## Testing African Violets Using ELISA INSV ImmunoStrips®

By Ronn Nadeau

In mid-May of 2013 a novice grower (NG) of African violets (AVs) gave me several plants to inspect. She had obtained them from sellers at an AV show/sale. At the time of purchase, the plants were blooming and appeared healthy. Her son built plant stands for her and for awhile all was good. However, after a few months, most of her plants were very sick. She asked, "What am I doing wrong!?"



A very sad looking specimen of "Kiwi Dazzler." The numbers indicate leaves that were removed for ELISA ImmunoStrip INSV testing.

NG's plants, which showed classic cyclamen mite symptoms, were tested using ImmunoStrips® purchased from Agdia, Inc. The numbers on the plant in Photo 1 show the five leaves that were used in five separate ELISA INSV tests. The leaf used in test 1, which was cut from the center of the plant and placed in front of the plant before taking the picture, is labeled "1". The INSV test of leaf 1 gave a strong positive result.

I could have stopped there and just reported that the plant was infected with INSV virus. However, because of my curiosity as to why some plants that have these symptoms test positive while some test negative, I conducted tests on some other leaves of this Kiwi Dazzler. Testing leaf 2 (Photo 1) gave another strong positive INSV result. Would all the leaves of the plant test positive? No! Leaves 3 and 4 were clearly negative! And, even more surprising, the senescent leaf 5 was strongly positive!

I discussed these results by phone and e-mail with Agdia scientist Dr. Francisco Assis, Senior Plant Pathologist, Vegetable Crops & Seed Health Specialist. To summarize, Dr Assis said that most virologists agree that virus distribution in infected plants is usually uneven, and that it is best to sample symptomatic leaf material. However, in these, 'Kiwi Dazzler' analyses, leaves 1 – 4 all showed symptoms (crinkled, brittle, distorted) but only 1 and 4 tested INSV positive. On the other hand, leaf 5, which was INSV positive, did not show "cyclamen mite symptoms" but did show the remnants of a necrotic ring-spot (upper left edge of leaf), which is a symptom of INSV.

In Photo 2, leaf 1 is shown after being cut away



A specimen of 'Ness' Red Velvet' with stunted center. The numbered leaves were tested by ELISA INSV.

and placed on top of the plant. Leaf-2 is smaller than normal, stunted. Leaf-3 represents two very small center stunted leaves that were analyzed together. Results: leaf 1 and leaf 2 were INSV negative; leaf 3 was positive. From that plant, only those leaves were analyzed.



A specimen of "Rob's Boolaroo"

Only one leaf was analyzed, shown near the base of the plant. It tested weakly positive.

One other of NG's diseased plants, labeled NQR (not pictured), was tested. Despite sampling the very center, plus three leaves, only negative INSV results were obtained from NOR.

## Conclusions

Results from these tests indicate that the distribution of virus particles in AVs showing INSV symptoms can be spotty and unpredictable. It seems likely that this accounts for INSV negative test results (false negatives?) even when it is suspected that INSV is present. However, there are other possible explanations. For example, this 'Kiwi Dazzler' might contain two viruses, an unknown virus (X) causing the so-called cyclamen mite symptoms, and the INSV virus causing the positive ELISA result. In that scenario, leaves 1 and 2 would have both viruses, leaves 3 and 4 would have only virus X, and leaf 5 would have only INSV. I consider that scenario unlikely. It seems more likely that the INSV concentrations in the plants described here were uneven, and that leaves 3 and 4 were INSV negative because the INSV concentrations therein were below the level of detection of the ELISA INSV method.

And what about the question NG asked: "What am I doing wrong?" I told her, "You did nothing wrong. The plants you obtained at the show/sale quite likely already contained INSV." (That is based on something I know but would not divulge here.) Viruses are well known for quiescent symptomless periods followed by periods of full blown viral expression. This is what makes INSV so pernicious and difficult to conquer. The plants sold to NG looked healthy at the time of sale and were not known to be contaminated. Propagation of AVs using symptomless, yet INSV containing leaves or suckers might be, in my opinion, the main way that AVs now become contaminated with INSV.

**Closing note**: I have given NG some 5-monthold pre-bloom seedlings. She is pleased and reports they are growing nicely. And I earlier did the same for another grower in the St Louis area, and she reports vibrant, healthy, blooming seedlings.

Please also read the next article.