Source: Acta Entomologica Sinica [Pin-Yin: Kunchong Xuebao, ISSN: 0454-6296]
(1993) v.36 p.143-149
Translated by Jian Wu , edited by Mohan Ramaswamy, Kansas State University, 2003

Analysis of factors inducing alatae in *Aphis glycines*

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Abstract

The effects of crowding, quality of host plant, temperature, and aphid types on the induction of alatae in soybean aphid (*Aphis glycines*) were investigated in this paper. This study shows that the crowding in the apterous adults is the major factor causing the formation of the alatae aphids in the next generation. In low aphid densities, the crowding effect is strengthened as the density increases, whereas overcrowding weakens the crowding effect. However, the crowding in young apterae would not develop them into alatae. The quality of host plant has an impact on the apterae's reaction to crowding. With the crowding density of two apterae per bottle, the percentage of winged offspring is higher when treated with mature leaflets than treated with young leaflets or control treatment (without leaflets). Furthermore, starvation cannot enhance the formation of alatae. *A. glycines* wing induction is also influenced by temperature in that 30^{o}C and 25^{o}C have a stronger inhibitory effect on the production of alatae than 21^{o}C. The ability of producing winged morphs varies among different types of parent *A. glycines*. Crowding in the alatae also can induce alatae in offspring, although the sensitivity to crowding is lower than that of apterae.

Key words: *Aphis glycines*, alatae dimorphism, crowding, quality of host plant, temperature

In 1745 Bonnet found the phenomena of viviparous parthenogenesis of aphids. After that, the polymorphism in aphids had drawn researchers' interest. After a long period of study, Bonnemaison discovered in 1951 that the interaction between individual aphids mainly accounts for the formation of alatae (Lees, 1966). Since then, researchers worldwide have conducted extensive and detailed studies, extending the known factors which influence the formation of alatae to crowding, photoperiod, temperature, host plant, and parent aphid types. Furthermore, the factors have distinct priorities of effects on different species of aphids. The causes of winged dimorphism in *A. glycines* were explored in this study.

Materials and methods

1. Tested insects

One wingless viviparous female aphid was cultivated on the leaflet of a soybean seedling planted in sand, which was collected from a soybean field in the Institute of Plant

Protection, Jilin Academia of Agriculture Science. Under conditions of 21 ^{o}C indoor temperature and illumination at 16 hours per day (16L:8D) we set up an apterous parthenogenesis colony of viviparous wingless aphid. We picked one baby viviparous wingless aphid from the colony and raised it separately on the young soybean. After this selected aphid gave the birth to nymphs, we collected 10 from the group and separated them (1 aphid per tiller) respectively as aphids to be tested in the experiment. When those 10 aphids had developed and had their offspring, we selected 10 aphids from the offspring as the experimental unit for future trials. During the experiment, the aphids were selected from aphids for testing and isolated respectively as the experiment unit.

2.Trial condition

The temperature of the experiment was 21 ^{o}C $\pm$ 1 ^{o}C, relative humidity was 60-80% and illumination time was 16 hours per day (16L: 8D) in the glass house. The soybean type of Jilin 20 was selected as the host plant, with the seeds grown in a sand bed, with the leaflets utilized as hosts in the experiment.

3. The isolation and transfer of the aphids in trial

We used soybean leaflets on which were settled individual or small colonies of aphids as the isolation unit. We took the insect incumbent (diameter of 36cm, height of 7cm) as the treatment or repeat unit. All the inspection and transfer operations were carried out in a special water-contained plate using soft wetted brush (water wiped off) to transfer the aphids.

4. Statistical method of the experiment.

We took 10-15 nymphs, which were offspring of the isolated tested aphids (parent aphids) and were cultivated on the soybean leaflet as the control (0 group). We moved parent aphids to the fresh young soybean plant after treatment to let them lay offspring for 24 hours (one day). The newborn nymphs were taken as the first group after treatment. We transferred the parent aphids again to the fresh soybean plant to lay new nymphs for 24 hours (one day), the new nymphs forming the second group. We moved the parent aphids continuously to give birth to new aphids which was the third group for another two days. We used this method until the 9th or 11th day after treatment (the fifth or sixth group) and ended the process of birth of the new aphids. The groups of the nymphs were cultivated to the 4th stage when the wing bud was visible. The amounts of the winged aphids and wingless aphids were counted.

Lee's method was used to analyze the experiment results (Lee, 1966). The preliminary results showed that the ratio of winged aphids in the third group of offspring after treatment could demonstrate soybean aphids' response intensity to different treatments, which was same as the *Megoura viciae'*s response to the crowding incentive in Lee's experiment. So we could use the ratio of winged aphids in the 3rd group and total offspringafter the treatment as a measurement of soybean aphids' response intensity to the different treatments. We used these two numbers to carry out the hypothesis test of significant difference between the two ratios. We used the (2 x 2) table to calculate the value of χ^2, the formula as follows:

$$\chi^2 = \frac{(ad - bc)^2 \cdot n}{(a + b)(c + d)(a + c)(b + d)}$$

The χ^2 table at 1 degree of freedom was referred. We compared the significant differences at the level of P=0.01 and P=0.05, using * to represent the significant differences ($P \leq 0.05$) between the results and ** to represent the larger significant differences ($P \leq 0.01$) between the results, with N.S. indicating no significant difference ($p \geq 0.05$).

5. Treatments in the experiment

Crowding experiment

We used Lee's crowding treatment for isolation from the host plant (Lees, 1966). The experiment was carried out in the 40 x 30 mm weighing flask and we selected the crowding density treatments of 1, 2, 5 10 and 20 aphids per flask for 24 hours.

Host plant quality experiment

We used the fully-opened dark green compound soybean leaves as the "mature" leaves and the full spread euphyla seedling in sand-beds as the "tender" leaves. We used leaf clip cages to clip the cages of one or two wingless viviparous aphids on the back of the mature or tender leaves. We set the leaves without clips as controls, with treatment time of 24 hours.

Temperature experiment

The bottles of 1, 2, 5 and 10 wingless viviparous aphids were treated under constant temperatures of 21 ^{o}C $\pm$ 1 ^{o}C, 25 ^{o}C $\pm$ 1 ^{o}C and 31 ^{o}C $\pm$ 1 ^{o}C respectively for 24 hours.

Parent aphids type experiment

The testing parent aphids were wingless viviparous aphids, the small cage modified from the insect-trapping tube net (diameter= 4 cm, length=12 cm) used to cover the aphids settled on the soybean plant to prevent escape. There were 12 aphids in each net and the treatment lasted 24 hours.

Results

1.Crowding effect on the generation of wingless viviparous soybean aphids

1). The crowding between the apterous viviparous adult aphids

The ratio of the winged aphids among offsring of the apterous viviparous aphids in the different crowding densities is shown in fig.1

When one parent aphid was in the bottle, there was no winged aphid generated in the

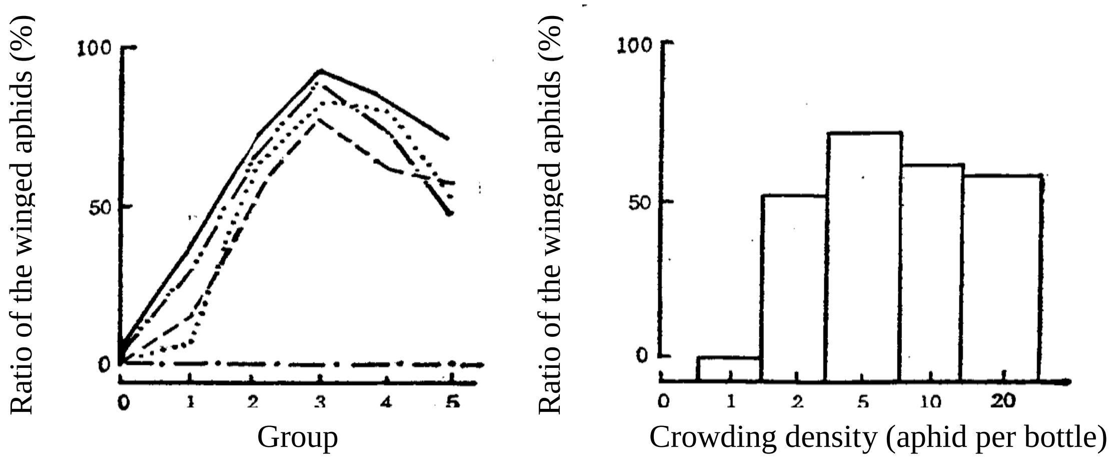


Figure 1. The ratio of the alatae among the offsring of the apterous viviparous aphids with different crowding densities

—·—· 1 aphid / bottle, - - - 2 aphids / bottle, — 5 aphids / bottle,

—· ·— 10 aphids / bottle, · · · 20 aphids / bottle, ☐general offsprings

offspring. The ratio of winged aphids in the offspring was 51.1% when there were two aphids in the bottle. The ratio became largest when the density was five aphids per bottle. The ratios of winged aphids among the third group and all of the offspring were both significantly higher than that of the treatment of 2 aphids per bottle. χ^2 =34.77, 120.5, P<0.01). When compared with the treatment of 10 aphids per bottle, the ratio of winged aphids in the 3rd group was not significantly different (χ^2=0.348 N.S.). The ratio in all offprings was significantly different (χ^2 =25.4, P<0.01). When the number of parent aphids per bottle rose sharply to 20 per bottle, the difference between the 3rd group compared with the treatment of 10 aphids per bottle was not significant (χ^2 =2.787 N.S.). But the difference in the ratios among all of the offspring was significant (χ^2 = 6.02, P<0.05). Compared with the density of 5 aphids per bottle, the response of the parent aphids to the crowding significantly declined (χ^2 =4.138, P<0.05, 53.7, P<0.01). The above results showed that the ratio of the winged aphids born from the crowded group of apterous viviparous parent aphids would increase with the augment of the density when the crowing density was low. The parent aphids' response to the crowding decreased when the crowding density was high.

2). The crowding between the apterous viviparous nymphs

To further study whether the crowding of nymphs had an effect on the generation of the present winged aphids, we isolated the nymphs (one per soybean plant), which were produced by the isolated apterous parent aphids within 12 hours, and waited for them to develop to a certain stage (according to the times of exuviations) to carry out the treatment at this stage. They were generally treated in 40 x 30 mm weighing flask for 24 hours. Since the first instar nymphs' ability to withstand starvation was weak, the treatment time was only 12 hours. We used the treatment that isolated the aphids and kept them on the host plant as the control. After the treatment the nymphs were transferred to the fresh soybean leaflet and examined when they developed into adults. The results are shown in the table 1.

The experimental results showed that though during every stage except the 3rd there were small amounts of nymphs that developed into winged viviparous aphids. The differences between the treatment groups and control were not significantly smaller than that of the control, which meant that the crowding among the apterous viviparous aphids would prevent them from developing into winged aphids.

Table 1.Crowding effect on the apterous viviparous baby soybean aphids

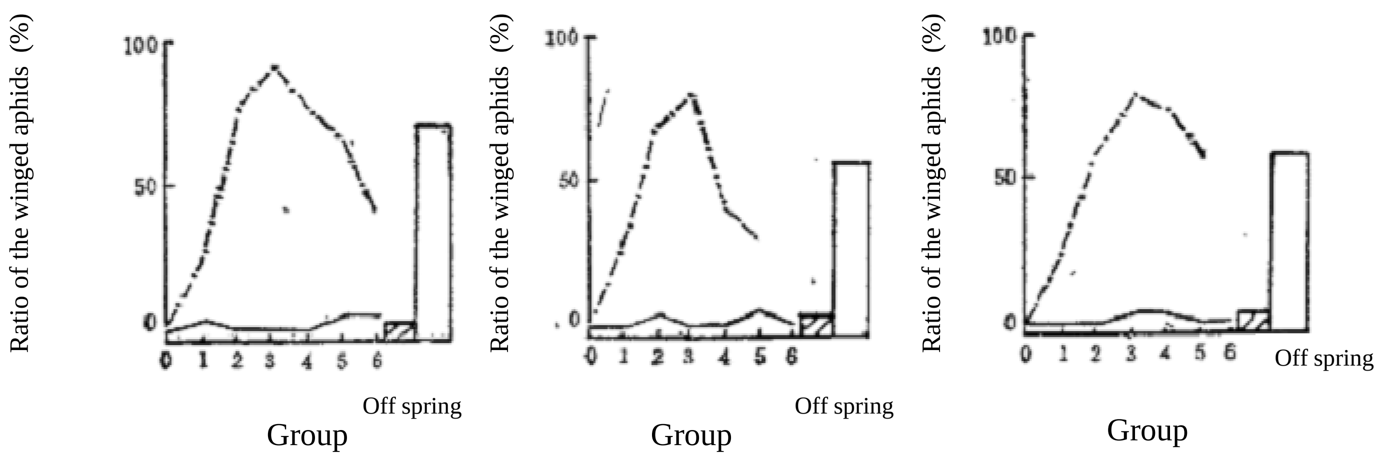
Stage of the tested aphids	Treatment		Repeat	Death rate in the treatment	The numbers of the different generation type of aphids			χ^2 Test
	Numbers of aphid (aphid/ bottle)	Time (hours)			AL (aphid)	Ap (aphid)	AL (%)	
1	50±	12	3	10.8	2	161	1.23	2.284 N.S.
2	45±	24	3	13.6	5	109	4.39	0.218 N.S.
3	30±	24	6	19.5	0	142	0.0	5.118*
4	25±	24	10	3.2	2	202	0.98	3.507 N.S.
CK (aphid/plant)	1	0	657	——	23	634	3.5	

1). AL (alatae) was winged viviparous aphids; Ap (apterae) was wingless viviparous aphids.
2). χ^2 Test was the comparison with the control (CK).

2.The effect of the quality of the host plant on the formation of the wingless viviparous aphids

The effect of host plant quality on the generation of wingless viviparous aphids is shown in figure 2.

The results show that after treatment of the aphids (2 aphids per bottle), among the 3rd (in the 4th and 5th day) offspring and general offspring the ratio of winged aphids produced by the apterous viviparous aphids treated by the mature leaves was higher than the ratio of those treated by the tender leaves (χ^2=10.59, 81.50, P<0.01) and the ratio of those in the control group (χ^2=12.93, 64.35, P<0.01). The differences were significant. But the difference between treatments of tender leaves and control was not significant (χ^2=1.44, N.S., 0.064 N.S.). If there was only one aphid per bottle, the ratio of winged aphids among the offspring of apterous viviparous aphids was only 0.1-1.5%. The above results showed that the mature leaves could increase the ratio of winged aphids among the offspring of the apterous viviparous aphids, but that hunger had no favorable effect on the generation of winged aphids among offspring of crowded aphids.



A. Mature leaf B. Tender leaf C. Control (no food)

Figure 2. The host plant quality effect on the generation of winged soybean aphids

—·—· □ 2 aphids / cage, — ▨ 1 aphids / bottle

3.The temperature effect on the generation of the wingless viviparous aphids

1). Crowding experiment under different constant temperature

The isolated parent aphids were treated with different densities under different constant temperatures. The results are shown in figure 3.

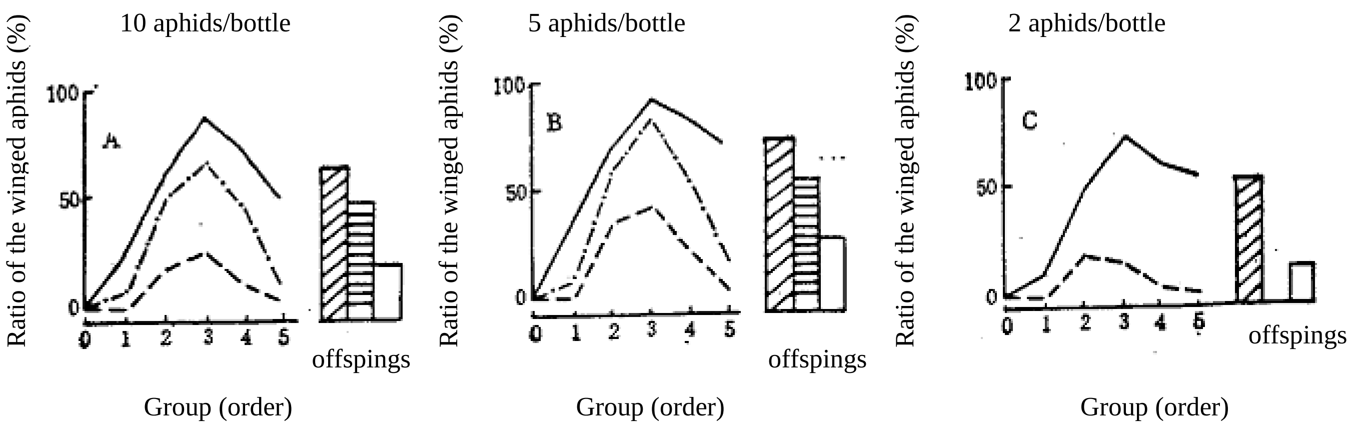


Figure 3. The ratio of alatae among the offsprings of the soybean aphids treated with different densities under different constant temperatures

— was 21 °C — · — was 25 °C , - - - was 30 °C

The experimental results show that the ratios of winged aphids among offspring of apterous viviparous aphids were different, with parent aphids treated under different constant temperatures of 30 °C , 25 °C and 21 °C. With the same density and as temperatures increased, the ratio of winged aphids born by the parent aphids with same density (2, 5 and 10 aphids per bottle in each trial) declined significantly ($P<0.01$). In the isolation condition (one aphid per bottle), there was no reproduction of winged aphids. It demonstrated that the higher temperature (30 °C, 25 °C relative to 21 °C) could inhibit the generation of winged aphids among offspring of parent aphids. The higher the temperature, the strong the restraining effect would be.

2). Crowding experiments under different changing temperature.

We applied the crowding treatment at the temperature of 21 °C for 24 hours to apterous viviparous aphids (10 per bottle), which were the parent aphids of aphids in the control group. Then we moved the parent aphids to fresh soybean plants to lay eggs of new aphids for 1 day. We transferred and isolated the parent aphids in a 40 x 30 mm weighing flask (1 aphid per flask), then placed the flask in 26°C ± 1 °C constant temperatures for 24 hours. After the treatment we placed the parent aphids on fresh soybean plants at the temperature of 24 °C to produce new aphids for 2 days. We transferred aphids in this way until the production of the aphids ended and examined the results.

There were 6 apterous viviparous aphids continuously producing aphids in the experiment. No winged aphids were born in the control group. The ratio of winged aphids after the crowding treatment was 41.5%. 81 nymphs were produced after two days of the treatment of temperature of 26°C ± 1 °C, only one of which was winged. The ratio of the apterous viviparous aphids among nymphs born in the 3rd and 4th day, 5th and 6th day and 7th and 8th day were 15.9%, 15.8% and 16.7% respectively. In the offspring born in the 9th and 10th day, there were 6 wingless nymphs. It demonstrated that higher temperatures could inhibit the generation of winged aphids. It could be concluded primarily that temperature affected the origination of wings in the embryo inside the apterous viviparous aphids' body and so influenced the generation of winged soybean aphids.

4.The aphid type effect on the generation of the winged viviparous aphids

Selected 12 of 4th-stages immature winged aphids from the soybean plant on with a large amount of winged aphids were produced. Isolated the selected aphids by the small cover cage (1 per plant per cage). After the birth of the control aphids, applied crowding treatment (12 aphids per bottle) to them and compared the result with the wingless viviparous aphids (10 per bottle). The result was showed in figure 4.

The result showed that the crowding of the winged soybean aphids can induce the generation of very small amount of winged aphids among their offspring. But compared with apterous viviparous aphids with the treatment of 10 aphids per bottle, the winged aphids' reaction to the crowding was weak. It demonstrated that the apterous viviparous aphids' sensitivity to the crowding was stronger than that of the alatae viviparous aphids.

Discussion

The factors influencing the winged generation differ with respect to different species of aphids. The crowding in the apterae is the main factor to induce alatae in aphids. It is a prenatal control and was reported in many species of aphids, such as *Megoura viciae, Acyrthosiphon pisum.* This kind of prenatal control is not caused by vision or olfaction; instead, it is caused by body touch among aphids (Lees, 1966; Sutherland, 1969a). In some species, the crowding in either prenatal stage or postnatal stage can increase the percentage of alatae in both the next generation and the current generation (Watt and Dixon, 1981). And some crowding reaction only occurs in postnatal stage for some species (Kidd and Tozer, 1984). Our results indicate that the crowding in soybean aphid postnatal stage will not induce alatae in the current generation. This study will not only facilitate the research on other wing inducing factors, but will also help in developing strategies to control the field population of aphid alatae.

Lees (1966) proposed that host plant quality was only an adjusting factor in wing induction, for it could only affect the strength of the crowding reaction. However, Johnson (1966a) and Sutherland (1969b) conducted detailed experiments and illustrated that the quality of host plant itself can determine the production of alatae. In this experiment, the reaction to the quality of host plant was not detected with one apterae, whereas two apterae in a bottle show a significant difference between treating with mature leaflets and treating with young leaflets. It indicated that the quality of host plant influences the crowding reaction by apterae, and mature leaflets strengthen the reaction.

There are a number of reports that lower temperature suits for alatae induction, whereas higher temperature plays an inhibitory role (Johnson and Birks, 1960; Schadfers and

Judge, 1971). And temperature has effects in both pre- and post-natal states. The target of the prenatal action was suggested to be located in the embryo. The initial study on temperature in aphids illustrates that higher temperature (30^{o}C and 15^{o}C) has a stronger negative effect on wing induction than 21^{o}C, and it suggests that the target of the inhibitory function is the wing tissue in embryo.

Many reports showed that different species of alatae differs in the reaction to crowding in the occurrence of alatae induction and the quantity of alatae (Lees, 1966; Shaw, 1970). For aphids, only a small number of alatae were produced by the crowding treatment of parent alatae. This phenomenon was also reported in other species (Watt and Dixon, 1981).

In the life cycle of soybean aphids, photoperiod plays an important role in alatae induction, especially in sexual aphid induction. To elucidate its function requires further investigation.

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