The optimization of *Aphis gossypii* rearing and RNA extraction methods

Nicholas Mueller[1,], and Kathleen M. Martin[2]*

[1] Undergraduate Student, Department of Entomology and Plant Pathology, Auburn University
[2] Assistant Professor, College of Agriculture, Auburn University

Introduction

Aphis gossypii, the cotton-melon aphid, has an international distribution, being found on all continents except Antarctica. In warm weather, these aphids give live birth, and reproduce by parthenogenesis in the spring through fall, producing winged and wingless forms (Fig. 1) (Berim, n.d.). Cotton leafroll dwarf virus, or CLRDV, is transmitted in a circulative and persistent manner by *A. gossypii* (Michelotto & Busoli, 2003) and does not replicate inside the aphid (Heilsnis, 2020). CLRDV is the cause of Cotton Blue Disease, which causes leaf curling, stunted growth, reddening of petioles and veins, and reduced cotton yield (Conner et al., 2021). In 2018, CLRDV was first identified in Alabama, where it caused an estimated loss of $19 million (Conner et al., 2021). CLRDV is a positive-sense single-stranded RNA polerovirus, meaning its genome is made of a single strand of RNA (Avelar et al., 2020).

The RNA extracted from *A. gossypii* can be used in research of CLRDV with applications such as detection PCR, CLRDV quantification, transmission assays, RNA sequencing, and protein-protein interaction assays. Optimizing these *A. gossypii* rearing and RNA extraction methods can aid in the research of CLRDV and can one day help us discover new virus management techniques.

Methods

Two *A. gossypii* rearing methods on the Deltapine 1646 cotton variety were compared, a caged method (Fig. 2.A) imitating aphid greenhouse rearing methods on seedlings, and a confined method (Fig. 2.B) imitating field trial or transmission assay methods for larger plants that are difficult to cage. For the caged method, *A. gossypii* were reared on a tray of one-to-two week old cotton in a cage (Fig. 2.A). After one week, aphids were collected from three-to-four seedlings (wingless adults and nymphs) or the wall of the cage (winged adults). For the confined method, 50 wingless adult aphids were confined to one leaf on a mature cotton plant, older than six months, with a cone, coated with Fluon to prevent aphid escape, around

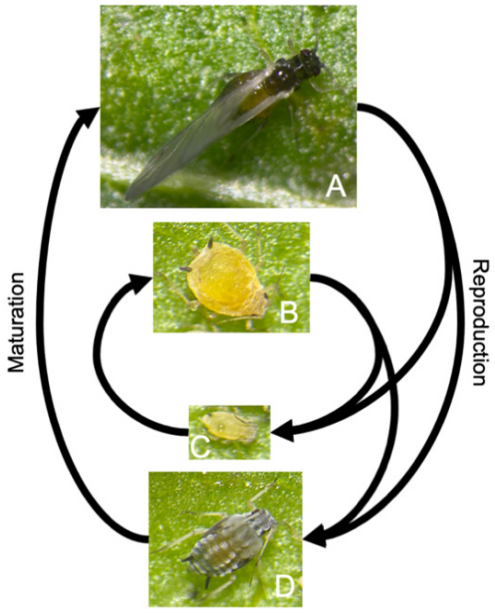

Fig. 1 *A. gossypii* parthenogenic life cycle with (A) winged adult, (B) wingless adult, (C) nymph of wingless adult, and (D) nymph of winged adult. Made in Microsoft PowerPoint.

Fig. 2 Caged method (A) and confined method (B) of *A. gossypii* rearing.

*Corresponding author: mnm0059@auburn.edu

the petiole and a mesh bag enclosing the leaf and cone (Fig. 2.B). The aphids were collected after 10-14 days from only the confined leaves. The rearing methods were assessed by the number of complete samples (100 adults, wingless or winged, or 300 nymphs) collected after 10 collections, as well as the ease of the rearing method.

Two common RNA extraction methods were compared, a chemical only RNA extraction method and a spin column assisted RNA extraction method. The chemical only extraction method used TRIzol Reagent, following the user guide (Invitrogen, 2023). The chemical only extractions were done with sample sizes of 25-75 mixed wingless and winged adult A. gossypii and 25-200 nymphs. The spin column assisted extraction method used the NucleoSpin RNA Kit, following the kit manual (Macherey-Nagel, 2023). The spin column assisted extractions were done with sample sizes of 50-150 winged or wingless adult *A. gossypi* and 50-300 nymphs. The RNA extraction methods were assessed by the quantity and purity of the RNA extracted for different aphid numbers, as well as the ease of the method. The quantity and purity were measured using a NanoDrop Microvolume Spectrophotometer (Thermo Scientific, 2023). The A260/280 ratio, or absorbance ratio at 260 nm and 280 nm, was used to measure the purity. Pure RNA should have an A260/280 value of ≥2.0.

Results

Between the two rearing methods, the caged method resulted in more complete samples collected across all three *A. gossypii* forms (Table 1). It is important to note that several of the leaves that aphids were confined to had died or fallen off the plant before any aphids were collected. As for the ease of the two rearing methods, the caged method was less time consuming to set up and easier to maintain.

Overall, the chemical only extraction method was able to extract more RNA per aphid on average than the spin column assisted extraction method, but the spin column assisted extraction method had a much higher average A260/280, indicating a higher average purity (Table 2). Once the spin column assisted extraction method was seen to result in purer RNA, separate wingless and winged samples were collected for RNA extraction to compare the two adult forms, which showed that the wingless adult aphids had on average more RNA extracted from them than the winged adult aphids (Table 2). Both extraction methods had inconsistent amounts of RNA extracted across samples of the same number and form of aphids. For example, the chemical only extraction with 25 mixed adult *A. gossypii* resulted in a range of total RNA extracted of 6.25-18.484 µg per sample. For the spin column assisted extractions with 100 wingless adults and 100 winged adults, the range of total RNA extracted was 16.664-59.265 µg and 9.02-23.32 µg per sample, respectively. To get similar amounts of RNA from adults, the chemical only extraction method required only a quarter the number of aphids as the spin column assisted extractions. For samples of 200 nymphs, the chemical only extraction resulted in 15.357 µg of total RNA while the spin column assisted extraction resulted in 4.496 µg of total RNA.

Table 1 Number of complete collections of A. gossypii forms per rearing method.

Aphid Form	Caged Method	Confined Method
Winged Adult	5	0
Wingless Adult	4	2
Nymph	3	1

Table 2 Number of complete collections of A. gossypii forms per rearing method.

	Chemical Only		Spin Column Assisted	
	Average RNA per Aphid (µg)	Average A260/280	Average RNA per Aphid (µg)	Average A260/280
Adult	0.382794	1.92	0.327098 (Wingless)	2.174 (Wingless)
			0.21312 (Winged)	2.136 (Winged)
Nymph	0.1711442	1.59525	0.0611283	2.1716666

Discussion

The caged method of rearing *A. gossypii* resulted in more complete aphid collections and is ideal for the greenhouse setting where smaller, younger cotton plants can be used. The softer tissues of the young plants may be preferable to the aphids, which would contribute to the

higher aphid numbers. The mature cotton plant used in the confined method of rearing had older, tougher leaves. The age of the leaves on the mature plant may have also contributed to the early death and abscission of the leaves the aphids were confined to. Despite the lower number of complete aphid collections for the confined method, this method is still useful for field trials, transmission assays, or aphid confinement on large, uncaged plants. It may be necessary to use an excess of aphids and more confined leaves to be able to get the final number of aphids that is needed using the confined method.

Due to the amount of both adult *A. gossypii* forms needed for the spin column assisted extraction method needed in comparison to the chemical only extraction method, the chemical only extraction method may be ideal for experiments with low aphid numbers. It is possible to correct for the low purity of the chemical only extraction method with further purification steps if necessary. The spin column assisted extraction method may then be ideal for experiments where the purity of the RNA is a priority and there are more aphids available to extract the RNA from. The difference in RNA quantity across the replicated extractions with the same number and form of aphids is likely due to the natural differences in aphid size within each aphid form. The difference in average RNA quantity across adult aphid forms is expected due to the function of each form. The function of the wingless adult *A. gossypii,* which had the most RNA, is to reproduce rapidly, having multiple developing nymphs inside of them at once. The function of the winged adult *A. gossypii* prioritizes long distance movement over reproduction (Braendle et al., 2006).

By weighing the pros and cons of both *A. gossypii* rearing methods and both RNA extraction methods, one can determine the best methods to use for future experiments in the investigation of *A. gossypii*-CLRDV interactions. This was a critical first step towards a larger study aimed at blocking transmission in the field.

Statement of Research Advisor

Nick Mueller was able to identify the best method of rearing the cotton-melon aphid, Aphis gossypii, as a key first step in the ongoing studies of the transmission of Cotton leafroll dwarf virus (CLRDV) in the field. He was also able to identify a method of extraction of aphid RNA which will further our work on identification of aphid proteins that interact with the proteins of CLRDV. Nick's experiments represent a crucial first step at the beginning of a large and complex project about how this virus can move through the tissues of A. gossypii. Understanding this process will enable better recommendations as to how to stop this process to halt the spread of this virus in the field.

- Kathleen Martin, Department of Entomology and Plant Pathology, College of Agriculture

References

Avelar, S., Ramos-Sobrinho, R., Conner, K., Nichols, R. L., Lawrence, K., & Brown, J. K. (2020). Characterization of the Complete Genome and P0 Protein for a Previously Unreported Genotype of Cotton Leafroll Dwarf Virus, an Introduced Polerovirus in the United States. *Plant disease*, 104(3), 780–786.

Berim, M. N. (n.d.). Pests: *Aphis gossypii Glov. - cotton aphid*. Interactive Agricultural Ecological Atlas of Russia and Neighboring Countries. Retrieved on December 19, 2023. http://agroatlas.ru/en/content/pests/Aphis_gossypii/

Braendle, C., Davis, G. K., Brisson, J. A., & Stern, D. L. (2006). Wing dimorphism in aphids. *Heredity*, 97, 192-199.

Conner, K., Strayer-Scherer, A., Hagan, A., Koebernick, J., Jacobson, A., Bowen, K., Sikora, E., Graham, S., & Brown, S., Cotton Leafroll Dwarf Virus. Alabama Cooperative Extension System. Retrieved November 2, 2023, from https://www.aces.edu/blog/topics/crop-production/cot ton-leafroll-dwarf-virus/ (2021, August 25).

Heilsnis, B. J., Transmission and Host Plant Resistance of Cotton Leafroll Dwarf Virus (CLRDV) in the Southeastern United States, M.S. Thesis, Auburn University, (2020).

M.D. Michelotto, M. D. & Busoli, A.C. "Caracterização da transmissão do vírus do mosaico-das-nervuras do algodoeiro pelo pulgão Aphis gossypii com relação à persistência e ao tempo necessário para inoculação," Bragantia, Campinas, 66 (2007), pp. 441-447.

Authors Biography

Nicholas Mueller is a recent Auburn University graduate with a B.S. in Applied Biotechnology from the College of Agriculture. Nicholas has worked for the Dept. of Entomology and Plant Pathology since May 2021, and worked in the Martin Lab from May to December of 2023 where he worked with *Aphis gossypii* and CLRDV. In June and July of 2022, Nicholas participated in the Summer Scholars Program at AgriTech at Cornell University investigating the transmission of Grapevine red blotch virus by *Spissistilus festinus*. His research interests include vector entomology and plant virology.

Dr. Kathleen Martin is an Assistant Professor in the Department of Entomology and Plant Pathology. She started her lab in vector entomology at Auburn in 2019. She works on the molecular aspects of insect transmission of plant viruses in the field. Her work focuses on Cotton leafroll dwarf virus, Soybean vein necrosis virus and Tomato spotted wilt virus. She started working on plant viruses during her Master's program at the University of Arizona and continued to work on viruses that also infect their insect hosts/vectors at the University of Kentucky where she completed her PhD in 2011.