Plant-Mediated Changes in the Feeding Behavior of an Invasive Whitefly

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ABSTRACT The invasive whitefly *Bemisia tabaci* (Gennadius) is a worldwide pest of agricultural crops that feeds on a wide variety of host plants. Although host plant preference is known to vary among *B. tabaci* biotypes, far less is known about the potential for intraspecific divergence caused by long-term isolation on a single species of host plant. We tested the hypothesis that multigenerational isolation of *B. tabaci* B, a biotype that has been well-established in China for nearly two decades, on three different host plants would lead to population-level divergence in feeding behaviors. We used individuals from a cabbage-feeding (*Brassica oleracea* L.) population of *B. tabaci* B to create three populations reared exclusively on *B. oleracea*, cucumber (*Cucumis sativus* L.), or tomato (*Lycopersicon esculentum* Mill.) for >80 generations. We then used electrical penetration graph techniques to investigate the feeding behavior of the three *B. tabaci* populations on each of the three host plants (nine total treatments). Across all three host plants, the cabbage-specific population equaled or exceeded the performance of the cucumber-specific (CuSP) and tomato-specific (ToSP) populations. Strikingly, neither CuSP nor ToSP ever had the best feeding performance on their natal hosts. Our results support the hypothesis that feeding differentiation has occurred, but we found no evidence that these changes increased the feeding performance of either CuSP or ToSP. Although confirming that rapid interpopulation divergence is possible, our findings nonetheless suggest that this differentiation did not yield highly adapted populations that might pose problems for future efforts at pest management.

KEY WORDS *Bemisia tabaci* B, host plant isolation, feeding behavior, differentiation, electrical penetration graph

The *Bemisia tabaci* (Gennadius) species complex (Dinsdale et al. 2010, De Barro et al. 2011) is a destructive pest of agricultural and horticultural crops worldwide. These whiteflies cause serious damage to their host plant both by direct injury (stylet insertion, resource depletion, etc.) and by acting as vectors for a wide range of plant viruses (Perring 2001, Jones 2003). Despite being members of the same species complex, different *B. tabaci* biotypes vary widely in their impact on plant health. Although native Chinese *B. tabaci* biotypes were first documented in 1949, they were not a crop pest until the entry and establishment of the East-Minor Asia 1 'B' biotype in the 1990s (Luo et al. 2002). In the 2000s, the invasion and rapid range expansion of the Mediterranean 'Q' biotype in China sharply increased the amount of agricultural damage by acting as a particularly effective vector (Pan et al. 2012b, Liu et al. 2013) of *Tomato yellow leaf curl virus* (*Begomovirus;* Family: Geminiviridae) and other plant geminiviruses (Lazarowitz and Shepherd 1992). Although whitefly populations can be controlled with an array of chemical (Ma et al. 2007), biological (Qiu et al. 2004, Qiu and Ren 2006), and physical control measures (Gu et al. 2008), *B. tabaci* continues to pose a major threat to greenhouse vegetable cultivation.

Although *B. tabaci* is capable of feeding on a wide variety of host plants, individual biotypes often perform much better on some plant species (Powell and Bellows 1992, Tsai and Wang 1996, Drost et al. 1998, Muñiz 2000, Nava-Camberos et al. 2001). The Q biotype, for instance, feeds more readily than the B biotype on tomato, cotton, and poinsettia, whereas the B biotype feeds better on cabbage and cucumber (Liu et al. 2012). Some of these biotype-specific differences may be attributed to the host plant's response to feeding. Even commercially grown plants possess an array of induced and constitutive defenses (Bellotti and Arias 2001) and can mediate the interaction between whiteflies and their symbionts (Pan et al. 2012a), viruses (Lapidot et al. 2001, Colvin et al. 2006), and natural enemies (Inbar and Gerling 2008). The impact of host plant choice on *B. tabaci* behavior and performance likely plays a major role in explaining why

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morphologically indistinguishable *B. tabaci* biotypes and/or sibling species differ substantially with respect to host range (Saxena and Barrion 1987, Brown et al. 1995). As a result, a better understanding of the *B. tabaci*–host plant interaction may yield improved pest management practices (Inbar and Gerling 2008).

Although whiteflies in crop systems can access a range of different host plants, tightly controlled greenhouse systems may confine *B. tabaci* to a single host plant for multiple insect generations. Such confinement may select for host-plant-specific feeding behaviors and other life-history traits favorable for colonizing novel habitats. We hypothesized that long-term confinement of *B. tabaci* on a single host plant might lead to differentiation in feeding behaviors. We tested this hypothesis by using *B. tabaci* B, a biotype that has been established in China for almost 20 yr.

We confined individuals from a common *B. tabaci* B population on three different host species for 6 yr (>80 whitefly generations). Following this period of host plant isolation, we tested them for evidence of host plant specialization. Because feeding behavior is indicative of host fitness and whitefly population growth (Lei et al. 1998), we used electrical penetration graph (EPG) technique (Tjallingii 1978, Walker 2000) to investigate the feeding behaviors of each host-specific population on each of the three host plants. By providing a better understanding of the impact(s) of host plant isolation on whitefly feeding, our work may help develop more effective pest management strategies.

Materials and Methods

Laboratory Whitefly Populations. *B. tabaci* B was collected on cabbage (*Brassica oleracea* L. variety 'Jingfeng1′) in Beijing, China, in 2004 and reared on four cabbage plants in a screen cage within a glasshouse to establish a parental population. The parental population was identified as *B. tabaci* B by sequencing the mitochondria cytochrome oxidase 1 (*mtCO1*) gene marker. After two generations of *B. tabaci* reproduction, two infested cabbage leaves, each with ≈200 whiteflies on it, were picked from the parental population and put into two screen cages (one cabbage leaf per cage). One screen cage contained four cucumber plants (*Cucumis sativus* L., cultivar 'Zhongnong 12′), and the other contained four tomato plants (*Lycopersicon esculentum* Mill., cultivar 'Zhongza 9′). For the past 6 yr, the three screen cages (and their associated *B. tabaci*), with four host plants per cage, have been maintained in a glasshouse under natural lighting and controlled (26 ± 2°C) temperatures. We ensured that the populations did not intermix by rearing them in three double-cages within the glasshouse. Each double-cage consisted of a large insect-proof nylon screening and a small cage nested within it. The small inner cage, where the insects were kept, had a sealable opening that was kept closed, except during necessary access. The larger outer insect-proof nylon screening enclosed the inner cage and had a small sealable opening that could be closed around the researcher's upper body. Whenever access was required (watering, exchanging plants, collecting insects, etc.), researchers would put their upper body into the outer nylon screening, seal it around them, and then open the inner cage. To minimize the possibility of whitefly disturbance, work was conducted at night whenever possible (when whiteflies are less active). Double-cages were rotated within the glasshouse to minimize the potential influence of site-specific factors, and whiteflies quickly grew in abundance (>500 whiteflies per plant) in each inner cage. For convenience, we refer hereafter to the host-plant-specific *B. tabaci* populations as the cabbage-specific population (CaSP), cucumber-specific population (CuSP), and tomato-specific population (ToSP). By the time of our study, each of the three populations had been isolated for >80 generations under identical abiotic conditions and without exposure to any chemical insecticides. We used newly emerged (2–5 d) female whiteflies from each of the three host-specific *B. tabaci* populations for our experiment.

Plants. The host plants for this experiment were cabbage (*B. oleracea* L., cultivar Jingfeng 1), cucumber (*C. sativus* L., cultivar Zhongnong 12), and tomato (*L. esculentum* Mill., cultivar Zhongza 9). Seedlings were individually cultivated in 1.5-liter plastic pots enclosed in separate screen cages under natural lighting and controlled temperature (26 ± 2°C) in a glasshouse. Host plants at the two- to three-true-leaf stage were used for the EPG experiments.

Experimental Design. We conducted a fully factorial experiment in which each of the three *B. tabaci* host-specific populations (CaSP, CuSP, and ToSP) were allowed to feed on each of the three host plants (cabbage, cucumber, and tomato). There were nine treatments in total, with 30 whiteflies tested per treatment. Because some whiteflies died, escaped, or became detached from the probes over the 360-min experimental period, we collected usable data from ≈24 replicates per treatment (mean [SE]: 24.1 ± 1.0, range: 19–28 replicates). Each replicate consisted of a fresh whitefly–plant combination that was EPG-monitored for 6 h. To avoid any treatment biases, EPG experiments were conducted in random order.

EPG Recording. Whitefly EPGs were recorded by using a Giga-8 DC-EPG system with 10^9 Ohm input resistance (Wageningen University, The Netherlands). Before recording, a whitefly was immobilized in a small ice-chilled glass dish. We then attached a 1.5-cm × 12.5-μm gold wire to the whitefly's dorsum by using a droplet of water-based silver glue (for details, see Rodríguez–López et al. 2012). The wired whitefly was then connected to the input of the Giga-8 probe and placed on the lower surface of the bottom leaf of the appropriate host plant. Each replicate whitefly–plant–probe combination was placed into an electrically grounded Faraday cage to shield the setup from electrical noise. Six hours of EPG signals were digitized with a DI710-UL analog-to-digital converter (DATAQ Instruments, Akron) and acquired with PROBE3.4 software (Wageningen University, The Netherlands). All experiments were carried out at

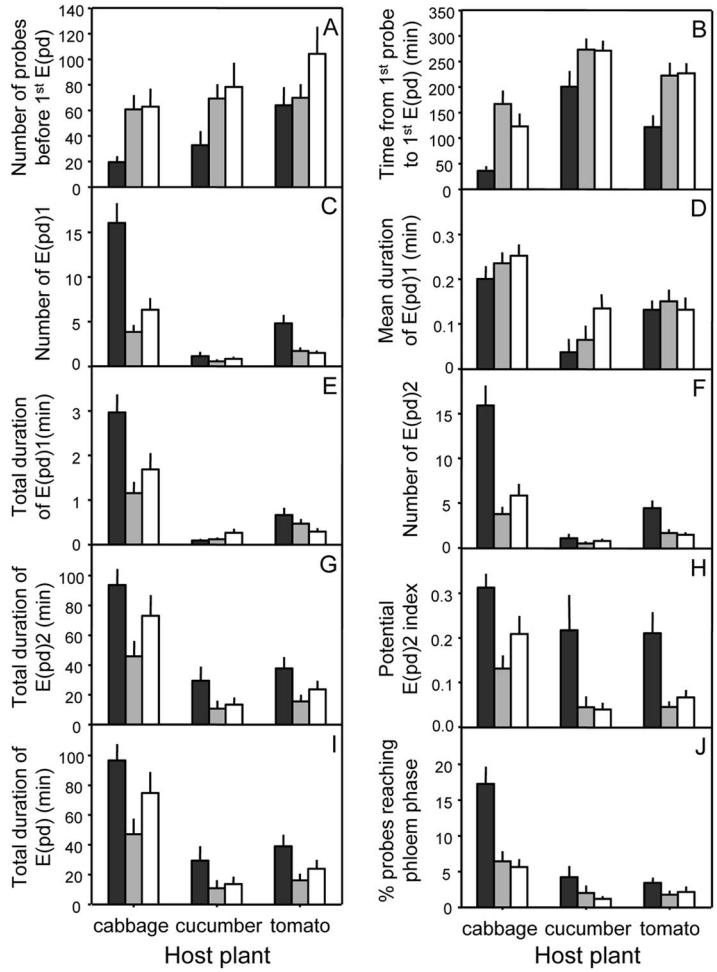

Fig. 1. Mean + SE values for EPG parameters (A–J) of three host-specific *B. tabaci* B populations, each feeding on three host plants. Black bars: cabbage-specific populations (CaSP); gray bars: cucumber-specific populations (CuSP); white bars: tomato-specific populations (ToSP).

26 ± 2°C, 70% RH, and under artificial light (1,500 lux) with a photoperiod of 14:10 (L:D) h.

EPG waveforms were identified according to standard classification patterns (Jiang et al. 1999, 2000). Five waveforms were identified: nonprobing (NP), pathway (C), potential drop (pd), and the phloem phases E(pd)1, salivation into a sieve element, and E(pd)2, ingestion of sieve element sap. Waveforms F (presumed penetration difficulties) and G (xylem sap ingestion) were rare and grouped into waveform C. We used PROBE3.4 software to record the time from the start to the end of each waveform. We used this information to calculate 10 feeding parameters (Fig. 1). Because we assumed that whiteflies that did not record an E(pd) within the experimental period would eventually have attempted to reach phloem phase, we recorded their "time from record beginning to first E(pd)" as 360 min (i.e., the total experimental duration). Whiteflies that did not reach phloem phase had zeros for all other phloem-related parameters (number and total duration of E(pd)1 and E(pd)2, % of probes reaching phloem phase, etc.). Each parameter was calculated for each replicate and the replicates averaged to derive treatment-level means and standard errors.

Statistical Analysis. Because the feeding data were non-normally distributed, we chose to use the nonparametric Wilcoxon rank sum test for our analyses. We tested whether the means for "host plant" (cabbage, cucumber, and tomato) and "*B. tabaci* population" (CaSP, CuSP, and ToSP) differed for each of the 10 feeding parameters. Analyses were conducted in JMP 9.0.

Results

Of the 217 whiteflies tested during this experiment, 142 reached the phloem phase (i.e., recorded an E(pd) within the 360-min experiment). When on cabbage, 23 of 23 CaSP whiteflies recorded an E(pd), 20 of 25 CuSP recorded an E(pd), and 22 of 25 ToSP recorded an E(pd). When on cucumber, 8 of 19 CaSP

Table 1. Results of Wilcoxon rank sum test for each of 10 feeding parameters

Feeding parameter	Plant		Population	
	χ^2, df = 2	P	χ^2, df = 2	P
A. No. probes before first E(pd)	10.01	**0.007**	17.21	**0.002**
B. Time from first probe to first E(pd)	44.39	**<0.001**	29.56	**<0.001**
C. No. E(pd)1	70.45	**<0.001**	24.27	**<0.001**
D. Mean duration of E(pd)1	46.15	**<0.001**	1.76	0.414
E. Total duration of E(pd)1	75.08	**<0.001**	9.27	**<0.001**
F. No. E(pd)2	69.82	**<0.001**	24.55	**<0.001**
G. Total duration of E(pd)2	52.52	**<0.001**	18.46	**<0.001**
H. Potential E(pd)2 index	40.93	**<0.001**	28.39	**<0.001**
I. Total duration of E(pd)	53.76	**<0.001**	18.68	**<0.001**
J. Percentage of probes reaching phloem phase	50.29	**<0.001**	21.89	**<0.001**

The factors analyzed were "host plant" (cabbage, cucumber, and tomato) and "*B. tabaci* pop" (CaSP, CuSP, and ToSP).

Values in bold are significant at $P < 0.05$.

whiteflies recorded an E(pd), 8 of 28 CuSP recorded an E(pd), and 10 of 25 ToSP recorded an E(pd). When on tomato, 19 of 20 CaSP whiteflies recorded an E(pd), 14 of 25 CuSP recorded an E(pd), and 18 of 27 ToSP recorded an E(pd). When summed across all *B. tabaci* populations, 89% of whiteflies recorded an E(pd) on cabbage, 36% on cucumber, and 71% on tomato.

Whiteflies fed differently on the three host plants ($P < 0.05$ for all 10 parameters; Table 1), with cabbage showing the "best" feeding performance (i.e., lowest time to first E(pd), most E(pd)1s, etc.) for all 10 parameters (Fig. 1). The *B. tabaci* populations also differed in their feeding performance ($P < 0.05$ for 9 of 10 parameters; Table 1). Across all of the host plants and feeding parameters, the feeding performance of CaSP either equaled (feeding parameters A and D in Table 1; Fig. 1) or exceeded (feeding parameters B, C, and E–J) the feeding performance of CuSP and ToSP. The difference between CaSP and the other populations was largest when feeding on cabbage; CaSP was 76% faster to start probing than CuSP and ToSP (Fig. 1B), had 3.2× as many probes that reached E(pd1) and 3.3× as many that reached E(pd)2 (Fig. 1C and F), and spent 59% more time in phloem-probing behaviors (Fig. 1I).

Despite having been confined for >80 generations on a single host plant, neither CuSP nor ToSP showed evidence of host plant specialization. On cucumber, CuSP was significantly less effective ($P < 0.05$) than CaSP in four feeding parameters; CuSP's potential E(pd)2 index, for example, was 80% lower than that of CaSP (Fig. 1H). ToSPs feeding performance on tomato was similarly poor, with CaSP outperforming it in four feeding parameters. CaSP had 3× more probes reaching the phloem-ingestion phase than ToSP (i.e., E(pd)2; Fig. 1F), and spent 62% more time in the phloem phase on tomato (Fig. 1I).

Although they did not feed as well on cabbage as CaSP, both CuSP and ToSP fed better on cabbage than on their natal host plant. Both populations spent more time salivating (Fig. 1E) and ingesting (Fig. 1G) on cabbage than on their natal host plants. Strikingly, neither CuSP nor ToSP was ever the best-performing population on their host plants; this held true for all 10 feeding parameters. In contrast, the relative feeding performance of CaSP was clearly higher on cabbage, its natal host plant; it spent 2.5× more time in the phloem phase on cabbage than the other two plants (Fig. 1I).

Discussion

We found clear evidence of feeding differentiation among separate populations of a single *B. tabaci* population confined for >80 generations on three different host species. Such a result is consistent with a number of studies that have documented rapid (<100 generations) differentiation in a species whose populations are confined to different host plants (e.g., Magalhaes et al. 2009). Given that the three host plants we tested differed substantially in their taxonomy (cabbage in the Brassicaceae family, cucumber in the Cucurbitaceae, and tomatoes in the Solanaceae), it is perhaps unsurprising that each host plant selected for different feeding behaviors.

We were surprised to find that the observed host differentiation apparently did not produce local adaptation. Across virtually all of the feeding parameters for the three host plants, the CaSP either equaled or exceeded the performance of the two other host-specific populations (Fig. 1A–J). Even more strikingly, neither CuSP nor ToSP ever outperformed the other populations on their own host plant, and both fed better on cabbage than on their natal hosts (Fig. 1). This result is at odds with previous work by Ramírez and Niemeyer (2000), who found that populations of the aphid *Sitobion fragariae* confined to wheat for a single generation fed more effectively on wheat than did aphids previously confined to oats. More generally, our results seem to run counter to a substantial body of literature showing that host-plant-mediated reproductive isolation selects for those individuals best suited to the novel resource (Via 1999). Because we (A) transferred several hundred *B. tabaci* individuals to each of the novel host plants at the start of the experiment, and (B) the populations built quickly from there and remained high, our findings are unlikely to be the result of genetic drift resulting from a population bottleneck (Allendorf 1986). We can also reject the hypothesis that tomatoes and cucumbers were unsuitable hosts, as the *B. tabaci* populations on both plants appeared to be thriving, and previous research has shown that biotype B feeds well on these plants (Liu et al. 2012). In the following text, we discuss several possible explanations for the apparent loss of adaptation to cabbage and lower relative feeding performance of CuSP and ToSP.

The fact that CuSP and ToSP were equaled or outperformed by CaSP on both cabbage and their natal host plants suggests that CuSP and ToSP lack something that cabbage-reared *B. tabaci* possesses. In this regard, our findings may be similar to those of other

studies of host-driven differentiation. Work on two host-specific races of the soapberry bug *Jadera haematoloma* (Herrich-Schaeffer), for example, found loss of adaptation to the ancestral host even in cases where there was no obvious adaptation to the new host (Carroll et al. 2001). One possibility is that different host plants alter the presence and/or prevalence of whitefly symbionts, organisms tightly linked to feeding behavior in many insects (Miller et al. 2010). Treating pea aphids (*Acyrthosiphon pisum* (Harris)) with the antibiotic chlortetracycline, for example, increases the amount of time aphids spend salivating but reduces the amount of time spent ingesting phloem (Wilkinson and Douglas 1995). *B. tabaci* harbors both primary and secondary symbionts that play important ecological and evolutionary roles. The P-symbiont *Portiera*, for instance, is essential for whitefly survival and development (Baumann 2005), whereas S-symbionts such as *Wolbachia*, *Cardinium*, *Hamiltonella*, and *Rickettsia* can also alter various aspects of host physiology (Gottlieb et al. 2006, Chiel et al. 2007, Gueguen et al. 2010). Recent work (Pan et al. 2012a) assessing the symbiont assemblages of the three populations of host-specific *B. tabaci* B populations used in this study found a number of among-population differences. In addition to CaSP having far higher rates of *Cardinium*-only infection (100%) than either ToSP (61%) or CuSP (25%), it also had higher rates of *Cardinium–Hamiltonella* and *Cardinium–Rickettsia* coinfections than the other two host-specific populations (Pan et al. 2012a). These findings were confirmed in a subsequent analysis of the symbiont assemblages of CaSP and CuSP (Pan et al. 2013), which found that CaSP harbored more *Cardinium* and *Rickettsia* than did CuSP. Although *Cardinium* infection has been shown to have male-killing properties in wasps (Perlman et al. 2008), its impact on *B. tabaci* is unknown. Infection of *A. pisum* by *Hamiltonella* provides protection against a parasitic wasp (Oliver et al. 2008), however, and *Rickettsia* has been shown to increase whitefly survival and decrease development time in southwestern U.S. *B. tabaci* populations (Himler et al. 2011). It could be that increases in whitefly feeding efficiency mediated by *Cardinium* and other S-symbionts reduce the fitness costs of these organisms to *B. tabaci.*

A second possibility is that host plant isolation alters *B. tabaci* digestive processes in ways that broadly affect their feeding (Terra and Ferreira 1994). Recent work analyzing the transcriptomes of the three host-specific *B. tabaci* populations used in this study found that a precursor to the enzymatic protein cathepsin B was more strongly up-regulated in the CaSP than in the other two host-specific populations (Xie et al., in preparation). Cathepsin B is found in the midguts of many invertebrate herbivores (Murdock et al. 1987, Foissac et al. 2002), where it acts as a digestive enzyme and reduces the herbivore's susceptibility to plant-derived protease inhibitors (Bown et al. 2004, Kollien et al. 2004, Koo et al. 2008). Regardless of the host plant, higher cathepsin B levels in CaSP could act generally to reduce the impact of plant defenses and increase the speed and efficiency of whitefly feeding. Although we cannot explain why ToSP and CuSP, both of which were derived from cabbage-feeding lines, express lower levels of cathepsin B, this possibility would explain our results.

It is important to note that our measurements of feeding behavior do not necessarily reflect the overall fitness of each *B. tabaci* population. Although the amount of whitefly feeding should broadly correlate with increased fitness, moderate decreases in sap ingestion may lessen *B. tabaci* exposure to plant defenses and improve overall survival. If so, the "low performing" ToSP and CuSP populations may nonetheless have population growth rates equal to or greater than CaSP when feeding on their natal host plants. Although this scenario is plausible, we did not collect the demographic data necessary to test it. It is worth noting, however, that this possibility would not explain why all three populations spent more time in the phloem phase on cabbage than the other two host plants (Fig. 1I).

Regardless of the explanation, our findings suggest that the common agricultural practice of intensive single-species greenhouse cultivation can lead to host plant differentiation in *B. tabaci* B feeding behavior. Although the role played by host plants in the invasion and rapid spread of *B. tabaci* has been extensively investigated (De Barro et al. 2011), population-level differentiation in feeding behavior has been far less studied. This lacuna is notable because, as feeding behavior is an important factor in the intraspecific competition between *B. tabaci* B and Q (Liu et al. 2012), such plant-driven behavioral changes may alter their competitive interaction. In addition, *B. tabaci*'s efficacy as a vector for tomato yellow leaf curl virus and other begomoviruses is a function of the time spent salivating (viral inoculation) and ingesting phloem sap (viral acquisition) (Jiang et al. 2000). Changing these parameters could affect the virus–vector interaction (Inbar and Gerling 2008) and alter the role played by whiteflies in facilitating plant disease outbreaks in agricultural systems. At a minimum, more attention should be paid to the correlation between plant-driven changes in *B. tabaci* feeding traits, something likely to occur in the greenhouse model of agricultural production, and the outbreaks of Tomato yellow leaf curl virus disease and other plant diseases. Ultimately, the differentiation in feeding behavior of the three host-specific populations may provide ecological and evolutionary information useful for better understanding the invasion of *B. tabaci* and its management.

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