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Performance of *Aphis glycines* Matsumura (Hemiptera: Aphididae) reared under different methods

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Abstract

Soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae), is an important soybean pest in North America. In this study, *A. glycines* was fed on detached leaves of *Glycine max* (L.) Merrill (Fabaceae) at 20, 23, 26, 29, and 32 °C under 3 rearing methods: leaf-disc method, filter paper method, and moisturized cotton method. *Aphis glycines* life parameters, including nymph stage duration, adult lifespan, adult fecundity, adult body size, and intrinsic rate of increase were measured. There were no significant differences in nymph stage duration among these rearing methods at 20 to 32 °C. Within this temperature range, the filter paper method and the moisturized cotton method resulted in similar or greater adult lifespans, adult fecundities, adult body sizes, and intrinsic rates of increase in comparison to the leaf-disc method. When nymphs of *A. glycines* were reared at 20 to 29 °C, the experimental labor required for the moisturized cotton method was less than that for the filter paper method and the leaf-disc method. Considering the development, reproduction, and experimental labor, the moisturized cotton method is recommended as a good detached leaf method for rearing individual *A. glycines*. These conclusions will be useful and informational for researchers who study *A. glycines*, especially when selecting methods to rear *A. glycines* individually in the laboratory.

Key Words: soybean aphid; filter paper method; leaf-disc method; moisturized cotton method

Resumen

El pulgón de la soja, *Aphis glycines* Matsumura (Hemiptera: Aphididae), es una plaga importante de la soja en América del Norte. En este estudio, *A. glycines* fue alimentado con hojas desprendidas de *Glycine max* (L.) Merrill (Fabaceae) a 20, 23, 26, 29 y 32 °C bajo 3 métodos de crianza: método de disco de hoja, método de papel de filtro y método de algodón humedecido. Se midieron los parámetros de vida del *A. glycines*, incluyendo la duración de la estadio del ninfa, la duración de vida de los adultos, la fecundidad, el tamaño corporal adulto y la tasa intrínseca de aumento. No hubo diferencias significativas en la duración del estadio de ninfa entre estos métodos de crianza a 20 a 32 °C. Dentro de este rango de temperatura, el método del papel de filtro y el método del algodón humedecido dieron resultados similares o mayores sobre la duración de vida de los adultos, la fecundidad, el tamaño corporal de adultos y la tasa intrínseca de aumento en comparación con el método del disco de hoja. Cuando se criaron las ninfas a una temperatura de 20 a 29 °C, el trabajo experimental requerido para el método del algodón humedecido fue menor que los métodos del papel de filtro y del disco de hoja. Teniendo en cuenta el desarrollo, la reproducción y el trabajo experimental, se recomienda el método del algodón humedecido como un buen método de hojas sueltas para criar los individuales de *A. glycines*. Estas conclusiones serán útiles e informativas para los investigadores que estudian *A. glycines*, especialmente al seleccionar métodos para criar *A. glycines* individualmente en el laboratorio.

Palabras Claves: pulgón de la soja; método del papel de filtro; método hoja-disco; metodo de algodon humedecido

Soybean aphid, *Aphis glycines* Matsumura (Hemiptera: Aphididae), is the second most important soybean pest in Asia (Ragsdale et al. 2011). In 2000, *A. glycines* invaded North America (Hunt et al. 2003; Venette & Ragsdale 2004) and has become one of the most important local soybean pests in areas where it has established. Studies of these aphids have received increased attention following the invasion into these new regions. Until now, there have been many reports describing studies on population dynamics (Fan et al. 2017; Leblanc & Brodeur 2018), natural enemies (Liu et al. 2012; Ode & Crompton 2013; Hesler 2014; Wang et al. 2016a; Hopper et al. 2017), host adaptability (Chen et al. 2015, 2017), economic threshold (Marchi-Werle et al. 2017), and control measures (Regan et al. 2017).

To study development and reproduction of *A. glycines* on host plants, it is necessary to determine some of their life-history parameters. In these studies, *A. glycines* should be reared individually on live plants of soybean, *Glycine max* (L.) Merrill (Fabaceae), and checked daily (Xu et al. 2011). However, it is difficult to search for a single aphid or its exuviae on a whole plant. To facilitate individual rearing, soybean aphids have been reared on live soybeans with clip-cages (Li et al. 2004; Michel et al. 2010). However, clip-cages were not practical for some nymphs, especially for first instars. There are many resilient villi on the surface of soybean leaves. No matter how tight a clip-cage is set on a leaf, there are still some tiny gaps between the clip-cage and the leaf, which are wide enough for a first instar to escape. If a clip-cage is set so tightly on a leaf to prevent first instar nymphs from escaping, the leaf is prone to damage, which would affect the development of nymphs in the cages.

In contrast to using live plants, the detached leaf method also is practical for studies on development and production of *A. glycines*. Vi-

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sual assessments of ecdysis are more efficient when adults and nymphs are reared on a detached leaf in a container. The leaf-disc method is one of the most widely used detached leaf methods for rearing aphids. With this method, soybean leaves are placed on the surface of an agar-medium in beakers (Yang et al. 2010; Wang et al. 2014; Chen et al. 2017). However, it was found that some types of unidentified fungi could grow rapidly on the agar-medium with the leaf-disc method at temperatures above 25 °C in some laboratories. Bound by fungal hyphae, nymphs may die accidentally when they are traversing the surfaces of the leaf and medium within the beaker.

The filter paper method also was used in some studies of *A. glycines* (Michel et al. 2010) and other aphids (Bai et al. 2014; Wang et al. 2016b). This method was an improvement over the leaf-disc method, with agar-medium being replaced by filter paper. When nymphs are reared with the filter paper method, there are fewer nutrients in the filter paper for fungal growth in comparison to the agar-medium. As a result, the problem of fungal growth is avoided, but daily application of water onto the filter paper surface is necessary to keep the leaves fresh; this daily water application is laborious and time consuming.

To avoid accidental death of aphids caused by growing fungi on medium and to save time during experiments, we developed the moisturized cotton method. A piece of square moisturized cotton is placed at the bottom of a beaker, then a piece of round filter paper is placed on the surface of the moisturized cotton. When water is dropped onto the surface of the filter paper, it is partially absorbed by and stored in the cotton square. Thus, addition of water daily is no longer necessary and fungal growth still is avoided. There were significant differences in some life parameters of *A. glycines* reared on the leaf-disc method and the filter paper method (Tian et al. 2018), but the question remains as to whether life parameters of *A. glycines* reared with the moisturized cotton method differs from those of *A. glycines* reared on the leaf-disc method or the filter paper method.

In this study, *A. glycines* was fed on detached leaves of *G. max* with the leaf-disc, filter paper, and moisturized cotton methods at 20, 23, 26, 29, and 32 °C to determine relevant life parameters of *A. glycines* through rearing. By comparing life parameters, the effects of different rearing methods on development and reproduction of *A. glycines* were studied. If there were significant differences in these life parameters, it would demonstrate that subtle differences between similar rearing methods could affect experimental results. These data will be useful and instructive for researchers studying *A. glycines*, especially when they are selecting methods for individual rearing of *A. glycines* in the laboratory.

Materials and Methods

INSECTS

Twenty wingless virginoparae *A. glycines* were taken from a soybean field in Northeast Agricultural University, Harbin, Heilongjiang Province, northeast China (126.72°E, 45.74°N). Only 1 individual adult was retained as the mother aphid to build a monoclonal population resulting in greater fecundity and body size; this also eliminated potential population differences among treatments, thereby ensuring a homogeneous population for experiments. The colony was maintained on soybean seedlings (variety 'Heinong 51') in a growth chamber at 25 ± 1 °C, 70 ± 5% relative humidity (RH), and a photoperiod of 14:10 h (L:D) with artificial light of 12,000 Lux.

HOST PLANTS

Seeds of *G. max* (variety 'Heinong 51') were purchased from Fangyuan Agriculture Corporation, Wuchang, Heilongjiang Province, China. Soybeans were grown in a growth chamber with 6 to 10 seeds per pot in plastic pots of 10 × 10 cm (diam × height) at 25 ± 1 °C, 70 ± 5% RH, and a photoperiod of 14:10 h (L:D). Seedlings of 15 to 20 cm tall (stage V2; Fehr et al. 1971) were used for experiments.

LIFE PARAMETERS OF *APHIS GLYCINES* REARED WITH DIFFERENT METHODS

To rejuvenate the population of *A. glycines*, apterous adults were removed from the monoclonal population and placed onto 4 pots (Tianfeng Technology Company, Harbin, China) of *G. max* plants (1 aphid per pot). The plants were placed in a growth chamber at 25 ± 1 °C, 70 ± 5% RH, and a 14:10 h (L:D) photoperiod for a 2-wk reproduction period. Afterwards, 50 apterous adults were transferred from these 4 soybean plants onto another 5 pots containing soybean plants (10 adults per pot). These plants were placed in a growth chamber under the same conditions for a 24-h reproductive period, after which all adults were removed. Newly deposited nymphs were removed individually from the plants with a small brush for experiments.

Each of the 3 methods used to rear nymphs consisted of 5 groups, with 50 newly deposited nymphs in each group; these groups were reared at 20, 23, 26, 29, and 32 °C. When *A. glycines* was reared with the leaf-disc method, detached leaves of *G. max* were cut into square 1.5 cm^2 pieces using a pair of scissors. Solid agar media was prepared in 45 mL, 4 × 4.5 cm (diam × height) glass beakers (Tianfeng Technology Company, Harbin, China). Each nymph was placed on the reverse side of a square piece of soybean leaf adhered to the surface of medium. To adhere the leaf to the medium, a small drop of water was dropped on the surface of medium, then the square piece of soybean leaf was put on the surface of the medium. With the addition of slight pressure using a small brush, the leaf was adhered on the surface of medium due to the surface tension of water. The beaker was then placed upside down on a 5 cm diam Petri dish (Chen et al. 2017) (Fig. 1A). When *A. glycines* was reared with the filter paper method, detached leaves of *G. max* were cut into square 1.5 cm^2 pieces. Pieces of round 4.2 cm diam filter paper were placed at the bottom of 45 mL, 4 × 4.5 cm (diam × height) glass beakers and were wetted by adding 400 µL water using a pipette. The filter papers were cut slightly large to keep them firmly fixed within the beakers. Each nymph was placed on the reverse side of a square piece of soybean leaf adhered to the surface of the filter paper. A small drop of water was placed on the surface of the filter paper, then the square piece of soybean leaf was put on the surface. With slight pressure, the leaf was adhered on the surface of filter paper. The beaker was then placed upside down on a 5 cm diam Petri dish (Fig. 1B). When *A. glycines* was reared with the moisturized cotton method, a piece of square moisturized cotton (facial cleansing cotton), 2 × 2 × 0.4 cm (length × width × height), was placed on the bottom of a 45 mL, 4 × 4.5 cm (diam × height) glass beaker, and a piece of filter paper of 4.2 cm in diam was placed on the surface of the moisturized cotton. The filter paper was then wetted with 2,200 µL water by dropping it onto the surface with a pipette. Detached leaves of *G. max* were cut into square 1.5 cm^2 pieces. Each nymph was placed on the reverse side of a square piece of soybean leaf adhered to the surface of filter paper. A small drop of water was placed on the surface of the filter paper, then the square piece of soybean leaf was put on the surface. With slight pressure, the leaf was adhered. The beaker was then placed upside down on a 5 cm diam Petri dish (Fig. 1C). Individual nymphs were checked daily for ecdysis and survival. When they developed into adults, nymphs deposited by each female were counted and removed daily. Adult survival was recorded daily until the death of each adult. Media used in the leaf-disc method were replaced every 5 to 7 d or upon observation of fungal growth. Filter papers used in the filter paper

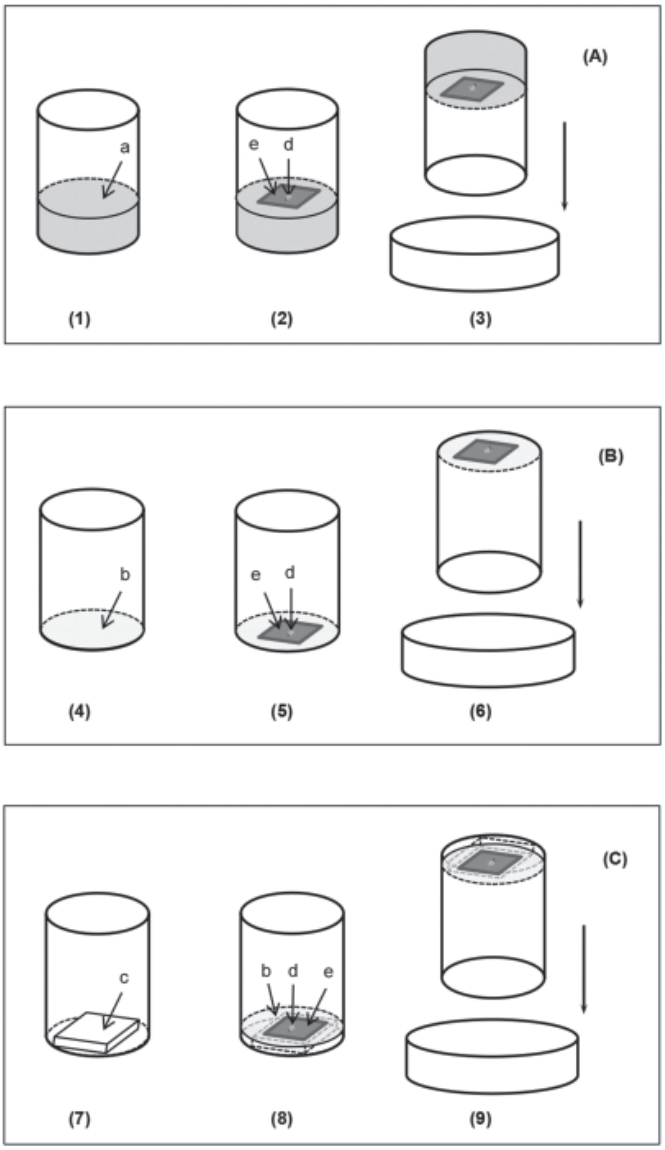

Fig. 1. Schematics of the 3 methods used to individually rear *Aphis glycines*: (A) leaf-disc method; (B) filter paper method; and (C) moisturized cotton method. (1) A 50 mL glass beaker with solid agar medium: 'a' indicates the 10 mL of solid agar medium. (2) *Aphis glycines* was placed on the reverse side of a piece of soybean leaf that was adhered to a surface of solid agar medium in a glass beaker: 'd' indicates *Aphis glycines*; 'e' indicates a square 1.5 cm^2 piece of soybean leaf. (4) A 50 mL glass beaker with a piece of filter paper: 'b' indicates the 4 cm diam filter paper. (5) *Aphis glycines* was placed on the reverse side of a piece of soybean leaf that was adhered to the surface of filter paper in a glass beaker. (7) A 50 mL glass beaker with a piece of moisturized cotton: 'c' indicated the moisturized cotton (2 L × 2 W × 0.4 H cm). (8) A piece of 4 cm diam filter paper was placed on the surface of the moisturized cotton. *Aphis glycines* was placed on the reverse side of a piece of soybean leaf that was adhered to the surface of filter paper in a glass beaker. (3, 6, 9) A glass beaker was placed upside down on a Petri dish (5 cm diam).

method were wetted daily by adding 400 µL water using a pipette. In the moisturized cotton method, water was added onto surface of filter paper every 7 to 10 d (400 µL each time). In these 3 methods, soybean leaves were replaced every 5 to 7 d or when they became yellowish.

BODY SIZE OF ADULT REARED WITH DIFFERENT METHODS

After adults died during a life parameter trial, they were preserved in a 70% alcohol solution. Adult body length and body width were measured with an optical microscope and a micrometer (Shanghai Cewei Company, Shanghai, China). The dorsal view of an adult aphid is regarded as a regular ellipse, and the ellipse area formula, $\pi \times a \times b / 4$ (where 'a' is the body length, and 'b' is the body width; $\pi \approx 3.14$), was used to calculate and evaluate the body size of adults (Tian et al. 2020).

RELATIVE HUMIDITY IN BEAKERS USED IN DIFFERENT METHODS

The relative humidity in beakers containing media, leaves, and aphids as described above was measured using the same rearing method and temperature settings as described above. A 0.8 cm diam circular hole was made with an electrical drill (Hankai Intelligent Technology Company, Zhejiang, China) at the center of each plastic Petri dish. Then the hole was covered by a 2 × 2 cm plastic piece outside and the piece was fixed onto the surface of the Petri dish by a piece of transparent adhesive tape. The beaker was still placed upside down onto the Petri dish. Relative humidity in beakers was measured with a temperature and humidity recorder (Yuan Hengtong Hardware Shop, Zhejiang, China,). To measure relative humidity, the beaker and Petri dish were picked up carefully to keep the 2 parts together. The plastic piece and adhesive tape were carefully removed and the detector of the temperature and humidity recorder, which was linked to the recorder with wires, was slowly put into the glass beaker through the hole. Then the beaker and Petri dish with detector were carefully put back into the growth chamber, and the relative humidity in beaker was recorded after 2 min (He et al. 2016). The relative humidity in beakers used in different rearing methods was recorded daily for 7 d.

EXPERIMENTAL TIME SPENT ON DIFFERENT REARING METHODS

For each rearing method, 5 groups of newly deposited nymphs and 5 groups of adults (10 individuals per group) were used for testing, and were reared at 20, 23, 26, 29, and 32 °C, 70 ± 5% RH, and a 14:10 h (L:D) photoperiod. After 24 h, they were evaluated for ecdysis, survivorship, and offspring production. The nymph ecdysis and nymphs deposited by each female were counted and removed with a small brush. Any dead aphids also were removed and recorded. The following trial steps were done: (1) in the filter paper method, 400 µL water was applied onto the surface of the filter paper; (2) in the leaf-disc method, media was replaced upon observation of fungal growth. The daily experimental labor time spent in each method to rear each nymph or adult was recorded with a stop-watch.

DATA ANALYSIS

Raw data of nymph stage durations, adult lifespans, and adult fecundities of *A. glycines* reared with different methods, along with their means and standard errors, were calculated with TWOSEX-MSChart software (Chi & Liu 1985; Chi 2017). Differences in nymph stage duration, adult lifespans, and adult fecundities of *A. glycines* reared with the 3 rearing methods at each temperature were analyzed by analysis of variance (PROC GLM) and Tukey's honest significant difference (HSD) tests (SAS 8.1) (Wang et al. 2019). The relative humidity data in beakers were arcsine-square root transformed for normal distribution. Differences in body size of adults, relative humidity in beakers, and experimental time to deal with nymphs or adults were analyzed by PROC GLM and HSD tests.

Means and standard errors of intrinsic rate of increase (r), net productive rate (R_0), finite rate of increase (λ), and mean generation time (T) were calculated using the bootstrap technique (Efron & Tibshirani 1993) using the computer program TWOSEX-MSChart (Chi 2017). Because bootstrap analysis uses random resampling, a small number of replications will generate variable means and standard errors; thus,

200,000 bootstrap iterations were used to reduce the variability of the results. Differences in these 4 parameters of *A. glycines* reared with different rearing methods at each temperature were analyzed by a paired bootstrap test (Huang & Chi 2011; Yu et al. 2013).

Results

NYMPH STAGE DURATION, ADULT LIFESPAN, AND ADULT FECUNDITY OF *APHIS GLYCINES* REARED WITH DIFFERENT METHODS

Nymph stage duration of *A. glycines* varied from 4.81 to 7.81 d when they were reared by different methods at a temperature range of 20 to 32 °C. At each of the temperatures, there were no significant differences in nymph stage durations of *A. glycines* reared with leaf-disc method, filter paper method, and moisturized cotton method (20 °C: df = 2, 140; $F = 0.06$; $P = 0.9442$; 23 °C: df = 2, 144; $F = 0.23$; $P = 0.7935$; 26 °C: df = 2, 137; $F = 1.22$; $P = 0.2984$; 29 °C: df = 2, 141; $F = 2.09$; $P = 0.1281$; 32 °C: df = 2, 137; $F = 3.00$; $P = 0.0530$) (Table 1).

Adult lifespans of *A. glycines* were the same when they were reared with the filter paper method and with the moisturized cotton method at 20, 23, and 29 °C, but *A. glycines* had shorter lifespans when they were reared with the leaf-disc method at those temperatures (20 °C: df = 2, 140; $F = 17.21$; $P < 0.0001$; 23 °C: df = 2, 144; $F = 40.58$; $P < 0.0001$; 29 °C: df = 2, 141; $F = 37.15$; $P < 0.0001$). At 26 °C, adult lifespans of *A. glycines* was longest (21.72 ± 0.74 d) when reared with filter paper method, followed by aphids reared with moisturized cotton method (13.04 ± 1.09 d) and then the aphids reared by the leaf-disc method (7.76 ± 0.48 d) (df = 2, 137; $F = 70.76$; $P < 0.0001$). At 32 °C, adults had longer lifespans when reared with the filter paper method (9.10 ± 0.57 d) than adults reared with the moisturized cotton method (5.98 ± 0.58 d), but their lifespans were not significantly different from those reared with the leaf-disc method (7.98 ± 0.48 d) (df = 2, 137; $F = 8.26$; $P = 0.0004$) (Table 1).

At 20 °C, there were no significant differences in adult fecundity among the 3 rearing methods (df = 2, 140; $F = 4.80$; $P = 0.0096$). At 23 °C, adults reared with the filter paper method produced a similar number of offspring (58.49 ± 1.65 offspring per female) as those reared with the moisturized cotton method (56.00 ± 2.10 offspring per female), but those reared with the leaf-disc method produced significantly fewer offspring (38.31 ± 2.21 offspring per female) (df = 2, 144; $F = 30.16$; $P < 0.0001$). At 29 °C, adults reared with the filter paper method produced the most offspring (53.40 ± 1.64 offspring per female), while those reared with the moisturized cotton method produced significantly fewer offspring (38.38 ± 2.01 offspring per female), and those reared with the leaf-disc method produced the fewest offspring (30.00 ± 2.03 offspring per female) (df = 2, 141; $F = 39.87$; $P < 0.0001$). At 26 and 32 °C, adult fecundity of *A. glycines* reared with the moisturized cotton method were as high as those reared with the leaf-disc method, which were both less than those reared with the filter paper method (26 °C: df = 2, 137; $F = 28.54$; $P < 0.0001$; 32 °C: df = 2, 137; $F = 15.96$; $P < 0.0001$) (Table 1).

LIFE TABLE PARAMETERS OF *APHIS GLYCINES* REARED WITH DIFFERENT METHODS

At 20 °C, there were no significant differences in intrinsic rates of increase, finite rates of increase, and mean generation times among the 3 methods. The moisturized cotton method resulted in a higher net reproductive rate than the leaf-disc method, whereas neither method resulted in significant differences in net reproductive rate when compared with the filter paper method. At 23 °C, intrinsic rate of increase, net reproductive rate, finite rate of increase, and mean generation time of *A. glycines* reared with the filter paper method were similar to those for *A. glycines* reared with the moisturized cotton method, and they were all higher than those for *A. glycines* reared with the leaf-disc method. At 26 °C, there were no significant differences in intrinsic rates of increase and finite rates of increase of *A. glycines* reared with different rearing methods. At 26 °C, net productive rate and mean generation time of *A. glycines* reared with the moisturized cotton method were similar to those for *A. glycines* reared with the leaf-disc method, and they were all lower than those for *A. glycines* reared with the filter paper method. At 29 °C, intrinsic rate of increase and finite rate of increase of *A. glycines* reared with the filter paper method were similar to those for *A. glycines* reared with the moisturized cotton method, and they were all higher than those for *A. glycines* reared with the leaf-disc method. Net productive rate of *A. glycines* reared with the filter paper method was the highest, whereas the net productive rate was higher for *A. glycines* reared with the moisturized cotton method than that for *A. glycines* reared with the leaf-disc method. Mean genera-

Table 1. Nymph stage duration, adult lifespan, and adult fecundity (mean ± SE) of *Aphis glycines* reared with 3 methods at different temperatures.

Temperature (°C)	Rearing method	*n*	Nymph stage duration (d)	Adult lifespan (d)	Adult fecundity (offspring)
20	Filter paper	47	7.74 ± 0.10 a	23.45 ± 1.62 a	53.02 ± 1.73 a
	Moisturized cotton	48	7.77 ± 0.11 a	27.12 ± 1.67 a	52.56 ± 1.82 a
	Leaf-disc	48	7.81 ± 0.10 a	15.42 ± 0.94 b	45.65 ± 2.09 a
23	Filter paper	49	6.43 ± 0.08 a	23.76 ± 1.13 a	58.49 ± 1.65 a
	Moisturized cotton	49	6.35 ± 0.09 a	23.55 ± 1.35 a	56.00 ± 2.10 a
	Leaf-disc	49	6.39 ± 0.09 a	11.37 ± 0.79 b	38.31 ± 2.21 b
26	Filter paper	46	5.26 ± 0.07 a	21.72 ± 0.74 a	61.83 ± 1.34 a
	Moisturized cotton	49	5.18 ± 0.07 a	13.04 ± 1.09 b	43.14 ± 2.55 b
	Leaf-disc	45	5.11 ± 0.06 a	7.76 ± 0.48 c	42.16 ± 2.04 b
29	Filter paper	50	4.86 ± 0.08 a	16.62 ± 0.74 a	53.40 ± 1.64 a
	Moisturized cotton	47	4.81 ± 0.07 a	14.70 ± 0.85 a	38.38 ± 2.01 b
	Leaf-disc	47	5.00 ± 0.05 a	8.04 ± 0.61 b	30.00 ± 2.03 c
32	Filter paper	50	5.24 ± 0.08 a	9.10 ± 0.57 a	4.68 ± 0.39 a
	Moisturized cotton	43	5.21 ± 0.10 a	5.98 ± 0.58 b	1.93 ± 0.31 b
	Leaf-disc	47	5.49 ± 0.09 a	7.98 ± 0.48 ab	2.60 ± 0.37 b

Sample size (*n*) is number of *A. glycines* used to calculate nymph duration, adult lifespan, and adult fecundity. Nymph stage is the total number of d that aphids take to develop from the first instar to adults. Means within a column at each temperature followed by the same letter do not differ significantly (HSD, $P < 0.01$).

tion times of *A. glycines* reared with the moisturized cotton method and the leaf-disc method were of similar length but both were shorter than mean generation time of *A. glycines* reared with the filter paper method. At 32 °C, intrinsic rates of increase, net productive rates, and finite rates of increase of *A. glycines* reared with the moisturized cotton method and the leaf-disc method were similar, and all were lower than those for *A. glycines* reared with the filter paper method. Mean generation time of *A. glycines* reared with the leaf-disc method was the longest, and mean generation times of *A. glycines* reared with the filter paper method and the moisturized cotton method were successively shorter (Table 2).

BODY SIZE OF *APHIS GLYCINES* REARED WITH DIFFERENT METHODS

At 20 °C, adults reared with the moisturized cotton method were larger in body size (0.89 ± 0.01 mm^2) than those reared with the leaf-disc method (0.78 ± 0.02 mm^2), and both were as large as those reared with the filter paper method (0.83 ± 0.02 mm^2) (df = 2, 130; $F = 10.71$; $P < 0.0001$). At 23 and 26 °C, there were no significant differences in body sizes of *A. glycines* adults reared with different rearing methods (23 °C: df = 2, 124; $F = 1.15$; $P = 0.3188$; 26 °C: df = 2, 127; $F = 2.29$; $P = 0.1057$). At 29 and 32 °C, body sizes of *A. glycines* adults reared with the moisturized cotton method were 0.63 ± 0.02 and 0.47 ± 0.01 mm^2, and both were as large as those reared with the filter paper method, 0.65 ± 0.01 and 0.47 ± 0.02 mm^2, and they were all larger than those reared with the leaf-disc method, 0.56 ± 0.01 and 0.40 ± 0.01 mm^2 (29 °C: df = 2, 118; $F = 10.47$; $P < 0.0001$; 32 °C: df = 2, 115; $F = 7.44$; $P = 0.0009$) (Fig. 2).

RELATIVE HUMIDITY IN BEAKERS USED IN DIFFERENT METHODS

There were no significant differences in relative humidity in beakers when *A. glycines* was reared with different rearing methods at 20 °C and 26 °C ($P > 0.05$) (Fig. 3A and 3C, respectively). When *A. glycines* was reared for 3 d at 23 °C, the relative humidity in beakers used in the filter paper method and leaf-disc method was 73.77 ± 0.18% and 73.47 ± 0.31%, respectively, which were both higher than that in the moisturized cotton method, 72.17 ± 0.26% (df = 2, 27; $F = 10.89$; $P = 0.0003$). When *A. glycines* was reared with the filter paper method and the leaf-disc method for 4 d at 23 °C, the relative humidity in beakers was 74.33 ± 0.38% and 73.09 ± 0.50%, respectively, which was also higher than that in the moisturized cotton method, 70.63 ± 0.44% (df = 2, 27; $F = 17.86$; $P < 0.0001$). There were no significant differences in relative humidity in beakers when *A. glycines* was reared with different rearing methods for 1 to 2 or 5 to 7 d at 23 °C ($P > 0.05$) (Fig. 3B).

When adults were reared at 29 °C, the relative humidity in beakers varied among different d with regard to different rearing method. On the first d, the relative humidity was higher in beakers used in the moisturized cotton method (72.87 ± 0.83%) than that in the filter paper method (68.38 ± 0.83%), which were both as high as that in the leaf-disc method (69.60 ± 0.53%) (df = 2, 27; $F = 9.70$; $P = 0.0007$). On the second d, relative humidity in beakers used in the moisturized cotton method was as high as that in the leaf-disc method, with values of 70.43 ± 0.68% and 70.65 ± 0.75%, respectively, which were both higher than that in the filter paper method, 66.64 ± 0.61% (df = 2, 27; $F = 10.92$; $P = 0.0003$). On the fifth d, relative humidity in beakers used in the filter paper method was as high as that in the moisturized cotton method, with values of 66.84 ± 0.43% and 68.68 ± 0.65%, respectively, which were both lower than that in the leaf-disc method, 73.22 ± 0.49% (df = 2, 27; $F = 38.06$; $P < 0.0001$). On the sixth d, relative humidity in beakers used in the leaf-disc method (69.49 ± 0.79%) was higher than that in the filter paper method (65.00 ± 0.58%), which were both as high as that in the moisturized cotton method (67.34 ± 0.54%) (df = 2, 27; $F = 12.10$; $P = 0.0002$). On d 3 to 4 and 7, there were no significant differences in relative humidity in beakers among different rearing methods ($P > 0.05$) (Fig. 3D).

When *A. glycines* adults were reared for 1 to 5 and 7 days at 32 °C, there were significant differences in relative humidity in beakers used in different rearing methods at the determining dates ($P < 0.05$). When *A. glycines* adults were reared for 6 days at 32 °C, there were no significant differences in relative humidity in beakers used in different rearing methods (df = 2, 27; $F = 3.98$; $P = 0.0305$) (Fig. 3E).

EXPERIMENTAL TIME SPENT IN DIFFERENT REARING METHODS

When *A. glycines* nymphs were reared at 20 and 26 °C, experimental time spent during the filter paper method was 13.69 ± 0.48 s and 11.72 ± 0.86 s, respectively, which was as long as that spent during the leaf-disc method, 11.87 ± 1.04 s and 13.41 ± 0.74 s, respectively, and

Table 2. Life table parameters (mean ± SE) of *Aphis glycines* reared with 3 methods at different temperatures.

Temperature (°C)	Rearing method	Intrinsic rate of increase (r) (d^{-1})	Net productive rate (R_0) (offspring)	Finite rate of increase (λ) (d^{-1})	Mean generation time (T) (d)
20	Filter paper	0.3137 ± 0.0045 a	49.84 ± 2.40 ab	1.3685 ± 0.0062 a	12.46 ± 0.12 a
	Moisturized cotton	0.3123 ± 0.0050 a	50.46 ± 2.26 a	1.3665 ± 0.0069 a	12.56 ± 0.14 a
	Leaf-disc	0.3032 ± 0.0050 a	43.82 ± 2.35 b	1.3542 ± 0.0068 a	12.47 ± 0.16 a
23	Filter paper	0.3768 ± 0.0055 a	57.32 ± 1.97 a	1.4576 ± 0.0080 a	10.74 ± 0.14 a
	Moisturized cotton	0.3745 ± 0.0054 ab	54.88 ± 2.33 a	1.4543 ± 0.0078 ab	10.69 ± 0.13 a
	Leaf-disc	0.3591 ± 0.0060 b	37.54 ± 2.27 b	1.4320 ± 0.0086 b	10.10 ± 0.14 b
26	Filter paper	0.4413 ± 0.0075 a	56.88 ± 2.67 a	1.5548 ± 0.0117 a	9.16 ± 0.10 a
	Moisturized cotton	0.4496 ± 0.0072 a	42.28 ± 2.62 b	1.5677 ± 0.0113 a	8.33 ± 0.14 b
	Leaf-disc	0.4484 ± 0.0086 a	37.94 ± 2.55 b	1.5658 ± 0.0134 a	8.11 ± 0.10 b
29	Filter paper	0.4534 ± 0.0059 a	53.40 ± 1.63 a	1.5736 ± 0.0093 a	8.77 ± 0.10 a
	Moisturized cotton	0.4407 ± 0.0089 a	36.08 ± 2.27 b	1.5538 ± 0.0133 a	8.13 ± 0.12 b
	Leaf-disc	0.4108 ± 0.0076 b	28.20 ± 2.13 c	1.5080 ± 0.0117 b	8.13 ± 0.13 b
32	Filter paper	0.2273 ± 0.0124 a	4.68 ± 0.38 a	1.2552 ± 0.0155 a	6.79 ± 0.09 b
	Moisturized cotton	0.0820 ± 0.0285 b	1.66 ± 0.28 b	1.0854 ± 0.0307 b	6.18 ± 0.28 c
	Leaf-disc	0.1257 ± 0.0213 b	2.44 ± 0.35 b	1.1339 ± 0.0240 b	7.10 ± 0.13 a

The initial number of aphids used in each method was 50. Means within a column at each temperature followed by the same letter do not differ significantly (paired bootstrap test, $P < 0.05$).

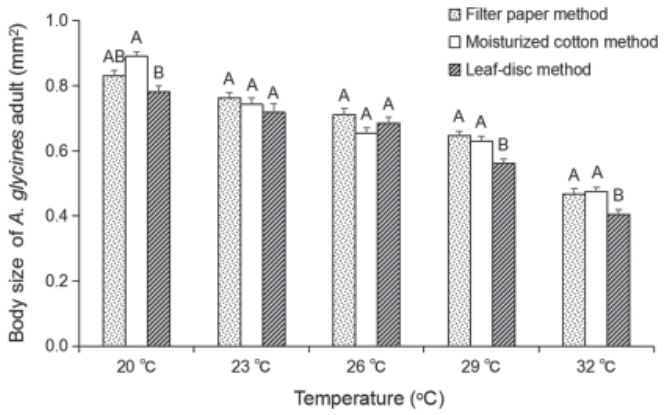

Fig. 2. Body size of *Aphis glycines* adults reared with 3 methods. Means at each temperature followed by the same letter do not differ significantly (HSD, $P < 0.01$).

both required more time than the moisturized cotton method, 4.50 ± 0.13 s and 6.74 ± 0.33 s, respectively (20 °C: df = 2, 27; $F = 53.77$; $P < 0.0001$; 26 °C: df = 2, 27; $F = 25.96$; $P < 0.0001$). At 23 and 29 °C, the leaf-disc method required the most time, 13.58 ± 0.90 s and 16.82 ± 0.90 s, respectively, whereas successively less time was required during the filter paper method (10.39 ± 0.44 s and 13.26 ± 0.26 s, respectively) and the moisturized cotton method (5.95 ± 0.22 s and 6.30 ± 0.84 s, respectively) (23 °C: df = 2, 27; $F = 41.71$; $P < 0.0001$; 29 °C: df = 2, 27; $F = 54.2$; $P < 0.0001$). At 32 °C, experimental time spent during the leaf-disc method was 14.06 ± 1.23 s, which was longer than time spent during the moisturized cotton method (8.46 ± 0.95 s), and neither required more time than the filter paper method (11.20 ± 0.37 s) (df = 2, 27; $F = 9.21$; $P = 0.0009$) (Table 3).

When *A. glycines* adults were reared from 20 to 32 °C, experimental time spent during the moisturized cotton method was similar to time spent during the leaf-disc method, and these required less time than the filter paper method ($P < 0.05$) (Table 3).

Discussion

Aphids are an important pest of crops, and they have resulted in large economic losses in many different commodities. During some aphid research, it is necessary to rear them prior to experimentation. In past studies, researchers have needed to select from different *A. glycines* rearing methods, because there is no universal method capable of meeting all needs. When life parameters of *A. glycines* were determined initially, the leaf-disc (Chen et al. 2017) and filter paper (Michel et al. 2010) methods were used. Inevitably, slight differences in rearing environments have likely resulted in differences in results between studies. In this study, *A. glycines* was reared with 3 different methods, and some of their life parameters were determined for comparison among methods; the focus of this study was to determine the best detached leaf method for rearing *A. glycines* individually.

At 20 to 32 °C, when compared with the leaf-disc method, the filter paper method and the moisturized cotton method resulted in greater or similar measures in adult lifespan, adult fecundity (Table 1), adult body size (Fig. 2), and intrinsic rate of increase of *A. glycines* (Table 2). When the leaf-disc method was used to rear *A. glycines* adults, and no fungal hyphae occurred on the media surface, the required experimental time was similar to that spent in the moisturized cotton method; these methods required less time than the filter paper method (Table 3). As far as experimental time is concerned, the moisturized cotton

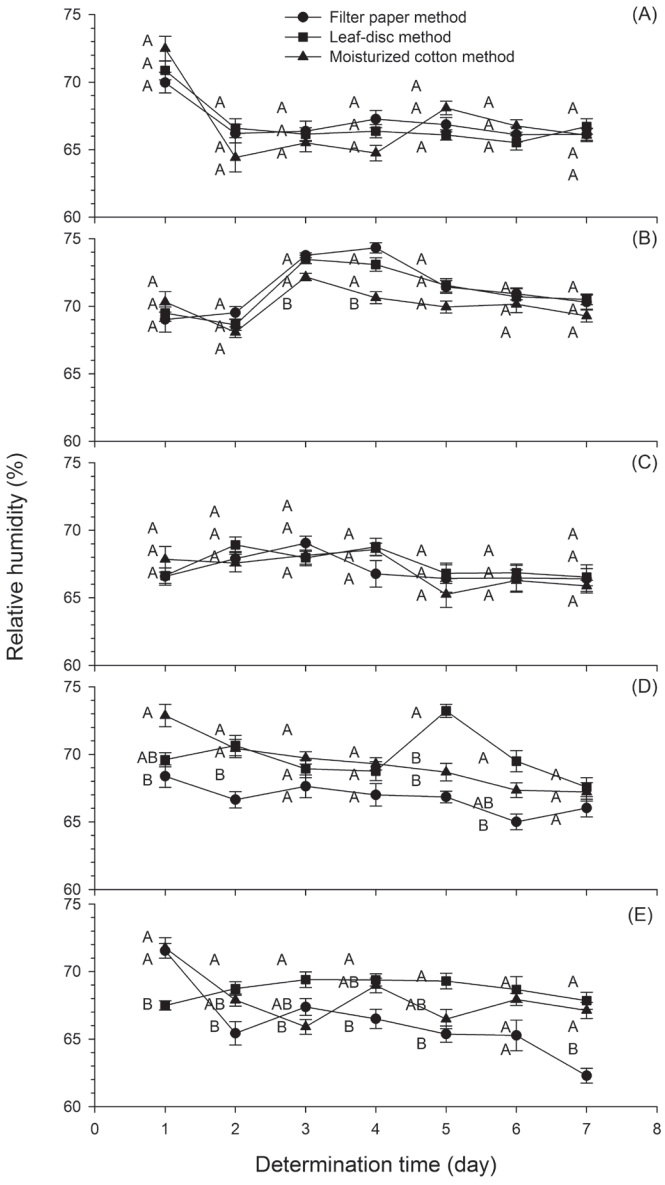

Fig. 3. Relative humidity in beakers used in different methods to rear *Aphis glycines*: (A) 20 °C; (B) 23 °C; (C) 26 °C; (D) 29 °C; (E) 32 °C. At each temperature, the means at each determining d followed by different letters are significantly different (HSD, $P < 0.01$).

method is recommended as a good method for rearing *A. glycines* individually.

When the moisturized cotton method is used to rear *A. glycines*, no fungal spores in the air can germinate and no hyphae can grow in beakers, because there is only water in the moisturized cotton and filter paper, and there are insufficient nutrients for germination of fungal spores or for hyphal growth. When water is dropped onto the surface of the filter paper, it is partially absorbed and stored in the moisturized cotton. Thus, daily addition of water is no longer necessary and much time is saved. However, cutting square cottons for moisturization, circular filters, and assembling them into beakers and wetting them prior to use for rearing aphids is labor intensive.

This is the first report of *A. glycines* being reared with the moisturized cotton method, and there are no previously reported data for comparison. However, the leaf-disc method has been used widely for

Table 3. Percentage of beakers where fungi hyphae occurred on the surface of the media or filter paper used in different methods and the experimental time (s per beaker, mean ± SE) spent during each method for rearing *Aphis glycines*.

			Nymphs of *A. glycines* reared		Adults of *A. glycines* reared	
Temperature (°C)	Rearing method	*n*	Hyphae percent (%)	Experimental time (s per beaker)	Hyphae percent (%)	Experimental time (s per beaker)
20	Filter paper	10	0	13.69 ± 0.48 a	0	15.31 ± 0.57 a
	Moisturized cotton	10	0	4.50 ± 0.13 b	0	7.91 ± 0.82 b
	Leaf-disc	10	100%	11.87 ± 1.04 a	0	9.42 ± 0.63 b
23	Filter paper	10	0	10.39 ± 0.44 b	0	15.27 ± 1.21 a
	Moisturized cotton	10	0	5.95 ± 0.22 c	0	7.01 ± 0.37 b
	Leaf-disc	10	100%	13.58 ± 0.90 a	0	8.59 ± 0.48 b
26	Filter paper	10	0	11.72 ± 0.86 a	0	16.11 ± 0.82 a
	Moisturized cotton	10	0	6.74 ± 0.33 b	0	11.84 ± 0.74 b
	Leaf-disc	10	100%	13.41 ± 0.74 a	0	11.52 ± 1.00 b
29	Filter paper	10	0	13.26 ± 0.26 b	0	15.86 ± 1.33 a
	Moisturized cotton	10	0	6.30 ± 0.84 c	0	9.55 ± 0.45 b
	Leaf-disc	10	100%	16.82 ± 0.90 a	0	10.37 ± 0.67 b
32	Filter paper	10	0	11.20 ± 0.37 ab	0	11.92 ± 0.52 a
	Moisturized cotton	10	0	8.46 ± 0.95 b	0	7.89 ± 0.75 b
	Leaf-disc	10	100%	14.06 ± 1.23 a	0	7.18 ± 0.47 b

The sample size (*n*) is the number of aphids used at the beginning of the study. Means within a column at each temperature followed by the same letter do not differ significantly (HSD, $P < 0.01$).

rearing soybean aphid in many studies, and these data are comparable. When *A. glycines* was reared with the filter paper, moisturized cotton, and leaf-disc methods at 20 °C, the duration of nymph stages (Table 1) were similar to that reported for rearing of *A. glycines* nymphs on *G. max* using the leaf-disc method, which was 7.39 ± 0.15 d (Wang et al. 2019). When *A. glycines* was reared with the leaf-disc method at 20 °C, the adult lifespan and fecundity (Table 1) were similar to previously reported data of 14.45 ± 0.89 d and 41.02 ± 2.12 offspring per female (Wang et al. 2019). Intrinsic rates of increase of *A. glycines* reared with 3 different methods at 20 °C (Table 2) were all similar to a previously reported result of 0.3130 ± 0.0051 one per d (Wang et al. 2019). When *A. glycines* was reared with the leaf-disc method at 23 °C in this study, the adult lifespan and fecundity (Table 1) values were both less than those previously reported, which were 15.10 ± 1.10 d and 46.10 ± 2.10 offspring per female (Chen et al. 2017), respectively. Intrinsic rate of increase of *A. glycines* reared with the leaf-disc method at 23 °C (Table 2) also was lower than the previously reported value of 0.4000 ± 0.0062 one per d (Chen et al. 2017). In our study, *A. glycines* was taken from a soybean field in the Northeast Agricultural University, Harbin, Heilongjiang Province, China. In the previous study, *A. glycines* was taken from a soybean field at the Xiangfang Experiment Station, Northeast Agricultural University, Harbin, Heilongjiang Province, China (126.75°E, 45.72°N) (Chen et al. 2017). When *A. glycines* was taken from these different places, they could have been from different biotypes. There are 4 biotypes of *A. glycines* in America, which differed in survival, development, and reproduction when they were fed on soybeans with different Rag (Resistance to *Aphis glycines*) genes (Hill et al. 2006). If the *A. glycines* used in this study and the previous study (Chen et al. 2017) were from different biotypes, the differences in their adult lifespan, adult fecundity, and intrinsic rates of increase would probably differ as well. Unfortunately, it remains unknown if there are distinct biotypes of *A. glycines* in northeast China.

When *A. glycines* was reared with the moisturized cotton method at 20 °C and 32 °C, the nymph stage duration (Table 1) were both slightly longer than those of soybean aphid being reared on live plants, with the reported 6.60 ± 0.10 (McCornack et al. 2004) and 3.80 (Hirano et al. 1996) d, respectively. At 20 °C, adult fecundity (Table 1) and net productive rate (Table 2) of *A. glycines* reared with the moisturized cotton method were smaller than those of soybean aphid being reared on live soybean plants, with 63.5 ± 2.20 and 75.48 offspring per female, respectively (McCornack et al. 2004). Based on the results mentioned above, it showed that nymphs of *A. glycines* reared with the moisturized cotton method survived longer and the adults produced less offspring than those being reared on live plants. But we still could not draw a conclusion on whether the moisturized cotton method is worse than the live plant method, because the *A. glycines* used in these trials were reared on different soybean varieties. Effects of different soybean varieties on development and reproduction of *A. glycines* is significant (Li et al. 2004). If the *A. glycines* could be comparatively reared with the moisturized cotton method and the live soybean plant method in a trial, and their various life history characteristics were compared using the same soybean aphid biotype and soybean variety, the question as to whether the moisturized cotton is as good as the live plant method probably could be answered.

There was evidence that resistance of a soybean variety to *A. glycines* was lost when soybean aphids were reared with a detached leaf method (Michel et al. 2010). Prior to exploring soybean germplasm for aphid resistance, a trial test should be carried out using the moisturized cotton method to rear a few *A. glycines*. If it showed that the resistance of the tested soybean to *A. glycines* was retained, then the method could be used for further study on the resistance level of the soybean as a rapid and practical assay to access host plant resistance.

In this study, the beakers used in different rearing methods were put into the same growth chamber at each temperature. In each chamber, the temperature and photoperiod fluctuated slightly wherever the beakers were placed. So, the fluctuation of life parameters of *A. glycines* reared with different rearing methods could be attributed partially to the different relative humidity in each beaker used in the different methods. Then the relative humidity in beakers used in the leaf-disc method, filter paper method, and the moisturized cotton method were determined, respectively. There were almost no significant differences in relative humidity among beakers in the same chamber at 20 to 26

°C, but there were significant differences in relative humidity among beakers in the same chamber at 29 or 32 °C (Fig. 3). Studies on effects of relative humidity in beakers used in the method on life parameters of *A. glycines* at temperatures above 29 °C should be conducted.

The detached leaves of soybean were yellowish when they were used for 5 to 7 d in the 3 methods. It was found that the speeds at which the leaves turn yellow were different (unpublished data), which probably means that the rates of loss of leaf nutrients were different. In further studies, the nutritional component of detached soybean leaves used in different rearing methods should be determined. If there were major significant differences in the loss rates of some leaf nutrients, which are necessary for *A. glycines* development when they are used in trials, this would likely answer the question as to why there were significant differences in various life parameters of *A. glycines* reared with leaf-disc methods, filter paper methods, and moisturized cotton methods. Other species of aphids also should be reared with the 3 methods in further studies. If results with other aphid species showed that the moisturized cotton method is better than other 2 methods, we would have greater confidence in recommending this method as a good method for rearing aphids individually in general.

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