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Stylet penetration behavior of soybean aphid *Aphis Glycines* on host and non-host plants.

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Abstract The stylet penetration and feeding behavior of the soybean aphid, *Aphis glycines* Mat., on host and non-host plants was monitored using the Electrical Penetration Graph (EPG). The results showed that phloem ingestion was reduced or did not happen on the non-host plants, *Gossypium hirsutum*, *Cucumis sativa* and *Luffa cylindrica*, compared with that on the host plant, *Glycines max*. The non-host plants possess resistance against *A. glycines*. The factors affecting ingestion and their position on the plant were different along with the difference of the non-host plants.

Key word *Aphis glycines*, piercing-sucking mouthparts, feeding behavior, host plant selection, EPG technique

Soybean aphid *Aphis glycines matsumura* is a type of oligophagous soybean pest. Its currently known summer hosts are only soybean *Glycine max*, black soybean *G. sp.* and wild soybean *G. soja* Sieb. et Zucc., which all belong to the genus *Glycines*. The winter host is only cascara *Rhamnus davurica* ^[1]. As with most aphids, the course of searching for hosts, from flying in the sky to finding appropriate host plants, includes three steps: landing on plant surface; detecting plant surface and ecto tissue; sting and “evaluating” tissue on which it feeds ultimately. Following this comes a choice of remaining (for host plant) or leaving (for non-host plants). The behavior of searching for hosts and the restriction of host ranges in phytophagous insects are well known, which are closely associated with the secondary metabolism substances in the plant. Tests done by Du Yongjun using the insect antennae electric potential method and determination of olfaction behavior have proven that the volatile secondary substances in winter host, summer host, and some non-host plants play an important role on the Aphid’s orientation in seeking host plants.^[2] This paper will study how the non-volatile material at the exterior and interior of the plant influences aphids to finally choose the host. Differences of stylet probing behavior of the soybean aphid on host plant (soybean) and non-host plant (cotton *Gossypium hirsutum*, cucumber *Cucumis sativa* and loofah *Luffa cylindrical*) were investigated using the Electrical Penetration Graph, EPG, so that factors affecting ingestion and their position on the plant were understood. This would provide a theoretical basis for further investigation of the mechanism of host selection of soybean aphids and research into new control methods.

1 Materials and methods

1.1 Soybean aphids were collected from cascara trees in the Qinghe Township, a suburb of Beijing, in April and reared on soybean plants in the lab.

1.2 A low and early-maturing soybean variety from Shangyu County, Zhejiang Province, was used. The seeds of non-host plants cotton, cucumber and loofah came from markets in Beijing. All plants were planted in the minitype-cup. The experiment was carried out when plants had grown 3-5 leaves.

1.3 A Giga-2 amplifier, which amplifies electronic signals of insect probing, connected with a 12v transformer, was supplied by the Entomology Department, Agricultural University, Wageningen. The electric connection to the aphid was a gold wire of 0.02mm diameter. A Gould recording unit with 220 pressure came from USA.

1.4 Methods: A gold wire (0.02mm diameter, 2-3cm long) was attached to the dorsum of strong apterous aphids with silver conductive paint, with the other end connected to the Giga-2 amplifier. The ground-wire of the amplifier was covered by the soil in the minicup. The output wire of the amplifier was connected to the recorder, which ran for 2 h upwards with 2.5cm/minute chart speed. After wiring, an aphid was placed on the back side of a soybean leaf. The experiment was carried out in a screening cage. Definition of waveforms referring to *tjallingii*^[3-6] will be elucidated in the Discussion section. Conclusions were obtained according to the analysis of recording waveforms.

2 Results

2.1 Comparison of staying time before stylet probing of soybean aphid both on host and non-host plants.

The results of Table 1 showed that staying time before stylet probing of soybean aphid on the soybean and loofah were the same. The T test showed no significant difference. But staying time before stylet probing of soybean aphid on cotton and cucumber were significantly different compared with that on the soybean, the latter being longer. ($t=1.757$ $p<0.05$ $t=-1.887$ $p<0.05$)

Table 1 Comparison of staying time before probing of soybean aphids on host and non-host plants

Species & repeat (n)	Soybean (9)	Cotton (9)	Cucumber (9)	Loofha (10)
The time before probing (min)+standard error	2.01 ± 0.63	4.16 ± 1.01	4.28 ± 1.44	2.08 ± 0.62

2.2 Comparison of numbers and frequency of probing of soybean aphids on host and non-host plants

Numbers and frequency (total probing numbers/duration of C wave) of probing of soybean aphids on host and non-host plants were recorded for 2 h upwards respectively. The results (T test) showed no significant difference between them (Table 2).

Table 2 Comparison of numbers and frequency of probing of soybean aphids on host and non-host plants

Species & repeat (n)	Soybean (9)	Cotton (9)	Cucumber (9)	Loofha (10)
Number of probes+standard error	43.2±11.12	37.1±6.18	50.7±11.19	40.8±11.28
Probing frequency+standard error	0.84±0.10	0.73±0.06	1.10±0.16	0.68±0.10

2.3 Recording of different waveforms duration

Duration of C (a+b+c), E (pd), F and G wave as well as NP (without probe activity) were calculated on host and non-host plants respectively. The results showed E (pd) wave duration reflecting aphid feeding activity was significantly different between host (soybean) and non-host (cotton, cucumber and loofha). The difference was significant $t=2.159 P<0.05$ $t=-2.318 P<0.05$ $t=2.959 P<0.01$. Although C, F and G wave duration were different on host and non-host plants, the T test was not significant. NP duration on cucumber leaf was longer than that on soybean. It occupied 42.2% and 19.6% of total duration respectively. The T test was significant. ($t=2.529 P<0.05$).

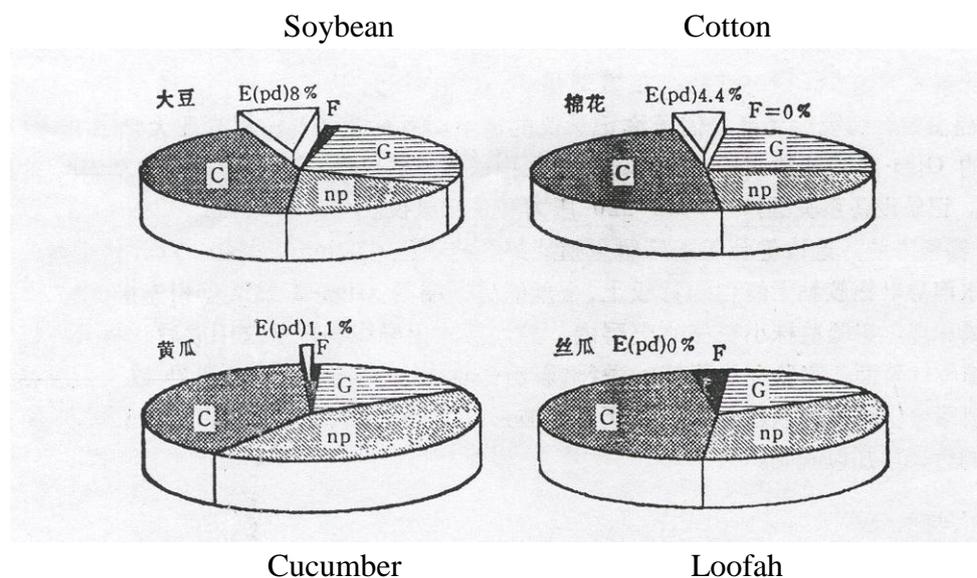


Fig 1 Proportion chart of different waveforms C, E (pd), F, G and NP (non-probing) of soybean aphids on each plant

3 Discussion and conclusion

Research on insect feeding behavior should ultimately reveal the hidden details of insect feeding, in order to offer clues for disruption of host selection by insect pests and to help design other methods of pest control. For a decade of research on insect feeding behavior, the EPG technique for recording the process of stylet probing has attracted great attention. Using the electric wave record of host stylet probing combined with isotope labelling, histology and image observation, pioneering researchers have proved the relativity between waveform and stylet trackway. All of this has established a foundation for applied research of EPG today.

From the aphid being placed on the leaf through different stages of sucking or

probing, there are seven kinds of waveforms recorded by this electronic instrument (Figure 2).^[7-9] The meanings of each are as follows:

A, B wave: It appears before wave C, maintains a shorter time and stands for initial stylet movement and saliva secretion.

C wave: It stands for stylet movement through the epidermis and mesophyll of the plant.

pd wave: It follows pattern C immediately and stands for a type of movement waveform penetrating through cell membrane.

E (pd) wave: It is associated with passive fluid ingestion from phloem sieve elements and secretes saliva at the same time.

F wave: Mechanical stylet activity in intercellular spaces or cell walls.

G wave: Active fluid ingestion from xylem elements.

Using the above-mentioned summary with this research showing recorded waveforms and the associated aphid behavior, the piercing-sucking condition of soybean aphids on host and non-host plants could be identified. Thereby the factors affecting ingestion and their feeding position on the plant could be judged.

3.1 A slight difference of staying time before stylet probing of soybean aphid between host (soybean) and non-host plants (loofah) indicates that substances on the leaf surface of loofah did not play a role in resisting soybean aphid. In addition the fact that soybean aphids hesitate to probe the leaves of cotton and cucumber, we could presume that surface substances on cotton and cucumber are not suitable for soybean aphid or are resistant to soybean aphid. The author extracted surface substances on the leaves of soybean, cotton, cucumber and loofah and analyzed these substances by gas chromatograph. The results indicated that numbers and kinds of chemicals from the leaf surface of loofah had a significant difference compared to the other three plants (unpublished data). Advanced tests need to be done to reveal its mechanisms.

3.2 The probe frequency reflects the times of stylet entering into a cell. The difference of probing frequency of soybean aphids between host and non-host plants was not significant except on loofah. It might be considered that the internal structure of loofah confers resistance to the soybean aphid, by existence of an arrestant or longer intercellular space that deters the tip of stylet from accessing the cell membrane. But this kind of resistance seems not to exist on the cotton and cucumber.

3.3 The duration of A+B+C wave (total C wave) on soybean was not significantly different than that of the other three plants. A slight difference of the F wave among four plants did not show a statistical difference. But C and F waves reflect soybean aphid stylet activity in the pathway, therefore in internal structure (such as mesophyll, cell wall and intracellular space). Of the cotton and cucumber plants there was no resistance or only slight resistance. But the longer duration of the F wave on loofah shows its resistance to aphid. The results are the same as those in summary 3.2. The reason for the longer duration of NP on cucumber than other plants might relate to the shorter duration of C, F waves on the cucumber plant, but this conclusion would be

premature.

The E (Pd) wave, the most important wave for studying plant resistance, showed a more complex condition among different plants (Fig.2).

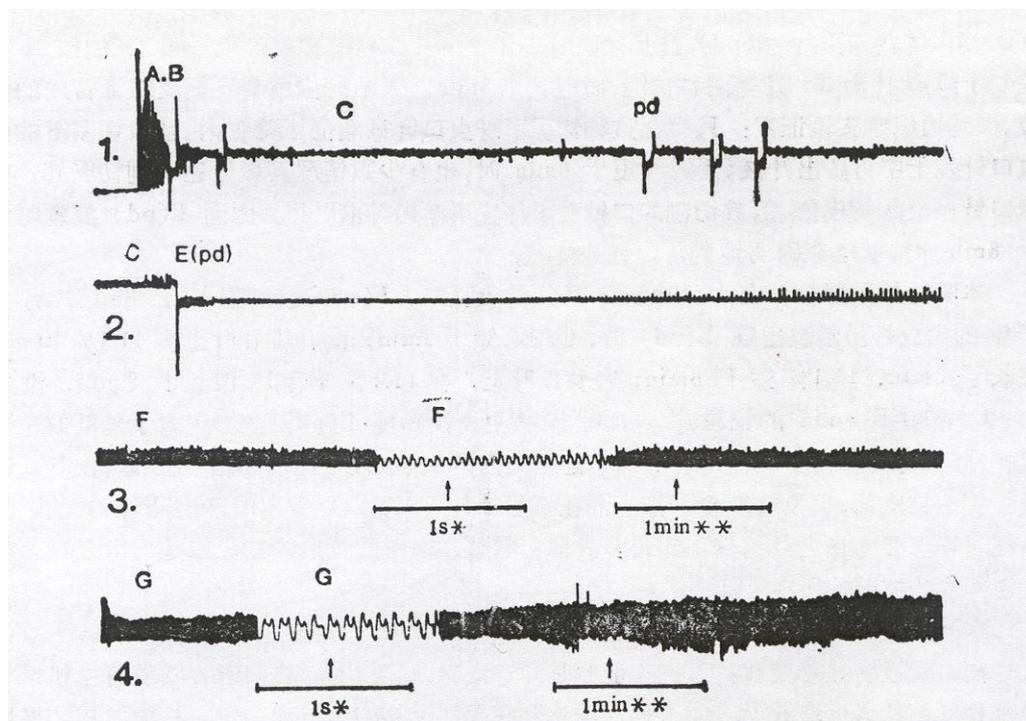


Fig 2. Demonstration of different waveforms of EPG

1. A, B, C, and pd waves; 2. E (pd) wave after C wave; 3.F wave; 4.G wave;
* the wave shown at 2.5mm/s chart speed; ** the wave shown at 2.5mm/min chart speed

Kimmins and Tjallingii verified that when there is an E (pd) waveform, in most situations the tip of the aphid stylet has entered into the sieve of phloem and has sucked sap in the phloem. Therefore the E (pd) wave is a key parameter reflecting aphid feeding. But pattern E (pd) could be divided into two occurrences, lasting less than or longer than 8 min. Longer than 8 min could definitely be considered as ingestion from phloem sieve elements, based on histology evidence that showed the tip of stylet located in the sieve here, and cut stylet stumps contained excretive sap. But in the less than 8 min situation, some of them did not get to the sieve of phloem or the stylet stumps when cut did not exude sap.^[10,11] Therefore it is difficult to verify exactly the position of the stylet in this situation.

Our experiment results showed that there were 25% soybean aphids which made E (pd) >8min on soybean, but 25% soybean aphids made E (pd) < 8 min on soybean. But in the cotton plant, 11% soybean aphids made E (pd) > 8min, 11% soybean aphids made E (pd) < 8min. In the cucumber plant, 11% soybean aphids made E (pd) > 8min, and there were no E (pd) < 8min. In the loofah, there was no E (pd) wave at all. It appears that internal structure and chemical component of loofah leaf are not all

suiting to the soybean aphid. Its stylet also did not reach the phloem and suck sap. Although soybean aphids feeding on the cotton and cucumber could make E (pd) wave, the E (pd) wave duration was shorter than that feeding on the soybean. It seems that phloem in cotton and cucumber are major factors producing resistance.

The G wave reflected the feeding condition of aphid stylet in xylem. These results indicated that G wave was not significantly different between host and non-host plant. This is consistent with the results of Tjallingii 1987^[12]. However, they had indicated that G wave duration increased along with degree of starvation and dehydration.

In this experiment, G wave duration of aphid also increased significantly in the hot noon or afternoon. But no statistical data was given. However, which chemical materials are playing this role need to be further studied by chemical analysis and other tests. Feeding behavior of aphids is a complex activity. Resolving these questions deeply and explaining it soundly requires a combination of this with other methods and instruments.