Fungal Genetics and Biology 96 (2016) 25–32

Contents lists available at ScienceDirect

Fungal Genetics and Biology

journal homepage: www.elsevier.com/locate/yfgbi

Regular Articles

Compression tests of *Fusarium graminearum* ascocarps provide insights into the strength of the perithecial wall and the quantity of ascospores

Ray F. David [a], Michael Reinisch [a], Frances Trail [b], Linsey C. Marr [a], David G. Schmale III [c,*]

[a] Department of Civil and Environmental Engineering, 411 Durham Hall, Virginia Tech, Blacksburg, VA 24061, USA
[b] Departments of Plant Biology, and Plant, Soil and Microbial Sciences, Michigan State University, East Lansing, MI 48824, USA
[c] Department of Plant Pathology, Physiology, and Weed Science, 413 Latham Hall, Virginia Tech, Blacksburg, VA 24061, USA

ARTICLE INFO

Article history:
Received 30 March 2016
Revised 2 September 2016
Accepted 25 September 2016
Available online 28 September 2016

Keywords:
Fungus
Fusarium graminearum
Fusarium head blight
Perithecium
Ascospore
Mechanical properties
Forcible discharge

ABSTRACT

The plant pathogenic ascomycete *Fusarium graminearum* produces perithecia on corn and small grain residues. These perithecia forcibly discharge ascospores into the atmosphere. Little is known about the relationship among the strength of the perithecial wall, the age of the perithecium, and the quantity of ascospores produced. We used a mechanical compression testing instrument to examine the structural failure rate of perithecial walls from three different strains of *F. graminearum* (two wild type strains, and a mutant strain unable to produce asci). The force required to compress a perithecium by one micrometer (the mean perithecium compression constant, MPCC) was used to determine the strength of the perithecial wall. Over the course of perithecial maturation (5–12 days after the initiation of perithecial development), the MPCC was compared to the number of ascospores contained inside the perithecia. The MPCC increased as perithecia matured, from 0.06 N μm^{-1} at 5 d to 0.12 N μm^{-1} at 12 d. The highest number of ascospores was found in older perithecia (12 d). The results indicated that for every additional day of perithecial aging, the perithecia become more resilient to compression forces. Every additional day of perithecial aging resulted in ~900 more ascospores. Knowledge of how perithecia respond to external forces may provide insight into the development of ascospores and the accumulation of turgor pressure. In the future, compression testing may provide a unique method of determining perithecial age in the field, which could extend to management practices that are informed by knowledge of ascospore release and dispersal.

1. Introduction

The fungus *Fusarium graminearum* causes Fusarium head blight (FHB) of wheat and barley (Goswami and Kistler, 2004). Between 1990 and 2000, FHB caused more than $3 billion in crop losses in the United States and $220 million in Quebec and Ontario (McMullen et al., 1997; Paulitz, 1999; Schmale III and Bergstrom, 2003; Windels, 2000). Because *F. graminearum* produces trichothecenes such as deoxynivalenol, the fungus causes adverse health effects (e.g., vomiting and nausea) in swine and humans if ingested in feed or finished food products, respectively (Desjardins et al., 1993; Snijders, 1990; Sutton, 1982). *Fusarium graminearum* forcibly discharges ascospores from perithecia at high acceleration rates from sources of inoculum such as crop debris (Goswami and Kistler, 2004; Guenther and Trail, 2005; Trail et al., 2005). The ascospores can be transported >500 m in the atmosphere to susceptible crop fields (Prussin II et al., 2014a; Sutton, 1982).

Perithecia of *F. graminearum* measure 150–175 μm in diameter and have a perithecial wall about 25–50 μm thick (Seifert, 1996). The perithecial wall is composed of three distinct layers: an 8–25 μm outer wall, a 6.5–13 μm middle layer, and a 4–7 μm inner layer. The cells transition from ellipsoidal in the outer wall to more elongated in the inner layer (Seifert, 1996). The ostiole (pore through which ascospores are discharged) is delineated by cells in the upper wall (Trail and Common, 2000). Inside the wall, the centrum develops, forming asci from rounded cells of the ascogenous system, and the apical paraphyses (sterile hyphae) that grow down from the upper wall (Trail and Common, 2000). Ascospore-containing asci develop within the inner portion of each perithecium between paraphyses that collapse as the asci develop (Trail and Common, 2000). The asci stretch upward in the perithecium, and ascospores develop within the asci in two rows (biseriate) with eight ascospores per ascus (Seifert, 1996; Trail and Common, 2000). The

* Corresponding author.
E-mail address: dschmale@vt.edu (D.G. Schmale III).

http://dx.doi.org/10.1016/j.fgb.2016.09.003
1087-1845/

ascospores discharge through a pore at the end of the ascus that extends through the ostiole (Trail and Common, 2000).

A conceptual model for ascospore release includes four essential steps, including a cue to release mature ascospores and the accumulation of sufficient turgor pressure (Trail and Seminara, 2014). High levels of relative humidity (Inch et al., 2005; Paul et al., 2007; Paulitz, 1996; Trail et al., 2002) and low air temperature (Del Ponte et al., 2009; Fernando et al., 2000; Sutton, 1982) have been correlated with *F. graminearum* ascospore discharge. Additionally, several meteorological conditions have been identified as causal agents of ascospore release (David et al., 2016a), and the numbers and distances of ascospore release have been investigated using 3D-printed discharge devices indicating differences based on temperature and relative humidity (David et al., 2016b). A laboratory-based study of ascospore discharge identified forces of 870,000 g during ascospore release and suggested that fluctuations in Cl^- and K^+ ions may play a role in ascospore release (Trail et al., 2005).

Little is known about the relationship between the strength of the perithecial wall, the age of the perithecium, and the quantity of ascospores produced (Sikhakolli et al., 2012). Paraphysis degeneration is necessary as perithecia mature to provide space within the structure to accommodate all relevant perithecial structures (Sikhakolli et al., 2012; Trail and Common, 2000). Paraphyses maintain functional membranes such that changes in humidity may result in the swelling of the perithecia and pressure changes within the asci and perithecia (Trail and Seminara, 2014). An unresolved issue is whether differences in perithecia age would result in differences in numbers of ascospores released under similar meteorological conditions. These factors would affect the pressure within the perithecium that may drive spore release. Research into these questions could extend to other model fungi such as *Neurospora crassa* (Davis and Perkins, 2002; Galagan et al., 2003) and *Venturia inaequalis* (Aylor and Anagnostakis, 1991; Stensvand et al., 1997).

The mechanical testing of biological materials represents a unique approach to help advance fundamental understanding of biological processes, improve the design of biological applications, and inform the development of bio-inspired materials (Meyers et al., 2008). The investigation of biofilms of the bacterium *Pseudomonas aeruginosa* using a film rheometer identified a compression speed associated with biofilm failure, providing valuable information on its mechanical stability (Körstgens et al., 2001) that would be useful when designing systems to prevent biofouling of membranes (Flemming, 2002). Compression testing has been used on materials ranging from the horseshoe crab *Limulus polyphemus* exoskeletons to shells of the abalone, *Haliotis refescens*, highlighting compressive forces that result in failure (Chen et al., 2008). Atomic force microscopy was used to analyze elastic properties of hyphae of the fungus *Aspergillus nidulans*, showing that the elasticity of the cell wall may be impacted by conditions within the growth medium (Zhao et al., 2005). Force-deformation relationships were obtained to the point of cell failure by mechanically compressing cells of *Saccharomyces cerevisiae*, and a correlation was found between deformation and compression force at failure (Smith et al., 2000a,b).

We hypothesized that older perithecia would be able to resist greater amounts of compressive force and would contain greater numbers of mature ascospores. The specific objective of this study was to determine the force-deformation relationship of *F. graminearum* perithecia at different ages. Perithecia ranging from 5 days old to 12 days old after the initiation of perithecial development were tested to structural failure by compression forces. The relationship between age of perithecia, compression force-deformation values, and ascospore number was determined. Enhanced understanding of the association between age of the perithecium and ascospore number provides additional information on ascospore emission rate (Prussin II et al., 2014b) that will be valuable for models of the spread of FHB. The results from this study, combined with knowledge about the effect of meteorological conditions on ascospore release (David et al., 2016a; Inch et al., 2005; Paulitz, 1996; Reis, 1990; Tschanz et al., 1975), could improve predictions of ascospore release under field conditions and the management of FHB. In the future, compression testing may provide a unique method of determining perithecial age in the field, and could inform management practices that depend on knowledge of ascospore release and dispersal.

2. Materials and methods

2.1. Generation of perithecia of Fusarium graminearum

Perithecia were generated from three strains of *Fusarium graminearum*: wild-type strain Fg_Va_GPS13N4_3ADON (hereafter referred to as FgVa) used in prior field studies in Virginia (Prussin II et al., 2014a,b), wild type PH-1 (NRRL 31084) (Cuomo et al., 2007; Trail and Common, 2000; Trail et al., 2002), and mutant of gene FGSG_04417 (a mutant of PH-1 with no asci but a fully developed perithecial wall).

Perithecia were generated on carrot agar (Burgess and Sydney, 1994; Klittich and Leslie, 1988; Leslie et al., 2006). Prior to the initiation of the cultures, a sterile 7-cm diameter filter paper (09-801A, Fisherbrand, Waltham, MA) was placed on the surface of carrot agar medium in 100-mm Petri dishes. The cultures were incubated at ambient room temperature (23 °C) and relative humidity (RH, 40%). After ~6 d, the plates were flooded with 1 mL of 2.5% Tween 60 solution (P1629, Sigma, St. Louis, MO) and the aerial mycelium was flattened using a sterilized rod. The cultures were then placed under a 12 h/12 h light/dark cycle at room temperature and RH until perithecia formed on the filter paper.

Experiments took place with perithecia ranging in age from 5 d to 12 d (measured following addition of 2.5% Tween 60 to the plates at 0 d). Prior to each compression trial, an individual perithecium was extracted from the carrot agar at the specified age using a scalpel and forceps. The sample was then placed onto a testing tip (described below) for compression tests.

2.2. Uni-axial compression testing instrument

A uni-axial compression testing instrument (Kammarth & Weiss, Tensile/Compression Module 5kN, Dortmund, Germany) was used to determine the force required to compress the perithecia and ultimately result in failure of the perithecial wall. The instrument is capable of applying a load of up to 5000 N. Traditional compression tests produce two distinct regimes: a linear elastic regime indicating the relationship between stress and strain that is due to bending of the material (Flores-Johnson et al., 2008) and a plastic regime where permanent deformation occurs and the stress is reduced to zero (Lubarda and Lee, 1981). The transition from the elastic to plastic regime is known as the yield point (Lade, 1977) and defined as the collapse of the perithecial wall.

Each perithecium was tested in a load frame, which was modified using two 1.27-mm slotted-head, 0.32-mm pin, aluminum mounts designed for use in scanning electron microscopy (SEM) (commonly known as SEM stubs) (16111, Ted Pella Inc., Redding, CA), as shown in Fig. 1. The failure surface was the wide, flat top of the stub, while the pin end was secured to one end of the load frame. The testing surface was composed of two specimen mounts that were adhered together head to head. One pin end was secured to the opposite end of the load frame from the failure surface. The other pin end was modified using a rotary grinder to create a