ. Ghent 62: 581 - 588). Therefore, there is a need to determine which pesticides are compatible with *PFR* 97™ to optimize its performance, especially in high value ornamental crops with low economic injury levels. A combination of control strategies including early detection of adult leafminers using the yellow sticky traps as a monitoring technique and application of the fungus, *I. fumosorosea* could lead to improved leafminer population management. This strategy may also slow pesticide resistance through reduced insecticide application frequencies or removal of resistant individuals.

The reduction in the number of leaf mines where *PFR* 97™ was applied demonstrates that incorporation of a biological control agent in pest control programs, especially in high value crops that are frequently sprayed, may minimize resistance problems, promote activity of other natural enemies and possibly lower production costs. *PFR* 97™ is sold for control of ornamental and greenhouse pests in Europe and Japan (Faria and Wraight 2001, Crop Prot. 20: 767 - 778), and although it lacks label approval in fruits and vegetables in the USA (label expected in 2011), it offers great promise for use in greenhouse conditions and may give more satisfactory results in leafminer control than most insecticides used for the control of this pest.

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