Attraction, Repellence, and Predation: Role of Companion Plants in Regulating *Myzus persicae* (Sulzer) (Hemiptera: Aphidae) in Organic Kale Systems of South Texas

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Abstract.   Annual outbreaks of green peach aphid, *Myzus persicae* (Sulzer), limit commercial production of brassica crops, especially in organic systems in subtropical areas such as South Texas. We assessed the influence of companion plants that emit attractant, repellent, and natural enemy-attractant stimuli (i.e., insectary plants) on abundance of green peach aphid in organic kale (*Brassica oleracaea* L. var. acephala) fields in South Texas.   We also monitored the assemblage of arthropod predators and parasitoids on kale and evaluated the response of numerically dominant species to companion plants. Indian mustard (*Brassica juncea* (L.) Vassiliĭ Matveievitch Czernajew; attractant), dill (*Anethum graveolens* L.; repellent), fennel (*Foeniculum vulgare* Mill.; repellent), and buckwheat (*Fagopyrum esculentum* Moench; insectary) were interplanted in 0.4-ha fields of organic kale varieties 'Lacinato' and 'Vates'. Manual, sticky-trap, and pitfall sampling were used to repeatedly assess green peach aphids and arthropod natural enemies on kale immediately neighboring test companion plants. Numerically dominant (more than 60% of all individuals) natural enemies were *Pterostichus* sp. in pitfall samples, and larval and adult convergent lady beetle, *Hippodamia convergens* (Guérin-Méneville), in manual, sticky trap, and pitfall samples. The presence of nearby attractant, repellent, insectary plants did not significantly alter abundance of green peach aphid or numerically dominant natural enemy species on kale plants. Our findings might indicate limited potential of companion plants in regulating aphids in South Texas, but we make recommendations for future research that considers spatial relationships between crops and companion plants and effects on aphids and natural enemies.

Introduction

The green peach aphid, *Myzus persicae* Sulzer (Hemiptera: Aphidae), is a serious threat to organic brassica production in the Lower Rio Grande Valley of South Texas (Liu and Sparks Jr. 2001). Direct feeding and honeydew excretion causes extensive cosmetic damage and reduces overall plant productivity in many cruciferous vegetables. The ability of green peach aphid to vector more than 100 plant viruses poses a greater problem to brassica production, because transmission of potyviruses can generate severe economic losses and potential crop failure (Hill

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1987, Wang et al. 1996, Blackman and Eastop 2008). Efforts to regulate green peach aphid in the Lower Rio Grande Valley are complicated by persistent warm and semi-humid weather of the South Texas subtropics that permits continuous parthenogenetic reproduction and rapid proliferation (e.g., as many as 30 generations a year) (Devonshire et al. 1998). The dominance of conventional agriculture in South Texas also means organic producers contend with more pests because increasing agricultural intensification can drive herbivorous pests to congregate and flourish in crops not treated with synthetic pesticides (Bianchi et al. 2006, Thies et al. 2011, Winqvist et al. 2011, but see Rand et al. 2014).

Agroecosystem diversification has been associated with reduction in numbers of many herbivorous pest species, including aphids (Altieri et al. 1990, Parajulee et al. 1997, Landis et al. 2000). While the nature of the relationship between biodiversity and pest suppression remains somewhat equivocal, there is ample evidence that increasing functional diversity within managed systems can limit pest abundance and decrease the possibility of outbreaks (Swift and Anderson 1994, Wilby and Thomas 2002, Gurr et al. 2003). One such prominent functional diversification strategy is by adding secondary hosts or companion plants that alter interactions between neighboring crops and associated assemblages of pests and natural enemies (Parolin et al. 2012).

The spatial integration of a companion plant species that dissuades pests away from a susceptible crop (repellent) with another species that draws pests (attractant) is the underlying mechanism behind *push-pull* approaches to pest management (Brewer and Elliott 2004, Zhou et al. 2013). While system-specific factors such as pest mobility and diet complicate the design and incorporation of push-pull technologies in agroecosystems, this approach has produced spectacular successes in natural pest control in tropical and subtropical regions of the world (Pyke et al. 1987, Cook et al. 2006, Fathipour and Sedaratian 2013). In Kenya, for example, push-pull strategies using attractant Napier grass (*Pennisetum purpureum* Schumach.) and repellent *Desmodium* sp. in maize (*Zea mays* L.) effectively suppressed economically important *Chilo partellus* (Swinhoe) and improved yields by 230% (Khan and Pickett 2004, Khan et al. 2008). Another type of companion plant used extensively in conservation biological control is insectary plants that attract generalist predatory and parasitic arthropods through nectar and pollen provisions (Barbosa 1998). While the underlying assumption of conservation biological control that enhanced numbers of naturally-occurring generalist natural enemies provide effective pest management is still unclear, the incorporation of insectary plants within fields and along field margins has been shown to substantially increase parasitism and mortality of multiple species of aphids (Brennan 2013, Balzan and Moonen 2014).

In South Texas, little is known of the role of companion plants in managing green peach aphid in organic brassica. Our objective was to assess the influence of intercropped Indian mustard (*Brassica juncea* (L.) Vassiliĭ Matveievitch Czernajew), dill (*Anethum graveolens* L.), fennel (*Foeniculum vulgare* Mill.), and buckwheat (*Fagopyrum esculentum* Moench) on abundance of green peach aphid and generalist predator and parasitoid species in organic kale (*Brassicae oleracaea* L. var. acephala). Indian mustard is an aphid attractant, while dill and fennel have aphid-repellent traits (Srinivasan and Krishna Moorthy 1992, Singh and Kothari 1997, Bender et al. 1999, Ramalho et al. 2012). Nectar and pollen from buckwheat plants attract and can improve establishment of multiple species of aphid natural

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enemies in crops, including coccinellid predators (Smith 1971, Lee et al. 2004, Tylianakis et al. 2004, Spellman et al. 2006).

Materials and Methods

In January 2014, kale varieties 'Lacinato' and 'Vates' were planted in neighboring plots (approximately 16,000 and 21,100 m^2) on an organic farm at Lyford, TX (Willacy County; latitude 26°22'4.42"N, longitude 97°55'1.51"W). All seeds and inputs were from organically certified sources, and management practices were compliant with USDA organic standards. Kale rows were approximately 1 m wide and contained a double bed of plants, with 25 cm between plants in a row and 1.1 m between plants across rows. In each plot, one attractant plant (Indian mustard), two repellent plants (dill and fennel), and one insectary plant were intercropped every 15 rows in a randomized complete block design (three replications per plot). Each block had a check grid with no other kind of plant between kale plants. Each treatment was planted in a 3 m x 3 m grid of companion plant strips, with five plants in each 30-cm strip. Companion plant strips were planted between the kale double beds in early February (Fig. 1). Within and across blocks, treatment grids were separated by 15 m. After planting, treatment crops were hand watered every 2 weeks, and both plots were flood irrigated in early April.

Abundance of green peach aphids and arthropod natural enemies in treatment grids were sampled twice during the growing season. Vates kale was sampled on 24 March and 24 April, and Lacinato on 3 and 10 April 2014. Green peach aphid and natural enemies on foliage were manually collected on central kale plants in each treatment grid (Fig. 1). On each sample date, a single plant (on the west-facing bed on the first date and on the east-facing bed on the second date) was inspected for 1 minute. Fine-tip brushes were used to place aphids and all foliar arthropod individuals into vials containing 70% ethanol. Maximum plant height (i.e., distance from the base of the plant to upper boundary of photosynthetic tissues) of all sampled kale plants was measured on each sampling date.

To assess the aerial assemblage of predators and parasitoids in each companion plant, individual 22 x 27-cm yellow sticky traps (Trece Inc., Adair, OK) were set up on the west-facing bed of each treatment grid (near the first manually sampled plant) 2 days before each sampling date. To assess ground-dwelling predators, individual pitfall traps were put into east-facing beds in each treatment grid 2 days before each sampling date. Traps were 9-oz plastic cups containing 30-ml of soap-water solution covered by a 22-cm diameter plastic dish (Solo, Newport News, VA). All sampled natural enemies were identified at least to family.

Significant differences in green peach aphid abundance between companion plant treatments and checks were assessed for both kale varieties using fixed effects ANCOVA in R version 3.1.0 (R Foundation for Statistical Computing 2014). Treatment row and date of sampling were used as spatial and temporal blocks, respectively, with maximum plant height included as a covariate. To satisfy ANCOVA assumptions, a square-root transformation was used on green peach aphid abundance on Vates variety and log-base 10 transformation on Lacinato. All pairwise comparisons were assessed by Tukey-adjusted post hoc tests.

In evaluating the response of foliar, aerial, and ground-dwelling generalist predators and parasitoids to companion plants and checks, we focused on the most abundant (numerically dominant) species in our samples. Numerically dominant predators and parasitoids comprised those species that represented more than 60%
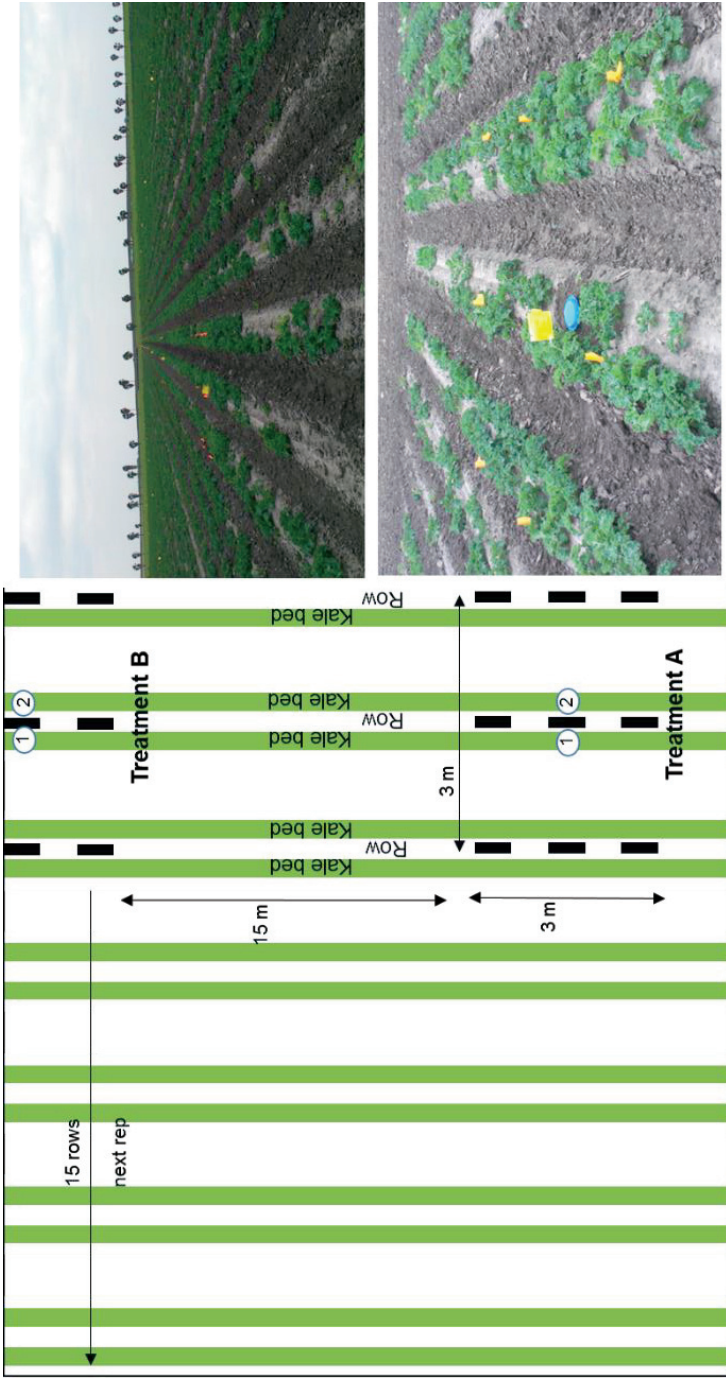
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Fig. 1. Schematic of a 3 x 3 m treatment grid across three double-bedded rows of kale. Black rectangles represent companion plant strips within the treatment grid, and white circles represent locations of (1) sticky traps and first manually sampled plants and (2) pitfall traps and second manually sampled plants. Treatment grids were separated by approximately 15 m. Photograph at top right shows treatment grids (red flags and yellow sticky trap) in the distance in one kale plot (Vates), while photograph at bottom right shows an individual treatment grid with yellow sticky trap on west-facing bed and pitfall trap on east-facing bed.

of all individuals in manual, sticky trap, and pitfall samples. Significant differences in abundance of numerically dominant species between companion plant and check treatments in Vates and Lacinato were assessed using repeated measures ANOVA, with Kruskal-Wallis rank sum tests used for non-normally distributed species.

For sticky traps, only numerically dominant species sampled in Vates were analyzed, because many traps in the Lacinato kale were blown away or damaged by wind. A log-base 10 transformation was applied to abundance of numerically dominant convergent lady beetle, *Hippodamia convergens* Guérin-Méneville (Coleoptera: Coccinellidae), to satisfy ANOVA assumptions. Because of the large percentage of manually sampled plants with no predators or parasitoids, chi-square tests were used to determine whether the presence/absence of numerically dominant foliar predators and parasitoids differed among companion plant and check treatments.

Results and Discussion

Of the 17 generalist predators and parasitoids sampled across trap types, two qualified as numerically dominant species (Table 1), following a well-documented pattern of abundance distribution in biological communities wherein only a few species are abundant while most are scarce (Sugihara 1980, Brown 1984, Hubbell 1997, Hill and Hamer 1998, Magurran and Henderson 2003). Convergent lady beetle, either larval or adult, was the most commonly sampled numerically dominant species in all sampling types, including pitfalls. While manual and pitfall sampling captured mostly convergent lady beetle larvae, sticky traps were effective in catching adults. The carabid predator *Pterostichus* spp. was a numerically dominant taxon in pitfall traps.

Of the two numerically dominant natural enemy species, convergent lady beetle is the most likely to kill green peach aphids, because both adults and larvae are voracious predators in tobacco, *Nicotiana tobacum* L.; potato, *Solanum tuberosum* L.; and crucifers (Weires and Chiang 1973, Horn 1981, Hamilton et al. 1999, Katsarou et al. 2005). While some species of *Pterostichus* climb plants in pursuit of prey, aphids might be less-preferred food relative to larger Coleoptera and Lepidoptera larvae (Sunderland and Vickerman 1980, Symondson et al. 2000, Alvarez et al. 2013).

On Vates kale, there were significant differences in green peach aphid abundance between companion plant treatments (overall $F_{4,27}$ = 3.98, p = 0.02). Aphid abundance on kale neighboring buckwheat was significantly less than on kale neighboring fennel (t = 4.16, p < 0.001; Fig 2A). Overall, however, no tested companion plant had significantly fewer aphids than the check treatment. In Lacinato, there were no significant differences in aphid abundance across any companion plants nor the check treatment (overall $F_{4,27}$ = 1.48, p = 0.25; Fig. 2B). While not statistically significant, the trend across both varieties of kale was that more green peach aphids tended to be on kale surrounded by fennel, which is surprising considering that intercropped fennel repels other aphid pests such as *Lipaphis erysimi* (Kaltenback) in brassica systems (Singh and Kothari 1997).

On average, convergent lady beetle larvae assessed through manual sampling were more abundant on kale surrounded by dill (Vates) and Indian mustard (Lacinato) (Table 2). Nevertheless, numbers of lady beetle larvae on foliage of Vates and Lacinato kale were not significantly different between the tested companion plants and the check (Vates: X^2 [4, N = 30] = 3.54, p = 0.47; Lacinato:

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Table 1. Arthropod Predator and Parasitoid Taxa in Manual, Sticky Trap, and Pitfall Samples in Vates and Lacinato Kale.

Variety	Sampling method	Taxa	Total	Avg./ sample	% total individuals	No. samples
Vates	Manual	**Hippodamia convergens (larvae)**	15	0.6	**75**	30
		Hippodamia convergens (adult)	4	0.1	20	
		Thomisidae 1	1	0.04	5	
	Sticky trap	**Hippodamia convergens (adult)**	14	0.32	**82.3**	30
		Hahniidae 1	3	0.06	17.6	
	Pitfall	**Hippodamia convergens (larvae)**	41	1.1	**36.3**	25
		***Pterostichus* spp.**	28	0.7	**24.8**	
		Hippodamia convergens (adult)	11	0.3	9.7	
		Lyniphiidae 3	10	0.3	8.9	
		Trochosa spp.	8	0.1	7.1	
		Lyniphiidae 2	4	0.08	3.5	
		Hahniidae 1	3	0.05	2.7	
		Pardosa spp.	2	0.05	1.8	
		Ichneumonidae 1	2	0.05	1.8	
		Solenopsis invicta	1	0.02	0.9	
		Nabis spp	1	0.02	0.9	
		Loxiscelidae 1	1	0.02	0.9	
		Carabidae 2	1	0.02	0.9	
Lacinato	Manual	**Hippodamia convergens (larvae)**	10	0.45	**90.9**	30
		Araneidae 1	1	0.05	9.1	
	Sticky trap	N/A	N/A	N/A	N/A	N/A
	Pitfall	***Pterostichus* spp.**	46	2.19	**50.5**	24
		Hippodamia convergens (larvae)	34	1.62	**37.4**	
		Trochosa spp.	6	0.29	6.6	
		Pardosa spp.	1	0.05	1.1	
		Hippodamia convergens (adult)	1	0.05	1.1	
		Lyniphiidae 3	1	0.05	1.1	
		Syrphidae 1	1	0.05	1.1	
		Zoodariidae 1	1	0.05	1.1	

Totals and averages of each species were pooled over both sample dates. Numerically dominant (>60% of individuals sampled) taxa are highlighted in bold.
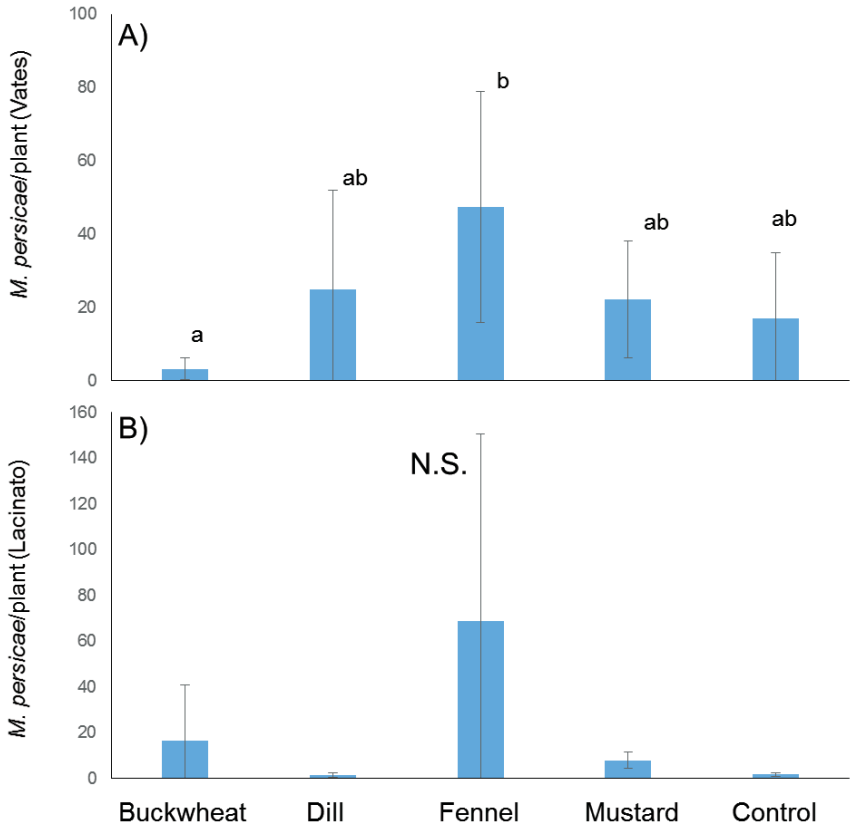
Fig. 2. Mean *Myzus persicae* abundance on Vates (A) and Lacinato (B) kale near buckwheat (insectary), dill (repellent), fennel (repellent), mustard (attractant), and check treatments. Vertical bars represent 95% confidence intervals, with significant differences between treatments indicated by different lowercase letters as determined by Tukey's post-hoc tests ($\alpha = 0.05$). Densities were transformed to square root (Vates) and log-base 10 (Lacinato) before ANCOVA. Densities shown are untransformed.

X^2[4, N = 30] = 4.61, p = 0.33). In analyzing the presence/absence of convergent lady beetle larvae on kale plants, we found no significant differences between companion plant treatments and the check (Vates: X^2[4, N = 30] = 3.67, p = 0.45; Lacinato: X^2[4, N = 30] = 4.52, p = 0.39).

In pitfall traps, convergent lady beetle larvae were more abundant in check, buckwheat, and Indian mustard for both varieties of kale (Table 2). While not statistically significant, the higher abundance of convergent lady beetle larvae in buckwheat and check treatments than in Indian mustard, fennel, and dill was of biological relevance in Lacinato kale (overall $F_{4,20}$ = 2.89, p = 0.06). In Vates kale, no statistical nor biological differences in abundance of lady beetle larvae in pitfall

Table 2. Mean Abundance (± SE) of Numerically Dominant Natural Enemies *Hippodamia convergens* (Larvae and Adults) and *Pterostichus* spp. in Vates and Lacinato Kale Plants (Determined through Manual, Sticky Trap, and Pitfall Sampling) in Check and Companion Plant Treatments.

Companion plant treatment	*H. convergens* larvae (±SE)				*H. convergens* adult (±SE)	*Pterostichus* spp. (±SE)	
	Manual		Pitfall		Sticky	Pitfall	
	Vates	Lacinato	Vates	Lacinato	Vates	Vates	Lacinato
Control	0.2 (±0.2)	0	1.29 (±0.3)	3.0 (±0.9)	0.28 (±0.2)	0.71 (±0.3)	3.33(±0.8)
Buckwheat	0	0.2 (±0.2)	1.43 (±0.3)	3.0 (±1.4)	0.57 (±0.1)	0.71 (±0.3)	2.0 (±1.4)
Dill	2.81 (±2.52)	0.68 (±0.51)	0.86 (±0.2)	0.83 (±0.2)	0.5 (±0.1)	1.14 (±0.3)	2.0 (±0.4)
Fennel	0	0.2 (±0.2)	0.33 (±0.1)	0.75 (±0.4)	0.22 (±0.04)	0.22 (±0.07)	2.0 (±0.6)
Mustard	0	1.22 (±0.73)	1.63 (±0)	1.83 (±0.3)	0.17 (±0.03)	1 (±0.2)	2.0 (±0.5)

Differences in abundance between treatments were not statistically significant for any natural enemy species ($\alpha = 0.05$).

traps were observed between treatments (X^2[4, N = 25] = 0.64, p = 0.96). More convergent lady beetle adults were caught in sticky traps in buckwheat, but overall differences in abundance between treatments were not statistically nor biologically significant (Vates: X^2[4, N = 30] = 1.86, p = 0.76). This finding was consistent with the other numerically dominant species in pitfall traps (*Pterostichus* spp.), with no significant differences in abundance between companion plant and check treatments (Vates: X^2[4, N = 25] = 3.03, p = 0.55; Lacinato: overall $F_{4,21}$ = 0.38, p = 0.82).

Companion plants reduce colonization, retention, feeding, and survival of pests on crops, either through direct (e.g., reduced palatability, host plant masking) or indirect (e.g., attraction/retention of natural enemies) effects (Parolin et al. 2012). While there was significant variation in green peach aphid abundance across companion plants, overall we found no evidence that companion plant intercropping directly reduced green peach aphids on neighboring kale plants. The indirect effects of companion plants on aphid abundance were also likely negligible, because none of the four tested companion plants succeeded in increasing abundance of numerically dominant predators, *H. convergens* and *Pterostichus* spp., in kale. Our results suggested that Indian mustard, dill, fennel, and buckwheat companion plants might have limited potential for managing green peach aphids in organic kale systems in South Texas.

While the benefits of intercropping and agroecosytem diversification on pest management are well documented (Andow 1991, Stein et al. 2010, Letourneau et al. 2011, Ratnadass et al. 2012), effects of companion planting on pests can be variable and often dependent on system-specific factors (Finch et al. 2003, Held et al. 2003). Spatial relationships between crops and secondary plants, for example, can influence how herbivores locate and colonize hosts. Crop patch size, the proportion of land with attractant and repellent secondary plants, and perimeter-to-area ratios of crops and secondary plants play a role in pest immigration and emigration rates in diversified crop systems (Altieri et al. 1983, Hannunen 2005). Responses of pest and natural enemies to insectary plants can also be influenced by the size of the crop area, with larger fields less attractive than smaller ones (Banks 2000, Bommarco and Banks 2003).

Dispersal and host plant-tracking abilities also influence how pests respond to attractant and repellent companion plants (Gómez Jiménez and Poveda 2009). It has been surmised that pest species with poor ability to detect host plants and with passive dispersal (such as aphids) are less impacted by diversified cropping systems (Potting et al. 2005). Nevertheless, evidence suggests intercropping strategies can successfully reduce colonization and propagation of aphids (Helenius 1990, Parajulee et al. 1997, Ma et al. 2006).

For future research on companion plants and pest management in South Texas agroecosystems, we suggest assessing natural enemy attraction in insectary plants. While our study focused on natural enemy migration into neighboring crops, it remained unclear whether natural enemies were more abundant in buckwheat, dill, fennel, and Indian mustard companion plants than in neighboring kale plants. It is possible that companion plants did attract natural enemies, but spill-over effects were not enough to reduce green peach aphids on nearby kale. Different spatial configurations of intercropped companion plants also should be tested on green peach aphid abundance in kale. Studies should assess patch size and distribution of attractant, repellent, and insectary plants in relation to crops, because the high ratio of crop-to-companion plant area in our study might have limited the effects on

pest and natural enemy abundance on kale. It is important to assess companion plants in other brassica plants and on non-aphid pest species to better understand the appropriateness and effectiveness of push-pull technologies in subtropical agroecosystems.

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