# Tectus Matrix® Bio Assay Evaluation (Preventative and Curative Applications) for *Fusarium oxysporum* f.sp. *cubense* (TR4) in Cavendish Banana

## Conducted at Texas A&M AgriLife Extension, College Station, Texas

Tested and prepared by Dr. Young-Ki Jo, Texas A&M AgriLife Extension at the Plant Pathology and Microbiology Building, College Station, Texas

July 29, 2020 | Final Report

to

SALVEO, INC.

Georgetown, Texas

# Tectus Matrix® Bio Assay Evaluation for *Fusarium oxysporum* f.sp. *cubense TR4*

## Background of Panamá Disease

**Panama** disease (or Fusarium wilt) is a plant disease that infects banana plants (Musa spp.). It is a wilting disease caused by the fungus *Fusarium oxysporum* f. sp. *cubense* (FOC). The pathogen is resistant to fungicides and its control is limited to phytosanitary measures.

During the 1950s, the first outbreak of Panama disease almost wiped out commercial Gros Michel banana production. The Gros Michel banana was the dominant cultivar of bananas, and Fusarium wilt inflicted enormous costs and forced producers to switch to other, disease-resistant cultivars. Currently, a new outbreak of Panama disease caused by the strain Tropical Race 4 (TR4) threatens the production of today's most popular cultivars, Cavendish.

***Gros Michel Devastation Era***

Gros Michel was the only type of banana eaten in the United States from the late 19th century until after World War II. From the beginning in the 1950's however, a serious disease was present in the banana plantations of Central America. The problem was first diagnosed in Panama after which it was named. Over several decades, the fungus spread from Panama to neighboring countries, moving north through Costa Rica to Guatemala and south into Colombia and Ecuador.

The banana industry was in a serious crisis, so a new banana thought to be immune to Panama disease was found and adopted, the Cavendish. In a few years, the devastated plantations resumed business as usual, and the transition went smoothly in the American market. Shortly thereafter, Malaysia entered the banana-growing business. Cavendish banana plantations were new to that country in the 1980s, but they rapidly expanded to meet the demand. Thousands of acres of rain forests and former palm oil plantations were shifted to banana production. Within a few years, though, the new plants began to die. While it took several years to find, the cause was ultimately attributed back to Panama disease. Although the Cavendish was then thought to be immune, it was immune only to the strain of the fungus that destroyed the Gros Michel. The version that annihilated the Gros Michel was found only
in the Western Hemisphere, but the version found in Malaysian soil was different, and the Cavendish is susceptible to it. It killed and spread faster, inspiring more panic than its earlier counterpart in Panama. The newly discovered strain of *F. oxysporum* was
named tropical race 4 (TR4).

INC

### *Commercial Loss*

The previous outbreak of the Panama disease in the Gros Michel cultivars led to a staggering loss of $2.3 billion worldwide. Presently, Panama disease threatens to have a devastating impact on the global banana yield, affecting the lives of millions of people depending on these bananas for their consumption and livelihoods. For example, over 100 million people in Africa depend on bananas as an important source of food and livelihood. The disease that is already raging across the African banana plantations thus threatens the well-being of all these people. A major banana-producing company in Mozambique, Matanuska, has already suffered an economic loss of $7.5 million USD because of this disease.

The negative public perception of bananas grown in the Panama disease afflicted countries is also adversely affecting the export of bananas to other countries. Over 10,000 hectares of banana plantations have been lost in the countries of China, Malaysia, Indonesia, and the Philippines since 1992, and over $400 million dollars have been lost due to the recent outbreak of Panama disease (TR1).

Source: https://www.worldatlas.com/articles/panama-disease-fungal-pariah-of-the-global-banana-industry.html

### *TR4 Devastation Era*

FAO. (The Food and Agriculture Organization of the United Nations):

According to the FAO: Banana Fusarium Wilt disease **(**Banana Fusarium Wilt disease, aka. Panama Disease, FOC TR4), which has been severely affecting banana plantations in several growing regions since the late 19th century, continues to be of serious concern to the global banana industry. The currently expanding strain of the disease, described as Tropical Race 4 (TR4), poses particularly elevated risks to global banana supplies as it can affect a much broader range of banana and plantain cultivars than other strains of Fusarium wilt. In addition, ***there is currently no effective fungicide or other eradication method that is capable of eliminating TR4***.

In affected plants, the disease can quickly cause a total yield loss. According to official information, TR4 is currently confirmed in 17 countries, predominantly in South and Southeast Asia. In August 2019, the fungus was officially reported for the first time on banana plantations in Latin America, in the northeastern region of La Guajira, Colombia. The discovery of Fusarium wilt TR4 in the world's largest exporting region, Latin America and the Caribbean, as well as its enduring occurrence in Asia, has caused considerable alarm in the banana export industry. Given the current annual value of production for export and the importance of Cavendish bananas for smallholders in the region, TR4 threatens to cause substantial losses to the sector. To date, few estimates of the additional disease-related expenses to producers are available, but it is clear that Latin American producers and exporters will be faced with significantly higher costs to shield their production from TR4.

A recently conducted assessment of the potential economic impact of the TR4 disease on global banana production and trade showed that a further spread of TR4 would, internationally, entail considerable loss of income and employment in the banana sector in the affected countries as well as significantly higher consumer costs in importing countries, at varying degrees contingent on the actual spread of the disease. These costs would add to the extra costs that are expected to be generated by the adverse impacts of climate change.

Source: http://www.fao.org/economic/est/est-commodities/bananas/en/

# Procedures

**Greenhouse Conditions:**

- Greenhouses are located in the penthouse of the Plant Pathology and Microbiology Building
- Light period: 16 hours each day
- Temps: 28 ± 2°C
- 2 weeks for plant acclimatization and monitor disease assessments for at least 7 weeks after inoculation
- Watering as needed to field capacity, eventually 500 ml per day
- Maintained a relative humidity between 70 to 85 percent
- Slow-release pellet fertilizer was incorporated in the soil media by the grower who provided the banana plants for the experiments. No additional fertilizer was applied during the experiment.

**Cultivars used:**

- Cavendish Dwarfs (avg. height 24+")

**Inoculation with *Fusarium oxysporum* var. *cubense Tropical* Race 4 (ATCC® 96289™):**

- Cultivate FOC in-vitro for a week on PDA at 25°C to produce mycelial plugs.
- Add FOC plugs in 1L Erlenmeyer flasks with 500 ml of water, 2 grams of mung bean (pre-boiled and autoclaved) and covered with a cotton plug top to each flask.
- Incubate flasks at 25°C and mixed in a rotary shaker at 150 rpms for 6 days.
- Make a direct pour solution into each pot with a concentration of spores at $1 \times 10^6$ at the rate of 200 ml solution per 1-liter pot.

**Experimental Design:**

- Four plants for each treatment
- Completely randomized block design

# Experiments

| Experiments | Objective | Conditions |
|---|---|---|
| Curative | Inoculation two weeks prior to Tectus Matrix® treatment to test curative ability | 4 strength doses (A, B, C, D) including positive, negative and absolute controls |
| Preventative | Tectus Matrix® treatment one day prior to fungal inoculation to test preventative capabilities | 4 strength doses (A, B, C, D), including positive, negative and absolute controls |

## Treatment Methodology Bananas with Tectus Matrix®

**Fungicides:**
Soil drench application of 500 mL in volume per individual pot with fungicide Tectus Matrix® solution at varying concentrations (A, B, C, D) to soil field capacity.

**Assessments of Plant Health:**
Entire plants were photographed in front of a black velvet canvas alongside a meter stick for reference prior to further analysis.

**Healthy Leaf Count:**
Prior to the cutting of the pseudostem, healthy banana leaves were counted and recorded for each replicate per treatment. Leaf counts across trials did not show significant differences between treatments.

**Pseudostem examination:**
Pseudostems were first cut 3 inches above the soil line and photographed. Then plants were removed from the pot, and shaken until a majority of the soil was removed and photographs were taken alongside a meter stick. The remaining pseudostem/corm below the soil line was cut vertically for photographs of the inside of the corm.

**Image analysis:** The photographs of dissected pseudostems were cropped within a circular image, closely framing each side of the corm. Photographs were analyzed using an open source photograph analysis software (ImageJ). Images in ImageJ were adjusted from the colored circular image to a 32-bit grayscale for analysis. The images were then adjusted using thresholds to highlight the lesions on the corm. These thresholds were kept consistent within each treatment group using the best representative plant as the threshold baseline. These thresholds were determined for each group depending on light conditions within the images in order to gain readings of the percentages of infected corm, while negating the presence of soil particles on the corms and in

the surrounding image.

The percentage of disease in each corm was measured using this process for the left and right sides of the corm for each plant and averaged in the tables in the pages below.

# Results of Curative Treatment

**Summary Table for the Curative Trial. Disease severity in Pseudostem (Table 1 below).**

*Table 1: Treatment and Disease Severity*

| Treatment # | Rep | Inoculation | Treatment | % disease | Stat (alpha = 0.05) |
|---|---|---|---|---|---|
| 1 | 4 | Yes | A: strength | 6.63 | BC |
| 2 | 4 | Yes | B: strength | 9.22 | BC |
| 3 | 4 | Yes | C: strength | 11.84 | B |
| 4 | 4 | Yes | D: strength | 19.72 | A |
| 5 | 3 | No | None | 2.15 | D |
| 6 | 3 | Yes | None | 21.86 | A |
| 7 | 4 | No | D: strength | 3.86 | CD |

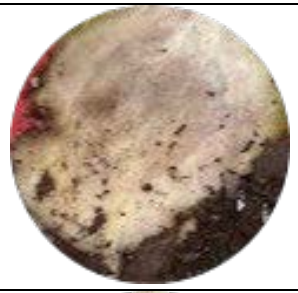
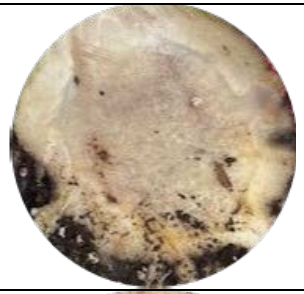
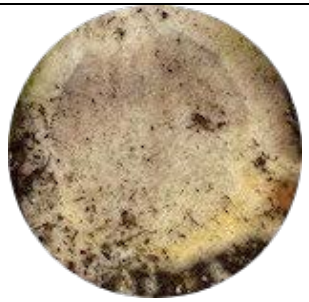
*The same letter in the Stat column indicates no significant differences at P-value < 0.05.*

## Curative Notes

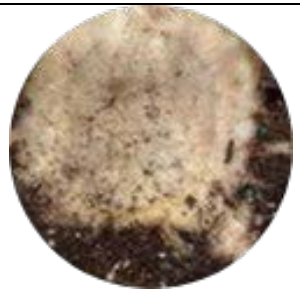
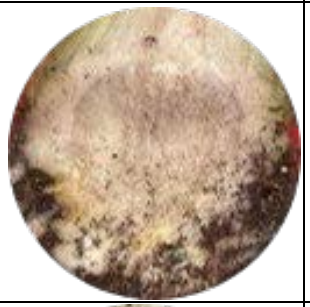
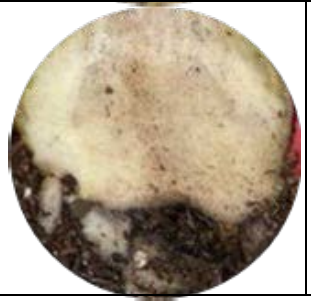
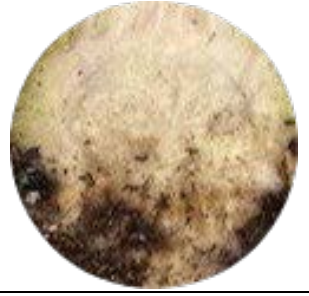
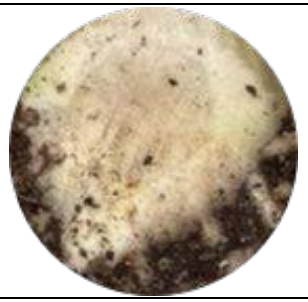
Tectus Matrix® treatments (> D strength) significantly reduced the FOC symptom development compared with the positive control (treatment # = 6; FOC inoculation and no Tectus treatment). The Tectus D treatment was similar to the positive control in disease severity. The disease symptoms were not statistically different among, A, B or C strengths.

We did not observe phytotoxicity effects by Tectus Matrix® at D strength (Treatment 7).

*Table 2. FOC symptoms developed in banana pseudostems after the curative trial.*

| Treatment | Replication 1 | Replication 2 | Replication 3 | Replication 4 | Average % Disease |
|---|---|---|---|---|---|
| 1<br>A strength |  |  |  |  | 6.63% |
| 2<br>B strength |  |  |  |  | 9.22% |
| 3<br>C strength |  |  |  |  | 11.84% |

INC

| | | | | | |
|---|---|---|---|---|---|
| 4<br><br>D strength |  |  |  |  | 19.72% |
| 5<br><br>No FOC + No tmt |  |  |  | Harvested at an earlier date | 2.15% |
| 6<br><br>FOC + No tmt |  |  |  | Harvested at an earlier date | 21.86% |
| 7<br><br>No FOC + D strength |  |  |  |  | 3.86% |

Disease symptoms were highest in treatment 6, followed by 4, and then 3. Notice the darkening/degradation of tissue in treatments 6, 4, and 3 compared to other treatments in which the symptoms were <10%.

INC

# Results of Preventative Treatment

**Summary Table for the Preventative Trial. Disease severity in pseudostem (Table 3 below).**

*Table 3: Preventative Trial Treatment and Disease Severity*

| Treatment # | Rep | Inoculation | Treatment | % disease | Stat (alpha = 0.05) |
|---|---|---|---|---|---|
| 1 | 4 | Yes | A strength | 3.48 | CD |
| 2 | 4 | Yes | B strength | 4.73 | C |
| 3 | 4 | Yes | C strength | 3.93 | C |
| 4 | 4 | Yes | D strength | 8.34 | B |
| 5 | 4 | No | No | 0.99 | D |
| 6 | 4 | Yes | No | 15.92 | A |
| 7 | 4 | No | D strength | 2.22 | CD |

The same letter in the Stat column indicates no significant differences at P-value < 0.05.

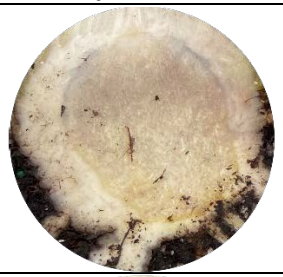
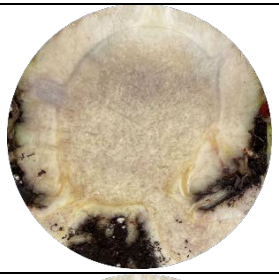
## Preventative Notes

Tectus Matrix® treatments (≥ C strength) significantly reduced the FOC symptom development compared with the positive control (treatment # = 6; FOC inoculation and no Tectus treatment). The Tectus Matrix® treatments at A, B and C strengths inhibited the disease severity more effectively than the D strength treatment. The disease symptoms were not statistically different among A, B and C strengths.

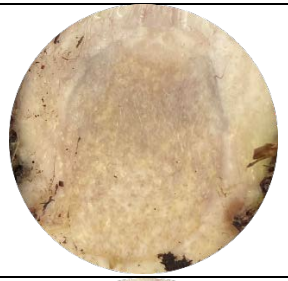
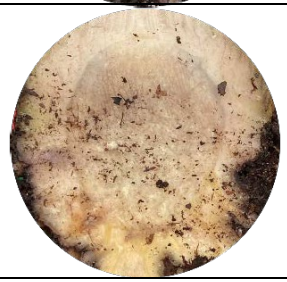
We did not observe phytotoxicity effects by Tectus Matrix® at C strength (Treatment 7).

INC

*Table 4. FOC symptoms developed in banana pseudostems after the preventative trial.*

| Treatment | Replication 1 | Replication 2 | Replication 3 | Replication 4 | Average % Disease |
|---|---|---|---|---|---|
| 1<br>A Strength |  |  |  |  | 3.57% |
| 2<br>B Strength |  |  |  |  | 4.82% |
| 3<br>C Strength |  |  |  |  | 4.01% |
| 4<br>D Strength |  |  |  |  | 8.52% |

INC

| | | | | | |
|---|---|---|---|---|---|
| 5<br><br>No FOC +<br>No tmt |  |  |  |  | 1.08% |
| 6<br><br>FOC +<br>No tmt |  |  |  |  | 15.76% |
| 7<br><br>No FOC +<br>D<br>strength |  |  |  |  | 2.33% |

**Note**

Treatment 6 was the only treatment that showed clear disease presence, above 10%, with visible lesions and darkened tissue on corms in the preventative trial. Compared to treatment 6 which was inoculated with FOC and was not treated with Tectus Matrix®, all other treatments were recorded with < 10% disease presence with visibly healthy corms.

## Salveo, Inc.

Salveo, Inc. (Salveo Naturals) founded in July 2016, USA was created to make natural fungicides and bactericides. The above report was made at Texas A&M University and the company donated funds to the university for the test's costs.

Tectus Matrix® is a patent-pending, broad-spectrum natural (can be classified as organic) fungicide and bactericide liquid that controls and mitigates fungal and bacterial diseases in food as well as ornamental crops and does not cause pathogenic mutations. The inventor believes Tectus Matrix® can be effective on the most economically important plant pathogens. However, more testing on fungi, bacterium and viral pathogens is preferred. Currently there are few successful treatments for plant bacterial or viral pathogens. It has been tested on ornamental crops such as greenhouse roses and tomatoes, hemp, and turf grass.

More information visit: www.SalveoNaturals.com or call +1.512.988.6012.