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Research Article

Preference of the Waterlily Aphid, *Rhopalosiphum nymphaeae* (Hemiptera: Aphididae) on Four Hostplants

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ABSTRACT

Waterlily aphid *Rhopalosiphum nymphaeae* is one of the aphids that have many hosts, including *Azolla filiculoides*, *Limnobium laevigatum*, *Monochoria vaginalis* and *Spirodela polyrhiza*. The aim of this study was to study the effect of host shift and confirm the results of previous studies on the effect of nitrogen and carbon factors among *A. filiculoides, L. laevigatum, M. vaginalis* and *S. polyrhiza* on the level of aphid preference and number of offspring. Analysis of the nitrogen and carbon content of plants was also carried out to confirm the preference and number of offspring produced by aphids. The study began with maintaining aphids on the four tested hosts, up to the 4th generation. Twenty five individuals were randomly selected from each host, then released on the inner wall of the plastic container (14 ×7.5 × 15 cm^3) which was filled with four hosts arranged side by side. Observations were made every 24 hours up to 97 hours starting from the first hour after treatment. Observations after 97 hours showed that waterlily aphids adult preferred *L. laevigatum* the most (49.28%), then on *M. vaginalis* (20.43%), *S. polyrhiza* (16.33%), and *A. filiculoides* (1.75%). Meanwhile, the number of offspring produced by each group of aphids that selected on four hosts were: 46.65 individuals on *L. laevigatum*, 37.8 individuals on *M. vaginalis*, 19 individuals on *S. polyrhiza*, and 0.6 individuals on *A. filiculoides*. The analysis showed that the highest nitrogen content was found in *M. vaginalis* (4.16%), followed by *S. polyrhiza* (3.71%), *L. laevigatum* (2.33%), and *A. filiculoides* (2.08%).

Keywords: offspring; plant preference; *Rhopalosiphum nymphaeae*

INTRODUCTION

The waterlily aphid (*Rhopalosiphum nymphaeae*) is capable of living below the surface of water (Blackman & Eastop, 1994; Holman, 2009). This species can be found in about 45 plant species (Center *et al.*, 2002). *R. nymphaeae* can be found in Prunus in winter, and inhabits various plants in wetlands and freshwater as secondary hosts in summer (Atousa *et al.*, 2015), including aquatic plants. This species has been declared a pest, for example damaging the aquatic plant *Ferox euryale* by 17.04-23.36% in India (Nath *et al.,* 2018). On the other hand, this species has the potential to be used to control weeds, for example *Heteranthera limosa* (Oraze & Grigarick, 1992).

Waterlily aphid is also found in several aquatic plants, i.e. *Azolla filiculoides*, *Limnobium laevigatum*, *Monochoria vaginalis* and *Spirodella polyrhiza* (Atousa *et al.*, 2015, Subramanian & Turcotte, 2020), which are common in rice fields. *A. filiculoides* and *S. polyrhiza* are used as biological fertilizers because of their ability to fix nitrogen (de Vries & de Vries, 2018), and increase rice yields. In contrast, *L. laevigatum* is a rice competitor in North America and Africa that is even more dangerous than water hyacinth and *M. vaginalis* (Cheng *et al.*, 2010; Howard *et al.*, 2016; Kadono, 2004).

Meanwhile, oligophagous insects tend to lay eggs on hosts which can improve offspring performance (Gripenberg *et al.*, 2010). Furthermore, the selection of hosts by aphids is based on performance of hosts which includes nutrient content, toxic metabolites, and plant resistance (Cao *et al.,* 2018; Hao *et al.,* 2019). For example, *Brevicoryne brassicae* and *Myzus persicae* show a preference for hosts with high nitrogen content (Ahmed *et al.,* 2019; Zarghami *et al.,* 2010). However, recent studies of waterlily aphid on *Spirodela polyrhiza*, *Landoltia punctata*, *Lemna minor* and *Wolffia brasiliensis* indicate that this aphid species prefers hosts with the lowest nitrogen content (Storey, 2007; Subramanian & Turcotte, 2020).

Therefore, this study was conducted to confirm the results of previous studies on the effect of nitrogen and carbon factors on the host, i.e. *Azolla filiculoides*, *Limnobium laevigatum*, *Monochoria vaginalis* and *Spirodela polyrhiza* which are common in rice fields, on host preferences and the number of offspring produced by *R. nymphaeae*. The test was carried out with a host shift behavior approach, that is from the host used for mass breeding for four generations to the four potential hosts mentioned earlier.

MATERIALS AND METHODS

Experimental Site

The research was conducted in August 2020 at the Laboratory of Animal Ecology, Faculty of Agriculture, Yamagata University, Japan.

Host Plant

Four host plants, *A. filiculoides*, *L. laevigatum*, *S. polyrhiza* and *M. vaginalis*, were initially used to mass breed *R. nymphaeae*. The first three species of host plants were propagated in plastic boxes (37 cm long × 25 cm wide × 11.5 cm high) filled with 500 g of soil and 7.4 liters of tap water, while *M. vaginalis* was bred in small plastic cups (3 cm diameter × 3 cm high). The host culture system was placed in an incubator with a relative humidity of about 20 ± 2°C, 40–60 ± 5% and under 10W of LED light (16:8) L/D photoperiods for two weeks. Watering is carried out every week.

Waterlily Aphid Rearing Method

Fifty waterlily aphid adults were developed each on four host plants for four generations before being used for the test. The breeding container was covered with a net, then placed in a room with a temperature of 20 ± 2°C, a relative humidity of 40-60 ± 5%, and under light / dark (16:8) photoperiod. Temperature and humidity are measured with the Dretec Thermo-Hygrometer O-230.

Preferences of Aphids Among Host Plant Species

The preference test for waterlily aphid on four plants was carried out in a plastic container (14 cm long × 7.5 cm wide ×15 cm high) divided into four parts (arenas), which each planted with *A. filiculoides, L. laevigatum, S. polyrhiza* and *M. vaginalis*. The first three hosts are planted covering 90% of the total area of the arena, thus providing room for plants to grow. Meanwhile, two individuals of *M. vaginalis* with 5 leaves were planted in the fourth arena. Furthermore, 25 individual aphids were randomly selected from the colony bred on four host plants, and placed on the wall of container. Waterlily aphid were allowed to select a host, then observed for the first time 1 hour after release.

Observation of the position of the aphids in the host species, which showed preference for aphids in a particular host, was then continued at 25, 49, 73, and 97 hours after release, so that five observational data were obtained. Observations were done at 9 pm (local time). At each observation time, the number of offspring produced by each aphid colony on each host was also counted.

Plants Nutrient Analysis

Analysis of nitrogen and carbon content in host plants was carried out to confirm the suitability of host quality with aphids preferences and ability to produce offspring. Analyzes were performed on plants 14 days after initial planting. Samples of *S. polyrhiza* and *L. laevigatum* were 3 leaves each plant, *M. vaginalis* used the five youngest leaves, and samples of *A. filiculoides* were taken randomly from fresh plant populations. The parts of the plants analyzed are the leaves and stems. The samples were oven-dried at 70°C for 3 days. The samples were dried and ground using a blender until they were powdered. 200 mg of sample per plant were injected into SUMIGRAPH NC 220F to obtain the nitrogen and carbon content (in%) of each plant species.

Data Analysis and Statistics

The host preferences of waterlily aphid and the number of offspring produced in four host plants were analyzed by the Kruskall-Wallis method using R software. Meanwhile, differences in plant nitrogen and carbon content in each host were analyzed by ANOVA, and if significant differences were found, then continued with Tukey's HSD Multiple Range Test (P ≤ 0,05) with application R version 4.0.2.

RESULTS AND DISCUSSION

Preferences of Aphids among Host Plant Species

The aphid colonies initially reared on four different hosts tended to prefer *L. laevigatum* as the best host, and in contrast, tended to avoid *A. filiculoides* (Table 1). Thus, waterlily aphid preferred the first host species and less preferred to the second host. Meanwhile, the other two tested hosts, i.e. *M. vaginalis* and *S. polyriza*, were categorized as moderate hosts or at the level of preference between the first two host species.

These results also indicated that waterlily aphid had a tendency to shift hosts, if better hosts were available. This is shown by the decline in the aphid population which was originally maintained using *A. filiculoides*, *M. vaginalis*, and *S. polyriza* which still chose these hosts at the beginning of the observation, but then moved to *L. laevigatum* at the end of the observation.

Preferences of Aphid Offspring among Host Plant Species

L. laevigatum was the best host that supported the production of aphid offspring the most, although it was originally bred on other hosts (Table 2). The table two prove that *L. laevigatum* is the best host that supports feeding activities as well as offspring income by adults. Meanwhile, *A. filiculoides* was not suitable to offspring production in all treatments.

Furthermore, Table 3 shows that the highest nitrogen concentration was found in *M. vaginalis*, while the nitrogen concentration in *L. laevigatum* was actually much lower, even when compared to the nitrogen concentration in *S. polyrhiza*.

Selection of hosts by insects is influenced by several factors, one of which is the quality of the host which includes the availability of nitrogen and carbon compounds, as well as secondary metabolites (Awmack & Leather, 2002). Furthermore, this study showed that the species of host had a strong effect on *R. nymphaeae*, as shown by selecting a particular host when faced with four different species of hosts.

Table 1. Number of aphid preference (± SE) on four species host plant

Natality	Preference	Number of aphid (Hour after investment)				
		1	25	49	73	97
Azolla filiculoides	*A. filiculoides*	4.80 ± 0.79 de	3.40 ± 0.62 fg	2.60 ± 0.70 fg	1.30 ± 0.37 g	1.00 ± 0.33 d
	L. laevigatum	8.60 ± 1.25 abc	8.40 ± 1.34 abcd	8.90 ± 1.24 abc	10.90 ± 0.89 a	11.30 ± 1.25 a
	M. vaginalis	4.20 ± 0.49 e	6.20 ± 0.55 cde	6.00 ± 0.70 de	6.00 ± 0.60 bc	5.40 ± 0.76 b
	S. polyrhiza	7.30 ± 0.78 bc	6.30 ± 0.86 de	6.30 ± 0.72 cde	5.30 ± 0.76 cde	4.50 ± 1.01 bc
Limnobium laevigatum	*A. filiculoides*	1.40 ± 0.48 f	2.80 ± 1.09 fg	1.00 ± 0.39 g	0.40 ± 0.16 g	0.30 ± 0.15 d
	L. laevigatum	8.90 ± 0.91 ab	7.50 ± 0.83 abcde	10.20 ± 0.87 a	12.20 ± 0.81 a	13.20 ± 0.88 a
	M. vaginalis	6.30 ± 0.63 cd	7.10 ± 1.10 bcde	8.90 ± 0.82 ab	6.80 ± 0.70 bc	5.00 ± 0.63 b
	S. polyrhiza	8.00 ± 0.68 abc	6.40 ± 0.75 bcde	4.40 ± 0.90 ef	4.30 ± 0.65 def	4.50 ± 0.62 bc
Monochoria vaginalis	*A. filiculoides*	0.90 ± 0.23 f	1.10 ± 0.55 g	1.00 ± 0.45 g	0.40 ± 0.16 g	0.20 ± 0.13 d
	L. laevigatum	8.80 ± 1.07 abc	9.30 ± 1.19 abc	10.40 ± 0.93 a	11.90 ± 0.97 a	13.40 ± 1.36 a
	M. vaginalis	10.60 ± 1.03 a	10.80 ± 1.04 a	10.10 ± 0.98 a	7.20 ± 0.63 b	4.90 ± 0.50 b
	S. polyrhiza	3.70 ± 0.73 e	2.50 ± 0.65 g	1.90 ± 0.53 g	3.20 ± 0.59 f	3.20 ± 0.65 c
Spirodela polyrhiza	*A. filiculoides*	1.00 ± 0.31 f	1.13 ± 0.55 g	1.00 ± 0.37 g	0.38 ± 0.21 g	0.25 ± 0.15 d
	L. laevigatum	9.13 ± 1.57 abc	10.75 ± 1.95 ab	10.88 ± 1.62 ab	11.75 ± 1.48 a	11.38 ± 1.59 a
	M. vaginalis	6.75 ± 1.10 bcd	8.00 ± 1.33 abcd	7.38 ± 1.13 bcd	5.63 ± 0.53 bcd	5.13 ± 0.67 b
	S. polyrhiza	7.13 ± 1.08 bc	5.13 ± 0.99 ef	4.63 ± 1.00 ef	4.38 ± 1.09 ef	4.13 ± 0.94 bc

Remarks: Within columns, values followed by different letters are significantly different (P<0.05, Post Hoc)

Table 2. Number of offspring preference (± SE) on four species host plant

Natality	Preference	Number of aphid (Hour after investment)				
		1	25	49	73	97
Azolla filiculoides	*A. filiculoides*	0.00 ± 0.00 a	0.00 ± 0.00 f	0.00 ± 0.00 f	0.00 ± 0.00 f	0.00 ± 0.00 f
	L. laevigatum	0.00 ± 0.00 a	4.40 ± 1.03 ab	10.40 ± 2.38 abc	23.40 ± 6.40 a	38.40 ± 7.14 a
	M. vaginalis	0.00 ± 0.00 a	2.20 ± 0.66 bcd	5.00 ± 0.71 de	8.80 ± 1.24 b	13.40 ± 1.96 b
	S. polyrhiza	0.00 ± 0.00 a	3.00 ± 0.77 abc	3.40 ± 0.93 ef	3.00 ± 1.34 c	3.40 ± 1.08 e
Limnobium laevigatum	*A. filiculoides*	0.00 ± 0.00 a	0.00 ± 0.00 f	0.00 ± 0.00 i	0.00 ± 0.00 d	0.00 ± 0.00 f
	L. laevigatum	0.00 ± 0.00 a	4.80 ± 1.24 a	11.80 ± 2.63 a	26.80 ± 1.39 a	45.60 ± 4.83 a
	M. vaginalis	0.00 ± 0.00 a	3.20 ± 0.80 abc	5.40 ± 0.93 bcde	7.60 ± 1.96 b	7.60 ± 2.73 cde
	S. polyrhiza	0.00 ± 0.00 a	1.40 ± 0.68 cde	1.80 ± 0.49 fg	2.00 ± 0.32 c	3.40 ± 0.93 e
Monochoria vaginalis	*A. filiculoides*	0.00 ± 0.00 a	0.20 ± 0.20 ef	0.20 ± 0.20 hi	0.20 ± 0.20 d	0.00 ± 0.00 f
	L. laevigatum	0.00 ± 0.00 a	8.20 ± 3.89 ab	17.00 ± 6.46 ab	30.40 ± 7.19 a	50.80 ± 5.70 a
	M. vaginalis	0.00 ± 0.00 a	4.40 ± 1.21 ab	6.40 ± 2.25 cde	9.20 ± 2.20 b	10.60 ± 3.70 bc
	S. polyrhiza	0.00 ± 0.00 a	1.20 ± 0.58 def	1.40 ± 0.60 gh	2.80 ± 0.86 c	8.20 ± 2.33 bc
Spirodela polyrhiza	*A. filiculoides*	0.00 ± 0.00 a	0.00 ± 0.00 f	0.00 ± 0.00 i	0.00 ± 0.00 d	0.60 ± 0.60 f
	L. laevigatum	0.00 ± 0.00 a	5.40 ± 0.98 a	11.80 ± 1.32 a	34.00 ± 2.77 a	51.80 ± 5.32 a
	M. vaginalis	0.00 ± 0.00 a	4.40 ± 0.51 a	6.40 ± 0.75 abcd	7.00 ± 0.84 b	6.20 ± .39 bcd
	S. polyrhiza	0.00 ± 0.00 a	2.00 ± 0.63 cd	2.00 ± 0.32 fg	2.20 ± 1.02 c	4.00 ± 1.64 de

Remarks: Within columns, values followed by different letters are significantly different (P<0.05, Post Hoc)

Table 3. Percentage of carbon and nitrogen in each plant species (±SE)

Plant species	Concentration (%)	
	Carbon	Nitrogen
Azolla filiculoides	34.42 ± 0.01 c	2.08 ± 0.14 d
Limnobium laevigatum	38.66 ± 0.00 b	2.33 ± 1.10 c
Monochoria vaginalis	34.88 ± 0.01 c	4.16 ± 0.02 a
Spirodela polyrhiza	42.87 ± 0.00 a	3.71 ± 0.25 b

Remark: Within columns, values followed by different letters are significantly different (p<0.05, Tukey HSD).

Furthermore, this study also proved the suitability of preference and performance of aphids against choice of a particular host, as shown in previous studies, for example by Craig *et al.* (1989) on *Euura lasiolepis*. Tables 1 and 2 show that the offspring production performance of the adult will be high on the most suitable host. For example, *R. nymphaea* growth on *L. laevigatum*, and even on other hosts; if then selected *L. laevigatum* as the final host, the number of offspring was also higher than that of aphids who chose the other three hosts.

However, the results of this study also indicated that waterlily aphid preference in the tested host was not always consistent with the nitrogen content of that host. For example, *L. laevigatum* was the most preferred host by adult *R. nymphaeae*, and also supported the production of offspring the most, although the nitrogen concentration in this host species was low, when compared to *M. vaginalis* and *S. polyrhiza*.

Several studies have shown that the structural characters of host plant can also be a determinant factors of insect preferences (Banerjee, 1987; Peeters, 2002; Caldwell *et al.* (2016). Peeters (2002) proved that leaf structure has a stronger impact than other characteristics, for example the compounds present in the tissue. Several sucking insects such as aphids tend to prefer plant tissue with a thin cuticle layer and high stomata density. This evidence is also supported by Caldwell *et al.,* (2016), which shows that herbivorous insects with different feeding guilds will show different reactions to the morphological characters of host plants. Furthermore, they demonstrated that sucking insects had a fairly strong correlation with morphological characters, but not the nutritional content of the host. Meanwhile, Hasanuzzaman *et al.*'s (2016) study showed that trichomes on the body surface of the *Solanum melongena* plant were a determinant of *Bemisia tabaci*'s eating preferences and egg-laying.

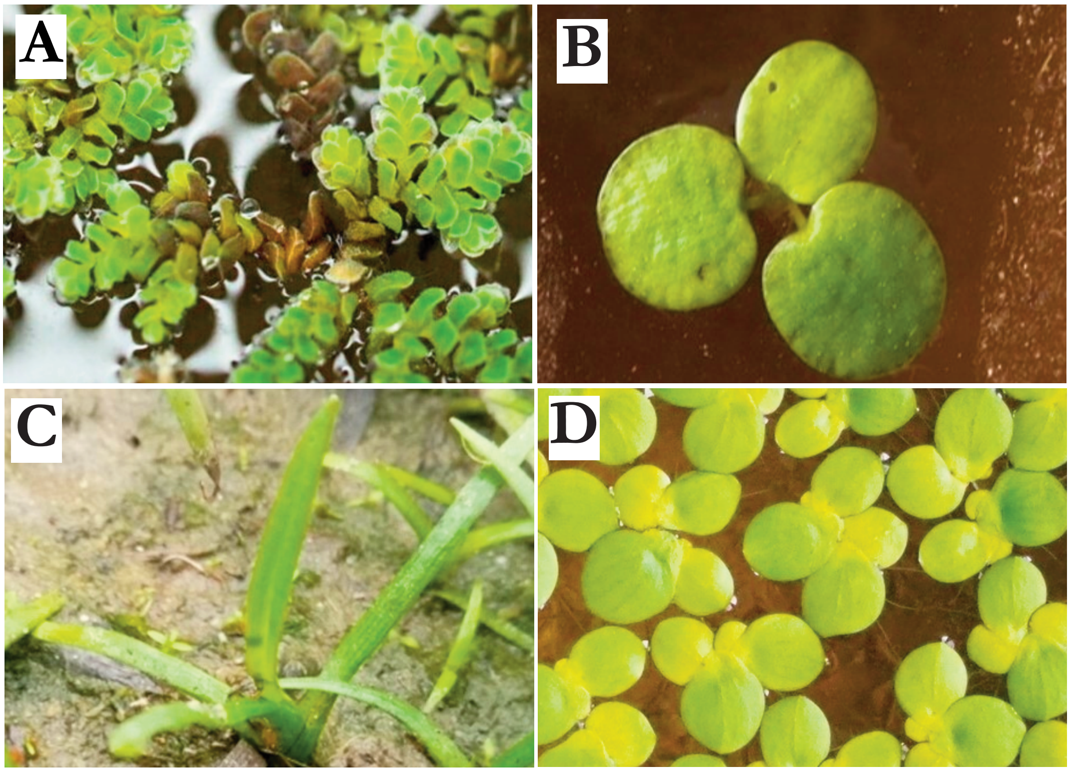

Figure 1. *Rhopalosiphum nymphaeae* host plant (A) *Azolla filiculoides,* (B*) Limnobium laevigatum,* (C) *Monochoria vaginalis*, and (D) *Spirodela polyrhiza*

Banerjee's (1987) found that tea varieties with leaf architecture (erect, semi-erect, horizontal) and varying hair density determined feeding guild of herbivore insects. Sucking insects feed on all tea varieties, but most preferred on tea varieties with erect leaves.

Results of those study explain the possible determinant of structural traits of tested host on the preference-performance of *R. nymphaeae*. Although it has a relatively low concentration of nitrogen in tissue, *L. laevigatum* has soft surface of tissue, compared to the *S. polyriza* and *M. vaginalis* (Fahmi, 2020; personal observation). Meanwhile, *A. filiculoides* has a hard tissue texture, in addition to it contains low nitrogen concentration. That might be the reason why waterlily aphid allegedly chose *L. laevigatum* because of its structural characteristics that are easier to penetrate to obtain phloem fluid (Figure 1). Therefore, studies that discuss the relationship between plant morphology and plant metabolite content are of great interest in explaining the preference-performance of *R. nymphaeae* in several host plants.

CONCLUSION

This study proved that the feeding preferences and offspring production of waterlily aphid were not only depend on the nitrogen content of the host, but were also possibly caused by the structural characters of the host. Therefore, research to discuss the relationship between the structural characteristics of the host, such as tissue surface thickness, the content of food-inhibiting compounds on the tissue surface, the density and length of trichomes, and other morphological characters with the performance-preference of waterlily aphid is interesting to do.

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