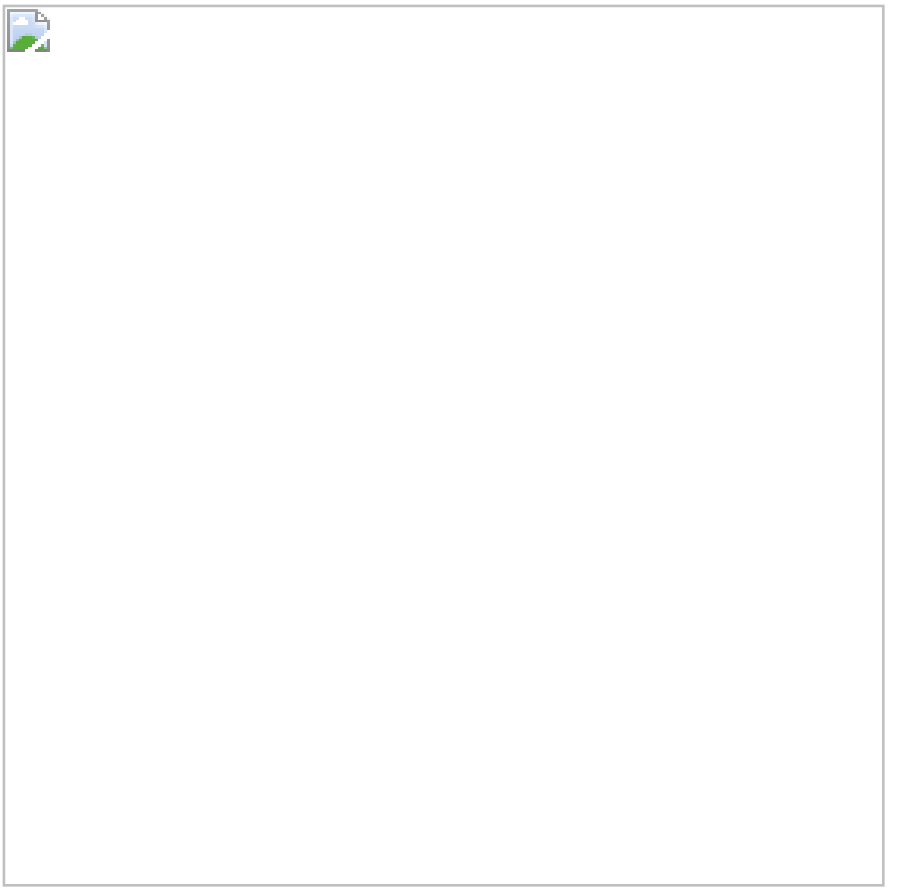

Brachygastra mellifica (Hymenoptera: Vespidae): Feeding Behavior and Preferential Predation on Diaphorina citri (Hempitera: Liviidae) Life Stages in México

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BRACHYGASTRA MELLIFICA (HYMENOPTERA: VESPIDAE): FEEDING BEHAVIOR AND PREFERENTIAL PREDATION ON *DIAPHORINA CITRI* (HEMPITERA: LIVIIDAE) LIFE STAGES IN MÉXICO

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ABSTRACT

In a lemon grove located at Rio Bravo, Tamaulipas, Mexico, during 2011-2012, a study was conducted through direct observations and video films to determine the predatory behavior of the Mexican honey wasp, *Brachygastra mellifica* (Hymenoptera: Vespidae), on the various developmental stages of the Asian citrus psyllid, *Diaphorina citri,* one of the most devastating citrus pests in the world. New flushes infested by eggs and the 5 instars of *D. citri* were manually exposed to this predator to study its preference for the different developmental stages. Other observations on its feeding behavior were made directly on infested flushes in the trees, during the foraging activity of the vespid. Results indicated that *B. mellifica* preferred the 4th and 5th instar *D. citri* nymphs more than the 2nd and 3rd instars. Eggs and first instar nymphs were mostly passed over because of their size and position in the flush. Occasionally, *B. mellifica* consumed *D. citri* adults. This predator represents a potential tool for natural control of *D. citri* in northern Mexico and southern USA through a program of management and conservation of beneficial insects.

Key Words: *Diaphorina citri, Brachygastra mellifica*, feeding behavior, Mexican honey wasp

RESUMEN

En un huerto de Limon Mexicano ubicado en Río Bravo, Tamaulipas, Mexico, durante 2011-2012, se realizó el presente estudio mediante observaciones directas y video filmaciones, para determinar el comportamiento de depredación del Vespido *Brachygastra mellifica* sobre estados de desarrollo del psilido Asiatico de los cítricos *Diaphorina citri*, una de las plagas más devastadoras de los cítricos en el mundo. Brotes tiernos infestados con huevos y ninfas de diversos instares fueron manualmente expuestos al depredador para estudiar su preferencia por los diferentes estados de desarrollo. Otras observaciones acerca de su comportamiento de alimentación se realizaron directamente en brotes tiernos de los arboles durante la actividad de forrajeo del depredador. Los resultados indicaron que *B. mellifica* prefirió depredar ninfas de 4° y 5° instar y después, ninfas de 2° y 3° instar. Huevos y ninfas de primer instar fueron prácticamente discriminados debido a su tamaño y posición en el brote. Ocasionalmente, *B. mellifica* depredo adultos. Este depredador representa una herramienta potencial para control natural de *D. citri* en el norte de Mexico y sur de EUA, a través de un programa de manejo y conservación de insectos beneficos.

Palabras Clave: *Diaphorina citri, Brachygastra mellifica*, comportamiento de alimentacion

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The Asian citrus psyllid *Diaphorina citri* Kuwayama (Hemiptera: Liviidae), is one of the world's more destructive pest of *Citrus* spp. (Sapindales: Rutaceae) in part because the adult is an efficient vector of the bacteria '*Candidatus* Liberibacter spp.' (Xu et al. 1998; Garnier & Bové 2000) the putative causal agent of the huanglongbing or greening disease that renders the fruit unpalatable and gradually kills the trees. A strategy and tactics eradicate *D. citri* have not been devised, but biological control of the psyllid should contribute to the suppression of their populations.

In Mexico, the main species attacking *D. citri* include the parasitoids, *Diaphorencyrtus* sp. (Hy-

menoptera: Encyrtidae) and *Tamarixia radiata* (Waterston) (Hymenoptera: Eulophidae), and the predators *Ceraeochrysa* sp. nr. *cincta* (Schneider), *Ceraeochrysa valida* (Banks), *Chrysoperla comanche* (Banks), *Chrysoperla externa* (Hagen), *Chrysoperla rufilabris* Burmeister (Neuroptera: Chrysopidae); *Azya* sp., *Brachiacantha decora* Casey, *Cycloneda sanguinea* (L.), *Harmonia axyridis* (Pallas), *Hippodamia convergens* Guérin-Méneville, *Olla v-nigrum* (Mulsant) (Coleoptera: Coccinellidae); *Allograpta obliqua* (Say), *Pseudodoros clavatus* (F.), *Toxomerus marginatus* (Say), *Toxomerus politus* (Say) (Diptera: Syrphidae); *Brachygastra mellifica* (Say, 1837) (Hymenoptera: Vespidae); and species of spiders. Also, adults of *D. citri* infected by the fungus *Hirsutella citriformis* Speare have been found (Lopez-Arroyo et al. 2008). Even so, there is relatively little known about the extent to which these beneficial organisms reduce infestations of *D. citri,* but some are regarded as important biological control agents.

Recently, the Mexican honey wasp *B. mellifica* was reported to be a predator of *D. citri* in a citrus grove in Tamaulipas, Mexico, where individual wasps were found to consume up to 31 fourth or fifth instar nymphs in just one session (Reyes et al. 2011).

Although a few studies on the biology and ethology of *B. mellifica* have been reported (Sugden & McAllen 1994; Hastings et al. 1998), scant information exists about its behavior as a predator of *D. citri*. This study was aimed to determine the preferences of *B. mellifica* for the various developmental stages of *D. citr*i, and to elucidate its feeding behavior.

Materials and Methods

Investigations were conducted during 2011-2012 at Rio Bravo, Tamaulipas, Mexico (N 25° 56' 39" W 98° 07' 9" and 28 m asl) in a lemon (*Citrus limon* (L.) Burm.f.) grove infested by *D. citri*. A nest of *B. mellifica* (30 × 40 cm) was found 50 m from trees where these studies were conducted, and this nest appeared to be the major source of the *B. mellifica* wasp population in this grove.

Preferential Predation of *B. mellifica* on Various Developmental Stages of *D. citri*

New leaf flushes, 5-10 cm in length, infested by eggs and various nymphal instars of *D. citri*, were removed from a lemon tree, and each infested leaf was detached from the flush. This was done to facilitate the filming of the movements and other behaviors of *B. mellifica* wasps by a video camera. As reported by Reyes et al. (2011) under natural conditions *B. mellifica* the wasp is adept at searching and feeding on *D. citri* in tree flushes, but the acquisition of adequate numbers film records of this aggressive wasp required special procedures.

Each infested leaf was secured with a short length of wire to square wood block was inserted in a wire which was introduced to a 2 × 4 × 5 cm wood square for an easy handling (Fig. 1). The wooden blocks facilitated the manipulation of the infested leaves for efficient recording of the numbers *D. citri* nymphs and other life stages consumed by the wasp and the times (seconds) required to do so. In some trials some of the developmental stages of *D. citri* were eliminated from the leaf by a small brush so that only a given stage or a few stages could be offered to the wasps. Groups of 20 flushes were utilized in the study, one group consisted of eggs and first instar nymphs, a second group consisted of second and third instar, and a third groups consisted of fourth and fifth instars. Each group was 1 treatment and the treatments were managed separately from each other in order to facilitate the registration of data. Without such simplification, the rapid movements of the wasp made data collection too difficult. The number of nymphs varied from 41 to 73 per flush. Data of the 3 treatments was analyzed together. Because of the difficulties of handling *D. citri* adults, their predation by *B. mellifica* was studied in situ in intact tree flushes.

The infested flushes were exposed to *B. mellifica* wasps when they were searching for prey in the grove. The duration of time that each wasp was active on a leaf flush varied depending on whether the wasp had already fed when it arrived at the flush and on whether it reacted to the manipulation of the leaf attached wood block. The movements and other behaviors of *B. mellifica* were recorded by a Canon® camera Power Shot S3IS, 6.0 Megapixel.

Feeding behavior of *B. mellifica* on *D. citri*.

The feeding behavior of *B. mellifica* was studied on 10 new flushes intact on the tree in order to elucidate the natural behavior of the wasp at a low population density. As before, all unwanted *D. citri* stages were eliminated, leaving only 3 to 5 fourth or fifth *D. citri* instars. If present, natural enemies were eliminated from every flush, and a sticker (Tanglefoot®) was applied to the petiole of the flush to exclude ants. Whenever the flush was visited by *B. mellifica*, the number of wasps, and the number of *D. citri* nymphs consumed during a 48 h period were registered. The associated behaviors of *B. mellifica* were filmed by the Canon® Power Shot camera.

Results

Preferential Predation of *B. mellifica* on Various Developmental Stages of *D. citri*

When flushes infested with eggs and *D. citri* first instars were exposed to *B. mellifica,* a maximum of 3 eggs or nymphs out of 100 individuals were consumed; these developmental stages were

Fig. 1. *Brachygastra mellifica* (indicated by the white arrow) feeding on *Diaphorina citri* on a lemon petiole secured with a short length of wire to square wood block.

practically passed over as a prey (Table 1). The time taken to consume 1 of these very immature developmental stages was 6 ±1 seconds (Fig. 2). In contrast when B. mellifica wasps were exposed to 2nd and 3rd instars a great increase in consumption per wasp occurred. Thus individual wasps consumed up to 42 second and third instar nymphs, and spent 17 seconds to predate each one (Fig. 2). Further one wasp was able to consume up to 27 fourth or fifth *D. citri* instars in 14 min, averaging 31 ± 2 seconds per nymph (Fig. 2); in some cases up to 37 seconds were required to consume 1 nymph, but others were consumed in less than 20 seconds. On most occasions *B. mellifica* consumed the entire prey. These developmental stages most preferred by *B. mellifica* were fourth and fifth instars (Table 1).

Because of difficulties in manipulating *D. citri* adults, the predation of *B. mellifica* on melanized or newly emerged *D. citri* adults was recorded by video film in intact flushes in the lemon grove. No control was exercised on the arrival or departure of the *B. mellifica* wasps, and none were marked. The film revealed that *B. mellifica* consumed more newly emerged adults (Fig. 3) than completely melanized adults. Probably this occurred because newly emerged adults they do not jump, or fly and their general movements are slow, whereas completely melanized adults have a consistently

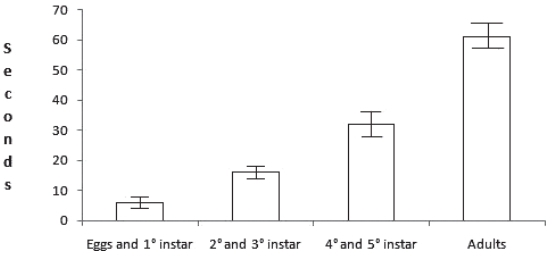

Fig. 2. Average time spent by *Brachygastra mellifica* to predate each individual of the various developmental stages of *Diaphorina citri* at Rio Bravo, Tamaulipas, Mexico during Jul 2012.

hard ornamented body, and they can jump and fly. Even though some predation of melanized adults (Fig. 4) was observed, mostly the newly emerged adults were consumed. Thus we were unable to count the number of *D. citri* adults that were consumed by *B. mellifica*, but the mean duration of feeding was 61 ± 4 s per adult *D. citri*.

Feeding behavior of *B. mellifica* on *D. citri*.

The appearance of *B. mellifica* wasps in the lemon grove did not always coincide with the ini-

TABLE 1. NUMBERS OF EGGS AND VARIOUS INSTARS OF THE CITRUS ASIAN PSYLLID, *DIAPHORINA CITRI,* CONSUMED PER MEXICAN HONEY WASP, *BRACHYGASTRA MELLIFICA* ON NEWLY FLUSHED LEMON LEAVES IN JULY 2012 AT RÍO BRAVO, TAMAULIPAS, MEXICO.

4th and 5th instars	21.75 ± 4.91 a
2nd and 3rd instars	9.8 ± 9.41 b
Eggs and 1st instars	0.45 ± 0.89 c

*Means followed by the same letter are not significantly different (Tukey $\alpha = 0.05$).

tial appearance of *D. citri* nymphs on the flushes. On the contrary the wasps arrived gradually at any time of the day. Their major foraging activity was observed to be between 800 and 1200 h, and from 1600 to 1900 h during the spring and summer seasons, and from 1000 to 1600 h during the fall and winter seasons, when the incidence of foraging by these wasps was diminished.

Major incidence of *B. mellifica* in the lemon grove was during Jul, Aug and Sep, coincident with the higher abundance of *D. citri* populations in the region (Reyes et al. 2010). During this period, copious rainfall occurred, and the daily temperature reached around 35 °C, and the lemon trees flushed profusely. In all cases during this period, the occurrence of the wasps in the grove was associated with the presence of *D. citri*, but when the psyllids were absent, the wasps practically disappeared until the next spring. However the reappearance of the wasps in the spring was associated with the presence of the citrus leaf miner *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillaridae).

Direct observations on *B. mellifica*, indicated that they spent 10 seconds or less, per session, searching for *D. citri* in flushes, and that they always checked new intense green flushes, and avoided mature leaves. When each flush contained 3 nymphs, *B. mellifica* consumed all of them in 7 out of 10 observed flushes; and when each flush contained 5 nymphs, *B. mellifica* consumed all of them in 9 out of 10 observed flushes. Not all nymphs were consumed in one session. *Brachygatra mellifica* visited each flush from 0 to 6 times per 1 h.

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When feeding, a *B. mellifica* dislodged *D. citri* nymphs from the newly flushed leaf with its mandibles, and chewed them rapidly and always used its fore legs to manipulate the prey (Fig. 5). Analysis of a video revealed that the *B. mellifica* wasp feeds not only on the fluids extracted from the nymph, but may also partially or totally consume the exoskeleton. While feeding, the wasp constantly and gently rubbed the prey with its antennae (Fig. 5). The wasp used its hind and middle legs to hold onto citrus plant parts, and often while feeding used its posterior legs to clean wax particles from the body of the prey and to discard remnants of its exoskeleton.

Some wasps behaved in different ways while feeding. Commonly they consumed *D. citri* as mentioned above, and rarely allowed a nymph to fall away. Yet occasionally some wasps behaved in an agitated manner, and apparently clumsily, when manipulating the nymphs, which were chewed for only a few seconds and then discarded; this behavior was shown repeatedly until the wasp was satisfied or moved away. The only time when numerous *D. citri* nymphs fell from the leaf was during the foraging movements of *B. mellifica*.

The predatory activity of a *B. mellifica* wasp was interrupted only when the wasp encountered a non-identified species of honey dew-harvesting ant (Hymenoptera: Formicidae) protecting the colony of *D. citri* nymphs. Whenever a *B. mellifica* wasp encountered an ant guarding a psyllid colony, the wasp appeared to attempt to drive away the ant with trains of wing beats each of 10 to 100 s in duration, and when this failed, the wasp departed and moved to another flush.

Although we did not make quantitative measurements on the predatory behaviors of other species of *D. citri* predators, we that gained the impression that *B. mellifica* explored each flush and moved between flushes more rapidly than other *D. citri* predators including the Japanese lady beetle, *Harmonia axyridis* (Pallas), and the red spotless lady beetle, *Cycloneda sanguínea* (L.) (Coleoptera: Coccinellidae).

DISCUSSION

Brachygastra mellifica populations were very abundant during Jul and Sept when they were associated with *D. citri* populations, which were also abundant in citrus groves during these months (Reyes et al. 2010). Occasionally, this predator was present during Feb and Mar when infestations of *P. citrella,* the citrus leafminer occur, but we have not made observations to determine if *B. mellifica* feeds on this leafminer.

The wasps never hesitated to attack and consume *D. citri* psyllids located on flushes when they were manually manipulated as mentioned above (Fig. 1). The video film revealed that *B. mellifica* did not behave differently when consuming psyllids on naturally infested flushes than on artificially manipulated infested flushes in the citrus trees. A video camera is especially useful for filming behaviors of predators in relation to

Figs. 3-6. 3. A non-melanized *Diaphorina citri* adult showing its characteristic white color, 4. Melanized adults of *D. citri,* 5. *Brachygastra mellifica* feeding on *D. citri* nymphs, and 6. Size comparison between a 1st instar (arrow) and a 5th instar (circled) *D. citri* nymph at Río Bravo, Tamaulipas, México.

relatively sedentary prey such as *D. citri* (Suderland et al. 2005).

During Jul-Aug uncontrolled infestations of *D. citri* in Mexican lemon, C. *limon* (L.) Burm. F., tend to be heavy, we found about 100 nymphs and 30 adults per flush, and this high density of prey precluded the ready study of the feeding behavior of *B. mellifica*. However in May and in Sep, when *D. citri* infestations were low, and flushes with known numbers of *D. citri* individuals were exposed to the wasps, we recorded a high percentage of predation, i.e., 70% when flushes had 3 nymphs each and 90% when flushes had 5 nymphs. The wasps required several sessions in the same day to consume all of the nymphs. There are reports on *B. mellifica* consuming other insects, such as larvae of *Anthonomus aeneolus* Dietz, and a moth of the family Alucitidae (Sugden & McAllen 1994), however, very little is known about the predatory action of *B. mellifica* on *D. citri*.

The only previous record of *B. mellifica* feeding on fourth and fifth nymph instars of *D. citri*, and spending an average of 30 s per individual was the report of Reyes et al. (2011), whose results were very similar to those of the present study. In the present study, *B. mellifica* was a main predator of fifth instar nymphs as well as fourth instars. Eggs and first instars were almost passed over, probably as a response to their size, which for eggs are 0.3 and 0.14 mm in width and length, respectively, and 0.3 and 0.17 mm for the first instar (García-Darderes 2009). These dimensions are too small for facile manipulation by the mandibles of *B. mellifica*, which are 1.1 mm in long and 0.6 mm wide. In contrast *B. mellifica* can readily manipulate the older instars which are 1.02 mm wide and 1.6 mm long (García-Darderes 2009). Moreover the relatively small size of eggs and first instars provides the possibility to shelter between the petioles or stems of the flushes, and hence out of the reach of the wasps. Also, it is more energy efficient and easier to find and consume large sized prey than small ones (Fig. 6). Our results show that *B. mellifica* can feed on all developmental stages of *D. citri* including adults, which move evasively once they detect this enemy.

Ants are attracted to *D. citri* colonies because of the psyllid excretes sugary honey dew (Chien & Chiu 1996). It is known that ants play an important role in protecting these hemipterans as well as aphids (Aphidae) and white flies (Aleyrodidae) from their natural enemies (Queiroz et al. 2001; Kaplan & Eubanks 2002).

An outstanding attribute shown by *B. mellifica* was its preference to feed on last instar nymphs. As much as 80%, or more, of insect mortality occurs during the first developmental stages (Rodríguez et al. 2000; Naranjo et al. 2004), but high levels of mortality of the early stages have much less impact on the population density of the next generation than comparable levels of mortality of the last developmental stages (Southwood 1978; Rodríguez et al. 2000). Thus because of the preference of *B. mellifica* for late stage nymphs, this predator may have a greater impact on *D. citri* control than other predators and parasitoids of eggs or first instars.

Brachygastra mellifica ranges from Central America to southern USA (Sugden & McAllen 1994). We confirmed the finding of Hastings et al. (1998) that one mature nest of *B. mellifica* may host between 3,496 to 18,698 individual wasps (Hastings et al. 1998); and if one half of them behave as predators and each consumes 30 *D. citri* nymphs per day, as we observed, then the daily consumption of *D. citri* nymphs by wasps individuals from 1 nest should vary from 52,440 to 280,470. Unfortunately we were not able to register the number of predation sessions of a given wasp per day. Under the conditions of our experiments, we could not establish from where each wasp was coming, nor how much food it had already consumed. Since the individual wasps were not uniquely, the data that we registered were only those pertaining to the wasp that was being filmed or directly observed.

During 2009, *B. mellifica* wasps were found consuming *D. citri* in a lemon grove in our region but we did not locate a nearby nest. However we postulate that in or near citrus groves where this wasp is present, there is good control of *D. citri* at the beginning of the infestation. Thus we suggest that *B. mellifica* has high potential for biological control of *D. citri*, and that the research on its mass rearing or the relocation of its nests into citrus groves should be undertaken.

Conclusion

Brachygastra mellifica predated all developmental stages of *D. citri,* however, fourth and fifth instar nymphs were preferred, while eggs and first instar nymphs were almost discriminated.

Per session or visit, it can consume more than 40 second and third instars, and from 27 to 31 of fourth and fifth instar. *Brachygaster mellifica* required the average times of 6 s to consume one *D. citri* egg or first instar, 17 s to consume one second or third instar, 31 s to consume one fourth or fifth instar and one min to consume one adult. Finally *B. mellifica* was observed to be an effective predator both of heavy and low *D. citri* infestations, a desirable of a biological control agent of use in an integrated pest management program.

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