Study of mass production of predatory bugs *Deraeocoris* sp. for use in biological control against sucking pests in different crops

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ABSTRACT

The present research aimed to study the biology and mass production of the predatory bug Deraeocoris lutescens Schilling (Hemiptera: Miridae) at 25±1°C temperature, relative humidity of 60±10% and a photoperiod of 16:8 h (L:D). Predatory bug reared on broad bean leaves in the round plastic Petri dishes or on bean plants. For the predator, a small measure of Sitotroga cerealella (Olivier) (Lepidoptera: Gelechiidae) eggs was offered as food every two days. The fecundity experiments on different plant species were investigated among four groups of plants, five crop plant species, three greenhouse plant species, six orchard plant species and five ornamental plant species. Among different plant species, most oviposition was on broad bean leaves with a total of 41.7±3.7, sweet pepper with 31.1±3.4, grape leave with 13.9±2.8 and begonia with 39.3±3.0 eggs in each group. In order to determine preference of D. lutescens for oviposition, broad bean leaves which infested with different nutritional sources, Aphis fabae, eggs of S. cerealella, 10% honey emulsion and 10% honey emulsion + yeast extract were used. Among these leaves as substrates of oviposition, the adult females showed higher preference for leaves infested with eggs of S. cerealella than with other nutritional sources.

Key words: Predatory bug, *Deraeocoris lutescens*, biology, mass production, oviposition.
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INTRODUCTION

Interest in biological control has increased considerably as a response to the various effects of pesticides on the environment and as a result of new international trends, which favors conservation and the sustainable use of biological resources. International food production policies increasingly demand alternatives to the use of chemical control, and biological control resurfaces with new energy in this scenario by means of techniques that make it viable to be used economically. According to [30] often populations of predators and parasitoids that are naturally present in the agroecosystems are insufficient to maintain the density of a pest organism below the economic injury level. Thus, mass-production and release of natural enemies is needed to obtain sufficiently low pest populations.

Primarily through the study of many insects, it has become clear that oviposition behaviour, and choice of oviposition sites in particular, can increase the performance and survival of insect progeny. Such oviposition behavior is largely driven by variation in the environment. Choice of oviposition sites and dispersion of eggs by adult insects can vary among host species, among individuals within a host population, and within an individual of a particular host population.

A positive correlation between female preference for oviposition sites and offspring performance was detected in many studies, primarily for herbivorous insects [4, 5,6, 10,13,21,27,28]. Females of the gall aphid *Pemphigus betae* indeed maximise their overall fitness; stem mothers prefer to establish galls at sites on the leaves that support more

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offspring [31,32]. Determinants of oviposition choice by phytophagous insects include allelochemicals, quantity and/or quality of resources, plant morphology and natural enemies [29]. Such factors can lead to variation in performance and survival of insect progeny for eggs deposited in different locations [5,16,19,20,25,26]. Because variation in oviposition behaviour can be genetic and heritable [11,12,29], natural selection may favour the choice of oviposiition sites that facilitate growth and survival of offspring.

Moreover, oviposition site selection by predatory bugs to be correlated positively with offspring performance at the substrate of oviposition. This is supported by the significantly higher hatching success of eggs deposited at the preferred vein origin site as compared with those deposited on other parts of the leaf [8]. When selecting an oviposition site, omnivores are expected to respond to both prey availability and, even more strongly, to plant traits that affect both females and their offspring [8]. The relation between omnivore oviposition preference and offspring performance was tested on two spatial scales, between plants of different nutritional value, and between areas within a leaf [8]. Some predators deposit their eggs where prey is concentrated [9]. Others oviposit away from prey [22], possibly relying on the high mobility of the hatching young. However, a rigorous exploration of the relationship between offspring mobility and oviposition strategy in predators has not yet been attempted. Even less is known about the oviposition preference–offspring performance relationship in omnivorous insects that feed on both prey and plant food sources [2]. Omnivory is widespread in nature, and may be exhibited by most consumers during at least one of their life stages [3,18,33]. These consumers are therefore expected to respond to both plant characteristics and prey availability when choosing an oviposition site.

Deraeocoris lutescens Schilling (Hemiptera: Miridae) is apredatory bug found commonly on a wide variety of plants across Middle East and Europe, that feeds on a wide range of arthropod pests such as aphids, small caterpillars, mites and insect eggs [15]. Females insert their eggs in leaf tissue. The females' choice of oviposition site is important for the subsequent distribution of nymphs on prey's host plants. Oviposition behaviour of many insects has been investigated by others on anthocorids [1,7,8,23,29]. Oviposition behaviour of this predator and others of the genus *Deraeocoris* is not well known. This research investigates the acceptability and preferences of a range of plants for oviposition by the predatory bug *D. lutescens* in the laboratory. Moreover, we examined oviposition preference on the same plant with different nutritional sources.

MATERIALS AND METHODS

Insect rearing
For obtaining of individuals in the desired age, aphid species of *Aphis fabae* Scopoli was kept in a climatically controlled chamber at 25±1°C temperature, relative humidity of 60±10% and a photoperiod of 16:8 h (L:D) on broad bean leaves in the round plastic Petri dishes (6 cm diameter) that were filled with 2 cm-thick-layer of 0.7% agar gel.

The predatory bug, *D. lutescens* were originally obtained from the experimental teaching garden of Shahid Bahonar University of Kerman, Iran. This species was identified by department of insect taxonomy research, Iranian research institute of plant protection, Tehran, Iran. The predatory bugs were reared on freshly excised broad bean leaf discs 5 cm in diameter (as substrate of oviposition) which were placed in the abovementioned round Petri dishes and bean plants in Plexiglas cages. Broad bean leaves were infested with eggs of *Sitotroga cerealella* (Olivier) (Lepidoptera: Gelechiidae) as food. The cages were held in a controlled climate and adults were transferred to new cages every two days. Broad bean leaves with eggs were incubated until egg hatching. These leaves and first nymphs were placed into new Plexiglas cages (7.5 × 15 × 4.5 cm), with a mesh-covered hole in the lid, to start the pre-imaginal rearing. Food and water were supplied up to adult emergence.

Experimental conditions
The biology of *D. lutescens* was comprehensively investigated in the laboratory at 25±1°C temperature, relative humidity of 60±10% and a photoperiod of 16:8 h (L:D). For all the experiments on embryonic and nymphal development, mortality, longevity as well as fecundity, round plastic Petri dishes 6 cm in diameter.

The preference oviposition of *D. lutescens* was determined by multiple-choice experiments in a Plexiglas cages (21×12×9 cm), with three mesh-covered holes in the lid in a climatically controlled chamber.

The oviposition preference by *D. lutescens* on different plant species was investigated among four groups of plants. During each experiment, leaf discs (5 cm in diameter) of five crop plant species (bean, potato, broad bean, sugar beet and cabbage), three greenhouse plant species (cucumber, egg plant and sweet pepper), six orchard plant species (grape, walnut, pistachio, cherry, peach and pear) and five ornamental plant species (geranium, coleus, Begonia, Stonecrop and giant dumb cane) were placed upside down onto, the round Petri dishes 5 cm in diameter were partially filled with 2 cm thick layer of 0.7% Agar gel. The round plastic Petri dishes containing different leaves of

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each group were randomly positioned in the abovementioned Plexiglas cages during a trial. The adult females and males of *D. lutescens* (1-day-old) were transferred for 7 days into the Plexiglas cages, containing leaves of different plant species of each group infested with eggs of *S. cerealella*. This step was necessary in order to reduce the possibility that *D. lutescens* might get adapted to a certain plant species and to give the adult females and males the chance to mate. After 8 days, three mated females were transferred together into another Plexiglas cage with different leaves and offered eggs of *S. cerealella* as food. The oviposition substrates were daily replaced by new ones and the numbers of laid eggs on the leaves were recorded. A trial lasted seven days from 9th till 15th days of longevity and replicated 12 times.

In order to determine preference of *D. lutescens* for oviposition on broad bean leaves, which infested with different nutritional sources, six freshly excised broad bean leaf discs (5 cm diameter) placed in the abovementioned round plastic Petri dishes were used. The round plastic Petri dishes containing *A. fabae* (3-4-days-old), 10% honey emulsion, 10% honey emulsion + yeast extract and eggs of *S. cerealella* on broad bean leaves were randomly positioned in the Plexiglas cages. Three *D. lutescens* females (mated, 9-days-old) were kept together in each Plexiglas cage. After that, the females were transferred to another Plexiglas cage containing new leaves every 24 hours. The leaves in the old Plexiglas cage were checked under a binocular for recording of the eggs. The experiment was continued for one week and replicated 12 times.

Statistical analysis

For statistical comparison among several means, all the data from the laboratory studies on biology and egg laying of the predatory bug were subjected to a one-way analysis of variance (ANOVA) followed by a Tukey Test (StatPlus, 2007).

RESULTS AND DISCUSSION

This study's part deals with the results of the experiments, which had been carried out to investigate of mass rearing, the embryonic and nymphal development, mortality, longevity and fecundity of *D. lutescens* with eggs of *S. cerealella* as food at a temperature of $25\pm1°C$, relative humidity of $60\pm10\%$ and a photoperiod of 16:8 h (L:D) on broad bean leaves or bean plants in the laboratory.

In fact the artificial rearing could be used for many purposes that could be grouped into two general objectives: academic research and practical use.

The mass production for utilization of parasitoid or predator insects in biological control strategies is the most evident aim. There are some advantages such as simplification of the production line, higher flexibility of the production, reduction or suppression of allergy problems generated by moth scales (from factitious hosts or prey), lower costs expected with scaling up effect. But, insect colonies are also used by many commercial companies for pesticide testing, production of insect derivates (pharmaceutical products). Of course there is a growing sector concerning the multiplication of beneficial insects. Especially the bio-control strategy using inundative releases needs huge quantities of insects. The basic food chain for entomophagous insects is the "natural" tritrophic system, with plant, pest insect (host or prey), and parasitoid or predator. One of the first ideas to improve this production line was to try to reduce the number of compartments. A first step consisted in using bitrophic systems, with two possibilities of substitutes. Artificial food could replace plant to feed host or prey insects: there are many examples with lepidopterous or coleopterous larvae used as hosts for parasitoid rearing. Also alternative host/prey, easier to produce in laboratory conditions than natural ones, could be used, such as coleopterous larvae of *Tenebrio molitor* L. and lepidopterous larvae of *Galleria mellonella* (L.), or eggs of *Ephestia kuehniella* Zeller and *S. cerealella*.

The mean duration of embryonic development of eggs was 10.7 ± 0.1 days. Percentage hatching of eggs was $80.4\pm2.7\%$.

D. lutescens included five nymphal instars. The mean development duration of the nymphal instars is summarized in table 1. There were not differences in the mean developmental duration of the five different nymphal instars of both sexes (P<0.05).

The percentage of nymphal mortality calculated 22.7%. The highest percentage of nymphal mortality was in the N1.

The longevity of females and males were respectively 21.8 ± 1.1 and 22.5 ± 2.4 that was not observed significant difference among them (P<0.05).

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Table 1: Mean developmental duration of nymphal instars of _D. lutescens_ on broad bean at 25±1°C.

n	sex	Duration of nymphal instars (days) mean±SE					Total nymphal duration
		N1	N2	N3	N4	N5	
22	♀♀	3.8±0.1	3.0±0.3	2.7±0.3	3.1±0.1	5.7±0.3	18.4±0.4 a
	♂♂	3.9±0.1	2.9±0.3	3.0±0.2	2.9±0.3	5.1±0.2	17.7±0.4 a

Significant difference among of nymphal duration both sexes= a

Egg-laying of _D. lutescens_ on different plants
The daily and total number of eggs laid by three _D. lutescens_ females from the 9th till the 15th days of longevity on three species of greenhouse plants, cucumber (_Cucumis sativus_), egg plant (_Solanum melongena_) and sweet pepper (_Capsicum annuum_) with eggs of _S. cerealella_ as food at 25±1°C are summarized in Table 2. The predatory bug was not able to lay eggs on greenhouse plant species used in the experiment equally.

The adult females showed most oviposition on Sweet pepper leaves with a total of 31.1±3.4 eggs and least oviposition on Cucumber with 7.1±1.5 eggs. However, among the three greenhouse plant species used, Sweet pepper had significantly the highest counts of the predator eggs (P<0.01).

Table 2. Mean daily and total number of eggs laid by _D. lutescens_ females on different leaf of greenhouse plant species at 25±1°C.

Host plant	n	Mean number of laid eggs on the day							Total
		9th	10th	11th	12th	13th	14th	15th	
Cucumber	12	0.9±0.3	1.2±0.8	0.6±0.3	0.3±0.2	1.6±0.8	1.2±0.8	1.3±0.5	7.1±1.5 a
Egg plant	12	3.7±1.4	2.7±0.8	2.2±1.1	2.7±1.5	3.0±1.1	2.5±1.6	1.4±0.5	18.2±3.3 b
Sweet pepper	12	8.1±2.7	4.9±1.8	6.9±1.9	2.7±1.0	4.2±1.3	2.3±1.1	2.0±0.6	31.1±3.4 c

The Broad bean leaves with a total of 41.7±3.7 eggs showed significantly (P<0.05) the highest number of _D. lutescens_ eggs among the crop plant species. No clear tendency in oviposition of the predator females was to be distinguished among the other crop plant species that are showed in Table 3.

Table 3. Mean daily and total number of eggs laid by _D. lutescens_ females on different leaf of crop plant species at 25±1°C.

Host plant	n	Mean number of laid eggs on the day							Total
		9th	10th	11th	12th	13th	14th	15th	
Bean	12	3.3±1.6	0.4±0.3	1.1±0.9	1.1±0.7	1.1±0.9	0.6±0.3	0.0±0.0	8.2±2.3 a
Cabbage	12	1.2±0.7	1.5±1.0	0.5±0.5	0.2±0.2	0.2±0.2	0.4±0.3	0.1±0.1	4.2±1.1 a
Broad bean	12	5.8±2.2	8.8±1.8	6.6±1.8	3.2±1.5	8.2±2.1	5.8±2.0	3.2±1.2	41.7±3.7 b
Sugar beet	12	2.2±1.0	2.9±1.0	1.2±0.8	2.0±1.0	1.7±0.6	1.0±0.7	0.2±0.1	11.2±1.7 a
Potato	12	1.0±0.7	2.7±1.2	4.3±1.4	2.6±1.1	0.9±0.7	1.2±0.5	0.1±0.1	12.6±2.5 a

Among six species of orchard plant, pear, walnut, pistachio and peach leaves were not as a suitable substrate for oviposition. Although among these leaves, cherry leaves (with a total of 1.0±0.5 eggs) were observed significant difference in total eggs laid by the predator. But the adult females showed significantly higher oviposition for grape leaves (with a total of 13.9±2.8 eggs) than other orchard plant species (P<0.005) (Table 4).

Table 4. Mean daily and total number of eggs laid by _D. lutescens_ females on different leaf of orchard plant species at 25±1°C.

Host plant	n	Mean number of laid eggs on the day							Total
		9th	10th	11th	12th	13th	14th	15th	
Cherry	12	0.2±0.2	0.2±0.2	0.0±0.0	0.0±0.0	0.2±0.2	0.2±0.1	0.0±0.0	1.0±0.5 b
Pear	12	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0 a
Grape	12	2.2±1.5	4.0±1.5	2.2±1.4	1.6±1.0	1.7±0.9	0.6±0.3	0.3±0.2	13.9±2.8 c
Walnut	12	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0 a
pistachio	12	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0 a
Peach	12	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0 a

Begonia leaves with a total of 39.3±3.0 eggs showed significantly (P<0.005) the highest number of _D. lutescens_ eggs among the ornamental plant species (except geranium) were used (Table 5).

Among stonecrop, giant dumb cane and coleus leaves as substrate of oviposition, no significant difference in egg-laying was observed (P<0.01). No clear tendency in oviposition of the predator females was to be observed between coleus and geranium (P<0.005).

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Table 5. Mean daily and total number of eggs laid by *D. lutescens* females on different leaf of ornamental plant species at 25±1°C.

Host plant	n	Mean number of laid eggs on the day							
		9th	10th	11th	12th	13th	14th	15th	Total
Begonia	12	3.6±1.5	8.4±1.7	7.2±1.8	6.4±2.9	4.7±1.3	5.7±1.0	3.2±0.7	39.3±3.0 c
Geranium	12	2.2±1.0	5.4±2.2	2.2±0.9	5.7±1.4	5.4±1.8	5.2±2.0	5.2±2.0	33.2±4.2 bc
Stonecrop	12	0.0±0.0	0.0±0.0	0.2±0.2	0.4±0.4	0.1±0.1	0.0±0.0	0.0±0.0	0.7±0.6 a
Giant Dumb Cane	12	0.2±0.1	0.8±0.6	0.8±0.7	0.0±0.0	1.2±0.7	1.1±0.8	0.7±0.6	4.8±1.9 a
Coleus	12	0.7±0.3	0.9±0.7	1.4±1.1	3.3±1.6	2.3±1.3	1.5±0.7	0.7±0.2	10.9±2.0 ab

Oviposition of *D. lutescens* to different nutritional sources

Table 6 represents the daily and total number of eggs laid by three *D. lutescens* females on the leaves from the 9th to 15th days of longevity. Among these leaves as substrates of oviposition, the adult females showed higher oviposition for leaves infested with eggs of *S. cerealella* than with other nutritional sources with a total of 19.3±3.3 eggs. No significant tendency in oviposition of the predator females was to be distinguished among the nutritional sources ($p < 0.05$).

Table 6. Mean daily and total number of eggs laid by *D. lutescens* females on broad bean leaves infested with different nutritional source portions at 25 ±1°C.

Nutritional source	n	Mean number of laid eggs on the day							
		9th	10th	11th	12th	13th	14th	15th	Total
A. fabae	12	0.6±0.5	2.7±1.2	0.6±0.6	1.0±0.9	1.6±0.9	3.7±1.5	1.3±1.1	11.6±2.6 a
S. cerealella (egg)	12	1.2±1.2	3.9±1.9	1.3±1.1	2.0±1.0	4.2±1.6	1.1±0.7	5.9±2.6	19.3±3.3 ab
10% Honey emulsion	12	0.0±0.0	2.0±1.4	0.8±0.4	0.9±0.6	2.3±1.4	1.4±0.7	1.4±0.6	8.9±2.4 a
10% Honey emulsion +yeast extract	12	1.2±1.2	5.6±3.0	0.9±0.5	0.7±0.4	1.7±1.0	1.3±0.8	1.0±1.0	12.6±3.9 a

The oviposition preference is a way of maternal investing; the females spend time and energy in such activity, which may result or not in providing the offspring development [14]. The oviposition of *D. lutescens* on Greenhouse, crop, ornamental plants is higher than orchard plants. The current study showed that the predator bug between plant groups has distinctive preference for different species. It dependent to physical and chemical structure of leaves, so oviposition-preference patterns of *D. lutescens* are supposed to correspond to host suitability for egg hatching and offspring development because females are assumed to maximize their fitness by oviposition on high-quality hosts. The major hypothesis of the evolution of oviposition behavior is that the females would choose species of plants that could maximize offspring survival and growth [29]. The predators and their herbivorous prey may respond in similar ways to variations in plant quality, resulting in a spatial or temporal overlap in their distributions [8]. *Anthocoris confusus* Reuter (Hemiptera: Anthocoridae) females insert their rostrum into plant tissue to determine the suitability of the plant for oviposition [7]. Moreover, *Orius insidiosus* (say) (Hemiptera: Anthocoridae) can obtain water from the xylem, and may ingest small amount of starches, sugars and amino acids from the mesophyll of plants [1]. Both moisture and nutrient levels could be detected by the females. Therefore, the females of the predator could detect both moisture and nutrient levels. The females select oviposition sites that maximize the hatch rate of deposited eggs rather than to optimize nymph performance may be related to the high mobility of the neonates and their ability to search for food away from the egg-hatching site. A similar oviposition strategy is probably exhibited by at least some of the predators that were found to lay egg near prey aggregations [8]. Microhabitat selection for oviposition by *Anthocoris nemorum* is affected by plant quality and prey availability as well as exudates from prey [24]. The abilities of *D. lutescens* females to distinguish between different nutritional sources and preferentially deposit their eggs into leaves with eggs of *S. cerealella* appears to be correlated with nymphal development success. The predators may therefore respond primarily to variations in food or prey quality, which may be a better predictor of nymph performance. In choice oviposition experiments by *Anthocoris nemoralis* and *A. nemorum*, honeydew-treated pear leaves attracted more oviposition than honeydew free leaves [24]. The predators rely on a nitrogen-rich prey diet, and oviposition of females may therefore respond to prey availability. In many cases, however, prey availability varies greatly in space and time, making it difficult for oviposition of females to predict prey availability for their offspring [8].

Detectable cues to assess microhabitat quality are a selective advantage for oviposition of females [17]. According to these results, the choice for oviposition sites by *D. lutescens* females is influenced by different factors of mentioned above. The preference of this predator to oviposit on different plant species and with different nutrition source is one of the major factors in determining its ability to successfully control the pests of these plants.

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CONCLUSION

In the laboratory, *D. lutescens* was able to successfully feed, develop and reproduce when fed on eggs of *S. cerealella* as food on broad bean leaves or on bean plants at an optimal temperature. The predator can smoothly adapt to fluctuating prey offer and will maintain its capability of oviposition at different environmental conditions. Moreover, the predatory bug seems to be a generalist predator. Consequently, this predatory bug seems to be a promising predator to be used alone or in IPM programs.

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