Didymella bryoniae Isolates from Watermelon Seedlings in Florida Transplant Houses and Their Sensitivity to Boscalid

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Gummy stem blight caused by the ascomycete *Didymella bryoniae* (anamorph *Phoma cucurbitacearum* (Fr.:Fr.) Sacc.) was first observed in the field on Florida watermelons in 1917. It is one of the most destructive pathogens of cucurbits, producing primarily foliar, stem, and vine symptoms (gummy stem blight, GSB) on watermelon and cantaloupe in Southwest Florida, and fruit symptoms (black rot) on pumpkin and winter squash. The gummy stem blight pathogen is dispersed within and between geographical areas via wind and on or in seed. Once established, *D. bryoniae* is soil-borne, surviving on crop residue and weeds such as wild citron, balsam pear, or volunteer cucurbits. In transplant houses, GSB may begin with a single infected seedling and disperse throughout the tray through seedling-to-seedling contact (Keinath 2013) and within the transplant house by contaminated equipment and water. Control measures for the disease include the use of pathogen-free seeds, phytosanitary programs during transplant production, crop rotation, deep tilling of crop residue, and fungicides to reduce inoculum. In the fields, fungicides are the primary method of GSB control; however, the development of fungicide resistance limits the number of effective chemicals available for control (Keinath et al., 2007; Keinath and Zitter 1998; Malathrakis and Vakalounakis 1983; Stevenson et al. 2004). In Florida, boscalid-resistant *D. bryoniae* isolates have been noted in watermelon fields in Desoto, Hendry, Collier, Suwannee, and Lafayette counties (Stevenson et al. 2012). The purpose of this study was to examine why GSB was not being controlled in watermelon fields without a previous history of boscalid use and whether or not boscalid resistance could have originated from the transplants. Therefore, we evaluated *D. bryoniae* isolates collected from three Florida transplant houses for resistance to boscalid and compared the molecular mechanism of this resistance to that in the literature on isolates from North and South Carolina as well as Georgia.

In 2012, watermelon seedlings displaying typical symptoms and signs of GSB were collected from three commercial transplant producers in southwest Florida (Fig. 1). Thirty-nine isolates of the pathogen were obtained and tested for boscalid resistance using a mycelia growth assay on boscalid-amended media. The succinate dehydrogenase (SDHB) subunit from five select boscalid-sensitive and six select boscalid-resistant isolates was sequenced (Interdisciplinary Center for Biotechnology Research (ICBR) at the University of Florida) for further characterization. Briefly, mycelia plugs taken from the edge of 7-day-old cultures were transferred to PDA amended with 3.0 µg/ml technical grade boscalid (dissolved in acetone) (Stevenson et al. 2008; Thomas et al. 2012). Control media consisted of PDA amended with 1.0 ml/liter acetone. Plates were incubated at 25°C in the dark and relative colony growth (RCG) determined on day 4. Boscalid-resistant isolates were defined as having a RCG greater than 0.2. Both boscalid-sensitive and -resistant isolates were found in the three transplant houses, and recovered isolates resistant to boscalid ranged from 27–50% per house. Overall, 16 isolates (41%) were resistant to boscalid with RCG ranging from 0.59–0.91. *D. bryoniae* isolates from seedlings in transplant houses 1, 2, and 3 yielded three (3/11; 27%), four (4/10; 40%), and nine (9/18; 50%) isolates that were insensitive to boscalid, respectively (Fig. 2). Sequencing of the SDHB subunit indicated that the Fe-S cluster [4Fe-4S] was conserved between all isolates; however, resistant isolates displayed a single nucleotide polymorphism in the iron-sulfur cluster [3Fe-4S] (Fig. 3) resulting in H277Y or H277R consistent with reference sequences HQ156462.1 and HQ156464.1, respectively (Avenot et al., 2012).

Because boscalid-resistant isolates were found in this study, it is clear that transplants play a role in the introduction of boscalid-resistant *D. bryoniae* isolates into the field. Knowledge of the sensitivity or resistance of *D. bryoniae* isolates on transplants to boscalid prior to field transplanting will allow cucurbit producers to make informed disease management decisions. This knowledge will allow producers, researchers, and chemical companies to reduce the population of resistant isolates through effective control with fungicides other than boscalid. Additionally, the development of disease screening assays prior to seed grow-out will further add another level of protection to cucurbit producers and the industry at large.

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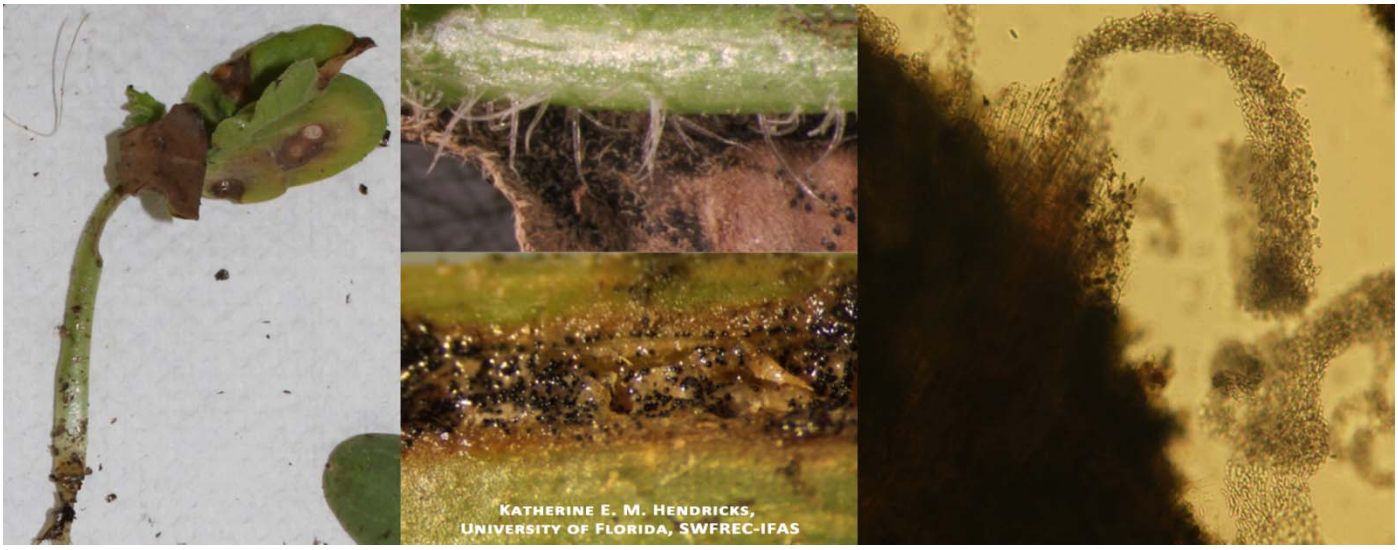

FIGURE 1

Watermelon seedling (left) exhibiting water-soaked lesions on the leaves and stem characteristic of gummy stem blight; leaf (center, top) and stem (center, bottom) showing small, black fruiting bodies (pycnidia or pseudothecia); and "spore horns" (right) containing thousands of conidia oozing from pycnidia.

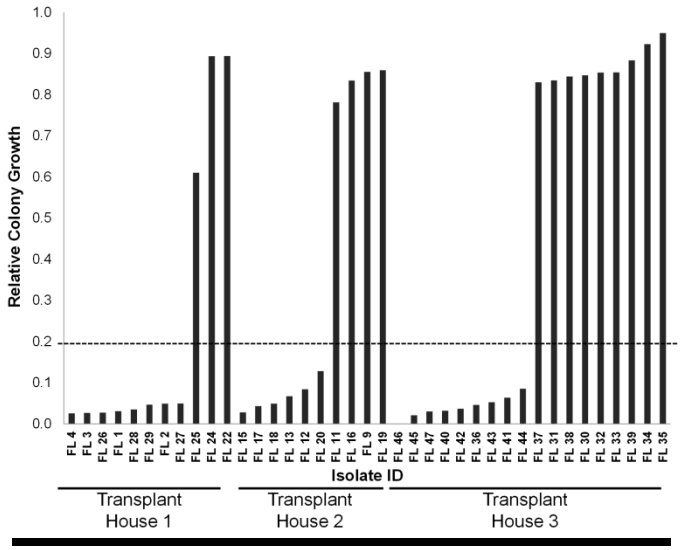

FIGURE 2

Relative colony growth (RCG) of *Didymella bryoniae* isolates on media amended with 3 mg boscalid per liter (3 µg/ml), organized according to increasing RCG and Florida watermelon transplant house. Isolates were considered resistant if RCG > 0.2

FIGURE 3

Partial alignment of the deduced amino acid sequence of the [4Fe-4S] and [3Fe-4S] cysteine-rich clusters of the iron sulfur gene SDHB (highlighted in gray) from 11 *D. bryoniae* isolates selected based on their sensitivity (S) and resistance (R) to boscalid; HQ156462.1 and HQ156464.1 are resistant to boscalid; HQ156461.1 is sensitive. Amino acid residues H, Y and R represent histidine, tyrosine and arginine respectively with an H-to-Y or -R substitutions within the [3Fe-4S] cysteine-rich cluster conferring resistance to Boscalid.

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