Figure 1. Stem-end rot on grapefruit in the field

Controlling diplodia stem-end rot before harvest

By Mark A. Ritenour, Jiuxu Zhang, Liliana M. Cano and Megan M. Dewdney

The decay of fresh citrus fruit in Florida is usually caused by fungal pathogens that grow and develop in the hot and wet conditions typical to the state. While green and sometimes blue *Penicillium* molds and sour rot can cause Florida fruit to decay, the subtropical conditions of Florida favor diplodia (*Lasiodiplodia theobromae*) stem-end rot (SER), phomopsis SER (*Diaporthe citri*), anthracnose (*Colletotrichum gloeosporioides*) and, less frequently, brown rot (primarily *Phytophthora palmivora* and *P. nicotianae*).

Of these, diplodia SER is the most common postharvest decay in fresh Florida citrus. Although *L. theobromae* infects the fruit calyx primarily during the summer months, the infections usually remain dormant until after harvest, where careful handling, pre-cooling and the appropriate use of postharvest fungicides traditionally controlled postharvest disease development.

A PREHARVEST PROBLEM

However, after huanglongbing (HLB) became prevalent, diplodia SER substantially increased and is now observed on hanging fruit preharvest (Figure 1). This might be due to HLB-induced tree stress and related increases in the amount of weakened and/or dead wood in the trees, which promotes growth and sporulation of *L. theobromae* and subsequent fruit decay.

Traditional postharvest methods to control fruit decay become ineffective if it begins to develop in the fruit before harvest. Researchers at the U.S. Department of Agriculture demonstrated a strong relationship between HLB symptoms, fruit detachment force (abscission), ethylene production in the abscission zone, *L. theobromae* development before harvest, and ultimately, fruit decay. Improved methods to control fruit decay that can be employed in the field before the fruit is harvested are needed.

Preharvest application of effective fungicides could reduce fruit infection and colonization of fungal pathogens in the grove, also reducing postharvest decay. Preharvest fungicides such as Benlate (benomyl) and later Topsin (thiophanate-methyl) were once available and provided excellent decay control during harvest and postharvest

2020–21 Preharvest Trials on Red Grapefruit

- Control - Water
- Topsin 4.5 FL*
  – **thiophanate-methyl (45%)**
- Graduate A+*
  – **fludioxonil (20.6%) + azoxystrobin (20.6%)**
- Switch 62.5 WG
  – **fludioxonil (25%) + cyprodinil (37.5%)**
- Miravis Prime*
  – **fludioxonil (21.4%) + pydiflumetofen (12.8%)**
- Miravis Top
  – **difenconazole (11.5%) + pydiflumetofen (6.9%)**
- Headline
  – **(Peraclostrobin) pyraclostrobin (23.6%)**
- Thyme Guard (Thyme)
  – **Thyme (23%)**
- Citrus Fix (2,4-D)*
  – **2,4-D (45%)**
- Quadris Top
  – **azoxystrobin (18.2%) + difenoconazole (11.4%)**

***not labeled for preharvest use in Florida grapefruit**

Table 1. Materials sprayed preharvest on red grapefruit during 2020–21 to evaluate their ability to reduce postharvest fruit decay. Label or manufacturer-recommended rates were used based on 250 gallons per acre volume.

handling and distribution. However, Benlate production was discontinued in 2001, and efforts to move Topsin from an EPA Section 18 emergency-use exemption to a full label were abandoned by the registrant in 2009.

There have been no known effective preharvest treatments with residual postharvest decay control for Florida citrus since then. Results from preharvest materials evaluated up through 2010 for their ability to control postharvest decay can be reviewed at journals.flvc.org/fshs/article/view/86240 and journals.flvc.org/fshs/article/view/83990. Alternative products need to be identified and registered for commercial use.

NEW STUDIES

Studies during the 2019–20 and 2020–21 seasons evaluated the preharvest application of relatively new fungicides, a plant growth regulator (2,4-D) and an essential oil extract (thyme) for their ability to reduce postharvest decay on grapefruit (Table 1). Topsin was included to compare

Tansgenic Possibilities

By Rick Dantzler, CRDF chief operating officer

This is the second in a series of columns on "rifle-shot" research, which I define as high-risk, high-reward projects that could lead to a cure for HLB. They are not fast and not inexpensive, but they could get this disease behind us once and for all.

Last month's column discussed using the citrus tristeza virus to carry peptides, genes or other liberibacter-neutralizing agents into the phloem of a citrus tree, where the disease resides. This month's column focuses on transgenics, the introduction of one or more genes of one species into another.

Upon discovery of HLB in Florida, a first hope was to create transgenic citrus that would be resistant to the disease. Several years later, when scientists learned how to transform mature citrus, the possibility arose of greatly speeding the process of getting these transgenics into the field.

This brought significant financial investment in the building and staffing of a mature transformation facility at the University of Florida Institute of Food and Agricultural Sciences (UF/IFAS) Citrus Research and Education Center. The expectation was that researchers from around the world would identify many genes that would protect citrus from HLB, either by resistant or tolerant trees or prevention of spread by psyllids. That expectation has not been met, but transgenic citrus resistant to canker and tolerant to HLB has been made (some are under field tests).

Excitement in the industry about transgenics has not been great, primarily because the fruit from these trees would be considered genetically modified organisms (GMOs), and the time and cost of approvals would be substantial, perhaps in the $10 million to $40 million range. To justify this cost, a transgenic likely would have to be resistant enough to eliminate HLB.

Another approach is cisgenics. With cisgenics, only sequences that could be conventionally bred into citrus by making crosses with other citrus species are introduced. While still genetically modified, it is modified with citrus sequences, which should be more acceptable and less expensive because the regulatory requirements would be less onerous.

Perhaps a more practical approach could be to use transgenic rootstocks that can actively protect the aboveground non-transgenic scion against HLB. In such a scenario, the transgenic rootstock would produce potent compounds that could either move up to the scion through the graft union or remain in the rootstock. In either scenario, liberibacter spread could be checked, and the protected scion would remain non-transgenic. This technology is still in its infancy, but early results are encouraging.

In the meantime, new technologies have arisen. With CRISPR, small, targeted mutations are created at a specific site in the citrus genome. Because there is no new foreign sequence introduced, the expectation is that resulting trees would not be regulated or considered GMOs. The Wang, Mou, Gmitter and Dutt labs of UF/IFAS, along with other labs around the world, are in the process of producing HLB-resistant or tolerant trees using this method.

The Citrus Research and Development Foundation is funding work in all these areas. Even if just one works, it could put HLB in the rearview mirror and allow us to move on to other things.

CRDF Grower Dollars at work for you

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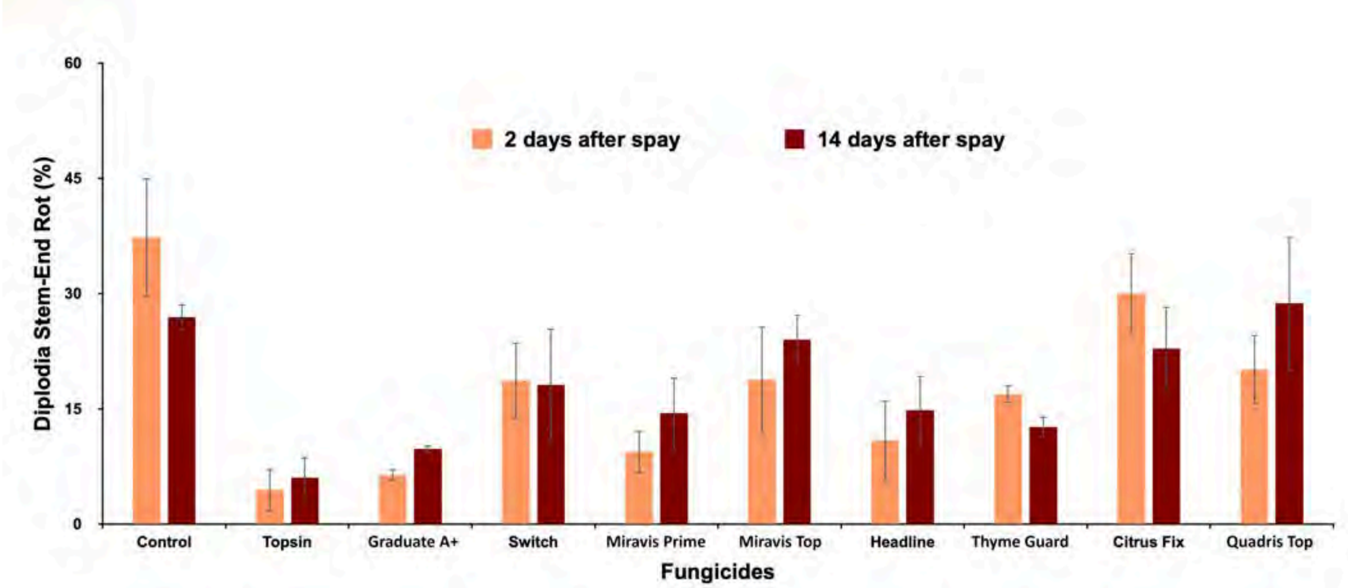

Figure 2. Incidences of diplodia stem-end rot on red grapefruit after preharvest treatment with the indicated materials, harvested two or 14 days after treatment, and then exposed to 5 parts per million ethylene for five days at 85° F, plus three weeks at 75° F without ethylene. Trees were sprayed on Feb. 23, 2021. Vertical bars represent standard deviation.

with the others as a likely "best case" control method.

The materials were sprayed on red grapefruit two or 14 days before harvest, and the fruit were exposed for 26 days to postharvest conditions that are known to promote diplodia SER development. The experiments were conducted six times using different red grapefruit blocks.

Figure 2 shows an example of commonly observed results. Topsin or Graduate A+ gave the greatest and most consistent reductions in fruit diplodia SER after harvest and storage. Graduate A+ was included because it is already registered for postharvest use and was effective in controlling postharvest diplodia SER if the decay did not start in the field.

Of the other materials, Headline and Miravis Prime occasionally reduced decay. Headline is the only material currently registered for preharvest use that at least gave occasional reductions in postharvest diplodia SER. Graduate A+ is not registered for preharvest use and is still rarely used postharvest in Florida because traditional postharvest fungicides are still effective.

This is unlike many other fresh citrus packing regions where pathogen resistance to the traditional fungicides required switching to new fungicides like Graduate A+. However, these studies used rates of Graduate A+ (fludioxonil + azoxystrobin) approved for postharvest use but that are higher than currently allowed in preharvest formulations utilizing these same fungicide components. Thus, future work will focus on evaluating combinations of fludioxonil, azoxystrobin and pyraclostrobin that will provide continuing postharvest decay control at rates allowed for preharvest use. Recommended use patterns will also be evaluated to minimize the development of fungal resistance.

LOOKING TO THE FUTURE

Continued research into better preharvest control methods is needed because of HLB's ongoing impact on tree health and increasing postharvest decay. Work is also progressing to develop additional postharvest control methods such as chlorine dioxide gas-releasing sachets and biological products. Researchers are hopeful that further development of an integrated systems approach to controlling diplodia SER and other postharvest diseases will better assure decay-free citrus fruit from Florida.

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