Genetic Fingerprinting for St. Augustinegrass

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The discovery of any new plant disease triggers a basic sequence of responses. The initial focus is on diagnosis, coupled with the search for management options. Is there an existing chemical that will control or eradicate it? Is there a vector, such as a host pest, that can be targeted? Is there a cultural practice that can be altered to prevent the disease or improve plant health? For plant viral diseases, there are often few ways to treat or cure infected plants, so the emphasis is placed on preventing infection and identifying alternative plants that meet the landscape need and are resistant to or unaffected by the new pathogen.

This is the sequence that's been followed as we've worked with what's now known as Lethal Viral Necrosis in St. Augustinegrass. The full diagnosis and understanding of the disease has evolved over the past two years and continues to do so. Work by Dr. Phil Harmon and Dr. Jane Polston originally identified *Sugarcane mosaic virus* (SCMV) as a component of the disease, potentially in combination with a secondary virus identified on affected samples. Further research is needed to understand the disease process and exactly how LVN causes the decline and eventual death (necrosis) of lawns.

One thing noted early on was that Floratam was the most dramatically impacted St. Augustinegrass cultivar. Where all cultivars in affected areas showed mosaic symptoms corresponding to SCMV infection, only Floratam developed necrosis eventually resulting in plant death, which is associated with LVN. This observation shifted the focus to finding out which existing cultivars were resistant and susceptible. The problem? Many St. Augustinegrasses look similar, so knowing with certainty which was which was an issue.

Because St. Augustinegrasses have generally been used as lawn and multi-purpose grasses, they've not been widely genetically sequenced, as the costs were significant.

Some commonly used St. Augustine varieties, including Floratam (developed by the University of Florida and Texas A&M and released in the mid-1970's) have outlived their patents. In these cases, certification programs may be discontinued when demand for certified product disappears and market prices can no longer support certification costs. (TPF provided oversight for the Certified Floratam program until about 10 years ago, when the last on-farm source returned the certified material to UF.)

To add to the confusion, other St. Augustines are much older, possibly closer to landraces (local cultivars improved by traditional agricultural methods) than clonal cultivars, and there is no genetic map of those cultivars as well. Identification largely depends on written descriptions, drawings, and the expertise of individuals who have worked with these cultivars over a long period of time.

Fortunately, genetic sequencing is helpful for differentiating known cultivars and as we build a database of samples, for developing a template for identifying older cultivars. For the past two years, Dr. Kevin Kenworthy and I have been working on samples sent in by growers to determine the cultivar and build a baseline of St. Augustinegrass genetic comparisons.

As growers, it may help to know a bit about how this is done, the time required, and the process involved. Here's how it works.

Genetic Fingerprinting of St. Augustinegrass Using Simple Sequence Repeats (SSRs)

Samples received from growers are planted in the greenhouse. If there are morphologically distinct stolons, we separate those stolons into two new pots, creating subsamples. *(Fig. 1)*

Figure 1. Sample showing two distinct stolon types. These are divided into separate subsamples.

Healthy actively growing leaves are collected from a single sample. We grind the samples and use a kit to filter out the DNA from other plant parts (cell wall components, proteins, RNA, etc.). Typically, these extractions are done in batches of up to 50 samples/subsamples, but we have to be very careful to keep each sample separate. If DNA from one tube gets into another tube, it can lead to major complications downstream.

Next, we test the quality of the DNA. Specifically, we want to make sure the DNA that was extracted was clean (no RNA or protein contamination), a high concentration (lots of DNA was extracted), and in good condition (not degraded). *(Fig. 2)*

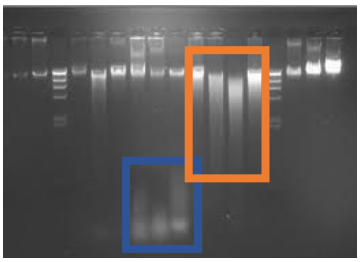

Figure 2. A sample showing both RNA contamination (blue box and DNA degradation (orange box).

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Using the extracted DNA, we run a Polymerase Chain Reaction (PCR). This process allows us to take a single segment of DNA and make millions of copies of it, like a copy machine. *(Fig. 3)*

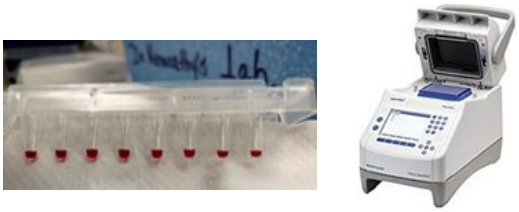

Figure 3. Reagents + Thermocycler = Genetic Photocopier!

We use primer pairs - small specific sequences of DNA - to target a specific section of the genome that we want to make copies of. It's like specifying the exact paragraph of a book you want copied (page 67, paragraph 2), instead of copying the entire book.

Each primer pair targets a different region of the genome. The more segments of the genome you look at, the more confidently you can say whether two samples are the same or different (aka polymorphic).

In St. Augustinegrass, we usually look at 5-20 regions, depending on how thorough we need to be. We check the quality of the PCR product to make sure a high number of copies were made.

The PCR products are then sent to a lab on campus where they measure the total size of our product, i.e., how many base pairs make up a segment. Using the book metaphor, it's analogous to determining how many characters are in the paragraph that we copied. It's important that we have a lot of copies of our fragment to be able to accurately estimate its size.

Next, we create a genetic fingerprint for the sample. A genetic fingerprint is a group of DNA segments than can be uniquely associated with a specific plant. The genetic fingerprints of each sample along with several reference cultivars are then compared to see how similar or dissimilar they are. We typically use Floratam, Palmetto, Seville and other cultivars for reference. In some cases, we may run up to 23 cultivars for comparison. *(Fig. 4)*

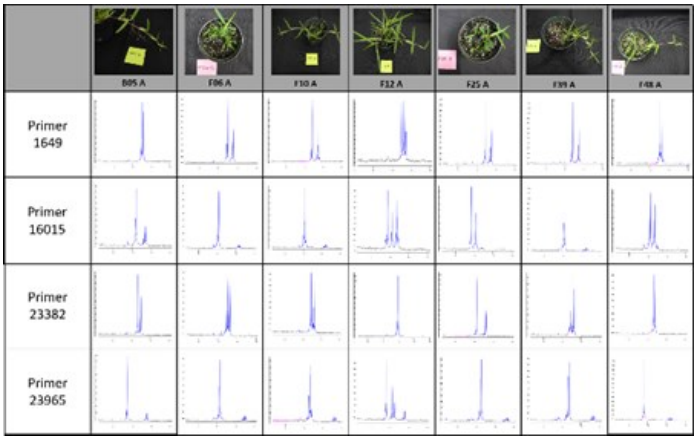

Figure 4. A binary matrix is normally used to convey the data shown in the picture above.

Finally, we use some old fashion common sense to check to make sure that our genetic fingerprint matches what we see growing in the greenhouse.

Sample Looks like Floratam

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Genetic Markers Match Floratam

Confirmed as **Floratam**

There are some limitations:

- Prior testing is needed to know which regions of the genome need to be looked at to differentiate cultivars from one another.
- We may or may not be able to differentiate closely related lines.
- If the presence of multiple genotypes in a sample is missed during the initial subsampling, this genotype will go untested in downstream processes as well.