



## Evaluation of Host Stinging Behavior of the Larval Ectoparasitoid *Diglyphus isaea* on Some Selected Host Plants

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### Authors' contributions

This work was carried out in collaboration among all authors. Author ARE designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors HAS and ARE managed the analyses of the study. Author AAAH managed the literature searches. All authors read and approved the final manuscript.

### Article Information

DOI: 10.9734/AJAAR/2020/v14i330134

#### Editor(s):

(1) Dr. Wuyi Liu, Fuyang Normal College, China.

#### Reviewers:

(1) Ekhlash M. Idan, University of Baghdad, Iraq.

(2) Fatimah J. Al-Hasani, University of Technology, Iraq.

Complete Peer review History: <http://www.sdiarticle4.com/review-history/62346>

Original Research Article

Received 18 August 2020  
Accepted 23 October 2020  
Published 21 November 2020

### ABSTRACT

**Background:** In parasitoid insects, beside parasitism, there are two other behaviors that may increase leaf miner mortality: Feeding on the host (host feeding) and, host penalization without oviposition or feeding (host stinging).

**Objectives:** The present study aimed to evaluate (host stinging) behavior of the larval ectoparasitoid *D. isaea* in field on some selected host plants.

**Methods:** The present study was carried out in Alejelat region. Four selected host plants were studied, chick pea (*Cicer arietinum*) and broad bean (*Vecia faba*) as a Winter host plants and, Cow pea (*Vigna unguiculate*) and kidney bean (*Phaseolus vulgaris*) were selected as summer host plants during the growing seasons 2018 and 2019. Samples were kept in plastic bags and transferred to be examined in the laboratory. Number of living *L. trifolii* larvae, and number of killed larvae according to feeding (no oviposition) by *D. isaea* were counted and recorded.

**Results:** On cow pea host stung larvae recorded (156 individuals/ 100 infested leaflets) occurred in 10<sup>th</sup> of August, with ( $r = +0.69^{**}$ ) between host stung larvae and the population of *L. trifolii*

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recorded). On kidney bean host stung larvae recorded (101 individuals/ 100 infested leaflets) occurred in 24<sup>th</sup> of August, with ( $r = + 0.85^{**}$ ) value between host stung larvae and the population of *L. trifolii* recorded. On the other hand, the percentage of host stung larvae ranged between (5.67% and 28.22%) and (15.88% and 42.30%) for cow pea and kidney bean respectively. On broad bean host stung larvae recorded (159 individuals/ 100 infested leaflets), with ( $r = +0.59^*$ ) between host stung larvae and the population of *L. trifolii*. On chick pea host stung larvae recorded (82 individuals/ 100 infested leaflets) with ( $r = +0.20^{ns}$ ) between host stung larvae and the population of *L. trifolii*. On the other hand, percentages of host stung larvae ranged between (8.45% and 36.60%) and (21.80% and 87.65%) for broad bean and chick pea respectively.

**Conclusion:** The highest percentage of host stung larvae recorded on chick pea 44.65% followed by kidney bean 24.21%, broad bean 18.45% and cow pea 12.97% respectively. On the other hand, the correlation coefficient value ( $r$ ) between numbers of *L. trifolii* larvae and, host stung larvae recorded the highest on kidney bean  $+0.85^{**}$  followed by cow pea  $+0.69^{**}$ , Broad bean  $+0.59^*$  and Chick pea  $+0.20^{ns}$  respectively.

**Keywords:** *D. isaea*; host stinging; *L. trifolii*.

## 1. INTRODUCTION

*Diglyphus* (Eulophidae) is an economically important genus of solitary ectoparasitoids against Agromyzid leaf miners, with a wide distribution worldwide (39 species) [1]. The female adult wasps of *Diglyphus* species show three types of host-killing behavior, reproductive parasitism (parasitism), non-reproductive host killing by feeding (host feeding), and host stinging without oviposition or feeding (host stinging), with the non-reproductive host-killing behavior (host feeding and host stinging) the biocontrol potential is significantly increase [2,3,4] reported that, for these species that express different types of host killing, it is very important to evaluate all of them separately and cumulatively to acquire information on their precise biocontrol potential.

*D. isaea* is the most common synovigenic idiobiont parasitoids of Agromyzid leaf miner species, parasitizing host larvae and feeding on host hemolymph (Zhang et al. 2014). *D. isaea* is a biparental, arrhenotokous ectoparasitoid, and a non-concurrent and destructive host feeder [5]. In various parasitoids, and particularly ectoparasitoids like *D. isaea*, induced host nonreproductive mortality, through host feeding and stinging, have been reported to be very critical in parasitoids' performance measurement [6].

Nicoli and Pitrelli [7] reported that, Besides the mortality induced by larval parasitic activity, *D. isaea* females can also cause host mortality by host feeding behavior. Females of the parasitoid sting host larvae (normally, 1<sup>st</sup> and 2<sup>nd</sup> instar larvae) with their ovipositor, feed on the body

fluids that come out and kill them. On the other hand, *D. isaea* can be seasonal inoculative release, of which control on pest population is obtained over many pest generations and can have a long-term impact if crops are grown for a season-long period [8].

Some parasitoids use hosts of different sizes, either as a substrate for egg laying or to feed upon [9]. The magnitude of the mortality due to host feeding may be similar to, or even higher than, that caused by the parasitism itself [10]. However, the effect of host feeding, functionally comparable to predation, is frequently ignored. This may greatly underestimate the levels of mortality caused by parasitoid species [11]. In some leaf miner parasitoid species, particularly belonging to the genera *Diglyphus*, penalization and death of leaf miner larvae are often observed without them being used as an oviposition substrate or to feed on [12]. Fu-YuYe et al. [13] reported that, *D. isaea* females fed on 94.4 hosts during a lifetime and killed 12.2 hosts per day. The host-feeding events accounted for 66.5% of the host-killing events. The trend in daily host-feeding events gradually increased and there was a small decrease between the 4<sup>th</sup> day and the 5<sup>th</sup> day. The host-feeding event was highest on the 4<sup>th</sup> day at 14.9 hosts.

## 2. MATERIALS AND METHODS

The present study was carried out in Alejelat region. Four selected host plants were studied, chick pea (*Cicer arietinum*) and broad bean (*Vicia faba*) as a winter host plants, and Cow pea (*Vigna unguiculate*) and kidney bean (*Phaseolus vulgaris*) were selected as summer host plants during the growing seasons 2018 and

2019. Hundred leaflets infested with *L. trifolii* were collected. Samples were kept in plastic bags and transferred to be examined in the laboratory. Number of living *L. trifolii* larvae, and number of killed larvae according to feeding (no oviposition) by *D. isaea* which were distinguished by the absence of the parasitoid larvae and the presence of a brown and black scars on their cuticle as described by [14], were counted and recorded. Normal agricultural practices of fertilizing and irrigation were followed and no chemical control measurements were applied. Samples took place from the appearance of the emergence of the first leaves and continued weekly until harvest.

### 3. RESULTS

Results illustrated in Fig. 1 showed the numbers and percentages of host stung larvae of cow pea and, kidney bean during the growing season 2019.

#### 3.1 On Summer Crops

##### 3.1.1 Numbers of host stung larvae

###### 3.1.1.1 On cow pea

Host stung larvae recorded low numbers in the beginning of the season in early June, then the population increased recording its peak of abundance (156 individuals/ 100 infested leaflets) occurred in 10<sup>th</sup> of August. On the other hand, correlation coefficient (r) between host stung larvae and the population of *L. trifolii* recorded (+ 0.69\*\*).

###### 3.1.1.2 On kidney bean

Host stung larvae recorded low numbers in the beginning of the season in early June, then the population increased recording its peak of abundance (101 individuals/ 100 infested leaflets) occurred in 24<sup>th</sup> of August. On the other hand, correlation coefficient (r) between host stung larvae and the population of *L. trifolii* recorded (+ 0.85\*\*).

##### 3.1.2 Percentages of host stung larvae

###### 3.1.2.1 On cow pea

Percentage of host stung larvae ranged between (5.67% and 28.22%) recording its peak in 17<sup>th</sup> of August.

###### 3.1.2.2 On kidney bean

Percentage of host stung larvae ranged between (15.88% and 42.30%) recording its peak in 14<sup>th</sup> of September.

#### 3.2 On Winter Crops

##### 3.2.1 Numbers of host stung larvae

###### 3.2.1.1 On broad bean

Host stung larvae recorded low numbers in the beginning of the season in December, then the population increased recording its peak of abundance (159 individuals/ 100 infested leaflets) occurred in 11<sup>th</sup> of Mars. On the other hand, correlation coefficient (r) between host stung larvae and the population of *L. trifolii* recorded (+0.59\*).

###### 3.2.1.2 On chick pea

Host stung larvae recorded low numbers in the beginning of the season in December, then the population increased recording its peak of abundance (82 individuals/ 100 infested leaflets) occurred in 25<sup>th</sup> of Mach. On the other hand, correlation coefficient (r) between host stung larvae and the population of *L. trifolii* recorded (+0.20<sup>ns</sup>).

##### 3.2.2 Percentages of host stung larvae

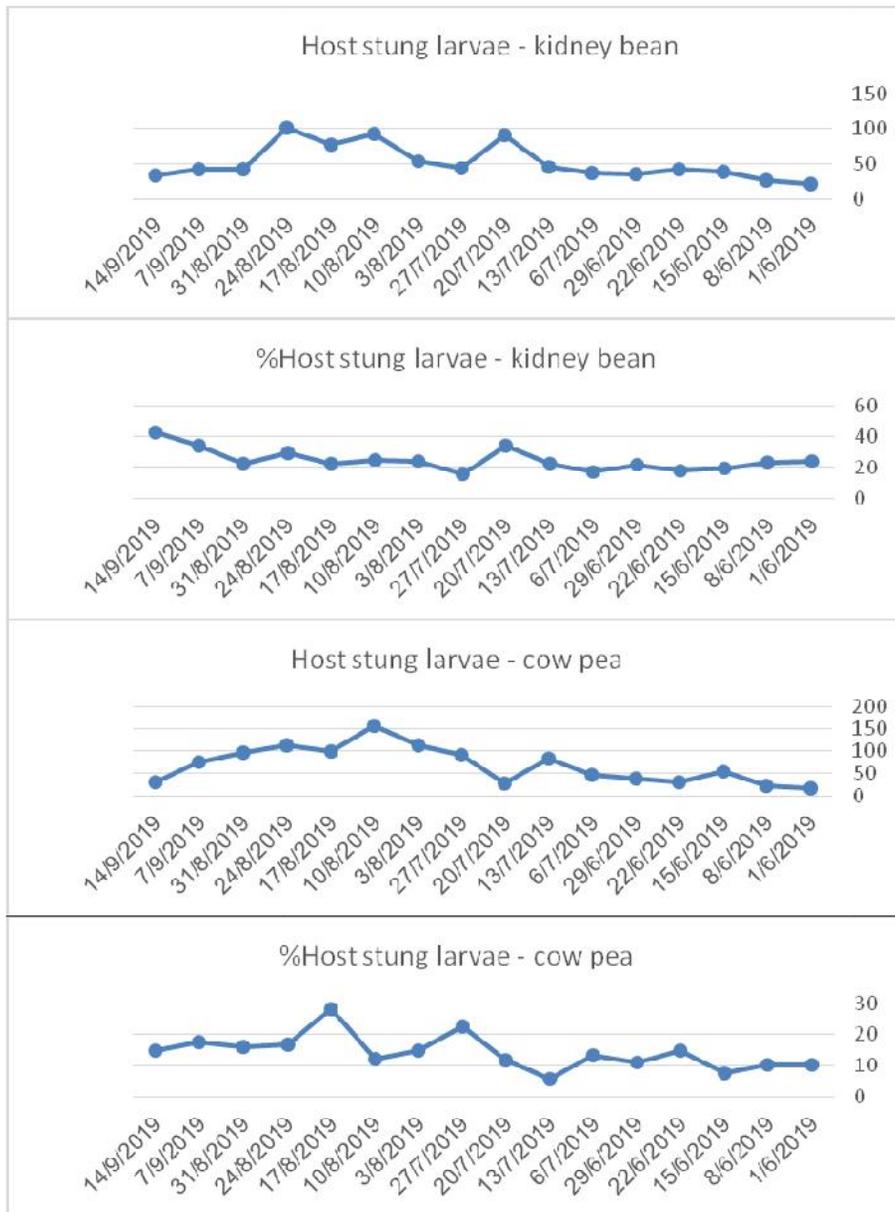
###### 3.2.2.1 On broad bean

Percentage of host stung larvae ranged between (8.45% and 36.60%) recording its peak in 8<sup>th</sup> of April.

###### 3.2.2.2 On chick pea

Percentage of host stung larvae ranged between (21.80% and 87.65%) recording its peak in 8<sup>th</sup> of April.

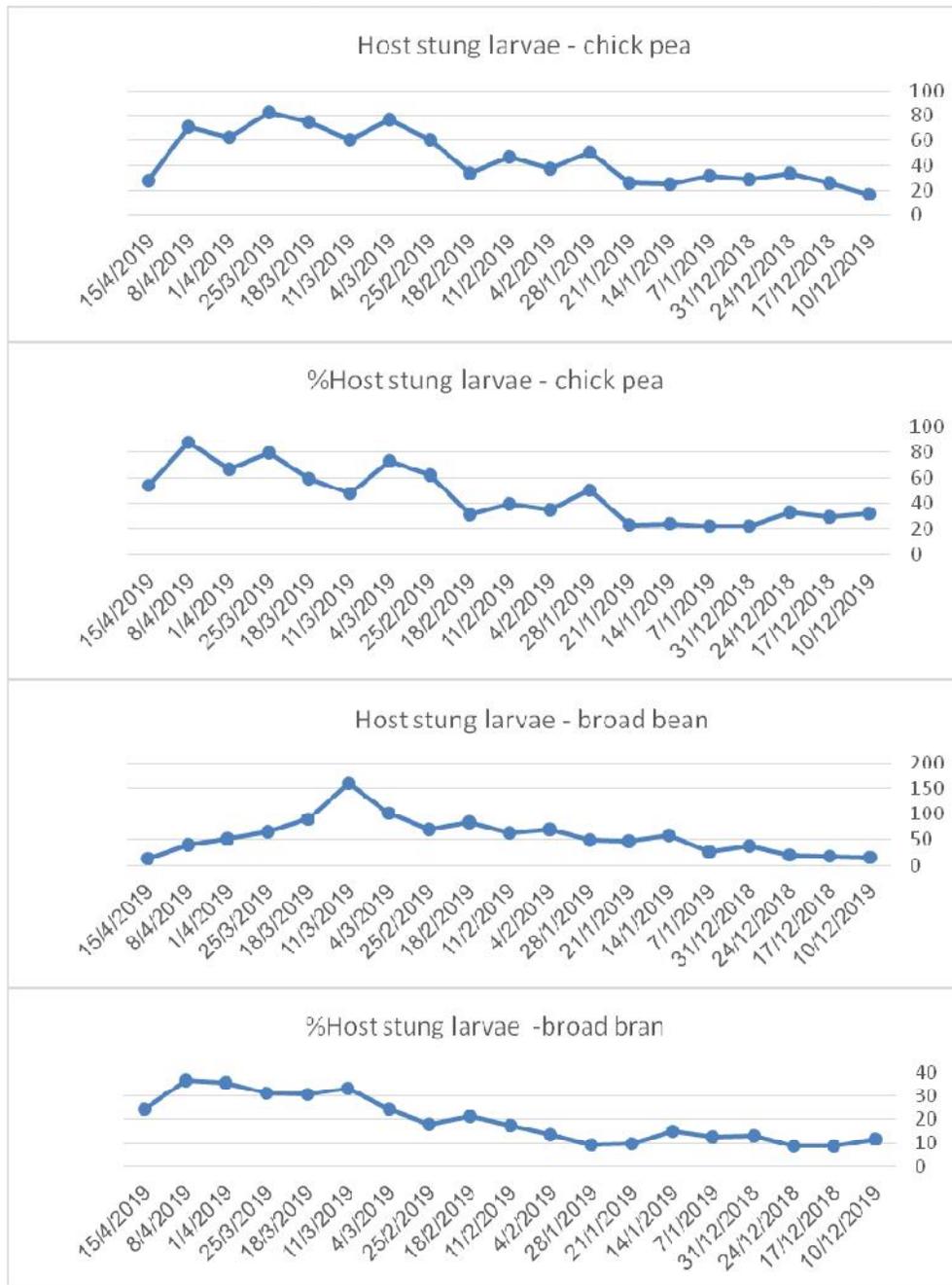
As shown in Table 1 the highest percentage of host stung larvae recorded on chick pea 44.65% followed by kidney bean 24.21%, broad bean 18.45% and cow pea 12.97% respectively. On the other hand, the correlation coefficient value (r) between numbers of *L. trifolii* larvae and, host stung larvae recorded the highest on kidney bean +0.85\*\* followed by cow pea +0.69\*\*, Broad bean +0.59\* and, Chick pea +0.20<sup>ns</sup>.



**Fig. 1. Numbers and percentages of host stung larvae on cow pea and, kidney bean during the growing season 2019**

**Table 1. Numbers of *L. trifolii* larvae, host stung larvae, % host stung larvae and correlation coefficient value (r) between numbers of *L. trifolii* larvae and, host stung larvae**

Host plants	Season	No. of <i>L. trifolii</i> larvae	No. host stung larvae	% Host stung larvae	r
Cow pea	Summer	8535	1107	12.97	+0.69**
Kidney bean	Summer	3387	820	24.21	+0.85**
Broad bean	Winter	5862	1082	18.45	+0.59*
Chick pea	Winter	1955	873	44.65	+0.20 <sup>ns</sup>



**Fig. 2. Numbers and percentages of host stung larvae on broad bean and, chick pea during the growing season 2018/2019**

#### 4. DISCUSSION

Although cow pea hosted the highest population of *L. trifolii* combined with the highest numbers of host stung larvae, the lowest percentage of host stung larvae were recorded on it 12.97%. On the other hand, a significantly positive high

correlation +0.69\*\* between *L. trifolii* population and the numbers of host stung larvae was recorded. A possible explanation is that, *D. isaea* females had a short life span during the summer season so, they prefer to feed and oviposit on the same targeted larvae, we cannot also rule out the heavy infestation by *L. trifolii* which also

combined with a high abundance of *D. isaea*. While kidney bean recorded (24.21%) with (r) value +0.85\*\*. The low abundance of *D. isaea* on kidney bean, the low preference as a host plant and short life span during the summer season may explain these results. On the other hand, broad bean had the lowest percentage of host stung larvae as a winter host plant recording (18.45%) with (r) value +0.59\*. This result may be explained by the high preference towards broad bean by the ectoparasitoid *D. isaea* for oviposition and the effective competition occurred by the endoparasitoids *Chrysocharis parksi* and *Opius pallipes* that could successfully encourage the parasitoid females to deposit more eggs on the host larvae instead of stinging it with no oviposition these results are in agreement with those of [15]. Chick pea had the highest percentage of host stung larvae as a winter host plant recording (44.65%) with (r) value +0.20<sup>ns</sup>. This result may be explained by the low abundance of *D. isaea* compared with broad bean and the very small leaflet size of chick pea the hosted low numbers of *L. trifolii*. Moreover *D. isaea* showed low preference towards chick pea compared with broad bean [15].

Benuzzi and Raboni [16] reported that, Besides the mortality induced by larval parasitic activity, *D. isaea* can also cause host mortality by the adult activity of host feeding. Females of the parasitoid sting host larvae normally, 1<sup>st</sup> and 2<sup>nd</sup> instar larvae) with their ovipositor, feed on the body fluids that come out and kill them. This behavior could occur whether the female deposit eggs or not and could successfully keep the population of *L. trifolii* at low densities these results are also in agreement with those of [17] who mentioned that, female with immature eggs or which has laid many of her eggs can gain more from host feeding [18].

## 5. CONCLUSION

The host stinging-to-total mortality of *D. isaea* attained 39.9% suggested that the proportions of different host-killing events depended on the density of leaf miner larvae on individual leaflets. With regard to such data, it could be concluded that, *D. isaea* could successfully keep the population of leaf miner at low densities and no further releases will be needed when the population of *D. isaea* is higher.

Our results are also in agreement with those of (Jervis et al. 2001) who mentioned that, in parasitoid insects, as well as parasitoid itself,

there are two other behaviors that may increase leafminer mortality: 1)feeding on the host (host feeding) and 2)host paralyzation without oviposition or feeding (host stinging). In the first case, adult wasps feed on a certain proportion of leafminer larvae, which may or may not be a previous requisite for egg laying.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:

The peer review history for this paper can be accessed here:  
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