

# Comparison of Plant Species as Host for Cabbage Leaf Miner in Khuzestan Province

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## ABSTRACT

Cabbage leaf miner *Scaptomyza flava* (Fallen) (Diptera: Drosophilidae) is a native and oligophagous leaf miner insect on cruciferous plants (Brassicaceae). It occurs in many parts of the world and throughout Khuzestan. The relationship between feeding and ovipositional preferences of insect, and host plant suitability on seven host plant species (cauliflower *Brassica oleracea* var. *botryds*, green cabbage *B. oleracea* var. *capitata*, hedge mustard *Sisymbrium officinale*, brussels sprout *B. oleracea* var. *germmifers*, turnip *Brassica rapa*, cucumber *Cucumis mtivits*, wheat *Triticum aestivum* and rice *Oryza saliva*) was evaluated. Ovipositional preference of *S. flava* was determined by measuring feeding punctures and egg density after adult female flies were given a simultaneous choice and non-choice of all host plants for feeding and oviposition. Studies were performed under greenhouse conditions. The experimental design was a randomized complete block one with 8 treatments and 5 replications of each treatment. *S. flava* showed distinct hierarchical ordering in its ovipositional preference, with turnip, green cabbage, and hedge mustard being preferred over all others. Approximately 1.05 and 5.8 times more eggs were deposited on green cabbage than on turnip and cauliflower respectively. When the percentage of punctures with eggs was calculated for each species, cauliflower had by far the highest value at 10% and 19.7% in choice and non-choice tests respectively. For all other species on which eggs were laid it was less than half this. In non-choice tests, females laid more eggs on green cabbage and turnip than other brassicas.

Keywords: Insect-plant relationship , Choice test , Non-choice test , Drosophilidae , Feeding and Ovipositional preference , *Scaptomyza flava*.

## INTRODUCTION

Dipteran leaf miners are a large and widespread group of small flies, most with larvae attacking a wide range of plants. Most simply mine leaves but a few are gall makers (7). Some species are cosmopolitan, others solely temperate and some restricted to the tropics. The range of host specificity is great, from broad polyphagy to restricted monophagy

on a single genus of host plant. About 150 species are regularly associated with cultivated plants, and these were the subject of a monograph by Spencer (23), the total number of species recorded is about 1800 (7).

Most leaf mining Diptera are from family Agromyidae but the larvae of some drosophilids have the leaf habit.

The crops most likely to suffer multiple leaf miner fly infestation (including Agromy-

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zidae and Drosophilidae) are those belonging to the families Leguminosac, Gramineac, Solanaceae, Cucurbitaceae, Chcnopodiaceae, and Brassicaceae.

Leaf mining Diptera are very common on seedlings, young plants and in some cases mature plants of most cultivated cruciferae in Khuzestan, The cabbage leaf miner, *Scaptomyza flava* (Fallen) (Diptera: Drosophilidae), is one of the most common leaf miners in Khuzestan, These minute flies have been greatly neglected in this region. *S. jlava* commonly infests many cruciferous vegetables especially at the young or seedling stages. It occurs as a pest in temperate and tropic zones. *S. flava* is widespread in Europe, Asia. North America, Australia and throughout Khuzestan.

The insect has become increasingly important within the last few years as a pest in Khuzestan. Other *Scaptomyza* species are probably present in Khuzestan, but little is known of their biology.

Damage by *S. flava* is caused by mining of the larvae within the leaf tissue and also by the feeding and ovipositional habits of the adult female. Under favourable environmental conditions, its population may reach high levels. The literature concerning *S. jlava* is very limited, although Holloway (B.A. Holloway, 1998. Personal communication) reported some details of host plants.

The identification of drosophilid leaf miner is extremely difficult at species level, many species being only distinguishable by the male genitalia, but at generic level there are some differences in acrostichal hairs on the thorax (6).

*S. flava* adults have four longitudinal rows of acrostichal setae in front of anterior dorsocentral bristles. For the identification of larvae, Holloway suggests a key according to the shape of posterior spiracles. Posterior spiracles of *S. flava* are light, with elongate and parallel apices.

The symptoms and extent of damage caused and the relationship of insects to their host plants are of fundamental importance to understanding pest biology and abundance and critical to the development of pest management systems. This research has been directed towards determining the behavior of *S. flava* on selected hosts, the economically important brassica species.

One of the most crucial events in the life cycle of phytophagous insects is the selection of a suitable site for oviposition. Newly hatched larvae of many insects are relatively immobile (e.g., leaf miners), and depend on the ability of their mother to find the best source of food for their successful growth and development. The driving forces affecting host finding and acceptance or rejection of a host are governed largely by surface and nutritional qualities of the plant, competition and coincidence of favourable conditions that ensure success of offspring (3).

Host selection by phytophagous insects can be considered as "choice behaviour" with the choice being made at different stages in the sequence leading up to host acceptance (24). Some insects "choose" solely after contact with the plant. Here the frequency with which different plant hosts and non-hosts, are visited depends only on their apparency and relative abundance. Other insects perceive plant characteristics at a distance and initially "choose" according to these impressions (4 and 24). However, in this case, after this initial choice is made, it should be noted that the insect probably continues to "choose" as it proceeds through the sequence leading up to oviposition (M. Harris. 1998. Personal communication).

In this study the selection by *S. flava* for feeding and oviposition from among eight plant species including five within the family Brassicaceae was examined. The specific objectives of this study were (1) to determine if *S. flava* will accept plants other

than Brassicaceae and (2) compare a range of brassicas as host plants for *S. flava*.

## MATERIALS AND METHODS

The experiments were conducted under ambient greenhouse conditions in the Plant Growth Unit, Shahid Chamran University. Feeding and oviposition preferences of adult females were determined for eight plant species (five first ones from Brassicaceae, sixth plant from cucurbitaceae and the last two plants from gramineae) (Table 1). Preference was measured by the number of feeding punctures and the number of eggs laid on the plants, in choice and non-choice tests. Temperature in the experimental chamber was maintained at 14±2°C (night) and 22±2°C by day. Natural daylength at a 11:13 (L:D) hours photoperiod with photophase from 07:10 (sunrise) to 17:15 (sunset) was that in December 1997. The experiments began between 09:00 and 11:00 hours when the flies were introduced to the test arenas.

Table 1. Eight plant species evaluated in choice and non-choice test with *S. flava*.

Common name	Scientific name
1. Cauliflower	<i>Brassica oleracea</i> (L.) var. <i>botrytis</i>
2. Green cabbage	<i>Brassica oleracea</i> (L.) var. <i>capitata</i>
3. Hedge mustard	<i>Sisymbrium officinale</i> (L.) Scop.
4. Brussels sprout	<i>Brassica oleracea</i> (L.) var. <i>gemmifera</i>
5. Turnip	<i>Brassica rapa</i> (L.)
6. Cucumber	<i>Cucumis sativus</i> (L.)
7. Wheat	<i>Triticum aestivum</i> (L.)
8. Rice	<i>Oryza sativa</i> (L.)

All plants were raised from seed in a greenhouse with the exception of hedge mustard which was collected from the field (plant about 10-15 cm high) and placed into pots. Seeds were germinated in greenhouse

media (peat, sand, pumice, dolomite, lime and superphosphate) in plastic pots (20 cm diameter) in an outdoor growth chamber (controlled environment with 20±1°C temperature and 70±10% RH) with enough light and fertilizer for plant growth to continue, except for wheat and rice seedlings which were transplanted at cotyledon opening into pots (10 cm diameter). A single transplant was planted in each pot except for wheat and rice where plants were not transplanted and each pot contained a group of plants (5 plants for rice and 10 for wheat). Plants were overhead watered on alternate days for the first four weeks and were also kept moist by capillary matting and perforated plastic film on the bench. Plants selected for choice and non-choice experiments were mostly at the 3-4 true-leaf stage (26-30 days after sowing, ca. 24 cm tall).

Newly emerged adult *S. flava* used in choice and non-choice experiments were treated identically before exposure to test plants. Flies were sexed (by obvious saw form ovipositor of females) under a binocular microscope after being anaesthetised by CO<sub>2</sub>.

The insects used in experiments were about 15 to 20 generations removed from field populations and were from a laboratory colony raised on green cabbage.

When the experiments were terminated, plant foliage was examined for the presence of feeding punctures and eggs with transmitted light under a dissecting microscope. To measure oviposition, each leaf was divided into four quadrants by marking four segments equidistant along the midvein, then drawing a line perpendicular to the midvein. The number of eggs (or feeding punctures) occurring in each quadrant were then counted. Test plants were stored in a refrigerator (4°C) until assessments could be made.

### Choice Tests

In these tests, experiments were conducted

in the test chamber described above, in five arenas (cylindrical cage, 85 cm in diameter and 90 cm in height made from net fine cloth), each of which contained eight potted plants (one of each plant species) arranged at random in a circular formation 10 cm apart and 14 cm from the cage walls. Groups of four malefemale pairs of newly emerged adult *S. flava* were introduced into each cage and left for four days to permit mating (21 and 25), feeding and egg laying (under laboratory and green-house conditions, the majority of males and females male within 24-48 hours of emergence and most gravid females began oviposition within 12-36 hours after mating). The Hies were then removed, and the distribution of feeding punctures and eggs among leaves was recorded,

The experimental design was a randomized complete block one. The strata of variation were among the five blocks (cages) and the eight treatments within blocks.

The experimental "whole treatment" units were pots of one plant and the allocation of treatments (different plant species) within blocks was randomized and their order was changed for each replicate. The results were subjected to two-way analysis of variance. When the analysis of variance (ANOVA) showed significant treatment effects (PS0.05), Fisher's protected least significant difference (LSD) test was used to separate means at PS0.01 (11). Regression analysis (SAS, 20) was used to evaluate the relationship between numbers of feeding punctures and eggs per leaf.

### **Non-Choice Tests**

In the non-choice test, eight single pots of each plant species were placed separately in each test arena (square cages (30 by 30 cm) of terylene net). There were three arenas (replications) for each species. The arenas

were assigned at random to positions in the greenhouse. The flies were sexed and paired up at random. Four newly emerged (3 hours old) male+female adult flies were placed in each cage and left for 4 days.

## **RESULTS**

The results of choice tests are presented in Table 2 and of non-choice tests in Table 3.

### **Choice Tests**

In a choice situation, on the basis of feeding punctures and number of eggs laid, plant species fell into three groups:

1) brussels sprout, green cabbage, turnip and hedge mustard with large number of feeding punctures and moderate number of eggs, 2) cauliflower with few feeding punctures and fewer eggs than the first group, and 3) cucumber, wheat and rice (non-brassicaceous plants) with no feeding punctures or eggs.

There were significant differences in total number of feeding punctures on the cruciferous plant species, most for brussels sprout, least for cauliflower and intermediate for turnip, hedge mustard and green cabbage (table 2). Some differences among species within this group in the total number of eggs laid were also significant. Turnip received significantly more eggs than brussels sprout and cauliflower, and cauliflower received fewer than all other species. There was no significant correlation between number of feeding punctures and eggs deposited.

Significant differences in developmental time occurred only between flies reared on turnip and cauliflower. Development was most rapid on turnip, slowest on green cabbage and intermediate on cauliflower.

### **Non-Choice Tests**

Two major differences were observed

**Table 2.** Mean number of feeding punctures, eggs, lifespan and developmental lime on eight plant species in choice tests with *S. flava*.

Plant species	Mean No. punctures per pot	Mean No. eggs per pot	% of punctures with eggs	% of egg emergence	ALS (days)	DT (days)
Cauliflower	24.7±2.3c	2.1±0.05bc	10	100	11	41a
Green cabbage	200.1±5.2b	3.2±0.9b	2.8	100	13	36ab
Hedge mustard	153.7±5.6b	7.7±0.8ab	3.4	89	12.5	35ab
Brussels sprout	402.9±8.1a	5.3±0.1b	1.1	92	12.3	34ab
Turnip	184.2±7.2b	12.5±0.9a	4.2	84	10.6	31b
Cucumber	Od	0c	-	-	-	-
Wheat	Od	0c	-	-	-	-
Rice	Od	0c	-	-	-	-

Treatments accompanied by the same letter are not significantly different at  $P \leq 0.05$  (ANOVA followed by LSD [ $l_{sd \text{ punctures}} = 67.29$ ,  $l_{sd \text{ eggs}} = 8.4$ ] test for separation of means). <sup>a</sup> Adult life span: Days from adult emergence to adult death. <sup>b</sup> Developmental Time: Days from egg to adult death.

**Table 3.** Mean number of feeding punctures, eggs, lifespan and developmental time on eight plant species in non-choice tests with *S. flava*.

Plant species	Mean No. punctures per pot	Mean No. eggs per pot	% of punctures with eggs	% of egg emergence	ALS" (days)	DT* (days)
<b>Cauliflower</b>	10.4±1.5d	2.7±0.9b	19.7	95	11.3	38a
Green cabbage	272.5±4.4ab	15.6±2.5a	4	98	12.6	35a
Hedge mustard	406.2±12.7a	1.7±0.1b	1.2	75	12.3	35a
Brussels sprout	32.5±1.7cd	1.9±0.9b	8.1	83	<b>11.5</b>	39a
Turnip	198.4±3.4b	<b>14.8*1.18</b>	3.2	97	12	34a
Cucumber	5.6±0.3d	0.3b	1.1	<b>0</b>	-	-
Wheat	98.3±3.2c	1.8±0.1b	4.3	0	-	-
Rice	Od	0b	-	-	-	-

Treatments accompanied by the same letter are not significantly different at  $P \leq 0.05$  (ANOVA followed by LSD [ $l_{sd \text{ punctures}} = 49.9$ ,  $l_{sd \text{ eggs}} = 3.8$ ] test for separation of means). <sup>a</sup> Adult life span: Days from adult emergence to adult death. <sup>b</sup> Developmental Time: Days from egg to adult death.

between the non-choice tests and choice tests. First, brussels sprout received significantly fewer punctures than turnip, hedge mustard and green cabbage, and was not significantly different from cauliflower. Secondly, an appreciable number of feeding punctures were made on wheat (significantly more than

cauliflower) and eggs were deposited in 4.3% of these. Numerically, per pot (10 plants per pot), wheat received as many eggs as cauliflower, hedge mustard and brussels sprout.

No significant difference ( $P > 0.05$ ) occurred between turnip and green cabbage for feeding

or oviposition but there was a significantly higher number of feeding punctures on hedge mustard, green cabbage and turnip as compared to cauliflower (table 2). Approximately 1.05 and 5.8 limes more eggs were deposited on green cabbage than on turnip and cauliflower respectively.

When the percentage of punctures with eggs was calculated for each species, cauliflower had by far the highest value at 10% and 19.7% in choice and non-choice tests respectively. For all other species on which eggs were laid it was less than half this.

In the presence of brussels sprout or cucumber only (non-choice test), 40 and 6 feeding punctures per pot were made by *S. jlava* as compared with 347 and 0 respectively when those and other plants were provided simultaneously (choice test). On green cabbage, most eggs were laid near the midrib of the leaf. In contrast, on cauliflower the majority of feeding punctures were around the leaf margin,

## DISCUSSION

A detailed knowledge of an insect's host finding and oviposition behaviour can suggest ways of modifying behaviour to man's advantage. Understanding the factors affecting feeding and oviposition of *S. jlava* would be important for the development of cruciferous crop varieties that are restricted to Brassicaceae.

The results reveal that apart from some anomaly plants, *S. jlava* female accepted only plants from Brassicaceae family. In the choice experiment reported here *S. jlava* typically selected only plants from the family Brassicaceae for feeding and egg laying. However, under non-choice conditions poor and moderate acceptance of cucumber and wheat respectively, also occurred. The eggs on the leaves of these non-host plants collapsed within a day or two and failed to

develop.

In choice and non-choice tests *S. jlava* adult Hies fed and oviposited on all cruciferous plants but showed distinct ovipositional preferences for some species. When *S. jlava* were given a simultaneous choice of seven plant species for feeding and oviposition, there was a distinct hierarchical ordering in their ovipositional preference, with turnip and then green cabbage and hedge mustard being highly preferred over all others.

Leaves of turnip, hedge muslard and green cabbage gave higher fitness indices based on larval growth rate, and survival (unpublished data) and were the most preferred for egg laying while cauliflower and brussels sprout gave the poorest performance and were the least preferred.

Thus, in accordance with field records of plant species affected (15), *S. jlava* showed clear preference for plants within the Brassicaceae based on number of feeding punctures and eggs laid. On green cabbage, the majority of feeding punctures were void of eggs (2.8% and 4.8% of punctures with eggs in choice and non-choice tests respectively). In contrast on cauliflower, 10% and 19.7% punctures contained eggs in choice and non-choice tests respectively. Thus, although the leaves of cauliflower appear to be less suitable than other cruciferous plants for feeding, they are well accepted for egg laying.

Non-choice tests addressed several questions unanswered by choice tests. In choice tests, reduced number of eggs laid on cauliflower could have resulted from a) fewer females accepting treatments, b) females accepting treatment but laying fewer eggs, or c) some combination of these factors. The first possibility begs the question of why even a small number of females would accept altered treatments, when the more host plants were available. A possible explanation is that some females were less discriminating because they a) had matured eggs more rapidly than

other females and therefore, were showing behavior effects of "time-dependent responsiveness" (14) or b) were stimulated to oviposit on the unaltered host, but reacting to proximity of other treatments or crowding on the unaltered cauliflower (or wheat), laid eggs on wheat would normally be a less stimulatory treatment (22).

In these experiments *S. flava* Hies preferred cauliflower least if the five brassicas offered. There is no obvious reason for this but turnip and green cabbage have a much softer leaf than cauliflower and may be easier to puncture with the insect's ovipositor. On the other hand, selection of plants for oviposition is determined both by the physical nature of their surfaces and by chemical factors which are detected only on contact (5). Surface texture of substrates may play an important role in oviposition preference of *S. flava*. Soft or wax-textured surfaces, *i.e.* leaves of cauliflower (cauliflower leaves have a very different leaf wax structure from turnip and green cabbage) were less suitable than other brassicas for feeding, but they were accepted for oviposition sites. Although percentage of punctures with eggs on cauliflower was relatively high, damage to leaves was relatively low. No data are available to explain this but it could be due simply to physical condition of cauliflower leaves as compared with green cabbage and turnip.

General morphology of leaves may also influence oviposition preference. However, leaf hair density does not appear to have an effect on feeding and oviposition of *S. flava*. relatively high leaf hair densities of turnip compared to green cabbage do not apparently inhibit penetration of the leaf surface for feeding and egg-laying.

Although it seems obvious that adult female insects should select individual host-plants, or part(s) of host-plants on which their larvae do best, the empirical evidence is equivocal: sometimes they do (16), but

sometimes they do not (1). Intraspecific variation in host-plant suitability seems to be much greater for leaf-miners than for free-living insects (8). Adult females *S. flava* not only oviposit in cruciferous plant leaves, but also host-feed, creating characteristic feeding punctures in the leaf. It cannot be said whether females select plants on which to feed themselves (thereby enhancing fecundity, *e.g.*, Minkenbergh and Fredrix, 1989) and simply stay to oviposit, or whether host-feeding provides them with information about the suitability of plants for oviposition. Both may be involved.

Glucosinolates are a group of compounds found in all cruciferous plants and their principal volatile hydrolysis products are isothiocyanates, sometimes referred to as mustard oils (2). Isothiocyanates are insect attractants (18) and feeding stimulants (9, 12 and 13) for many crucifer-feeding insects. Glucosinolates are known to stimulate feeding (2) and oviposition (19) in a number of insect species that feed exclusively on crucifers. At the same time, though, glucosinolates are feeding deterrents and toxins for many polyphagous insects. Changes in glucosinolate composition of plants might reduce the attractiveness of the crop to cruciferous specialists (2). Reed *et al.* (18), believed that the types of glucosinolates vary in different plants and change rapidly as seedlings develop (9, 10 and 17).

*S. flava's* distinct preferences between species within the family Brassicaceae suggests that development of host plant resistance might be a possible control strategy for this pest in the future. Also these results support the hypothesis that host preference of ovipositing females is an important factor in the utilization of cruciferous crop by *S. flava* flies. It is possible that some populations of *S. flava* have broadened their host range beyond cruciferous plants. A factor conducive to host plant expansion is limited access to

preferred hosts (10). The absence of other leaf miners on green cabbage, turnip, brussels sprout and cauliflower in the Khuzestan area has allowed *S. flava* to exploit this niche without competition.

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## مقایسه گونه‌های مختلف گیاهی به عنوان میزبان مینوز کلم در استان خوزستان

### *Scaptomyza flava* (Diptera: Drosophilidae)

#### چکیده

مینوز کلم آفتی چند خواره و بومی خوزستان است که روی گیاهان خانواده کلمیان فعالیت تغذیه‌ای دارد. خسارت این آفت، از سراسر خوزستان و مناطق بسیاری در جهان گزارش شده است. ترجیح تغذیه‌ای و تخم‌ریزی حشره مزبور روی هفت گیاه در شرایط آزمایشگاهی براساس تعداد سوراخهای ایجاد شده و تعداد تخم روی برگ، مورد ارزیابی قرار گرفت. گیاهان مورد بررسی از خانواده‌های مختلف بودند: کلم پیچ، کلم بروکسل، گل کلم، خردل و شلغم از کلمیان، خیار از خانواده کدوئیان و برنج و گندم از خانواده گندمیان. آزمایش در دو مرحله انجام شد: در مرحله اول (آزمایش انتخاب)، هفت گیاه توأم (همزمان و زیر یک قفس) در معرض تغذیه و تخم‌ریزی حشره قرار گرفت و در مرحله دیگر (آزمایش عدم انتخاب)، گیاهان مزبور بطور جداگانه (ولی همزمان) در اختیار آفت گذاشته شد. آزمایش براساس بلوکهای کامل تصادفی با ۸ تیمار و ۵ تکرار (برای هر تیمار) انجام شد. حشره آفت در آزمایش انتخاب، گیاهان خانواده کلمیان به ویژه سه گونه کلم پیچ، خردل و شلغم را جهت تخم‌ریزی بر سایر گیاهان ترجیح داد. کلم پیچ به عنوان مناسبترین میزبان آفت مزبور شناخته شد، به طوری که میزان تخم‌ریزی حشره روی این گیاه به ترتیب ۱/۰۵ و ۵/۸ برابر میزان تخم‌ریزی روی شلغم و گل کلم بود. درصد نسبت تعداد تخم به سوراخهای تغذیه‌ای ایجاد شده توسط حشره روی گل کلم به ترتیب ۱۰٪ و ۱۹/۷٪ در آزمایش انتخاب و عدم انتخاب ارزیابی شد که این میزان برای سایر گیاهان کمتر از نصف بود. در آزمایش عدم انتخاب، حشرات ماده بیشتر تخمهای خود را روی کلم پیچ و شلغم در مقایسه با سایر گیاهان میزبان، قرار دادند.