Host-age effects and the efficiency of the pupal parasitoid *Dirhinus giffardii* (SILVESTRI, 1913) (Hymenoptera: Chalcididae) against the melon fly *Bactrocera cucurbitae* (COQUILLET, 1849) (Diptera: Tephritidae)

SHAHID MEHMOOD^{1,2*}, KAMRAN SOHAIL^{2*}, MUHAMMAD QASIM³,
SHUPENG XU¹, HAIYANG GENG¹, MUHAMMAD USMAN⁴, ZELLE HUMA⁵

¹Lab of Honeybee Molecular Breeding & Health, College of Bee Science, Fujian Agriculture and Forestry University, Fuzhou-350002, China

²Department of Entomology, The University of Agriculture Peshawar-25000, Pakistan

³Insect Ecology Lab, College of Plant Protection, Fujian Agriculture and Forestry University, Fuzhou-350002, China

⁴Department of Entomology, Bacha Khan University Charsadda-24420, Pakistan

⁵Department of Plant Protection, The University of Agriculture Peshawar-25000, Pakistan

ABSTRACT. *Dirhinus giffardii* is an efficient and aggressive pupal parasitoid of tephritid flies, including *Bactrocera cucurbitae*. Here we report on the various biological aspects of *Dirhinus giffardii*, mainly host-age preference, potential as well as the survival of this parasitoid from the pupae of different ages and its *in vivo* release against *Bactrocera cucurbitae*. The emergence rate of parasitoids was higher in old pupae than in fresh pupae. Similarly, the lifespan and potential of parasitoids emerged from older pupae were higher than those emerged from younger ones. The parasitoids reared on older pupae significantly controlled melon fly infestation *in vivo*. These findings suggest that parasitoids reared on older pupae of *B. cucurbitae* have a greater potential regarding fecundity, parasitism performance and survival, as these parasitoids effectively suppressed populations of *B. cucurbitae* under greenhouse conditions.

KEY WORDS: Cucurbit, Parasitoid wasp, Greenhouse conditions, Organic agriculture, Pesticide free, *Bactrocera cucurbitae*.

* Corresponding authors: shahidbee@aup.edu.pk, sohailagrian@hotmail.com

INTRODUCTION

The melon fly *Bactrocera cucurbitae* (COQUILLET, 1849) (Diptera: Tephritidae), one of the most destructive pests of vegetables, was first reported from India; it is also widely distributed in Pakistan, China, Nepal, New Guinea, the Philippines and the Hawaiian Islands, and all over most of southern Asia (WEEMS & HEPPNER 2001). It damages over eighty-one (81) host plants and is reported to be a major pest of cucurbitaceous vegetables (DHILLON et al. 2005). In Pakistan, the melon fly causes 50-90% losses of fruit. Guava, mango, citrus, melon and cucurbits are seriously affected (SAJJAD et al. 2017). The female fly lays her eggs in the soft, tender tissues by piercing them with the ovipositor; this results in a watery fluid oozing from the puncture and maggots developing inside the fruits (JACKSON et al. 2003). This species can also attack non-cucurbit hosts, damaging their flowers, stem and root tissues (OKE 2008).

The biological control of tephritid flies is gaining importance and succeeding in tropical and subtropical regions; successful applications have been reported from Hawaii, Florida, the USA, Fiji and Europe, and these are encouraging researchers to rear parasitoids and carry out field tests (PURCELL 1998). On Reunion Island, *Fopius arisanus* (SONAN, 1932) decreased the infestation and population rate of *Bactrocera dorsalis* (HENDEL, 1912), *B. tryoni* (FROGGATT, 1897) and *B. kirki* (FROGGATT, 1911) in guava fields: this is considered the classical biological method for the control of fruit flies outside Hawaii (VARGAS et al. 2007). The native Australian braconid parasitoids *Diachasmimorpha tryoni* (CAMERON, 1911) was applied against *Bactrocera tryoni* in the Australian region and was used to great effect in Hawaii and other locations (ZAMEK et al. 2012). *Pachycrepoideus vindemmiae* (RONDANI, 1875) can also be used as a biocontrol agent against *Bactrocera dorsalis*; it also has the capability to parasitize melon fly pupae at every stage (ZHAO et al. 2013a, 2013b). The hymenopteran *Spalangia endius* (WALKER, 1839) is the dominant pupal parasitoid of fruit flies and was successfully used against different-aged pupae of *Bactrocera cucurbitae* *in vitro* (TANG et al. 2015).

Dirhinus giffardii (SILVESTRI, 1913) (Hymenoptera: Chalcididae) is a general parasitoid that attacks a wide range of fruit-infesting tephritids, including *Bactrocera cucurbitae*, *B. dorsalis*, *B. tryoni* and *B. oleae* (ROSSI, 1790) (WHARTON & YODER 2018). This parasitoid is native to West Africa, and its original host being *Ceratitis capitata* (WIEDEMANN, 1824). Besides tephritids, *Dirhinus giffardii* has the ability to parasitize dipterous houseflies (MOHAMED et al. 2006, WHARTON & YODER 2018). It has also been used for the first time in West Africa to control Black Soldier fly (DEVIC & MAQUART 2015).

Dirhinus giffardii penetrates the puparial wall of the fruit flies and oviposits inside the puparium, emerging from the pupa after completing its larval stage. It can produce larger offspring when females attack larger host species (WANG & MESSING 2004a). The egg

laying rate of *D. giffardii* starts to decline as it grows older, so for efficient mass rearing, it has been suggested that the parasitoids be discarded after 15 days (SHAH et al. 2014). Earlier, studies were undertaken to discover the efficacy of *D. giffardii* on differently aged pupa, but little research has been done on the potential, survival and the parasitism performance of this chalcid parasitoid wasp. Here we present these outcomes *in vitro* and *in vivo*; furthermore, the present study was planned to evaluate the performance of *D. giffardii* in controlling melon fly infestation in a summer squash field.

MATERIALS AND METHODS

Rearing of melon flies and *Dirhinus giffardii*

The experiment was carried out at the Nuclear Institute of Food & Agriculture (NIFA), Peshawar, Pakistan. Individual melon flies were obtained from a culture maintained in the fruit fly laboratory ($27 \pm 1^\circ\text{C}$, $60 \pm 5\%$ RH with 14:10 h photoperiod); fruits of summer squash (*Cucurbita pepo* L.) were used as a food source or host. Fresh cucurbits were provided on a regular basis and were placed in the melon fly cage ($1 \times 1 \times 1$ m). After two to three days, the infested fruits were transferred from the melon fly cage to a new wooden container covered with wheat bran (3 cm) for easy collection of pupae using plastic sieves. The newly emerged larvae lived inside the fruit and fed for 5-6 days until maturity; on completion of the larval stage, the larvae jumped into the wheat bran container for pupation. Pupae were sieved from this container and placed in the fruit fly cage for culturing. Sugar, yeast, honey and water were regularly supplied as a food supplement in Petri dishes. The cotton swabs in the Petri dishes were replaced regularly to prevent contamination.

Dirhinus giffardii adults were brought from Tando Jam, Punjab, Pakistan. Adults (3-5 mm) were reared and maintained in large glass cages ($30 \times 30 \times 30$ cm); the ambient temperature was $15 \pm 1^\circ\text{C}$. Cotton swabs dipped in a syrup of honey and water were placed in the cage as food supplements. The previous rearing technique described by WANG & MESSING (2004b) was followed and modified for the *in vitro* tests: we used small Plexiglas cages ($15 \times 15 \times 15$ cm, easy to observe) with 70-80 parasitoids per cage. Two- to four-day old melon fly pupae were supplied to the parasitoids in the Petri dish; after an exposure time of 8-24 h, the Petri dish was taken from the cage and placed in a new one for culturing.

Host-age preference, survival and parasitism potential of emerged parasitoids

After completing the maggot stage inside the fruit, the melon fly larva jumped from the pupae for pupation, which took around 8-9 days (MIR et al. 2014). To investigate host-age preference, pupae of different ages (12, 24, 48 and 72 h) were selected during the pupal period. These were transferred to 7 cm diameter Petri dishes containing a layer of wet tissue paper, and a 1 cm layer of wheat bran to prevent the pupae from drying out. Later, the different groups of melon fly pupae were provided to *Dirhinus giffardii* for 24 h, after which the pupae were moved from the *D. giffardii* cage to new, small glass jars for recording the emergence rates of parasitoids. In addition, 20 newly emerged parasitoids from melon fly pupae of different ages were transferred to new, small glass jars for screening and observing their survival and mortality levels. In addition, 200 melon fly pupae (age not recorded) were supplied for two days to parasitoids emerged from fresh pupae and older pupae in order to record the parasitism efficacy of each group (≥ 24 h and ≤ 48 h) *in vitro*. The cages were shuffled, and the experiment was repeated three times. All the data were collected on a daily basis.

In vivo performance of *Dirhinus giffardii* emerged from pupae of different ages

To investigate the *in vivo* performance and infestation control rate between parasitoids emerged from pupae of different ages, a field experiment was carried out at the NIFA experimental block. The summer squash transplants were hand-planted in three different field plots, i.e. the ≥ 24 h parasitoids plot (parasitoids emerged from 12 and 24 h pupae), the ≤ 48 h parasitoids plot (parasitoids emerged from 48 and 72 h pupae) and the control plot (plot with no parasitoids or untreated). The basic experimental design and techniques according to NESMITH (1993) were followed but modified for the summer squash plantation. Each well-drained soil plot was 20 rows wide (20 m, row-row distance 1 m) and 10 m long (10 transplants per row, plant-plant distance 1 m). In addition, the field plots were cultivated with pre-control measures and were covered completely with thin white sieving cloth from the very first day to prevent the entry of other insect pests. Honey bees (*Apis mellifera* LINNAEUS, 1761) in a small hive box (10×10×10 cm) were used as pollinators in each field plot. After several weeks, the field plots were inspected for fresh fruiting. At the same time 40 adult melon flies (19 to 21 aged individuals) were released in each field plot. After 7 days' exposure of the melon flies in the fields, 600 *Dirhinus giffardii* individuals (3 per plant manually) were released in each treated field plot (excluding the control field). The presence and activities of *D. giffardii* were observed on a daily basis. The experiment was carried out using a completely randomized block design with three replicates. 200 fruits (one from each plant) were selected randomly every week in order to acquire infestation data.

Data analysis

All the experiments were performed at least three times. The emergence and survival rates of *Dirhinus giffardii* and infestation control rates of *Bactrocera cucurbitae* were recorded at different time points. All values are expressed as means $\pm$ standard error. Analysis of Variance (ANOVA) and correlation analysis were used to evaluate the correlation between the number of parasitoid emergences and pupal age (12, 24, 48 and 72 h). ANOVA was also used to assess the significant differences between the fresh-pupae group (12 and 24 h) and the old-pupae group (48 and 72 h), and the significant differences of infestation among the three groups in each week. Pairwise comparisons for significant differences were evaluated using Tukey's post-hoc test. The Kaplan-Meier Survival Function was used to evaluate the survival of parasitoids emerged from pupae of different ages. Finally, pairwise comparisons for significant differences were evaluated using Log Rank (Mantel-Cox) and Breslow (Generalized Wilcoxon) tests.

RESULTS

***Dirhinus giffardii* host-age preference**

Melon fly pupae of different ages were provided to *D. giffardii*, the emergence results were nearly the same in each treatment. The correlation was significant at the 0.01 level (two-tailed) with a Pearson correlation value of 0.971 (Table 1). Furthermore, the emergence of parasitoids in fresh 12 h pupae showed the lowest rate of emergence in all replicates; this was followed by the 24 h pupae group with mean values of 28.00 ± 0.58 and 29.33 ± 0.33 , respectively. Although a significant difference was observed in older pupae ($p < 0.0001$), the highest emergence rate of parasitoids was recorded with 72 h followed by 48 h old pupae (mean values of 39.00 ± 0.58 and 36.30 ± 0.33 , respectively). These results indicated a high emergence rate in the 72 h pupae among the different age-groups in all replicates (Fig. 1). The parasitism performance of parasitoids emerged from the older pupae group (48 and 72 h) was good enough in comparison with the fresh-pupae group (12 and 24 h). Moreover, the number of emergences recorded in the fresh group was significantly lower than in the old pupae group, with an F value of 73.778 and mean values of 113.67 ± 3.712 and 150.67 ± 2.186 , respectively (Fig. 2).

Table 1. Correlation between parasitoid emergence and age of pupae.

		Age	Number
Age	Pearson correlation	1	**0.971
	Sig. (2-tailed)		0.000
	N	12	12
Number	Pearson correlation	**0.971	1
	Sig. (2-tailed)	0.000	
	N	12	12

** Correlation is significant at the 0.01 level (2-tailed).

Fig. 1. The emergence rate of *Dirhinus giffardii* individuals from *Bactrocera cucurbitae* pupae of different ages. The columns marked 12, 24, 48 and 72 represent the age of the pupa (in hours) provided to *Dirhinus giffardii* for parasitism. The different capital letter represent significant difference at the 0.01 level, the different lowercase letter represent significant difference at the 0.05 level.

Survival of emerged parasitoids from host pupae of different ages

The survival of newly emerged parasitoids from parasitized pupae of different ages (12, 24, 48 and 72 h) was observed during 1-20 days. The mean survival times were 10.567 ± 0.805 , 10.783 ± 0.796 , 14.550 ± 0.800 and 13.783 ± 0.798 days, respectively. Pairwise comparisons showed that the longevity of newly emerged parasitoids from the old-pupae group (48 and 72 h) was significantly longer than fresh-pupae group (12 and 24 h), with $P < 0.01$ (Table 2, Fig. 3).

Fig. 2. The parasitism level of parasitoids, emerged from groups of different ages. ≤ 24 h represent the parasitoids emerged from 12 and 24 h old pupae, while ≥ 48 represent the parasitoids emerged from 48 and 72 h old pupae. The different capital letter represent significant difference at the 0.01.

Table 2. Pairwise comparisons for significant differences with Log Rank (Mantel-Cox) and Breslow (generalized Wilcoxon). The figures 12.00, 24.00, 48.00 and 72.00 represent the parasitized pupa; the parasitoids emerging from lower age-pupae live shorter than the other group (48 and 72 h).

Group		12.00		24.00		48.00		72.00	
		χ^2	Sig.	χ^2	Sig.	χ^2	Sig.	χ^2	Sig.
Log Rank (mantel-Cox)	12.00			0.027	0.869	16.028	0.000	9.450	0.002
	24.00	0.027	0.869			14.631	0.000	8.323	0.004
	48.00	16.028	0.000	14.631	0.000			1.141	0.285
	72.00	9.450	8.323	8.323	0.004	1.141	0.285		
Breslow (generalised Wilcoxon)	12.00			0.047	0.829	13.630	0.000	8.523	0.004
	24.00	0.047	0.829			12.329	0.000	7.392	0.007
	48.00	13.630	0.000	12.329	0.000			0.875	0.350
	72.00	8.523	0.004	7.392	0.007	0.875	0.350

Fig. 3. Survival rate of parasitoids emerged from parasitized pupae of different ages (12, 24, 48 and 72 h). The figures 0.00 - 20.00 on the x-axis represent survival time in days. The different capital letter represent significant difference at the 0.01 level, the different lowercase letter represent significant difference at the 0.05 level.

The effectiveness of parasitoids in controlling the infestation rate of *Bactrocera cucurbitae* in vivo

In the first three weeks after the parasitoids had been released, the results showed a roughly similar infestation in each field plot (≤ 24 h parasitoid plot, ≥ 48 h parasitoid plot and control), which started to increase after the third week. A significant variation in infestation was recorded among three groups after the third week. The parasitoids emerged from the 48 h and 72 h pupae exhibited a significant level of infestation control in comparison with parasitoids reared on 12 h and 24 h individuals. The highest level of infestation was recorded in the untreated or control field plot (Fig. 4).

Fig. 4. *In vivo* efficacy of *Dirhinus giffardii* against the melon fly; ≤ 24 h represents the parasitoids emerged from 12 and 24 h old pupae, while ≥ 48 represent the parasitoids emerged from 48 and 72 h old pupae used in the different field plots. “Control” means that the field plot was untreated or that no parasitoids were released. The different capital letter represent significant difference at the 0.01 level, the different lowercase letter represent significant difference at the 0.05 level.

DISCUSSION

As host age plays a very important role in the development of parasitoids, it is important for a parasitoid to select a host of suitable age for its development. Parasitoids can discriminate between host pupae of different ages, and then select a suitably aged host for parasitization; this offers an apparent advantage for the survival of the parasitoid population (DAHLMAN & BRADLEIGH 1976). The present study confirmed that the pupal parasitoid *Dirhinus giffardii*, used to parasitize older-aged melon fly pupae, preferred older puparia to fresh ones throughout this study (WANG & MESSING 2004b, NAVEED et al. 2014). Different hosts, different host instars or different host pupal ages may have different nutritional qualities (HARVEY 2000, HARVEY & STRAND 2002). We provided pupae of different ages

to *D. giffardii* individuals, and the results showed the highest level of parasitism in the oldest pupae. An identical maximum level of parasitism was recorded when *D. giffardii* individuals were used against *Bactrocera zonata* (SAUNDERS, 1842) at various depths of plant debris (ALI et al. 2016). Similarly, some other parasitoid wasps prefer older puparia for oviposition when they have a choice (HE & WANG 2006, HE et al. 2011, TANG et al. 2015).

For a number of pupal parasitoids, the age at which the host pupa is parasitized influences offspring survival and parasitism performance. The present findings of the longevity and potential of parasitoids (*Dirhinus giffardii*) have been confirmed by those of EL-HUSSEINI et al. (2008), who stated that old parasitoid pupae are better than young ones. They lived longer and developed more quickly in comparison with parasitoids emerged from smaller pupae. Furthermore, a high mortality rate was recorded in younger parasitoids emerged from fresh pupae in the first week of emergence. The lower rate of parasitism on young pupae was associated with the relatively higher mortality of host pupae and emerging insects, suggesting that young pupae are more susceptible to injury caused during oviposition, including venom injections or stings or both. In contrast, parasitism did not seem to induce mortality in older pupae. Food quality is one of the most important factors, as pupae of different ages may not have the same nutritional quality. During pupal development, internal tissues undergo histolysis, histogenesis and differentiation to form adult internal organs and sclerotized appendages. Thus, older pupae may contain fewer resources for developing parasitoids (ECKENRODE & CHAPMAN 1971). The oviposition decisions (i.e. host selection and sex allocation) of female parasitoids are expected to correspond with host quality, as the fitness of their offspring is dependent on the amount and quality of resources provided by a single host (STEPHENS & KREBS 1986). Previously, different hymenopteran and braconid parasitoids were tested on host-pupae of different ages for their development, potential and progeny; this showed that older hosts or pupae or instars can increase the progeny level and development of parasitoids (IMANDEH 2006, WANG et al. 2008, TANG et al. 2015, SITTHICHAIAKUL & AMORNSAK 2017). This suggested that there were some cues that allowed the parasitoids to discriminate between the hosts of different ages. Most parasitoids have the ability to determine host quality during oviposition (WANG & LIU 2002, CHINWADA et al. 2003, LI et al. 2006, RORIZ et al. 2006); however, some prefer host pupae of medium age for their development (PFANNENSTIEL et al. 1996, COLINET et al. 2005). The ability of parasitoids to cope with hosts of variable quality would have the overall effect of determining host range.

Biological control using parasitoid wasps is an effective and safe approach for controlling tephritid flies. The method is successful and worth applying in the control of these pests (PURCELL 1998, VARGAS et al. 2007, ZAMEK et al. 2012, TANG et al. 2015). The parasitoid individuals in the summer squash field significantly controlled the fruits

infestation level (Fig. 4). They parasitized *Bactrocera cucurbitae* pupae in the soil and were completely developed in the field conditions. We used *Dirhinus giffardii* for the first time *in vivo* against melon flies in cucurbit fields; however, this parasitoid has been successfully used and developed in field conditions against different fruit fly species in mango orchards (SHAH et al. 2014, SARWAR et al. 2015). Like other parasitoid species parasitizing tephritid flies (WONG et al. 1992, ARGOV & GAZIT 2008).

The symptoms of melon fly disease in the cucurbit fruits are quite apparent: this must affect the market value of vegetables, with consequent capital losses to farmers. The experimental findings showed that this notorious pest can be effectively controlled, and it gave an easy direction to control *Bactrocera* MACQUART, 1835 species mainly melon fly. Furthermore, environmentally-friendly control of insect pests for the human wellbeing and environmental conservation is necessary.

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Received: 24 November 2017

Accepted: 6 February 2018