

Phenology of Asian Citrus Psyllid (Hemiptera: Liviidae) and Associated Parasitoids on Two Species of *Citrus*, Kinnow Mandarin and Sweet Orange, in Punjab Pakistan

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ABSTRACT The population phenology of Asian citrus psyllid, *Diaphorina citri* Kuwayama, was monitored weekly for 110 wk on two species of *Citrus*, kinnow mandarin and sweet orange, at two different research sites in Faisalabad, Punjab Pakistan. Citrus flush growth patterns were monitored and natural enemy surveys were conducted weekly. Flush patterns were similar for kinnow and sweet orange. However, flush on sweet orange was consistently more heavily infested with Asian citrus psyllid than kinnow flush; densities of Asian citrus psyllid eggs, nymphs, and adults were higher on sweet orange when compared with kinnow. When measured in terms of mean cumulative insect or Asian citrus psyllid days, eggs, nymphs, and adults were significantly higher on sweet orange than kinnow. Two parasitoids were recorded attacking Asian citrus psyllid nymphs, *Tamarixia radiata* (Waterston) and *Diaphorencyrtus aligarhensis* (Shafee, Alam and Agarwal). The dominant parasitoid species attacking Asian citrus psyllid nymphs on kinnow and sweet orange was *T. radiata*, with parasitism averaging 26%. *D. aligarhensis* parasitism averaged 17%. Generalist predators such as coccinellids and chrysopids were collected infrequently and were likely not important natural enemies at these study sites. Immature spiders, in particular, salticids and yellow sac spiders, were common and may be important predators of all Asian citrus psyllid life stages. Low year round Asian citrus psyllid densities on kinnow and possibly high summer temperatures, may, in part, contribute to the success of this cultivar in Punjab where *Candidatus Liberibacter asiaticus*, the putative causative agent of Huanglongbing, a debilitating citrus disease, is widespread and vectored by Asian citrus psyllid.

KEY WORDS *Citrus reticulata*, *Citrus sinensis*, *Diaphorencyrtus aligarhensis*, flush growth, *Tamarixia radiata*

Asian citrus psyllid, *Diaphorina citri* Kuwayama (Hemiptera: Liviidae), initiates economic losses in citrus due to its feeding activities, which cause either direct (i.e., feeding injuries to immature foliage) or indirect (i.e., transmission of a plant pathogen) crop damage. Asian citrus psyllid is notorious for indirect damage to citrus, notably the transmission of a phloem-dwelling bacterium, *Candidatus Liberibacter asiaticus* (CLAs). This bacterium causes a debilitating and incurable disease in citrus, Huanglongbing (HLB), which is also known as citrus greening (Halbert and Manjunath 2004, Bové 2006, Grafton-Cardwell et al. 2013). Asian citrus psyllid and HLB are likely native to the Indian subcontinent (Husain and Nath 1927, Halbert and Manjunath 2004) and collectively have been a serious perennial problem for citrus production

where they co-occur (Grafton-Cardwell et al. 2013, Halbert and Manjunath 2004).

Within this putative native range, it has been estimated that Asian citrus psyllid and, by association, HLB have caused ≈90% losses in citrus production in parts of Pakistan (Ahmad 1961), and similar high losses have been reported in India (Shivankar et al. 2000). However, Chohan et al. (2007) caution that estimates of citrus losses to HLB in Pakistan are difficult to quantify in the absence of molecular confirmation of CLAs because of other competing maladies, such as citrus tristeza, canker, and phytophthora. The combined impact of these diseases, including HLB, is generally referred to as citrus decline. This argument can likely be applied to estimates of HLB losses in India.

Despite these impediments to production, citrus is an important crop in Pakistan. Over 2010–2011, citrus production in Pakistan covered 194,500 ha on which 1,982,200 tons of fruit was produced of which 493,000 tons was exported with a net worth of ≈US\$232 million (Anon. 2011). Citrus production is concentrated in Punjab and accounts for 184,200 ha (≈95% of production area) from which 1,912,000 tons (≈96%) of

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total citrus fruit is produced (Anon. 2012). More than 75% of this production is from kinnow (*Citrus reticulata* L.), a variety of mandarin (Hoddle 2010, Razi et al. 2014). In Florida, the citrus industry is worth ≈US\$9 billion annually (Hodges and Spreen 2012), and Asian citrus psyllid (first detected in 1998 [Halbert and Manjunath 2004]) and HLB (first discovered in 2005 [Bové 2006]) are invasive in Florida. It has been estimated that since 2005, ≈60,000 acres of citrus (≈10% of total production acreage) have been lost to HLB (Salifu et al. 2012). The subsequent economic impact of CLAs-induced mortality of citrus in Florida has been significant, with an estimated 6,600 jobs lost, US\$1.3 billion in direct losses, and an increase in production costs of ≈40% (Salifu et al. 2012).

Significant research effort in Florida citrus orchards has been directed toward enhancing our understanding of Asian citrus psyllid biology and ecology (Tsai et al. 2002, Hall et al. 2008), natural enemies associated with this pest, in particular, predators (Michaud 2001, 2004) and introduced parasitoids (Qureshi and Stansly 2009, Qureshi et al. 2009), and the development of chemical control programs (Qureshi and Stansly 2007, 2010). Asian citrus psyllid was first discovered in urban areas of southern California in 2008, and the presence of CLAs in this region was confirmed molecularly in 2012 (Kumagai et al. 2013). Consequently, research efforts similar to ongoing programs in Florida have commenced in California in an attempt to lessen the threat Asian citrus psyllid–HLB poses to the state's ≈US\$1.5 billion citrus industry. One area of focus in California's emerging Asian citrus psyllid management program is classical biological control with host-specific parasitoids (Hoddle 2010, Hoddle and Pandey 2014). Foreign exploration for natural enemies has focused on Punjab Pakistan because this is likely part of the native range of Asian citrus psyllid (Husain and Nath 1927) and natural enemy diversity is consequently expected to be highest here. Further, Punjab has ≈70% climate match with important citrus-producing areas in California, which should benefit biological control efforts because introduced natural enemies should be preadapted to prevailing climates in the new range (Hoddle 2012, Hoddle and Hoddle 2013).

Husain and Nath (1927) published the first treatise on Asian citrus psyllid and the association of this insect with citrus trees in Punjab Pakistan. As part of the results of their studies, Husain and Nath (1927) hinted at a rich fauna of parasitoids attacking Asian citrus psyllid, with up to nine possible species having been reared from Asian citrus psyllid nymphs, which collectively accounted for parasitism rates of up to 95%. Just one of these parasitoids is mentioned by name, *Tamarixia radiata* (Waterston) (Hymenoptera: Eulophidae) (referred to as *Tetrastichus radiatus* Waterston by Husain and Nath [1927]). *T. radiata* is an ectoparasitoid that preferentially parasitizes fourth and fifth instar nymphs and females will host feed on younger nymphal life stages (Chien et al. 1995, Gómez-Torres et al. 2012). This parasitoid has been used for classical biological control of Asian citrus psyllid in

different countries and regions within the United States including Florida (Qureshi and Stansly 2009) and California (Hoddle and Hoddle 2013, Hoddle and Pandey 2014). The identities of the eight other Asian citrus psyllid parasitoids mentioned by Husain and Nath (1927) were not given. However, the identity of one additional species attacking Asian citrus psyllid nymphs has been confirmed, the endoparasitoid *Diaphorencyrtus aligarhensis* (Shafee, Alam and Agarwal) (Hymenoptera: Encyrtidae) (Hoddle et al. 2013). Husain and Nath (1927) noted that unnamed species of hyperparasitoids were associated with primary parasitoids of Asian citrus psyllid in Pakistan, four species of which have been identified (Hoddle et al. 2013, Bistline-East and Hoddle, 2014).

Despite Husain and Nath's (1927) work, the phenology of Asian citrus psyllid and its nymphal parasitoids on citrus in Punjab is poorly documented, as there appear to be no published studies monitoring pest and natural enemy population fluctuations through time in this part of the native range. In support of the classical biological control program in California, a project to better understand Asian citrus psyllid phenology and the parasitoid complex associated with Asian citrus psyllid in Punjab Pakistan was undertaken. Weekly surveys were conducted to monitor Asian citrus psyllid eggs, nymphs, and adults, parasitoid populations attacking Asian citrus psyllid nymphs, and citrus growth phenology in Faisalabad, Punjab Pakistan. This city, formerly known as Lyallpur, was one of the original areas Husain and Nath (1927) conducted their Asian citrus psyllid surveys. Here we report on the phenology of Asian citrus psyllid and associated parasitoid activity on two different species of *Citrus*, kinnow mandarin (*C. reticulata*) and sweet orange (*Citrus sinensis* Osbeck), across two different study sites in Faisalabad over March 2011 to April 2013.

Materials and Methods

Study Sites and Study Duration. Two study sites at the University of Agriculture Faisalabad (UAF) were selected for monitoring. Site one, Square 9 (31° 25' 50.4" N; 73° 03' 40.2" E; elevation 190 m), is 10 ha of which 6 ha is citrus composed of four blocks of kinnow, two blocks of sweet oranges, one block of grapefruit (*Citrus × paradisi*), two blocks of sweet limes (*Citrus limetta* Risso), and one block of *C. reticulata* 'Feutral's Early,' and one block of germplasm stock composed of various Rutaceae. Two blocks, one sweet orange and one kinnow block, composed of ≈30–40 trees each were selected for phenology studies, and within each block, 15 trees were randomly selected and flagged for weekly monitoring. Site 2, PARS (Postgraduate Agricultural Research Station; 31° 23' 35.20" N; 73° 01' 27.0" E; elevation 210 m), also part of the UAF campus, is located 12 km from Square 9. This site is 182 ha of which 90 ha is divided into two widely separated sections of citrus. This study was conducted in the 30-ha citrus section that is composed of eight blocks of citrus; two kinnow, two sweet orange, two grapefruit, one Feutral's Early, and one block of sweet lime. For

Asian citrus psyllid phenology studies at PARS, 15 kinnow (all in one block) and 15 sweet orange trees (in one block) were randomly selected and flagged in appropriate blocks for monitoring. The studies conducted at these research sites spanned 26 March 2011–20 April 2013. All trees at both study sites were 8–10 yr of age, 4.5–5.0 m in height, and scions were grafted to sour orange (*Citrus aurantium* L.) rootstocks. Trees were flood irrigated on an as needed basis, no fertilizers or pesticides were applied to experimental plots, weeds were hand cut with scythes, and no trees were pruned during this time period.

Tap-Sample Monitoring of Adult *D. citri* and Generalist Predators. The tap method (Qureshi and Stansly 2007, Hall and Hentz 2010) was used to sample adult Asian citrus psyllid and generalist predators inhabiting citrus foliage. Each experimental tree was divided into four quadrants and branches within a quadrant were struck five times to dislodge adult Asian citrus psyllid and predators. A white plastic tray (38 cm in length by 27 cm in width) was placed under the foliage being tap-sampled, and adult psyllids and predators landing on the tray were counted and recorded per quadrant for each tree. Potential Asian citrus psyllid predators were grouped as spiders, coccinellids [larval and adult *Coccinella septempunctata* (L.) and *Menochilus sexmaculatus* (F.)], and lacewing larvae (Chrysopidae). The mean weekly number of adult psyllids and total number of predators counted were calculated across each citrus variety for each study site. Mean weekly adult Asian citrus psyllid counts were converted to cumulative insect days (Ruppel 1983) per tree. This approach combines time and the number of insects observed as a way of estimating the area under the population curve for each tree (Ruppel 1983). Cumulative totals per tree were log transformed and compared statistically with 2-way ANOVA (SAS Institute 2008) to determine if differences in mean cumulative adult Asian citrus psyllid days differed between kinnow and sweet oranges within and across sites.

Monitoring Citrus Flush Phenology, Asian Citrus Psyllid Eggs, Nymphs, and Parasitoids. Asian citrus psyllid females only oviposit on flush growth, and colonies of nymphs develop exclusively on this young plant material (Hall et al. 2008). Because Asian citrus psyllid nymphs only develop on flush growth, the presence of flush was estimated each week by randomly selecting 10 branches around each experimental tree at shoulder height and assessing them for the presence or absence of flush growth which was then recorded by tree, site, and date. These data were used to determine percentage flush growth (i.e., number of branches with flush growth/[number of flush growth branches + nonflush growth branches] \times 100) per tree at each sampling site and date. Flush growth was composed of three age-sequential stages and comprised: 1) “feather” growth where immature leaves were very small, and tightly folded lengthwise; 2) leaves were partially expanded, soft, and light green, and 3) leaves were almost fully or fully expanded, soft, and light green. In comparison, nonflush consisted of mature leaves that were stiff and had mature dark

green coloration. If sampled branches had flush growth with Asian citrus psyllid eggs or nymphs, it was excised and returned to the laboratory. In the laboratory, the length of flush was measured and the mean number of Asian citrus psyllid eggs and nymphs per cm of flush was calculated for each tree by sampling date and location. Mean weekly egg and nymph counts per centimeter of flush were converted to cumulative Asian citrus psyllid days per tree (Ruppel 1983) for each life stage, which provided an estimate of the total egg or nymph exposure on flush that individual citrus trees experienced. Cumulative totals for eggs or nymphs per tree were log transformed and compared statistically with two-way ANOVA in SAS (2008) to determine if differences in average cumulative egg and nymph Asian citrus psyllid days differed between kinnow and sweet oranges within and across sites. Flush infested with Asian citrus psyllid nymphs was held in the laboratory for parasitoid emergence and percentage nymphs parasitized was calculated (i.e., number of emerged parasitoids/[number of emerged parasitoids + number of emerged adult Asian citrus psyllid] \times 100). The sex ratio of emerged parasitoids was calculated (i.e., number of females/[number of females + number of males]).

Meteorological Data. Maximum, minimum, and average temperatures, relative humidity, and rainfall were recorded at the meteorological observatory maintained by the Crop Physiology Department on the main campus at UAF. These data were used to prepare average weekly climatic summaries for temperature, humidity, and rainfall for the duration of this study.

Results

Climatic Data and Citrus Flush Patterns. Average weekly climatic trends are presented in Fig. 1A. Maximum weekly temperatures averaged close to 45°C over May–July in 2011 and 2012, and in December–January, minimum weekly temperatures were lowest, around 5°C. Measurable rainfall was detected from May–September in 2011, and was substantially lower in 2012 over the same time period (Fig. 1A). Percentage flush data for kinnow and sweet orange were combined across sites, and both cultivars exhibited major flush events starting in February. This major flush period tended to end in early April for kinnow but continued at substantially lower levels for sweet orange until a second less pronounced flush period occurred in June–October in 2011 and 2012 for kinnow and sweet orange (Fig. 1B). Overall, flush patterns and intensity were similar between kinnow and sweet orange. However, sweet orange flush growth tended to start a little sooner, lasted longer, and produced more flush, especially during periods when kinnow flush activity was very low or nonexistent (e.g., November 2011 to February 2012; Fig. 1B).

Asian Citrus Psyllid Phenology on Kinnow and Sweet Orange, Comparison of Mean Cumulative Asian Citrus Psyllid Days, and Percentage of Flush Growth Infested With Asian Citrus Psyllid. Asian citrus psyllid life stage count data for kinnow and

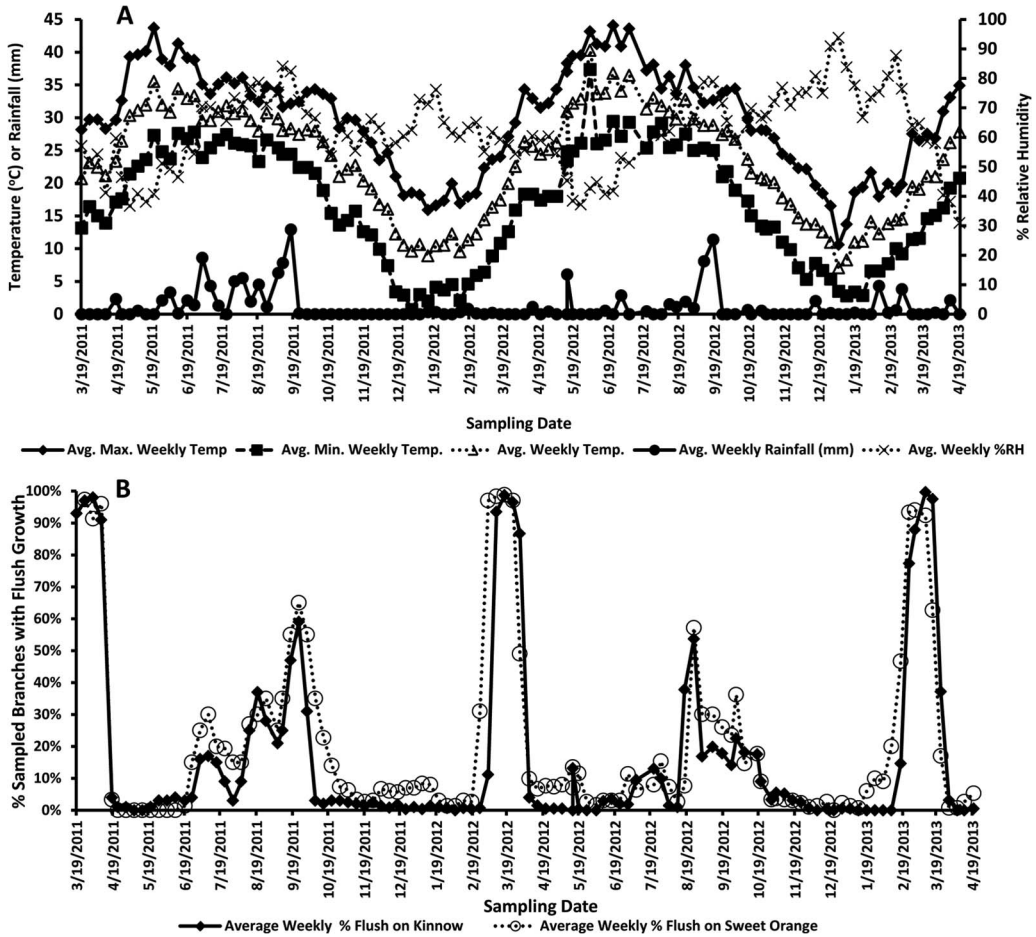


Fig. 1. (A) Mean maximum, minimum, and average weekly temperatures, mean weekly % relative humidity, and rainfall at the University of Agriculture Faisalabad, Punjab Pakistan. (B) Weekly flushing patterns for kinnow and sweet orange combined across both study sites, Square 9 and PARS, University of Agriculture Faisalabad, Punjab Pakistan.

sweet orange were combined across Square 9 and PARS. Asian citrus psyllid egg production was most pronounced on sweet orange (Fig. 2A) and coincided with periods of flush growth (Fig. 1B) as expected (Hall et al. 2008). Similarly, Asian citrus psyllid nymph densities per centimeter of sampled citrus twig were substantially greater on sweet orange and coincided with flush activity over June–October 2011 before decreasing and persisting on sporadic flush into January 2012. This pattern repeated itself in 2012, and sweet orange flush was again consistently infested with more Asian citrus psyllid nymphs (Fig. 2B). This trend for sweet orange continued with adult Asian citrus psyllid, and the mean numbers of tap sampled adults were consistently higher on sweet orange over March–August in 2011 and 2012 when compared with kinnow (Fig. 2C). Adult Asian citrus psyllid were conspicuously absent from kinnow and sweet orange from approximately early September 2011–early March 2012 and late September 2012–early March 2013.

Mean cumulative Asian citrus psyllid days for eggs was significantly different between kinnow and sweet orange ($F = 45.82$; $df = 1, 56$; $P < 0.001$; Fig. 3); the effect of site was not significant ($F = 0.32$; $df = 1, 56$; $P = 0.32$). However, the interaction between citrus cultivar and site was significant ($F = 6.63$; $df = 1, 56$; $P = 0.13$), indicating that the difference in mean cumulative Asian citrus psyllid egg days between kinnow and sweet orange was influenced by site. However, Asian citrus psyllid egg days were significantly higher on sweet orange when compared with kinnow regardless of site (PARS [$F = 43.65$; $df = 1, 56$; $P < 0.01$] vs. Square 9 [$F = 8.80$; $df = 1, 56$; $P = 0.04$]). Because Asian citrus psyllid days were consistently higher on sweet orange, cumulative Asian citrus psyllid days for eggs on kinnow or sweet orange were averaged across sites (Fig. 3).

Mean cumulative Asian citrus psyllid days for nymphs was significantly different between kinnow and sweet orange ($F = 465$; $df = 1, 56$; $P < 0.0001$; Fig. 3); the effect of site was not significant ($F = 0.18$;

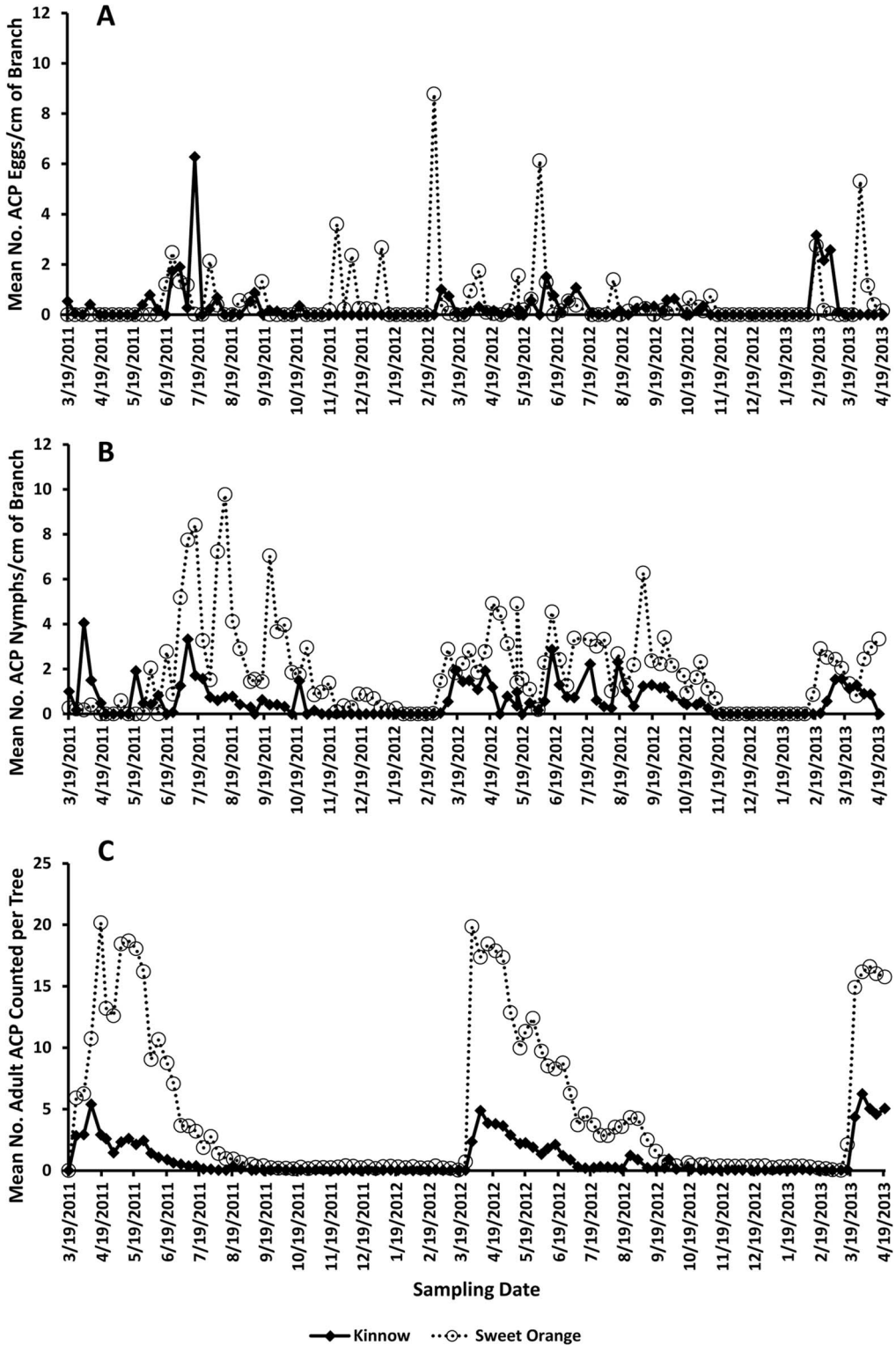


Fig. 2. (A) Mean number of Asian citrus psyllid eggs per centimeter of citrus twig exhibiting flush growth, (B) mean number of Asian citrus psyllid nymphs per centimeter of twig exhibiting flush growth, and (C) mean number of adult Asian citrus psyllid per tree from tap sampling foliage. Data to prepare these graphs were combined across PARS and Square 9, University of Agriculture Faisalabad, Punjab Pakistan.

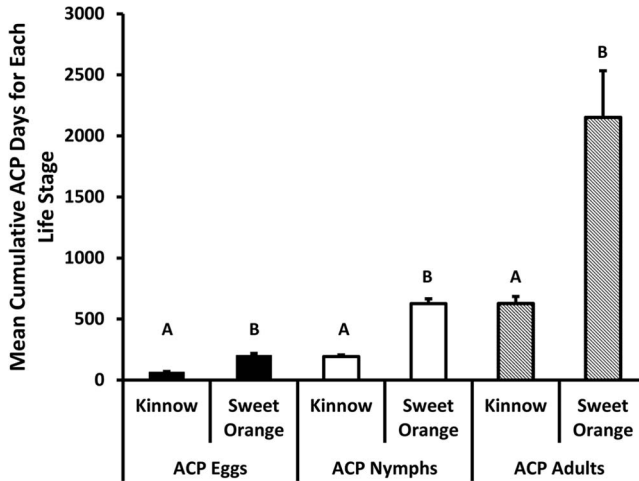


Fig. 3. Mean (\pm SE; untransformed) cumulative Asian citrus psyllid egg days (A), nymph days (B), and (C) adult days for kinnow and sweet orange. Different letters within a life stage indicate significant differences between citrus cultivars at the 0.05 level. Asian citrus psyllid days for each citrus cultivar were averaged across PARS and Square 9, University of Agriculture Faisalabad, Punjab Pakistan.

$df = 1, 56; P = 0.67$). A significant interaction between citrus cultivar and site existed ($F = 126; df = 1, 56; P < 0.001$); however, regardless of site, mean cumulative Asian citrus psyllid days for nymphs was significantly higher on sweet orange at PARS ($F = 537; df = 1, 56; P < 0.0001$) and Square 9 ($F = 53.47; df = 1, 56; P < 0.0001$) when compared with kinnow. Cumulative Asian citrus psyllid days for nymphs on kinnow or sweet orange were therefore averaged across sites (Fig. 3). Mean cumulative Asian citrus psyllid days for adult psyllids were significantly different between kinnow and sweet orange ($F = 51.51; df = 1, 56; P < 0.001$; Fig. 3); the effect of site was not significant ($F = 0.52; df = 1, 56; P = 0.10$). A significant interaction between site and citrus variety was detected ($F = 9.04; df = 1, 56; P = 0.004$), but mean Asian citrus psyllid days for adult psyllids was significantly greater on sweet orange at PARS ($F = 51.86; df = 1, 56; P < 0.0001$) and Square 9 ($F = 8.70; df = 1, 56; P = 0.005$) when compared with kinnow. Cumulative Asian citrus psyllid days for adult psyllids on kinnow or sweet orange were averaged across sites (Fig. 3).

The percentage of citrus flush growth infested with Asian citrus psyllid eggs (Fig. 4A), nymphs (Fig. 4B), and adults (Fig. 4C) was consistently greater on sweet orange when compared with kinnow. Flush infested with Asian citrus psyllid eggs was greatest for sweet orange in mid-February to mid-March in 2012 and 2013, reaching around 20% (Fig. 4A). For kinnow, flush was noticeably infested in late-February to late-March in 2013 but only reached around 10% infestation at the peak in early March 2013 (Fig. 4A). Kinnow and sweet orange flush was also infested with Asian citrus psyllid eggs in late-June to August 2011, and from April to July 2012, with additional minor infestations being observed in August–September 2012 (Fig. 4A). Infestation levels for Asian citrus psyllid nymphs on sweet orange flush were highest over

early-March to early-April in 2012 and from mid-February to late-March in 2013 when peak levels approached 50% of flush sampled. Peak infestations of nymphs on kinnow were observed 1–2 wk after peak infestations on sweet orange and were substantially lower; the highest infestation recorded being $\approx 30\%$ of flush sampled in March 2013 (Fig. 4B). Infestation of flush growth by Asian citrus psyllid adults was most pronounced on sweet orange in terms of percentage flush infested and duration of infestation when compared with kinnow (Fig. 4C). Percentage flush infested with Asian citrus psyllid adults on sweet orange was significant over mid-March 2011 to late-August 2011 when compared with kinnow where adult Asian citrus psyllid were almost undetectable on flush (Fig. 4C). Similar high infestations were observed on sweet orange from March to October 2012 and over this time adult Asian citrus psyllid were detected on kinnow flush, but the percentage of flush infested did not exceed 10% (Fig. 4C). This pattern of higher percentage flush infested with Asian citrus psyllid adults on sweet orange in comparison to kinnow was repeated in March 2013 before sampling was terminated in late April 2013 (Fig. 4C).

Surveys for Generalist Predators and Estimates of Parasitism Rates. Generalist natural enemies, in particular, coccinellids (larval and adult *C. septempunctata* and *M. sexmaculatus*) and lacewing larvae (Chrysopidae), were collected too infrequently from tap sampling to provide insight into their relationship with Asian citrus psyllid phenology (data not shown). In total, 1,552 spiders (adults and immatures combined) were collected from tap sampling, and these specimens represented 15 families and 26 genera, of which 16 were confirmed to species level (see Vetter et al., 2013 for a complete inventory of spiders collected as part of this project). However, there was little congruence between spider abundance and

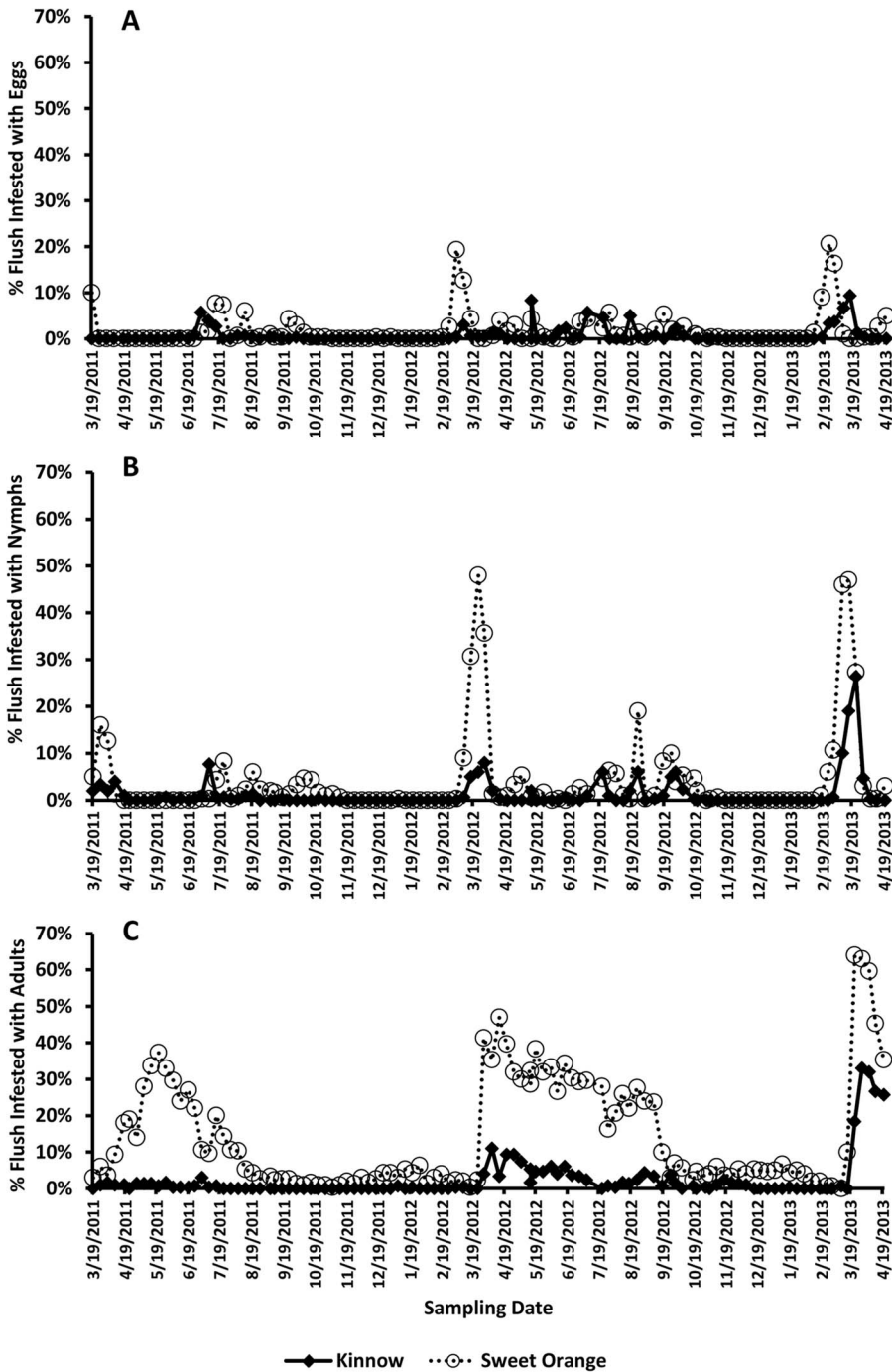


Fig. 4. Percentage of kinnow or sweet orange flush growth infested with Asian citrus psyllid eggs (A), nymphs (B), and adults (C). Percentage flush growth infested with Asian citrus psyllid life stages was combined for each citrus cultivar across PARS and Square 9, University of Agriculture Faisalabad, Punjab Pakistan.

Asian citrus psyllid phenology, and all adult spiders collected were too large to feed on any Asian citrus psyllid life stage. Immature spiders, especially those of cursorial predators such as salticids and a yellow sac spider, *Cheiracanthium insulanum* (Thorell), may be

potential natural enemies of all Asian citrus psyllid stages (Vetter et al. 2013).

In total, 3,396 parasitoids were reared from field-collected Asian citrus psyllid nymphs. Two parasitoid species were recovered, *T. radiata* and *D. aligarhensis*.

T. radiata was the most common parasitoid reared with 2,341 specimens composed of 1,271 females and 1,070 males (sex ratio was 54% female). In total, 1,055 *D. aligarhensis* were reared from Asian citrus psyllid nymphs of which 480 were females and 575 were males (sex ratio was 45% female). On kinnow, parasitism of Asian citrus psyllid nymphs by *T. radiata* averaged 26% (range, 1–100%) and for *D. aligarhensis* mean parasitism was 14% (range, 1–41%). On sweet orange, *T. radiata* inflicted an average parasitism rate of 26% (range, 1–100%) and for *D. aligarhensis* parasitism averaged 19% (1–100%). Major parasitoid activity on kinnow was observed from June to October in 2011 and August to October in 2012, and reduced activity was observed from March to April in 2011, 2012, and 2013 (Fig. 5A). A similar trend was observed for sweet orange, and parasitism was most pronounced over July–November 2011 and August–November 2012 (Fig. 5B), with minor parasitism events observed over April–May 2012 and April 2013 when sampling ended (Fig. 5B).

Discussion

This work is the first major study to document Asian citrus psyllid phenology, natural enemy diversity, abundance, and phenology, and flush phenology on two *Citrus* species, kinnow and sweet orange, in Punjab Pakistan, part of the presumed native range of this pest (Husain and Nath 1927, Halbert and Manjunath 2004). Survey results obtained from two research sites, PARS and Square 9, over 26 March 2011–20 April 2013 clearly indicated that sweet orange consistently supported higher populations of Asian citrus psyllid when compared with kinnow. The reasons for higher Asian citrus psyllid densities on sweet orange in Punjab are not well understood, but could be due in part to flush events that were slightly more pronounced on sweet orange, especially at times when flush activity on kinnow was comparatively lower or nonexistent. Flush growth is critical for Asian citrus psyllid egg laying and the subsequent development of nymph populations (Hall et al. 2008). In comparison to kinnow, flush growth on sweet orange supported significantly higher infestations of Asian citrus psyllid eggs, nymphs, and adults, up to 3.17, 3.24, and 3.43 times more, respectively, when measured in terms of mean cumulative Asian citrus psyllid days for these life stages. Additionally, the percentage of flush growth infested with Asian citrus psyllid eggs, nymphs, and adults was generally higher on sweet orange when compared with kinnow, especially for nymphs and adults.

Because Asian citrus psyllid oviposits and develops almost exclusively on citrus flush, its life cycle is tightly linked to the temporal availability of this resource (Sétamou et al., 2008). Consequently, visual and olfactory stimuli associated with citrus flush likely play an important role in the detection, location, and evaluation of potential host plants by Asian citrus psyllid (Patt and Sétamou 2010). The ability to integrate information from these two different sensory modalities, odor and color, may facilitate Asian citrus psyllid's

ability to recognize preferred signature stimuli from host plants and to be more attracted to certain competing signatures when choosing between potential hosts (Patt and Sétamou 2010). Additionally, flush growth quality in terms of nutritional value and physical characteristics has important influences on the fitness of Asian citrus psyllid (Tsai and Liu 2000, Nava et al. 2007, Tsagkarakis and Rogers 2010, Teck et al. 2011, Westbrook et al. 2011, Alves et al. 2014). For mandarins, fitness effects measured in the laboratory are significant and Asian citrus psyllid does not perform well on 'Cleopatra' (*Citrus reshni* Hort. ex Tanaka) (Tsagkarakis and Rogers 2010) or 'Sunki' (*Citrus sunki* Tanaka) (Nava et al. 2007) varieties when compared with 'sour orange' (*C. aurantium*), Rangpur lime (*Citrus limonia* Osbeck), or orange jasmine (*Murraya exotica* L.). In comparison to Cleopatra mandarin, sour orange is a superior host plant for Asian citrus psyllid. Females lay 3.32 times more eggs on sour orange, egg to adult survivorship rates are higher (4% [Cleopatra] vs. 62% [sour orange]), and egg to adult development times are significantly shorter on sour orange (Tsagkarakis and Rogers 2010). Additionally, population growth parameters (e.g., net reproductive rate [R_0] and intrinsic rate of increase [r_m]) for Asian citrus psyllid are significantly lower on Ponkan mandarin when compared with sweet orange (i.e., Valencia; Alves et al. 2014). The practical implications of reduced fitness for Asian citrus psyllid on mandarins could result in lower Asian citrus psyllid populations. Vector reduction is a significant factor in HLB management because transmission rates of bacteria increase with an increasing number of CLAs-infected psyllids feeding on a plant and the likelihood of disease development subsequently increases (Pelz-Stelinski et al. 2010). Consequently, reduced psyllid population densities can lead to a decrease in the incidence of HLB (Etienne et al. 2001) because fewer bacteria-carrying psyllids capable of inoculation are feeding on trees (Bassanezi et al. 2013).

The dominant natural enemies associated with Asian citrus psyllid nymphs sampled in this study were parasitoids, in particular *T. radiata*, which accounted for 69% of reared material, with *D. aligarhensis* comprising the remaining 31%. *T. radiata* has been widely used in classical biological control programs targeting invasive Asian citrus psyllid populations (Halbert and Manjunath 2004). The impact of *T. radiata* on Asian citrus psyllid has been subjected to intensive study in Florida where field studies indicate average parasitism rates are ≈ 1 –3% (Tsai et al. 2002, Michaud 2004, Qureshi and Stansly 2009), and may sometimes reach $\approx 20\%$ (Qureshi et al. 2009). In this study, average parasitism by *T. radiata* was $26 \pm 2\%$ and for *D. aligarhensis* average parasitism was $17 \pm 2\%$ across kinnow and sweet orange combined. Lower rates of parasitism observed in Florida maybe due, in part, to the use of pesticides to control Asian citrus psyllid that are incompatible with natural enemies (Qureshi and Stansly 2009). *T. radiata* released in Florida were originally sourced from Taiwan and south Vietnam (Qureshi and Stansly 2009). Low genetic diversity due

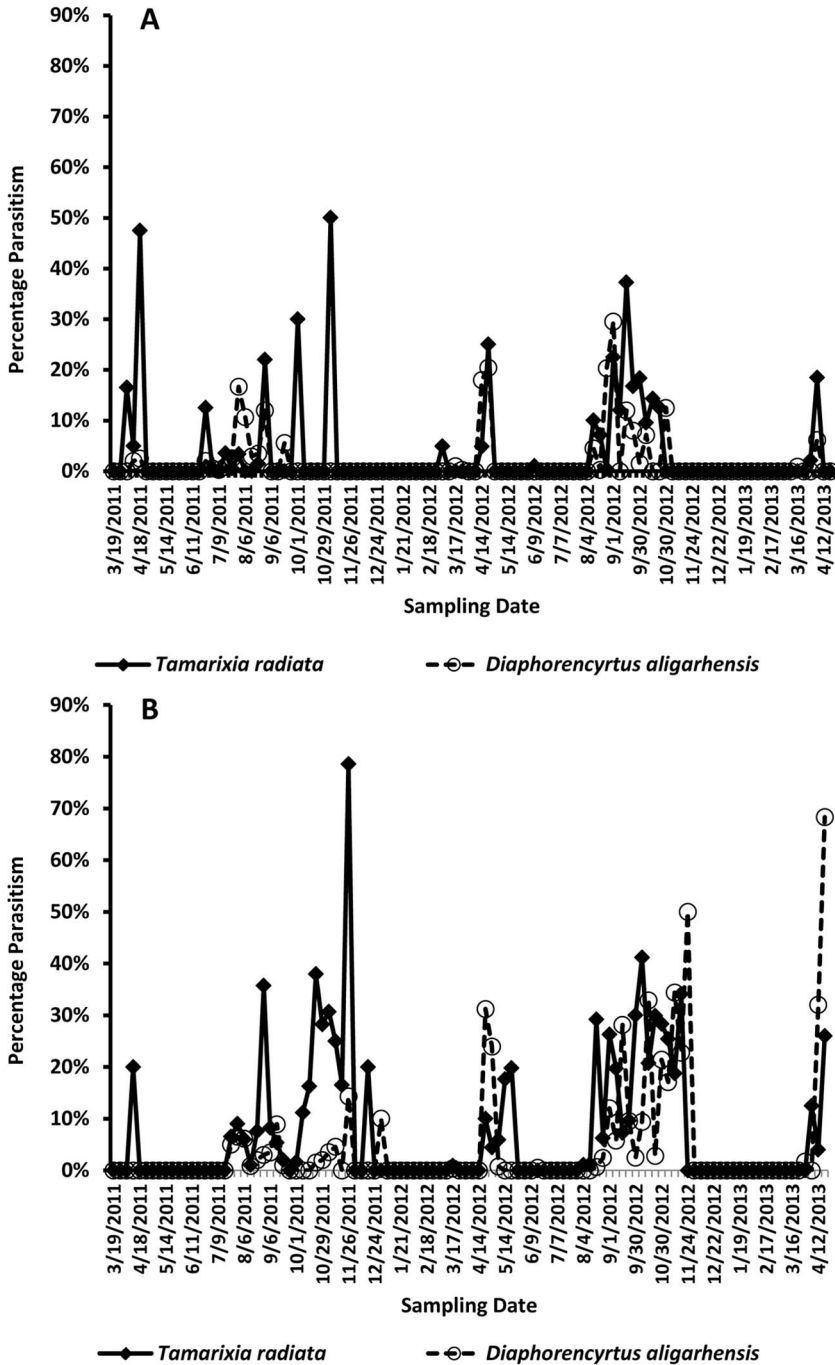


Fig. 5. Weekly percentage parasitism trends for *T. radiata* and *D. aligarhensis* attacking Asian citrus psyllid nymphs on kinnow (A) and sweet orange (B). Parasitism data were combined for each citrus variety across PARS and Square 9, University of Agriculture Faisalabad, Punjab Pakistan.

to too few parasitoids being collected and imported during foreign exploration and subsequent bottleneaking and inbreeding in quarantine before field releases could have reduced *T. radiata* efficacy. A potential indicator of loss of vigor during quarantine rearing may have manifested itself in adult sex ratios.

Skelley and Hoy (2004) reported that during quarantine rearing the sex ratio for *T. radiata* was consistently 1.8 females to 1 male, while in Taiwan, the source area for the parasitoid, the field sex ratio was 3.2 females to 1 male. Establishment of additional parasitoid species in Florida for biological control of

Asian citrus psyllid has been recommended (Qureshi and Stansly 2009). However, efforts to establish a uniparental strain of *D. aligarhensis* for classical biological control of Asian citrus psyllid in Florida have been unsuccessful (Rohrig et al. 2012). Host range testing has been completed for a biparental strain of *D. aligarhensis* sourced from Punjab Pakistan, which is being considered for potential release in California. The Environment Assessment Report for the Punjabi *D. aligarhensis* is currently under review by U.S. Department of Agriculture–Animal and Plant Health Inspection Service (USDA-APHIS).

The role of predators attacking Asian citrus psyllid eggs and nymphs was not elucidated in this study, and these natural enemies may be important even though their impacts were not documented. Life table studies would be very useful in assessing the importance of predators for Asian citrus psyllid suppression in Punjab. Weekly tap sampling indicated that predatory coccinellids and lacewing larvae were not commonly sampled and did not appear to be closely synchronized with immature or adult Asian citrus psyllid populations on experimental citrus trees (data were not presented here). Tsai et al. (2002) reported a similar finding for Asian citrus psyllid infesting *M. exotica* in Florida. However, this lack of predator activity is markedly different to that observed in citrus in Florida where generalist predators, especially coccinellids, are important natural enemies of Asian citrus psyllid (Michaud 2001, 2004; Qureshi and Stansly 2009). The reason for this discrepancy is unclear but could potentially be due to prey density and duration of availability which could be more favorable in Florida citrus for supporting persistent populations of generalist predators when compared with study sites used in Pakistan. The role of spiders in Punjab citrus, especially immature salticids and yellow sac spiders, whose life stages are small and abundant enough to feed on Asian citrus psyllid eggs, nymphs, and adults, is unresolved and needs to be investigated with feeding bioassays in the laboratory and through observation and manipulative experiments in the field (Vetter et al. 2013).

When taken together, data from this study suggest that kinnow and sweet orange vary significantly in their ability to host Asian citrus psyllid populations, and flush attractiveness and quality could be responsible for differences in Asian citrus psyllid densities observed on these citrus varieties over time in Punjab Pakistan. However, if this suggestion is correct, how flush characteristics influence Asian citrus psyllid phenology and abundance on kinnow mandarin and sweet orange in Punjab Pakistan is unknown and should be studied. Understanding the influence of these plant growth variables could provide insight into Asian citrus psyllid–HLB dynamics, which could explain why kinnow is the dominant citrus cultivar in Punjab. Abiotic influences, especially high temperature effects over summer on HLB persistence and expression in kinnow and sweet orange, need to be investigated. High temperatures (24–38°C) appear to limit CLAs development in flush growth of Valencia sweet or-

anges (Lopes et al. 2013), and fewer adult Asian citrus psyllid test positive for CLAs when temperatures are high (Razi et al. 2014). The therapeutic effect of high heat (i.e., inactivation of CLAs replication or acquisition by adult Asian citrus psyllid [Razi et al. 2014]) may be more pronounced in kinnow than sweet orange in Punjab where mean weekly summer temperatures can reach $\approx 45^{\circ}\text{C}$. This detrimental thermal effect on CLAs persistence in trees when coupled with lower year round Asian citrus psyllid densities harboring this bacterium (Razi et al. 2014) could give kinnow a production advantage over sweet orange in Punjab.

Kinnow was introduced into Pakistan from California in 1944 and has survived for ≈ 70 yr in Punjab under relatively simple management practices that include flood irrigation, disking of weeds that damage root systems, intercropping with wheat and maize, lack of certified disease-free planting material, unsophisticated use of pesticides and fertilizers, poor extension services, and no IPM programs. Despite these shortcomings, the outcomes of this natural field experiment have resulted in kinnow becoming the dominant citrus cultivar, accounting for $>75\%$ of citrus production which satisfies Pakistan's national citrus demands, and the fruit accounts for 90% of citrus exports with a net worth of \approx US\$232 million (Anon. 2011; Hoddle 2010). Other citrus varieties and species, such as sweet orange, grapefruit, lemons, and limes are relatively uncommon in comparison to kinnow which is suggestive that this mandarin is a superior commercial variety in Punjab. Additionally, acreage planted to kinnow in Punjab is increasing and research in Pakistan developing low seed varieties of kinnow is ongoing (Hoddle 2010). These observations suggest that there is commercial confidence in growing kinnow in Punjab despite Asian citrus psyllid–HLB and other plant maladies. Kinnow, like two other mandarins, Sunki and Cleopatra (and possibly King mandarin [*C. reticulata* \times *maxima*] a parent of kinnow grown commonly in Vietnam where Asian citrus psyllid and HLB are widespread), appears to be a low-quality host for Asian citrus psyllid (and potentially CLAs under field conditions?) in comparison to sweet orange. Improved understanding of plant characteristics (e.g., flush attractiveness and nutritional quality), the role of environmental conditions (e.g., high temperatures), and their interactions would increase our understanding as to why kinnow is the dominant citrus cultivar in Punjab despite relatively unsophisticated farming practices and high pressure from Asian citrus psyllid–HLB and other maladies (e.g., citrus tristeza, canker, and phytophthora) that collectively cause citrus decline and eventual tree death. Lower year round Asian citrus psyllid densities on kinnow as documented here may be one important reason as to why this citrus variety has performed well in this region. We suggest that answering this seemingly simple question, “why is kinnow still alive in Punjab Pakistan?” could provide useful insight for the development of programs attempting to manage this invasive insect–disease combination.

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