Fusarium oxysporum second attack on bananas

Food & Diet

Fusarium oxysporum Second Attack on Bananas: Preventative Measures through Soil Microbiome Change, Elimination by Tissue Culture, Creation of Resistance, and In-Field Technology Applications

Bananas are one of the top staple foods around the world with one of the largest industries, based on volume. The Gros Michel banana industry was wiped out in the Central Americas during the mid-twentieth century by the plant pathogen, *Fusarium oxysporum* race 1, also known as " Panama Disease" (Dita *et al.* , 2018). To prevent the collapse of the banana industry in Central America, there was a switch from Gros Michel to the current Cavendish banana cultivar. While this saved the banana industry, farmers and exporters took huge economic losses.

Today's banana exports consist of 99% Cavendish with most cultivars being a sterile cross between the diploid wild species *Musa acuminata* and *Musa balbisiana* (Siamak & Zheng, 2018). Now the Cavendish banana industry is being threatened by another race of *Fusarium oxysporum* . *Fusarium oxysporum* f. sp *. cubense* or tropical race 4 that was isolated to only East and Southeast Asia, but with increased globalization and banana exports, it has begun to spread. Through various methods, *Fusarium oxysporum* has since spread to South Asia, the Middle East, Africa, and Australia.

Fusarium oxysporum has the ability to destroy large numbers of banana plants in a short period of time, resulting in up to 20-80% loss in yields (Chand *et al.* , 2016). It is a necrotrophic and hemibiotrophic pathogen, infecting the xylem and inducing wilt, ultimately resulting in plant death (Dita *et al.* , 2018). The fungus produces chlamydospores that are resistant

to desiccation and poor environmental conditions. This makes *Fusarium oxysporum* especially resilient and attributes to its long soil residence time of up to 20 years, which facilitates its continued spread. Contaminated soils are a major problem for farmers because they can be spread to *Fusarium oxysporum* free plots via vehicles, people, and water. Areas with rainy seasons and/or typhoons/hurricanes have major issues with *Fusarium oxysporum* spread. With such easy mobility, it is especially important to determine contamination sources and quarantine areas to slow fungal spread.

Once contaminated, suckers used for plant propagation also contain *Fusarium oxysporum* . Planting infected suckers results in infected plants and plant loss. This makes traditional methods of plant propagation fruitless. The suckers are asymptomatic due to *Fusarium oxysporum'* s long latent period, making them an excellent method for spreading *Fusarium oxysporum* between farmers sharing plant materials. Fruit crowns, old leaves, and other plant materials can also contain fungal structures and can cause further spread. *Fusarium oxysporum* species can also infect healthy humans local to the cornea and nails, causing onychomycosis, keratomycosis, or mycotic keratitis (Namboothiri *et al.,* 2014). In immunocompromised individuals disseminated infections can occur, causing skin lesions with erythematous papules, nodules with central necrosis, or subcutaneous nodular lesions (Kaur & Maheshwari, 2013). In rare cases meningospondylodiscitis is possible (Namboothiri *et al.,* 2014).

In other cultures where the banana leaves are used in food packaging, pathogen attachment to leaves becomes an issue. The Chua & Dykes (2013)

study found that bananas could carry pathogens, such as *Staphylococcus aureus, Salmonella enterica, Escherichia coli,* and several fungal pathogens. Use of agar coating can reduce the number of pathogens on banana fruits and increase their shelf-life (Ziedan *et al.* , 2018). Reduced product weight loss is attributed to the agar producing a barrier against gas exchange and water vaporization. Agar is ideal in this situation because it is cheap and nondegradable by microorganisms. This method reduces both human and food contact with *Fusarium oxysporum.*

Old methods of control included the use of chemical fungicides, but often resulted in negative environmental impacts and never fully eliminated *Fusarium oxysporum* from the area (Dita *et al.* , 2018). Fungicides also contributed to reduced levels of beneficial organisms in the soils (Siamak & Zheng, 2018). Other methods require the removal and burning of infected tissues, which have not been successful in fungus eradication (Chand *et al.,* 2016). Research has shifted to other interventions to control fungusspread.

Soils lacking or suppressing *Fusarium oxysporum* have been studied to determine what the microbial environment consists of (Dita *et al.,* 2018). In suppressive soils there are large populations of *Rhizobium, Bhargavaea, Pseudolabrys* , and *Sinorhizobium* species. Crop rotations can function as a method for naturally changing the microbial community found in soils (Siamak & Zheng, 2018). Chinese leek has been found to successfully reduce *Fusarium oxysporum* in soils and rice paddies (Wang *et al.,* 2015). The Wang *et al.* (2015) compared the microbial communities after rotations with either pineapple or maize, looking to see if more lucrative crops have the same suppressive effects on *Fusarium oxysporum.* Pineapple rotations showed a

significant decrease in *Fusarium oxysporum* levels. Communities of *Acidobacteria* , *Planctomycete* , and *Chloroflexi* bacteria increased, while species of *Actinobacteria* and *Firmicutes* decreased. Fungal species of *Basidiomycota, Glomeromycota, Trechispora, Zopfiella,* and *Gymnopilus* increased and decreases in *Sordariomycetes* were seen. The increased abundance of *Gymnopilus* helped create an antifungal environment due to the species' fungal suppressive properties. By removing *Fusarium oxysporum* 's host and replacing with another crop, the beneficial bacterial and fungal communities in the soil increase. Most of the suppression is attributed to the changing of the fungal community abundances.

With the increased spread of *Fusarium oxysporum* to vulnerable banana populations and the Cavendish industry in jeopardy, the need for new technologies is apparent. While changing the environment that the banana plants are grown greatly reduces *Fusarium oxysporum* soil residence, it does not completely remove the threat it poses. Since Cavendish and most edible banana species are triploid, they are often left sterile, but capable of being propagated via suckers (Dita *et al.,* 2018). Susceptibility continues as susceptible plants are used for propagation. The need for resistant plants is high, but due to long generation times, various ploidy levels, and little genetic variability resistance is rare (Mahadev *et al.,* 2011).

Through the use of plant tissue culture, the most *Fusarium oxysporum* resistant plants can be selected and cultured to remove pathogens (Remakanthan *et al.,* 2013). Plant tissue culture can also be used to generate resistant transgenic plants via introduction of extraneous DNA. It has been deemed cost efficient that eliminates pathogens and reduces

contamination of plants. Studies using different *Musa* cultivars can determine what is necessary for plant regeneration and which cultivars have the most promise for in-field survival. Several methods for culturing Cavendish bananas exist but change based on the explant type being used and later experiment applications. Mahadev *et al.* (2011) was able to initiate shoot production from explants using different combinations of 6-benzylaminopurine, coconut water, naphthaleneacetic acid, and gibberellic acid in Murashige & Skoog basal medium. Explants had to be sterilized before culturing to reduce microbial levels using 70% ethanol for 30 seconds, 0. 1% mercuric chloride three times for five minutes, and autoclaved, double-distilled water. The most efficient medium for shoot initiation contained 5 mg l^{-1} 6-benzylaminopurine and 15% coconut water. Shoot elongation was achieved using 5 mg l^{-1} 6-benzylaminopurine, 1 mg l^{-1} naphthaleneacetic acid, and 1. 5 mg l^{-1} gibberellic acid. Plants regenerated from the shoot elongations had a 100% survival rate.

Embryogenic cultures require different protocols to generate plantlets. This is the preferred method for transforming Cavendish banana plants. In embryogenic, suspensions absent genes can be introduced and then used in large scale propagation (Remakanthan *et al.,* 2013). Remakanthan *et al.* (2013) achieved embryogenesis in 15-day to 90-day old cultures, using low levels of 2, 4-Dichlorophenoxyacetic acid, 6-benzylaminopurine, and thidiazuron. Results showed that younger 15-day-old cultures had shorter treatment response times, producing embryos sooner than older 60-day-old and 90-day-old cultures. Agar levels were also experimented with and showed that the ratio of agar to plant growth regulators greatly determines

whether a culture will become embryogenic. The Uma *et al.* (2012) study showed that embryos stored for up to three months could still be germinated, aided by the use of gibberellic acid. With scalability of production and long storage times, embryogenic culture for Cavendish banana plant regeneration is a viable route.

Using embryogenic cultures alongside *Agrobacterium* , transgenic plants can be generated (Siamak & Zheng, 2018). The co-culturing of the two allows for *Agrobacterium* -mediated transformation of embryos. This is the method used by Hu *et al.* (2013) to introduce the antifungal gene, chitinase (*chit42*), to generated plants. *Chit42* secretes an endo-chitinase from *Trichoderma harzianum* that can cleave the chitin in the fungal cell wall. In total 186 plants were generated and seven contained chit42. From these, seven lines were created and tested for antifungal activity. Lines were inoculated with *Fusarium oxysporum* and examined over the course of two months for signs of disease. Transgenic plants in comparison with wild type plants showed negligible disease symptoms, but some later developed advanced symptoms. One of the seven lines showed no signs of *Fusarium* wilt throughout the entire study. From this line, propagated plants will have resistance to *Fusarium oxysporum.* This makes their in-field application more advantageous.

Other methods of inducing resistance in Cavendish banana tissue culture plants have been explored. Methods of inoculating the tissue culture plants have been experimented with. Paparu *et al.* (2008) studied the reintroduction of endophytes to plants and the rates of colonization of plant roots. They found that reintroduction occurred 84. 4% in roots and 100% in

rhizomes in four weeks. It was observed in experimental plants that *Fusarium oxysporum* levels decreased faster from rhizomes than roots. Jie *et al.* (2009) used extracts from healthy Cavendish banana plants instead of pure fungal mixtures. This method combines both beneficial fungi and bacteria communities. Extracts were found to contain several endophytic microbes – *Bacillus pumilus, Bacillus subtilis, Pseudomonas fluorescens* , and *Streptomyces* – with a concentration of 7. 1 log CFU/g. Plants that received extract treatments showed reduced infection rates, reduced severity of disease symptoms, and increased growth parameters. Chand *et al.* (2016) wanted to determine if live or dead hosts of *Fusarium oxysporum* , root knot nematodes, from infected plants induced resistance in Cavendish banana plants, effectively immunizing plants and allowing them to accumulate defense enzymes. It was observed that plants treated by live or dead root knot nematodes showed elevated defense related enzymes. Plants inoculated with live root knot nematodes showed signs of disease, while those inoculated with dead root knot nematodes showed no signs of infection. The observed resistance conferred in the three studies could have been due to the added microbes inducing or increasing expression of intrinsic host defenses or they provided extrinsic sources of defense. It is possible that this method applying healthy microorganism populations confers and induces resistance that does not require the persistence of the fungi or bacteria populations.

As new technologies are developed, how can it be ensured that they are reaching the largest amount of the population that it can? Studies examining how to increase agricultural technology, such as plant tissue culture,

adoption have been increasing. The Kabunga *et al.* (2012) study combined Average Treatment Effects (ATE) framework and Joint Exposure and Adoption (JEA) parameters to determine what factors affected the rates of tissue culture banana adoption in the central and eastern Kenya provinces. They differentiated between awareness exposure – whether the farmer knew of tissue culture bananas – and knowledge exposure – whether the farmer had sought out detailed information sources. With a total of 385 farmers, 223 adopters and 162 nonadopters, they determined that the rate of technology adoption could be increased from 15. 4% based on ATE data to 28% after correcting for knowledge exposure. The factors that played the largest role in whether the farmer adopted or did not adopt tissue culture bananas were education, information access, and peers/social circles. The research focusing on social circles wanted to prepare the foundation for a community engagement strategy using game theory and qualitative description (Bandewar *et al.,* 2017). The Bandewar group found that exposure visits, group learning, input from community trainers, and field schools functioned as excellent methods for increasing the community's confidence in tissue culture and facilitating organizations.

As *Fusarium oxysporum* continues to spread, more and more Cavendish banana plants, and farmer livelihoods are at risk. It is necessary to use new technologies to develop reduced *Fusarium oxysporum* levels and produce resistant cultivars. Through the use of crop rotations *Fusarium oxysporum* levels can be reduced without the use of harmful chemical fungicides. Pathogen-free plants can be produced through tissue culture. The use of Agrobacterium-mediated transformation can insert antifungal genes to give

the transgenic plant resistance. The application of healthy microbial populations can help induce resistance to *Fusarium oxysporum* . Combinations of different methods of *Fusarium oxysporum* suppression will give farmers new ways to protect their crops and for the industry to survive.

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