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Effect of chickpea genotypes on *Liriomyza cicerina* oviposition preference and insect performance

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Abstract

Chickpea Leaf miner, *Liriomyza cicerina* Rond., behavior was investigated regarding the oviposition preference and insect performance. In choice situation, the insect was able to discriminate among the presented cultivars of chickpea and showed an oviposition preference. The resistant and the susceptible plants had the lowest and highest number of eggs per plant, respectively. In the no-choice test, the insect laid fewer eggs on the improved lines and oviposition was significantly correlated to the insect performance in terms of larval survival and adult emergence. In both the choice and no-choice tests, oviposition was also correlated with the leaf surface area. The female incentive to produce eggs was affected by the presented plants as they laid more eggs in the presence of susceptible plants. High attraction for oviposition could be used in attract-and-kill pest management designs.

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Introduction

Chickpeas leaf miner *Liriomyza cicerina* (Rondani, 1875) (Diptera: Agromyzidae) is the most threatening insect pest to chickpea crops in Morocco (Andaloussi *et al*., 2015), causing approximately 20% and 40% yield losses during winter and spring cropping seasons, respectively (Sabraoui *et al*., 2019). The insect is wildly distributed in North Africa, West Asia and South Europe with 30% to 40% yield losses (Reed *et al*., 1987; El-Bouhssini *et al*., 2008; Ali *et al*., 2015). Generally, two to three overlapping generations occur during one cropping season. The losses are mainly caused by the larvae, which mine inside the leaflets and consume the mesophyll tissues, thus causing leaf weakness and, ultimately, drop under heavy infestation (Lahmar & Zeouienne, 1990; Changizi *et al*., 2012). The larva passes through three instars before exiting the leaf and pupating in the soil (Lahmar & Zeouienne, 1990; Changizi *et al*., 2012). Because of the lack of the larva ability to move from a leaf to another, the species performance and fitness depend solely on the female oviposition choice and the plant characteristics (Parrella, 1987; Hering, 2013).

Oviposition preference could be defined as an active choice that the ovipositing female makes among alternatives. An insect specimen prefers a plant A over B if the plant A is more likely to receive more eggs whenever it is presented at the same time with plant B to the same insect (Singer, 1986). This behavior is affected by multiple factors that are related to the insect, the plant, and the environment (Singer, 1986).

In the case of the chickpea Leaf miner, there is a huge gap in resistant cultivars that were selected based on field evaluation of intensity and extent of plant damage under natural infestation. Generally, a quantitative 1 to 9 visual damage scale is used to categories the level of resistance which could be summarized to; plants with less than 20%, 20-30%, 30-40% and more than 40% mined leaflets are considered as resistant, moderately resistant, moderately susceptible, and susceptible, respectively (Weigand, 1990). Although this approach is a very practical way to screen a huge number of germplasms every year, it doesn't help much in explaining the observed differences in the infestation level among those cultivars. However, it is known that plants with multipinnate and small leaflets suffer less damage than those with large leaves (Singh *et al*., 1996; Malhotra *et al*., 2007; Toker *et al*., 2010a,b; Toker *et al*. 2012). The resistant cultivar ILC 5901 received fewer eggs in comparison to the susceptible one ILC 3397 under artificial infestation and had a lower adult emergence rate (Khoja *et al*., 2012).

The present study aims to contribute to a better understanding on the *L. cicerina* oviposition behavior under choice and no-choice conditions, and its relation to the leaf size and insect performance.

Materials and methods

Seeds

Four improved chickpea lines with different levels of resistance to chickpea leaf miner (different level of visual damage score): LMR 133 (ILC 3805×ILC 5309), LMR 202 (ILC 3805×ILC 5309), ILC 86 (ILC 5309) and ILC 93 (ILC 5901) were used along with two varieties commonly grown by Moroccan farmers Arifi (FLIP98-50C) and Moubarak (F84-182C) and one local commercial cultivar (Table 1). Seeds were provided by the genetic resources' unit and chickpea breeding program of ICARDA.

Flies

Because of the difficulty of maintaining a colony of *L. cicerina* in the laboratory, a small field was sown near the greenhouse to get enough flies to conduct the experiments below. In this field, the commercial cultivar was used for its high susceptibility and ability to produce higher insect density for fly collection. The adults were collected early morning using a hand aspirator and transported to the greenhouse where they were checked and counted before infestation.

Oviposition assays

Multi-choice test

The seven genotypes were presented simultaneously to insects for egg laying in a multi-choice test. Plants were sown in 20×40cm (length × width) multi-pot-trays containing a mixture of soil with peat and watered on need. The experiment was done on a completely randomized design with four trays as replication and each tray had three plants of the same genotype. Once plants reached 20cm of height, they were infested and covered with white fine tissue. A total number of 84 files were released on each tray with a ratio of 2 males and 2 females per plant and were allowed to mate and lay eggs freely. After 48h of infestation, the cover was removed, and plants were scored for the number of eggs deposited on each plant. The experiment was carried out under glasshouse conditions as the temperature, humidity and photocycle were 25±2°C, 50% and 14L:10D, respectively.

Dual-choice test: The four improved lines that showed lower scores during the multi-choice test (LMR 133, LMR 202, ILC 86 and ILC 93) were tested against the control (the commercial cultivar) in a dual-choice. A control *vs* control was also included for appropriate comparisons. Plants were sown in small pots of 5×5cm (length × width) containing a mixture of soil and peat and watered on need. Once reached 20cm of height, the plants were taken to a glass cage of 90×90×90cm (length × width × height) and infested by 2 males and 2 females per plant. Each cage had three pots of the tested line and three pots of the control and thus, 24 adults were released to mate and lay eggs on each cage. After 48h of infestation, eggs laid on each genotype were counted and expressed as frequencies.

No-choice test and insect performance

Females had contact with only one plant and were forced to oviposit or not oviposit their eggs on it. This experiment was carried out to measure the degree of acceptance of the four improved lines that showed to be less preferred in the multi-choice test. The plants were cultivated as described in the dual choice and were infested individually with 2 males and 2 females per plant and covered with white fine tissue. After 48h of infestation, the cover was removed, the number of eggs was recorded for each genotype and the plants were observed daily until larvae became ready for pupation. Leaves were cut, collected in petri dishes and supplemented with watered cotton for pupae collection. Emerged adults were checked daily and counted. The experiment was done under laboratory conditions using a completely randomized design with three repetitions of each genotype. The insect performance was scored for larval mortality and adult emergence.

Leaves surface area

Plants used in the multi-choice and no-choice tests were subjected to leave area measurement using WinRhizo scanner in the ICARDA Crop Physiology Laboratory. Images of the leaves were analyzed using ImageJ software.

Statistical analysis

Statistical analyses were carried out using the software Genstat 64-bit Release 19.1 (PC/Windows 8) Copyright 2018, VSN International Ltd. Means were compared and analyzed using ANOVA one-way test followed by Student-Newman-Keuls (p=0.05).

Table 1. Characteristics of the studied chickpea genotypes.

Genotypes	Leaf type	Leaflet size	Pigmentation	Susceptibility to leaf miner
ILC 93	Multipinnate	Very small	Absent	Resistant
ILC 86	Normal	Intermediate	Absent	Moderate resistant
LMR 133	Normal	Small	Absent	Moderate resistant
LMR 202	Normal	Small	Absent	Moderate resistant
Arifi	Normal	Intermediate	Absent	Susceptible
Moubarak	Normal	Intermediate	Absent	Susceptible
Commercial cultivar	Normal	Intermediate	Absent	Susceptible

Results

In the multi-choice test, all plants received eggs in a range between 1 and 15 eggs per plant and a total of 249 eggs were counted. Only few eggs were laid on ILC 93 followed by the other three improved lines, while there were no significant differences between the local cultivars and the susceptible control based on the Student-Newman-Keuls test (Table 2). Egg distribution was correlated with the leaf surface as shown in Figure 1.

The oviposition score represents the average of total eggs for each genotype per tray; Values followed by the same letters in the same raw are not significantly different based Student-Newman-Keuls (p=0.05)

In the dual-choice, the same behavior was observed; the females laid significantly more eggs on the susceptible control than on the tested plants (P<0.001). However, ILC 86 received fewer eggs than LMR 133 and LMR 202 (Figures 2 and 3).

During the no-choice test, all plants were accepted for oviposition and ILC 93, LMR 133 and LMR 202 received significantly fewer eggs than the control (P=0.005). Similarly, to the multi-choice test, the distribution of the eggs was correlated with leaves surface area as shown in Figure 4.

Table 3 highlights the results of the insect performance on each tested plant. Significant differences were noted for the improved lines compared to the control (the commercial cultivar) in both larval survival and adult emergence (P<0.001). A strong correlation was observed between the number of eggs and the survival of larvae and adult emergence with $R^2 = 0.8939$ and $R^2 = 0.9065$, respectively (Figure 5). Values followed by the same letters in the same line are not significantly different based on Student-Newman-Keuls (P=0.05).

Discussion

Egg deposition is a result of a sequence of visual, chemical, and tactical encounters-behaviors that an ovipositing female passes through before making any decision whether accept or reject the encountered host plant (Singer, 1986). During the first stages of host-finding, olfaction and vision (or both) play a major role in the selection process especially from long distances (Bernays & Chapman, 1994; Thiéry *et al*., 2013a). Once at close range, multiple factors such as plant chemicals, coloration, texture, size and others combine together to influence the female's oviposition behavior (Harris & Rose, 1990; Thiéry *et al*. 2013b). A general illustration for *Liriomyza* species was given by Bethke and Parrella (1985). The female punctures several holes for feeding and ovipositing, however, the final decision whether to lay an egg comes after feeding and the plant still could be rejected at any moment during the process (Bethke & Parrella, 1985).

Even though in the present study *L. cicerina* was observed to

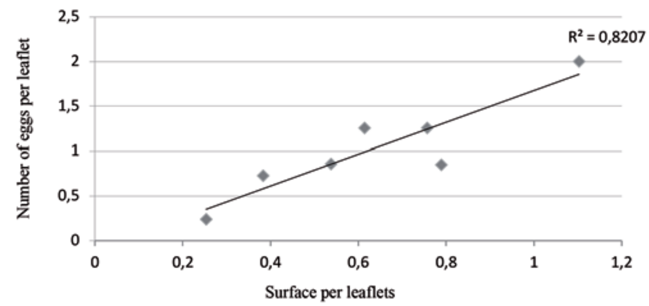

Figure 1. The relation between the number of eggs laid by *L. cicerina* on each genotype and their leaflets surface area in multi-choice test.

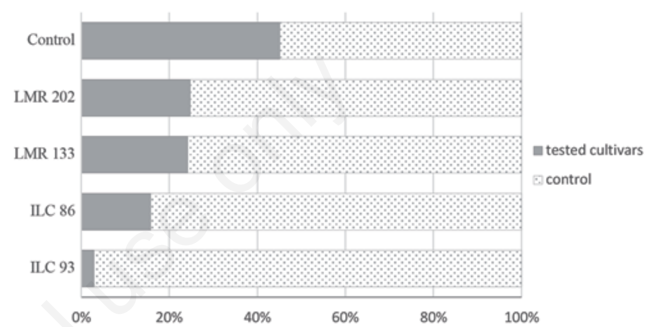

Figure 2. Frequencies of eggs laid on each genotype vs control (commercial cultivar) in dual-choice test.

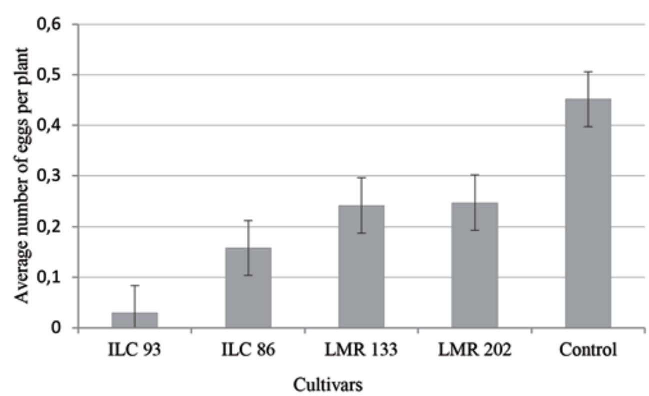

Figure 3. Effect of chickpea genotypes on the oviposition scores per plant in the dual-choice test.

Table 2. Effect of chickpea genotypes on the oviposition scores in the multi-choice test.

plant	ILC 93	LMR 133	LMR 202	ILC 86	Arifi	Moubarak	Control
Mean	2.75^a	7.25^b	7.5^b	8^b	11^c	12.75^c	13^c
SD	0.5	0.5	1	0.81	2	1.7	2.44
SED	1.1						
LSD	2.31						

Different letters mean different significance.

be feeding and ovipositing on all the tested plants, it was shown that in choice situations the females exhibited a preference for oviposition towards some plants more than others. Similarly, to previously reported results (khoja *et al*., 2012), the tested local cultivars received a higher number of eggs compared to the improved lines which explain their susceptibility in field situation (Sabraoui *et al*., 2019). Oviposition preference was also reported for other *Liriomyza* species (Neder de Roman *et al*., 1993; Gomez & Rodriguez, 1994; Hawthorne *et al*., 1992; Fernandes *et al*., 2012). Videla *et al*. (2012) reported that *L. huidobrensis* (Blanchard) showed a preference for oviposition only when two hosts were simultaneously presented, while no significant differences of eggs laid on the same plants in a no-choice test.

Furthermore, *L. cicerina* was more induced to lay eggs in the presence of some cultivars than others. It is relevant to note that all adults used during this study were collected from the commercial cultivar and this might have an influence on oviposition behavior.

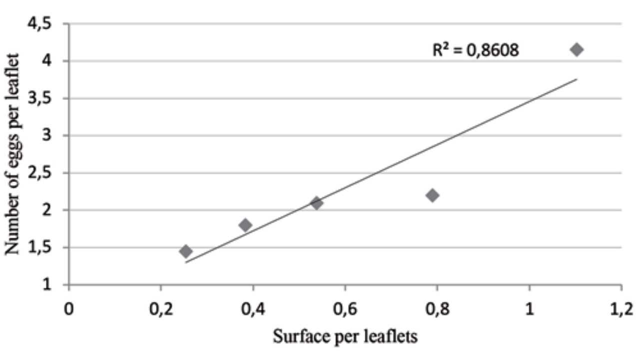

Figure 4. The relation between the number of eggs laid by *L. cicerina* on each genotype and their leaflets surface area in no-choice test.

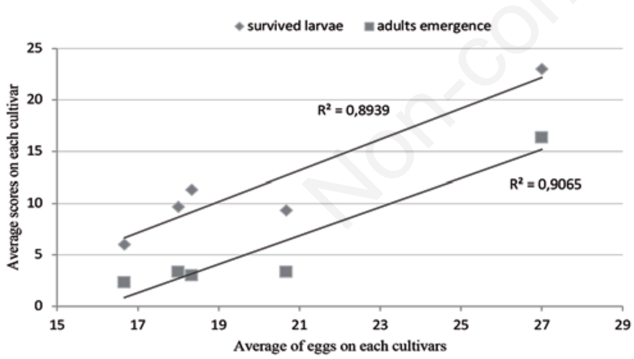

Figure 5. The relation between the numbers of eggs laid on each genotype and larval mortality and adult emergence.

If it is true, we assume that a smaller number of eggs should be expected to be laid during the no-choice test in particular on the ILC 86 genotype. Field adults of *Liriomyza trifolii* (Burgess) were found to have different oviposition preference than a laboratory colony due to their higher genetic variability (Trumble & Quiros, 1988).

During the dual-choice test, significant differences in total eggs were scored in control vs control compared to control vs other plants. This indicates the recognition of a stimulant/inhibitor for oviposition by the insect. However, this could be explained by the strong correlation that was found between the numbers of eggs laid on each plant and its leave surface area. Leaves with small leaflets and a high number of trichomes were associated with fewer eggs deposition (Khoja *et al*., 2012). Leaf tissue structure was found to be influencing the feeding and the probing behavior of *L. huidobrensis* (Blanchard) on pea plants (Wei *et al*., 2000), and *L. sativae* (Blanchard) on melon plants (de Oliveira *et al*., 2021). Other studies examined the role of acid exudates in the resistance/susceptibility suggested malic and oxalic acids as markers of selection against chickpea leaf miner (Rembold *et al*., 1989; Ali *et al*., 2020). A recent study has suggested a potential implication of secondary metabolites in the chickpea host plant resistance as *L. cicerina* infestation induced an increase in total phenol and flavonoids contents particularly at the vegetative stage (Soltani *et al*., 2020). However, *L. cicerina* tends to prefer fully grown leaves from the plant base rather than the newly formed leaves from the upper part of the plant (Pimbert, 1989; Soltani *et al*., 2017). A similar tendency was reported for other *Liriomyza* spp. on other hosts (Facknath, 2005). Such behavior from the females might avoid competition for food for their offspring (Auerbach & Simberloff, 1989; Aparicio *et al*., 2015).

Our results have shown a correlation between egg deposition and insect performance. Higher larval mortality and fewer adult emergences were scored on the less preferred plants. The ILC 86 scores were of an exception to this conclusion; no significant differences were found between egg deposition on ILC 86 and the control (the commercial cultivar) when no alternatives were available. However, higher insect performance was observed for the local cultivars Moubarak and Arifi that were highly preferred for oviposition (Oubayoucef, data not included). These results suggest that the studied improved lines can exhibit both antixenosis and antibiosis mechanisms of host plant resistance according to Painter classification (Painter, 1951).

Chickpea resistant cultivars were shown to suffer less mining damage (Ali *et al*., 2015) and were highly recommended to be incorporated into IPM strategies (El-Bouhssini *et al*., 2008; Ali *et al*., 2015). Understanding the mechanism of resistance and the plant-insect interaction under natural conditions is crucial for a better long-term deployment of the improved cultivars (Rembold & Winter, 1981). Other factors such as the insect behavior and the role played by the natural enemies also play an important role in insect management (El-Bouhssini *et al*., 2008; Soltani *et al*., 2018). The level of infestation was reported to be higher in border plants compared to plants from inside the plots on field situation (Soltani

Table 3. Effects of chickpea genotypes on the number of eggs laid per plant in the no-choice test, larval mortality and adults emergence.

	ILC 93	LMR 133	LMR 202	ILC 86	Control	S.E.D	L.S.D
Eggs	16.67[a]	18[a]	18.33[a]	20.67[ab]	27[b]	1.84	4.25
Larvae	6[a]	9.67[a]	11.33[a]	9.33[a]	23[b]	1.93	4.46
Adults	2.33[a]	3.33[a]	3[a]	3.33[a]	16.33[b]	1.96	4.52

Different letters mean different significance.

et al., 2017). In this context, our results suggest a potential use of highly susceptible cultivars all along with resistant ones either in a variety mixture or attract-and-kill pest management designs (Karungi *et al*., 2010; Grettenberger & Tooker, 2017). The great attraction of the susceptible plants could avoid egg deposition on the resistant plants. However, this strategy should be banded with strict monitoring that will allow spraying on susceptible plants in case of heavy infestation and thus reducing economical costs. Selective insecticides showed encouraging results and their use must be enhanced to reduce their negative effects on the natural enemies (El-Bouhssini *et al*., 2008; Çikman *et al*., 2011).

Conclusions

The present study has clearly shown that *L. cicerina* oviposition behavior varied depending on the available plants in terms of incitement and preference. Furthermore, the number of oviposited eggs was correlated with leaflet size and offspring performance. Results also suggest that resistant plants possess both antixenosis and antibiosis mechanisms of resistance.

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