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Effects of water regime, crop residues, and application rates on control of *Fusarium oxysporum* f. sp. *cubense*

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ABSTRACT

Biological soil disinfestation is an effective method to control soil-borne disease by flooding and incorporating with organic amendments, but field conditions and resources sometimes limited its practical application. A laboratory experiment was conducted to develop practice guidelines on controlling *Fusarium* wilt, a widespread banana disease caused by *Fusarium oxysporum* f. sp. *cubense* (FOC). FOC infested soil incorporated with rice or maize straw at rates of 1.5 tons/ha and 3.0 tons/ha was incubated under flooded or water-saturated (100% water holding capacity) conditions at 30°C for 30 days. Results showed that FOC populations in the soils incorporated with either rice or maize straw rapidly reduced more than 90% in the first 15 days and then fluctuated till the end of incubation, while flooding alone without organic amendment reduced FOC populations slightly. The rapid and dramatic decrease of redox potential (down to −350 mV) in straw-amended treatments implied that both anaerobic condition and strongly reductive soil condition would contribute to pathogen inactivation. Water-saturation combined with straw amendments had the comparable effects on reduction of FOC, indicating that flooding was not indispensable for inactivating FOC. There was no significant difference in the reduction of FOC observed in the straw amendments at between 1.5 and 3 tons/ha. Therefore, incorporating soil with straw (rice or maize straw) at a rate of 3.0 tons/ha under 100% water holding capacity or 1.5 tons/ha under flooding, would effectively alleviate banana *Fusarium* wilt caused by FOC after 15-day treating under 30°C.

Introduction

Banana is one of the most important agricultural crops in the world and serves as a major source of income in many developing countries (Dita et al., 2010). *Fusarium* wilt of banana (*Musa* spp.) is considered to be the most serious fungal disease attacking banana varieties (O'Donnell et al., 1998; Wang et al., 2013). The disease is known to be commonly caused by *F. oxysporum* f. sp. *cubense* (FOC) (Snyder and Hansen, 1940). Symptoms of *Fusarium* wilt begin with yellowing and wilting of the older leaves, and then spread to the younger leaves till the death of whole plant. FOC is a typical soil-borne pathogen that produces chlamydospores as a resting propagule. They are thick-walled and resistant to environmental fluctuations, enabling the pathogen to survive in soil a long time

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without host plants (Momma et al., 2010). Once being infested by FOC in soil, the susceptible banana varieties cannot be successfully replanted for nearly decades (Stover and Ploetz, 1990). Hence, the banana production has been dramatically reduced in many countries, ranging from the Central America to Asian (Ploetz, 2006).

Aiming to control FOC, many attempts have been conducted, such as planting resistant banana varieties, applying chemical pesticides and bio-control agents, and rotating crops (Stover and Ploetz, 1990; Alabouvette et al., 2009; Helslop-Harrison and Schwarzacher, 2007). A new approach, called biological soil disinfestations (BSDs) or anaerobic soil disinfestations (ASDs) or reductive soil disinfestations (RSDs) is proposed to control a broad spectrum of soil-borne pathogens (including FOC) and nematodes (Blok et al., 2000; Momma et al., 2006; Shennan et al., 2007; Mowlick et al., 2013). This approach is characterized by incorporating easily decomposable organic amendments, flooding soil through irrigation, and covering with plastic film for a period to create anaerobic conditions.

While the BSD is promising in controlling banana wilt disease, several aspects of problems hinder its practical application. First, flooding requires large amount of water and limited to fields, which are able to maintain standing water, layer and are equipped with convenient hydraulic and irrigation facilities (Blok et al., 2000; Gamliel et al., 2000; Katan, 2000). Soil texture and profile structure can influence the flooding effect. Organic soils with fine texture have a tendency to retain water, while coarse texture soils tend to drain water (Snyder, 1987). Flooding might not be practical in hilly areas where standing water layer could not be easily formed and in fields whose soils are permeable intensively with limitation of water resource. Moreover, considering water is a valuable resource, a more efficient and conservative way should be developed. Previous researches have revealed that saturating the soil to its maximum water holding ability is an effective alternative approach, because it can not only fill soil voids with water and restrict gas exchange as flooding does, but also conserve water and reduce nutrient runoff (Momma et al., 2010; Muramoto et al., 2008; Snyder, 1987). However, other studies found that merely saturating the soil was not effective in inhibiting some soil pathogens such as *Pythium* spp. (Snyder, 1987). Therefore, further work is needed to clarify this point.

Second, BSD requires the incorporation of a great number of organic amendments into soil. The incorporation rate of 10 tons/ha or even more was reported (Butler et al., 2012a; Momma, 2008; Momma et al., 2006, 2013; Subbarao et al., 1999). Although a variety of organic materials, including molasses, wheat bran, broccoli, ethanol, cover crops, animal and green manure could be used for BSD approach (Momma et al., 2011; Núñez-Zofío et al., 2011; Shinmura, 2002), they are not easily accessible all the time and are not economical due to transportation and purchase expense. On the other hand, crop residues have long been regarded as waste agricultural byproducts and even as contamination sources. Open-field burning of crop residues has induced serious atmospheric pollution in China (Zhang and Zhang, 1999). Also, *in situ* incorporation of crop residues is not adoptable, because the duration of interval between two crops is too short in the areas where multiple cropping is practiced, and it may increase the conductive to foliar diseases and reduce rough crop yields (Buresh and Sayre, 2007; Gadde et al., 2009; Hrynchuk, 1998). Therefore, if crop residues could be used as organic amendments in BSD, it would be possible to establish an environment-friendly straw disposal strategy to take the place of open-field burning.

In this study, in order to develop a more practical and economical BSD method to control *Fusarium* wilt, a laboratory experiment was conducted to determine: (1) Whether maize or rice straw has the potential to be used for BSD approach for controlling banana *Fusarium* wilt disease? (2) Whether water-saturation can replace flooding to reduce population of FOC, a pathogen of banana *Fusarium* wilt disease? and (3) How many days of water-saturation is the minimum to reduce the FOC population maximally?

1. Materials and methods

1.1. Soil and crop residues

The soil for the laboratory experiment was collected from the farm of Hainan Wanzhong Co., Ltd., Hainan, China. The field had been planted with banana for more than 10 years and *Fusarium* wilt disease incidence had spread most area of the farm, which was no longer suitable for banana growth. Because of undulating terrain, a standing water layer was hard to be formed for BSD treatment. The soil sample was collected from a field, which was abandoned for two years due to the *Fusarium* wilt disease incidence more than 50%. The soil was sand loamy, developed from red dry soil, with pH value of 6.55, organic matter content of 4.95 g/kg, and total N content of 1.29 g/kg. Before the experiment, the soil was sieved through a 2 mm sieve. Locally collected rice and maize straw were selected as organic materials for BSD treatment. The rice straw contained 42.5% C and 0.77% N (C:N ratio of 55:1, *m*/*m*), and the maize straw contained 47.1% C and 0.68% N (C:N ratio of 69:1, *m*/*m*). Both two kinds of straw were air dried for about one week, chopped into approximately 2-3 cm pieces, grounded into powders and finally sieved through a 2 mm sieve.

1.2. Experiment design

In the laboratory, a set of pots (1.5 L volume) were filled with 500 g prepared soil (soil depth: 5.5 cm, surface area: 78.5 cm^2) and then treated as follows: (1) non-amended and non-flooded (control CK); (2) non-amended but flooded (flood control); (3) flooded and amended with rice straw at a rate of 1.5 tons/ha (Flood + LR) (The conversion between weight and area units was based on the soil bulk density of 1.2 g/cm^3); (4) flooded and amended with rice straw at a rate of 3.0 tons/ha (Flood + HR); (5) flooded and amended with maize straw at a rate of 1.5 tons/ha (Flood + LM); (6) flooded and amended with maize straw at a rate of 3.0 tons/ha (Flood + HM); (7) water saturated and amended with rice straw at a rate of 3.0 tons/ha (Saturate + HR); (8) water saturated and amended with maize straw at a rate of 3.0 tons/ha (Saturate + HM). Each treatment had three replicates. For the incorporation rate of 1.5 tons/ha and 3.0 tons/ha, 0.32 g and 0.64 g straw was respectively added into each pot with 500 g soil. After thoroughly mixed with designed amount of crop residues, tapwater was added to soil/water ratio of 1:1 in flooded pots and to reach the water holding capacity (100% WHC) in water-saturated pots. Then, each pot was packed in a plastic self-seal bag and incubated in an incubator at 30°C for 30 days. All treatment pots were completely randomly arranged. Incubated soil was analyzed for soil properties and FOC population on days 0, 5, 10, 15, 20 and 30. Among them, the samples incubated after 30 days was divided into two parts, one was analyzed soil properties immediately and another was air dried at room