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# Symbiont infection affects whitefly dynamics in the field

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## Abstract

Inherited intracellular insect endosymbionts may manipulate host reproduction or provide fitness benefits to their hosts in ways that result in their rapid spread throughout a host population. Fitness benefits in particular can result in the increased pest potential of agriculturally important insects. While benefits due to endosymbiont infection have been well studied in laboratory assays, very little is known about how these benefits translate to insect performance in the field. Laboratory experiments have shown that the maternally inherited bacterial endosymbiont, *Rickettsia*, increases the fitness of its whitefly host, *Bemisia tabaci*, through improved fecundity, faster development times and female-biased sex ratios. We conducted field population cage studies to determine whether the benefits conferred by *Rickettsia* to whiteflies in the laboratory were evident on one of its major hosts, cotton, under field conditions in Arizona, USA. In cages with either *Rickettsia*-infected or uninfected whiteflies, we observed up to ten-fold higher whitefly egg and nymph densities when whiteflies were *Rickettsia*-infected compared with uninfected whiteflies throughout the season. We also observed a steep initial increase in *Rickettsia* frequency in population cages started with either 25% or 50% *Rickettsia*-infected whiteflies, with the 50% cages approaching fixation within three generations. Using growth rates obtained in the density cages, we calculated and compared an expected trajectory of the frequencies of *Rickettsia* infection with the observed frequencies. Results showed similar observed and expected frequencies of *Rickettsia* in the first two generations, followed by a significantly lower than expected frequency in three of four treatment/sample combinations at the end of the season. Taken together, these results confirm the patterns of fecundity and population growth observed in laboratory assays, under field conditions, as well as provides preliminary empirical support for a *Rickettsia* equilibrium frequency of less than 100%.

## Zusammenfassung

Vererbte intrazelluläre Endosymbionten von Insekten können die Fortpflanzung des Wirtes manipulieren oder ihren Wirten in einer Weise Fitnessvorteile bieten, die in ihrer schnellen Ausbreitung in der Wirtspopulation resultiert. Insbesondere Fitnessvorteile können zur Folge haben, dass das Schadenspotential von landwirtschaftlich wichtigen Insekten zunimmt. Während solche durch Endosymbionten vermittelten Vorteile unter Laborbedingungen gut untersucht sind, ist wenig darüber bekannt, wie sich diese Vorteile auf die Performanz von Insekten im Freiland auswirken. Laborversuche haben gezeigt, dass der maternal

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vererbte bakterielle Endosymbiont, *Rickettsia*, die Fitness seines Wirts, der Mottenschildlaus *Bemisia tabaci*, erhöht. Dies geschieht durch gesteigerte Fekundität, höhere Entwicklungsgeschwindigkeit und ein zu den Weibchen hin verschobenes Geschlechterverhältnis. Wir führten Käfigversuche durch, um zu ermitteln, ob die von *Rickettsia* im Labor vermittelten Vorteile auch im Freiland (Arizona, USA) auf Baumwolle, einem der wichtigsten Wirte von *B. tabaci*, zutage traten. In Käfigen mit entweder mit *Rickettsia* infizierten oder nicht infizierten Mottenschildläusen beobachteten wir über die Saison bis zu zehnmal höhere Eier- und Nymphendichten bei infizierten *B. tabaci* als bei nicht infizierten. Wir beobachteten auch einen steilen anfänglichen Anstieg der *Rickettsia*-Infektionen bei Käfigpopulationen, die mit 25%- oder 50% igem Befall gestartet waren, wobei die 50%-Populationen sich innerhalb von drei Generationen der Fixation annäherten. Mit den Wachstumsraten aus den Käfigversuchen berechneten wir eine Kurve der zu erwartenden Infektionsraten und verglichen diese mit den beobachteten Infektionsraten. Die Ergebnisse zeigten ähnliche Beobachtungs- und Erwartungswerte für die beiden ersten Generationen, gefolgt von signifikant geringeren Beobachtungswerten in drei von vier Behandlung/Probetermin-Kombinationen am Ende der Saison. Zusammengenommen bestätigen diese Ergebnisse aus dem Freiland die Muster von Fekundität und Populationswachstum, die im Labor beobachtet wurden, und sie stellen eine vorläufige empirische Unterstützung für eine Gleichgewichtsfrequenz von unter 100% bei *Rickettsia*-Infektionen dar.


***Keywords:*** Endosymbionts; *Rickettsia*; Competition; *Bemisia tabaci*; Whiteflies; Cotton; *Wolbachia*; Insect pest; Invasive species

## Introduction

Maternally inherited, bacterial endosymbionts are prevalent and influential in arthropod biology. Primary bacterial symbionts are obligate mutualists and play a nutritional role by providing essential nutrients lacking in the diet (Baumann 2005; Douglas 1998). Secondary symbionts are facultative, and may contribute directly to host fitness, or manipulate host reproduction in ways that increase the proportion or fitness of (infected) daughters (Bull 1983; O'Neill, Hoffmann, & Werren 1997; Werren, Baldo, & Clark 2008). The benefits to hosts include ecological mediation such as thermal tolerance (Henry et al. 2013; Russell & Moran 2006), host range expansion (Ferrari, Scarborough, & Godfray 2007; Henry et al. 2013; Tsuchida, Koga, & Fukatsu 2004; Tsuchida, Koga, Matsumoto, & Fukatsu 2011), insecticide resistance (Berticat, Rousset, Raymond, Berthomieu, & Weill 2002) and resistance to parasitoids (Oliver, Russell, Moran, & Hunter 2003; Oliver, Degnan, Hunter, & Moran 2009) and pathogens (Hedges, Brownlie, O'Neill, & Johnson 2008; Scarborough, Ferrari, & Godfray 2005). In spite of concerted efforts to understand the effects of secondary symbionts on hosts in laboratory and growth chamber studies, very little is known about how these effects translate to the field. Given the marked differences in laboratory and field conditions, and the sometimes-unexpected dynamics of symbionts in host populations (Oliver, Campos, Moran, & Hunter 2008; Xi, Khoo, & Dobson 2005), it is important to understand how symbionts influence the population and field ecology of their hosts.

Dramatic spread of insect endosymbionts in host populations has been documented in a handful of cases (Duplouy, Hurst, O'Neill, & Charlat 2010; Himler et al. 2011; Hoshizaki & Shimada 1995; Jaenike, Unckless, Cockburn, Boelio, & Perlman 2010; Kriesner, Hoffmann, Lee, Turelli, & Weeks 2013; Turelli & Hoffman 1991). The mechanisms behind spread have typically been assessed in laboratory assays where the infection status of hosts can be controlled and directly compared (Himler et al. 2011; Hoffmann & Turelli 1988; Hoffmann et al. 2011; Jaenike et al. 2010). Between these studies and field studies, however, are a wealth of abiotic differences, including temperature extremes and fluctuations, precipitation and wind, and expected biotic differences in plant quality, intraspecific density-dependent responses and competitive interactions of the insect hosts, alternative host plants, spatial complexity, and the host's natural enemies. In general, the effects of symbiont infection in the laboratory are unlikely to be equivalent to those in the field. For example, Xi et al. (2005) observed differences in fitness costs of *Wolbachia* infection in mosquitoes between population cage and individual assays, results that underlined the importance of population level assessments of symbiont effects on the host. Understanding the population consequences of symbiont infection for host biology in the field is of even more critical importance as the applied use of symbionts for pest or vector control moves from the laboratory to the field (Hoffmann et al. 2011; Walker et al. 2011).

Bacterial endosymbionts are common in one of the worst global agricultural pests, the complex of species collectively known as the sweetpotato whitefly *Bemisia tabaci* (Hemiptera: Aleyrodidae). Within these is the especially invasive species provisionally called "B" species or Middle East-Asia Minor 1 (MEAM 1) species (Dinsdale, Cook, Riginos, Buckley, & De Barro 2010; Liu, Colvin, & De Barro 2012). Heavy whitefly infestations cause plant stunting and chlorosis. Perhaps even more significantly, *B. tabaci* is the vector for dozens of plant viruses that affect many different crops around the world (Oliveira, Henneberry, & Anderson 2001). In addition to the primary nutritional symbiont *Portiera aleyrodidarum* (Sloan & Moran 2012; Thao & Baumann 2004), secondary

symbionts found among members of the *Bemisia* complex include *Wolbachia*, *Hamiltonella*, *Arsenophonous*, *Fritschea*, *Cardinium*, *Hemipteriphilus* and *Rickettsia* (Bing, Yang, Zchori-Fein, Wang, & Liu 2013; Gottlieb et al. 2006; Moran, Russell, Koga, & Fukatsu 2005). Although their roles are generally unknown, some of these symbionts appear to increase the whitefly's pest status. For example, *Hamiltonella* may improve transmission of plant viruses (Gottlieb et al. 2010). A survey of *B. tabaci* [MEAM 1] in Arizona, USA, indicated that *Rickettsia* sp. nr. *bellii* spread rapidly within the *B. tabaci* host population in just 6 years, with infection rates rising from 1% in 2000 to 97% by 2006 (Himler et al. 2011). In laboratory comparisons, *Rickettsia*-infected *B. tabaci* were associated with increased fecundity, reduced development time, increased survival and a female-biased sex ratio (Himler et al. 2011). These characteristics, individually or collectively, could have contributed to the rapid spread of *Rickettsia* in *B. tabaci* populations in Arizona.

The performance of symbiont-infected whiteflies in the field is especially difficult to observe directly against the backdrop of the intensive management of the agricultural crops in the southwestern USA, especially in cole crops, cucurbits and cotton, on which these whiteflies depend. Changes in planting dates, regional suppression of other pests in the system, e.g. *Lygus hesperus* and *Pectinophora gossypiella*, as well as improvements in conservation of natural enemies by selective insecticides all occurred during and after the spread of *Rickettsia* in whiteflies (Ellsworth & Martinez-Carrillo 2001; Naranjo 2005; Naranjo & Ellsworth 2009a; Naranjo, Ellsworth, & Hagler 2004) and have resulted in effective management of this pest (Naranjo & Ellsworth 2009a, 2009b). In fact, despite the clear benefits of *Rickettsia* infection in laboratory experiments, whitefly pest problems in desert grown cotton have not generally increased since the spread of *Rickettsia* (Naranjo et al. 2009a, 2009b). This study was conducted to determine the cause of this apparent contradiction. Are whiteflies no worse pests because the effects of one change (in performance of whiteflies) are negated by a series of other management changes that tipped the scales toward whitefly suppression? Or is the performance of whiteflies infected with *Rickettsia* no better than that of uninfected whiteflies under field conditions? Here we examined the effects of *Rickettsia* sp. nr. *bellii* infection on population performance of *B. tabaci* (B species) in the field on cotton in Arizona, USA.

## Materials and methods

### Insect cultures

The whiteflies used for this study were obtained from a laboratory culture in which the nuclear background of *Rickettsia*-infected ($R^+$) and uninfected ($R^-$) lines were homogenized by introgression. Whiteflies were collected and established from Maricopa, AZ in 2006 and the introgression series occurred in 2009 (Himler et al. 2011). In the spring of 2012 prior to the summer field season, a second introgression series was conducted between $R^+$ females and $R^-$males for four generations to homogenize any genetic differences that may have arisen in these cultures via genetic drift, and to reduce any culture-specific environmental differences. These lines differed by cytotype but were >98% similar in nuclear genes. Laboratory cultures were maintained on cowpea (*Vigna unguiculata*). Prior to introduction of whiteflies in the field cages, separate cultures of both $R^+$ and $R^-$ lines were established on cotton (*Gossypiun hirsutum* L.) in growth chambers and maintained for four generations.

### Field experiments

#### Population growth field experiment

Both field experiments were conducted at the University of Arizona Campus Agricultural Center, Tucson, AZ, USA. Cotton plants of the variety DP164B2RF were planted in a half-acre plot on 24th April 2012 and managed according to standard agronomic practices for cotton in central and southern Arizona. Because *B. tabaci* is common in cotton in AZ, and the frequency of *Rickettsia* in the local population is ~95%, our study comparing the performance of $R^+$ and $R^-$ whiteflies necessitated the use of whitefly-proof fabric cages around growing cotton plants. Plants to be caged were chosen from interior rows of the cotton field, at least 6 m apart. After cages were put in place, treatments were randomly assigned to cages. Throughout the growing season, all plants were treated identically. For the population growth experiment, cages were seeded with either $R^+$ or $R^-$ whiteflies. Thirty-two cages were placed over individual plants at the five-leaf stage (16 cages per treatment). Each cage was made of two inverted U-shaped metal rods sunk into the ground to which a fine polyester (voile) fabric cage (rectangular, with a fabric sleeve for introducing and sampling whiteflies) was tied. The fabric was anchored at the bottom by attachment to a 10 cm diameter PVC pipe sunk into the soil around the plant with a plastic cable tie. This design prevented most insects from getting into the cages, although some ants and flies were later found, presumably entering via the soil. Two weeks after setting up cages, when the plants had approximately 10 leaves, introductions of either *Rickettsia*-infected ($R^+$) or uninfected ($R^-$) whiteflies were started and continued weekly for three weeks to mimic the staggered natural immigration of whiteflies into cotton fields. On June 21, June 28 and July 3, 2012 we introduced 10, 10 and 24 pairs of males and females, respectively, of either $R^+$ or $R^-$ whiteflies into each cage. Sampling was conducted two weeks following the last introduction date and every two weeks thereafter, for a total of four sampling dates. At each sampling date, single main stem leaves were collected from each cage at an interval of four nodes beginning with the top most leaf (e.g. leaves 1, 5, 9, etc.). Because the plants at the time of first sampling had approximately 50–100 leaves, the leaves removed represented a relatively small proportion of the total leaf

surface of the plant. The harvested leaves were placed in Ziploc bags and taken to the lab where whole leaf counts of whitefly eggs and total nymphs were made for each leaf. Leaf areas were measured using a Leaf Area meter (LI-COR, LI-3100C, Lincoln, NE. USA) to obtain whitefly densities (numbers/cm$^2$).

## Competition population cage experiment

The population cage experiment was set up concurrently in the same cotton plot, using more of the cages described above. Two treatments varied in initial frequencies of $R^+$ whiteflies (approximately 25% and 50%), each with eight cages. Treatments were set up using a replacement series (De Wit 1960) in which the relative proportions of $R^+$ and $R^-$ whiteflies varied but the density of whiteflies released remained the same across treatments. For the 25% $R^+$ treatment, three introductions of combinations of $R^+$ and $R^-$ whiteflies were made on the same dates as the previous experiment as follows: (i) 4 pairs of $R^+$, 11 pairs of $R^-$; (ii) 3 pairs of $R^+$, 12 pairs of $R^-$; (iii) 6 pairs of $R^+$, 18 pairs of $R^-$. For the 50% $R^+$ treatment, the three introductions consisted of (i) 8 pairs of $R^+$, 7 pairs of $R^-$; (ii) 7 pairs of $R^+$, 8 pairs of $R^-$; (iii) 12 pairs each of $R^+$ and $R^-$ whiteflies. Sampling was conducted every 3 weeks, an interval that approximates a generation for whiteflies in the field. At each sampling date, 20–30 whitefly adults were collected from each cage. Adults were collected from the interior of the cage netting and from the undersides of multiple leaves. Adult whiteflies were stored in 95% EtOH until diagnostic polymerase chain reaction (PCR) was conducted to determine the relative frequency of *Rickettsia* infection status.

### Diagnostic polymerase chain reaction (PCR)

To determine *Rickettsia* infection frequency in the population cage experiment, a chelex DNA extraction protocol was used (Chiel et al. 2009) and PCR amplification was conducted on single whitefly females. The PCR was conducted using *Rickettsia*-specific 16S rDNA primers (Chiel et al. 2009) and included a negative control of sterile water and a positive control of a *Rickettsia*-infected whitefly. PCR amplifications were carried out with a program used for *Rickettsia* diagnostic PCR in whiteflies (Himler et al. 2011). Four μl of PCR product was visualized on a 1% agarose gel (1X TAE) stained with 0.64 μl 20× SYBR Green (Molecular Probes, Eugene, OR). For the samples that showed no *Rickettsia* infection, DNA extraction quality was verified using whitefly mtDNA (COI) primers (Frolich, Torres-Jerez, Bedford, Markham, & Brown 1999) and the 16S rDNA PCR was repeated to ensure these were true negatives. The proportion (frequency) of infected whiteflies was calculated as the percentage of infected whiteflies to total number of female whiteflies sampled in each cage. In all but 3 instances, 20 adult females were used to determine *Rickettsia* frequency; fewer females were used (10) when the number of adults in the cages was very low.

## Data analysis

A mixed model, repeated-measures ANOVA (Littell, Milliken, Stroup, & Wolfinger 1996) was used to examine the effects of *Rickettsia* infection on the density of immature whiteflies. The replicates and their associated interactions with infection status and date were entered into the model as random effects and the Kenward–Roger method was used to estimate the corrected degrees of freedom. The covariance structure of the repeated measures was estimated using the first order autoregressive function (AR1), because it consistently maximized both Akaike's information and Schwarz' Bayesian criteria (Littell et al. 1996). The significant main effects of infection status and sampling date were examined by mean separations using the DIFF option of the LSMEANS statement. All densities were log transformed (ln) and frequency data was arcsine-transformed to meet normality and homogeneity of variance assumptions, although untransformed means are presented. Analyses were conducted separately for whitefly eggs and nymphs and all analyses were conducted in the PROC MIXED platform of SAS.

## Expected vs. observed *Rickettsia* infection frequencies

We calculated the seasonal per capita population growth rate of immature whiteflies in each of the $R^+$ or $R^-$ cages for each whitefly stage using the formula $dN/Ndt = (\ln[N_2] - \ln[N_1])/(t_2 - t_1)$, where $N_2$ and $N_1$ represent the total density of immatures (eggs and nymphs) at $t_2$ (final sampling date) and $t_1$ (initial sampling date) respectively.

The mean seasonal per capita growth rate of each type of whitefly ($R^+$ or $R^-$) was then used to determine an expected number of $R^+$ and $R^-$ in each of the population cage treatments (with 25 or 50 initial $R^+$ whiteflies) using the formula $N_t = N_{t-1} + (N_{t-1} \times \text{per capita growth rate})$ where $N_t$ is the expected density of either $R^+$ or $R^-$ at day $t$ and $N_{t-1}$ is the density on the previous day. We used a generational interval of 21 days for whiteflies to determine the expected densities at each generation. The expected $R^+$ frequency was then determined using the formula $N_{R+}/N_{R+} + N_{R-}$ where $N_{R+}$ is the expected density of $R^+$ whiteflies and $N_{R+} + N_{R-}$ is the expected total number of whiteflies. We then tested the statistical significance of differences between expected and observed frequencies of *Rickettsia* infection at each generation of the population cage experiment by calculating a 95% binomial confidence interval for each observed frequency and comparing it to the expected frequency. Because binomial confidence intervals may be inexact especially when they are close to 0 or 1 (Newcombe 1998), five types of binomial confidence intervals were generated (asymptotic, exact binomial, Wilson, Agresti-Coull and Jeffreys) (Sergeant 2014), and the largest one chosen for each sample point.

## Results

### Population growth field experiment

*Rickettsia*-infected whitefly populations grew approximately 10 times faster than uninfected populations. There were highly significant effects of *Rickettsia* infection, sampling date and a *Rickettsia*-by-sampling-date interaction on whitefly egg densities ($F_{1,12.9} = 52.3$, $P < 0.0001$; $F_{3,80.5} = 28.79$, $P < 0.0001$; and $F_{3,80.5} = 18.75$, $P < 0.0001$ respectively). Egg densities were significantly higher in the *Rickettsia*-infected compared to the uninfected whiteflies on all but the first sampling date with densities up to 10 times higher in the $R^+$ plots by the end of the season. ($P < 0.05$; Fig. 1A). Similarly, there were significant effects of *Rickettsia* infection, sampling date and a *Rickettsia*-by-time interaction on whitefly nymph densities ($F_{1,12.8} = 39.58$, $P < 0.0001$; $F_{3,80.6} = 19.79$, $P < 0.0001$; and $F_{3,80.6} = 12.97$, $P < 0.0001$ respectively). Nymph densities were also significantly higher in the *Rickettsia* infected compared to the uninfected whiteflies on all but the first sampling date ($P < 0.05$; Fig. 1B).

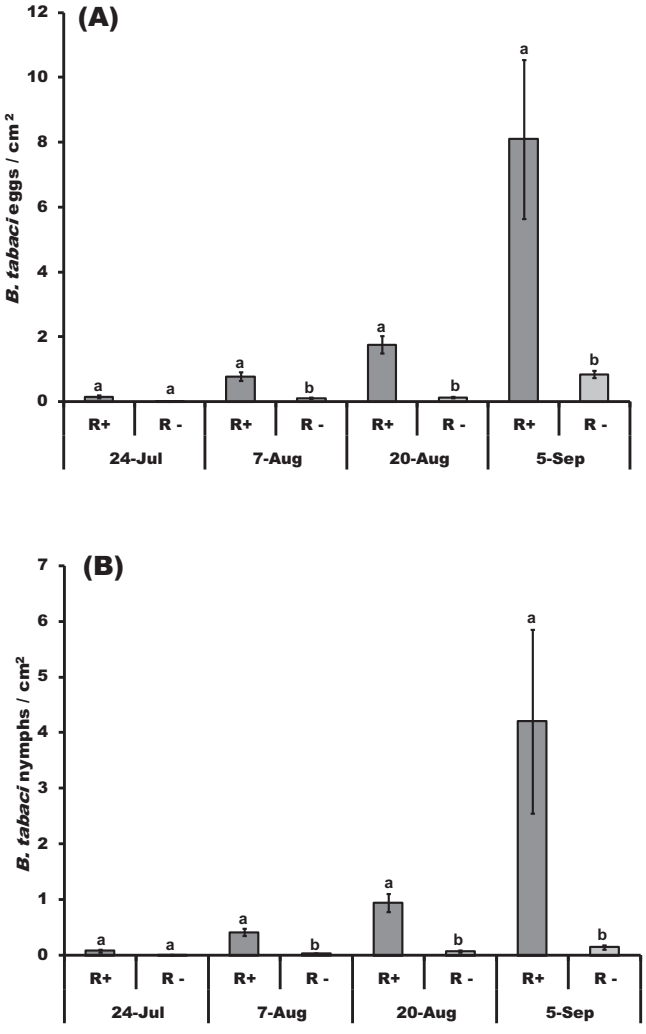


**Fig. 1.** Mean (±SE) densities of *B. tabaci* eggs (A) and nymphs (B) in cotton field cages infested with *Rickettsia*-infected ($R^+$) or uninfected ($R^-$) whiteflies. Bars with different letters indicate statistically significant differences ($P < 0.05$) at each sampling date.

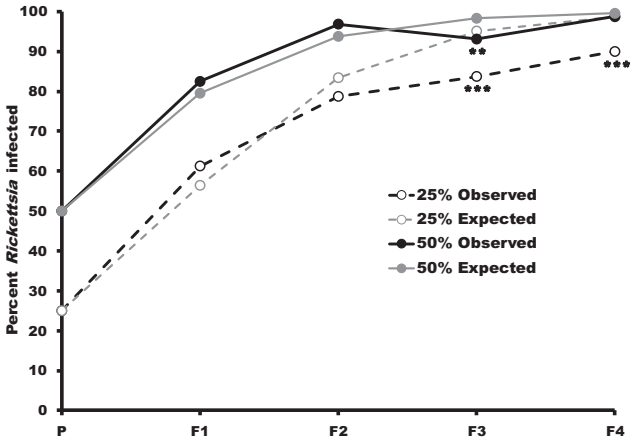


**Fig. 2.** Expected and observed proportions of *B. tabaci* infected with *Rickettsia* in cotton field cages inoculated with 25% and 50% initial *Rickettsia* infected whiteflies. Expected frequencies were calculated using the relative growth rates of $R^+$ and $R^-$ whiteflies observed in the single whitefly type cages. Asterisks indicate where 95% binomial confidence intervals around observed proportions do not include expected values (**$P < 0.01$, ***$P < 0.001$).

### Competition population cage experiment

The frequency of *Rickettsia* infection increased dramatically in population cages initiated with either 25% or 50% *Rickettsia*-infected whiteflies. There were significant effects of initial frequency and generation on the frequency of *Rickettsia*-infected whiteflies ($F_{1,28} = 35.04$; $P < 0.0001$ and $F_{3,21} = 6.59$; $P = 0.002$ respectively). The interaction between initial frequency and generation was not significant ($F_{3,28} = 0.5$; $P = 0.6821$). Perhaps unsurprisingly, the two treatments differed in the frequency of *Rickettsia* infection overall, with the treatment starting at 50% maintaining a higher infection rate than the treatment starting at 25% ($t_{1,28} = -5.92$; $P < 0.0001$). Among generations, significantly higher frequencies were only observed in the F2 compared to the F1 ($t_{1,21} = -2.63$; $P = 0.0157$), where the rise in frequency is most dramatic. All other subsequent pairwise comparisons between generations were not significantly different, although they were consistently greater at the 50% initial frequency ($P > 0.05$) (Fig. 2).

### Expected vs. observed frequencies of *Rickettsia* infection

A comparison of the expected *Rickettsia* infection frequencies derived from average growth rates of $R^+$ and $R^-$ whiteflies with the observed frequencies showed a reasonably close fit in the first two generations, with 95% binomial confidence intervals for the observed values including the

expected frequency. In the last two generations, however, expected frequencies were significantly greater than the observed for three of the four treatment/date combinations. In the third generation of the 50% treatment, the expected frequency was 98% while the observed frequency was 93% (95% confidence interval (CI): 88%, 97%; $P < 0.01$). However, this difference was greater for the 25% treatment where the expected frequencies approached 100%, while the observed frequencies were significantly lower in both third (expected 95%, observed 84%, 95% CI: 77%, 89%; $P < 0.0001$) and fourth generation (expected 99%, observed 90%, 95% CI: 84%, 95%; $P < 0.001$).

## Discussion

In field cages in cotton, we found that growth rates of *Rickettsia*-infected whiteflies were up to 10 times higher than those of uninfected whiteflies. In population cages as well, the frequency of *Rickettsia* infection increased rapidly. Although similarly beneficial effects of *Rickettsia* on whitefly performance have been documented in laboratory studies (Himler et al. 2011), this study documents that *Rickettsia* can also increase performance in the field. In cages with only $R^+$ whiteflies, protected from natural enemies, the high densities observed by the mid to late season were associated with observations of sooty mold on the leaves and sticky cotton lint. When only $R^-$ whiteflies were present, whitefly densities did not reach the densities necessary to cause these effects. High amounts of sooty mold growing on whitefly honeydew when the crop is at peak growth interfere with the amount of photosynthate being channeled to the fruiting structures, ultimately leading to smaller bolls and a reduction in yield, while honeydew in the lint reduces cotton quality (Oliveira et al. 2001). We note that these results pertain to a single field season in Tucson, Arizona, USA. Repetitions of this experiment in different years and locations would be a valuable confirmation of the generality of the results. However, high frequencies of *Rickettsia* across the desert southwestern USA suggest that the greater growth rates of *Rickettsia*-infected whiteflies may be widespread in this area.

The competitive ability of insects is driven by several life history characteristics, of which fecundity, development time and survivorship are some of the most important (Dean 2006). In competition population cages, *Rickettsia* infection increased dramatically from frequencies of either 25% or 50% *Rickettsia*-infected whiteflies. It is possible that horizontal transmission of *Rickettsia* from infected to uninfected whiteflies could have contributed to this result as well as differential rates of reproduction of $R^+$ and $R^-$ whiteflies. However, while horizontal transmission of *Rickettsia* has been shown in *B. tabaci* in Israel (Caspi-Fluger, Mozes-Daube, Inbar, Katzir, Portnoy, Belausov, Hunter, Zchori-Fein), it appears to play a negligible role in this interaction in the USA (Himler et al. 2011). In contrast, the population growth cage experiment in the current study showed large differences in growth rates of $R^+$ and $R^-$ whiteflies in isolation that we expect drive the increase in *Rickettsia* frequency when these whiteflies compete. Further, the competitive superiority of the $R^+$ whiteflies in the population cages can be attributed to the presence of *Rickettsia*, since differences in whitefly nuclear genes were largely removed by introgression.

The mechanism by which *Rickettsia* infection improves whitefly fitness is unknown. While a nutritional function for this symbiont is possible, we would not ordinarily expect nutritional benefits to be conditional (see discussion below) and instead consider plant manipulation to be a more likely, if still untested, possibility.

Whatever the mechanism, the increase in *Rickettsia* infection frequency in our controlled caged-whitefly experiments also qualitatively echo the pattern seen at a much larger geographic scale over several years. Surveys conducted in the southwestern USA showed an increase in *Rickettsia* infection from 1% to near fixation in six years (approximately 80 generations) and *Rickettsia* continues to persist at high frequencies since then (Himler et al. 2011; Cass et al., unpublished data). *Rickettsia* frequency in our experiments appears to climb even more rapidly than this; the equilibrium rate of infection seen across the region (within 5% of fixation) would likely have been reached in the cages in less than 10 generations even with a far-lower starting frequency. Large differences in spatial complexity as well as environmental and genetic heterogeneity of open field populations of whiteflies are perhaps most likely to be responsible for the differences in rate of increase of *Rickettsia* between the field cage experiments and the open field. In addition, the environment whiteflies experienced in closed cage experiments was in many ways intermediate between the laboratory and the open field conditions. The field experiment differed markedly from laboratory conditions in climate and plant physiology, both factors potentially important in the host-symbiont interaction. For example, while our laboratory experiments are typically run at constant 27 °C, temperatures during the period of the field experiment ranged from 20 to 42 °C with a mean temperature of 38 °C (AZMET, 2013). Also, while we have used seedling cotton in laboratory experiments, the cotton plants in this study had between 50–100 leaves. On the other hand, open field conditions are subjected to greater effects of abiotic and biotic natural control, for example wind, rain, predators and parasitoids, all of which could influence the rate of increase of $R^+$ whiteflies.

The rapid rise in *Rickettsia* frequency in the first two generations of the population cage experiments was well predicted by the growth rates of $R^+$ and $R^-$ whiteflies in isolation (Fig. 2). Interestingly, however, in the last two samples both the 25% and 50% treatment had at least one date in which the observed frequencies were lower than expected. Incomplete vertical transmission rates can cause a lower equilibrium frequency. While *Rickettsia* shows near perfect vertical transmission rates under laboratory conditions (Himler et al. 2011), transmission could potentially be

lower under the higher temperatures experienced by whiteflies in the field. Additional abiotic and biotic factors could have caused a change in the cost/benefit ratio of *Rickettsia* infection in this study of caged whiteflies on cotton over the season, including humidity, cryptic pathogens, conspecific density, the frequency of *Rickettsia* infection of whiteflies on a plant, and plant size and physiology. More generally, however, the "slow to fixation" effect we saw in this experiment appears to reflect the pattern seen at a much larger scale in multiple field samples; while *Rickettsia* may reach very high frequencies of infection (particularly in the southwestern United States), the equilibrium frequency appears to be less than 100% (Himler et al. 2011; Cass et al., unpublished data). In the open field, even more factors could be important in causing the conditional benefits one might associate with high but not fixed frequencies, including the effects of *Rickettsia* on whiteflies' ability to migrate, or on the susceptibility of whiteflies to natural enemies or pathogens. These high but not fixed frequencies observed for *Rickettsia* differ from those seen in host population surveys of endosymbionts that are nutritional mutualists or of reproductive manipulators that cause parthenogenesis (Stouthamer 1997) where fixation is generally the rule. It also differs from cytoplasmic incompatibility (CI) symbionts, in which frequencies of infection that rise above an unstable equilibrium determined by CI strength, vertical transmission and fitness costs also generally rise to fixation (Caspari & Watson 1959; Hoffmann & Turelli 1997).

Despite the dramatic effects of *Rickettsia* infection on whitefly performance observed in this study, these effects might not be apparent to cotton growers in the desert southwestern USA. Cotton in this region is attacked by two major pests, *B. tabaci* and *L. hesperus*, both of which typically cause economic losses. Over the last 15 years, an Integrated Pest Management approach centered on the use of selective insecticides has resulted in dramatic reductions in pest densities while boosting the natural control (through predation and parasitism) of these pests in cotton (Naranjo & Ellsworth 2009a). Additionally, more intensive management of whiteflies in spring crops that serve as a source of whiteflies in early season cotton (Castle, Palumbo, & Prabhaker 2009) has resulted in reduced whitefly densities in cotton. In the context of multiple management changes, the potentially damaging effects of increased whitefly fitness due to *Rickettsia* infection might be masked. It remains to be determined, however, whether *Rickettsia* causes qualitative changes in whiteflies that may have more serious consequences. The *B. tabaci* complex of whiteflies vector over 100 plant viruses (Jones 2003), and symbionts may interact with plant viruses (Gottlieb et al. 2010). In this context, the rise of the semi-persistent whitefly-vectored Cucurbit Yellow Stunting Disorder Virus (CYSDV) (Castle et al. 2009) in melons and vegetables in the mid 2000s raises the question of whether *Rickettsia* infection could have a role in higher whitefly densities and/or virus transmission in southwestern USA vegetable production.

## Conclusions

*Rickettsia* sp. nr. *bellii*, a facultative symbiont of the invasive whitefly *B. tabaci* species "B," caused marked increases in population growth and *Rickettsia* frequency in whiteflies on cotton in the field. These findings corroborate observations of rapid increases in *Rickettsia* frequency across the southwestern USA, as one would expect for a symbiont associated with fitness benefits (Himler et al. 2011), and are also consistent with current observations of high frequencies of *Rickettsia* in whiteflies across the USA (Cass et al., unpublished data), and in some populations around the world (Bing, Ruan, Ra, Wang, & Liu 2013). In field population cages, observed *Rickettsia* frequencies generally fall short of expected frequencies, and this also resembles the pattern seen in samples from cotton across the USA, where regional frequencies of *Rickettsia* are generally not fixed, but range from 71 to 93% (Cass et al., unpublished data). Lastly, while pest managers have not seen a marked increase in whitefly densities in cotton since the spread of *Rickettsia*, many changes in pest management over the same period may mask biological differences in whiteflies during the same period. It also remains to be seen whether *Rickettsia* infection plays a role in whitefly-vectored virus transmission in the alternative vegetable host crops in southwestern USA.

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