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Long term dynamics of aphelinid parasitoids attacking *Bemisia tabaci* ☆

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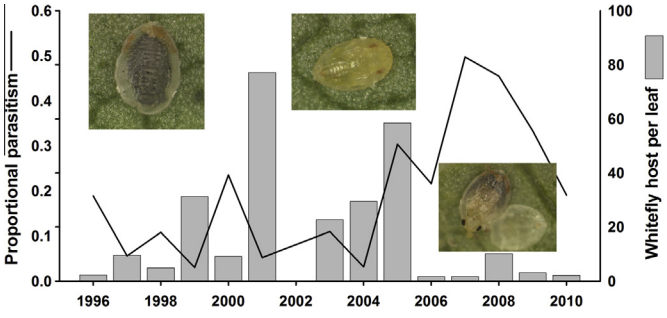
HIGHLIGHTS

- We examined aphelinid parasitoids attacking *Bemisia tabaci* over a 15 year period.
- Native aphelinid species were displaced by introduced exotic species.
- Decreasing pest abundance was associated with increasing apparent parasitism.
- Introductions do not appear to have impacted pest control in Arizona cotton.
- Multi-habitat life table studies are needed to assess success of introductions.

GRAPHICAL ABSTRACT



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ABSTRACT

Aphelinid parasitoids are widely known natural enemies of *Bemisia tabaci* (Gennadius), a serious pest of agriculture globally. Here we examine pest–parasitoid interactions and dynamics in Arizona cotton from 1996 to 2010, during which a classical biological control program was implemented. Two native species, *Eretmocerus eremicus* Rose and Zolnerowich and *Encarsia meritoria* (Gahan) were either largely or completely displaced by exotic *Eretmocerus* sp. (Ethiopia) and *Encarsia sophia* (Gahan) in the early 2000s. Further, *E. sophia* became the dominant parasitoid of *B. tabaci* in cotton after many years of predominance by native and exotic *Eretmocerus*. Apparent rates of parasitism were highly variable within and between years and averaged ≈17% overall. In some years there was evidence that *B. tabaci* populations declined as apparent parasitism increased. Lower pest abundance was associated with higher rates of apparent parasitism over the entire 15-year period but this pattern was not supported by long term life-table based measurements of parasitism. Detailed life table studies within the entire agro-ecosystem will be needed to fully assess the impact of the classical biological control program.

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1. Introduction

Most herbivorous insects host a diverse array of insect parasitoids that affect population dynamics of the host and overall community organization (Hochberg and Hawkins, 1994). Aphelinid parasitoids belonging to the genera *Eretmocerus* and *Encarsia* are common members of natural enemy communities associated with *Bemisia tabaci* (Gennadius) populations in agricultural systems worldwide (Hoelmer, 1996; Gerling et al., 2001; Arnó et al., 2010; Liu et al., 2015). In some instances they can be responsible

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for high levels of parasitism in *B. tabaci* populations (Gerling, 1967; Bellows and Arakawa, 1988; Kajita et al., 1992; McAuslane et al., 1993; Stansly et al., 1997) and may even be key factors associated with variations in mortality across generations (Asiimwe et al., 2007; Karut and Naranjo, 2009).

B. tabaci Biotype B (Middle East-Asia Minor Group 1; Dinsdale et al., 2010; De Barro et al., 2011) invaded the low desert regions of Arizona and California in the early 1990s and quickly became a key economic pest of many agronomic and horticultural crops in the region, including cotton (Oliveira et al., 2001). Some early work attempted to assess the associated natural enemy fauna in the southwestern USA (Hagler and Naranjo, 1994a,b; Hoelmer, 1996; Gerling and Naranjo, 1998; Naranjo et al., 2003, 2004; Hagler and Naranjo, 2005; Naranjo and Ellsworth, 2005; Hoelmer et al., 2008). These studies showed that while numerous arthropod predator species were known to attack *B. tabaci* in southwestern USA cotton and other crops, the parasitoid fauna was more limited and comprised of a single native species of *Eretmocerus* (*Eretmocerus eremicus* Rose and Zolnerowich), and several native species of *Encarsia*, including *Encarsia luteola* Howard and to a greater extent *Encarsia meritoria* Gahan. These species are primarily parasitoids of native whitefly species such as *Trialeurodes abutiloneus* (Haldeman) (Hoelmer et al., 2008), and the indigenous biotype of *B. tabaci* (Biotype A, Costa and Brown, 1991) that has been present in Arizona since at least 1926 (Russell, 1975). Shortly after the invasion of *B. tabaci*, significant emphasis was placed on classical or introductory biological control (Faust, 1992). This was precipitated in part due to past success in using classical biological control for other whitefly species (Onillon, 1990; Williams, 1996), and the general lack of parasitoid diversity, especially in some key crops comprising the seasonal cycle of *B. tabaci* in parts of the USA (Hoelmer, 1996). From 1992 to 1999, over 50 populations of *Encarsia* spp. and *Eretmocerus* spp. were imported into quarantine in the USA and many of these were cultured and released in the southern tier of the country (Goolsby et al., 2005; Kirk et al., 2008). During this period, approximately 30 exotic populations of aphelinid parasitoids were released in the low desert agricultural production areas of California, including some releases in Yuma on the western border of Arizona (Roltsch et al., 2008a). Four species of *Eretmocerus* were released in large numbers in the Phoenix, Arizona area in the mid to late 1990s (Gould et al., 2008b). These included *E. hayati* Zolnerowich and Rose, *E. mundus* Mercet, *E. emiratus* Zolnerowich and Rose, and *Eretmocerus* sp. from Ethiopia. Several populations of the heteronomous hyperparasitoid *Encarsia sophia* (Girault and Dodd) were intentionally released in southern California and Yuma (Roltsch et al., 2008a), and also were released as contaminants of the *Eretmocerus* cultures in the Phoenix, Arizona, area (SEN, personal observation).

Between 1996 and 2010 we have amassed data on the composition and relative abundance of *Eretmocerus* and *Encarsia* parasitoids in Arizona cotton as part of numerous studies to examine population dynamics and biological control of *B. tabaci*. Our objectives here are to describe the long-term patterns of parasitoid community composition and levels of parasitism in *B. tabaci* populations in cotton. This collection of studies overlapped with the classical biological control program for *B. tabaci* and affords us the opportunity to examine how species composition and levels of parasitism have changed in a key summer crop affected by the pest.

2. Materials and methods

2.1. Study sites

All study sites were located on the ≈900 ha University of Arizona, Maricopa Agricultural Center farm in Maricopa, Arizona, USA. Data on parasitoid and *B. tabaci* abundance were primarily collected from control (untreated) plots of cotton that were part of various experimental studies to evaluate and assess insecticide selectivity, whitefly dynamics, and biological control from 1996 to 2010. Cotton, *Gossypium hirsutum* L., was planted in mid-April to early-May each year and grown according to standard agronomic practices for the area. Details of the experimental studies each year are summarized in Table 1. Similar experimental designs were used in all years and consisted of a randomized complete block replicated four times in all years but 1996 where there were three replicate blocks. The means and SEM presented are based on this experimental design structure.

2.2. Insect sampling

Leaf samples from the 7th node below the mainstem terminal were collected to estimate the abundance of *B. tabaci* and aphelinid parasitoids (*Eretmocerus* spp. and *Encarsia* spp.) attacking *B. tabaci*. Samples (20–30 whole leaves per plot) were randomly collected weekly to bi-weekly from early July through late September or early October each year. In the laboratory, all larval and pupal parasitoids of *Eretmocerus* spp. and *Encarsia* spp. and all unparasitized fourth instar whitefly nymphs were counted on the entire leaf using a dissecting microscope. The presence of visible larvae (C-shaped or not) within the host or the presence or absence of meconia associated with pupal stage parasitoids in the mummy was used to discriminate *Encarsia* spp. from *Eretmocerus* spp. Displacement of the host's bacteriomes was used to determine the presence of young parasitoid larvae, but in these cases the genus of the parasitoid could not be discerned. The number of *B. tabaci* exuviae and

Table 1
Summary of cotton studies, 1996–2010, Maricopa, AZ.

Year	Variety	Individual plot size ha	Total experimental area ha (% sprayed)[a]	No. sample dates	Associated study
1996[b]	Deltapine NuCOTN33B	1.2–2.0	72 (100)	10	Naranjo et al. (2003)
1997–1999	Deltapine NuCOTN33B	0.11–0.13	1.8–2.1 (75)	9–11	Naranjo et al. (2004)
1999–2001, 2003	Deltapine NuCOTN33B & Deltapine 5415 [isoline of 33B]	0.12–0.17	1.0–1.4 (0–50)	8–9	Naranjo (2005)
2004	Deltapine 449BR	0.095	0.8 (0)	10	Current study
2005	Deltapine 449BR	0.13–0.40	5.3 (90)	9	Current study
2006	Deltapine 449BR	0.13–0.29	5.4 (90)	8	Current study
2007	Deltapine 164B2RF	0.09–0.29	3.7 (90)	3	Current study
2008	Deltapine 164B2RF	0.06	3.8 (90)	4	Current study
2009	Deltapine 164B2RF	0.06	2.6 (91)	4	Current study
2010	Deltapine 1044 B2RF	0.05	2.2 (91)	4	Current study

[a] Proportion of experimental plot area sprayed with selective (for *B. tabaci*) or non-selective insecticides.
[b] The commercial-scale experiment in 1996 did not have an untreated control plot. Thus, results are based on combined data from all plots in each replicate block.

the empty cadavers left behind following parasitoid emergence were also counted on each leaf. Whole leaf samples were lost in 2002 due to a refrigerator malfunction.

2.3. Parasitoid identification

Each year from 1996 to 1999 and 2002 to 2010, subsamples of leaves from each plot were held to determine genera and species composition from emerged adults. In 2000 and 2001 we did not set aside samples for species identification, but did determine genera. Although we lost whole-leaf samples in 2002, there were sufficient parasitoids collected from on-going life-table studies (Naranjo, 2005) to estimate genera and species composition for that year. For the studies prior to 2000, Kim Hoelmer (now USDA-ARS, Delaware) helped identified parasitoids in these subsamples. From 2002 to 2003 Mike Rose (Montana State University, now deceased) aided with identification of *Eretmocerus* spp. and this coincided with the first appearance of exotic species from the classical biological control program of the mid to late 1990s (Gould et al., 2008a).

To further verify these identifications and identify specimens after 2003, we used a RAPD-PCR technique initially developed by USDA-APHIS (Vacek et al., 2008) to help catalog and track multiple species of exotic aphelinids imported into the USA in the 1990s. For these analyses, single parasitoids were crushed in a 0.5 ml tube containing extraction buffer (5 mM Tris–HCl PH 8.0, 0.5 mM EDTA, 1% Tween 20, 1 mg/ml proteinase K and sterile ddH_2O). The homogenate was incubated at 60 °C for 60 min, then boiled at 95 °C for 10 min to inactivate the proteinase K. Extracted DNA was stored at −20 °C until use. The RAPD-PCR reaction mix containing dNTPs, $MgCl_2$, buffers, 10-mer primer (OPC-04 or OPA-10), and Taq DNA polymerase was prepared in 0.5 ml tubes and then 2 μl of extracted DNA was added for a final volume of 25 μl. The PCR procedure (Black et al., 1992) used here was as follows: (1) an initial step of 80 °C for 25 min, (2) 94 °C for 1 min, (3) 45 cycles of amplification with 92 °C for 1 min, 35 °C for 1 min, slope to 72 °C at 1 °C steps every 8 s, and 72 °C for 2 min, (4) a final step at 72 °C for 2 min, then held at 4 °C indefinitely. The PCR products were separated by agarose gel electrophoresis. The amplified PCR products were loaded into a 1.5% agarose gel and run for sufficient time for the amplicons to separate into distinct bands. All gels were digitally scanned and archived with an analytical imaging system (Alpha Innotech, San Leandro, California USA). The association of specific banding patterns and species were determined based on Vacek et al. (2008).

2.4. Data summary and analyses

Although *B. tabaci* and parasitoid samples were collected in most treatment plots in most years, summary and analysis here are restricted mainly to those samples collected in control plots receiving no insecticidal sprays. Data from parasitoid identification were compiled to examine the changing patterns of species and genera composition as two exotic aphelinid parasitoid species became established in central Arizona. To examine parasitoid-host interactions two indices of apparent parasitism were estimated. The first was based on live insects and was calculated as the proportion of fourth instar nymphs parasitized (parasitized nymphs/(4th instar nymphs + parasitized nymphs). The definition of parasitism here is broad and included both larval and pupal parasitoid stages. The second index was based on whitefly exuviae and empty whitefly mummies. Apparent parasitism here was calculated as empty whitefly mummies/(whitefly exuviae + empty whitefly mummies). Spearman's rank correlation was used to test for the association of *B. tabaci* host abundance and rate of apparent parasitism each year. This analysis used the 3–4 replicate measures for each sample date (n = 12–44). The Benjamini and Hochberg (1995) method, with the false discovery rate set at 5%, was used to correct hypothesis testing for multiple correlation tests within each parasitism estimation method. Spearman's rank correlation was further used to examine patterns between *B. tabaci* abundance and parasitism over the entire 15-year period. For this analysis, seasonal means for apparent parasitism rates and host density were averaged over multiple sample dates for each replicate plot in each year (n = 55). Estimates of marginal parasitism from on-going field based life tables (Naranjo, 2005; Naranjo and Ellsworth, 2005; Naranjo et al., 2009; unpublished) also were examined in relation to host density (n = 38). Each marginal estimate of parasitism via life-tables was based on an average of 2–6 cohorts with more than 50–200 individuals per cohort each year (see Naranjo and Ellsworth (2005) for methodological details). Finally, associations among the various methods of estimating parasitism were examined. All analyses were performed with JMP V9 (SAS Institute, Cary, NC, USA).

We did not attempt to examine density-dependence in rates of parasitism in this study because apparent rates of parasitism are not based on whitefly generations but are general measures of parasitoid and host interactions that would involve overlapping generations of the host.

3. Results

3.1. Parasitoid composition

From 1996 to 1999 only native *E. eremicus* and native *Encarsia* (primarily *E. meritoria*) were found in leaf samples from cotton (Fig. 1). However, by 2002, two exotic species, *Eretmocerus* sp. (Ethiopia) and *E. sophia* had become well established and by 2004 the native *E. eremicus* had been completed displaced in cotton. The displacement of the native *Encarsia* spp. was less complete with just over 20% of the parasitoid complex represented by these species as late as 2010. The community transition from native to exotic species appears to have initiated sometime around 2000–2001, the two years in which subsamples were unfortunately not collected to determine species composition.

The introduction and establishment of the exotic aphelinid species in the Arizona cotton system also were associated with a rather sudden shift in the overall parasitoid community. *Eretmocerus* spp. (native and/or exotic) were the dominant parasitoids of *B. tabaci* from 1996 until 2005. From 2005 to 2006 there was a sudden transition to strong dominance by *Encarsia* species, primarily the exotic *E. sophia* (Fig. 1). This dominance persisted through 2010 and this pattern does not appear to have changed in the last few years (SEN personal observation).

3.2. Dynamics of parasitism

Patterns of apparent parasitism based on the combination of all species and on live whitefly and parasitoid stages varied over the season and in relation to abundance of *B. tabaci* hosts (Figs. 2 and 3). Rates of parasitism varied greatly from year to year. Peak rates were moderate (30–40%) in some years (1996, 1998, 2000, 2006, 2009, 2010) and high (>60%) in several years (2005, 2007, 2008). In contrast, parasitism rates were low in other years, rarely exceeding 20% in 2003 or 10% in 1997, 1999, 2001 and 2004. Host density was equally variable over the years of this study with very low densities in 1996 and very high densities from 1999 to 2005, followed by low densities from 2006 until the end of monitoring in 2010. The highest whitefly densities were associated with the highest rates of parasitism in 2005. In this year, the crop was planted relatively late and there were issues with weather and

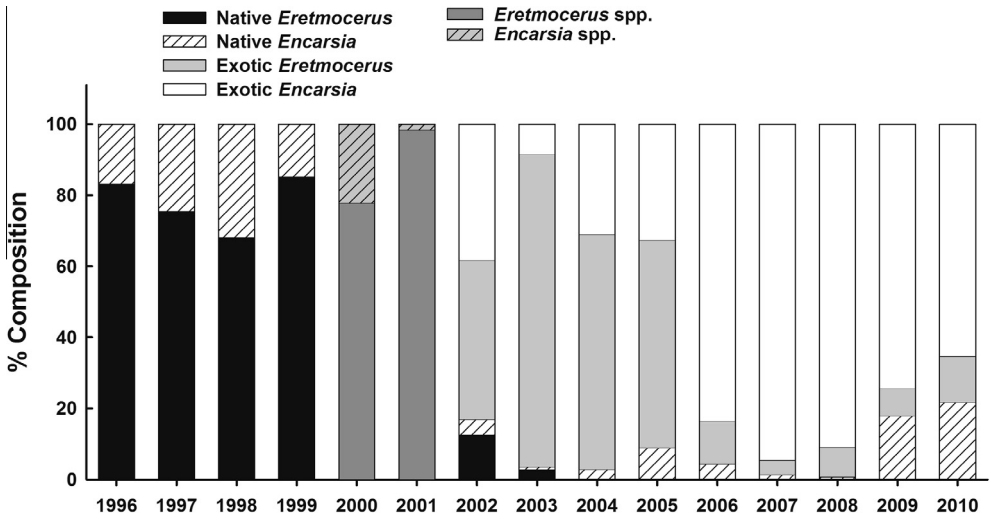

Fig. 1. Relative composition of native and exotic *Eretmocerus* spp. and *Encarsia* spp. attacking *Bemisia tabaci* in cotton from 1996 to 2010, Maricopa, Arizona, USA. $n = 75$–17,281 per year. In 2000 and 2001 only parasitoid genus was determined.

timely insect management decisions in surrounding treated cotton plots. In the majority of years parasitism rates generally increased as the season progressed but the relationship of these increases to host density was not consistent (Fig. 3). In other years, parasitism peaked mid season and in several years there was no distinct pattern (e.g., 1997, 2004). Parasitism rates based on insect exuviae were generally lower than those based on live insects but were equally variable and showed similar temporal patterns (data not shown).

In the majority of years, there were numerically negative associations between host abundance and rates of apparent parasitism, regardless of how parasitism was estimated (Fig. 3). This suggests that higher rates of parasitism were associated with declining host abundance. In some years the associations were positive, suggesting that rates of parasitism increase with host abundance. Overall, associations (negative or positive) were statistically significant in half of the years examined based on both methods of estimating parasitism (Fig. 3).

A final analysis was conducted to examine the association between parasitism and host density over the 15-year period of this study. As noted from individual year analyses above, rates of parasitism and whitefly density varied widely over time (Fig. 2). Analyses showed that host density was negatively correlated with apparent parasitism based on both live insect stages (Spearman's = −0.457; $P = 0.0006$, $n = 55$) and mummies and exuviae (Spearman's = −0.280; $P = 0.042$, $n = 55$). There was no correlation between host density and marginal rates of parasitism derived from cohort-based, field life tables ($P = 0.877$, Fig. 4). The two methods of estimating apparent parasitism were significantly correlated with one another (Spearman's = −0.760; $P < 0.0001$, $n = 55$), but neither apparent estimate was correlated with marginal rates of parasitism from life-tables ($P > 0.162$).

4. Discussion

The aphelinid parasitoid community attacking the invasive *B. tabaci* (MEAM1) in the cotton system in Arizona is low in diversity and has undergone dramatic changes since the pest first invaded this region. Initially, *E. eremicus* and two species of *Encarsia*, predominantly *E. meritoria*, likely moved from native whitefly hosts such as *T. abutiloneus* (Hoelmer et al., 2008) or the indigenous Biotype A of *B. tabaci* (Costa and Brown, 1991) to parasitize the invasive *B. tabaci*. Levels of parasitism due to these species were generally low in cotton fields and played a minor role in the dynamics of the pest (Naranjo and Ellsworth, 2005). Following multiple introductions of a number of exotic aphelinid species (Roltsch et al., 2008a; Gould et al., 2008b) into Arizona and California, two new species (*Eretmocerus* sp. [Ethiopia] and *E. sophia*) established sometime between 2000 and 2002 in Arizona. These specialists of the invasive *B. tabaci* quickly displaced the three native aphelinid species but did not spill over into other non-*Bemisia* native whitefly species, at least based on surveys up to late 2001 (Hoelmer et al., 2008). The role of competition for limited hosts in this transition from a native to exotic dominated community appears weak because overall rates of parasitism remained relatively low during this period. Follow-up surveys to look at longer-term non-target effects of exotic parasitoids on native hosts or the return of native parasitoids to native hosts have not been done in this region. More changes occurred from the mid 2000s onward when *E. sophia* became the prevailing parasitoid in the community after many years of predominance by native, and then exotic *Eretmocerus*. Overall, this displacement of native aphelinid parasitoids suggests that the exotic specialists were perhaps more efficient at exploiting the invasive *B. tabaci*. A similar pattern in displacement of native *Eretmocerus* spp. by exotic *E. mundus* Mercet occurred in the Central Valley of California (Pickett et al., 2013). In this case it appears that *E. mundus* was primarily attacking *B. tabaci* while the native species were attacking native whitefly species.

There has been considerable discussion around the impact of heteronomous hyperparasitic *Encarsia* spp. parasitoids in biological control (Williams, 1996; Zang et al., 2011), with some models suggesting that inclusion of such species has the potential to destabilize biological control (Mills and Gutierrez, 1996; Briggs and Collier, 2001), but with experimental studies suggesting that pest control may not be negatively affected (e.g. Bogran et al. (2002), Hunter et al. (2002)). Williams (1996) showed that *E. tricolor* Förster drove the primary parasitoid *E. inaron* (Walker) to very low levels in experimental arenas when the two co-existed. Based on a literature review, he also showed that autoparasitic aphelinids were dominant in about 70% of natural communities, but only in about one-fourth of instances where they were introduced for biological control. These findings are somewhat consistent with the patterns we observed, where *Eretmocerus* sp. (Ethiopia) has been nearly eliminated from the parasitoid community in the cotton

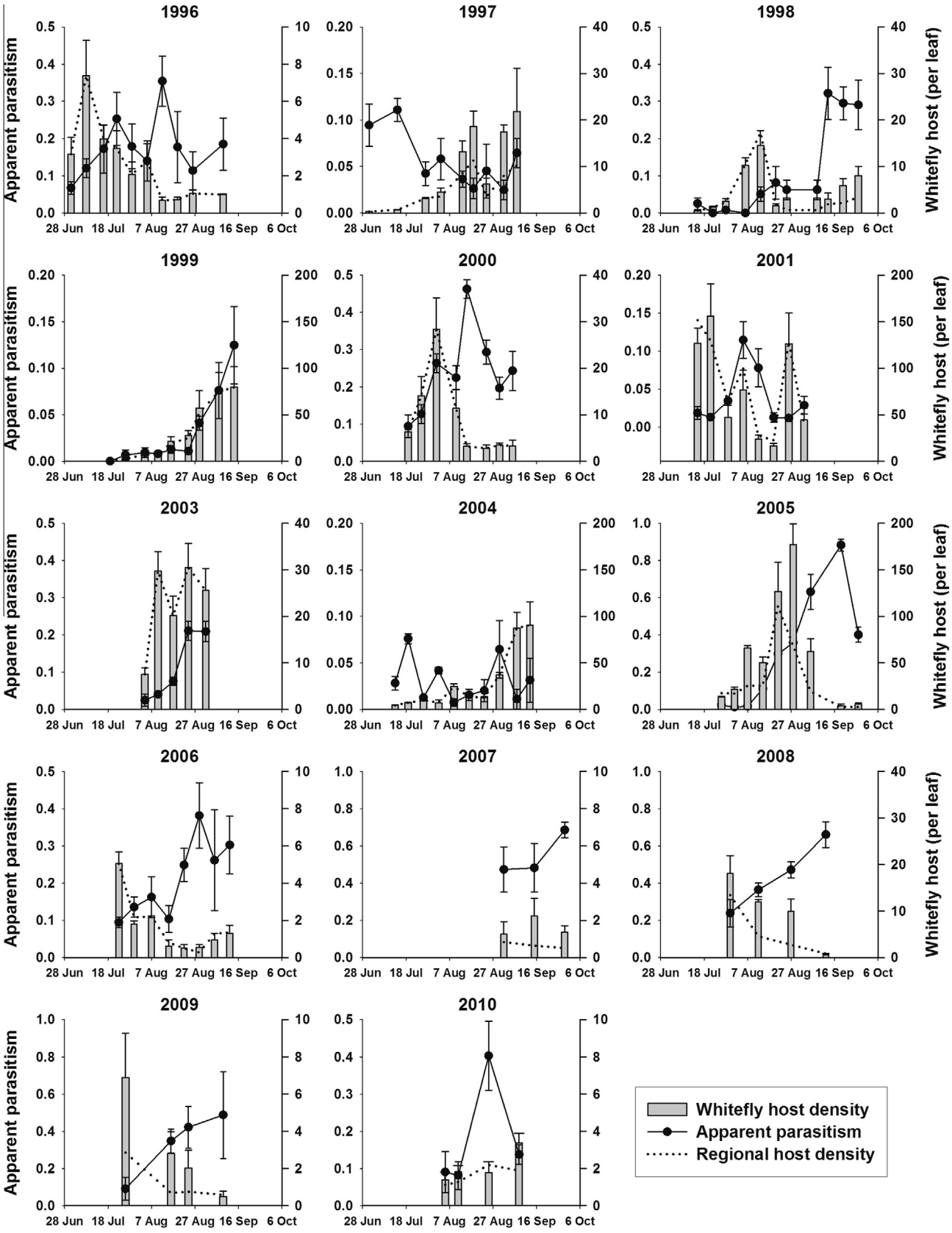

Fig. 2. Temporal dynamics of apparent aphelinid parasitism and host, *Bemisia tabaci*, abundance over the season for each year from 1996 to 2010. Apparent parasitism was estimated as the proportion of fourth instar nymphs parasitized (parasitized nymphs/(4th instar nymphs + parasitized nymphs). Error bars are SEM. The dotted line represents mean whitefly host abundance over the entire experimental area in each year. Note changes in *y*-axes scales.

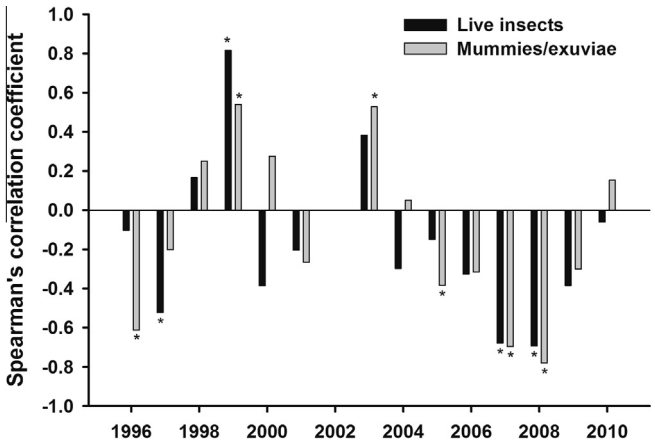

Fig. 3. Association between *Bemisia tabaci* host density and apparent parasitism measured using live host and parasitoid stages or using mummies and exuviae. Asterisks denote significant correlations (Spearman, $P < 0.05$).

system after an initial dominance for the first few years following its establishment. To our knowledge, interactions between these two species have not been examined in more detail. However, it appears that the dynamics we observed likely reflects the utilization of *Eretmocerus* sp. (Ethiopia) by *E. sophia* for the production of their males that resulted in a decline in abundance of this host parasitoid species. Whether this has disrupted biological control of *B. tabaci* is unclear. Life table studies with the native complex showed that the contribution of parasitoids to biological control of the pest in Arizona cotton was minor (Naranjo and Ellsworth, 2005). Based on apparent parasitism rates here and more accurate marginal parasitism rates from life table studies following establishment of the exotic aphelinids (Naranjo, 2008; unpublished), it appears that parasitism rates on average have not changed much since the exotics became established and not much since populations of the exotic *Eretmocerus* declined precipitously in 2005–2006. This would suggest that *E. sophia* is now contributing more to overall parasitism than it was when *Eretmocerus* spp. were dominant. In the Imperial Valley of California, *E. sophia* also rose in prominence after its establishment in the late 1990s and experimental cage studies demonstrated that the populations of this species originating from Pakistan had comparatively high rates of reproduction (Hoelmer and Roltsch, 2008). The DNA fingerprints that we used here do not allow us to discriminate the different *E. sophia* populations introduced, but based on the dominance pat-

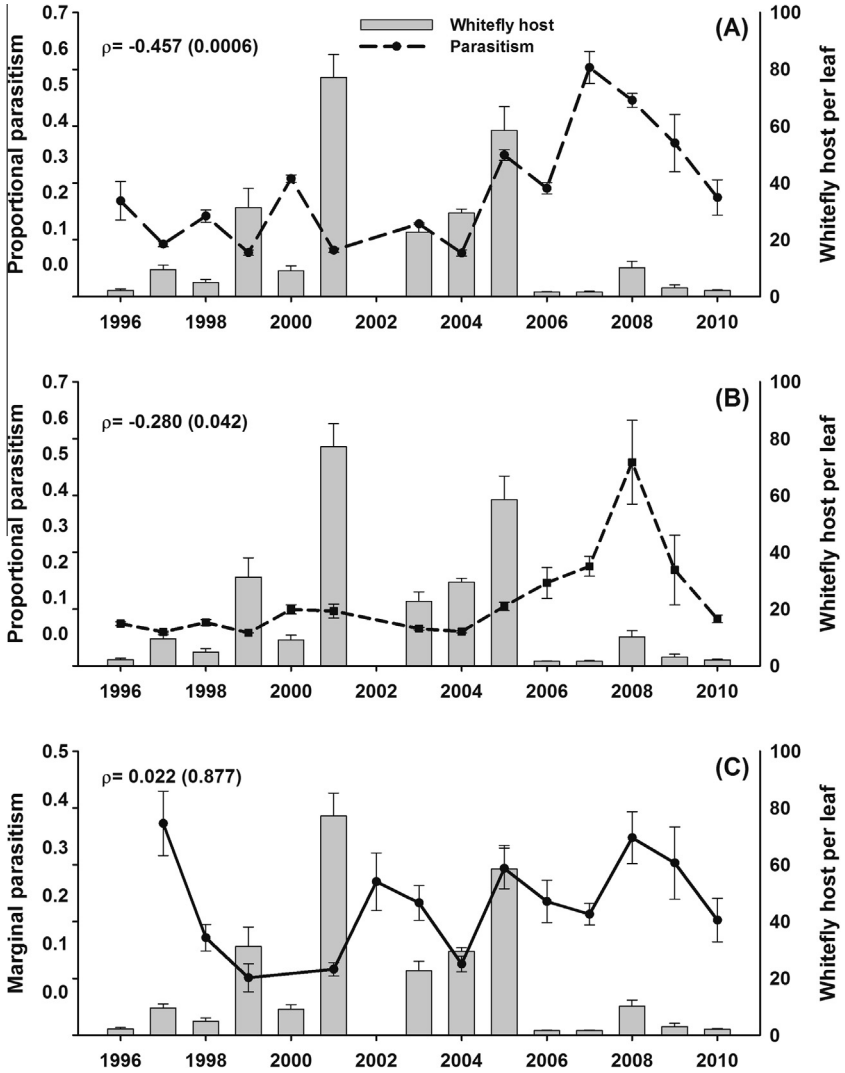

Fig. 4. Summary of aphelinid parasitism and host *Bemisia tabaci* abundance from 1996 to 2010, Maricopa, Arizona, USA using three measures of parasitism (A) apparent rates based on live parasitoid and whitefly stages, (B) apparent parasitism based on whitefly mummies and exuviae and (C) marginal parasitism from cohort-based field life tables (Naranjo and Ellsworth, 2005; Naranjo, 2005; Naranjo et al., 2009, unpublished). Values are seasonal means and SEM based on the experimental replicate structure ($n = 3–4$) for A and B and on life tables conducted on 2–6 generations per year for C.

terns observed here we hypothesize that it is the better adapted Pakistan population that has become established in Arizona.

We found that levels of apparent parasitism by the combined action of all aphelinid species varied greatly within each season and from year to year. Over the 15 years of this study, apparent parasitism varied from <10% to >80% depending on year and time of the season in any given year (see Fig. 2). Seasonal means ranged from 4–54% and averaged about 17% over the entire 15-year period. Similar variations have been observed in other systems (e.g. Stansly et al. (1997), McAuslane et al. (1993), Kajita et al. (1992), Simmons et al. (2002)), including cotton (Gerling, 1967; Bellows and Arakawa, 1988). Such dynamics in parasitism are not unexpected. Like their whitefly hosts, parasitoids must colonize crops such as cotton anew each year. Mark recapture studies show that aphelinid parasitoids do not appear to be strong dispersers (Hagler et al., 2002; Byrne and Bellamy, 2003) and studies in which different host crops of *B. tabaci* were in close proximity to one another showed that levels of parasitism may be enhanced in the cropping system overall (Naranjo et al., 2009). Parasitism by aphelinids has been shown to be a key factor affecting *B. tabaci* populations in several systems including cotton in Turkey (Karut and Naranjo, 2009). We speculated that this pattern was facilitated by lower overall summer temperature and more diverse cropping systems in Turkey. The habitat management approach used in the Imperial Valley, CA to aid the success of exotic parasitoid establishment (Roltsch et al., 2008b) is consistent with the positive effects of diverse cropping systems on whitefly parasitoid populations.

A common technique used to gauge the impact of a classical biological control program is to examine longer-term changes in host density relative to changes in the activity of the biological control agent. Here, we examined these associations over short (yearly) time scales and over the course of 15 years. On a year by year basis we found that increasing levels of parasitism were sometimes significantly associated with declining host abundance. Further, there was a trend for these negative associations to be slightly more common after the establishment of the exotic parasitoid. One hypothesis would be that the increasing activity of the parasitoids was having a negative effect on the pest population, at least over a relatively small time period. Whether this represents a density-dependent response that could lead to pest population regulation is unknown, but prior life-table work before the exotic parasitoids became established suggests that temporal density-dependence is weak at best (Naranjo and Ellsworth, 2005). Life tables that examine generational mortality will be needed to test for density-dependence. We also found several years where parasitism increased in association with increased host abundance. This might suggest a numerical response to host density or perhaps greater efficiency in parasitism with higher host abundance.

We applied a similar correlation analysis to examine host and parasitism associations over the 15-year period of this study (see Fig. 4). Here we observed that a decline in *B. tabaci* populations in cotton were associated with an increase in the level of apparent parasitism measured using either of two different methods. This is suggestive of a potentially positive role for the exotic parasitoid introductions in lowering *B. tabaci* populations. However, this association did not hold if we used a more accurate measure of marginal parasitism from cohort-based life tables over the same 15-year period. In general, life table based estimates of marginal parasitism were more consistent from year the year, even in the face of generally declining *B. tabaci* populations. This overall decline in pest populations has largely been a result of significant improvements in management of cotton insects that have focused on conservation of natural enemies, particularly arthropod predators (Naranjo and Ellsworth, 2009). Many other facets in the system have also changed including better overall regional management of *B. tabaci* in the agricultural landscape. These effects cascade through the seasonal cycle of the pest and benefit all affected crops.

The differing results noted above force consideration of another factor that must be considered when assessing relationships between *B. tabaci*, and that is the way in which parasitism is measured. The frailty of using apparent parasitism to provide insight into parasitoid and host dynamics has been discussed generally (Van Driesche, 1983) and more specifically for whitefly parasitoids (Hoelmer, 1996; Naranjo, 2001). Hoelmer (1996) showed that a broad range of estimates of apparent parasitism could be derived from the same sample depending on what stages of the parasitoid and host were examined. The importance of this finding is that researchers need to be explicit in the methods they use for estimating apparent parasitism so that proper comparisons can be made across studies. Here we used the ratio of live larval and pupal stage parasitoids in 4th stage hosts or mummies to live *B. tabaci* 4th stage nymphs, a common approach. We also used the ratio of empty mummies to host exuviae, another common approach. The former resulted in slightly higher rates of parasitism, on average, but showed similar seasonal patterns to those based on mummies and exuviae. Nonetheless, the use of apparent parasitism may impose limitations on our understanding of pest and natural enemy dynamics.

Finally, the results of this study should be viewed through the lens of spatial scale and the potentially confounding effects of insecticides used both within the experimental plots areas and in the surrounding agricultural environment. Plot size was relatively small in some years although we have never found that this has compromised our ability to discern strong treatment effects for pest insects or their natural enemies (e.g. Naranjo et al. (2004), Naranjo and Ellsworth (2009), Asiimwe et al. (2013)). Quasi-commercial sized plots were employed in 1996 and it does not appear that the dynamics we observed there differ in any meaningful way from other years of the study. Insecticides are putatively problematic and certainly played a role in reducing larger scale densities of both the host and the parasitoids (see Fig. 2). However, Gerling and Naranjo (1998) showed that rates of apparent parasitism varied little between insecticide-treated and untreated plots. They reasoned that the insecticides caused similar proportional declines in both the parasitoids and the hosts. In any case, insecticides are part of agro-ecosystem associated with cotton in Arizona and all other cropping systems affected by *B. tabaci*. Thus, the dynamics we observed are likely to be more realistic and meaningful then a system where such disturbances were artificially controlled.

A more comprehensive analysis of the classical biological control program for *B. tabaci* based on life table analyses in cotton is currently underway (Naranjo, in prep). This present study was focused exclusively on cotton in a defined region of central Arizona. As noted, *B. tabaci* is highly polyphagous and a major insect pest of a number of other crops, including horticultural crops such as cucurbits. Many landscape ornamentals, native plants and weed hosts also serve as seasonal hosts that provide significant reservoirs and bridging hosts for *B. tabaci* populations to move between crops. Thus, broader surveys and assessments will likely be necessary to evaluate the full impact of the classical biological control program.

Conflict of interest

The authors declare that they have no conflicts of interest.

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