

2015

Insecticides promote viral outbreaks by altering herbivore competition

Huipeng Pan

Evan L. Preisser

University of Rhode Island, preisser@uri.edu

See next page for additional authors

Follow this and additional works at: https://digitalcommons.uri.edu/bio_facpubs

**The University of Rhode Island Faculty have made this article openly available.
Please let us know how Open Access to this research benefits you.**

This is a pre-publication author manuscript of the final, published article.

Terms of Use

This article is made available under the terms and conditions applicable towards Open Access Policy Articles, as set forth in our [Terms of Use](#).

Citation/Publisher Attribution

Pan, H. , Preisser, E. L., Chu, D. , Wang, S. , Wu, Q. , Carrière, Y. , Zhou, X. and Zhang, Y. (2015), Insecticides promote viral outbreaks by altering herbivore competition. *Ecological Applications*, 25: 1585-1595. doi:10.1890/14-0752.1 Available at: <https://doi.org/10.1890/14-0752.1>

This Article is brought to you for free and open access by the Biological Sciences at DigitalCommons@URI. It has been accepted for inclusion in Biological Sciences Faculty Publications by an authorized administrator of DigitalCommons@URI. For more information, please contact digitalcommons@etal.uri.edu.

Authors

Huipeng Pan, Evan L. Preisser, Dong Chu, Shaoli Wang, Qingjun Wu, Yves Carrière, Xuguo Zhou, and Youjun Zhang

1 **Insecticides promote viral outbreaks by altering herbivore competition**

2

3

4 Huipeng Pan^{a,d}, Evan L. Preisser^b, Dong Chu^c, Shaoli Wang^a, Qingjun Wu^a, Yves Carrière^d,
5 Xuguo Zhou^{*c}, and Youjun Zhang^{*a}

6

7

8 ^aDepartment of Plant Protection, Institute of Vegetables and Flowers, Chinese Academy of
9 Agricultural Sciences, Beijing, 100081, P. R. China

10 ^bDepartment of Biological Sciences, University of Rhode Island, Kingston RI 02881, USA

11 ^cCollege of Agronomy and Plant Protection, Qingdao Agricultural University, Qingdao,
12 266109, P. R. China

13 ^dDepartment of Entomology, University of Arizona, Tucson AZ 85721, USA

14 ^eDepartment of Entomology, University of Kentucky, Lexington KY 40546, USA

15 -----

16 *Corresponding Authors:

17 Dr. You-Jun Zhang: zhangyoujun@caas.cn

18 Dr. Xuguo "Joe" Zhou: xuguozhou@uky.edu

19

20 **RUNNING TITLE:** Insecticides alter whitefly competition

21

22 **ABSTRACT**

23 While the management of biological invasions is often characterized by a series of
24 single-species decisions, invasive species exist within larger food webs. These biotic
25 interactions can alter the impact of control/eradication programs and may cause suppression
26 efforts to inadvertently facilitate invasion spread and impact. We document the rapid
27 replacement of the invasive *Bemisia* MEAM1 cryptic species by the cryptic MED species
28 throughout China, and demonstrate that MED is more tolerant of insecticides and a better
29 vector of *tomato yellow leaf curl virus* ('TYLCV') than MEAM1. While MEAM1 usually
30 excludes MED under natural conditions, insecticide application reverses the MEAM1-MED
31 competitive hierarchy and allows MED to exclude MEAM1. The insecticide-mediated
32 success of MED has led to *TYLCV* outbreaks throughout China. Our work strongly supports
33 the hypothesis that insecticide use in China reverses the MEAM1-MED competitive
34 hierarchy and allows MED to displace MEAM1 in managed landscapes. By promoting the
35 dominance of a *Bemisia* species that is a competent viral vector, insecticides thus increase the
36 spread and impact of *TYLCV* in heterogeneous agroecosystems.

37

38

39 **KEYWORDS:** *Bemisia tabaci*, MEAM1, MED, biological invasions, competitive
40 displacement, plant virus, agriculture, insecticides

41 INTRODUCTION

42 Rapid increases in the speed and volume of international trade have led to biological
43 invasions becoming an increasingly serious problem worldwide. Invasions that bring together
44 previously disjunct taxa have the potential to profoundly alter both natural and managed
45 ecosystems. Despite a large body of literature on interactions between native and invasive
46 species, there has been less attention paid to the outcome and consequences of invasive-
47 invasive interactions (Simberloff and Von Holle 1999, Simberloff 2006). There are several
48 reasons why understanding such interactions, and the factors affecting them, is important.
49 First, invasive-invasive interactions should grow more common as the number and density of
50 these species increases. Second, the rapid population growth rates of many invasive species
51 (Sakai et al. 2001) should increase interspecific competition; a meta-analysis found that
52 competition was stronger between invasive herbivores than between native species (Denno et
53 al. 1995). Even in the absence of competition, newly-arrived exotic species might also alter
54 the spread and impact of other invasive species, a phenomenon called “invasional meltdown”
55 (Simberloff and Von Holle 1999, Grosholz 2005, Simberloff 2006).

56 Understanding the interaction between invasive species, and the factors affecting it, is
57 especially important when the species have different impacts on a shared resource. This can
58 happen when resource depletion is not the only (or even primary) means by which an
59 invasive herbivore impacts its host plant. For example, the effect of herbivory on a host plant
60 can be magnified if the herbivore acts as a disease vector (Miles 1999, Jones 2003) or
61 facilitates the entry of secondary pathogens (Wallin and Raffa 2001). Because organisms
62 often differ in their vector competence or feeding-related damage (Dorschner et al. 1987),

63 interactions between exotic species with disparate impacts may alter the ecological and
64 economic costs of invasions.

65 The whitefly ‘species’ *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae) is
66 actually a complex of genetically divergent but morphologically indistinguishable cryptic
67 species (De Barro et al. 2011). The two most invasive *Bemisia* species are the Middle East –
68 Asia Minor I ‘MEAM1’ and the Mediterranean ‘MED’. During the past two decades,
69 MEAM1 and MED have invaded nearly 60 countries and caused massive agricultural losses
70 (De Barro et al. 2011). Although their feeding itself is damaging, whiteflies also vector over
71 100 begomoviruses that can have catastrophic impacts on plant growth and survival (Jones
72 2003). The whitefly-mediated introduction of *tomato yellow curl leaf virus* (‘TYLCV’) and
73 other pathogens into agricultural ecosystems often produces outbreaks whose costs far exceed
74 those of the whiteflies themselves (Jones 2003). Although both species are viral vectors, the
75 feeding behavior of MED makes it more likely than MEAM1 to acquire and transmit TYLCV
76 and other viruses (Jiang et al. 2000, Pan et al. 2012); as a result, MED appears to be a more
77 competent viral vector than MEAM1 (Pan et al. 2012).

78 Although MEAM1 and MED are both major pests of agricultural crops, they vary in
79 their mating behavior and prefer different host plants (Crowder et al. 2010b, Crowder et al.
80 2011, Elbaz et al. 2011, Tsueda and Tsuchida 2011). They also differ in their susceptibility to
81 insecticides, with MED generally showing greater tolerance of neonicotinoids and other
82 insecticides (Crowder et al. 2010a, Jones et al. 2011). The outcome of MEAM1-MED
83 interactions can be affected by host plant and abiotic factors such as temperature and
84 humidity (Chu et al. 2012b); in general, however, the greater ability of MEAM1 than MED to

85 mate with conspecifics in mixed populations and greater effort devoted by MEAM1 to
86 reproduction leads to the competitive exclusion of MED in laboratory experiments (Pascual
87 and Callejas 2004, Pascual 2006, Crowder et al. 2010a, Crowder et al. 2010b, Tsueda and
88 Tsuchida 2011).

89 After MEAM1 entered China in the mid-1990s, it spread rapidly and caused serious
90 crop losses while displacing native *Bemisia* spp. (Liu et al. 2007). MEAM1 dominated
91 Chinese agricultural systems until 2003, when MED was first detected in Yunnan Province.
92 The new species spread quickly and by 2007 had supplanted MEAM1 as the dominant
93 whitefly in agricultural systems in China (Chu et al. 2010, Pan et al. 2011). The same pattern
94 of initial invasion and dominance by MEAM1, followed by the invasion and rapid
95 replacement of MEAM1 by MED, also occurred in Japan (MEAM1 in 1989 and MED in
96 2004; Ohto 1990, Ueda and Brown 2006) and South Korea (1998 and 2005, respectively; Lee
97 and De Barro 2000, Park et al. 2012). The rapid exclusion of MEAM1 in these countries is at
98 odds with previous laboratory studies demonstrating the competitive dominance of MEAM1
99 (Pascual and Callejas 2004, Pascual 2006, Crowder et al. 2010a, Tsueda and Tsuchida 2011).
100 This apparent contradiction begs the question: what factors explain the rapid displacement of
101 MEAM1 by MED throughout China?

102 We explore the factors affecting competition between invasive herbivore species and
103 link this interaction to an increase in herbivore-vectored viral outbreaks. Specifically, we
104 present the results of experimental work demonstrating that insecticide application reverses a
105 naturally-occurring competitive hierarchy and allows MED to exclude MEAM1. We also
106 document a rapid increase in the domestic use of both neonicotinoid- and pyrethroid-based

107 insecticides between 2000 and 2011 (Figure 1), and present the results of contemporaneous
108 landscape-level surveys documenting both the replacement of MEAM1 by MED throughout
109 China and the tight association between TYLCV and MED. The ascent of MED, a highly-
110 competent begomovirus vector, has led to damaging outbreaks of *TYLCV* in China and other
111 Pacific Rim nations. This is the first research to comprehensively address the hypothesized
112 connection between herbivore competition, insecticide resistance, and viral outbreaks. Instead
113 of reducing impacts, our results suggest that heavy insecticide use for whitefly suppression
114 has exacerbated the spread of *TYLCV* in heterogeneous agricultural landscapes.

115 **METHODS**

116 ***Bemisia* field survey.** Adult whiteflies were collected from agricultural fields in 26 of
117 34 province-level administrative units in China during 2003, 2007, 2009, 2011, and 2012
118 (Table S1). In the 2003-2011 surveys, ten adult whiteflies were collected at each site, each
119 individual from a different individual host plant. Adult whiteflies were placed alive into 95%
120 ethanol and stored at -20°C until DNA extraction. In the 2003-2011 surveys, genomic DNA
121 was extracted from the ten individuals from each site; in the 2012 survey, the decreasing costs
122 of genetic analysis allowed us to collect and determine the species identity of up to 30
123 individuals per site (Table S1). DNA was stored at -20°C until analysis of the *mtCOI* gene
124 (Shatters et al. 2009) for species determination.

125 ***TYLCV-Bemisia* field survey.** In 2011, adult whiteflies were collected from healthy
126 (i.e., without the ‘leaf curls’ symptomatic of *TYLCV* infection) or *TYLCV*-infected (i.e., with
127 leaf curling) fields of tomato, *Lycopersicon esculentum*. Correspondence between leaf-curling
128 symptoms and *TYLCV* infection was confirmed by screening sampled whiteflies for *TYLCV*

129 genes (detailed at the end of this paragraph); *TYLCV* was always detected in whiteflies
130 collected from ‘leaf-curling’ fields but never detected in whiteflies on plants from fields
131 where the symptomatic leaf curling was absent. A total of 48 fields from 26 province-level
132 administrative units in China were sampled (Table S2). At each site, at least 100 whiteflies
133 were collected from a cluster of ten plants (at least 10 whiteflies per plant). Whiteflies were
134 placed alive into 95% ethanol and stored at -20°C until DNA extraction. If none of the ten
135 plants exhibited the leaf-curling symptomatic of *TYLCV* infection, the corresponding
136 collections were considered to be from healthy plants; otherwise, they were classified as
137 *TYLCV*-infected. For each of the 48 collections, 10-20 individual whiteflies were randomly
138 selected for determination of species and *TYLCV* presence. DNA extraction and species
139 determination were conducted as in the *Bemisia* field surveys (previous paragraph). *TYLCV*
140 presence in each whitefly was determined using two primers that amplified the AV2 gene,
141 *TYLCV*-61 and *TYLCV*-473 (Ghanim et al. 2007).

142 ***Bemisia* laboratory colonies and host plants.** MEAM1 and MED laboratory
143 populations were collected on cabbage, *Brassica oleracea* (cv. Jingfeng1), and poinsettia,
144 *Euphorbia pulcherrima*, in Beijing, China in 2004 and 2009, respectively. Source populations
145 of MEAM1 and MED were maintained in separate whitefly-proof screen cages on tomato
146 plants (cv. Zhongza 9) in a glasshouse under natural light and controlled temperature (26 ±
147 2°C) for four generations. Fifteen adults per generation were randomly selected for *mtCOI*
148 sequencing to ensure the purity of each culture (Shatters et al. 2009).

149 Because MEAM1 and MED are both highly polyphagous, we explored their
150 interactions on multiple host plants. Five crop species, each widely cultivated in China, were

151 used as host plants in the experiments: tomato (cv. Zhongza 9), cabbage (cv. Jingfeng 1),
152 cucumber (*Cucumis sativus*, cv. Zhongnong 12), pepper (*Capsicum annuum*, cv. Zhongjiao
153 6), and cotton (*Gossypium hirsutum*, cv. DP99B). Seedlings were grown to the 5-7 true leaf
154 stage in individual 1.5 L pots with potting mix (peat moss, vermiculite, organic fertilizer, and
155 perlite; 10:10:10:1 ratio by volume). Plants were grown under natural light and controlled
156 temperatures ($26 \pm 2^\circ\text{C}$) in screen cages within a glasshouse.

157 **Insecticides.** We tested the impact of three insecticides on MEAM1 and MED:
158 Thiamethoxam (Syngenta China), Spirotetramat (trade name ‘Movento’; Bayer CropScience
159 China), and Bifenthrin (Ruidefeng Pesticide). Thiamethoxam, a neonicotinoid insecticide,
160 was introduced in China in 2000 for the control of *Bemisia* on field and greenhouse crops
161 (Wu et al. 2003). Spirotetramat, a spirocyclic tetramic acid derivative, is a systemic
162 insecticide that targets whiteflies and other phloem-feeding insects (Cheng et al. 2013); it was
163 introduced in China in 2008. Bifenthrin is a pyrethroid insecticide that has been widely used
164 against *Bemisia* and other crop pests in China since the mid-1990s (Ma et al. 2007).

165 **Insecticidal bioassays.** The effect of Thiamethoxam, Bifenthrin, and Spirotetramat on
166 *Bemisia* eggs was assessed using a slightly-modified version of the standard bioassay
167 protocol (Cahill et al. 1996). Fifteen mating pairs of MEAM1 or MED whiteflies were
168 transferred to separate cotton seedlings (first true leaf stage, ~11.4 cm in height) for egg
169 laying, then removed after 24 hours. After determining egg density on each leaf, seedlings
170 were dipped in different serial dilutions of insecticide for 20 seconds and dried naturally for
171 two hours. Because MEAM1 and MED differ in their susceptibility to insecticides (Crowder
172 et al. 2010a, Jones et al. 2011), we used one set of serial dilutions for MEAM1 (water only,

173 6.25, 12.5, 25, 50, 100, and 200 mg L⁻¹) and another for MED (water only, 100, 200, 400,
174 800, and 1600 mg L⁻¹). Seedlings were maintained in water-filled 50mL beakers and the
175 number of nymphs recorded. Hatching rate was calculated by dividing the number of nymphs
176 by the number of eggs. There were four replicates for each species x insecticide concentration
177 combination; water-only treatments were controls. Bioassays were conducted in an incubator
178 at 26±2°C and a 16:8 L:D photoperiod.

179 Nymphal bioassays were conducted using standard procedures (Li et al. 2012). After
180 determining the per-seedling density of late second-instar nymphs, seedlings were sprayed
181 with different insecticide concentrations. MEAM1 nymphs were exposed to water-only,
182 0.225, 0.45, 0.9, 1.8, 3.6, and 7.8 mg L⁻¹; MED nymphs were exposed to water-only, 3.6, 7.8,
183 15.6, and 31.2 mg L⁻¹. After six days, the per-seedling density of fourth-instar nymphs was
184 determined and used to calculate the mortality rate of third-instar nymphs. Replication and
185 control treatment were as in the egg bioassays.

186 Adult bioassays were conducted using standard procedures (Feng et al. 2009). Twenty
187 adult whiteflies (~5 days post-eclosion) in a glass tube were sprayed with different insecticide
188 concentrations. MEAM1 adults were exposed to water-only, 0.5, 1.5, 6, 24, and 96 mg L⁻¹;
189 MED adults were exposed to water-only, 1.5, 6, 24, 96, 384, 1536, and 6144 mg L⁻¹.
190 Mortality was assessed after 48 h. There were four replicates for each species x insecticide
191 concentration combination; water-only treatments were used as controls.

192 **Host plant effects on MEAM1-MED competition.** We conducted a laboratory
193 experiment assessing MEAM1-MED competition on five plant species: cabbage, cotton,
194 cucumber, pepper, and tomato. Each 0.6m x 0.4m x 0.8m screened cage (=replicate)

195 contained two plants of the same species. Each replicate was inoculated with 20 male-female
196 pairs of newly-emerged MEAM1 adults and 20 male-female pairs of MED adults (80 total
197 whiteflies). There were three replicates per plant host for cabbage, cotton, pepper, and
198 tomato, and five replicates for cucumber. The sampling protocol and timing is detailed below;
199 briefly, two days after the progeny of each *Bemisia* generation began emerging, we collected
200 100 whiteflies per replicate, identified each of them as either MEAM1 or MED, and
201 expressed the results as the proportion of MED present in the 100-*Bemisia* sample.

202 **Insecticide effects on MEAM1-MED competition.** We conducted a laboratory
203 experiment examining how insecticides affect MEAM1-MED competition on cucumber and
204 tomato. Cage design was as in the host plant experiment, with two plants per cage
205 (=replicate). In the cucumber experiment, 20 male-female pairs of newly-emerged MEAM1
206 adults and 20 male-female pairs of newly-emerged MED adults (80 total whiteflies) were
207 added per replicate. We added whiteflies to each replicate seven days before applying the
208 control and insecticide treatments (detailed below) to allow them to establish populations on
209 the host plants. At the beginning of the second whitefly generation, each replicate was
210 randomly assigned to one of the following four treatments: water spray (control),
211 Thiamethoxam spray, Spirotetramat spray, or Bifenthrin spray (applied at concentrations of
212 31.3, 20, and 96 mg L⁻¹, respectively). These concentrations reflect recommended spraying
213 rates for *B. tabaci* management on cucumber and tomato (<http://www.chinapesticide.gov.cn>).
214 Insecticides and water were applied by spraying each plant to run-off; each treatment was
215 replicated five times. The tomato experiment was identical except that it contained only two
216 treatments, water spray and Thiamethoxam spray (31.3 mg L⁻¹), with four replicates per

217 treatment. Insecticides and water were reapplied every seven days after the initial spraying.
218 The sampling protocol and timing is detailed in the following paragraph; briefly, two days
219 after the progeny of each *Bemisia* generation began emerging, we collected 100 whiteflies per
220 replicate, identified each of them as either MEAM1 or MED, and expressed the results as the
221 proportion of MED present in the 100-*Bemisia* sample.

222 **Sampling and identification of whiteflies in species exclusion experiments**

223 **(previous two paragraphs).** Whitefly samples for species determination were taken two
224 days after the progeny of each generation began emerging. Each sample consisted of 100
225 randomly-selected whiteflies per cage, each of which was identified as MEAM1 or MED via
226 *mtCOI* analysis (Shatters et al. 2009). After sampling, we removed one of the two plants in
227 each cage (and the whiteflies on it) and replaced it with a whitefly-free plant to prevent
228 overcrowding. In the experiment evaluating the impact of insecticides, each plant was
229 sprayed with the appropriate treatment (water or insecticide) before being placed in the cage.
230 Sampling ended when only one species was detected in the sample; the experiment ended
231 when all treatments consisted of a single species.

232 **Statistical analysis:** For the *TYLCV-Bemisia* survey, a Fisher Exact test was used to
233 compare the percentage of MEAM1 and MED at sites with and without *TYLCV*-infected
234 plants, and the percentage of *TYLCV*-infected MEAM1 and MED. We used logistic
235 regression for binary data to assess the effects of sample month, percentage of MED
236 individuals, and the percentage of *TYLCV*-infected MEAM1 or MED individuals on the odds
237 of tomato plant infection by *TYLCV*. Because there was evidence of a tight fit when the
238 percentage of *TYLCV*-infected MED was included in the model, regression parameters were

239 estimated using a Firth Adjusted Maximum Likelihood method. As there was no evidence
240 that month or the percentage of MED individuals was significantly associated with the odds
241 of plant infection (Drop-in-deviance test, $df = 4$, $\chi^2 = 3.92$, $p = 0.42$), we removed these two
242 factors in a reduced logistic regression model that investigated the effect of the percentage of
243 *TYLCV*-infected MEAM1 and MED individuals.

244 Bioassay data, including LC_{50} values (i.e., the pesticide concentration necessary to kill
245 50% of the population) and their 95% fiducial limits, were calculated from probit regressions
246 using POLO-PC. Mortality was corrected using Abbott's formula for individual probit
247 analyses. Resistance to an insecticide was considered significantly different between species
248 if the 95% fiducial limits associated with the LC_{50} values of each species did not overlap.
249 JMP v.9 was used for all statistical analyses.

250 RESULTS

251 **Changes in *Bemisia* species composition in China, 2003-2012 (Figure 2).** At the
252 start of our surveys in 2003, MED was absent from 17 provinces and occurred sporadically in
253 or around the flower markets of Yunnan, Henan, and Beijing (Fig. 2A). By 2007, MED was
254 present in 11/24 sampled provinces (46%; Fig. 2B); by 2009, MED was present in 14 of 20
255 sampled provinces (70%; Fig. 2C). At the time of the 2012 survey (Fig. 2D), MED occurred
256 in 25/26 provinces (96%) and had completely excluded MEAM1 from 13 of them. At the
257 province level, the mean proportion of MED in sampled *Bemisia* populations increased from
258 <5% in 2003 to >80% in 2012 (Fig. 2E)

259 **Landscape-level association between *TYLCV* and *Bemisia* species (Figure 3).** Of
260 the 48 *Bemisia* populations sampled in 2011, 25 collections from 11 provinces contained only

261 MED, three collections from three provinces contained only MEAM1, and 20 collections
262 contained both MEAM1 and MED (Table S2). MED was dominant (>50% of sampled
263 individuals) in 14 of 20 mixed-species samples. Across all collections, *TYLCV* was more
264 commonly detected in MED than MEAM1 (Figure 3; Fisher Exact test, $p < 0.001$). MED was
265 also more abundant at sites with *TYLCV*-infected tomato plants (85% MED) than at sites with
266 healthy tomato plants (70% MED) (Fisher Exact test, $p < 0.001$). Of 45 populations with
267 MED in 2011, 28 of them (62%; also see Fig. 3) had at least one *TYLCV*-positive MED
268 individual; of 23 populations of MEAM1 in 2011, nine of them had at least one *TYLCV*-
269 positive MEAM1 individual (39%; again, see Fig. 3).

270 The logistic regression model that included the effects of month, percentage of MED
271 individuals, and percentages of *TYLCV*-infected MEAM1 and MED adequately described the
272 variation in tomato plant infection (Goodness of fit $X^2 = 3.09$, $df = 13$, $p = 0.99$; Akaike's
273 Information Criterion adjusted for sample size (AIC_c) = 29.0). Of the four factors, only the
274 percentage of *TYLCV*-infected MED was positively and significantly associated with tomato
275 plant infection (slope = 3.42, SE = 1.66, $\chi^2 = 6.81$, $p = 0.009$). The reduced logistic
276 regression model that only included the percentage of *TYLCV*-infected MEAM1 and MED
277 also had a good fit but lower AIC_c (Goodness of fit $X^2 = 1.38$, $df = 17$, $p = 1$; $AIC = 10.3$).
278 This reduced model produced similar results: plant infection was associated with *TYLCV*
279 infection of MED (slope = 5.25, SE = 2.14, $\chi^2 = 12.96$, $p = 0.003$) but not with infection of
280 MEAM1 (slope = 2.55, SE = 2.60, $\chi^2 = 0$, $p = 1$).

281 ***Bemisia* susceptibility to insecticides (Table 1).** The LC_{50} of MEAM1 eggs exposed
282 to Thiamethoxam was 6.6-fold lower than of MED eggs. Nymphs of both species were far

283 more susceptible than eggs to Thiamethoxam; again, however, the LC_{50} of MEAM1 nymphs
284 was 4.9-fold lower than in MED. Resistance increased in both MEAM1 and MED adults, but
285 the LC_{50} of MEAM1 remained substantially (3.1-fold) lower than it was for MED adults.

286 The same pattern occurred when both species were exposed to Spirotetramat and
287 Bifenthrin. Eggs of MEAM1 were ~25-fold less resistant to Spirotetramat than those of
288 MED, while MEAM1 nymphs and adults were 6.5-fold and 7-fold less resistant, respectively.
289 Bifenthrin, a contact insecticide used primarily against adult whiteflies, was not toxic to
290 MEAM1 or MED eggs, and was similarly toxic to MEAM1 and MED nymphs; MEAM1
291 adults were, however, 3.7-fold less resistant than MED adults.

292 **Host plant effects on species competition (Figure 4).** MEAM1 competitively
293 excluded MED from four of five host plants, taking between four (on cabbage) and nine (on
294 cucumber) generations to eliminate MED. The only exception to this occurred on pepper,
295 where MED excluded MEAM1 in two generations.

296 **Insecticidal effects on species competition (Figure 5).** In the absence of insecticide,
297 MEAM1 excluded MED by the ninth generation on cucumber. Insecticide application
298 reversed this outcome, allowing MED to exclude MEAM1 within five generations (Figure
299 5A). This result held true on tomato, where MEAM1 excluded MED within six generations in
300 the absence of Thiamethoxam but MED excluded MEAM1 when the insecticide was used
301 (Figure 5B).

302 **DISCUSSION**

303 Our research provides strong support for the hypothesis that insecticide use in China
304 (Figure 1) reverses the competitive hierarchy between MEAM1 and MED, allowing highly-

305 resistant MED to displace moderately- resistant MEAM1 in managed landscapes throughout
306 the country (Figure 2). By promoting the dominance of a *Bemisia* species that is a superior
307 viral vector (Figure 3), insecticide use facilitated the spread of *TYLCV* in heterogeneous
308 agricultural landscapes. Increased chemical control of *Bemisia* and other pests (i.e., the use of
309 pyrethroid insecticides rose 72% between 2009 and 2011; Figure 1) thus appears to enhance
310 rather than reduce the likelihood and severity of future *Bemisia*-mediated viral outbreaks.

311 The application of three different insecticides (neonicotinoid, pyrethroid, and tetramic
312 acid) reversed the normal competitive hierarchy (Figure 4) and allowed MED to exclude
313 MEAM1 on both tomato (Figure 5A) and cucumber (Figure 5b). All MED life-history stages
314 were more resistant than MEAM1 to these insecticides (Table 1). Other researchers working
315 with Chinese MEAM1 and MED have found similar differences in resistance: one study
316 found that a Jiangsu MED population was 1900-fold more resistant to imidacloprid and 1200-
317 fold more resistant to Thiamethoxam than MEAM1 (Wang et al. 2010).

318 Our finding that MED is replacing MEAM1, and that this switch is related to
319 pesticide application, is consistent with observations from other parts of the world. In Israel,
320 for example, MED became dominant in mixed field populations treated with pyriproxyfen
321 and neonicotinoids (Horowitz et al. 2005). The ability of insecticides to mediate the
322 MEAM1-MED interaction was initially described by Crowder et al (2010a), who used
323 modeling and an experimental manipulation of North American *Bemisia* to demonstrate the
324 potential for MED to competitively exclude MEAM1. The present work, for the first time,
325 provides empirical evidences from both a 10-year field survey and laboratory experiments to
326 establish this framework, identifies the unexpected consequences of this reversal, and

327 confirms that three insecticides, each recommended for control of *Bemisia* and other plant
328 sucking pests, produce the same outcome.

329 The replacement of MEAM1 by MED in China (Figure 2) and neighboring countries
330 has been repeatedly linked to *TYLCV* outbreaks. In China, for instance, MEAM1 was first
331 detected in 1990, followed by MED in 2003 and *TYLCV* in 2006 (Figure 1; Hu et al. 2011,
332 Pan et al. 2011). Our survey (Figure 3) demonstrates the association between *TYLCV* and
333 MED in China. In addition, we found a positive relationship between *TYLCV* infection in
334 tomato and the percentage of *TYLCV*-infected MED; in contrast, there was no relationship
335 between plant health and the percentage of *TYLCV*-infected MEAM1. In sum, our findings
336 are in accord with previous surveys (Pan et al. 2012, Park et al. 2012) and experimental work
337 (Jiang et al. 2000, Liu et al. 2013a) indicating that vector-plant transmission of *TYLCV* in
338 Asia is predominantly associated with MED.

339 While the displacement of an established exotic species by another invader has been
340 previously documented (Reitz and Trumble 2002), the unintended economic consequences of
341 this insecticide-driven displacement for agriculture in China and other Pacific Rim nations
342 make this system unusual and perhaps unique. Although information on the economic impact
343 of *TYLCV* in China is not publicly accessible, data from other countries can provide a
344 perspective on the possible costs. Outbreaks of *TYLCV* in India, a country whose farming
345 infrastructure is similar to that of China, cause yield losses ranging from 50-100% (Reddy et
346 al. 2010). By way of comparison, *TYLCV* outbreaks in the early 2000s that reduced tomato
347 yields by 20% in North America and Europe had a cost of more than 300 million U.S. dollars
348 (Glick et al. 2009). Given the polyphagous nature of *B. tabaci*, the hundreds of viruses it can

349 vector, and the extensive agricultural sector in China, the total economic cost of *TYLCV* and
350 other viral pathogens are likely higher, perhaps dramatically so. Such impacts amply justify
351 *B. tabaci*'s selection as one of the world's hundred worst invasive species.

352 Our results provide strong support for the hypothesis that the insecticide-mediated
353 competitive replacement of MEAM1 by MED is linked to the spread of *TYLCV*. In other
354 countries, however, different outcomes have occurred. In Israel, for instance, the MEAM1-
355 MED competitive hierarchy fluctuates, with MEAM1 being relatively more abundant than
356 MED in field crops and MED more abundant than MEAM1 in glasshouses and other
357 protected environments where insecticides are intensively used (Kontsedalov et al. 2012). A
358 similar but more extreme situation is observed in the United States, where MED is only found
359 in greenhouses in 23 states while MEAM1 is present in both field crops and greenhouses
360 (McKenzie et al. 2012). Why has the MEAM1-MED interaction played out so differently in
361 China?

362 The most likely explanation for these divergent outcomes involves the infrastructure
363 and incentives in place for pest management. Chinese farming is dominated by the
364 'household responsibility' system of small family farms whose owners are often uneducated
365 and lack access to agricultural extension personnel. As a result, the vast majority of farmers
366 rely on high-dose chemical treatments for pest and weed management (Xu et al. 2008).
367 Although effective in controlling pest species, many of these insecticides also alter local food
368 webs. Alarm at the impact of neonicotinoid insecticides on pollinators, for instance, has led
369 the European Commission to approve a two-year ban on their widespread use. These
370 concerns notwithstanding, Chinese farmers continue to increase use of neonicotinoids and

371 other synthetic insecticides, with unknown consequences for the surrounding ecosystems.
372 This reliance has been exacerbated by China's investment in chemical production facilities in
373 the early 2000s; the global recession decreased exports and flooded the domestic market with
374 insecticides (Figure 1; CCM 2012). The low cost and high efficacy of these products further
375 encourages small farmers in China to 'spray' their way out of pest problems (Xu et al. 2008).

376 In contrast, crop production in the United States is dominated by large-scale
377 agricultural concerns acutely aware of the danger posed by insecticide-resistant *Bemisia*
378 (Osborne 2013). The integrated pest management strategy for *Bemisia* control in the U.S.
379 emphasizes non-chemical approaches and strict action-thresholds for chemical treatments in
380 field crops (Ellsworth and Martinez-Carrillo 2001). Organized and sustained grower
381 education facilitated the deployment and adoption of this plan, which has lowered both
382 *Bemisia*-targeted insecticide use and whitefly problems (Ellsworth and Martinez-Carrillo
383 2001). The emphasis on 'managing' for reduced insecticide resistance may have favored
384 MEAM1 in field crops in the U.S., keeping MED relatively rare and confined to high-
385 pesticide areas like greenhouses (McKenzie et al. 2012). These disparate approaches to pest
386 management likely explain why *TYLCV* has become a greater problem in China than the U.S.

387 While we consider insecticide use to be the most likely explanation of the rapid
388 spread of MED and *TYLCV* in China, other factors could influence success of this species.
389 For example, temporal changes in diversity of agricultural landscape could have affected
390 coexistence of MEAM1 and MED. In Israel, MEAM1 and MED dominated on different
391 agricultural plant families, although MED was the most abundant on a broader range of host
392 plants (Crowder et al. 2011). This wider resource niche of MED could foster coexistence by

393 providing a refuge against MEAM1, which is superior at reproductive interference
394 competition (Crowder et al. 2011). By contrast, our host plant experiment confirmed previous
395 research finding that MEAM1 was dominant over MED on most, but not all, plant species
396 (Figure 4; Iida et al. 2009, Tsueda and Tsuchida 2011, Liu et al. 2013b). Specifically, our
397 results agree with work (Tsueda and Tsuchida 2011) that found MEAM1 could not develop
398 on pepper but did better than MED on tomato, cabbage, and cucumber. Reasons for the
399 differences in host-plant mediated competition between Israel and China and currently
400 unknown, but could be related to geographical changes in the haplotypes of one or more
401 species (De Barro et al. 2011). Nevertheless, our results indicate that a shift towards MED-
402 preferred host plants could favor this species (and *TYLCV*) over MEAM1, although we are
403 unaware of any such large-scale change in cropping systems occurring during the last 10
404 years in China.

405 Although MED has spread through agricultural systems in China, it is unlikely to
406 extirpate MEAM1. Our competition experiments (Figures 4 & 5), for example, were carried
407 out under warm (26 ± 2 °C) conditions with no climatic variation. MEAM1 and MED respond
408 differently to temperature variation, however, with MED being more tolerant than MEAM1
409 of temperature extremes (Elbaz et al. 2011, Chu et al. 2012b). Although MED now dominates
410 agricultural systems, China contains a heterogeneous mix of landscapes that vary in their
411 abiotic, biotic, and anthropogenic (i.e., insecticide use) conditions. Prior to the entry of MED,
412 MEAM1 displaced native whiteflies from agricultural systems; these species persisted in
413 natural landscapes (Liu et al. 2007, Hu et al. 2011). MEAM1 may persist in a similar manner
414 in low-insecticide systems, or on vegetation growing near high-pesticide fields; adopting IPM

415 strategies to reduce chemical use and favor susceptible organisms might favor the resurgence
416 of MEAM1 and increase their likelihood of excluding MED (and, perhaps, their viruses)
417 from systems.

418 While our work provides a cogent explanation for the recent upsurge of whitefly-
419 vectored diseases in heterogeneous agricultural systems, other questions remain to be
420 answered. While *TYLCV* and MED are tightly linked in China and other Pacific Rim nations
421 (Pan et al. 2012, Park et al. 2012), *TYLCV* in Israel is primarily associated with MEAM1
422 (Gottlieb et al. 2010). These differences are driven by the geographic differences in the
423 composition of *Bemisia* endosymbiont communities (Gottlieb et al. 2010), and insecticide-
424 driven shifts in community structure may also alter vector competence. Recent research has
425 also identified five well-defined MED haplotypes (Chu et al. 2012a) whose endosymbiont
426 communities may well differ. Exploring the interaction between insecticide resistance and
427 endosymbiont communities may help develop management programs aimed at reducing viral
428 outbreaks.

429 One drawback of our work is that the comprehensive nature of our approach
430 (combining multiple surveys, experiments, and assays) precluded a completely factorial
431 design. In other words, we did not have sufficient resources to rear both *Bemisia* species on
432 all five host plants prior to the experiment, repeat the insecticide bioassays using whiteflies
433 reared on all five host plants, carry out the ‘insecticide-mediated competition’ experiment on
434 all five host plants, etc. While we would have preferred to test all host plants in all
435 experiments, there is no indication that any of these five plant species are, for instance,
436 capable of altering the response of *Bemisia* to insecticides. We thus believe it to be highly

437 unlikely that our results confound the effects of host plant and insecticides in a way that
438 invalidates our conclusion, an interpretation supported by the fact that our laboratory results
439 are substantially consistent with data emerging from our field surveys.

440 Our research demonstrates that insecticide use alters the competitive interaction
441 between two invasive whiteflies, and is the likely driver of MED's displacement of MEAM1
442 throughout China. The ascent of MED, an effective viral vector, has in turn been linked to
443 economically-damaging agricultural disease outbreaks. Our experiments and surveys strongly
444 suggest that the overuse of insecticides has, through food web interactions, inadvertently
445 exacerbated the problem it sought to solve. This result is especially important since a
446 substantial fraction of agricultural land worldwide is managed by small landholders rather
447 than by large-scale producers. As a consequence, phenomena observed in a heterogeneous
448 agricultural system like China may also occur in several Asian states (e.g., Japan and Korea)
449 and other countries worldwide. Given the larger context of increasing biological invasions
450 worldwide and the importance of species interactions in determining community structure,
451 policies of single-species management that do not account for the larger food web may
452 produce similarly counterintuitive outcomes that pose a major challenge to natural and
453 managed systems alike.

454 **ACKNOWLEDGEMENTS**

455 Thanks to M. Eubanks, C. Fox, C. Linnen, J. Obrycki, for comments on an earlier
456 draft. This work was supported by the National Science Fund for Distinguished Young
457 Scholars (31025020), the 973 Program (2012CB017359), the National Natural Science
458 Foundation of China (31171857), and the Beijing Key Laboratory for Pest Control and

459 Sustainable Cultivation of Vegetables. These agencies had no role in study design, data
460 collection/analysis, manuscript preparation, or the decision to publish. The authors declare no
461 competing financial interests.

462 DATA ACCESSIBILITY SECTION

463 Should this article be provisionally accepted, we commit to publishing the underlying
464 datasets in datadryad (www.datadryad.org) prior to final acceptance and publication.

465 REFERENCES

466 Cahill, M., I. Denholm, G. Ross, K. Gorman, and D. Johnston. 1996. Relationship
467 between bioassay data and the simulated field performance of insecticides against susceptible
468 and resistant adult *Bemisia tabaci* (Homoptera: Aleyrodidae). *Bulletin of Entomological*
469 *Research* **86**:109-116.

470 CCM. 2012. Survey of Pesticide Industry in China. CCM International Limited, New
471 York.

472 Cheng, J. L., X. R. He, Z. C. Wang, J. G. Zhang, J. H. Zhao, and G. N. Zhu. 2013.
473 Metabolism-based synthesis, biological evaluation and structure–activity relationship analysis
474 of spirotetramat analogues as potential lipid biosynthesis inhibitors. *Pest Management*
475 *Science* **69**:1121-1130.

476 Chu, D., X. S. Hu, C. S. Gao, H. Zhao, R. L. Nichols, and X. C. Li. 2012a. Use of
477 mitochondrial cytochrome oxidase I polymerase chain reaction-restriction fragment length
478 polymorphism for identifying subclades of *Bemisia tabaci* Mediterranean group. *Journal of*
479 *Economic Entomology* **105**:242-251.

480 Chu, D., Y. L. Tao, Y. J. Zhang, F. H. Wan, and J. K. Brown. 2012b. Effects of host,

- 481 temperature and relative humidity on competitive displacement of two invasive *Bemisia*
482 *tabaci* biotypes [Q and B]. *Insect Science* **19**:595-603.
- 483 Chu, D., F. Wan, Y. Zhang, and J. Brown. 2010. Change in the biotype composition of
484 *Bemisia tabaci* in Shandong Province of China from 2005 to 2008. *Environmental*
485 *Entomology* **39**:1028-1036.
- 486 Crowder, D. W., A. R. Horowitz, H. Breslauer, M. Rippa, S. Kontsedalov, M. Ghanim,
487 and Y. Carrière. 2011. Niche partitioning and stochastic processes shape community structure
488 following whitefly invasions. *Basic and Applied Ecology* **12**:685-694.
- 489 Crowder, D. W., A. R. Horowitz, P. J. De Barro, S. S. Liu, A. M. Showalter, S.
490 Kontsedalov, V. Khasdan, A. Shargal, J. Liu, and Y. Carrière. 2010a. Mating behaviour, life
491 history and adaptation to insecticides determine species exclusion between whiteflies. *Journal*
492 *of Animal Ecology* **79**:563-570.
- 493 Crowder, D. W., M. I. Sitvarin, and Y. Carrière. 2010b. Plasticity in mating behaviour
494 drives asymmetric reproductive interference in whiteflies. *Animal Behaviour* **79**:579-587.
- 495 De Barro, P., S. Liu, L. Boykin, and A. Dinsdale. 2011. *Bemisia tabaci*: A statement of
496 species status. *Annual Review of Entomology* **56**:1-19.
- 497 Denno, R., M. McClure, and J. Ott. 1995. Interspecific interactions in phytophagous
498 insects: competition reexamined and resurrected. *Annual Review of Entomology* **40**:297-331.
- 499 Dorschner, K., J. Ryan, R. Johnson, and R. Eikenbary. 1987. Modification of host
500 nitrogen levels by the greenbug (Homoptera: Aphididae): its role in resistance of winter
501 wheat to aphids. *Environmental Entomology* **16**:1007-1011.
- 502 Elbaz, M., M. Weiser, and S. Morin. 2011. Asymmetry in thermal tolerance trade-offs

- 503 between the B and Q sibling species of *Bemisia tabaci* (Hemiptera: Aleyrodidae). Journal of
504 Evolutionary Biology **24**:1099-1109.
- 505 Ellsworth, P. C. and J. L. Martinez-Carrillo. 2001. IPM for *Bemisia tabaci*: a case
506 study from North America. Crop Protection **20**:853-869.
- 507 Feng, Y. T., Q. J. Wu, B. Y. Xu, S. L. Wang, X. L. Chang, W. Xie, and Y. J. Zhang.
508 2009. Fitness costs and morphological change of laboratory-selected thiamethoxam resistance
509 in the B-type *Bemisia tabaci* (Hemiptera: Aleyrodidae). Journal of Applied Entomology
510 **133**:466-472.
- 511 Ghanim, M., I. Sobol, M. Ghanim, and H. Czosnek. 2007. Horizontal transmission of
512 begomoviruses between *Bemisia tabaci* biotypes. Arthropod-Plant Interactions **1**:195-204.
- 513 Glick, E., Y. Levy, and Y. Gafni. 2009. The viral etiology of *tomato yellow leaf curl*
514 *disease* - a review. Plant Protection Science **45**:81-97.
- 515 Gottlieb, Y., E. Zchori-Fein, N. Mozes-Daube, S. Kontsedalov, M. Skaljac, M.
516 Brumin, I. Sobol, H. Czosnek, F. Vavre, F. Fleury, and M. Ghanim. 2010. The transmission
517 efficiency of *tomato yellow leaf curl virus* by the whitefly *Bemisia tabaci* is correlated with
518 the presence of a specific symbiotic bacterium species. Journal of Virology **84**:9310-9317.
- 519 Grosholz, E. 2005. Recent biological invasion may hasten invasional meltdown by
520 accelerating historical introductions. Proceedings of the National Academy of Sciences USA
521 **102**:1088-1091.
- 522 Horowitz, A. R., S. Kontsedalov, V. Khasdan, and I. Ishaaya. 2005. Biotypes B and Q
523 of *Bemisia tabaci* and their relevance to neonicotinoid and pyriproxyfen resistance. Archives
524 of Insect Biochemistry and Physiology **58**:216-225.

525 Hu, J., P. De Barro, H. Zhao, J. Wang, F. Nardi, and S. S. Liu. 2011. An extensive
526 field survey combined with a phylogenetic analysis reveals rapid and widespread invasion of
527 two alien whiteflies in China. PLoS ONE **6**:e16061.

528 Iida, H., T. Kitamura, and K. Honda. 2009. Comparison of egg-hatching rate, survival
529 rate and development time of the immature stage between B- and Q-biotypes of *Bemisia*
530 *tabaci* (Gennadius) (Homoptera: Aleyrodidae) on various agricultural crops. Applied
531 Entomology & Zoology **44**:267-273.

532 Jiang, Y., C. de Blas, L. Barrios, and A. Fereres. 2000. Correlation between whitefly
533 (Homoptera: Aleyrodidae) feeding behavior and transmission of *tomato yellow leaf curl*
534 *virus*. Annals of the Entomological Society of America **93**:573-579.

535 Jones, C. M., M. Daniels, M. Andrews, R. Slater, R. J. Lind, K. Gorman, M. S.
536 Williamson, and I. Denholm. 2011. Age-specific expression of a P450 monooxygenase
537 (CYP6CM1) correlates with neonicotinoid resistance in *Bemisia tabaci*. Pesticide
538 Biochemistry and Physiology **101**:53-58.

539 Jones, D. 2003. Plant viruses transmitted by whiteflies. European Journal of Plant
540 Pathology **109**:195-219.

541 Kontsedalov, S., F. Abu-Moch, G. Lebedev, H. Czosnek, A. R. Horowitz, and M.
542 Ghanim. 2012. *Bemisia tabaci* biotype dynamics and resistance to insecticides in Israel
543 during the years 2008-2010. Journal of Integrative Agriculture **11**:312-320.

544 Lee, M. L. and P. J. De Barro. 2000. Characterization of different biotypes of *Bemisia*
545 *tabaci* (Gennadius) (Homoptera; Aleyrodidae) in South Korea based on 16S ribosomal RNA
546 sequences. Korean Journal of Entomology **30**:125-130.

- 547 Li, X. C., B. A. Degain, V. S. Harpold, P. G. Marcon, R. L. Nichols, A. J. Fournier, S.
548 E. Naranjo, J. C. Palumbo, and P. C. Ellsworth. 2012. Baseline susceptibilities of B- and Q-
549 biotype *Bemisia tabaci* to anthranilic diamides in Arizona. *Pest Management Science* **68**:83-
550 91.
- 551 Liu, B. M., E. L. Preisser, D. Chu, H. P. Pan, W. Xie, S. L. Wang, Q. J. Wu, X. G.
552 Zhou, and Y. J. Zhang. 2013a. Multiple forms of vector manipulation by a plant-infecting
553 virus: *Bemisia tabaci* and *tomato yellow curl leaf virus*. *Journal of Virology* **87**:4929-4937.
- 554 Liu, B. M., E. L. Preisser, X. Jiao, H. Pan, W. Xie, S. L. Wang, Q. Wu, and Y. J.
555 Zhang. 2013b. Plant-mediated changes in the feeding behavior of an invasive whitefly.
556 *Environmental Entomology* **42**:980-986.
- 557 Liu, S. S., P. J. De Barro, J. Xu, J. B. Luan, L. S. Zang, Y. M. Ruan, and F. H. Wan.
558 2007. Asymmetric mating interactions drive widespread invasion and displacement in a
559 whitefly. *Science* **318**:1769-1772.
- 560 Ma, D., K. Gorman, G. Devine, W. Luo, and I. Denholm. 2007. The biotype and
561 insecticide-resistance status of whiteflies, *Bemisia tabaci* (Hemiptera: Aleyrodidae), invading
562 cropping systems in Xinjiang Uygur Autonomous Region, northwestern China. *Crop*
563 *Protection* **26**:612-617.
- 564 McKenzie, C., J. Bethke, F. Byrne, J. Chamberlin, T. Dennehy, A. Dickey, D. Gilrein,
565 P. Hall, S. Ludwig, R. Oetting, L. Osborne, L. Schmale, and R. Shatters, Jr. 2012.
566 Distribution of *Bemisia tabaci* (Hemiptera: Aleyrodidae) biotypes in North America after the
567 Q invasion. *Journal of Economic Entomology* **105**:753-766.
- 568 Miles, P. 1999. Aphid saliva. *Biological Reviews* **74**:41-85.

- 569 Ohto, K. 1990. Occurrence of the sweetpotato whitefly, *Bemisia tabaci* (Gennadius),
570 on the poinsettia. *Plant Protection* **44**:264-266.
- 571 Osborne, L. 2013. *Bemisia tabaci* management - information on both the B and Q
572 biotype (<http://mrec.ifas.ufl.edu/lso/bemisia/bemisia.htm>). Mid-Florida Research and
573 Education Center, Apopka FL.
- 574 Pan, H. P., D. Chu, D. Q. Ge, S. L. Wang, Q. J. Wu, W. Xie, X. G. Jiao, B. M. Liu, X.
575 Yang, N. N. Yang, Q. Su, B. Y. Xu, and Y. J. Zhang. 2011. Further spread of and domination
576 by *Bemisia tabaci* (Hemiptera: Aleyrodidae) biotype Q on field crops in China. *Journal of*
577 *Economic Entomology* **104**:978-985.
- 578 Pan, H. P., D. Chu, W. Q. Yan, Q. Su, B. M. Liu, S. L. Wang, Q. J. Wu, W. Xie, X. G.
579 Jiao, R. Li, N. Yang, X. Yang, B. Y. Xu, J. K. Brown, X. G. Zhou, and Y. J. Zhang. 2012.
580 Rapid spread of *tomato yellow leaf curl virus* in China is aided differentially by two invasive
581 whiteflies. *PLoS ONE* **7**:e34817.
- 582 Park, J., S. M. H. Jahan, W. G. Song, H. Lee, Y. S. Lee, H. S. Choi, K. S. Lee, C. S.
583 Kim, S. Lee, and K. Y. Lee. 2012. Identification of biotypes and secondary endosymbionts of
584 *Bemisia tabaci* in Korea and relationships with the occurrence of TYLCV disease. *Journal of*
585 *Asia-Pacific Entomology* **15**:186-191.
- 586 Pascual, S. 2006. Mechanisms in competition, under laboratory conditions, between
587 Spanish biotypes B and Q of *Bemisia tabaci* (Gennadius). *Spanish Journal of Agricultural*
588 *Research* **4**:351-354.
- 589 Pascual, S. and C. Callejas. 2004. Intra- and interspecific competition between
590 biotypes B and Q of *Bemisia tabaci* (Hemiptera: Aleyrodidae) from Spain. *Bulletin of*

591 Entomological Research **94**:369-375.

592 Reddy, B. A., M. S. Patil, and T. Rajasekaram. 2010. Effect of *tomato leaf curl virus*
593 infection on plant growth and yield in tomato. Karnataka Journal of Agricultural Science
594 **23**:806.

595 Reitz, S. R. and J. T. Trumble. 2002. Competitive displacement among insects and
596 arachnids. Annual Review of Entomology **47**:435-465.

597 Sakai, A., F. Allendorf, J. Holt, D. Lodge, J. Molofsky, K. With, S. Baughman, R.
598 Cabin, J. Cohen, N. Ellstrand, D. McCauley, P. O' Neil, I. Parker, J. Thompson, and S. Weller.
599 2001. The population biology of invasive species. Annual Review of Ecology and
600 Systematics **32**:305-332.

601 Shatters, R., Jr., C. A. Powell, L. M. Boykin, H. Liansheng, and C. L. McKenzie.
602 2009. Improved DNA barcoding method for *Bemisia tabaci* and related Aleyrodidae:
603 development of universal and *Bemisia tabaci* biotype-specific mitochondrial cytochrome c
604 oxidase I polymerase chain reaction primers. Journal of Economic Entomology **102**:750-758.

605 Simberloff, D. 2006. Invasional meltdown 6 years later: important phenomenon,
606 unfortunate metaphor, or both? Ecology Letters **9**:912-919.

607 Simberloff, D. and B. Von Holle. 1999. Positive interactions of nonindigenous
608 species: Invasional meltdown? Biological Invasions **1**:21-32.

609 Tsueda, H. and K. Tsuchida. 2011. Reproductive differences between Q and B
610 whiteflies, *Bemisia tabaci*, on three host plants and negative interactions in mixed cohorts.
611 Entomologia Experimentalis et Applicata **141**:197-207.

612 Ueda, S. and J. K. Brown. 2006. First report of the Q biotype of *Bemisia tabaci* in

- 613 Japan by mitochondrial cytochrome oxidase I sequence analysis. *Phytoparasitica* **34**:405-411.
- 614 Wallin, K. and K. Raffa. 2001. Effects of folivory on subcortical plant defenses: can
615 defense theories predict interguild processes? *Ecology* **82**:1387-1400.
- 616 Wang, Z., H. Yan, Y. Yang, and Y. Wu. 2010. Biotype and insecticide resistance status
617 of the whitefly *Bemisia tabaci* from China. *Pest Management Science* **66**:1360-1366.
- 618 Wu, Q. J., B. Y. Xu, Y. J. Zhang, and G. R. Zhu. 2003. Toxicity and field efficacy of
619 thiamethoxam to *Bemisia tabaci* with different methods of treatment. *Chinese Journal of*
620 *Pesticide Sciences* **5**:70-74.
- 621 Xu, R., R. P. Kuang, E. Pay, H. Dou, and G. de Snoo. 2008. Factors contributing to
622 overuse of pesticides in western China. *Environmental Sciences* **5**:235-249.
- 623
- 624

625 **Table 1: Bioassay results of MEAM1 and MED to insecticides commonly used for**
 626 ***Bemisia* control in China.** Slope = increase in mortality as chemical concentration increases;
 627 LC₅₀ = concentration of chemical sufficient to cause 50% mortality in the target population;
 628 95% FL: 95% fiducial limit around the LC₅₀; RR: Resistance Ratio (the ratio of the LC₅₀ of
 629 MED and MEAM1). Lower-case letters following LC₅₀ values indicate differences
 630 significant at $p < 0.05$.

631

Species	Development Stage	Slope (SE)	LC ₅₀ (mg/L)	95% F.L.	RR
Thiomethoxam					
MEAM1	Egg	0.54 (0.073)	130a	73.2 - 230	6.6
MED		1.58 (0.125)	860b	670 - 1105	
MEAM1	Nymph	1.19 (0.105)	1.0a	0.8 - 1.4	4.9
MED		1.89 (0.201)	5.2b	3.1 - 8.3	
MEAM1	Adult	1.06 (0.112)	17.8	9.8 - 32.5	3.1
MED		0.60 (0.084)	54.9	19.2 - 157	
Spirotetramat					
MEAM1	Egg	1.63 (0.101)	0.18a	0.15 - 0.22	24.2
MED		0.47 (0.038)	4.34b	2.26 - 8.33	
MEAM1	Nymph	0.73 (0.048)	1.07E ⁻³ a	6.10E ⁻⁴ - 1.86E ⁻³	7.0
MED		1.88 (0.101)	7.51E ⁻³ b	6.20E ⁻³ - 9.11E ⁻³	
MEAM1	Adult	1.51 (0.209)	2450a	1628 - 3687	>6.5
MED		-	>16000b	-	
Bifenthrin					
MEAM1	Egg	-	no effect	-	n/a
MED		-	no effect	-	
MEAM1	Nymph	1.37 (0.080)	39.5	29.0 - 53.7	1.5
MED		1.59 (0.129)	58.2	46.7 - 72.6	
MEAM1	Adult	1.89 (0.149)	63.2a	50.9 - 78.4	3.7
MED		1.88 (0.144)	233b	187 - 293	

632

633 **FIGURE LEGENDS**

634 **Figure 1: Domestic use of neonicotinoid (2000-2009) and pyrethroid (2000-2011)**
635 **insecticides in China (metric tons).** Data reflects total pesticide use summed across all crop
636 types. 2006 data for both insecticide classes, and 2010 and 2012 data for neonicotinoids were
637 unavailable at time of request; all other data provided courtesy of China Pesticide
638 Information Network (<http://www.chinapesticide.gov.cn>).

639 **Figure 2: Replacement of MEAM1 by MED in China.** Field surveys were carried
640 out in 2003 (2A), 2007 (2B), 2009 (2C), 2011 (not shown), and 2012 (2D). See text for
641 survey details. In figures 2A-D, green denotes unsurveyed regions; colors of sampled
642 provinces denote differing percentages of MED (colors as in 2E). Figure 2E: summary data
643 indicating the mean province-level proportion of MED in sampled populations over time. The
644 number of *Bemisia* adults, sites, and provinces sampled per survey is listed above each point.

645 **Figure 3: Observed frequencies of TYLCV infection in MEAM1 and MED in**
646 **China in 2009 and 2011.** See text for survey details; the 2009 data was previously published
647 in Pan et al (2012) and is provided solely for the purpose of comparison. Left axis (circles):
648 Mean \pm SE percentage of sampled MEAM1 (green) and MED (blue) per population infected
649 with TYLCV. Right axis (bars): Percentage of MEAM1 and MED populations infected with
650 TYLCV.

651 **Figure 4: Changes in the proportion of MEAM1 and MED on five different host**
652 **plants over time.** Mixed cultures were reared on cotton (*Gossypium hirsutum*), cucumber
653 (*Cucumis sativus*), cabbage (*Brassica oleracea*), tomato (*Lycopersicon esculentum*), and
654 pepper (*Capsicum annuum*). See text for treatment details. Values are mean \pm SE from three

655 replicates (five replicates for *C. sativus*).

656 **Figure 5: Effect of insecticides on the proportion of MEAM1 and MED on**

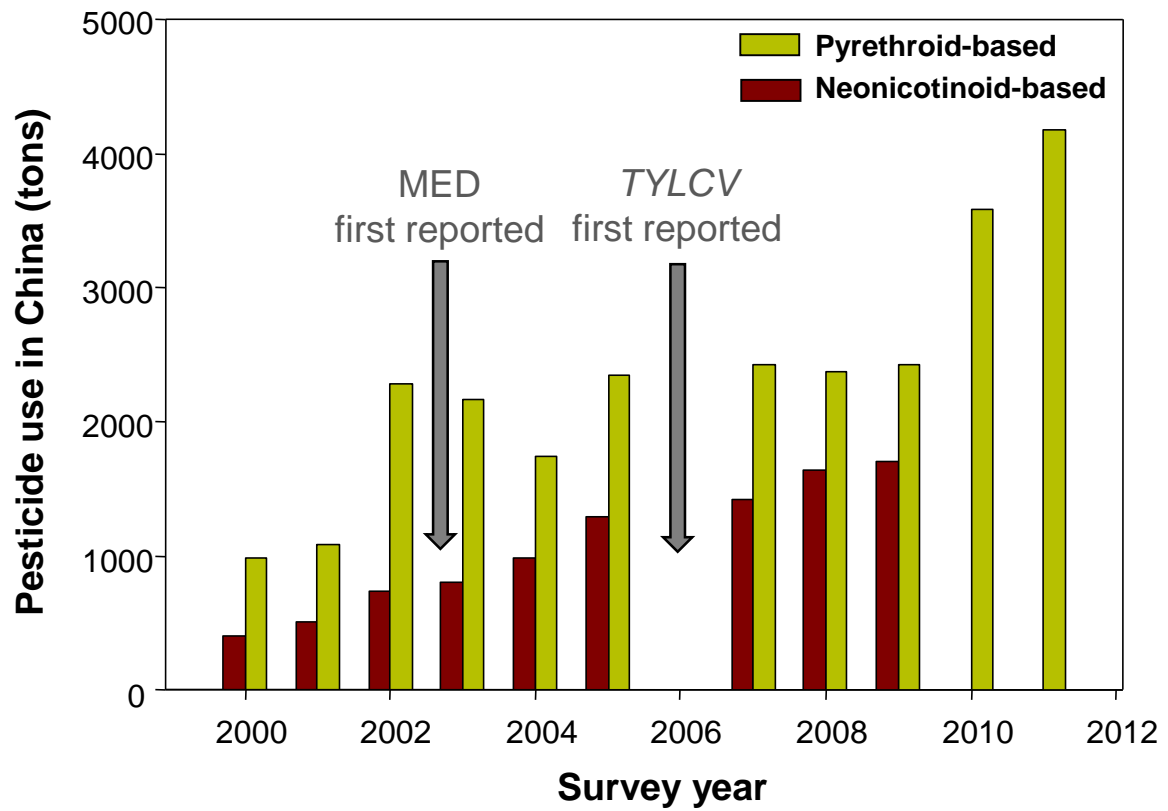
657 **cucumber (5A) and tomato (5B).** See text for treatment details. Values are mean \pm SE from

658 four replicates (5A) and five replicates (5B).

659

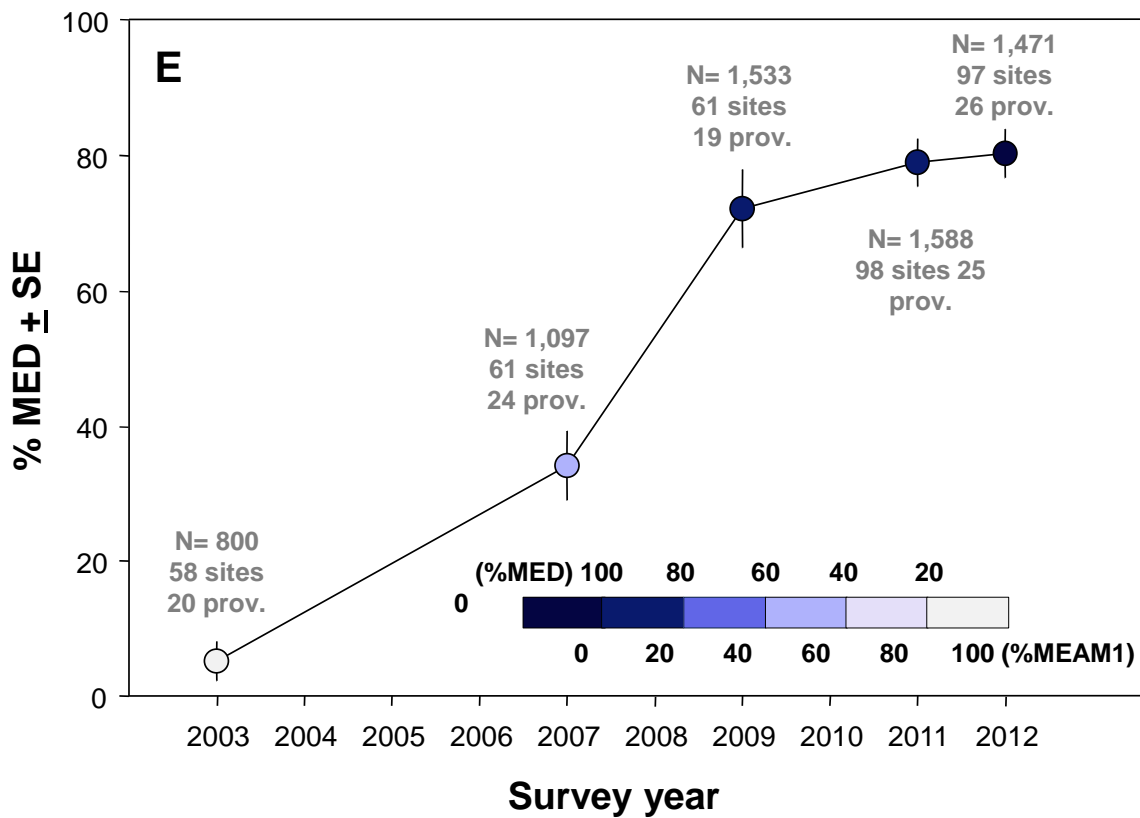
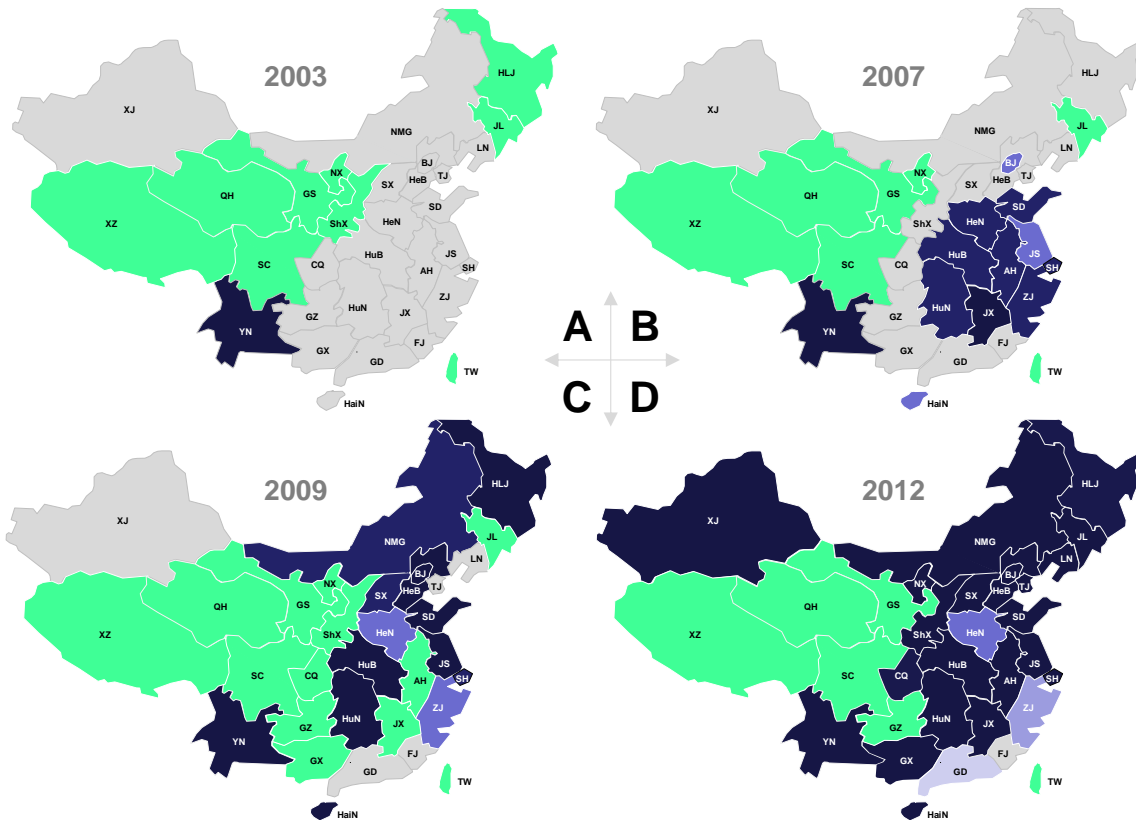
660 **Figure 1.**

661

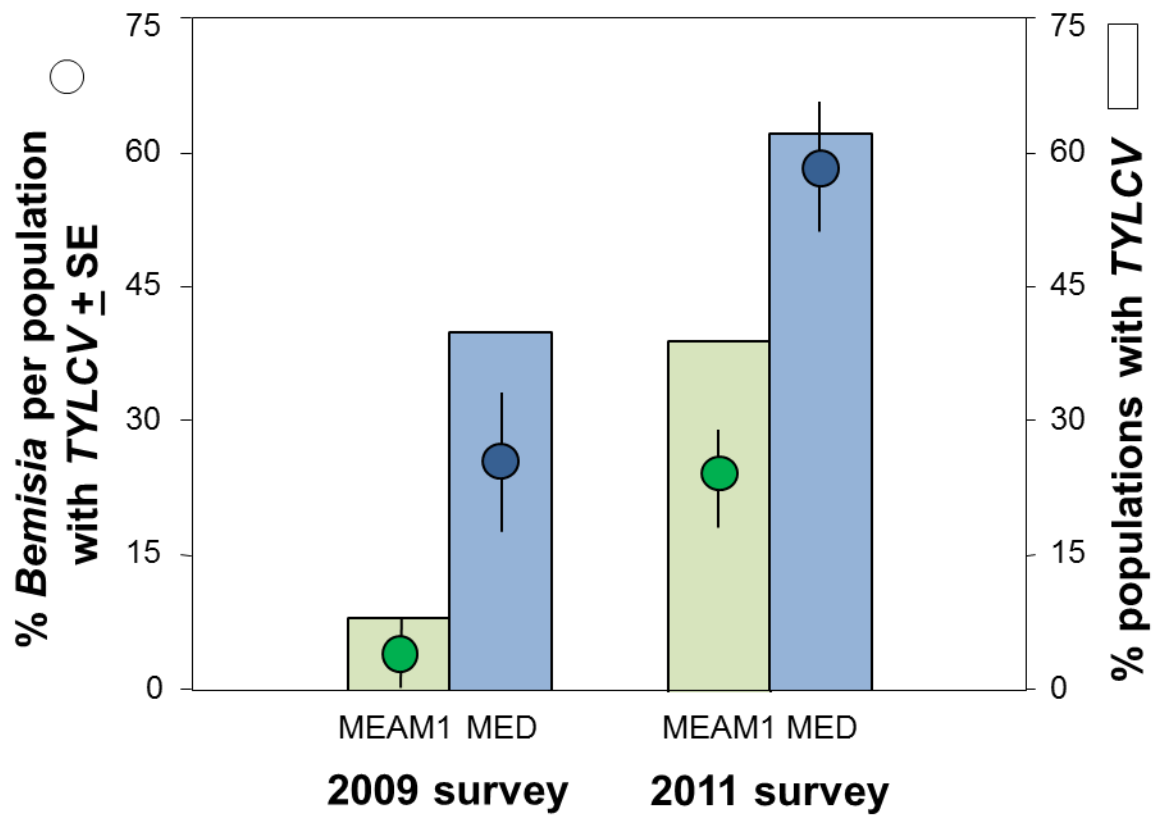


662

663 **Figure 2.**



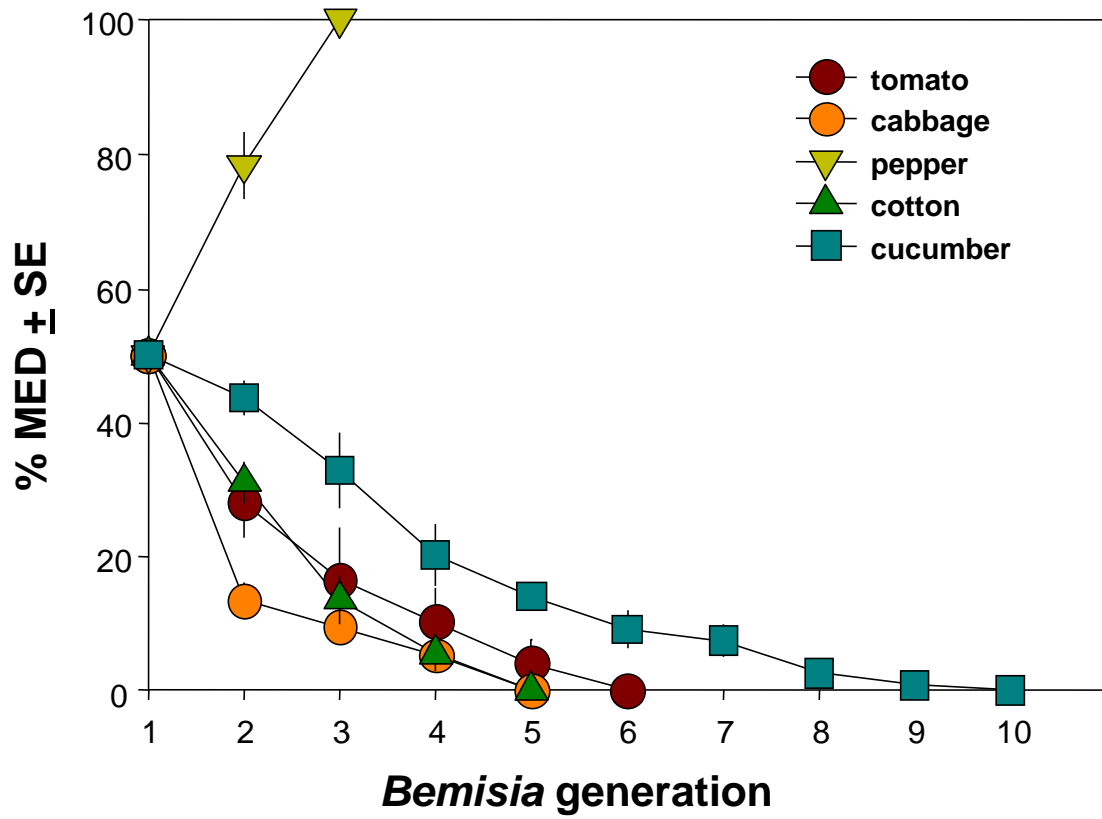
665 **Figure 3.**



666

667

668

669 **Figure 4.**

670

671 **Figure 5.**

