Inquiries into *Wheat Streak Mosaic Virus* and Other WSM Associated Viruses

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Abstract

Wheat Streak Mosaic (WSM) is a complex disease found to cause severe impact on wheat yield. *Wheat streak mosaic virus* (WSMV), *Triticum Mosaic Virus* (TriMV) and *Wheat Mosaic Virus* (WMoV) are all transmitted by *Aceria tosichella*, the wheat curl mite, and have been reported as the viruses associated with WSM. There is a research project on the genetic composition of the associated viruses as well as determining any novel viruses that may be involved in the disease. The process has begun with the virus assessment of field samples by RT-PCR and Sanger Sequencing. In a different study, the potential of a common insect in wheat fields, *Rhopalospihum padi*, the Bird cherry oat aphid, to be a vector for WSMV was investigated. *R. padi* was found to be not a vector of WSMV through RT-PCR. The other project screened some wild-relative wheat plants for WSMV resistance. Various lines of *Aegilops tauschii*, a landrace wheat, were tested for viral resistance by assessing the viral copy number by RT-qPCR. The initial result has demonstrated that one of the four selected lines may have promising tolerance. The preliminary tests performed for each inquiry provides insight to improving the respective experiment design for further understanding of how concerned parties such as farmers may better combat the disease.

Transmission Assay of WSMV in *Rhopalosiphum padi*

Rationale and Objective

- *Aceria tosichella,* Wheat Curl Mites, are currently the only known vectors of WSMV
- 87% of the known *Potyviridae* viruses are transmitted by aphids (Wylie et al., 2017)
- *Rhopalosiphum padi* L., Bird Cherry oat aphids, are one of the most abundant insect populations (Medina-Ortega, Bosque-Pérez, Ngumbi, Jiménez-Martínez, & Eigenbrode, 2009)
- Determine if *R. padi* can acquire and transmit WSMV
- Determine what makes WSMV preferential to *A. tosichella*

Methods

- Tomahawk wheat plants were inoculated with WSMV inoculum and monitored for the disease by RT-PCR
- Virus acquisition was tested by transferring ~100 aphids to each infected plant
- Aphids fed in intervals of 30 minutes, 24 hours, 48 hours on infected plant material
- 10 aphids were collected for virus detection by RT-PCR and 10 were transferred to each new tube cage for 30 minutes, 24 hours, 48 hours and 72 hours feeding on uninfected plants for virus transmission.
- Aphids were removed after the allotted time and plants were left untouched for two weeks
- Plant leaves were collected for virus detection by RT-PCR

Preliminary Results

- *R. padi* were found to be nonviruliferous which demonstrated that *R. padi* did not acquire the virus
- The wheat samples collected after the aphid feeding were found to be not infected with WSMV which shows that there was no virus transmission

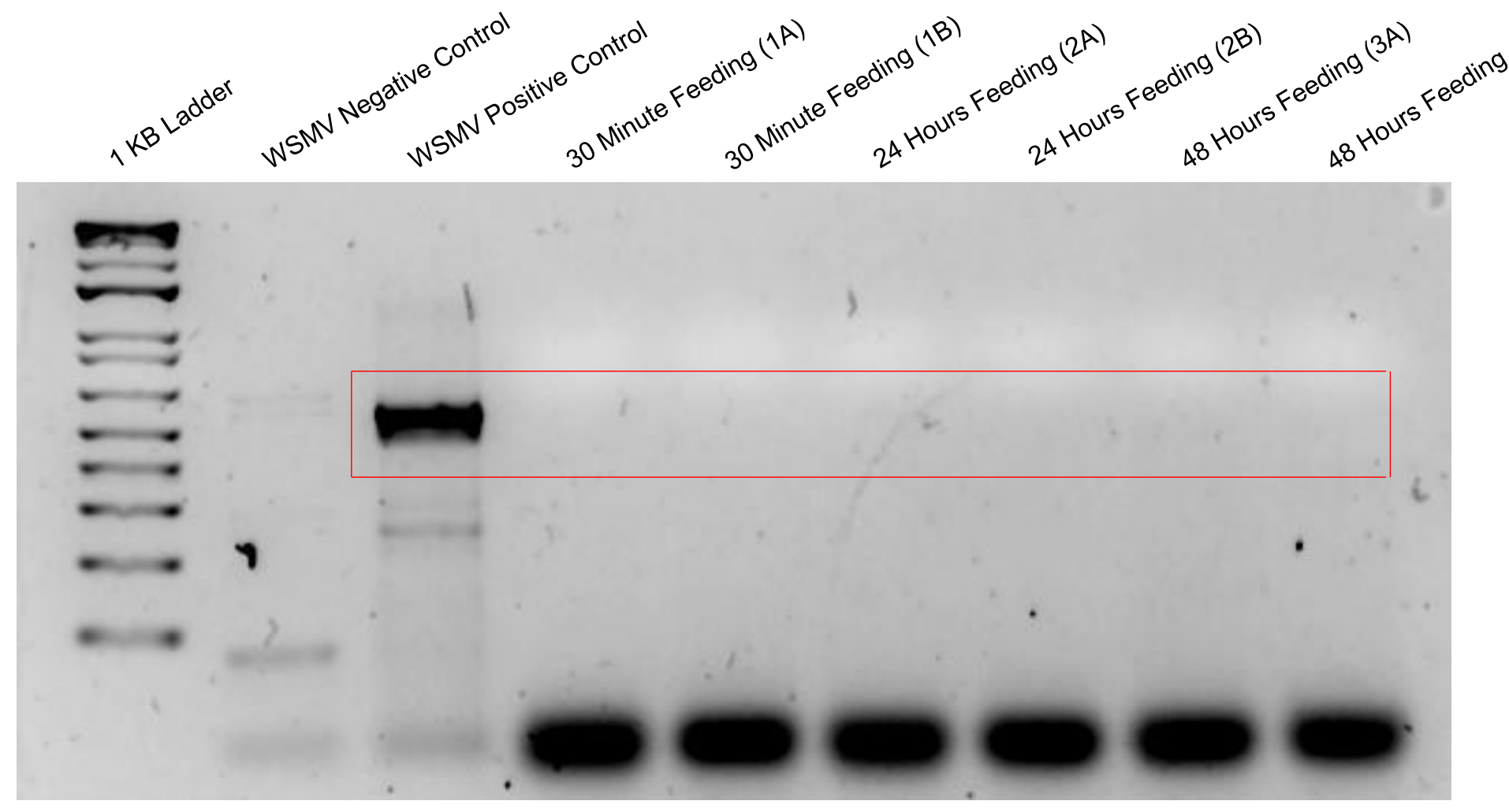

Figure 1. The bands observed in the *R. padi* samples collected from the various feeding times. The red box indicates where the band indicating WSMV would appear.

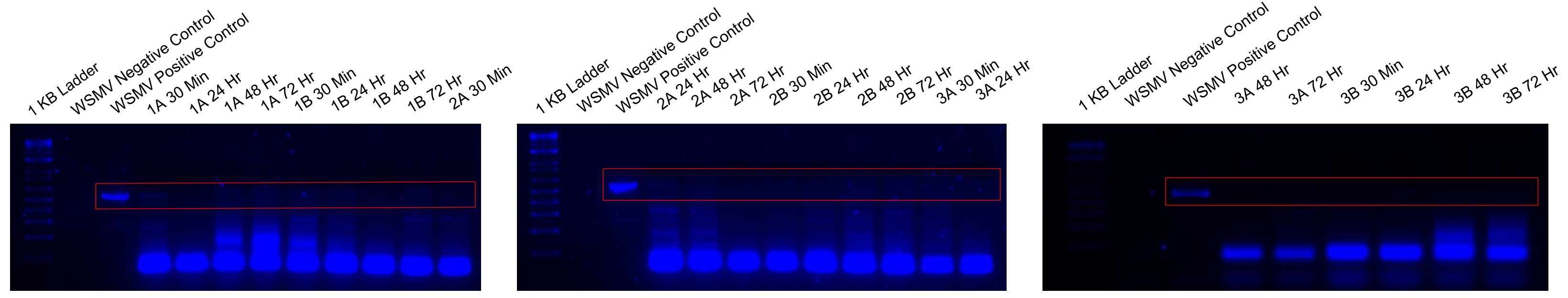

Figure 2. The bands observed in healthy Tomahawk wheat infested with the aphids that fed on WSMV infected Tomahawk wheat. The red box indicates where the band indicating WSMV would appear.

Figure 3. A field of wheat observed in Kansas. Photo by Carla Redila

Analyzing the Genetic Diversity of Known and Novel Viruses Associated with WSM

Rationale and Objective

- *Wheat Streak Mosaic Virus* (WSMV), *Triticum Mosaic Virus* (TriMV) and *Wheat Mosaic Virus* (WMoV), are known causal agents of the WSM complex.
- Little is known about genetics of these associated viruses in Kansas fields
- Assess the genetic variation and evolutionary mechanisms of WSM associated viruses and determine if there are any new potential viruses associated with WSM
- Provides a snapshot of the diversity of WSM in the field

Methods

- Collect wheat samples from numerous counties of Kansas
- Screen samples from counties with viral histories by RT-PCR and Sanger Sequencing
- Select 20 samples with infections from multiple viruses causing WSM for Total RNA deep sequencing

Figure 4. Savannah Phipps working on processing field samples. Photo by Carla Redila.

Preliminary Results

- Initial virus screening by RT-PCR has demonstrated a majority of the samples to be potentially positive for TriMV and WSMV
- Some samples have shown potential for mixed infections

Future Direction

- Prepare a Total RNA library with the selected samples for sequencing
- Perform a series of bioinformatic analyses for the genetic diversity of known viruses, genetic composition of novel viruses, and evolutionary mechanisms that may be involved in the observed populations.

Screening of WSMV in lines of *Aegilops tauschii*

Rationale and Objective

- *Aegilops tauschii* is a wild-relative wheat known for contributing to the genome of modern wheat
- Screening may provide insight to resistance to WSMV
- Potentially resistant lines may be able to later help understanding of the evolutionary capabilities of WSMV
- Genes may be incorporated into varieties that are used commercially

Methods

- *A. tauschii* lines TA 1578, 1605, 1664, and 1669 were propagated from seed and inoculated with WSMV inoculum.
- All lines had two biological replicates inoculated with an infectious inoculum and another inoculated with a mock buffer inoculum.
- For comparison and as a positive control, Tomahawk wheat plants were inoculated with an infectious and mock inoculum
- After two weeks, plants were tested for WSMV by RT-PCR
- Plants that demonstrated the positive band in gel electrophoresis were confirmed by Sanger sequencing
- Positive samples were then further evaluated for virus titer with absolute quantification (qPCR)

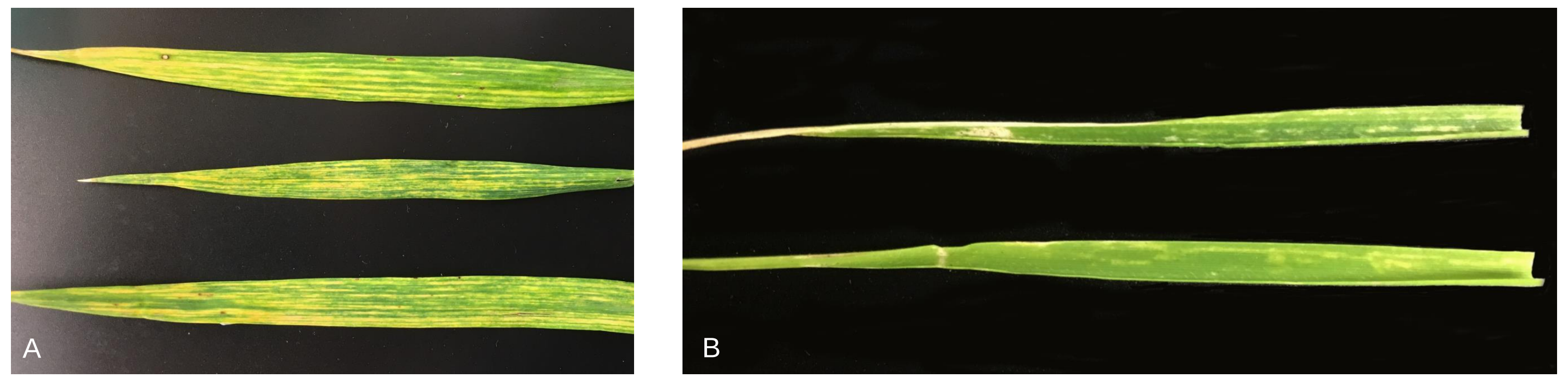

Figure 5. A: A photo of common wheat displaying WSMV symptoms. B: *A. tauschii* line, TA1669. The top leaf was inoculated with WSMV while the bottom leaf was inoculated with a mock solution.

Preliminary Results

- TA 1578 was found to have the lowest count of WSMV
- Other lines had high counts similar to virulent Tomahawk wheat

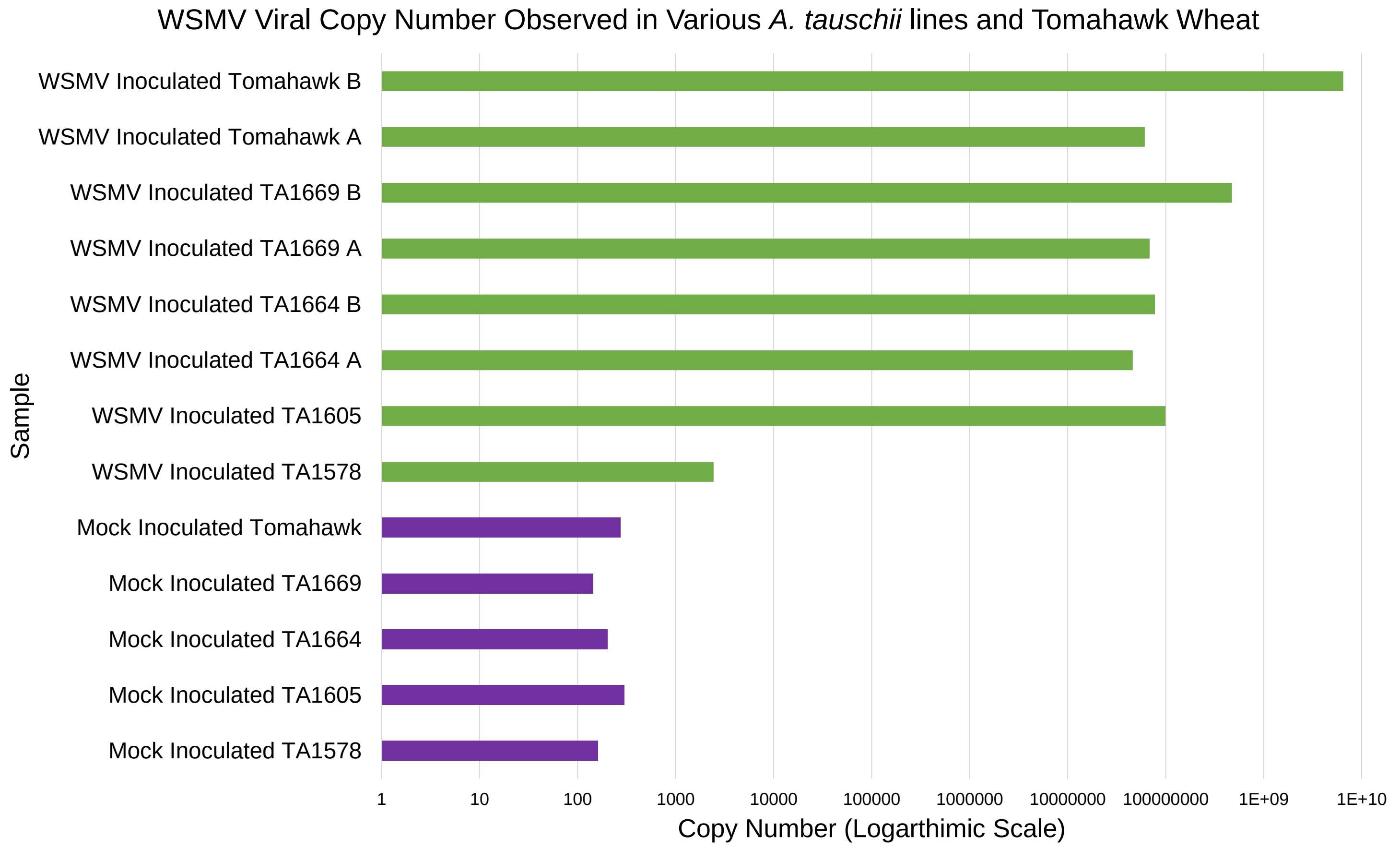

Figure 6. The resulting data of viral copy number achieved by absolute RT-qPCR of the mock and WSMV inoculated wheat plants.

References

Medina-Ortega, K. J., Bosque-Perez, N. A., Ngumbi, E., Jimenez-Martinez, E. S., & Eigenbrode, S. D. (2009, June). *Rhopalosiphum padi* (Hemiptera: Aphididae) Responses to Volatile Cues From Barley Yellow Dwarf Virus-Infected Wheat. *Environmental Entomology*, *38*(3), 836-845. Retrieved from https://doi.org/10.1603/022.038.0337.

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