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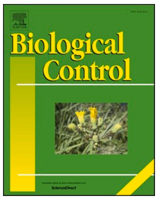

Novel insecticides and generalist predators support conservation biological control in cotton

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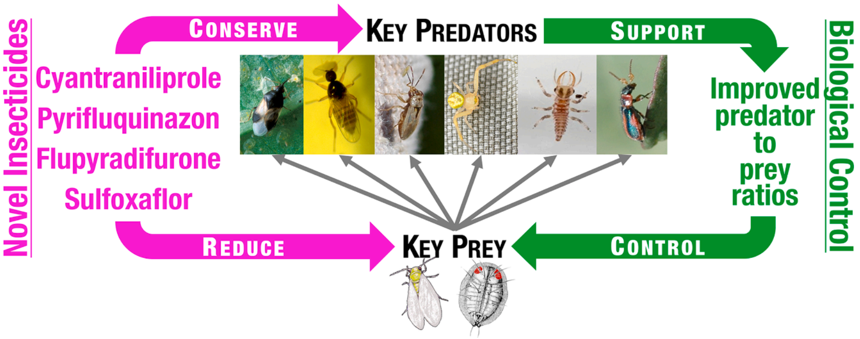
HIGHLIGHTS

- Conservation biological control is central to successful IPM in the cotton system.
- Field studies measured the selectivity of four recently introduced insecticides.
- Insecticides selectively conserved the arthropod predator community.
- Insecticides enabled predator to prey ratios favorable to biological control.
- Growers have additional options for pest management that conserve natural enemies.

GRAPHICAL ABSTRACT



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ABSTRACT

Arizona has a successful integrated pest management plan for arthropod pests of cotton including two key pests, *Bemisia argentifolii* (=*B. tabaci* MEAM1) and *Lygus hesperus*. Central to this plan is conservation of natural enemies through threshold-based use of effective and selective insecticides. Field studies were designed to test the selectivity of the insecticides cyantraniliprole, flupyradifurone, pyrifluquinazon and sulfoxaflor on the cotton arthropod community (27 taxa measured), which includes the key generalist predator taxa: *Collops* spp., *Orius tristicolor*, *Geocoris* spp., *Misumenops celer*, *Drapetis* nr. *divergens* and *Chrysoperla carnea s.l.* Compared with an untreated check and in contrast to acephate-treated positive controls, predator densities were rarely affected, and the overall arthropod predator community was conserved by all insecticides. Occasional significant reductions in predator abundances were likely associated with lower prey availability after insecticide sprays rather than direct toxic effects. The proportions of time that predator to prey ratios were at or above levels indicative of functioning biological control were either significantly higher or not significantly different from the untreated check for these insecticides. The cotton food web populated by generalist predators is resilient and flexible enough to accommodate temporary reductions in abundance of some species, periods of low prey densities, or other constraints on individual predator species function. Our study demonstrates that the insecticides tested are selective and compatible with sustainable pest management in the Arizona cotton system, representing new

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options for insect pest control that conserve natural enemies and support biological control through generally favorable changes to predator to prey ratios.

1. Introduction

The conservation of natural enemies in agroecosystems has come to be recognized as a practice that must be implemented for effective Integrated Pest Management (IPM). Natural enemies contribute to the mortality of key and secondary pests, however, the complexity of their interactions with pests and crops is challenging to understand and to apply in IPM (Zalucki et al., 2015). Limited research efforts have been made towards understanding biological control function and insecticide selectivity, especially with studies that investigate pests and natural enemies concurrently, and in field plots of sufficient size to properly test these IPM attributes. The lack of information on these subjects has impeded advances in IPM in many different crop systems (Naranjo, 2001; Furlong and Zalucki, 2010; Macfadyen et al., 2014, 2015; Zalucki et al., 2015).

The IPM research program for cotton in Arizona has succeeded in untangling predator and pest interactions and demonstrated practical use of this knowledge for growers by showing how integration of chemical and biological controls enabled with sampling and economic thresholds for the target pest is possible (Naranjo and Ellsworth, 2009b; Anderson et al., 2019; Romeis et al., 2019; Reisig et al., 2019). The main natural enemies and their biological control function have been studied, and results show that natural enemies strongly contribute to pest suppression (Asiimwe et al., 2016; Vandervoet et al., 2018). The selectivity of insecticides recommended for use in cotton has been determined in field trials, and these findings have been shared with growers (Naranjo and Ellsworth, 2009a, 2009b; Ellsworth et al., 2012b; Vandervoet et al., 2018). Natural enemies are conserved through implementation of well-designed sampling and threshold systems that guide the use of selective insecticides for control of our two key pests, whitefly, *Bemisia argentifolii* Bellows and Perring (=*B. tabaci* MEAM1), and Lygus bug, primarily *Lygus hesperus* Knight (Ellsworth et al., 1996, 2006, 2012a; Ellsworth and Martinez-Carrillo, 2001; Ellsworth and Barkley, 2001; Ellsworth, 2001). The introduction and proper use of selective insecticides was one of the main factors that contributed to this successful IPM plan (Naranjo and Ellsworth, 2009b; Anderson et al., 2019; Romeis et al., 2019; Reisig et al., 2019).

In the early 1990′s, Arizona growers suffered major outbreaks of whiteflies with negative impacts on yield and quality of cotton. At that time, only broad-spectrum insecticides were available to growers. The use of broad-spectrum insecticides caused pest resurgences and secondary pest outbreaks, because natural enemies were indiscriminately killed. The intense and broad-scale use of these insecticides also led to reductions in insecticide susceptibility (Dennehy and Williams, 1997; Ellsworth and Naranjo, 1999; 2002).

Today, Arizona produces high quality cotton through a comprehensive IPM program that integrates chemical and biological controls. One of the main features that enabled the establishment of the IPM program in Arizona was the conservation of natural enemies when two selective insecticides, buprofezin and pyriproxyfen, became available in 1996. Other factors that contributed to the conservation of natural enemies in cotton were the introduction of Bt cotton, genetically engineered to selectively control pink bollworm, *Pectinophora gossypiella* (Saunders), and other lepidopteran pests in 1996, and the registration of a selective Lygus feeding inhibitor insecticide, flonicamid, in 2006 (Anderson et al., 2019; Romeis et al., 2019). As a result, natural enemies have become a potent factor in suppressing whitefly populations below economic levels, and cotton IPM for whiteflies and Lygus has become based not only on insecticide efficacy towards pests but also on insecticide selectivity towards natural enemies (Ellsworth and Barkley, 2005; Naranjo and Ellsworth, 2009a).

The stability of this successful IPM plan is dependent, among other factors, on insecticide selectivity not just for these key pests, but for the control of other arthropod pests in the cotton system. The selectivity and efficacy of selective insecticides currently recommended for IPM in cotton is well documented in Arizona (Naranjo and Ellsworth, 2009b). However, for novel insecticides, selectivity towards natural enemies is unknown in our system. There are novel insecticides available for control of our key pests; however, grower decisions for using them have been mainly based on information provided by manufacturers and on product costs.

Research and use of selective insecticides in our system has allowed us to better understand and make practical use of a complex of key whitefly predators that populate the food web in cotton, and significantly contribute to the reduction of whitefly populations (Vandervoet et al., 2018). In light of this knowledge, we tested the selectivity and compatibility of these novel insecticides with the IPM program in Arizona using field trials that emulated grower's pest management practices, and evaluated whitefly and natural enemy relationships. We assessed insecticide effects on the arthropod community, on the individual abundance of key predators, and on known predator to prey ratios that indicate functioning biological control in our system.

2. Materials and methods

2.1. Experimental design

Studies were conducted at the University of Arizona's Maricopa Agricultural Center, Maricopa, AZ, United States, in 2017 and 2018. Cotton, *Gossypium hirsutum* L., was planted on 1 June 2017 and 4 May 2018, and grown according to agronomic practices for the area. A Bollgard II/XtendFlex variety (Monsanto Company, MO, USA) that confers resistance to lepidopteran insects and tolerance to herbicides was planted each year (DP1549B2XF). A randomized complete block design was used in both years. All plots were established in a single field site about 3 ha in size, subdivided into four blocks. Within blocks, treatments were randomly assigned to plots that were 18.3 m long by 18.3 m (18 rows) wide with 1.016 m row spacing, and 3 m unplanted alleys.

The trial was sprayed with a six row (6 m) tractor-mounted boom sprayer (TJ69-8003VS TeeJet spray tip) at a volume of 112.5 l/ha. To avoid drift, insecticides were sprayed directly to plots during calm weather conditions, using low spray boom heights and reduced sprayer ground speed.

Four candidate insecticides were tested. The active ingredient, rate and product name were as follows: (1) sulfoxaflor, 78 g a.i./ha (Transform® 50WG, Corteva Agriscience, Indiana, USA); (2) cyantraniliprole, 150 g a.i./ha (Exirel® 0.83SE, FMC Corporation, Pennsylvania, USA); 3) flupyradifurone, 202 g a.i./ha (Sivanto™ 200SL, Bayer Crop Science, North Carolina, USA); and (4) pyrifluquinazon, 52 g a.i./ha (PQZ™ 1.87SC, Nichino America, Delaware, USA). Sulfoxaflor is an insecticide used for *L. hesperus* control with suppressive effects on *B. argentifolii*, and the other insecticides target *B. argentifolii*. Insecticides were sprayed every 14 days for a total of 3 sprays at their highest labeled rates during the flowering period. Spray dates were 8/1, 8/15 and 8/29 in 2017, and 8/2, 8/16 and 8/30 in 2018. Candidate insecticides were compared to a negative control, the untreated check (water only, UTC) and to a positive control (a broad-spectrum insecticide) with known negative effects on the arthropod community (Asiimwe et al., 2013; 2016). This positive control enabled us to assess the ability of our experimental design to detect an expected effect on the arthropod community, and was implemented as acephate (Orthene® 97P, Amvac Chemical Corporation, California, USA) at 1120 g a.i./ha. Acephate has commercial activity against *L. hesperus*, but essentially no effect on *B. argentifolii*.

Maintenance sprays targeting *L. hesperus* and *B. argentifolii* were deployed when their levels reached economic thresholds in the untreated check and in treatments without commercial activity against one of these pests, based on well-established sampling methods for these pests (Ellsworth, 2001; Ellsworth and Barkley, 2001; Ellsworth et al., 2006; 2012a). The objective of maintenance sprays was to ensure approximate prey uniformity among treatments to prevent conditions where excess prey or prey resources, such as honeydew, led to conditions more attractive to arthropod predators, and to avoid gross changes across treatments that could possibly mask the effects of insecticides on non-target organisms (e.g., flower loss due to Lygus damage). Maintenance sprays targeting *L. hesperus* were deployed in all treatments, except the ones with known commercial activity against this pest. Both sulfoxaflor and acephate have commercial activity against Lygus and those treatments did not require sprays for Lygus, because they never exceeded threshold. The maintenance sprays for *L. hesperus* control were done with a selective insecticide twice in 2017 (8/10 & 8/24) and three times (8/2, 8/16 & 8/30) in 2018 (flonicamid, Carbine® 50WG, 98 g ai/ha, FMC Corporation, Pennsylvania, USA). The maintenance sprays targeting *B. argentifolii* were deployed once in the acephate treatment and in the untreated check both years. We sprayed the selective insecticides, pyriproxyfen (Knack® 0.86EC, 75 g ai/ha, Valent, California, USA) on 8/24/2017, and buprofezin (Courier® 3.6SC, 390 g ai/ha, Nichino America, Delaware, USA) on 8/30/2018. The other candidate treatments have whitefly activity and did not require additional maintenance sprays against whiteflies, because those levels never exceeded thresholds. A plant growth regulator (mepiquat pentaborate, Pentia™ 0.82l, BASF, Texas, USA) was sprayed in 2017 according to cotton commercial guidelines to manage the balance between vegetative and reproductive growth for cotton production. These growth regulator sprays were not necessary in 2018.

2.2. Arthropod sampling and predator:prey ratios

Densities of *B. argentifolii* eggs, nymphs and adults were sampled at three and seven days after each spray, for a total of six samples each year. Adult density was estimated by counting individuals on the underside of leaves from the fifth mainstem node below the terminal *in situ* (Naranjo and Flint, 1995), and nymph and egg densities were estimated by counting individuals in the laboratory under magnification on a 3.88 cm^2 disk taken from these leaves (Naranjo and Flint, 1994). Ten leaves per plot were randomly collected to estimate these densities with an average precision of 25% (Naranjo and Flint, 1994; 1995). Nymphs were classified as small (first and second instars) and large (third and fourth instars, including pharate adults or "pupae").

L. hesperus were sampled with a standard 38 cm diameter sweep net at three, seven and 13 days after spray (a total of 9 weekly dates over the season each year). 25 sweeps per plot were used in 2017 and two sets of 25 sweeps (50 total) per plot were used in 2018. All data were standardized to 100 sweeps, because this is the unit of measurement used for *L. hesperus* sampling in our system (Ellsworth, 2001; Ellsworth and Barkley, 2001). We counted the number of adults and nymphs of *L. hesperus*. Nymphs were considered as either small (first and second instars) or large (third to fifth instars).

Arthropod predators were sampled with a standard 38 cm diameter sweep concurrently with *L. hesperus* (see above). Densities of 27 taxa were measured, including key arthropod predators such as *Collops quadrimaculatus* (Fabricius)*, Collops vittatus* (Say), *Orius tristicolor* (White), *Geocoris* punctipes (Say)*, Geocoris pallens* Stål, *Misumenops celer* (Hentz), *Drapetis* nr. *divergens* Loew and *Chrysoperla carnea s.l.* (Stephens). Samples were frozen and later counted in the laboratory using a dissecting microscope. We also examined the effects of the candidate insecticides on western flower thrips, *Frankliniella occidentalis* (Pergande), the most dominant thrips species in cotton in our region. Western flower thrips are omnivorous and important mite predators in the western United States (Bibby, 1958; Trichilo and Leigh, 1986). They are likely whitefly predators, based on our observations in the field.

We calculated predator to prey ratios as the quotient of each of the species mentioned above, individually as means per 100 sweeps, to the number of *B. argentifolii* adults as means per leaf or large nymphs as means per leaf disc. These species are considered key predators in our system, because they significantly reduce densities of *B. argentifolii* (Vandervoet et al., 2018). We estimated eight predator to prey ratios as follow: *M. celer/B. argentifolii* adults, *M. celer/B. argentifolii* large nymphs, *D.* nr *divergens/B. argentifolii* adults, *D.* nr *divergens/B. argentifolii* large nymphs, *O. tristicolor/B. argentifolii* adults, *C. carnea* larvae/*B. argentifolii* adults, *Collops* spp./*B. argentifolii* large nymphs and *G. punctipes/B. argentifolii* large nymphs (we confined evaluation to *G. punctipes* because *G. pallens* Stål densities were very low throughout the years of these trials). We chose these ratios because they indicate functioning whitefly biological control in our system (Table 1) (Vandervoet et al., 2018; Ellsworth et al., 2019a; 2019b).

2.3. Statistical analyses

A mixed-model, repeated measures analysis of variance (JMP® Pro 14.2, SAS Institute Inc., Cary, NC) was used to test for treatment differences affecting the abundance of the six key predators, thysanoptera and key pests over the season in both years. The model included fixed effects of insecticide treatment, year and sampling date (repeated measure). Block and associated interaction terms were considered random effects. The covariance structure used was AR(1). In order to minimize expected year effects in abundance of the predatory arthropods and compare both years more consistently, we used the proportion of maximum scaling method (POMS), which transforms each scale (predators' scale in each year) to a common metric running from 0 (=minimum possible) to 1 (=maximum possible) (Moeller, 2015). Reduced mixed-models were used to investigate insecticide trends by sampling date within each year. Here, insecticide and sampling date were fixed effects and block was a random effect. The mean weekly abundance of the six key predators, thysanoptera and pests within each year were compared with the UTC using Dunnett's test. To meet normality and homoscedasticity assumptions, the arthropod predator data were transformed by $\mathrm{sqrt}(x + 0.05)$, and *B. argentifolii* and *L. hesperus* abundance were transformed using $\ln(x + 1)$ and $\mathrm{sqrt}(x + 0.5)$, respectively. Analyses were performed for sample dates after the first application of insecticides. Prior to trial initiation, pre-counts of arthropod densities were not statistically different. For clarity of viewing patterns, all pest and natural enemy abundance is graphically represented by cumulative arthropod-days over the season using the trapezoidal rule (Ruppel, 1983); however, all statistical analyses were conducted on abundances measured.

We calculated the proportion of dates that each of the eight predator to prey ratios were above functioning critical ratios known to enable biological control in our system (Table 1), using six whitefly and arthropods sampling dates for each year. Each of these ratios serves as a

Table 1
Predator to prey ratios associated with biological control of *B. argentifolii* in Arizona cotton systems (Vandervoet et al., 2018).

Arthropod predator	Number of predators per *B. argentifolii* nymph	Number of predators per *B. argentifolii* adult
M. celer	3.5	1
D. nr *divergens*	44	8
O. tristicolor		1.5
Collops spp.	2	
Geocoris spp.	0.75	
C. carnea larvae		0.5

See Material and Methods for *B. argentifolii* and predator sampling methods and units.

proxy for the function of the entire predator community (Vandervoet et al., 2018). The model included fixed effects of insecticide treatment and year; the block variable and associated interaction terms were entered as random effects. We used Dunnett's test to compare the proportion of dates that the ratios of each insecticide treatment were above or below functioning biological control ratios relative to the UTC.

We examined the main effects of insecticides on the entire arthropod predator community through Principal Response Curves (PRC), a time-dependent, multivariate analysis that depicts the arthropod community trend over time for each treatment relative to a standard (Van den Brink and Ter Braak, 1998, 1999; Ter Braak and Smilauer, 1998, 2012). We examined the main insecticides effects using the negative control (untreated check) as the standard. In addition, we also did these analyses using the positive control (acephate) as the standard. A distribution-free *F* type test based on sample permutation was used to test for statistical significance in patterns. Species with high weights more closely denote the shape of the resulting curve. Analyses were conducted with CANOCO v4.5 and 5 (Microcomputer Power, Ithaca, NY, USA).

3. Results

3.1. Predator abundance

Population densities of key predators along with thrips varied over the 18 sampling dates in 2 years of field testing. The temporal effects (week, year and related interactions) were important for all arthropods and reflected different seasonal patterns for each taxa. The interactions of insecticide with temporal effects were important for *M. celer* and *C. carnea* larvae (Table 2). With the exception of the positive control (acephate), the abundance of most taxa in the insecticide treatments was similar when compared with the UTC (Fig. 1). As expected, the acephate treatment was broadly destructive to the arthropod complex, supporting far lower predator densities compared with the other treatments. The results from the positive control provided strong evidence that the experimental design was able to measure a known destructive effect of acephate on the arthropod community. Densities of four of the six key predator species and total arthropods were reduced relative to the UTC on at least one date for pyrifluquinazon, while densities of two species were lower for the other three treatments on at least one date. The majority of the time—18 sample dates across 2 years—the candidate insecticides had predator densities similar to what was found in the UTC.

3.2. Non-target arthropod community dynamics

The Principal Response Curve (PRC) shows the effect of each insecticide relative to the untreated check. PRCs based on the first axis of redundancy analysis were significant ($P = 0.002$) and explained 24.2% of the variation due to insecticide treatment (Fig. 2). There was a significant treatment effect on eight dates in 2017 and on four dates in 2018. Eleven predator taxa followed the general patterns depicted by the PRC. One predator taxon, *C. carnea* larvae, followed a numerical trend opposite to the general arthropod community pattern of the PRC, as indicated by the highly negative species weight (Fig. 2).

We also examined each individual insecticide compared to the untreated check in each year to further understand community effects (Fig. S1). The arthropod community was significantly reduced in the acephate treatment (2017, $P = 0.03$; 2018, $P = 0.03$), with the exception of *C. carnea* larvae, which with a negative species weight shows a pattern opposite to the rest of the arthropod community. Again, the negative impact of acephate (positive control), showed that the experimental design was able to clearly measure a known effect. With the exception of pyrifluquinazon in 2017, there were no significant differences between candidate insecticide PRCs and the untreated check for 2017 or 2018, respectively: flupyradifurone ($P = 0.19$; $P = 0.22$), sulfoxaflor ($P = 0.07$; $P = 0.7$), pyrifluquinazon ($P = 0.03$; $P = 0.35$), and cyantraniliprole ($P = 0.09$; $P = 0.25$).

The PRC investigating the effects of the candidate insecticides (including the untreated check) compared to the positive control (acephate) was significant ($P = 0.02$) (Fig. S2). The PRCs individually comparing each insecticide to the positive control in each year also were significant ($P < 0.04$) (Fig. S3).

3.3. Whitefly and Lygus target pest abundance

Population densities of *B. argentifolii* varied over the sampling dates and years; however, they were higher in 2017 (Fig. 3). Generally, *B. argentifolii* populations were kept below economic thresholds for each insecticide treatment. The acephate positive control exceeded whitefly thresholds for *B. argentifolii* multiple times in 2017 and a few instances in 2018, however, *B. argentifolii* abundance was not at overwhelming outbreak levels in this treatment. The abundance of *B. argentifolii* was significantly higher in the acephate treatment than the untreated check at least twice in 2017, and only once in 2018. For the candidate insecticides, the abundance of *B. argentifolii* was significantly lower on a few sampling dates for the sulfoxaflor and cyantraniliprole treatments compared with the untreated check; however, whitefly densities were frequently lower and different from the untreated check for the pyrifluquinazon and flupyradifurone treatments (Fig. 3).

Population densities of *L. hesperus* were below threshold in the sulfoxaflor and acephate treatments most of the season, but only significantly lower than the untreated check, which received maintenance sprays for Lygus control, on three sampling dates. Generally, population densities for other insecticides were above threshold, especially in 2017, but statistically similar to Lygus levels in the untreated check (Fig. 4).

3.4. Predator:prey effects

We estimated the proportion of sample dates that key predator to prey ratios for each insecticide were above functioning biological control critical ratios (Table 3). Averaged for both years, the proportion of dates above functioning biological control critical ratios was significantly lower in four out of eight ratios in the acephate treatment compared with the UTC (Dunnett's, $P < 0.008$, Fig. 5). Most often, the proportion of sample dates above critical ratios for the candidate insecticides was not significantly different from the UTC. However, the proportion of dates above functioning biological control for the *C. carnea* larvae: *B. argentifolii* nymphs ratio was marginally lower than the UTC for flupyradifurone ($P = 0.06$) and cyantraniliprole ($P = 0.06$)

Table 2
Fixed effect F-values of mean seasonal (per 100 sweeps) arthropod predator abundance over two years.

Fixed Factors	DF	*M. celer*	*D.* nr *divergens*	*O. tristicolor*	*Collops* spp.	*G. punctipes*	*C. carnea* larvae	Thrips	Total Arthropods
Insecticide	5, 35.9	5.09**	8.79***	1.12^{NS}	0.86^{NS}	21.3***	13.5***	1.29^{NS}	2.53*
Week	8, 235.5	7.54***	27.2***	19.9***	4.33***	6.88***	1.68^{NS}	3.64***	31.1***
Year	1, 35.9	3.35^{NS}	269***	10.6**	9.63**	0.09^{NS}	0.29^{NS}	50.6***	5.04*
Insecticide*Week	40, 253.5	1.72**	0.83^{NS}	1.25^{NS}	0.65^{NS}	1.38^{NS}	1.91**	1.07^{NS}	1.55*
Insecticide*Year	5, 35.9	0.81^{NS}	0.98^{NS}	0.62^{NS}	0.56^{NS}	0.66^{NS}	0.52^{NS}	0.50^{NS}	0.91^{NS}
Week*Year	8, 235.5	1.18^{NS}	30.9***	8.08***	2.23*	4.03***	3.39**	15.0***	13.9***
Insecticide*Week*Year	40, 253.5	0.97^{NS}	0.71^{NS}	0.84^{NS}	0.97^{NS}	1.14^{NS}	1.83**	1.15^{NS}	0.50^{NS}

Repeated-measures ANOVA, * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; ^{NS}: not statistically significant, $P > 0.05$. DF are approximated.

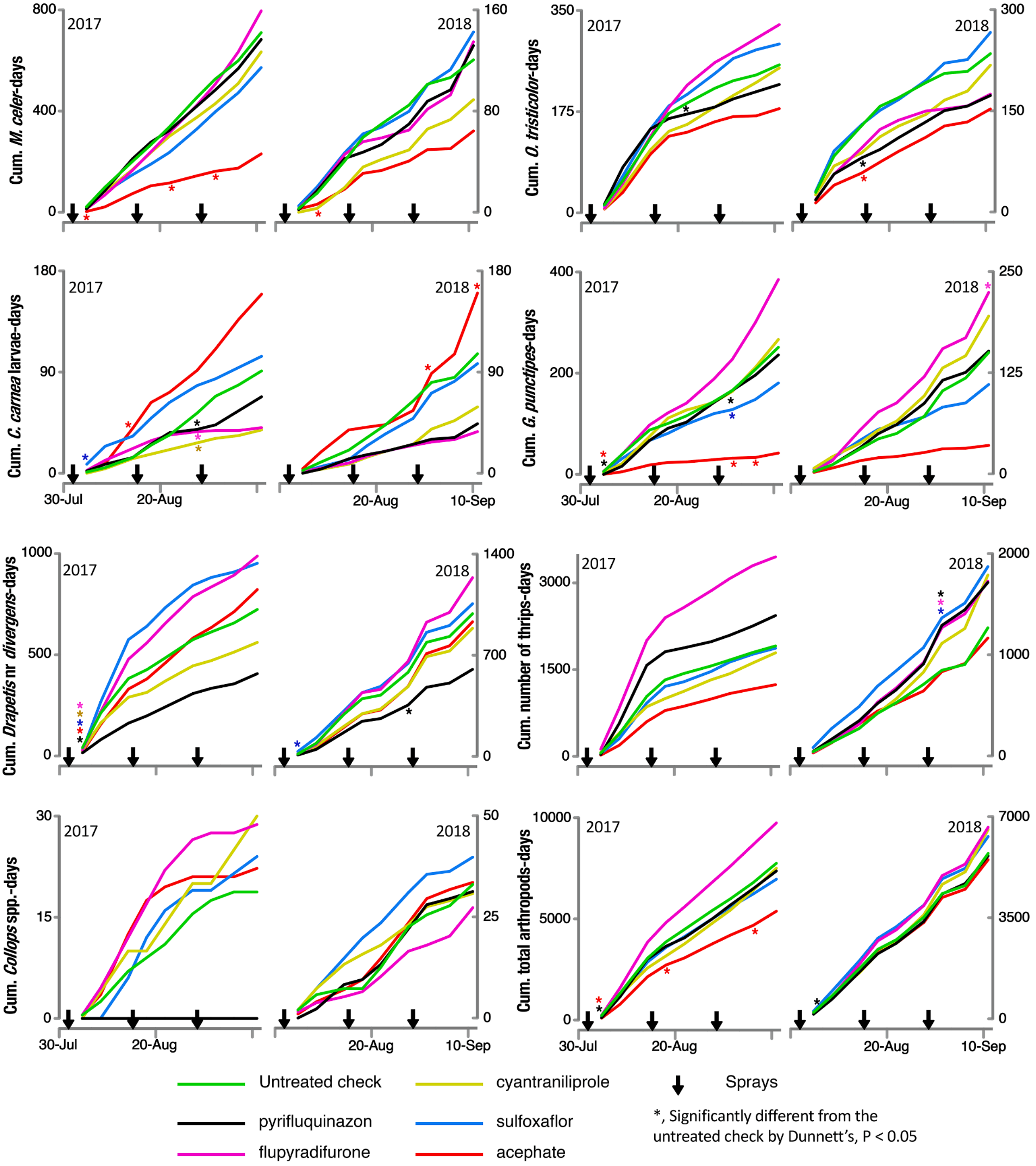

Fig. 1. Post-treatment, cumulative mean insect-days for arthropod predators per 100 sweeps during two growing seasons in Maricopa, AZ. Statistical analyses were conducted on abundance measured on each sampling date. Asterisk colors correspond to treatment means for insecticides that were significantly different from the untreated check by Dunnett's, $P < 0.05$.

treatments, and the pyrifluquinazon treatment was marginally higher than the UTC for the ratio *D. divergens*: *B. argentifolii* adults ($P = 0.08$). For flupyradifurone, the proportion of dates above critical ratios was significantly higher than the UTC ($P < 0.04$) for three ratios. For the remaining ratios, the insecticides were similar to the UTC ($P > 0.13$, Fig. 5).

4. Discussion

As novel insecticides near registration or are registered, their impacts on the arthropod community need to be assessed to secure the critical ecosystem services provided by predators in the Arizona cotton system. We have examined insecticide selectivity based on the entire predatory arthropod community and also within the context of key generalist predators that dominate the food web in Arizona cotton (Vandervoet et al., 2018). Our results identified insecticides with narrower spectrums of activity that promote biological control through generally favorable changes in predator to prey ratios.

We compared four new insecticides with an untreated check, and concluded that all are selective, based on four parameters: 1) individual predator species abundance was usually not different from the UTC; 2) the overall arthropod predator community was generally preserved and not different from the UTC; 3) the overall arthropod predator community of all candidate insecticides were significantly different from the

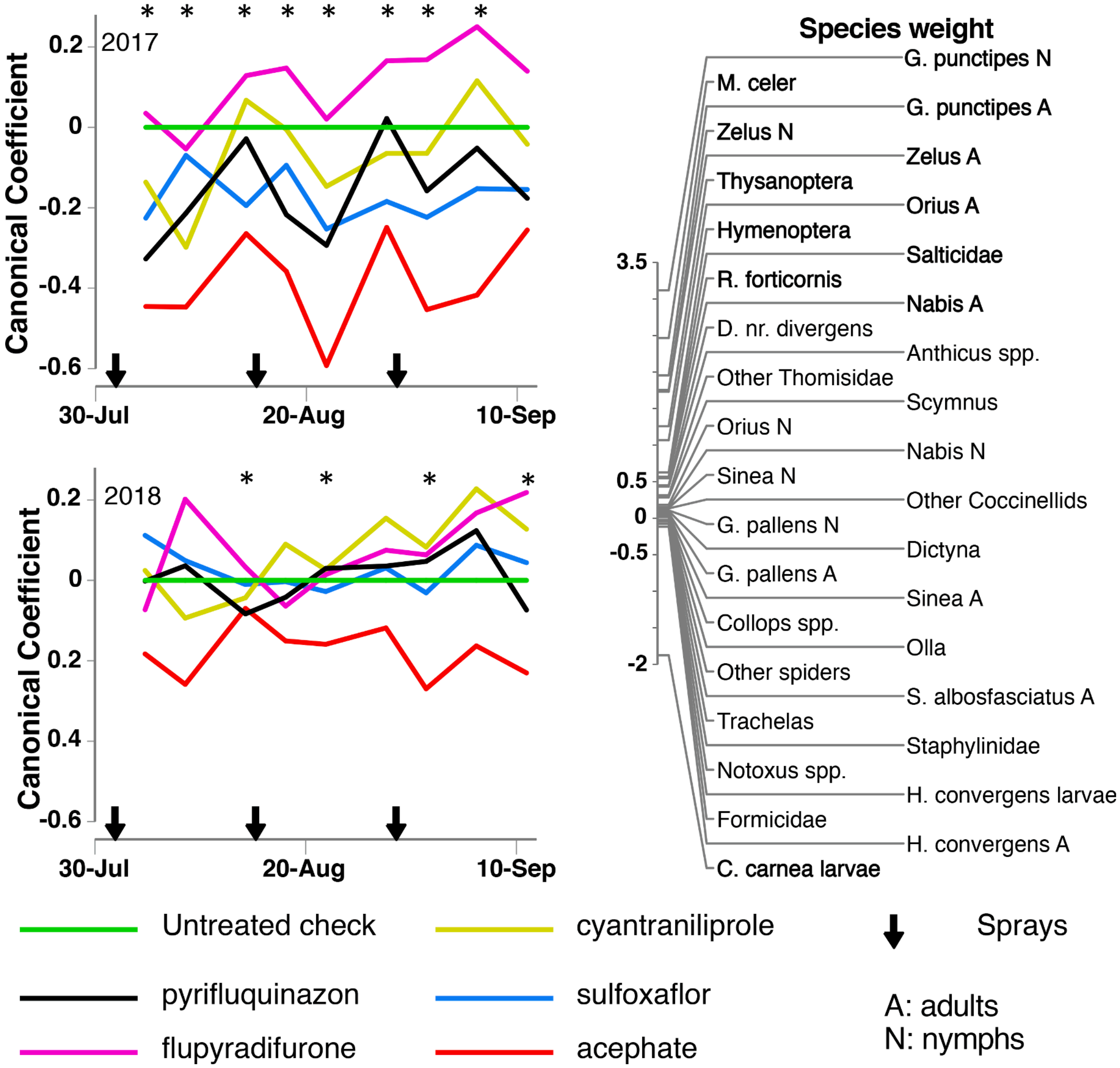

Fig. 2. Principal response curves (PRC) showing the main effects of insecticides on the arthropod community during two growing seasons in Maricopa, AZ. The PRC show the effect of each insecticide relative to the untreated check ($y = 0$ line). The product of the species weight and the canonical coefficient for a given insecticide and time estimates the natural log change in density of that species relative to the untreated check. The greater the species weight the more the response for that species resembles the PRC. Negative weights indicate an opposite pattern, and weights between −0.5 and 0.5 indicate a weak response or a response unrelated to the PRC. Weights >0.5 or <−0.5 are bolded for emphasis. The *P*-value, $P = 0.002$, denotes the significance of the PRC analysis over all dates based on an *F*-type permutation test. The "*" at the top of the charts indicate the significance ($P < 0.05$) of the insecticides compared to the untreated check on each date determined by *F*-type permutation test.

positive control acephate (Fig. S2-3); 4) predator to prey ratios were mostly improved or not different from the UTC, thus generally facilitating biological control. These patterns held true despite worst-case use scenarios of spraying candidate insecticides at their highest rates three consecutive times, likely far exceeding potential commercial practices in Arizona and in some cases U.S. label restrictions. The infrequent and ephemeral reductions in particular predator taxa associated with use of these insecticides (e.g., pyrifluquinazon) would likely be lessened in commercially managed fields.

The positive control, acephate, is a broad-spectrum insecticide that kills natural enemies along with one of our key pests, *L. hesperus* (Asiimwe et al., 2013; 2016). We were able to detect known disruptive effects of acephate on the arthropod community density, and on biological control through our analyses of predator to prey ratios, strongly suggesting that our experimental design was robust enough to allow the measurement of true insecticide effects (Jepson and Thacker, 1990; Pullen et al., 1992; Duffield and Aebischer, 1994; Kennedy et al., 2001; Prasifka et al., 2005; Macfadyen et al., 2014). Other research (Naranjo et al., 2004; Naranjo and Ellsworth, 2009a; Asiimwe et al., 2013; 2016;; Vandervoet et al., 2018) and a companion study on plot size for non-target studies (unpublished) support this conclusion.

Prey availability may influence interpretation of non-target effects; thus, target and non-target prey densities should be considered and managed. We minimized prey density disparities through maintenance sprays with insecticides having demonstrated selectivity for our key target pests (Naranjo et al., 2004; Ellsworth and Barkley, 2005; Naranjo and Ellsworth, 2009a). These sprays helped us to achieve similar prey densities throughout the experiment, especially in treatments that are likely to have higher prey densities over time, such as the UTC and the acephate positive control treatment.

Acephate has essentially no effect on *B. argentifolii*, thus precipitating resurgence of this pest that made it challenging to stabilize prey levels in this treatment even with whitefly-targeted maintenance sprays. Whitefly densities in our study were not at outbreak levels (Ellsworth

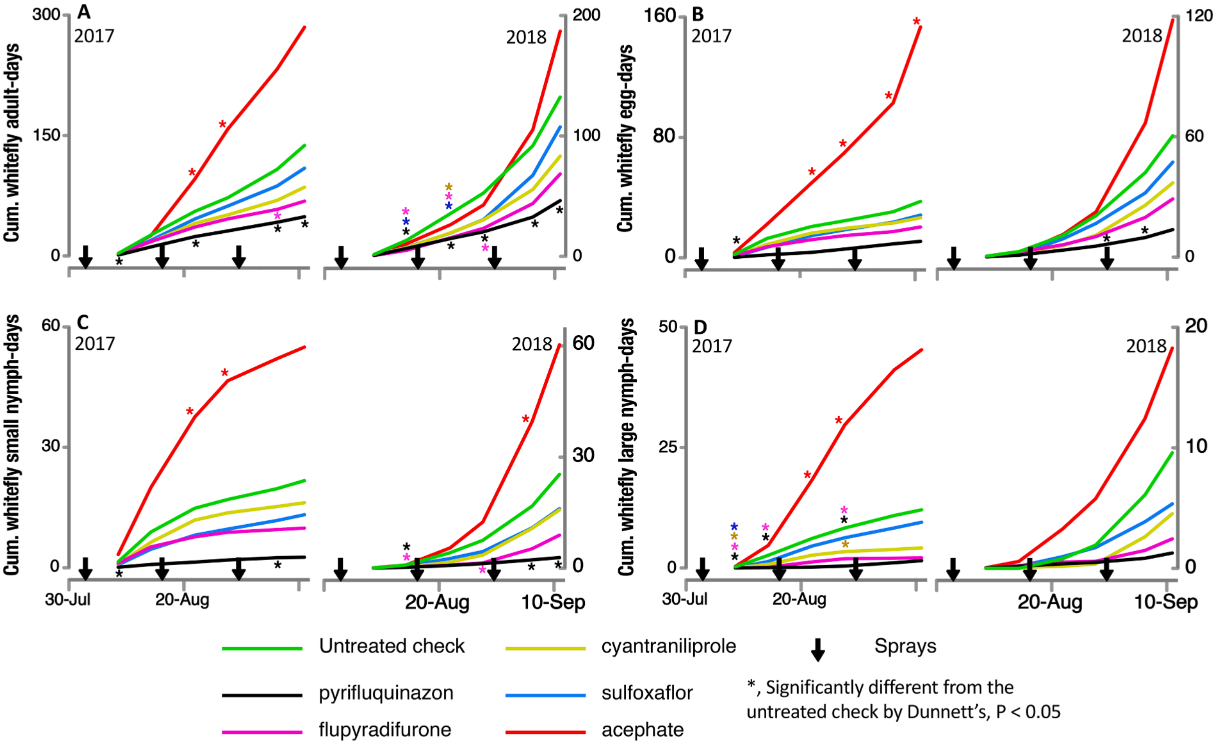

Fig. 3. Cumulative mean insect-days for *Bemisia argentifolii,* expressed as number of adults per leaf (A), eggs (B), small (C) and large (D) nymphs per 3.88 cm^2 leaf disc. Statistical analyses were conducted on abundance measured on each sampling date. Asterisk colors correspond to treatment means for insecticides that were significantly different from the untreated check by Dunnett's, $P < 0.05$.

et al., 2006) because they were effectively controlled by our maintenance spray(s) in UTC and positive control plots, resulting in moderate whitefly densities, particularly in 2018. However, when the acephate treatment significantly increased whitefly abundance and associated honeydew, there was potential for distorted natural enemy distributions. For instance, increases in densities of *C. carnea* in the acephate treatment were likely related to increased whitefly prey availability. Other factors and interactions could have contributed to these patterns, such as greater reproductive success of predators within plots unhindered by intraguild predation and greater reproduction due to honeydew stimuli (Evans and Swallow, 1993; Naranjo et al., 2004).

Despite efforts to stabilize prey densities among treatments, significant reductions in predator abundances still occurred occasionally and were likely associated with lower prey availability after insecticide sprays rather than direct toxic effects (Naranjo et al., 2004). Pyrifluquinazon was the treatment that most frequently had significantly lower whitefly densities in both years over multiple dates. There also were temporary, but significant reductions in abundances of *O. tristicolor* and *G. punctipes* in the pyrifluquinazon treatment that were likely associated with the concomitant reductions in availability of whitefly eggs and nymphs rather than direct toxic effects of the insecticide. The favorable predator to prey ratios of these species in the pyrifluquinazon treatment and comparison of community patterns for this insecticide showing significant differences against the positive control acephate (with known toxic effects) as the standard in PRC analyses (Figs. S3) support our conclusion that the effects measured are more likely due to low prey availability.

Predator to prey ratios also were used to account for prey disparities. Analogous to rates of parasitism that express proportion of hosts parasitized (without hosts there are no parasitoids) we examined the ratio of predator to prey. These ratios minimize issues with prey availability because the number of predators needed for biological control is expected to be proportional to prey abundance in the system. The critical ratios for each predator act as a proxy for the entire natural enemy community and indicate when biological control of whiteflies is possible (Vandervoet et al., 2018; Ellsworth et al., 2019a; 2019b). These ratios can verify how often each insecticide favored biological control throughout the season when compared with the untreated check. We demonstrated that all insecticides except acephate could support biological control through generally favorable changes in predator to prey ratios.

The use of selective insecticides has been critical in favoring biological control by maintaining the integrity of the food web in the Arizona cotton system (Naranjo and Ellsworth, 2009b). The food web is populated by generalist and omnivorous predators that feed on target prey as well as on each other (Hagler, 2006, 2011, 1994, 2004, 2005, 2015; Hagler et al., 1992; Hagler and Blackmer, 2013). Feeding habits of predators may contribute to their survival during periods of low prey density, for instance through intraguild predation and plant feeding. All these factors help to support a flexible and resilient food web that remains functional in pest control even when circumstances may reduce the abundance of several key natural enemies. Our results from the community analyses and the predator to prey ratios showed that the selective insecticides conserved most of the natural enemies, and served to fortify the food web and resulting biological control. While the eight critical predator to prey ratios denote functioning biological control of the entire community, they are independent from one another (Vandervoet et al., 2018; Ellsworth et al., 2019a; 2019b). Thus, one ratio at a level that supports biological control is sufficient to suppress whiteflies even when the abundance of other predators is low due to variable factors, such as seasonality and management practices. In contrast, broad-spectrum insecticides, such as acephate can collapse the food web because multiple predators are disrupted. As a result, pests rapidly reach threshold levels, and outbreaks of secondary pests are triggered (Asiimwe et al., 2013).

Toxicological effects of the candidate insecticides on some of our key predators or related species have been observed, usually under laboratory conditions. Laboratory tests on some of these insecticides detected

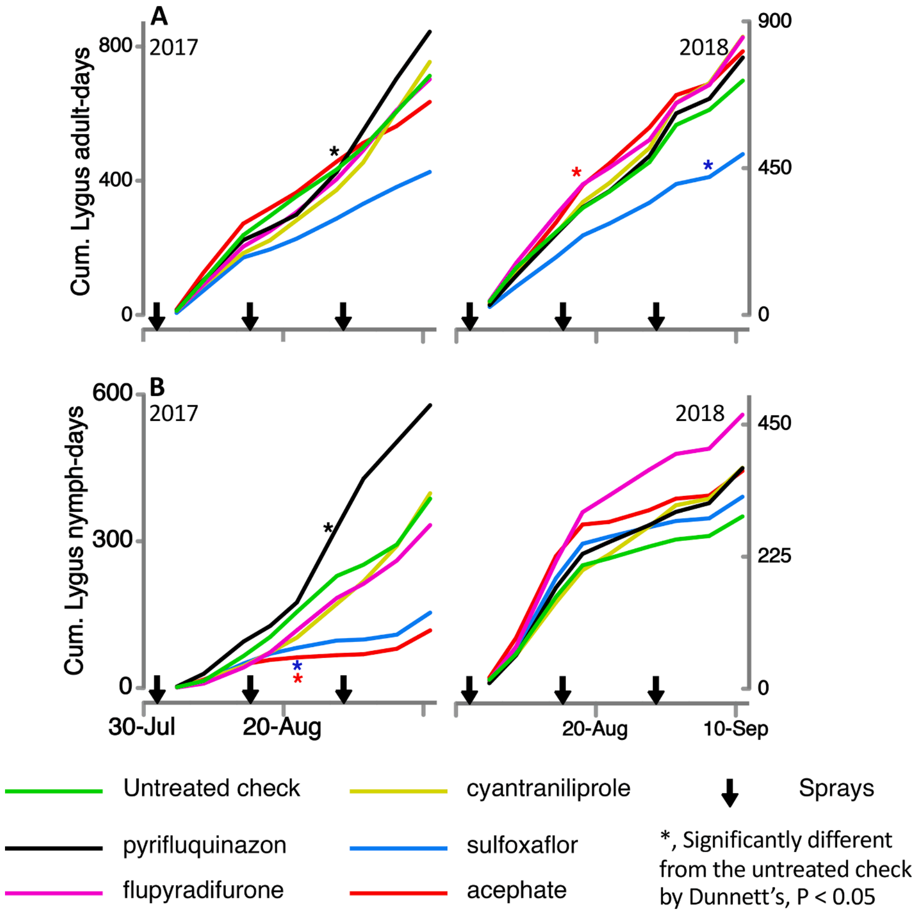

Fig. 4. Cumulative mean insect-days for *Lygus hesperus* adults (A) and nymphs (B) per 100 sweeps. Statistical analyses were conducted on abundance measured on each sampling date. Asterisk colors correspond to treatment means for insecticides that were significantly different from the untreated check by Dunnett's, $P < 0.05$.

Table 3

Fixed effect *F*-values of proportion of dates that each of the eight predator to prey ratios were above levels associated with biological control of *B. argentifolii* in the Arizona cotton system.

Fixed Factors	DF	*M. celer/B. arg. nymph*	*M. celer/B. arg. adult*	*D.* nr *divergens/ B. arg. nymph*	*D.* nr *divergens/ B. arg. adult*	*O. tristicolor/B. arg. adult*	*Collops* spp./ *B. arg. nymph*	*G. punctipes/B. arg. nymph*	*C. carnea* larvae/*B. arg. adult*
Insecticide	5, 36	9.71***	8.31***	10.31***	5.22**	4.0**	0.49^{NS}	8.35***	0.05^{NS}
Year	1, 36	47.21***	0.06^{NS}	25.02***	15.27***	0.01^{NS}	9.42**	0.79^{NS}	0.12^{NS}
Insecticide*Year	5, 36	0.61^{NS}	0.98^{NS}	0.02*	0.97^{NS}	0.29^{NS}	1.62^{NS}	0.61^{NS}	0.55^{NS}

Mixed-model ANOVA, * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; ^{NS}: not statistically significant, $P > 0.05$. *B. arg*: *B. argentifolii*. DF are approximated.

significant impacts on *Orius* spp. and *Chrysoperla* spp. (Amarasekare and Shearer, 2013; Amarasekare et al., 2016; Andorno et al., 2019; Tran et al., 2016; Prabhaker et al., 2017; Barbosa et al., 2017; Cloyd and Herrick, 2018; Dale and Borden, 2018; Dáder et al., 2019); however, other authors found minor or no impacts on these species in both field and laboratory trials (Funderburk et al., 2013; Srivastava et al., 2014; Garzón et al., 2015; Bacci et al., 2018; Barbosa et al., 2017; Herrick and Cloyd, 2017; Machado et al., 2019; Nichino America, 2019). A field trial detected significant reductions in the abundance of predators (*Nabis* spp., *Orius* spp., *Geocoris* spp.) only at the highest rate of flupyradifurone (Shimat and Bolda, 2016); however, this study did not attempt to stabilize prey resources or examine predator to prey ratios. Thus, it is possible that the lower abundance of predators measured at the highest rate of flupyradifurone was due to reductions in prey availability. Laboratory studies provide information on insecticide hazard, while field studies determine insecticide risk, a combination of hazard and exposure (Naranjo and Ellsworth, 2009b; Beers et al., 2016). Insecticides need to be validated in the system of interest, because their impacts are dependent on ecological context (Naranjo and Ellsworth, 2009b). Even though our conclusions might be system-specific, our approach for testing could be universally applied.

The inclusion of selective insecticides in the Arizona IPM plan for *B. argentifolii* and *L. hesperus* has enabled the conservation of arthropod predators that significantly contribute to suppression of our key pests below economic levels and vastly reduced the total number of arthropod-targeted sprays in Arizona (Naranjo and Ellsworth, 2009b; Anonymous, 2014; Ellsworth and Naranjo, 2017; Ellsworth et al., 2018). This elegant combination of selective chemical controls with biological controls is at the core of IPM, first articulated by Stern and colleagues (1959) as integrated control. Field trials on insecticide selectivity have

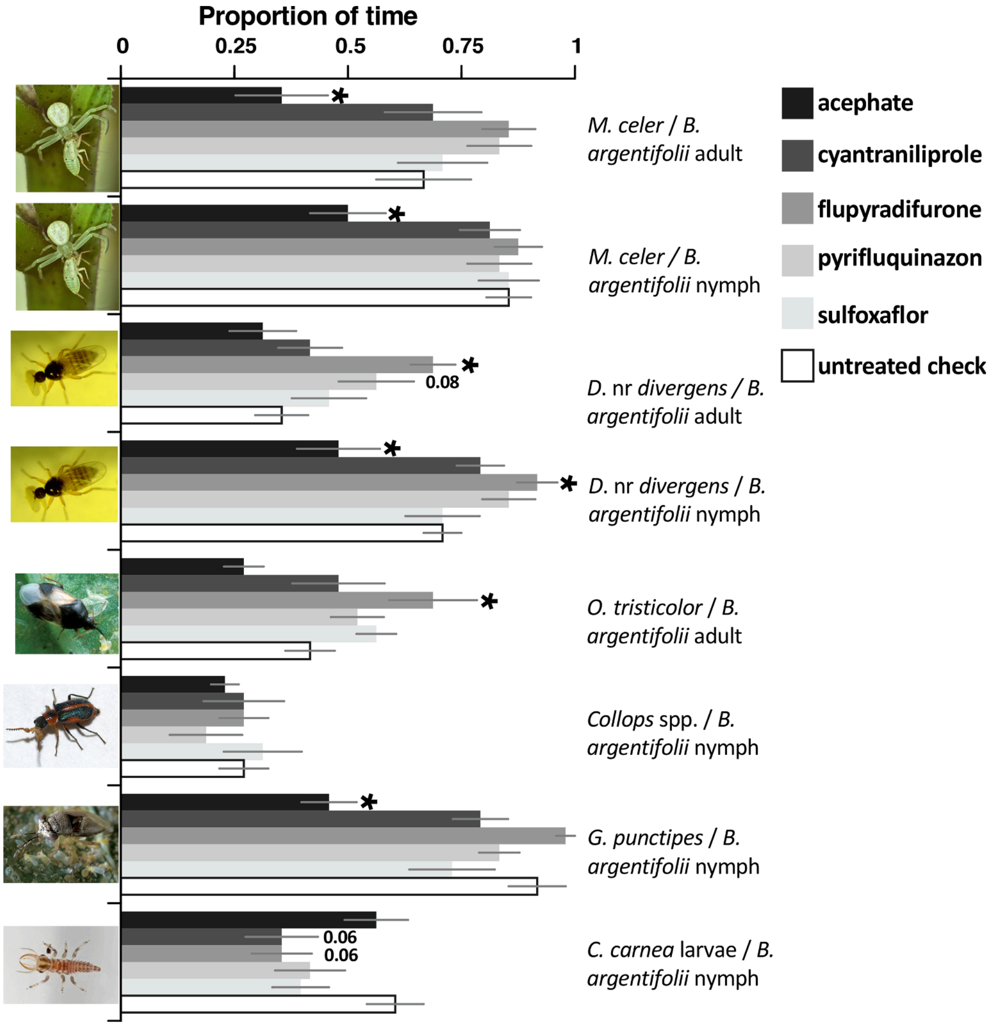

Fig. 5. Proportion of time each of the eight predator to prey ratios were above functioning biological control critical ratios known in our system (mean ± SE; Vandervoet et al., 2018). For each insecticide, these proportions were compared to the UTC by Dunnett's (*, $P < 0.05$); P-values < 0.10 are also provided.

helped to boost these advances in IPM and reduce risk to human health, non-target organisms, and the environment through adoption and proper use of selective insecticides (Naranjo and Ellsworth, 2009b; Anonymous, 2014; Ellsworth and Naranjo, 2017; Ellsworth et al., 2018). Our study demonstrates that the insecticides tested are selective and compatible with sustainable pest management in the Arizona cotton system, representing new options of insect pest control that conserve natural enemies and support conservation biological control.

CRediT authorship contribution statement

Isadora Bordini: Conceptualization, Data curation, Formal analysis, Funding acquisition, Methodology, Project administration, Visualization, Writing - original draft. **Peter C. Ellsworth:** Conceptualization, Data curation, Formal analysis, Funding acquisition, Methodology, Project administration, Visualization, Writing - original draft, Writing - review & editing. **Steven E. Naranjo:** Conceptualization, Formal analysis, Funding acquisition, Methodology, Visualization, Writing - original draft, Writing - review & editing. **Alfred Fournier:** Funding acquisition, Writing - review & editing.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biocontrol.2020.104502.

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