Many aspects of the mating behavior of Diaphorina citri Kuwayama (Hemiptera: Liviidae), the Asian citrus psyllid (ACP) are shared by other members of the Psylloidea. Adults are reproductively mature within about 2 days post-eclosion, and both sexes mate multiple times during their lifetime. Typically, courtship is mediated by short-range, substrate-borne vibrational communication and semiochemicals. In citrus orchards, *D. citri* courtship is facilitated by host-seeking and foraging behavior, as both sexes are attracted to green and yellow colors, as well as to volatiles of young flush shoots on the host tree, and short-range communication is sufficient for finding mates in aggregations that develop soon after the flush opens. Courtship behavior includes a series of duets, in which a searching male produces vibrational calls that elicit rapid replies from receptive females, enabling him to focus on willing partners. Both sexes produce vibrational communication signals by extending and fanning their wings while their legs hold on to the plant. The signal is transmitted to the host plant structures and then detected by vibration-sensitive, chordotonal organs in the legs of the receiving conspecific. During the duetting bouts, male *D. citri* call intermittently, with an interval of 9 ± 1.4 s (mean ± standard error) between calls, and females reply within 0.95 ± 0.09 s. Males produce signals ranging approximately 150–500 ms in duration, and females 331–680 ms. The spectra of communication signals produced by *D. citri* have prominent frequencies that are multiples (harmonics) of the 170–250 Hz wingbeat frequency, and both sexes respond behaviorally to synthetic signals containing three or more wingbeat harmonics. When the male finds the female, he moves alongside with their heads pointing in the same direction and grasps her with his adjacent legs, bringing his abdomen from underneath to meet the opening of her genital segment. They remain in copulation for about 48 min. Dispersal and mating behavior of *D. citri* is influenced by abiotic factors including light, temperature, storms and barometric pressure, and by biotic factors including host plant flush, host plant structure, aggregation behaviors and learning behaviors. Opportunities exist to co-opt *D. citri* mating behavior for purposes of detecting and managing populations, enabling reductions in the incidence and spread of the bacteria causing huanglongbing, a devastating disease of citrus. This chapter describes details of what is currently known about *D. citri* mating behavior and how such knowledge has been applied in development of methods that apply vibrational communication to disrupt mating or trap males.
3.1 Introduction

_Diaphorina citri_ Kuwayama (Hemiptera: Liviidae) native to India (Hollis, 1987), initially was reported as a significant psyllid pest of _Citrus_ spp. in China, Taiwan, Japan and the Philippines in the early 20th century (Crawford, 1912); however, little was reported about its ecology, life cycle and mating behavior (Husain and Nath, 1927; Catling, 1970; Pande, 1971) until it became important as a worldwide vector of huanglongbing (HLB), a devastating bacterial disease in citrus orchards (Halbert and Manjunath, 2004; Gottwald et al., 2007). The bacterium _Candidatus Liberibacter_ spp. (Alphaproteobacteria) resides in symbiotic bacteriomes within the _D. citri_ hemocoel that also contain nutritional and defensive symbionts in mutually indispensable associations, all of which are transmitted transovarially (Dan et al., 2017). Economic damage caused by HLB has stimulated interest in development of knowledge about _D. citri_ biology and mating behavior that could be used for an integrated, multidisciplinary approach to management of _D. citri_ and thereby HLB (Aubert, 1990; Grafton-Cardwell et al., 2013; Hall et al., 2013), as has occurred also with other economically important insect pests (Mankin, 2012; Benelli et al., 2014; Takanashi et al., 2019). In this chapter, we focus on aspects of mating behavior that can be co-opted to reduce _D. citri_ populations in citrus and other hosts in the Aurantioidae subfamily of Rutaceae (Halbert and Manjunath, 2004; Hall et al., 2017). The life stages of _D. citri_ on rough lemon _Citrus jambhiri_ Lush, sour orange _C. aurantium_ L., grapefruit _C. paradisi_ Macfadyen, and orange jessamine _Murraya paniculata_ (L.) Jack were described by Tsai and Liu (2000). These and other host species have a range of different physical and structural characteristics that, as discussed further below, strongly affect vibrational signal amplitudes and the efficacy of mate-seeking behaviors (Cocroft et al., 2006; Mankin et al., 2018).

3.2 General Aspects of Mating Behavior in _Diaphorina citri_ and Other Psylloids

Members of the Psylloidea, a group of about 3850 phloem-feeding Sternorrhynchan species (Burckhardt and Ouvrard, 2012; Martoni et al., 2017), share numerous aspects of reproductive biology and mating behavior (Lubanga et al., 2014, 2016a). Psyllids reproduce only sexually, unlike some hemipterans (Kennedy and Stroyan, 1959). Many male psyllids reach reproductive maturity within about 2 days post-eclosion, and both sexes mate several times (Burts and Fischer, 1967; Van den Berg et al., 1991; Wenninger and Hall, 2007; Guédot et al., 2012; Lubanga et al., 2018) during a typical lifetime of 49 days or longer (Wenninger and Hall, 2008). Refractory periods have been reported for females of some psyllid species (Lubanga et al., 2016a) but have not been reported in _D. citri_. Newly emerged _D. citri_ females have immature ovaries that remain without mature eggs until mating occurs (Dossi and Cônsoli, 2010). Mating stimulates vitellogenesis and rapid development of oocytes, and females often begin laying eggs on the day of mating (Wenninger and Hall, 2007). Because oocyte maturation is metachronous, with only one oocyte developing per oogenic cycle, the stimulatory effects of mating (Dossi and Cônsoli, 2010) may contribute to the polyandry (Wenninger and Hall, 2008) observed in this species. General aspects of several different male reproductive systems in the Psylloidea are described in Schlee (1969), Macharashvili and Kuznetsova (1997) and Kuznetsova et al. (1997); and the male _D. citri_ genitalia and reproductive system were described in Stockton et al. (2017b) and Alba-Alejandre et al. (2018). Dossi and Cônsoli (2014) and Stockton et al. (2017b) described the _D. citri_ female reproductive organs.

Short-range semiochemicals have been demonstrated to play a role in mate-finding of several (Wenninger et al., 2008; Brown et al., 2009; Guédot et al., 2009, 2010; Mann et al., 2013) but not all psyllids (Lubanga et al., 2016b). Wu et al. (2016) investigated the antennal and abdominal transcriptomes of male and female _D. citri_ to consider whether chemosensory proteins could be identified for development of attractants or repellents. It was found that a large proportion of chemosensory genes were similar in male and female antennae and terminal abdominal tissues, but two were expressed at higher levels in male than female antennae (Wu et al., 2016), which is consistent with a potential role of antennal chemosensilla in _D. citri_.
mate-finding or species identification (Onagbola et al., 2008).

In addition, substrate-borne vibrational communication is an important mechanism for mate location in many psylloids (Ossiannilsson, 1950; Virant-Doberlet and Čokl, 2004; Tishechkin, 2005; Percy et al., 2006; Lubanga et al., 2014, 2016b; Eben et al., 2015; Liao and Yang, 2015, 2017; Liao et al., 2016), as well as other Hemiptera (Cocroft and Rodríguez, 2005) including the Auchenorrhyncha (Percy and Day, 2005) and the Cicadellidae (Gordon et al., 2017). The capability to detect and produce vibrations is essentially ubiquitous in terrestrial invertebrates, which attests to the importance of vibrational cues for reproduction and predator avoidance (Cocroft and Rodríguez, 2005; Pollack, 2017; Takanashi et al., 2019). In many Hemiptera, sexual communication involves a duet, in which a searching male will call and a sedentary female will reply, which facilitates searching behavior as well as mate recognition (Bailey 2003; Derlink et al., 2014).

Unlike in air, a relatively uniform substrate, the physically non-uniform characteristics of the interiors and interfaces within plant structures strongly affect propagation of vibrations from their sources to the sensing insect (Michelsen et al., 1982). Reflections from surface edges, frequency-dependent attenuation and background noise make it difficult to locate or estimate the distance to a vibration source precisely (Michelsen et al., 1982; Mankin et al., 2011, 2018; Dent, 2017; Gordon et al., 2019). In addition, the small size of *D. citri* (and many other psylloids) as well as frequency-dependent attenuation observed in plant structures essentially reduces the ‘active space’, i.e. the maximum communication distance, of vibrational communication signals to 1–2 m (Ichikawa 1979; Michelsen et al., 1982; Mazzoni et al., 2014). Consequently, the information that vibrational communication provides is typically transmitted over only a short range.

Males of *Nezara viridula* (L.) and other large hemipteran species have been reported to use the time delay between vibrational signals that reach two different legs as a directional cue for locating the female (Čokl et al., 1999). The distances between the legs of psylloids, however, may be too small to use this delay effectively in search behavior (Tishechkin, 2007). Virant-Doberlet and Čokl (2004) suggested that even small insects can stretch their legs between branches at bifurcations, thereby increasing the time delay, which could provide directional cues at bifurcation points. Nevertheless, background noise from wind or other loud sound or vibration sources masks the weak signals produced by small insects and interferes with precise identification of the direction of a replying female psyllid (Tishechkin, 2013). To accommodate directional uncertainties and signal masking, many hemipterans augment their searching behavior with a ‘call-fly’ strategy under which a searching male produces substrate-borne vibrational calls spontaneously when it first lands on a host plant and then moves upward and/or towards a receptive female who has produced a duetting reply in response to his call (Hunt and Nault, 1991). Such a strategy is beneficial when the females aggregate towards the upper perimeter of the plant, as is discussed in Section 3.4 below.

### 3.3 Substrate-borne Communication in *Diaphorina citri*

Substrate-borne communications associated with mating behavior of virgin male and female ACP, 5–7 days post-eclosion, were characterized by Wenninger et al. (2009a). Males and females both produce low-amplitude vibrational signals, 140–700 ms in duration, by extending and flapping their wings rapidly while holding on to the substrate with their legs, as in many other psylloids (Tishechkin, 1989, 2005). The signal then is transmitted through the legs to the substrate and connected structures. The communication signal spectral frequencies are harmonics of the 170–250 Hz wingbeat frequency (i.e. the fundamental frequency) (Mankin et al., 2016), which is negatively correlated with body mass (Wenninger et al., 2009a). ACP production of sounds consisting of wingbeat fundamental and harmonic frequencies is similar to that observed in many flying insects such as mosquitoes and bumblebees, the aerodynamics of which are described in Bae and Moon (2008). Due to its small size, ACP produces only faint sounds and the signal is carried primarily through vibrations transmitted through the leaf or twig surfaces (Michelsen et al., 1982), which have signal attenuation and other structural characteristics
that vary considerably among the different plants that are ACP hosts (Mazzoni et al., 2014; Ebert et al., 2018). Several other psylloids have rows of teeth on the axillary cords of the wing mesoscutellum and metascutellum which can serve as a stridulatory organ for sound production (Heslop-Harrison, 1960; Taylor, 1985a; Liao et al., 2019). However, stridulatory organs usually produce chirps with high-amplitude fundamental frequencies and weak harmonics (e.g. Mankin et al., 2009; Grant et al., 2014) caused by the friction of the pars stridens scraping over the plectrum. Because such spectral patterns are not observed in its signals (Mankin et al., 2016), ACP possibly uses only wing-flapping and not stridulation as a sound-production mechanism.

ACP searching and mating activity on citrus occurs primarily on new leaf flush between 10:00 am and 3:00 pm (Wenninger and Hall, 2007). As with Cacopsylla pyri (L.) (Eben et al., 2015), male *D. citri* usually produce calls spontaneously within about 15 min after landing on a host plant, except during extreme changes in weather or barometric pressure (Zagvazdina et al., 2015). Receptive females produce a duetting reply within 0.95 ± 0.09 s (mean ± standard error) after the male call (Wenninger et al., 2009a). The female remains sedentary while the male moves intermittently towards the female. Durations of male calls range from approximately 148 to 544 ms, and female replies from 331 to 680 ms (Wenninger et al., 2009a). After detecting a female reply, a male that has begun searching on a leaf or branch of a citrus tree typically moves in the direction of the reply for a few seconds until it reaches a bifurcation or other visually identifiable transition point. There it pauses, calls again, and then continues or returns back along the original path. Males call intermittently during the duetting bouts, with an interval of 9 ± 1.4 s between calls (Lujo et al., 2016).

Typical movement speeds of males involved in searching behavior are > 9 mm/min (Zagvazdina et al., 2015). Several instances of reciprocating behavior may occur before the male finds the female or ceases searching. Lujo et al. (2016) reported that, in 17 mating tests on small citrus trees, a male placed on a separate branch from a female searched for 15.9 ± 2.4 min before finding the female. An example of a 45 s section of a duetting bout recorded from a small tree in an anechoic chamber is shown in Fig. 3.1.

It should be noted that only virgin *D. citri* males and females were tested in the Mankin et al. (2013), Rohde et al. (2013), Lujo et al. (2016), and Hartman et al. (2017) studies, and there is evidence that males learn female-produced olfactory cues and associated environmental odors.
during their first mating encounter and subsequently are attracted to such odors (Stockton et al., 2017a). Similar learning responses have been observed in mice (Remedios et al., 2017). Previously mated D. citri males may be more responsive than virgins to female odor and associated environmental odor cues, as well as to vibrational reply cues when searching for females. In addition, there is evidence that females can learn cues associating male color with subsequent reproductive success and thereby increase fecundity (Stockton et al., 2017b). Learning may be particularly beneficial (Dukas et al., 2006) when multiple movement biases or other factors result in aggregations with high encounter rates between males and females, the topic of the next section. There is evidence that male cuticular hydrocarbons, including dodocanoic acid (Mann et al., 2013), degradation products of citrus volatiles (George et al., 2016; Lapointe et al., 2016; Zanardi et al., 2018), and combinations of yellow, green and ultraviolet light (Paris et al., 2017a, b) may serve as significant cues in male searches for mates and host plants, as well as in female searches for host plants.

Once the male finds the female, he moves to her side with their heads pointing in the same direction, similarly as in Trioza erytreae (Del Guercio) (Van Den Berg et al., 1991). If she is receptive, he holds on to her abdomen with his nearest legs, bending his upward-pointing genital segment down to meet the opening of the female segment, while supporting himself on the substrate with his remaining legs, and begins copulation (Husain and Nath, 1927). They copulate while the male holds the female with legs on one side of his body and supports himself on the plant with his remaining legs (Husain and Nath, 1927). The mean duration in copula has been measured as 48.3 ± 8.4 min, ranging from 15.2 min to 98 min (Wenninger and Hall, 2007). The female usually begins ovipositing on the day of mating (Wenninger and Hall, 2007) and may lay up to 800 eggs over a lifetime of 2 months (Husain and Nath, 1927).

It has not been established whether differences among the temporal or spectral components of female replies affect ACP male mating preference although preference has been observed in fulgorid males (Mazzoni et al., 2015). It may be relevant, however, that ACP females infected with the CLas pathogen are more fecund and therefore have greater reproductive fitness than uninfected females, which may facilitate the spread of HLB (Pelz-Stelinski and Killiny, 2016). It is not known whether mating behavior itself is affected by CLas infection but increased fecundity could result from multiple matings with high-fertility partners, from changes in hormonal regulation of immune function and metabolic allocation (Harshman and Zera, 2007) or from increased movement leading the female to healthier flush (Martini et al., 2015).

In addition, there are numerous abiotic factors that could interfere with different aspects of communication, physical activity or physiological processes associated with mating behavior. These include weather extremes and barometric pressure extremes (Zagvazdina et al., 2015; Martini and Stelinski, 2017; Udell et al., 2017; Martini et al., 2018), high altitude (Jenkins et al., 2015) and high levels of wind and other interfering vibrational background noise (Tishechkin, 2013).

### 3.4 Movement Bias towards Light and Flush: Impacts on Mating Behavior

As with many psylloids, D. citri females and males exhibit phototaxis, both when flying (Sétamou et al., 2011; Anco and Gottwald, 2015; Paris et al., 2015) and walking (Pregmon et al., 2016; Paris et al., 2017a). They are attracted to green and yellow colors (Paris et al., 2015); yellow colors are known to induce settling of many hemipteran herbivores on host plants (Döring, 2014). There is evidence that citrus tree volatiles play a role in attraction to the host (Wenninger et al., 2009b). In addition, D. citri are attracted to new leaf flush (Catling, 1970; Hall and Albrigo, 2007; Patt and Sétamou, 2010; Sule et al., 2012; Sétamou et al., 2016; Hall and Hentz, 2016, Stelinski, 2019) on which eggs are laid and nymphs develop. Nutrient availability (Steinbauer, 2013), ability to easily probe the thinner structure of citrus leaf veins in young flush (Ammar et al., 2013) and phagostimulants (George et al., 2016; Lapointe et al. 2016) may play a role in such attraction. Oviposition cues detected by sensilla on the legs and female ovipositor also may play a role in
flush attraction (Zhang et al., 2019). Such movement biases can result in both-sex aggregations on upper canopies (Soemargono et al., 2008), border trees (Sétamou and Bartels, 2015) and flush (Tsai et al., 2000; Sétamou et al., 2008; Hall et al., 2015). Aggregation and social behaviors also have been documented in numerous other hemipterans (e.g. Kennedy et al., 1967; Way and Cammell, 1970; Lin, 2006). The occurrence of aggregations may reduce predation, partly by increased tending by ants (e.g. Navarrete et al., 2013) which would lead to more rapid increases of D. citri populations.

Given that the volume of upper-canopy flush is generally only a fraction of the volume of the complete canopy, mate-seeking males that move towards light and flush will, on average, have less distance to cover in searching for a female than males that search at random. Consequently, such biases are reproductively advantageous. Likewise, when the density of nymphs and adults increases to levels that reduce flush healthiness, it is reproductively advantageous to migrate to areas with lower ACP populations (Martini et al., 2015; Martini and Stelinski, 2017), as has been observed frequently in auchenorrhynchs (Taylor, 1985b).

A relatively unstudied impact of ACP aggregations is whether the presence of nearby conspecifics of both sexes may affect mating behavior. Until now, social interactions involving vibroacoustic communication have been studied primarily in social insects (Hunt and Richard, 2013) but interactions also have been documented in Delphacidae (Ott, 1994) and Cicadellidae (Hunt and Morton, 2001), and acoustic interactions are well documented in mating swarms of mosquitoes and midges (Mankin, 2012; Simões et al., 2016; Jakhete et al., 2017). Recordings from D. citri in infested orchard trees (Mankin et al., 2016) as well as in greenhouse trees with D. citri maintained for behavioral bioassays (Paris et al., 2013, and unpublished), suggest that social interactions in which multiple males and females take part in duets occur frequently in aggregations during the time of day when mating typically occurs, an example of which is shown in Fig. 3.2. The notable differences in the amplitudes and fundamental frequencies of the signals in Fig. 3.2 suggest that two different males and three different females had participated in the duets. Unpublished studies suggest that the duetting frequency per number of aggregated individuals decreases relative to the

![Fig. 3.2](image-url)
duetting frequency of isolated pairs, possibly because males can locate females in the aggregation readily by random movement without calling. Also, it has been observed that previously mated ACP males are less likely than virgin males to begin calling spontaneously (Wenninger et al., 2009a), which may reduce the rate of calling in aggregations.

### 3.5 Potential for Mimicking or Interfering with Vibrational Communication Signals to Trap Males or Disrupt Mating

Soon after *D. citri* vibrational signals were first characterized in 2009, interest developed in the possibility of devices that mimicked the female reply signal to attract and capture males or disrupt mating. An understanding of *D. citri* population densities and spatial distributions in citrus groves is important for development and timing of management decisions (Sétamou et al., 2008), but commonly used stem-tap and sweep-net sampling methods (Hall et al., 2013; Monzo et al., 2015) have limited efficiency at low population densities, and sampling with yellow sticky traps is costly and requires a relatively large time commitment (Hall and Hentz, 2010; Hall et al., 2010; Monzo et al., 2015). Mating disruption seemed feasible, having been demonstrated previously by Saxena and Kumar (1980) on cotton leafhopper *Amrasca devastans* Dist. and rice brown plant hopper *Nilaparvata lugens* Stål. Disruption of substrate-based communication is a natural competitive practice in the leafhopper *Scaphoideus titanus* Ball (Mazzoni et al., 2009), Therefore, a series of investigations was conducted to develop methods to trap and/or disrupt *D. citri* mating behavior.

Initially, recordings of duets (Rohde et al., 2013) as well as synthetic mimics (Mankin et al., 2013) were bioassayed for their potential to elicit female replies and male searching behavior in citrus trees. The bioassays demonstrated that males were attracted to the recorded replies as well as to synthetic mimics in which three or more harmonics of the fundamental frequency were present (Mankin et al., 2016). Such knowledge of the spectral and temporal patterns needed for *D. citri* species recognition and male attraction thereafter led to development of prototype signal-mimicking devices that disrupted mating (Lujo et al., 2016) and attracted male *D. citri* to traps (Mankin et al., 2016; Hartman et al., 2017).

A potentially useful result of the male *D. citri* trapping study (Hartman et al., 2017) was that males were found to be variably responsive to searching cues. Also, Zagvazdina et al. (2015) had previously reported variability in male searching behavior, demonstrating that changes in barometric pressure affected the proportions of males who moved either > 9 mm/min or < 9 mm/min towards the synthetic female reply from the prototype device. Similarly, variability in courtship behaviors of male *D. citri* was observed by Stockton et al. (2017b). A better understanding of such variability can help guide additional efforts to co-opt mating behavior for integrated pest management applications.

Mating disruption has been demonstrated in several different hemipterans since the initiation of studies with *D. citri*. Mating disruption of *S. titanus* (Eriksson et al., 2012) and *Homalo disca vitripennis* (Germar) has been accomplished in vineyards (Gordon et al., 2017; Krugner and Gordon, 2018). There is potential to disrupt mating in *Halyomorpha halys* (Stål), given that males have been attracted to synthetically produced signals (Mazzoni et al., 2017). Field testing and device improvements coupled with experimentation to identify the best locations from which to deploy such devices ultimately may result in *D. citri* being one of the first insects for which the co-opting of vibrational communication becomes an important component of an integrated pest management program. Knowledge of *D. citri* movement biases enables pest managers to increase the efficiency of vibrational traps and mating disruption devices by operating them near potential sites of aggregation.

It should be noted also that a potent modulator of transient receptor potential channels in insect chordotonal organs has been developed recently (Kandasamy et al., 2017) to which *D. citri* has been demonstrated to be susceptible (Chen et al., 2018). Given the importance of vibration sensing in *D. citri* courtship, insecticides that target chordotonal organ function, reducing the perception of vibrational signals, are likely to have strong negative impacts on *D. citri* mating behavior at sublethal dosages.
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