

Pawpaw Greetings! My attempt with this posting on September 17th, 2024 is to inform and educate the pawpaw community with an important issue concerning pawpaw tree disease susceptibility.

I have two main reasons for not being able to fulfill a very large number of pawpaw requests this season. Thanks to Google algorithm rankings, I am both blessed and cursed with a highly ranked website resulting in many inquiries related to purchasing fresh fruit. My backyard grove is small and is a hobby pastime. All tree care, harvesting, communication, and sales is done by myself. Ever since the Pandemic I have simply been overwhelmed with inquiries for pawpaw fruit. I have nightmares about how I could possibly make more than fifty people all happy with fruit purchases on a single day.

The second reason for a limited supply of pawpaw fruit this season is a health problem with my pawpaw trees. As of September 2024 many of my pawpaw trees in my grove are either dead or near death. The long-standing statement that pawpaw trees are highly disease resistant is false. Until a week ago, I had attributed my pawpaw tree deaths to a combination of factors including old age, sapsucker woodpecker trunk boring damage, water stress, jumping worms, and my poor and thin sandy soil on top of ledge. Two years ago, I lost a gorgeous and productive Shenandoah tree sourced from Neal Peterson in 2005. Last year I removed a dead Shenandoah tree also sourced from Neal Peterson in 2005. This year I noted with alarm the decline in the health of my oldest pawpaw tree planted in 1996 along with two younger productive trees originating from suckers. I observed that there must be a systemic cause affecting all three trees at the same time. I searched with Google Scholar came across these two publications that gave me new insight into the reasons my ailing pawpaw trees:

<https://apsjournals.apsnet.org/doi/full/10.1094/PDIS-11-22-2639-PDN>

Maclot FJ, Mandujano M, Nakasato K, Byrne J, Paudel S, Guyer D, Malmstrom C. First Report of Tobacco Ringspot Virus Infecting Pawpaw Orchard (*Asimina triloba* (L.) Dunal) in North America. *Plant Dis.* 2022

<https://www.mdpi.com/2223-7747/11/24/3565>

Choi, Jiyeong, Anya Clara Osatuke, Griffin Erich, Kristian Stevens, Min Sook Hwang, Maher Al Rwahnih, and Marc Fuchs. "High-Throughput Sequencing Reveals Tobacco and Tomato Ringspot Viruses in Pawpaw." *Plants* 11, no. 24 (2022): 3565.

I reached out with inquiries for testing information and resources. Thankfully, I received a prompt and helpful reply from a plant disease diagnostic company in Indiana as follows:

Thank you for reaching out to Agdia! We can certainly help you identify the cause of the Pawpaw decline that you are observing.

*Tobacco ringspot virus (TRSV) is one of the pathogens found in *Asimina triloba*. Other reported pathogens include Tomato ringspot virus (ToRSV) and *Phytophthora* (Phyt). I would recommend testing for all three pathogens at first to understand which one (or more) are causing the disease problem.*

There are two options for testing. One would be to send a small branch with leaves attached to our Testing Services laboratory and have us conduct the analysis. Shipping is prepaid via a printable UPS label on our website (after completing the sample submission form). Results are emailed within a few days of sample receipt. The cost of testing 1 sample for TRSV, ToRSV and Phyt would be \$97.90. Each additional sample would cost only an extra \$23.90.

Another option would be to purchase our rapid ImmunoStrip tests and perform the test yourself. We offer these rapid tests for all three:

TRSV: <https://orders.agdia.com/agdia-immunostrip-for-trsv-isk-64001>

ToRSV: <https://orders.agdia.com/agdia-immunostrip-for-torsv-isk-22001>

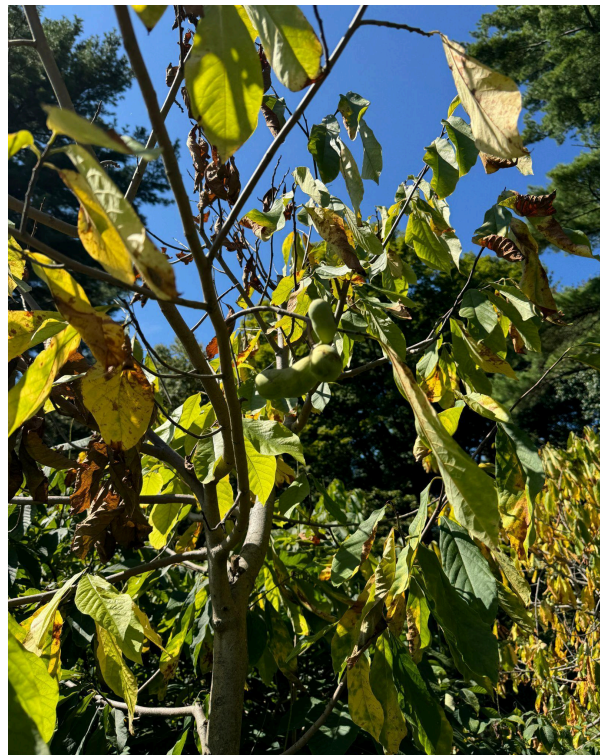
Phyt: <https://orders.agdia.com/agdia-immunostrip-for-phyt-isk-92601>

Some of our growers will send a sample to our lab first, and then purchase the ImmunoStrip tests based on those results. More information on how to submit samples can be found here: <https://www.agdia.com/testing-services/>.

In response to Agdia, I immediately ordered the rapid ImmunoStrip tests for the three viruses of concern. The total cost with shipping was \$229.86. I felt this was a very worthwhile investment that could give me answers and inform others in the pawpaw community.

I performed the viral diagnostic tests on five of my pawpaw trees. Two were sick and three were healthy trees. I followed the instructions for using the ImmunoStrip tests. For the sick trees I selected leaves that were not brown nor desiccated. You can see from the photos that I chose leaves with chlorotic patches. For the healthy trees I chose leaves that appeared stressed. I had to search for appropriate test leaves, since the healthy trees have mostly vibrant green foliage.

Here are the test details as images: Sick pawpaw tree, a productive sucker tree from a Sunflower seedling derived tree purchased from Tripple Brook Farm in 1996. I weighed out 0.15 grams of leaf tissue and added this to the buffer pouch.

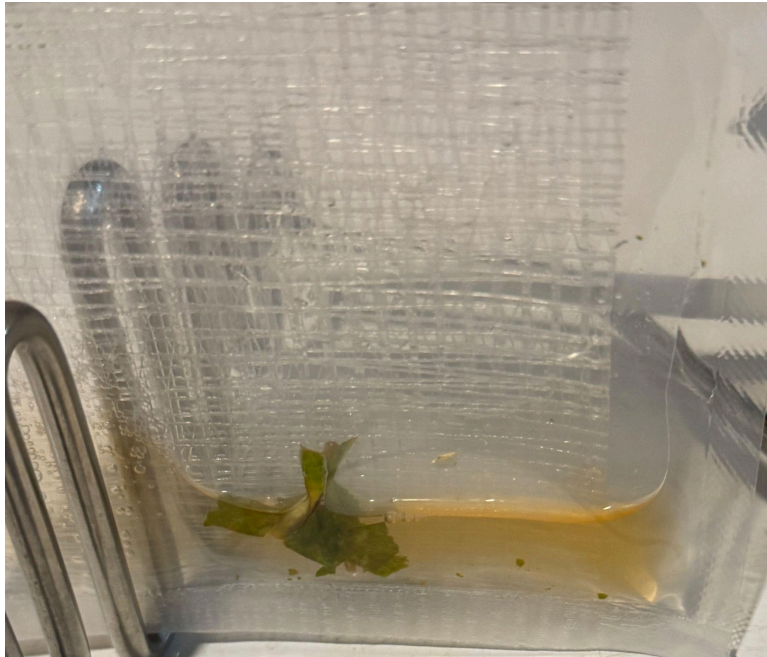




All handling and process was done with clean tools and frequent handwashing. I used hydrogen peroxide to disinfect scissors between leaf samples. The ceramic chopstick for macerating the tissue in the buffer pouch had been cleaned and sanitized in a dishwasher. After maceration, I supported the buffer pouch upright with a kitchen utensil.



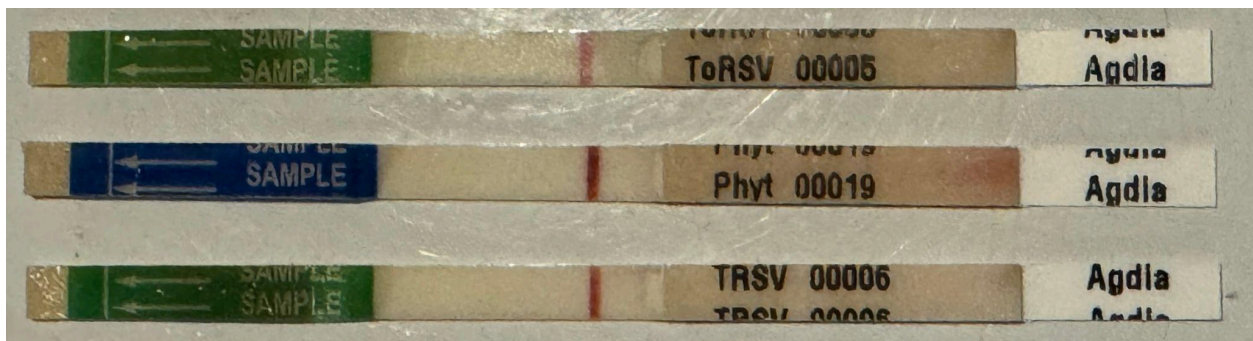
After letting the leaf debris settle for three minutes, I added each test strip by carefully only touching the end portion with the Agdia label. I removed the test strips after 30 minutes for analysis and photography.



As expected, I saw a positive test result for TRSV with the two sick trees sampled. You see this in the image below with the pink lines. The line on the left is the positive signal and the line on the right is the test control.



The great news was that all five pawpaw trees tested negative for ToRSV and Phyt. Also, the three healthy trees tested were negative for TRSV, too. These healthy trees are all grafted cultivars that include Overleese, Potomac, and Shenandoah. The image shows that all three test strips have only the test control line signal indicating that the procedure was done correctly.



I have many questions and concerns about having sick trees affected by the TRSV virus. Some statements from the Maclot *et al* paper are very worrisome. I question if I will still have any living pawpaw trees in my backyard in 2027.

We screened for TRSV infection in leaves from four symptomatic and three nonsymptomatic trees from the same site in 2022. RT-PCR revealed positive infection in all symptomatic samples and one (33%) nonsymptomatic sample, confirming TRSV infection in symptomatic pawpaw trees and emphasizing the importance of also monitoring nonsymptomatic trees. We confirmed graft transmission, with a 100% transmission rate in 200 trees grafted from a TRSV-infected pawpaw (Shenandoah cultivar); investigation of other transmission vectors is ongoing. Because of TRSV's wide host range (Tolin 2008), its broad transmission profile in other crops (via nematodes, thrips, seeds, sap inoculation, and grafting; Hill and Whitham 2014), and the notable decline observed in different infected pawpaw cultivars (10-35, NC-1, Overleese, Pennsylvania-Golden, Shenandoah, Sunflower, Wabash), TRSV appears to be a new threat to pawpaw orchards.

In conclusion, I think that nurseries have an ethical obligation to provide customers with trees that test negative for viruses such as TRSV. Buyers should ask for full disclosure on viral testing results. Pawpaw tree owners should be aware of early signs of viral infection that include leaf wilting despite frequent watering, premature leaf drop, and many small rock-hard fruits. Be wary about exchanging pawpaw pollen, seeds and grafting stock from pawpaw orchards affected by virus. Some statements from the Choi *et al* paper are important and helpful for new pawpaw tree growers.

There is no cure for TRSV and ToRSV once trees are infected in the orchard. Therefore, based on the expansion of pawpaw orchards in the United States and worldwide, a careful selection of clean pawpaw seeds and propagation material is critical for producing clean grafted cultivars and preventing the introduction of viruses, such as TRSV and ToRSV, in newly established orchards.

I will continue to do research on how best to manage pawpaw trees in a grove infected with TRSV. Perhaps soil amendments and sufficient water can help healthy trees ward off the virus or delay infection. I hope that sharing my pawpaw disease status can help others with sourcing and maintaining healthy pawpaw trees.

The final good news is that I still have healthy pawpaw trees negative for all tested viruses. I am excited about offering delicious pawpaw fruits for sale within a few weeks. Below is a photo of my grafted Overleese tree loaded with ripening pawpaw fruit as of September 17th, 2024.



Yours Truly to Pawpaw Lovers, Abbie