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Influence of Leaf Tissue Structure on Host Feeding Selection by Pea Leafminer *Liriomyza huidobrensis* (Diptera: Agromyzidae)

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Jianing Wei, Li Zou, Rongping Kuang and Liping He (2000) Influence of leaf tissue structure on host feeding selection by pea leafminer *Liriomyza huidobrensis* (Diptera: Agromyzidae). *Zoological Studies* **39**(4): 295-300. Host feeding selection by the female pea leafminer, *Liriomyza huidobrensis*, on 47 species of plants was studied. The leaves were sectioned by microtome, and 15 characteristics of the leaf tissue structure were measured under a microscope. Correlation analysis between host feeding selection and leaf tissue structure indicated that the preference of host feeding selection was positively correlated with the percentage of moisture content of leaves and negatively with thickness of the epidermis wall, and densities of the palisade and spongy tissues of leaves. Leaf tissue structure was influential in feeding and probing behavior of female *L. huidobrensis*. So, thickness of epidermis wall, densities of the palisade and spongy tissues can act as a physical barrier to female oviposition. Furthermore, higher densities of palisade and spongy tissues can be considered a resistant trait which affects mining of leafminer larvae as well. As a result, plants with lower leaf moisture content may not be suitable for the development of *L. huidobrensis*.

Key words: Correlation analysis, Pea leafminer, Host plants, Antixenosis.

Pea leafminer, *Liriomyza huidobrensis* (Blanchard) is a polyphagous species that has been recorded feeding on 14 plant families (Spencer 1990). In 1993, the leafminer species was first found in Yuanmou, one of the largest vegetable plantations in Yunnan Province, China. By 1997, this pest had spread to most regions in Yunnan, seriously damaging ornamental plants and vegetable crops. The main factor causing the outbreak of this pest in recent years is that there are large numbers of preferable host plants in Yunnan. In fact, adults have been reared on host plants of 29 families in this Province (unpublished data). Parrella (1987) indicated that *Liriomyza* leafminers could influence crops in at least 6 ways, among which the most significant 2 ways are causing reductions in crop yields and reducing the aesthetic value of ornamental plants. It is estimated that the agricultural industry of Yunnan Province lost approximately 50 million yuan (or renminbi ≈ US$ 6 million) annually from 1995 to 1998 because of the outbreak of *L. huidobrensis*, with a tendency of a consecutive economic loss (Zou et al. 1998).

Although interactions between *Liriomyza* and host plants are rather complicated, some studies of host selection by *Liriomyza* and plant resistance have been carried out. Host selection experiments were conducted in the past to explain the differences in preference of *Liriomyza* among several cultivars (Parrella et al. 1983, Zehnder and Trumble 1984, Herr and Johnson 1992). Distribution and density of leaf trichomes and trichome exudates of some plants were found to be the important factors in host selection by *Liriomyza*. High trichome density acts as a physical deterrent to *L. trifolii* (Fagoonee and Toory 1983, Knodel-Montz et al. 1985). Antibiosis and antixenosis of adult *L. trifolii* were found to be partially a result of the tomato plant's trichome exudates (Alanerb et al. 1993). Phenolic compounds in host plants are responsible for resistance to *L. brassicae* (Ipe and Sadaruddin 1984). *L. trifolii* preferred to feed and deposit eggs on plants with high nitrogen content (Minkenberg and Fredrix 1989,

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Minkenberg and Ottenheim 1990). Therefore, development and application of host plant resistance are potential tactics for effective and economic management.

Adult leafminer females use their ovipositor to penetrate the epidermis of host plant leaves and feed on the wounded part. Then, the feeding puncture, which looks like a white spot, is produced; the leaf cuticle is the only place where feeding punctures are produced. Larvae develop by feeding on the leaf mesophyll and producing serpentine mines. Obviously, the leaf physical structure, such as its thickness, thickness of the epidermis wall, densities of the palisade and spongy tissues, and so on, can play very important roles in the feeding and oviposting by female leafminers and mining and development of larvae leafminers. However, little information is available on how host feeding selection by *L. huidobrensis* is influenced by the leaf tissue structure of plants. It is fundamental to determine how the leaf tissue structure of host plants affects host feeding preferences of *L. huidobrensis*. According to the present paper, the 15 characteristics of leaf tissue structure that may be correlated with host feeding selection of females can decide the significant leaf physical antixenotic traits of plant resistance to *L. huidobrensis*.

MATERIALS AND METHODS

Pea leafminers and plants

Colonies of *L. huidobrensis* were obtained from an ornamental plant, *Gypsophila paniculata* L., from greenhouses, in Chenggong County, Yunnan Province. Leaves with larvae were collected and kept in plastic bags. After leafminers in leaves reached the pupal stage, the pupae were transferred to culture dishes. After emergence, 2-d-old females were used in host feeding selection tests. Forty-seven species of plants, belonging to 19 plant families, including 16 ornamental plants, seventeen agricultural crops, and 14 common weeds, were chosen in the study as test plants (Table 1). Upper leaves of test plants were selected in the host feeding selection experiments.

No-choice test

In the no-choice tests, a piece of leaf (5 x 2 cm) was cut from each plant and put into a glass tube (3.8 cm in diameter by 11 cm long). Then a pair of females was introduced into the glass tube, and after 12 h, the number of feeding punctures per piece of leaf was recorded. This study was replicated 5 times.

Choice test

In the no-choice tests, adults were allowed to feed on only 1 species of plant. So feeding punctures might have been produced in some plant species that were not the preferred host plants of adults. In order to remove this bias of the no-choice tests, choice tests were carried out. In choice tests, two different plants of the 47 species were randomly paired. For each group, leaves of the test plants were cut into discs (3 cm in diameter) and put into glass dishes (12 cm in diameter) with wet filter paper on the bottom. A pair of females was introduced into the glass dishes, and the numbers of feeding punctures on discs of the 2 plants were recorded after 12 h. Each experiment was replicated 5 times.

Based on the results of this choice test, test plants were sorted into 0-37 ranks. Plants with fewer feeding punctures were assigned lower rankings, and plants with more feeding punctures were assigned higher rankings. Plants with higher selection ranks were more favored by *L. huidobrensis*. Ten plant species had no feeding punctures and were ranked as 0.

Field studies

In field studies, the percentage of damaged leaves of the 47 species of plants was investigated. For each species of plant, twenty individual plants were randomly sampled, and the numbers of healthy and damaged leaves were recorded. Leaves with mines were considered as damaged leaves. For each species of plant, leaves weighing about 200 g were collected. These leaves were placed in an oven set at 60 °C for 24 h. Percentage of weight lost in the leaves was regarded as the leaf moisture content. The wet and dry weights of the leaves were measured with an electronic balance (Libror AEL-160, Shimadzu, Japan).

Examination of leaf tissue structure characteristics

Leaves of all test plants were sectioned by paraffin wax methods (Yang et al. 1995). The tissue structure characteristics of leaves were measured under a microscope at a magnification of 16 x 40 times. The quantitative characteristics of leaves measured involved the size of cells of the superior and inferior epidermis, the thickness of the superior and inferior epidermis, the thickness of the superior and inferior epidermis walls, the number of the palisade tissue layers, and the thickness and densities of the palisade and spongy tissues. Leaf trichome density and length were recorded under a dissection microscope. Each leaf characteristic was measured 5 times. Correlations between host feeding selection and these 15 characteristics of leaf tissue structure were analyzed by Pearson correlation test (SAS Institute 1989).

RESULTS

The number of feeding punctures, the selection rank, and percentage of damaged leaves of 47 plant species are listed in table 1. The number of feeding punctures was considered as an indicator of host feeding selection of female leafminers in the no-choice tests. Plants with more feeding punctures were more favored by female *L. huidobrensis*.

The results of correlation analysis between host feeding selection and leaf tissue structure of the 47 species of plants are listed in table 2. In the no-choice test, the characteristics having a negative correlation ($p < 0.05$ or $p < 0.01$) with feeding punctures were: thickness of the superior epidermis wall ($p = 0.006$), thickness of the inferior epidermis wall ($p = 0.012$), density of palisade tissue ($p = 0.033$), and density of spongy tissue ($p = 0.038$). In the choice test, the selection ranks were negatively correlated with thickness of the superior epidermis wall ($p = 0.002$), thickness of the inferior epidermis wall ($p = 0.003$), density of palisade tissue ($p = 0.029$), and thickness of the superior epidermis ($p = 0.044$). The correlations between density and length of leaf trichomes and host feeding selection were not significant ($p = 0.161$, $p = 0.295$ in the no-choice tests, $p = 0.131$, $p = 0.173$ in the choice tests, respectively). The correlation analysis between percentage of damaged leaves and leaf tissue structure showed that the density of palisade tissue ($p = 0.0001$) and density of spongy tissue ($p = 0.012$) were negatively correlated with percentage of damaged leaves in the field. However, densities of palisade ($p = 0.0001$) and spongy tissues ($p = 0.012$) were more significant than was thickness of the epidermis wall ($p = 0.065$, $p = 0.074$). Percentage of leaf moisture content was positively correlated with feeding punctures ($p = 0.006$), selection ranks ($p = 0.021$), and percentage of damaged leaves ($p = 0.014$).

Table 1. Host feeding selection of *Liriomyza huidobrensis* on 47 species of plants

Host plant (Selection rank 37-14)	Host plant (Selection rank 13-0)
Vicia faba L. (37a 57.8b 73.63c)	*Eustoma vussellianum* (13a 16.8b 17.2c)
Stellaria media (L.) Vill (36a 91.4b 45.35c)	*Conyza canadensis* Crong. (12a 4.25b 6.65c)
Gypsophila paniculata L. (35a 52.12b 28.14c)	*Gerbera jamesonii* L. 1(11a 16.5b 3.0c)
Daucus sativa DC. (34a 67.6b 2.0c)	*Mentha spicata* L. (10a 10.5b 0c)
Brassica pekinensis Rupr. (33a 61.6b 16.14c)	*Tagetes erecta* L. (9a 9.6b 21.8c)
Brassica chinese L. (32a 75.8b 0c)	*Alocasia macrorrhiza* Schott (8a 9.5b 0c)
Pisum sativum L. (31a 57.6b 23.5c)	*Zizania lalifdia* Turcz. (7a 8.5b 0c)
Lactuca asparagina Bail (30a 60.0b 10.0c)	*Trifolium repens* L. (6a 7.5b 0c)
Lactuca capitata L. (29a 63.0b 18.0c)	*Calystegia sepium* R. Br. (5a 5.6b 1.5c)
Brassica botrytis L. (28a 42.4b 12.0c)	*Iresine herbstii* Hook. F. (4a 4.33b 0c)
Brassica napus L. (27a 22.0b 3.0c)	*Helichrysum bracteatum* Willd. (3a 3.4b 4.45c)
Ranunculus sceleratus L. (26a 33.0b 24.51c)	*Chrysanthemum morifolium* Rt. (2a 2.75b 8.77c)
Galinsoga parviflora Cav. (25a 66.0b 20.77c)	*Rosa chinese* Jacq. (1a 0.5b 0c)
Apium graveolens L. (24a 55.88b 16.65c)	*Polygonum hydropiper* L. (0a 0b 0c)
Brassica juncea Czern. et Coss. (23a 43.33b 13.5c)	*Solanum melongena* L. (0a 0b 0c)
Vigna sinensis Endl. (22a 18.2b 7.36c)	*Statice sinuatum* (0a 0b 0c)
Allium fistulosum L. (21a 35.25b 16.88c)	*Lilium tigrinum* Ker. (0a 0b 0c)
Allium sativum L. (20a 42.75b 15.0c)	*Nephrolepis cordifolia* (L.) Presl. (0a 0b 0c)
Setaria viridis Beauv. (19a 11.25b 4.64c)	*Dianthus chinensis* L. (0a 0b 0c)
Callistephus chinensis Nees (18a 30.2b 9.34c)	*Limonium ferulaceum* (L.) Kuntze. (0a 0b 0c)
Tagetes patula L. (17a 13.66b 12.60c)	*Colocasia esculenta* Schott (0a 0b 0c)
Gladiolus hybridus Hort. (16a 13.0b 3.33c)	*Eupatorium odoratum* L. (0a 0b 0c)
Malva verticillata L. (15a 11.25b 9.99c)	*Alternanthera philoxeroides* Grisob. (0a 0b 0c)
Chrysanthemum coronarium L. (14a 19.5b 7.66c)	

[a]Selection ranks in choice test (for selection ranking system see "Materials and Methods").
[b]Number of feeding punctures in the no-choice test.
[c]Percentage of damaged leaves (%) in field studies.

DISCUSSION

Host selection behavioral procedures of female leafminers include host settling, probing, leaf puncturing, feeding, and oviposition. In this paper, we emphasize the leafminer female's host feeding selection. The results of this study show that the density and length of leaf trichomes are not the main factors that influence the host feeding selection of *L. huidobrensis* (Table 2). In previous studies (Fagoonee and Toory 1983, Knodel-Montz et al. 1985), the density and length of leaf trichomes were found to significantly influence host selection. Different observations in this study as compared to previous studies may partially result from different methods and analyses, and different plant materials used in the experiments. The previous studies were not designed to obtain additional data on leaf characteristics other than trichome density and length. Thus, the influence of thickness of the epidermis wall, for example, could not be evaluated. However, in this paper, a series of plants was tested including host and non-host plants, which can provide comprehensive information of the influence of leaf tissue structure on the host feeding selection of this pest species. Some tested plants, such as *Dianthus chinensis* L., with a thicker leaf epidermis wall without trichomes, had fewer feeding punctures and was less susceptible to *L. huidobrensis*. In addition, leaves of 14 of 47 test plants have these traits (trichome density and length) in this study. Therefore, correlations between density and length of leaf trichomes and host feeding selection may possibly be concealed by the process of statistical analysis.

Among characteristics of leaf tissue structure, thickness of the epidermis wall most significantly correlated with host feeding selection by female *L. huidobrensis* (Table 2). These results can be explained by the feeding and probing behavior of *L. huidobrensis*. Females frequently touch the surface of a leaf with their tarsi and probe the surface of the leaf with the ovipositor before feeding. If a leaf is acceptable, the female will bend her abdomen into such a position that the ovipositor can rapidly be inserted into the leaf perpendicularly. Once the surface of the leaf is penetrated, the female swings her abdomen right and left resulting in damage to mesophyll cells. Then the female sucks fluid from the wounds, and the feeding punctures are produced. If the epidermis wall of the leaf is too thick to be penetrated, the female will move to other leaves. Therefore, we drew the following conclusions. When the leaf epidermis wall of a plant is thick and the densities of the palisade and spongy tissues are high, the cuticle of the leaf is not easily penetrated, and the mesophyll tissue is not easily damaged by ovipositors.

The thickness of the epidermis wall and selection rank were significantly correlated, as were the thickness of the epidermis wall and number of feed-

Table 2. Correlation analysis[a] between host feeding selection and leaf tissue structure

Characteristics of leaf tissue structure	Number of feeding punctures (no-choice tests)		Selection rank (choice tests)		Percentage of damaged leaves (field experiment)	
	Coef.	Prob.	Coef.	Prob.	Coef.	Prob.
Size of cell of superior epidermis	0.133	0.372	–0.214	0.148	–0.063	0.674
Thickness of superior epidermis	0.214	0.149	–0.295	0.044[c]	0.017	0.912
Thickness of superior epidermis wall	–0.392	0.006[b]	–0.440	0.002[b]	–0.271	0.065
Size of cell of inferior epidermis	–0.036	0.809	–0.121	0.417	0.012	0.935
Thickness of inferior epidermis	–0.153	0.308	–0.116	0.265	–0.132	0.376
Thickness of inferior epidermis wall	–0.364	0.012[c]	–0.423	0.003[b]	–0.263	0.074
Number of layers of palisade tissue	–0.186	0.211	–0.051	0.734	–0.201	0.176
Thickness of palisade tissue	–0.157	0.291	–0.057	0.705	0.041	0.784
Density of palisade tissue	–0.312	0.033[c]	–0.319	0.029[c]	–0.539	0.0001[b]
Thickness of spongy tissue	–0.178	0.231	–0.149	0.316	–0.020	0.891
Density of spongy tissue	–0.304	0.038[c]	–0.288	0.050[c]	–0.364	0.012[c]
Percentage of leaf moisture	0.398	0.006[b]	0.335	0.021[c]	0.358	0.014[c]
Density of leaf trichomes	–0.208	0.161	–0.223	0.131	–0.129	0.388
Length of leaf trichomes	–0.156	0.295	–0.202	0.173	–0.107	0.476
Thickness of leaves	–0.237	0.109	–0.200	0.177	0.083	0.798

[a]Pearson correlation test (SAS Institute 1989).
[b]Correlation significant ($p < 0.01$).
[c]Correlation significant ($p < 0.05$).

ing punctures. But, thickness of the epidermis wall and percentage of damaged leaves were not closely related (Table 2). However, the densities of palisade and spongy tissues and percentage of damaged leaves were correlated more significantly than were the densities of palisade and spongy tissues and number of feeding punctures and selection rank (Table 2). These results were possibly due to different feeding behaviors between adults and larvae. The thickness of the epidermis wall of leaves was the most important characteristic for resisting penetration and damage from the females in both the no-choice and choice experiments. Nevertheless, in the field, the percentage of damaged leaves was considered to be partly affected by the mining of larvae. As larvae live by feeding from the spongy and palisade tissues in serpentine mines in the leaf, the activity and development of larvae can be obstructed when the densities of the palisade and spongy tissues are high. But, if the densities of the palisade and spongy tissues are low, the larvae will mine the mesophyll more easily and cause serious damage to the leaves. Therefore, the densities of palisade and spongy tissues should be considered as the most important factor influencing the mining by larvae. In conclusion, increasing thickness of the leaf epidermis wall and densities of the palisade and spongy tissues act as physical barriers to adult females. High densities of palisade and spongy tissues also should be considered an antixenotic character that affects the mining by larvae. Because the percentage of leaf moisture content was positively correlated with host feeding selection of *L. huidobrensis*, plants with lower leaf moisture contents are not suitable for leafminers.

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植物葉片組織對南美斑潛蠅寄主取食選擇性之影響

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本文研究了南美斑潛蠅對 47 種植物(*Liriomyza huidobrensis*)的寄主取食選擇性。對植物葉片進行了組織切片，對所選的 15 個葉片組織結構的特徵進行顯微測量。各葉片組織特徵與南美斑潛蠅寄主取食選擇性的相關性分析顯示，其取食選擇性與葉片含水率呈顯正相關，而與葉片表皮外壁厚度、柵欄組織和海綿組織的緊密程度呈顯負相關。南美斑潛蠅雌成蟲的刺探、取食行為受葉片組織結構的影響。葉片組織表皮外壁厚度、柵欄組織和海綿組織緊密程度高以及含水量低的植物，對南美斑潛蠅成蟲的選擇性有較強的物理阻隔作用。葉片的柵欄組織和海綿組織緊密程度高，對南美斑潛蠅幼蟲的取食活動有一定的限制作用。植物葉片含水量低可能不利於南美斑潛蠅幼蟲的生長發育。

關鍵詞：相關分析，南美斑潛蠅，寄主植物，抗性因子。

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