### Biological Attributes of *Diaphorina citri* Kuwamaya on *Citrus limetta* under Controlled Conditions

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**Abstract**

The present experiment was commenced under the laboratory condition to study the biological parameters of *Diaphorina citri* Kuwayama. The psyllid species, *Diaphorina citri* is potentially the most dangerous at the present time because it serves as vector for Citrus Huanlongbing which is the most serious disease of citrus in the world. *Diaphorina citri* is the only known vector of Huanglong-bing- a phloem-limited bacterial disease of international importance. Adults and nymphs suck sap from the phloem of tender shoots and buds, whilst adults also feed on the phloem of mature leaves. Severe infestations in orchards could lead to withering, distortion, and loss of immature leaves and irregular-shaped canopies. Citrus psylla are prevalent throughout the year but the high population coincides with the periods of new vegetative flushes on citrus plants during spring and summer. The population is sensitive to high rainfall and moisture condition and survives throughout the year without diapause, completing about 9-10 generations per year. The life cycle of Asian Citrus Psylla includes an egg stage and five nymphal instars. Study of citrus psyllid biology is necessary for decision making of appropriate management strategies and to reduce the economic damage.

**Keywords**

Psyllids, Huanlongbing, diapause and instars

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**Introduction**

*Citrus* spp. is susceptible to a number of destructive diseases and insects that are continuously emerging and which can severely limit production or totally decimate an industry of a country. The psyllid species, *Diaphorina citri* is considered as potentially the most dangerous at this time because it serves as vector for Citrus Huanlongbing. Citrus greening disease or Huanglongbing is the most serious disease of citrus in the
world (Aubert et al., 1996). The Asian Citrus Psylla, *Diaphorina citri* Kuwayama is worth noting as vector of an endocellular, phloem-restricted bacterium, associated with the Citrus greening disease (CGD). CGD is a destructive malady seriously affecting most commercially important Citrus cultivars in Africa and Asia. Yield reductions of 23% from 2006 to 2011, revenue losses of $1.71 billion and the losses of 8,257 jobs directly or indirectly related to this industry was estimated. NASS statistics show orange production in Florida decreased from 6.94 to 3.33 billion tones (67.4% reduction) over the 8-year interval from 2007-08 to 2015-16 seasons (NASS, United States Department of Agriculture, 2015-16). Six other species of *Diaphorina* are reported on citrus, but these are non-vector species of relatively little importance (Halbert and Manjunath 2004). About 823 species have been reported attacking on Citrus, among them 175 have been found in India (Ebeling, 1959), whereas 34 species are actively recorded from Punjab and Uttar Pradesh (Devi et al., 2014). In which asiatic citrus psyllid (*Diaphorina citri* Kuwayama [Hemiptera: Psyllidae]) was recognised as a major pest of citrus in subtropical and tropical Asia, initially in India and then elsewhere in the region (Halbert and Manjunath, 2004.) It is themost efficient vector of citrus greening bacterium (*Candidatus Liberobacter asiaticum*) throughout Asia and the Far East (Catling, 1970). Due to their jumping and feeding habits its status derives, not from the damage it causes, but from its role as the only known vector of Huanglongbing- a phloem-limited bacterial disease of international importance. Adults and nymphs suck sap from the phloem of tender shoots and buds, whilst adults also feed on the phloem of mature leaves. Severe infestations in orchards could lead to withering, distortion, and loss of immature leaves and irregular-shaped canopies. Growth of sooty mould fungi on honeydew excreted by nymphs leads to blemishing of foliage and fruit, and can reduce photosynthesis (Wang et al.: 2002). The disease can devastate orchards within a few years of planting. It also poses a major threat to endangered indigenous citrus germplasm in Asia. The population is sensitive to high rainfall and moisture condition and survives throughout the year without diapause, completing about 9-10 generations per year (Husain and Nath, 1927). Despite of its wide occurrence and serious damage to citrus crop, very little attention has been given to this pest especially in generating the pin point information on development and reproductive potential of citrus psylla, *D. citri*. Therefore, the present investigation has been aimed to study the biology of *D. citri*, as management strategies of an insect pest must be based on thorough ecological studies of the concerned insect pest.

**Materials and Methods**

The present work and experiment commenced in February 2019 under the laboratory condition at Department of Plant Protection, Faculty of Agricultural Sciences, Aligarh Muslim University, Aligarh, Uttar Pradesh, India. The biological parameters of *Diaphorina citri* Kuwayama, were studied on *Citrus limetta* at a temperature range of 25-27°C and 60-70% relative humidity in a BOD incubator. The adults of *D. citri* were collected from a mature, fruiting tree of *Citrus limetta* by an aspirator (Plate 4-K) and brought to laboratory conditions to maintain the stock culture (Plate 3-J). The adult females were singly aspirated into big glass jars and was covered by muslin cloth on top. Tender twigs wrapped with moist cotton and placed in the water plastic container, 2 X 5 cm (diameter X depth) served as a food source. Two males were also released into it. The jars were checked constantly for egg laying. For incubation study, shoots along with freshly laid eggs were cut and transferred in petri dishes after one day of adult release. Cut ends of detached shoots
were dipped in water and wrapped with moist cotton and placed in petri dishes. For nymphal period study, nymph immediately after hatching, were picked up with soft moist camel hairbrush and transferred to a fresh tender twig singly and placed in petri dish and cut ends were kept moist by wrapping moist cotton wool. Tender twigs were provided as food material and transferred twice, daily during the early instar and once during later instars. The hatching period, nymphal duration, oviposition period and adult longevity were recorded daily. Adults were also kept in clip cages made in laboratory to study the fecundity (Plate 4-L). Five such cases were hanged on a single tree.

**Results and Discussion**

It was found that psyllid’s life cycle included an egg stage and five nymphal instars (Plate 1). Eggs were oviposited in clusters on leaf folds, petioles, axillary buds, upper and lower surfaces of young leaves and tender stems (Plate 1). Pre-oviposition period lasted for 1.4±0.55 days (Table 1). The egg laying started on fourth day with an incubation period of 1.4±0.55 days and oviposition lasted for 18.22±1.30 days (Table 1). Eggs were anchored on a stalk-like process arising from the plant tissue. They were somewhat conical, almond shaped with a broad basal end and tapering towards its distal end (Plate 1-A). Light yellow coloured eggs deposited freshly, turned bright orange later with two distinct red eye spots at maturity. Egg shell turned transparent and membranous after hatching and remain attached to the plant tissue (Plate 1-B). All the nymphs reared underwent four moults, continuously secreted a copious amount of honeydew from anus and a thread like waxy substances from the circumanal glands resulting in the growth of black sooty mould on the lower leaves. The early instars were docile and moved slightly on disturbances while older nymphs and adults were highly mobile and jump frequently. They feed on tender and succulent leaves (Plate 2-I). Newly emerged nymphs were pale yellow in colour and developed wings can be clearly seen in the last instar (Plate 1-F). The duration for first, second, third, fourth and fifth instar were recorded as 3.20±0.84, 2.80±0.84, 3.60±0.89, 3.80±0.84, 4.20±0.84 days (Table 1). Fecundity of an adult female was recorded to be 321.2±52.51 eggs (Table 1). Adult longevity was recorded 27.40 ±1.67 days for female and 22.60±2.88 for male psylla (Table 1). Adults are adapted for sucking plant vessels, by the means of elongated mouth parts. The metathoracic legs are highly modified for jumping and the forewings remain folded in roof-like fashion (Plate 2-G and H). Life cycle of male and female were completed in 40.20±2.86 and 45.00±2.53 days respectively (Table 1). Husain and Nath, 1927 observed that nymphs are yellow in colour, flattened and circular in shape, remain congregated close to the site of oviposition for feeding and move to matured leaves, thick shoots and petioles. As described by Hall and Albrigo (2007), oviposition and development of immature *D. citri* are confined to young, tender flush leaves. Tsai and Liu, 2000 reported that the egg is anchored to plant tissue on a slender stock-like process on one end in an upright position, and large numbers of eggs may be found on a single flush shoot. Chhetry et al., 2012 reported that nymphs move slowly in early stage and body size increases at a very slow pace. Chhetry et al., 2012 also reported similar findings regarding nymphal periods. He observed the developmental range of 3.42±0.17 days, 2.17±0.21 days, 2.52±0.18 days and 3.07±0.31 days for first, second, third, fourth and fifth instars respectively. According to Richardson and Hall; 2013, female survives longer than the male. My findings revealed the same. Khan et al., 1989 and Xu et al., 1994 stated that *D.citri* completes 6-7 generations per year or even upto 16 generations in a single year.
### Table 1: Duration of different development stages of *Diaphorina citri*n citrus plant

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Parameters</th>
<th>Mean duration (days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(Insect stage/Duration)</td>
<td>Mean ± SD</td>
</tr>
<tr>
<td>1</td>
<td>Incubation period (eggs)</td>
<td>1.4±0.55</td>
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<tr>
<td></td>
<td><strong>Nymph</strong></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>1st instar nymphal period</td>
<td>3.20±0.84</td>
</tr>
<tr>
<td>3</td>
<td>2nd instar nymphal period</td>
<td>2.80±0.84</td>
</tr>
<tr>
<td>4</td>
<td>3rd instar nymphal period</td>
<td>3.60±0.89</td>
</tr>
<tr>
<td>5</td>
<td>4th instar nymphal period</td>
<td>3.80±0.84</td>
</tr>
<tr>
<td>6</td>
<td>5th instar nymphal period</td>
<td>4.20±0.84</td>
</tr>
<tr>
<td></td>
<td><strong>Adult</strong></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>Pre-oviposition period</td>
<td>1.4±0.55</td>
</tr>
<tr>
<td>8</td>
<td>Oviposition period</td>
<td>18.22±1.30</td>
</tr>
<tr>
<td>9</td>
<td>Post-oviposition period</td>
<td>6.8±0.84</td>
</tr>
<tr>
<td></td>
<td><strong>Adult longevity</strong></td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>Female</td>
<td>27.40±1.67</td>
</tr>
<tr>
<td>11</td>
<td>Male</td>
<td>22.60±2.88</td>
</tr>
<tr>
<td>12</td>
<td>Fecundity (eggs)</td>
<td>321.2±52.51</td>
</tr>
<tr>
<td></td>
<td><strong>Total life cycle</strong></td>
<td></td>
</tr>
<tr>
<td>13</td>
<td>Female</td>
<td>45.00±2.53</td>
</tr>
<tr>
<td>14</td>
<td>Male</td>
<td>40.20±2.86</td>
</tr>
</tbody>
</table>

Plate 1:  
A: Eggs of *D. citri*  
B: 1st instar nymph  
C: 2nd instar nymph  
D: 3rd instar nymph  
E: 4th instar nymph  
F: 5th instar nymph
Plate-3: G. Adults of D. citri
H. Male and Female
I. Attack on citrus plant by D. citri

Plate-3: J. Rearing glass jar in BOD
In conclusion, citrus psylla are prevalent throughout the year but the high population coincides with the periods of new vegetative flushes on citrus plants during spring and summer. The population is sensitive to high rainfall and moisture condition and survives throughout the year without diapause. The life cycle of Asian Citrus Psylla includes an egg stage and five nymphal instars. Eggs are oviposited in clusters on leaf folds, petioles, axillary buds, upper and lower surfaces of young leaves and tender stems. Pre-oviposition period lasted for 1.4±0.55 days. The egg laying started on fourth day with an incubation period of 1.4±0.55 days and oviposition lasted for 18.22±1.30 days. Eggs were anchored on a stalk-like process arising from the plant tissue. They were somewhat conical, almond shaped with a broad basal end and tapering towards its distal end. Light yellow coloured eggs deposited freshly, turned bright orange later with two distinct red eye spots at maturity. The early instars were docile and moved slightly on disturbances while older nymphs and adults were highly mobile and jump frequently. They feed on tender and succulent leaves. Newly emerged nymphs were pale yellow to light pink in colour. The duration for first, second, third, fourth and fifth instar were recorded as 3.20±0.84, 2.80±0.84, 3.60±0.89, 3.80±0.84, 4.20±0.84 days. Fecundity of an adult female was recorded to be 321.2±52.51 eggs. Adult longevity was recorded 27.40±1.67 days for female and 22.60±2.88 for male psyllid. Life cycle of male and female were completed in 40.20±2.86 and 45.00±2.53 days respectively.
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