EVALUATION OF RESISTANCE OF CUCUMBER CULTIVARS TO THE VEGETABLE LEAFMINER (Liriomyza sativae Blanchard) (DIPTERA: AGROMYZIDAE) IN GREENHOUSE

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The leafminer, Liriomyza sativae Blanchard, is distributed around the world and is an important pest of vegetables and ornamentals. Given the resistance potential of the leafminer to current insecticides, the use of resistant plant cultivars and parasitoids could be effective integrated pest management (IPM) strategies against it. Cucumber (Cucumis sativus L.) is a preferred host for this insect. Seventeen cultivars of cucumber have been evaluated to study resistance mechanisms to L. sativae. All cucumber cultivars were evaluated in screening tests in greenhouse with indices such as the number of leafminer stings, the number of larval mines, the proportion of larval mines to leafminer stings, and the rate of injury. Significant differences (p < 0.01) were found among cultivars, as well as significant correlations among all evaluated indices. Cucumber cultivars were ranked by the cluster method based on all measured characters and were classified into four groups: susceptible, semi-susceptible, semi-resistant, and resistant. Trials of antibiosis resistance of cucumber cultivars were conducted in a growth chamber and were evaluated with biological indices of insect activity including oviposition period, larval and pupal duration, percentage of larval and pupal mortality, and the sex ratio among selected cultivars. Significant differences were found for all indices except pupal weight and the sex ratio. The cultivars were analyzed by the cluster method based on all measured characters and were divided into three groups including sensitive, slightly resistant, and semi-resistant. No cultivar was immune to injury.

Key words: Cucumber cultivar, screening, antibiosis, Cucumis sativus.

Cucumber (Cucumis sativus L.), which is the main greenhouse vegetable in Iran, is attacked by different pests, including leafminers (Fathipour et al., 2006). Agromyzid leafminers are found worldwide and have an economically important impact on many agricultural crops (Kaspi and Parrella, 2005; Hondo et al., 2006). The leafminer Liriomyza sativae Blanchard is a major pest of greenhouse cucumbers in Iran and a wide variety of vegetables and ornamental crops throughout the world (Parrella, 1983; Reitz and Trumble, 2002). Damage is caused mostly by larvae that feed and mine the mesophyll and by the female feeding behavior, puncturing the leaf with its ovipositor and feeding on the leaf sap, which decreases photosynthesis (Parrella, 1983). As well, they may transmit plant pathogens during oviposition (Johnson et al., 1980; Minkenberg and Helderman, 1990). Chemical control of leafminers usually lasts only a short period of time and adult control with contact insecticides is often ineffective because flies can easily move around, and the treated field is subject to reinestation from adjacent untreated crops and weeds (LeStrange et al., 1999).

Leafminers have developed a high degree of resistance to a broad range of insecticides (Mason et al., 1987; Parrella and Trumble, 1989; Keil and Parrella, 1990). Therefore, it is essential to develop alternative strategies for leafminer management. Resistant varieties remain the most economical means of insect control. Their development could reduce pesticide use, which would be beneficial to growers, consumers, and the environment. Screening for antixenotic (nonpreferential) plants that have traits that make them unattractive to insect pests to feed or to lay their eggs is usually carried out in choice tests where insect can choose among different plant genotypes for feeding or oviposition (Mou and Liu, 2004). Mou and Liu (2004) screened more than 200 lettuce (Lactuca sativa L.) accessions and identified sources of antixenosis resistance. Mou (2008) screened 345 accessions of spinach (Spinacia oleracea L.) for evaluation of antixenosis resistance and found that resistant genotypes have fewer pores than commercial cultivars. Antibiosis and antixenosis resistance to Liriomyza trifolii (Burgess) in tomato (Lycopersicon esculentum Mill.) hybrids were reported by Erb et al. (1993). Studies of antixenosis resistance divided 19 bean (Phaseolus vulgaris L.) cultivars into three main groups, including semi-resistant,
regularly. The flies used in this study had been reared on cucumber for 5 months.

To rear vegetable leafminer, individuals were collected from cucumber greenhouses in suburbs of Tehran, Iran, in August 2008. *Liriomyza sativae* larvae were reared on cucumber, *C. sativus* cv. Adrian and maintained at 25 ± 1 °C, 65 ± 5% RH, and a 16:8 h photoperiod. The flies used in this study had been reared on cucumber for 5 months. To reduce any inbreeding effects, wild flies were added regularly.

Seventeen cultivars of cucumber were screened for leafminer resistance, including ‘Vikima’, ‘Korazh’, ‘Karim’, ‘Soletan’, ‘Green magic’, ‘Royal’, ‘Evergreen’, ‘Jiroft 1’, ‘Khasib’, ‘Zohal’ (greenhouse), ‘Service plus’, ‘Maximus F1’, ‘Victor’, ‘Super dominus’ (field), and local cultivars ‘Sanandaj’, ‘Gorgan’, and ‘Roodbar’. Seeds were sown in 10 × 10 × 10 cm plastic pots with a 2:1 sand:soil mix (by volume). Pots were placed in a growth chamber with supplemental fluorescent lighting.

### MATERIALS AND METHODS

#### Rearing methods

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### Screening experiments

Experiments were conducted at the Agricultural Research Station, Varamin, Iran, in 2009. Four weeks after planting, all cultivars were arranged in a randomized complete block design (RCBD) with five replicates, with each plot consisting of one plant in a pot. Spacing was 30 cm between plants and 100 cm between blocks in a greenhouse set at 25 ± 2 °C, 55 ± 5% RH and a natural photoperiod. The number of leafminer stings, the number of larval mines, the proportion of larval mines to leafminer stings, and the rate of injury were determined. The number of stings was counted in 4 cm² leaf areas with the highest sting density on a randomly selected leaf from each plant with the aid of a magnifying glass. The numbers of leafminer stings and larval mines and the rate of injury were evaluated on days 1, 4 and 15, respectively.

#### Antibiosis trials

Four weeks after planting, six cucumber cultivars were selected based on screening results in this research, plants were placed in 30 cm high × 30 cm wide × 40 cm deep insect cages covered with polypropylene fabric, and were arranged in a completely randomized design with 10 replicates. The experimental unit was a pot. All of them were kept in a growth chamber at 25 ± 1 °C, 55 ± 5% RH, and 16:8 h photoperiod. Four of same age fly pairs were released in each of the insect cages to feed on the plants. Flies were removed after day one and the plants were evaluated using biological indices of insects, including oviposition period, larval and pupal periods, percentage of larval and pupal mortality and sex ratio among selected cultivars.

### Leaf hairs in selected cultivars

For the assessment of this trait, a fully expanded healthy leaf from each cultivar was cut and examined with binocular microscope at 50X magnification. A four-stage scale was used to score hair density of short, long, and glandular hairs.

### Statistical analysis

Data were analyzed with the SPSS statistical program (SPSS, 2004) and were verified for normal distribution, except for rate of injury data, which were normalized by log10.

### RESULTS

#### Screening experiments

Differences among cultivars were found significant for measured traits (Table 1). The number of leafminer stings was significantly different among cultivars. The mean (±SE) number of leafminer stings per 4-cm² leaf area ranged from 2.80 ± 0.37 (Gorgan, a local cultivar) to 29 ± 0.71 (‘Karim’). The number of larval mines was significantly different among cultivars. Larval mines varied from 0.8 ± 0.49 (‘Gorgan’) to 7 ± 0.71 (‘Karim’). The proportion of larval mines to leafminer stings ranged from 0.19 ± 0.01 (‘Korazh’) to 0.55 ± 0.08 (‘Green magic’) (Table 2).

The correlation between leafminer stings and larval mines was positive and significant (*r* = 0.85, *p* < 0.01). The mean rate of injury ranged from 1.40 ± 0.40 (‘Gorgan’, the cultivar with the most stings and the least damage) to 9.60 ± 0.24 (‘Zohal’, the cultivar with the greatest damage) (Table 2). No cultivar was immune to injury.

Means were compared separately for each index (Table 2). Leafminer activity among cultivars was significantly

### Table 1. ANOVA of stings per 4-cm² leaf area, larval mines, and proportion of mines to leafminer stings.

| Source of variation | Degree of freedom | Leafminer stings | Larval mines | Proportion of larval mines to leafminer stings | Rate of injury |
|---------------------|------------------|------------------|--------------|-----------------------------------------------|---------------|
| Block               | 4                | 6.558*           | 3.944*       | 0.0474*                                       | 1.045         |
| Cultivars           | 16               | 319.347**       | 17.423**     | 0.057*                                        | 17.125**      |
| Error               | 64               | 2.352*           | 0.987*       | 0.032*                                        | 0.445         |
| CV                  | 12               | 12.973*          | 26.65*       | 0.097*                                        | 20.03         |

*Significant F tests at *P* = 0.05 and 0.01, respectively; ns: non significant.
Table 2. Means (± SE) of traits under study in leaves of 17 cucumber cultivars.

| Cultivar          | Number of leafminer stings | Number of larval mines | Proportion of larval mines to leafminer stings | Rate of injury |
|-------------------|----------------------------|------------------------|------------------------------------------------|---------------|
| Evergreen         | 17.80 ± 1.24b              | 5.60 ± 0.51b           | 0.30 ± 0.03abcd                                 | 5.40 ± 0.50c  |
| Karim             | 29.00 ± 0.71a              | 7.00 ± 0.71a           | 0.24 ± 0.02cd                                   | 7.20 ± 0.58b  |
| Korazh            | 28.00 ± 0.001a             | 5.20 ± 0.37b           | 0.19 ± 0.01d                                    | 7.00 ± 0.70b  |
| Vikima            | 14.60 ± 0.51c              | 5.40 ± 0.51b           | 0.37 ± 0.04abcd                                 | 5.20 ± 0.37c  |
| Zohal             | 18.20 ± 0.86b              | 6.80 ± 0.58a           | 0.38 ± 0.04bcd                                  | 9.60 ± 0.24a  |
| Khasib            | 12.60 ± 0.08c              | 4.40 ± 0.24bc          | 0.36 ± 0.03abcd                                 | 5.40 ± 0.50c  |
| Jiroft1           | 14.20 ± 0.86c              | 4.40 ± 0.40bc          | 0.32 ± 0.03abcd                                 | 5.20 ± 0.37c  |
| Royal             | 9.80 ± 0.37d               | 4.20 ± 0.37bc          | 0.43 ± 0.04abcd                                 | 4.20 ± 0.58c  |
| Green magic       | 5.20 ± 0.37e               | 2.80 ± 0.37d           | 0.55 ± 0.08a                                    | 4.20 ± 0.58c  |
| Super dominus     | 5.20 ± 0.37e               | 2.20 ± 0.37def         | 0.43 ± 0.07abcd                                 | 5.40 ± 0.50c  |
| Victor            | 5.00 ± 0.45e               | 1.80 ± 0.37def         | 0.36 ± 0.06abcd                                 | 4.20 ± 0.58c  |
| Maximums          | 5.20 ± 0.37e               | 2.20 ± 0.37def         | 0.45 ± 0.10abcd                                 | 4.40 ± 0.50c  |
| Service plus      | 8.40 ± 0.93c               | 4.20 ± 0.58bc          | 0.51 ± 0.06ab                                   | 5.40 ± 0.50c  |
| Soltan            | 18.80 ± 0.73c              | 3.00 ± 0.45cd          | 0.20 ± 0.03d                                    | 4.20 ± 0.37c  |
| Roodbar Local     | 5.40 ± 0.68e               | 2.40 ± 0.24de          | 0.50 ± 0.12abc                                  | 4.40 ± 0.24c  |
| Sanandaj Local    | 4.80 ± 0.37f               | 1.20 ± 0.37ef          | 0.25 ± 0.08bcd                                  | 2.20 ± 0.37d  |
| Gorgan Local      | 2.80 ± 0.37f               | 0.80 ± 0.49f           | 0.30 ± 0.04d                                    | 1.40 ± 0.40d  |

Means followed by the same letter are not significantly different at the 5% level according to Duncan’s multiple range test.

**Table 3. ANOVA for traits under study in leaves of six cucumber cultivars in antibiosis test.**

| Source of variation | dfz | Oviposition period | Larval period | Larval mortality | Pupal period | Pupal mortality | Sex ratio |
|---------------------|-----|--------------------|---------------|-----------------|--------------|----------------|-----------|
| Cultivars           | 5   | 1.069**            | 1.725**       | 1488.56**       | 0.029**      | 608.81**       | 0.013ns   |
| Error               | 54  | 0.039              | 0.092         | 9.138           | 0.008        | 8.112          | 0.034     |
| CV                  | -   | 5.70               | 6.26          | 13.96           | 3.29         | 16.38          | 19.32     |

dfz: degree of freedom; **Significant F tests at P = 0.01; ns: non significant.

**Table 4. Mean (± SE) for traits under study in leaves of six cucumber cultivars.**

| Cultivar          | Oviposition period | Larval period | Larval mortality | Pupal period | Pupal mortality |
|-------------------|--------------------|---------------|-----------------|--------------|----------------|
| Gorgan Local      | 3.47 ± 0.08bc      | 4.90 ± 0.05bc | 17.60 ± 0.74c   | 8.22 ± 0.11ab| 15.20 ± 0.41c  |
| Soltan            | 3.21 ± 0.04bd      | 4.53 ± 0.14cd | 12.50 ± 0.56de  | 8.06 ± 0.03ab| 12.00 ± 0.53cd |
| Victor            | 3.60 ± 0.06bd      | 5.04 ± 0.02b  | 31.50 ± 1.51b   | 8.27 ± 0.04ab| 21.30 ± 1.33b  |
| Jiroft1           | 3.35 ± 0.08cd      | 4.70 ± 0.12bcd| 15.70 ± 0.81cd  | 8.15 ± 0.06b | 13.80 ± 0.53cd |
| Super dominus     | 4.03 ± 0.01a       | 5.53 ± 0.07a  | 41.50 ± 1.24a   | 8.47 ± 0.07a | 31.40 ± 1.49a  |
| Karim             | 3.12 ± 0.05d       | 4.36 ± 0.10d  | 10.80 ± 0.29e   | 8.01 ± 0.02b | 10.60 ± 0.33d  |

Means followed by the same letter are not significantly different at the 5% level according to Duncan’s multiple range test.

**Figure 1. Ward’s clustering dendrogram of resistance of 17 cucumber cultivars against Liriomyza sativae.**

**Antibiosis trials**

Significant differences were found for all indices except sex ratio (Table 3). The oviposition period ranged from 3.12 ± 0.05 (‘Karim’) to 4.03 ± 0.01 (‘Super dominus’) (Table 4). The larval period varied from 4.36 ± 0.1 (‘Karim’) to 5.53 ± 0.07 (‘Super dominus’) and the maximum percentage of larval mortality was observed in ‘Super dominus’ (41 ± 1.24%) and minimum percentage of larval mortality was observed in ‘Karim’ (10.80 ± 0.29%). The mean of pupal period ranged from 8.06 ± 0.02 (‘Karim’) to 8.47 ± 0.07 (‘Super dominus’) and the maximum percentage of pupal mortality was observed in ‘Super dominus’ (31.4 ± 1.4%) and minimum percentage of pupal mortality was observed in ‘Karim’ (10.60 ± 0.33%) (Table 4). Finally, the cultivars were clustered using all parameters into three groups: sensitive (‘Sultan’,...
‘Jiroft’, ‘karim’), slightly resistant (local ‘Gorgan’, ‘Victor’) and semi-resistant (‘Super dominus’) (Figure 2).

**Trichomes in the cultivars**

The counts of trichomes in the cultivars are presented in Table 5. Local cultivars (‘Gorgan’, ‘Sanandaj’, ‘Roodbar’) and field cultivar (‘Super dominus’) have the highest number glandular hairs and greenhouse cultivars (‘Karim’, ‘Korazh’, ‘Zohal’) had no glandular hairs.

![Figure 2. Ward’s clustering dendrogram of the resistance of six cucumber cultivars against Liriomyza sativae in tests for antibiosis.](image)

Table 5. Characteristics of trichomes in cucumber cultivars.

| Cultivars | Short trichomes | Long trichomes | Glandular trichomes |
|-----------|-----------------|----------------|---------------------|
| Evergreen | Low             | Low            | Low                 |
| Karim     | Low             | Nil            | Nil                 |
| Korazh   | Low             | Low            | Nil                 |
| Vikima   | Low             | Low            | Low                 |
| Zohal      | Low             | Low            | Nil                 |
| Khasib     | Low             | Low            | Medium              |
| Jiroft 1   | Medium          | Medium         | Medium              |
| Royal      | Medium          | Medium         | Medium              |
| Green magic | Medium        | Medium         | Medium              |
| Super dominus | Medium    | High           | High                |
| Victor     | Medium          | Medium         | Low                 |
| Maximuns   | Medium          | Medium         | Medium              |
| Serviceplus | Medium        | Medium         | Medium              |
| Soltan     | Low             | Low            | Low                 |
| Roodbar Local | Medium  | High           | High                |
| Sanandaj Local | High     | High           | High                |
| Gorgan Local | High       | High           | High                |

**DISCUSSION**

All measured traits showed significant differences among the cultivars, suggesting that they differ in suitability to the leaffminer, and that there is resistance to leaffminer in cucumber germplasm. ‘Karim’ and ‘Gorgan’ had the most leaffminer stings and least larval mines respectively. The correlation (r = 0.85, p < 0.01) between both traits indicates a strong relationships, similar to studies of resistance to leaffminers by Mou and Liu (2003) and Zahiri et al. (2003).

No cultivar was immune to injury. Therefore comparison of results among leaffminer stings and larval mines to rate of injury showed that all of these traits are interdependent. Similar result was found in the study by Zahiri et al. (2003).

All traits measured (leaffminer stings, larval mines, larval mines/leaffminer stings, and rate of injury) to evaluate antixenosis were related. Thus, the number of stings per unit of leaf area is a feasible trait in selecting leaffminer-resistant plants. Mou and Liu (2003) suggested that the number of stings per unit leaf is a more reliable trait for investigation of plant resistance to leaffminers. As well, Zahiri et al. (2003) used four parameters (cited above), but leaffminer stings were introduced for evaluation of cultivars of *Apium graveolens* (L.) to *L. trifolii* (Trumble et al., 2000). So, the index of leaffminer stings has been confirmed by all researchers as the definitive criteria for evaluating resistance.

Local cultivars (Gorgan, Sanandaj, Roodbar) had significantly fewer leaffminer stings, larval mines and rate of injury than greenhouses cultivars (Vikima, Korazh, Karim, Soltan, Green magic, Royal. Evergreen, Jiroft 1, Khasib, Zohal) and field cultivars (Service plus, Maximus F1, Victor, Super dominus) (Table 1). There are similar results for resistance of other crops to leaffminers, for example, 46 lettuce genotypes were evaluated and results showed that wild species had significantly fewer leaffminer stings than cultivated lettuce (Mou and Liu, 2003). An accession from another wild species, *Apium nodiflorum* (L.), demonstrated substantial insect toxicity and few mines were observed (Trumble et al., 1990). Among 345 accesses of the U.S. spinach collection for leaffminer resistance, significant genotypic differences were found for leaffminer stings per unit leaf area and mines per plant (Mou, 2008). These results suggest that greenhouses cultivars are generally more susceptible to the leaffminer than the local cultivars and field cultivars.

In the choice test, fewer stings per unit leaf area suggest host non-preference (antixenotic resistance). Resistance based on antixenosis would be desirable because even the photosynthetic losses caused by adult feeding and oviposition would be reduced. This could prompt leaffminer movements to weeds or crops tolerant to insect damage, as suggested by Trumble et al. (1985).

Based on results of this research, in a stable temperature and without antagonistic insects, larval period and percentage of larval mortality are intensity affected by the host plant, and in this case, cucumber cultivars were related particularly to the larval period and the percentage of larval mortality. A study of resistance of *chrysanthemum* cultivars to *L. trifolii*, survival of 1st and 2nd stage larvae was affected by plant resistance (Bottrell et al., 1998). As well, in a free-choice study of sources of resistance in celery genotypes under assay, resistant cultivars showed fewer leaffminer stings and longer larval periods, indicating antixenosis and antibiosis resistance to *L. trifolii* (Trumble et al., 2000). In this research, significant differences were found in pupal period and pupal mortality. Similar results were found in evaluation of resistance of lettuce cultivars to *L. sativae* (Mou and Liu, 2004). However, in the study of resistance of bean varieties to leaffminers, no significant differences were observed in the pupal period and the percentage of pupal...
mortality (Zahiri et al., 2005). Our results for antibiosis revealed genetic diversity among cucumber cultivars. For example, the percentage of larval mortality ranged from 10.8% in ‘Karim’ (greenhouse cultivar) to 41.50% in ‘Super dominus’ (field cultivars). This result does not indicate resistance in excess to L. sativae in the resistant cv. Super dominus, such that use of this cultivar will not lead to generating a resistant population of the insect. The cucumber cultivars clustered according to all measured characters were classified into three groups, similar to those of Zahiri et al. (2005) in the study of antibiosis of bean cultivars to L. sativae. Principal component analysis indicated that there is considerable diversity in cucumber cultivars for antixenosis and antibiosis components of resistance to L. sativae. Cultivars were assigned to different groups, from which both antixenosis and antibiosis to L. sativae can be used in resistance breeding programs to diversify the basis of resistance to this pest.

Glandular hairs are one of the resistant factors in cucumber to this leafminer. The genetics of glandular hairs is complicated and requires more study in the selected cultivars. More density of glandular hair was observed in local cultivars and likewise, the lowest density was observed in cultivars mainly planted in greenhouses. Furthermore, local cultivars were more resistant than greenhouse ones to the leafminer when screening for antibiosis. There is probably a direct inverse relationship between resistance and sensitivity of the cultivars and the presence and density of trichomas. Further studies should be conducted on their chemical basis.

Resistant cultivars remain the most economical means of insect control. Their use cuts down on the costs of chemicals, machinery, fuel and labor associated with pesticide spray. It also reduces the exposure of workers to hazardous chemicals. Insecticide residues in plants resistant to insects are reduced, and result in increased consumer acceptance of produce. It may also reduce pesticide contamination of soil and ground water, alleviating the pressure on the environment. Cucumber products free from leafminer stings and mines have potentially greater quality and value (Mou, 2008).

CONCLUSION

Our results indicate that greenhouse cucumber cultivars were generally more susceptible to L. sativae than the local and field cultivars. Resistance of these cucumber cultivars appear to be related to the presence of glandular hairs. Herein, a wide range of genetic variation in traits related to leafminer resistance was found in cucumber germplasm.

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Palabras clave: Cultivar de pepino, screening, antibiosis, Cucumis sativus.
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