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Investigating the Role of Fungal Entomopathogens in Whitefly Landscape IPM Programs[1]

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The rugose spiraling whitefly, *Aleurodicus rugioperculatus* Martin, and Bondar's nesting whitefly, *Paraleyrodes bondari* Perracchi, are two recently introduced whitefly species affecting several plant hosts in Florida. Native to Central America, rugose spiraling whitefly was first detected in the Continental United States from Miami–Dade County, FL, in 2009 (Stocks and Hodges 2012, Pest Alert, DACS-P-01745). Since then its distribution range has expanded rapidly, and it has become a serious pest in residential, commercial, and municipal landscapes (Kumar et al. 2013, http://edis.ifas.ufl.edu/pdffiles/IN/IN101500.pdf). Rugose spiraling whitefly has been reported affecting more than 100 plant species (Stocks 2012, FDACS-DPI report. 6 p.) including landscape (coconut palm, black olive, gumbo limbo, weeping fig, live oak, rose, and sabal palm), agriculture (citrus, mango, avocado, and sugarcane), and natural areas (sabal palm, live oak, coconut palm, Brazilian pepper, and Virginia creeper). This insect has the potential for spreading into the northern parts of the State and beyond. Rugose spiraling whitefly reproduces throughout the year with multiple, overlapping generations.

Endemic to Brazil, Bondar's nesting whitefly was first reported from a ficus hedge in Lee County, FL, in 2011. Since then it has been found affecting at least five plant species in seven Florida counties (Stocks 2012, FDACS-DPI report. 6 p.). Feeding by these whiteflies causes stress to host plants resulting in premature leaf drop. Furthermore, the excessive production of wax and honeydew creates an enormous nuisance in infested areas. Black sooty mold fungi can then grow on the honeydew, reducing aesthetics of plants in the landscape.

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Whitefly management programs rely heavily on the use of traditional insecticides, both contact as well as systemic. Currently there are 32 registered insecticides and only one microbial product available for professional and homeowner use against whiteflies in Florida (Mannion 2013, http://trec.ifas.ufl.edu/mannion/pdfs/Rugose spiraling whitefly.pdf). Inappropriate and excessive applications of traditional insecticides harm natural enemies, both predators and parasitoids, and may lead to potential concerns for groundwater contamination, especially with soil-applied systemics. Also, the high risk of resistance developing in these pest species due to continuous use of chemical insecticides cannot be ignored.

Effective use of entomopathogens against several pest whiteflies has been reported by many researchers. *Isaria fumosorosea* Wize has been shown to be effective against different whitefly pests including *Trialeurodes vaporariorum* Westwood, *Bemisia tabaci* Bellows & Perring, and *A. dispersus* Russell (Sanchez and Castillo 2008, Biological Assessment Dossier, EPPO Guidelines, Commission of the European Communities, DOC. 7600/VI/95 rev. 6, 33 p.; Zimmermann 2008, Biocontrol Sci. Technol., 18: 865–901). This fungal entomopathogen also reduced egg hatch of *A. dispersus* by 50% (Sanchez and Castillo 2008). Both *I. fumosorosea* and *Beauveria bassiana* (Balsamo) Vuillemin were shown to reduce *B. tabaci* populations infesting poinsettias when compared to untreated check plants (Oetting 2006, http://mrec.ifas.ufl.edu/lso/DOCUMENTS/SLWF05-14%20Q%20biotype%20IR4%20funded.rpt.pdf; Oetting 2008, http://ir4.rutgers.edu/Ornamental/SummaryReports/WhiteflyDataSummary2014.pdf, p. 22.) In a recent study McKenzie et al. (2014, Pest Manag. Sci., 70: 1573–1587) reported *I. fumosorosea* provided about 51–73% reduction in *B. tabaci* adults, and *Metarhizium anisopliae* (Metchnikoff) Sorokin suppressed whitefly immatures 54–79% during different sampling weeks. Avery et al. (2011, Florida Entomol. 94: 696–698) found *I. fumosorosea* naturally infecting another whitefly pest, the fig whitefly *Singhiella simplex* (Singh), in a ficus hedge in St. Lucie County, FL. All these studies suggest that mycoinsecticides could be an efficient tool for at least short-term management of whitefly pests. In addition, there is no published information available on the negative impact of these products on natural enemies of whiteflies which include specific parasitoids (i.e., *Encarsia* sp.) and general predators (i.e., lady beetles, phytoseiid mites) when released several days post-application. Taravati et al. (2013, http://edis.ifas.ufl.edu/in1004) mentioned several natural enemies that have been observed attacking rugose spiraling whitefly including parasitoids (*Encarsia* sp., *Aleuroctonus* sp.) and predators (*Nephaspis* sp., *Chrysoperla* sp.); however, efficacy of these natural enemies against this whitefly still needs to be evaluated. In the current study, we evaluated the role of three fungal entomopathogens—*I. fumosorosea*, *B. bassiana*, and *M. anisopliae*—as tools in rugose spiraling whitefly management programs to preserve natural enemies and reduce the environmental load of chemical pesticides. Fungal entomopathogens selected were evaluated alone and with reduced rates of the standard neonicotinoid insecticide imidacloprid. This is the first attempt wherein three commercially available mycoinsecticides have been integrated in a pest management program for these two invasive whitefly species.

The study was conducted in two phases. Phase I was initiated in July 2013 and completed in November 2013 at the Davey Tree Expert Company facility in Naples, FL, using coconut (*Cocos nucifera* L.) plants grown in 27-L containers. Potting media consisted of 50% Florida peat, 30% 1.9-cm pine bark, and 20% cypress duff. Plants were inoculated three times with rugose spiraling whiteflies by placing naturally infested fronds among the containers over a 13-week period. Pre- and post-treatment counts of the masses of immature whitefly stages (nymph and egg) were taken from two fronds of similar size per plant. Treatments were arranged in a randomized complete block design with four replications. Post-treatment counts were taken 21 d after treatment (DAT). Treatments containing purified water were applied to runoff with hand-pressurized sprayers at 35–40 pounds per square inch (psi) (Table 1). Purified water was used as a control. Plants were watered as needed during the absence of precipitation.

In order to confirm the success of mycoinsecticides in regulating rugose spiraling whitefly, a second phase of the study was initiated in February 2014 and completed in April 2014. Landscape coconut trees growing on a golf course in Sanibel, FL, were used. Pre- and post-treatment counts were taken from 20 randomly selected leaflets per frond. Treatments were arranged in a completely randomized design with eight replications. Post-treatment counts were taken 30 d and 60 d after the initial application. Treatments were applied to wetness with a power backpack sprayer at 35–40 psi (Stihl Industries, Norfolk, VA). Purified water was used with all treatments as listed in Table 1.

In both phases, the fungal entomopathogens were either applied alone or with a reduced rate of imidacloprid. When applied alone, two foliar treatments were made 7 d apart. When applied with a reduced rate of imidacloprid, the latter was soil-drenched while the entomopathogen was foliar applied. The combination treatments were an attempt to evaluate the collective efficacy and pursuant reduction in overall use of imidacloprid. There were a total of 10 treatments: (1) NoFly + imidacloprid; (2) NoFly alone; (3) Preferal + imidacloprid; (4) Preferal alone; (5) BotaniGard + imidacloprid; (6) BotaniGard alone; (7) Met52 + imidacloprid; (8) Met52 alone; (9) Imidacloprid alone; (10) Untreated Check.

Fungal entomopathogen viability in the commercial preparations was determined at the University of Florida, Indian River Research and Education Center in Ft. Pierce, FL, and ranged from 85–91% for both studies. In order to confirm infection of specific entomopathogenic fungi to the whitefly immatures, samples of coconut leaflets with infected nymph masses were collected, cooled, and shipped overnight to the Crop Bioprotection Research Unit, USDA, in Peoria, IL.

Number of immature whiteflies was subjected to analysis of variance (ANOVA) after being square root transformed, and treatment means were separated by using the least significant difference (LSD) test at $\alpha = 0.05$. The data presented are the untransformed means. Because main effect of treatment $\times$ time was significant, separate ANOVAs were conducted for each sampling period. Treatment effects that were significant had means separated by the LSD test at $\alpha = 0.05$. Because the number of whitefly immatures in the efficacy trial was not uniform, Henderson-Tilton's formula was used to calculate corrected mortality. All statistical tests were conducted using PROC GLM procedures of the Statistical Analysis Systems (PROC GLM, SAS Institute, Cary, NC).

In Phase I, all treatments resulted in significant reduction of rugose spiraling whitefly population compared to the control at 21 DAT ($F = 5.17$; df $= 4, 30$; $P < 0.0001$) (Fig. 1). No differences with increased mortality were noticed when imidacloprid was combined with the fungal entomopathogen versus when the entomopathogenic agent was used

Table 1. Formulated pesticides evaluated in coconut palm landscape trials against rugose spiraling whitefly and Bondar's nesting whitefly in Naples and Sanibel, FL.

Pesticide Active Ingredient	Trade Name	% Active Ingredient (w/w)	Manufacturer	Rate per 100 L	Application* Method	IRAC**
*Isaria fumosorosea**** strain FE 9901	NoFly WP	18	Natural Industries	0.5 kg	F	NA
*Isaria fumosorosea**** Apopka 97 strain ATCC 20874	Preferal WP	20	SePRO	0.2 kg	F	NA
Beauveria bassiana Strain GHA	Botanigard (Mycotrol) ES	10.9	BioWorks, Inc.	253 ml	F	NA
Metarhizium anisopliae Strain F52	Met52 EC	11	Novozymes	127 ml	F	M
Imidacloprid	Merit 2F	21.4	Bayer Environmental Sciences	****	D	4a

* D=drench; F=foliar; foliar treatments were applied as a full-coverage foliar spray using a compressed air hand-held sprayer at 35–40 psi. Mycoinsecticides were mixed 5 min prior to application with regular agitation to insure the solution was thoroughly dissolved. Drenches were applied by pouring 3,785 ml of the solution evenly around the base of the tree.

** Insecticide Resistance Action Committee (IRAC) classification.

*** *Isaria fumosorosea* (= *Paecilomyces fumosoroseus*).

**** Imidacloprid applied alone at 20 ml/m of plant height (Phase I) or 2.4 ml/cm of diameter at breast height (dbh) (Phase II). Applied with an entomopathogen at 10 ml/m of plant height (Phase I) or 1.2 ml/cm dbh (Phase II).

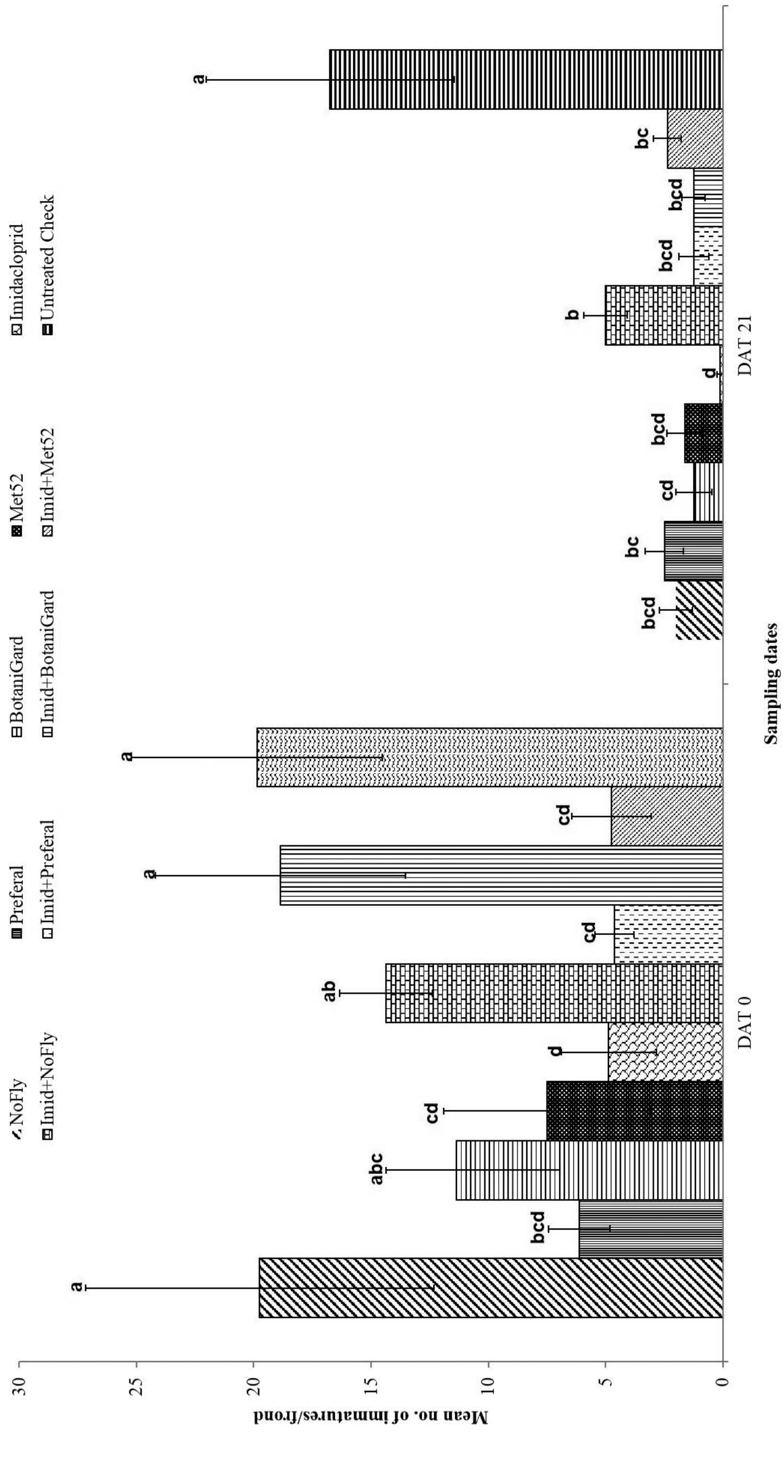

Fig. 1. Effects of selected fungal entomopathogens against the rugose spiraling whitefly infesting potted coconuts at various days after treatment (DAT), Naples, FL, Fall 2013. The bars represent the mean ± SEM for all treatments and the letters that are different above the bars indicate significance ($P < 0.05$, LSD test).

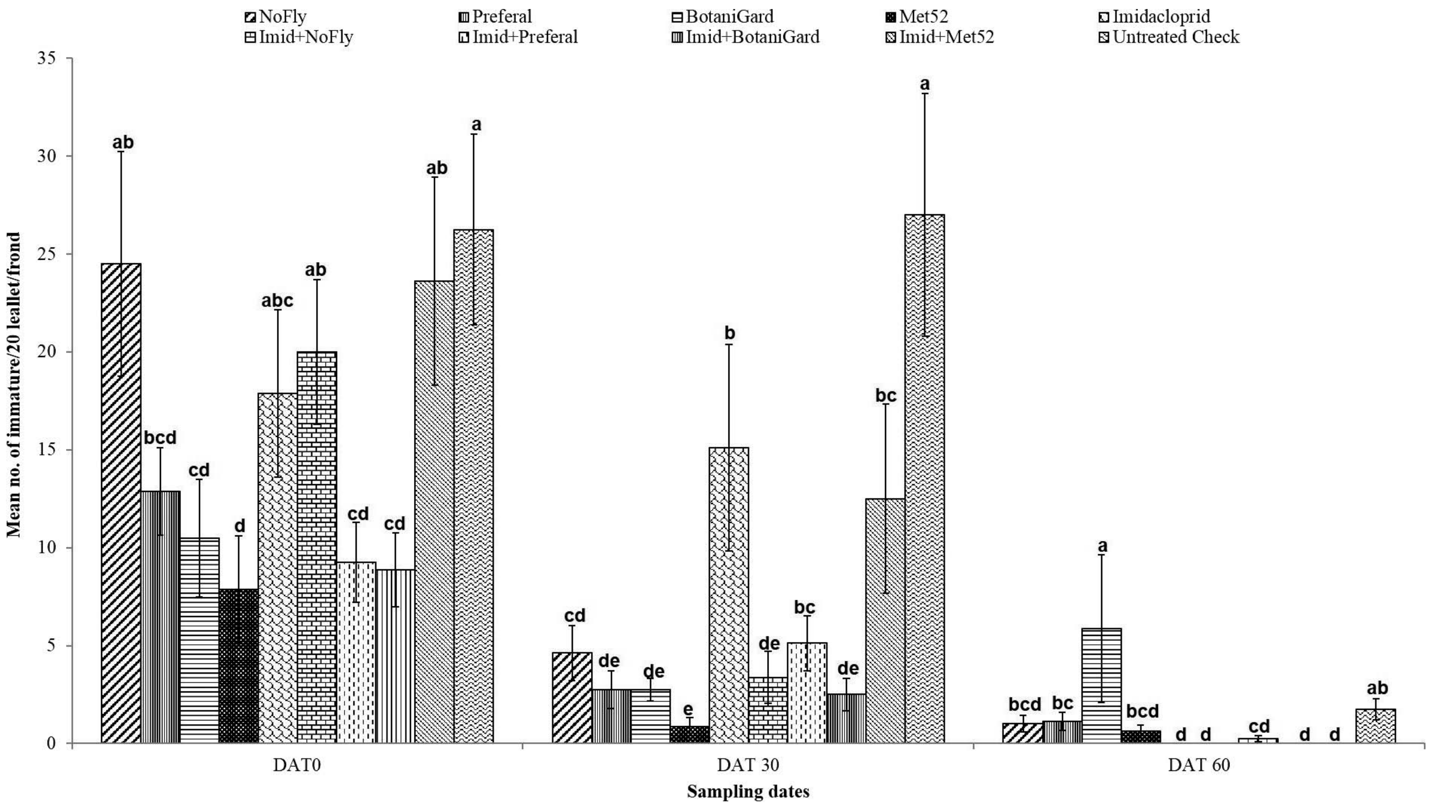

Fig. 2. Effects of selected fungal entomopathogens against whiteflies (rugose spiraling whitefly and Bondar's nesting whitefly) infesting landscape coconuts at various days after treatment (DAT), Sanibel, FL, Spring 2014. The bars represent the mean ± SEM for all treatments and the letters that are different above the bars indicate significance (*P* < 0.05, LSD test).

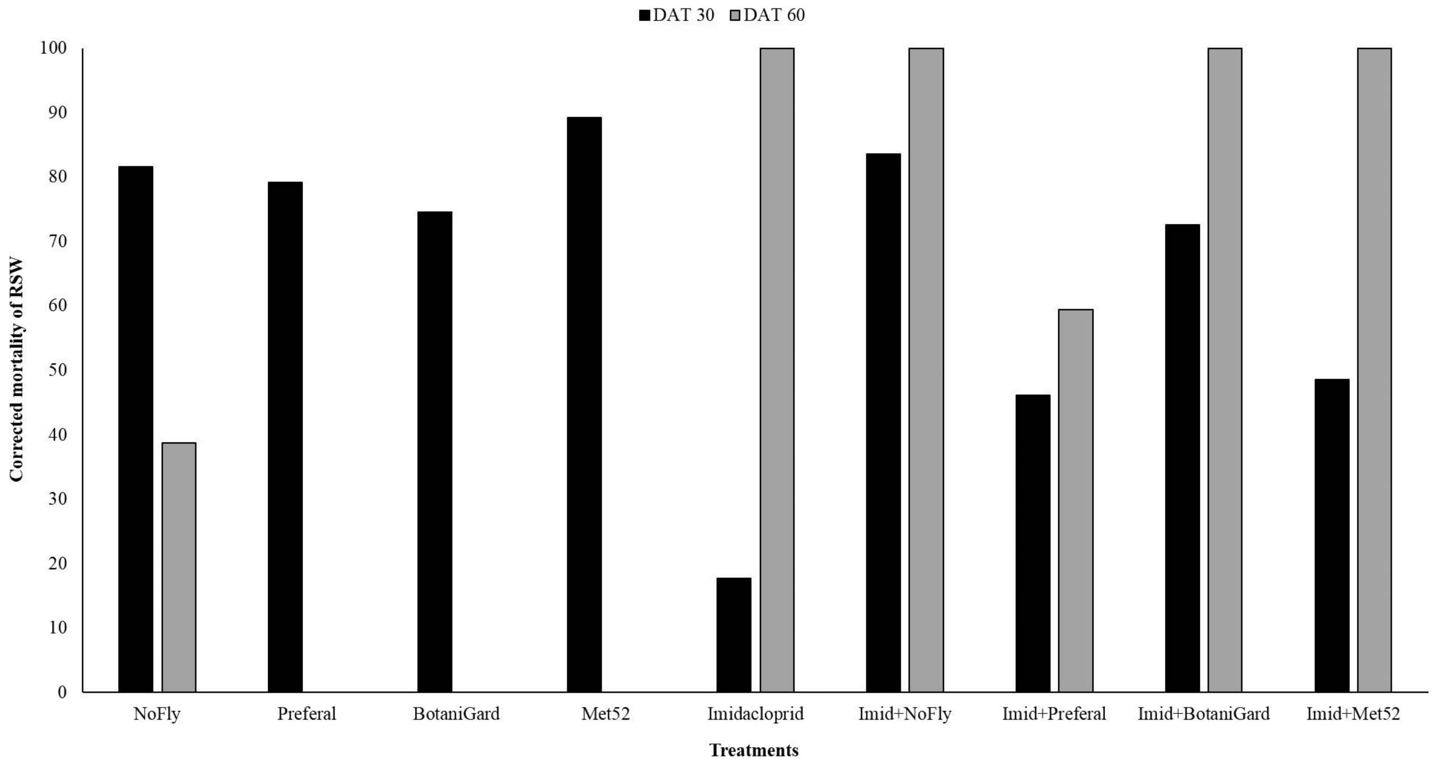

Fig. 3. Corrected percent mortality of whitefly nymphs (rugose spiraling whitefly and Bondar's nesting whitefly) infesting landscape coconuts using Henderson-Tilton's formula; the two series represent values taken 30 d and 60 d after treatment (DAT), respectively, Sanibel, FL, Spring 2014.

alone. Evaluation of the pest populations was conducted again at 42 DAT; however, the numbers were too low for meaningful results.

In Phase II, mostly rugose spiraling whitefly and some Bondar's nesting whitefly were noticed infesting the coconut trees; therefore, nymph and egg mass counts of both species were combined for data analysis. At 30 DAT, all treatments provided significant reduction in whitefly populations compared to the control ($F = 4.50$; df $= 9, 70$; $P < 0.0001$) (Fig. 2). At 60 DAT, there was a slight nymphal increase in the *B. bassiana* treatment; however, a significant reduction in whitefly populations compared to the control was observed in trees treated either with imidacloprid alone or in combination with entomopathogens ($F = 4.92$; df $= 9, 70$; $P < 0.0001$). Corrected nymphal mortality at 30 DAT and 60 DAT is shown in Fig. 3. During the first sampling among all treatments, mortality of whitefly life stages varied from between 17% (imidacloprid) to 89% (*M. anisopliae*). Effectiveness of fungal entomopathogens was found to be reduced by 60 DAT except for *I. fumosorosea* (strain FE9901; 38% mortality); however, none of the other three mycoinsecticides showed activity against whitefly populations. All the imidacloprid-treated trees, either alone or in combination with mycoinsecticides, showed 100% mortality except for the combination treatment (imidacloprid + *I. fumosorosea* [strain ATCC 20874]) where 59% mortality was observed. Overall population levels were lower at 60 DAT, likely due to natural enemies, especially parasitoids, because many nymphal and pupal cases were observed with parasitoid exit holes. Over these several months in southeast Florida, naturally occurring beneficials (both predators and parasitoids) appeared to be reducing rugose spiraling whitefly populations (C. M. Mannion, pers. comm.). This trend may have been occurring in southwest Florida where these trials were conducted as well, given the high mortality in the control. Thus, in order to develop a sustainable management program against these invasive whitefly species, it is important to safeguard natural enemies with the use of compatible insecticides.

In Phase I, temperature ranged from 15–33°C, while relative humidity ranged from 45–100%, whereas in Phase II, temperature ranged from 12–34°C while relative humidity ranged from 52–100%. In both phases of the study, fungal entomopathogens were effective in reducing the whitefly population comparable to the imidacloprid-treated plants until 21 DAT for Phase I and until 30 DAT for the Phase II study. Overall, in both trials, the high relative humidity appeared to have aided in the establishment, persistence, and efficacy of the fungal entomopathogens. In the landscape setting (Phase II), the combination treatments with a reduced rate of imidacloprid seems to have provided control slightly better than the entomopathogens alone. Our preliminary results suggest that fungal entomopathogens may be a useful tool for short-term control of the rugose spiraling whitefly and Bondar's nesting whitefly. In order to achieve long-term pest suppression, entomopathogenic fungi may be successfully integrated with a chemical control strategy. Benefits of such a strategy would include reduced environmental load of neonicotinoids, delayed onset of resistance in the pest populations, and a safer habitat for naturally occurring beneficial organisms.

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