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# Sugar cane processing residue, bagasse, enhances decomposition of citrus leaves and could contribute to citrus black spot management

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ABSTRACT

Citrus black spot (CBS) is a quarantine disease, reported in Florida in 2010. The pathogen, *Phyllosticta citricarpa* (*Pc*), survives in twigs and leaf litter during the off-season. Speeding up leaf litter decomposition could contribute to reducing disease pressure and potential spread. Objectives were to investigate (a) effects of bagasse, cellulolytic microbes and urea on leaf litter decomposition; (b) relations between litter composition, moisture content and decomposition rate; and (c) effects of the treatments on *Pc* survival. Two laboratory and two field experiments were conducted in Northern Florida, where the pathogen is absent. In microcosms, the effects of bagasse ± cellulolytic microbes, just microbes, urea, and a control treatment were compared with respect to decomposition of *Pc*-inoculated mature sweet orange leaves on soil, *Pc* reisolation and detection by qPCR. In two disease-free sweet orange groves, effects of bagasse and urea on decomposition of nylon-bagged sweet orange leaves on soil were compared to a control at various distances from micro-sprinklers. Bagasse resulted in a leaf dry weight reduction of 93% in microcosms and 67% in groves compared to 36% in both controls after two months. Dry weight reductions declined with distance from sprinklers, except in the bagasse treatment which maintained leaf moisture content. *Pc* was not reisolated, but its DNA was detected in all inoculated but not in non-inoculated samples after one month. Bagasse enhanced decomposition of sweet orange leaves on soil. Additional experiments are needed in CBS affected groves to investigate effects of bagasse on *Pc* survival in leaf litter.



## 1. Introduction

Florida is the largest citrus producing state, supplying 56% of the total citrus production in the United States, which is the third largest sweet orange producer in the world after Brazil and China (Hodges et al., 2014; USDA/NASS, 2015). The Florida citrus industry had been valued approximately at $ 10.8 billion in industry output, providing 62,000 jobs in 2012–13 (Hodges et al., 2014; USDA/NASS, 2015). However, citrus production has been threatened by several recently introduced pathogens in Florida, including *Xanthomonas axonopodis* pv. *citri*, causing citrus canker (Gottwald et al., 2001), *Candidatus* Liberibacter asiatus, associated with citrus huanglongbing (Narouei-Khandan et al., 2016; Shen et al., 2013), and since 2010, *Phyllosticta citricarpa* McAlp. van der Aa, teleomorph *Guignardia citricarpa* Kiely, causing citrus black spot (CBS) (Er et al., 2012, 2013; Schubert et al., 2012; Zavala et al., 2014). As a result, the citrus industry has declined over the past 14 years with reductions in revenues, employment, bearing acreage and yield (Hodges et al., 2014; USDA/NASS, 2015).

CBS was first reported in Collier County, South Florida, in March 2010 (Schubert et al., 2012). From there, CBS has spread to two neighboring counties (Hendry and Lee) in South Florida and was found once in another county (Polk) in mid Florida within six years (Er et al., 2013; FDACS, 2016). CBS infected fruits are not marketable as fresh fruit due to unsightly lesions (Er et al., 2012). When plants are severely infected the fruit can fall prematurely, leading to significant yield losses. Crop losses per season due to fruit drop prior to harvesting fruit with CBS have been estimated at 22% in Ghana (Brentu et al., 2012). Up to 80% fruit loss has been recorded in untreated groves in Australia and South Africa (Araújo et al., 2013; Kotzé, 1981). This disease is now present in Asia (EPPO, 2006;

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Wang et al., 2012), many countries in Africa (Baayen et al., 2002; Brentu et al., 2012), several countries in South America (Garrán, 1996; Kotzé, 2000), and in Florida in North America (Er et al., 2012; Schubert et al., 2012). The disease is not yet present in Europe, Japan and some other countries, and *P. citricarpa* is a quarantine pest there (EFSA Panel on Plant Health, 2014). Import into the EU of citrus from regions positive for the disease is allowed under the condition of specific phytosanitary requirements like strict control measures and inspections (Baayen et al., 2002; EFSA Panel on Plant Health, 2014; Paul et al., 2005). Quarantine zones have been established around affected areas in Florida to prevent further spread in Florida and the U.S. which would result in severe losses nationally and jeopardize the export of fresh fruit to overseas markets. The potential economic losses due to the establishment of this disease without implementation of appropriate control strategies were estimated between 116 and 847 millions of U.S. dollars (USDA/APHIS, 2010).

The pathogen *P. citricarpa* is often accompanied by nonpathogenic strains of *Phyllosticta*, mostly identified as *P. capitalensis* (Baayen et al., 2002; Baldassari et al., 2008; Er et al., 2012; Glienke et al., 2011; Zavala et al., 2014). These non-pathogenic strains are typical endophytes that can have various beneficial properties like fending off insect pests and pathogens or producing medicinal metabolites and hydrolytic enzymes (Compant et al., 2016; Rodrigues et al., 2000). They seem to be dominant in fallen citrus leaves (Brentu et al., 2012; Hu et al., 2014), and their growth could possibly be stimulated by addition of organic materials to the orchard floor. Organic materials also could enhance decomposition of leaves (Boopathy et al., 2001) and competition by a variety of nonpathogenic fungi (Santos et al., 2016). The composition of endophytic fungi, including *Phyllosticta* species, is likely to be dependent on soil health management (Johnston, 1998; van Bruggen et al., 2016).

The epidemiology of CBS is similar to that of the well-known disease apple scab caused by *Venturia inaequalis*. In most areas, the initial inoculum comes primarily from decomposing infected leaves on the orchard floor where pseudothecia are formed, which release sexual ascospores into the air after repeated wetting and drying cycles in winter and spring. Ascospores are deposited on the surfaces of twigs, young leaves and fruit where they germinate and infect underlying tissues under moist conditions (Aylor and Flesh, 2001; Kotzé, 1981). While leaf symptoms are common for apple scab, they are rare for CBS despite infection by *P. citricarpa*. Citrus fruits can remain susceptible to *P. citricarpa* for 4–7 months after petal fall (Brentu et al., 2012), and latent infection is common (Aguiar et al., 2012; Er et al., 2013). Black spot symptoms may appear 6 months after fruit set or even after harvest (Er et al., 2013). Asexual spores, conidia, are produced in pycnidia on fruits, twigs and sometimes on leaf litter, are splash-dispersed and can infect other fruits or leaves (Perryman et al., 2014). Early on, it was postulated that conidia do not play an important role in the epidemiology of CBS (Kotzé, 1981). However, more recently, conidia were considered important in the epidemiological cycle (Spósito et al., 2011). In Florida, the relative roles of conidia versus ascospores were unknown at the start of this research.

Citrus black spot is difficult to manage because of the extended period of leaf drop and potential ascospore release, the long period of fruit susceptibility, and the simultaneous presence of young and mature fruit on some cultivars. Currently, CBS is controlled primarily by using fungicides during critical periods for fruit infection (Agostini et al., 2006; Dewdney et al., 2013, 2016; Hincapie et al., 2014; Kotzé, 1981; Makowski et al., 2014; Possiede et al., 2009). However, the long period of fruit susceptibility makes successful disease control difficult and increases citrus production costs as a consequence of the high number of fungicide applications needed (Makowski et al., 2014). Therefore, alternative measures of CBS control that are more effective and minimize harmful side effects to the environment are needed.

Various alternative and complementary control measures that are part of integrated orchard management have been developed and used for apple scab control (Holb et al., 2006). Ascospore production in fallen leaves has been targeted by sanitation treatments, including plowing or leaf removal, or by enhanced decomposition of leaves, including treatment with urea, dolomitic lime, sugar beet pulp (vinasse), molasses, fungal antagonists, and shredding of leaf litter (Heijne et al., 2006a,b; Spotts et al., 1997; Vincent et al., 2004). Apple leaves were degraded faster by humus forming earthworms (*Lumbricus* spp.) when the leaves had been treated with vinasse compared to a urea or glucose treatment and an untreated control (Heijne et al., 2006a). Leaf degradation was accelerated and ascospore production by *V. inaequalis* was reduced at increasing rates of vinasse application (Heijne et al., 2006b). Urea had a negative effect on leaf consumption by earthworms, probably because ammonia is a repellent and toxic to earthworms. However, in some years, the urea treatment reduced the number of ascospores more than vinasse (Heijne et al., 2006b). Various microorganisms have been applied on intact leaves or leaf litter to enhance its decomposition (Carisse and Dewdney, 2002; Vincent et al., 2004). Finally, ascospore release to the atmosphere can be impeded by a mulch cover (Aylor and Flesh, 2001). Altogether, these alternative treatments have resulted in up to 95% reduction in initial apple scab inoculum (Holb et al., 2006). However, it is not known whether the methods that are successfully used for apple scab control will be effective for CBS management.

In citrus, application of urea (Bellotte et al., 2009) or dolomitic lime (Dewdney et al., 2016; Mondal and Timmer, 2003) has been recommended to enhance leaf litter decomposition. Urea and ammonium sulfate application on leaf litter were thought to reduce the pathogen without enhancing leaf decomposition (Dewdney et al., 2016; Mondal and Timmer, 2003). Urea applications on leaf litter are already made by citrus growers in Florida, but ammonia released from the urea may be toxic to earthworms that enhance litter decomposition (Holb et al., 2006). Thus, not much is known about the effects of the application of various compounds on citrus leaf litter decomposition. Contrary to sugar beet pulp (vinasse), sugar cane pulp (bagasse) is readily available in South Florida, at relatively short distances from citrus groves. This material has not been tested for potential enhancement of leaf litter decomposition. For different purposes, enhancement of the decomposition of sugar cane residues has been accomplished by a consortium of cellulolytic fungi and bacteria (Beary et al., 2002). This microbial consortium has not been tested to enhance the decomposition of citrus leaves with or without bagasse. Unlike decomposition of litter from forest trees (Tuomi et al., 2009) and grasses (Bloemhof and Berendse, 1995), decomposition of citrus leaf litter with or without amendments has not been studied. Litter decomposition rates are strongly dependent on chemical composition, moisture and temperature (Bloemhof and Berendse, 1995; Mondal and Timmer, 2002; Talbot and Treseder, 2012; Tuomi et al., 2009), but effects of these factors on citrus leaf decomposition have not been studied. The ultimate aim of the research presented here is to contribute to the integrated management of CBS. The immediate objectives were to (a) test the efficacy of waste products from the cane sugar industry (bagasse), a microbial consortium, and urea on leaf litter decomposition, (b) relate relative decomposition rates to chemical composition and moisture content of citrus leaves, and (c) determine the effects of various amendments on the survival of *P. citricarpa* on citrus leaves.