Collection and Hatching Methods of the Aphid, *Aphis spiraecola* PATCH, Eggs in the Laboratory

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(Received 19 May, 1994; Accepted 4 November, 1994)

The best method for collection and hatching of eggs of *Aphis spiraecola* was studied in the laboratory. Almost 80% eggs were deposited during the first seven days. Most eggs were obtained when the cotton cloth was used as the oviposition surface and aphids were maintained at 20°C. However, eggs on the cotton cloth did not pigment as fast as those on the filter paper, and the final percentage of pigmentation was lower on the cotton cloth than on the filter paper. In addition, eggs on the cotton cloth easily detached from surface when eggs were dipped in water. It is concluded that the filter paper is better surface for egg collection. The highest percentage of pigmented eggs (90%) gained when eggs on the filter paper were maintained at 14°C. Females with an artificial diet did not produce more eggs than those without it. Sterilization by 1% formaldehyde solution treated immediately after oviposition had the ill effect for the egg pigmentation. Prolonged pre-cooling incubation period at 14°C did not affect post-diapause development of eggs.

Key words: oviposition, sterilization, temperature

INTRODUCTION

There are several reports on the egg hatching methods in the laboratory for the aphids, *Aphis fabae* (BEHRENDT, 1967), *Sitobion avenae* (HAND, 1983; NEWTON and DIXON, 1987), *Myzocallis coryli* (MESSING and ALINIAZEE, 1991), *Schizaphis graminum* (WIPPERFÜRTH and MITTLER, 1986) and *Pterocallis alni* (GANGE and LLEWELLYN, 1988). The egg development is important factor for prediction of spring occurrence and for genetical analysis of populations because aphids mate only once a year and parthenogenetic generation does not produce genetic variation except of mutation (BLACKMAN, 1979; SUOMALAINEN et al., 1980; TOMIUK and WÖHRMANN, 1982). This report describes the best method of collecting and hatching *A. spiraecola* eggs.

MATERIALS AND METHODS

In the fall of 1987 and 1988, oviparae and males were collected from spirea trees, *Spiraea thunbergii* SIEB., at Okitsu Branch, Fruit Tree Research Station in Shimizu and confined in small glass cups. This strain is the spirea overwintering type (KOMAZAKI, 1990, 1991). The egg collection method was after WIPPERFÜRTH and MITTLER (1986) with small modification. The outline of apparatus is illustrated in Fig. 1. Ten to 25 females and two to four males are confined in a cup. A wet filter paper was set on the bottom of the cup and the upper side was covered with a filter paper or a piece of cotton cloth, and an

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Glass cup

Fig. 1. Outline of the apparatus used for egg collection. A: aluminum foil, B: oviposition surface, C: rubber band, D: wet filter paper.

aluminum foil cover was banded with a rubber band. Eggs deposited on glass and oviposition surface were counted daily.

Egg deposition by aphids with and without an artificial diet was also tested. The glass tube was used for supplying the diet. The diet was applied on under side of the tube. Diet composition and application method were the same as described in KOMAZAKI (1984) with 20% sucrose concentration.

Deposited eggs on oviposition surfaces were maintained in Petri dishes to prevent from drying during several days and sterilized by dipping in 1% formaldehyde solution for 2 min and washed five times by soaking in autoclaved distilled water. The surface with eggs was confined in the 9 cm sterilized plastics Petri dish that was sealed with a parafilm band to prevent drying.

Eggs were sterilized on different days after oviposition. Process of egg pigmentation was observed daily. All tests were carried out in growth chambers at 14.4°C constant temperature and under the natural photoperiod from December to February unless otherwise described.

How the length of pre-cooling periods affects the egg development was also tested. Eggs were incubated for 7, 14 and 21 d at 14.4°C before cooling at 6.3°C, and the temperature raised at 14.4°C after 50 d cooling period. Egg eclosion was observed daily after temperature raised.

RESULTS AND DISCUSSION

Egg collection

Number of aphids used for the oviposition experiment are listed in Table 1. The egg deposition curve is drawn in Fig. 2 from total eggs deposited at 14°C (555 eggs from 227 females). During first six days after confinement in cups, about 80% of total eggs were deposited. Egg deposition peaked on the third day and decreased to low level on the sixth day after confinement. This result is similar to that in *Pterocallis alni* that deposited 88% eggs during seven days after mating at 15°C (GANGE and LLEWELLYN, 1988). This result shows that it is better for efficient egg collection to discard aphids in five or six days after collected.

Total number of eggs and eggs per female under different conditions are listed in Table 1. Significant differences are detected between 14°C and 20°C and between filter paper

Table 1. Number of aphids used for the oviposition experiments

Temperature	Oviposition surface	Diet ^a	Female	Male	Eggs	Eggs/female
14°C	filter paper	—	30	6	55	1.83
	filter paper	+	93	18	194	2.09
	cotton cloth	—	74	6	241	3.26
16°C	filter paper	—	62	12	160	2.58
	cotton cloth	—	63	12	146	2.32
20°C	filter paper	—	200	27	598	2.99
	filter paper	+	63	13	157	2.49
	cotton cloth	—	254	30	848	3.34

^a +: with diet, —: without diet.

Fig. 2. Egg deposition curve in the glass cup at 14°C. Solid line: number of eggs per day per female, dotted line: cumulative number of eggs.

with diet and cotton cloth. These statistical analyses were done by SAS program of TURKY's studentized test (*SAS/STAT User's Guide Release 6.03 Edition*, SAS Press, Tokyo, 1078 pp. (1990)). The diet did not elevate oviposition on the filter paper. The most eggs (3.3 eggs/female) were obtained at 20°C on the cotton cloth. Newly deposited eggs were yellow, and turned black in a few days. Eggs on the filter paper turned black faster than those on the cotton cloth and 91% of eggs on the former while 83% of eggs on the latter pigmented. In addition, eggs on the cotton cloth were easy to be detached from the surface and floated on water in sterilization. Then it is concluded that the filter paper is the better material as oviposition surface than the cotton cloth though oviposition was the largest on the cotton cloth, and artificial diet was no need. In this method, total number of eggs deposited per female ranged from 1.8 to 3.3. This is smaller than the field data of 5.9 in *P. alni* (GANGE and LEWELLYN, 1988). This difference cannot be compared directly because the probability is not excluded that aphids had oviposited before collected from *Spiraea thunbergii* though young females were collected daily.

Sterilization

Eggs were sterilized with 1% formaldehyde after (7th and 5th days after oviposition) and before (immediately after oviposition) pigmentation. These eggs were maintained at

Fig. 3. Percentage of egg pigmentation at three temperatures, 14°C (circle), 16°C (triangle) and 20°C (square). The symbols accompanied by same alphabet do not differ in the Chi-square test.

Fig. 4. Hatching curves of eggs that were incubated for three different periods before cooling treatment.

14°C. Eggs had turned black at five days after oviposition unless sterilized immediately after oviposition. For each treatment, 96% (47/49), 94% (47/50) and 55% (22/40) eggs turned black respectively. Eggs aged zero to one day were sensitive to sterilization. These eggs remained yellow like as unfertilized eggs or incompletely pigmented.

Egg development in the pre-cooling period under different temperatures

Eggs turned black at different temperatures are illustrated in Fig. 3. Thirty-two, 156 and 46 eggs on the filter papers were used at 14, 16 and 20°C incubation respectively.

Eggs incubated at 16°C turned black slowly than those at 14°C, and final percentage of pigmented ones was smaller. Eggs at 20°C turned black earlier than those at 14°C but final percentage of pigmented eggs was smaller. It is shown that 14°C incubation resulted the best development in pre-diapause stage.

Effects of pre-cooling period on the egg development in post-diapause

Eggs that experienced different length of pre-cooling periods hatched almost the same time (Fig. 4). For the experiment, 102, 100 and 98 eggs were used 7, 14 and 21 d pre-cooling incubation periods respectively. Significant difference in median date of egg eclosion after transfer to 14°C was not detected by the MANN-WHITNEY test. This shows that the period from 7 to 21 d before cooling did not affect both the diapause and the post-diapause development of eggs. It also suggests that the egg period before cooling treatment does not need any attention for studying egg diapause.

With this method, where eggs were sterilized on the 7th day after oviposition, incubated at 14°C in pre-cooling period, chilled at 6.4°C for 50 days and incubated at 14°C after cooling, more than 88.8% of 300 eggs were hatched. This rate is very high comparing with other laboratory hatching methods for other species (HAND, 1983; WIPPERFÜRTH and MITTLER, 1986; NEWTON and DIXON, 1987; VIA, 1992) and with the result under natural conditions of *A. spiraecola* (KOMAZAKI, 1990). This method will be a good tool for studying on diapause of aphid eggs.

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