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

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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 to 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 $0.06 \text{ N } \mu\text{m}^{-1}$ at 5 d to $0.12 \text{ N } \mu\text{m}^{-1}$ 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.

Keywords: fungus; *Fusarium graminearum*; Fusarium head blight; perithecium; ascospore; mechanical properties; forcible discharge

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46 **1 Introduction**

47 The fungus *Fusarium graminearum* causes Fusarium head blight (FHB) of wheat and barley
48 (Goswami and Kistler, 2004). Between 1990 and 2000, FHB caused more than \$3 billion in crop losses in
49 the United States and \$220 million in Quebec and Ontario (McMullen et al., 1997; Paulitz, 1999; Schmale
50 III and Bergstrom, 2003; Windels, 2000). Because *F. graminearum* produces trichothecenes such as
51 deoxynivalenol, the fungus causes adverse health effects (e.g., vomiting and nausea) in swine and humans
52 if ingested in feed or finished food products, respectively (Desjardins et al., 1993; Snijders, 1990; Sutton,
53 1982). *F. graminearum* forcibly discharges ascospores from perithecia at high acceleration rates from
54 sources of inoculum such as crop debris (Goswami and Kistler, 2004; Guenther and Trail, 2005; Trail et
55 al., 2005). The ascospores can be transported > 500 m in the atmosphere to susceptible crop fields
56 (Prussin II et al., 2014a; Sutton, 1982).

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

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), will 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; Prussin II et al., 2014b), 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). The generation and characterization of this mutant has been reported elsewhere (Trail et al., *submitted*).

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/12h 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 5,000 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 **Figure 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 smaller, flat surface that served as the testing tip. During each trial, a single perithecium was adhered to the testing tip using a thin layer of nonconductive adhesive (16079, Ted Pella Inc., Redding, CA).

Perithecia were tested at each age ranging from 5 to 12 d ($n = 12$ for each age). Because testing was destructive, a different group of perithecia was used for each age of interest. The testing surface with the adhered perithecium was secured into the load frame, and a small clearance of ~ 0.15 mm between the perithecium and the failure surface was established prior to the start of the compression test. As illustrated in **Figure 1**, the perithecia were tested in the horizontal direction. The compression instrument had a displacement speed of $1 \mu\text{m s}^{-1}$. The trials ended once a load of 50 N had been applied to the sample, providing ample opportunity to observe the elastic regime, yield point, and plastic regime (i.e., rupture of the perithecial wall).

Four polymer samples with known hardness properties were used as controls. The materials tested (with durometer values indicating polymer hardness) included latex (38D to 40D), gum rubber (40D), santoprene (55D), and oil resistant vinyl (70D). Each polymer sample was 1.6 mm thick, and three replicates of each control material were subjected to compression testing according to the same protocol as used for the perithecia.

2.3 Analysis of compression data

The compression data was analyzed using MATLAB 8.4 (MathWorks Inc., Natick, MA). For each replicate, a plot of force applied (N) vs. deformation (μm) was created. An example plot is shown in **Figure S1**. At small deformations, the perithecium remained in the elastic region, where force increased linearly with deformation. As deformation increased, the perithecium reached the yield point, which indicated the amount of force required to rupture the perithecium. Large, non-linear changes in compression force versus displacement corresponded to the plastic zone, where the perithecium had been permanently damaged (i.e., ruptured).

Initially, the start and end of the elastic zone for each sample were determined, and the corresponding slope of the resulting line was calculated. The start was defined as where at least three sequential force measurements increased (indicating sample contact and compression), thus omitting data points corresponding to the initial void (~ 0.15 mm clearance) between the perithecium and the failure surface. The end of the elastic regime was defined by a displacement of $25\ \mu\text{m}$ following initial contact with the perithecium. This displacement span was selected because all tests had a displacement span of at least $25\ \mu\text{m}$ in the elastic regime, although data points beyond $25\ \mu\text{m}$ and still within the elastic regime were excluded from the analysis. A linear fit was applied to the elastic regime, and the slope of the fitted line was calculated. This slope was denoted the mean perithecium compression constant (MPCC) ($\text{N}\ \mu\text{m}^{-1}$), signifying the amount of compression force required to compress the perithecium by $1\ \mu\text{m}$. The MPCC was defined as positive.

The yield point (N) was obtained by determining the compression force at which the elastic zone transitioned into the plastic zone. This point was identified by initially determining the slope between the compression force and the deformation of perithecia over $20\text{-}\mu\text{m}$ segments of the curve. The difference in slope between successive segments was calculated, and the yield point was defined as the force at which the slopes changed by $>6\%$ over each of three adjacent segments. The yield point was visually verified to fall within the elastic-plastic transition area and not the noisy region at the start of most trials. Once the

location of the yield point was established, the corresponding force was determined by obtaining the median value of the three consecutive 20- μ m segments used to obtain the slopes.

2.4 Quantification of ascospores

The number of ascospores contained in each perithecium was determined as a function of age. Because the procedures were destructive, different perithecia were used from those subjected to compression testing. For each of the ages investigated, a perithecium was extracted from carrot agar using a scalpel and forceps. The perithecium was placed inside of a 0.6-mL microcentrifuge tube with flat cap (02-681-240, Fisher Scientific, Waltham, MA). A metal rod was used to rupture the perithecium in a similar manner to perithecial failure during compression testing. The perithecium and released ascospores were centrifuged at 2000 g for ~5 s. The ascospores were subsequently re-suspended in 12 μ L of sterile water. Ascospores were quantified using 10 μ L of the re-suspended ascospore solution ($n = 10$ for each maturation day) placed within a hemocytometer (3100, Hausser Scientific, Horsham, PA) and quantified using a light microscope at 40x magnification.

2.5 Statistical Analyses

Statistical analyses were conducted using JMP Pro 10.0.2 (SAS Institute Inc., Cary, NC). Statistical significance was defined at a level of 0.05 for all analyses. Tukey's pairwise comparisons test ($P < 0.05$) was used to determine whether differences were significant between all pairwise combinations of different ages of perithecia. Least-squares regression analysis was used to identify significant linear relationships between variables.

3 Results

3.1 Relationship between perithecial age and compression force

At day 5 after initiation of perithecial development, the perithecium wall was fully formed and the perithecia contained some asci with mature spores. This was the first day that forcible ejection of ascospores was observed. Ninety-six perithecia representing eight ages (5 d to 12 d) were subjected to compression tests (12 perithecia per time point, selected from a single petri plate). The perithecia selected did not exhibit cirrhous formation: the exudation of asci, *en masse*, without forcible firing. The MPCC ranged from 0.060 (5 d) to 0.12 N μm^{-1} (12 d), where a larger value indicates a larger compressive force required to squeeze perithecia by 1 μm . Differences were significant (Tukey's pairwise comparisons, $P < 0.05$) between younger ages (5 d to 8 d) and 12 d: 5-day-old vs. 12-day-old ($P < 0.01$); 6-day-old vs. 12-day-old ($P < 0.01$); 7-day-old vs. 12-day-old ($P < 0.01$); and 8-day-old vs. 12-day-old ($P = 0.029$). The strong linear fit ($R^2 = 0.77$) shown in **Figure 2** indicates that as perithecia matured from 5 d through 12 d, a larger compressive force was required to produce failure of the perithecial structure. The slope of the line indicates that for each additional day perithecia matured, they were able to withstand an additional 0.007 N of force to compress the perithecia structure by 1 μm .

Least-squares regression analysis identified a significant linear relationship ($P < 0.01$) between the MPCC of the isolate (FgVa) of *F. graminearum* and age of perithecia. The relationship between age of perithecia and MPCC was not significant for the mutant strain 4417 ($P=0.54$) and the parent strain PH-1 ($P=0.31$), as shown in **Figure 2**. The MPCC's for PH-1 (0.0013 N μm^{-1} per day) and FgVa (0.007 N μm^{-1} per day) had the same magnitude. The slope of the mutant strain with no developed asci was lower: 0.0009 N μm^{-1} per day. The wild-type strains of parent strain PH-1 (range of std. error values: 0.0026 to 0.012 N μm^{-1}) and FgVa (range of std. error values: 0.0064 to 0.016 N μm^{-1}) had similar variability within perithecial ages while the mutant 4417 had less variability (range of std. error values: 0.0029 to 0.0095 N μm^{-1}).

The yield point of the FgVa, PH-1, and 4417 strains generally increased with age (**Fig. S2**), though differences between ages and least-squares linear regression analysis were not significant. The 8-day-old yield point for the FgVa dataset was an outlier, and if it were removed from the least-squares regression analysis, a significant linear fit ($P = 0.04$) was identified.

The compression tests performed on polymers demonstrated the ability of the compression testing instrument to detect differences between materials (**Fig. S3**). There were significant differences between latex vs. oil resistant vinyl ($P < 0.01$); gum rubber vs. oil resistant vinyl ($P < 0.01$); and santoprene vs. oil resistant vinyl ($P < 0.01$). Differences were not detected among the materials with overlapping or close durometer values (latex vs. santoprene, latex vs. gum rubber, and santoprene vs. gum rubber).

3.2 Relationship between perithecia age and number of ascospores

The highest numbers of ascospores were found in older (more mature) perithecia. There was a significant difference in number of ascospores between the younger (immature) and older (mature) perithecia. The pairs identified as significantly different by Tukey's pairwise comparisons were 5-day-old vs. 12-day-old ($P < 0.01$); 6-day-old vs. 12-day-old ($P < 0.01$); 7-day-old vs. 12-day-old ($P < 0.01$); 8-day-old vs. 12-day-old ($P = 0.036$); and 5-day-old vs. 11-day-old ($P = 0.036$). **Figure 3** shows the strong linear relationship ($R^2 = 0.94$) between number of ascospores and perithecia age. As perithecia aged, greater number of ascospores were produced. For every additional day of perithecial aging, about 877 ascospores were produced in each perithecium.

3.3 Relationship between compression force and number of ascospores

There was a linear relationship between the number of ascospores within perithecia and the perithecial structure's MPCC. The lowest number of ascospores corresponded to the lowest MPCC, and ascospore count increased with MPCC as perithecia matured (**Fig. S4**). An additional 1,027 ascospores are contained within a perithecium for every increase in MPCC by 0.01 N per micrometer.

4 Discussion

Few published studies have investigated the mechanical properties of living cells. Studies on yeast cells (*Saccharomyces cerevisiae*) found a correlation between bursting force and the deformation at failure due to compression (Smith et al., 2000b). A study of *Zea mays* (maize) found that lignin concentrations increased with age and then plateaued (Morrison et al., 1998), indicating that this complex organic polymer known for supplying structural integrity (Buxton and Casler, 1993) develops over time. A light microscopy study of *F. graminearum* development observed distinct changes as **perithecial development** is initiated; distinct outer and inner wall layers appear around the ascogoneous system (Trail and Common, 2000). The centrum (central cavity) then develops with apical paraphyses forming from the upper wall, the ostiole establishing at one pole of the perithecium, the asci developing between collapsed paraphyses, and the ascospores arranging biserially within the asci (Trail and Common, 2000). Additionally, **perithecial development** includes development of chitin, which provides a resilient fibrous network, comparable to steel on a mass basis, enough to withstand external forces (Latgé, 2007; Lenardon et al., 2010; Munro and Gow, 2001). Here, we used compression testing to rupture perithecia of *F. graminearum*. We showed that the force required to rupture perithecia of strain FgVa was associated with the number of ascospores inside the ascocarp. To our knowledge, this is the first detailed report of compression testing of fruiting bodies of a fungus.

The force required to permanently deform perithecia increased as perithecia matured. This increase in uni-axial compressive force required to cause the failure of perithecia may be attributed to its maturation that culminates with the production of asci filled with ascospores (eight per ascus) (Seifert, 1996). As a perithecium matures, the density of the perithecial contents will likely increase, contributing to the overall resilience of the ascocarp. There is a potential loss of contents through spore firing and cirrhous formation with previous research indicating peak spore firing occurring 6 to 9 days after tween application (Trail et al., 2002). A potential explanation for the increasing tolerance of older perithecia is

the senescence of the cell walls and the change in perithecial wall structures as perithecia age. An investigation of the *F. graminearum* perithecial development visualized the three layers of the periderm and observed the developmental changes of each wall (Trail and Common, 2000). The light microscopy study observed the thick-walled and thin-walled cells of the middle and inner periderm layers compressed as perithecia aged (Trail and Common, 2000). The strength provided by the periderm has been noted in studies of carrot roots noting that removal of the periderm resulted in increased levels of damage due to tensile forces (Hartz et al., 2005). A study of cortical bones determined that orientation of fibers and density were important in determining tensile strength of the bone (Martin and Ishida, 1989). The aging of perithecium and the related flattening and compressing of the middle and inner periderm layers may be factors that result in the changes in MPCC at different ages. Future work should also include the impacts of continuous ascospore firing and cirrhi formation (our tests did not include perithecia that produced cirrhi). The potential loss of contents due to these events may change the strength of perithecia. While the current study aimed to determine the applicability of compression testing of perithecia and used a baseline of perithecia without cirrhi (to measure similar populations of perithecia), the measurement of perithecia producing cirrhi could provide additional information on the integrity of the ascocarp. It is also important to note that the activity of an ascus may impact the nature of neighboring asci (Cavinder et al., 2012; Trail et al., 2005). Though there are limitations in selecting perithecia without cirrhi (potential bias towards specific developmental stages), and using different strains that may discharge ascospores at different rates (this was not measured in our study), our work sets the stage for future work to carefully dissect these elements with additional compression testing experiments.

The MPCC and perithecia age were not correlated for both the wild-type parent (PH-1) and the mutant strain (4417) unable to produce asci. While the mutant strain's flatter slope was anticipated due to the lack of asci-related development that may contribute to perithecial strength, the variability of the MPCC for both the PH-1 and 4417 strains among the days provided less clarity. Chitin levels, attributed to providing strength to fungal cells (Bowman and Free, 2006; Levitz, 2010), decreased and rates of growth slowed in *Saccharomyces cerevisiae* when chitin synthase was disrupted (Shaw et al., 1991). A

study of the impact of chitin synthase genes in *Fusarium oxysporum* wild-type and mutant strains found that chitin content in hyphae was reduced by 10% for mutant strains that had disrupted *chs1* and *chs2* genes (Martín-Udíroz et al., 2004). The variation in MPCC and perithecial age for PH-1 and 4417 may be associated with differences in periderm development that contains layers that compress as the perithecium ages (Trail and Common, 2000), but additional compression tests on more mutants would be needed to test this hypothesis. We speculate that observed MPCC differences between the two wild-type strains were largely due to variations of the perithecial wall and structure. Differences in developmental time and the timing of ascospore release may also contribute to these differences, but we did not conduct experiments to dissect these potential factors. Additional work would benefit from the inclusion of many different wild-type strains that vary in perithecium size, cirrus formation, and ascospore release.

Ascospore numbers generally increased with age of perithecia for strain FgVa (ascospores were not quantified for PH-1). These findings are compatible with the study performed by Trail and Common (2000), who observed that meiosis and mitotic division produced the eight ascospores per ascus. A laboratory study found that the number of discharged ascospores increased up to approximately 5 d after perithecial maturity before decreasing until no release was observed (Prussin II et al., 2014b). The highest number of ascospores per perithecium corresponded with the largest compressive force required for perithecial-structure failure. This result suggests that the maturation of perithecia produces a more resilient structure and a higher potential risk of pathogen spread. The results seem to indicate a larger ascospore source at older ages, with the timing and number of the ascospores previously also linked to meteorological conditions (Del Ponte et al., 2009; Paulitz, 1996; Prussin II et al., 2014b; Sutton, 1982). Perithecial age and meteorological conditions appear to be important determinants of a release event and the severity of an event. Studies have shown the ascospore-containing asci are composed of long chitin fibrils and that covalent crosslinks between chitin and β -glucan provide a scaffold linked to the shape and strength of the cell wall (Lenardon et al., 2010; Munro and Gow, 2001; Ruiz-Herrera, 1991; Walker et al., 2010; Zhao et al., 2005). The forcible discharge of ascospores from asci at immense accelerations likely requires support from the rigid cell wall (Schmale III et al., 2005a; Schmale III and Ross, 2015; Trail,

2007; Trail et al., 2005). The perithecial wall may have the capability of withstanding outside compressive forces as well as the high forces experienced by the perithecia during ascospore release events (Trail et al., 2005).

The hardness of perithecia may provide useful information regarding the age of the perithecium, but potentially more valuably, the amount of turgor pressure and the number of developed ascospores contained within the structure. It has been shown in both field investigations (David et al., 2016a; Inch et al., 2005; Paulitz, 1996) and laboratory studies (Trail et al., 2002; Tschanz et al., 1976) that meteorological parameters such as light (Schmale III et al., 2005b; Schmale III et al., 2006; Trail et al., 2002), relative humidity (David et al., 2016a; Paul et al., 2007; Paulitz, 1996), and temperature (Del Ponte et al., 2009; Fernando et al., 2000; Paulitz, 1996; Sutton, 1982) may be required at specific levels to trigger ascospore release events. The active release of ascospores from an opening in the ascus has been associated with high levels of relative humidity (David et al., 2016a; David et al., 2016b; Paulitz, 1996; Reis, 1990). The impact of relative humidity, along with other meteorological parameters, on the required compression forces on perithecia warrants future investigation. It has been hypothesized that asci swell as they mature (De Bary et al., 1887; Trail and Seminara, 2014), but it may be possible that changes in relative humidity may contribute to the swelling of the asci, independent of the age of ascocarp. While the perithecial wall provides strength to environmental conditions, it also must allow for release of ascospores through a pore (the ostiole) at the end of the ascus (Trail and Common, 2000). Additional research is required to fully elucidate the relationship of perithecial age and meteorological conditions on ascospore release.

In consideration of the hypothesis that asci of mature perithecia swell prior to discharge (De Bary et al., 1887; Trail and Seminara, 2014) due to the accumulation of K^+ and Cl^- ions (Trail et al., 2005), the greater compression forces identified for the mature perithecia may indicate that a sufficient buildup of ions has resulted in swelling and that the exposure of the perithecia to favorable meteorological conditions may produce a release event. On the other hand, less mature perithecia may not contain a prerequisite level of turgor pressure for ascospore discharge. Thus, the exterior compressive forces

required to result in failure may reflect an array of information like age of perithecia, number of ascospores available for discharge, and the level of turgor pressure accumulated within the perithecia. Additional mutant strains of PH-1 (with variable regulation of K^+ and Cl^- ion accumulation) would likely be needed to test these hypotheses. Mechanical testing of such mutant strains, and those strains with and without asci and ascospores, would provide additional information to determine the extent the perithecial wall, the number of ascospores, the level of turgor pressure, or other parameters may impact compressive failure.

The relationship between the mechanical strength of a perithecium and the number of ascospores it contains offers a unique approach to the management of FHB. While this study is specific to the ascocarps of the fungus *F. graminearum*, information on wall strength and quantity of spores may be obtained for other fungi including *Neurospora crassa* (Davis and Perkins, 2002; Galagan et al., 2003), and *Venturia inaequalis*. Such information may be useful to generate models that predict the risk of disease to relevant hosts. One could envision a portable compression testing instrument for field use that could provide estimates of the nature and timing of spore release to inform timely field management decisions, such as the application of fungicides. In a wheat field with various areas impacted by *F. graminearum*, information on the age of perithecia on within-field residues may inform the timing of fungicide applications (e.g., a grower might apply a fungicide at or near the point of maximum spore discharge) (Prussin II et al., 2014b). This information could be incorporated into an improved understanding of the emission rate of spores of *F. graminearum* (Aylor, 2003; Aylor et al., 2001; Prussin II et al., 2015; Prussin II et al., 2014b; Schmale III and Ross, 2015), particularly in regions of the world where local sources of inoculum (i.e., within field) are predominant. Moreover, this study has the potential to inform the development of fungicides that could target the perithecial wall and/or the firing of ascospores, which could offer some unique disease management strategies (Broders et al., 2007; Chen and Zhou, 2009; Li et al., 2008).

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6 References

- Aylor, D. E., 2003. Spread of plant disease on a continental scale: role of aerial dispersal of pathogens. *Ecology*. 84, 1989-1997. <http://dx.doi.org/10.1890/01-0619>.
- Aylor, D. E., Anagnostakis, S. L., 1991. Active discharge distance of ascospores of *Venturia inaequalis*. *Phytopathology*. 81, 548-551.
- Aylor, D. E., Fry, W. E., Mayton, H., Andrade-Piedra, J. L., 2001. Quantifying the rate of release and escape of *Phytophthora infestans* sporangia from a potato canopy. *Phytopathology*. 91, 1189-1196. <http://dx.doi.org/10.1094/PHTO.2001.91.12.1189>.
- Aylor, D. E., Sutton, T. B., 1992. Release of *Venturia inaequalis* ascospores during unsteady rain: relationship to spore transport and deposition. *Phytopathology*. 82, 532-540.
- Bowman, S. M., Free, S. J., 2006. The structure and synthesis of the fungal cell wall. *BioEssays*. 28, 799-808. <http://dx.doi.org/10.1002/bies.20441>.
- Broders, K., Lipps, P., Paul, P., Dorrance, A., 2007. Evaluation of *Fusarium graminearum* associated with corn and soybean seed and seedling disease in Ohio. *Plant Dis*. 91, 1155-1160. <http://dx.doi.org/10.1094/PDIS-91-9-1155>.
- Burgess, L. W., Sydney, N., 1994. Laboratory manual for *Fusarium* research. *Fusarium Research Laboratory*, Department of Crop Sciences, University of Sydney,
- Buxton, D., Casler, M., Environmental and genetic effects on cell wall composition and digestibility. In: H. Jung, (Ed.), *Forage Cell Wall Structure and Digestibility*. American Society of Agronomy, Inc., Madison, WI, 1993, pp. 685-714 <http://dx.doi.org/10.2134/1993.foragecellwall.c25>.
- Cavinder, B., Sikhakolli, U., Fellows, K. M., Trail, F., 2012. Sexual development and ascospore discharge in *Fusarium graminearum*. *J. Visualized Exp.* <http://dx.doi.org/10.3791/3895>.

- Chen, P. Y., Lin, A. Y. M., Lin, Y. S., Seki, Y., Stokes, A. G., Peyras, J., Olevsky, E. A., Meyers, M. A., McKittrick, J., 2008. Structure and mechanical properties of selected biological materials. *J. Mech. Behav. Biomed. Mater.* 1, 208-226. <http://dx.doi.org/10.1016/j.jmbbm.2008.02.003>.
- Chen, Y., Zhou, M.-G., 2009. Characterization of *Fusarium graminearum* isolates resistant to both carbendazim and a new fungicide JS399-19. *Phytopathology*. 99, 441-446. <http://dx.doi.org/10.1094/PHYTO-99-4-0441>.
- Cuomo, C. A., Güldener, U., Xu, J.-R., Trail, F., Turgeon, B. G., Di Pietro, A., Walton, J. D., Ma, L. J., Baker, S. E., Rep, M., 2007. The *Fusarium graminearum* genome reveals a link between localized polymorphism and pathogen specialization. *Science*. 317, 1400-1402. <http://dx.doi.org/10.1126/science.1143708>.
- David, R. F., Bozorgmagham, A. E., Schmale III, D. G., Ross, S. D., Marr, L. C., 2016a. Identification of meteorological predictors of *Fusarium graminearum* ascospore release using correlation and causality analyses. *Eur. J. Plant Pathol.* 145, 483-492. <http://dx.doi.org/10.1007/s10658-015-0832-3>.
- David, R. F., Marr, L. C., Schmale III, D. G., 2016b. Ascospore release and discharge distances of *Fusarium graminearum* under controlled temperature and relative humidity. *Eur. J. Plant Pathol.* <http://dx.doi.org/10.1007/s10658-016-0891-0>. <http://dx.doi.org/10.1007/s10658-016-0891-0>.
- Davis, R. H., Perkins, D. D., 2002. *Neurospora*: a model of model microbes. *Nature Reviews Genetics*. 3, 397-403. 10.1038/nrg797.
- De Bary, A., Garnsey, H. E. F., Balfour, I. B., 1887. Comparative morphology and biology of the fungi, mycetoza, and bacteria. Clarendon Press, Oxford <http://dx.doi.org/10.5962/bhl.title.5372>.
- Del Ponte, E. M., Fernandes, J. M. C., Pavan, W., Baethgen, W. E., 2009. A model-based assessment of the impacts of climate variability on fusarium head blight seasonal risk in southern Brazil. *J. Phytopathol.* 157, 675-681. <http://dx.doi.org/10.1111/j.1439-0434.2009.01559.x>.
- Desjardins, A. E., Hohn, T. M., McCormick, S. P., 1993. Trichothecene biosynthesis in *Fusarium* species: chemistry, genetics, and significance. *Microbiol. Rev.* 57, 595-604.
- Fernando, W. G., Miller, J., Seaman, W., Seifert, K., Paulitz, T., 2000. Daily and seasonal dynamics of airborne spores of *Fusarium graminearum* and other *Fusarium* species sampled over wheat plots. *Can. J. Bot.* 78, 497-505. <http://dx.doi.org/10.1139/b00-027>.
- Flemming, H.-C., 2002. Biofouling in water systems—cases, causes and countermeasures. *Appl. Microbiol. Biotechnol.* 59, 629-640. <http://dx.doi.org/10.1007/s00253-002-1066-9>.
- Flores-Johnson, E., Li, Q., Mines, R., 2008. Degradation of elastic modulus of progressively crushable foams in uniaxial compression. *J. Cell. Plast.* 44, 415-434. <http://dx.doi.org/10.1177/0021955X08095113>.
- Gadoury, D. M., Stensvand, A., Seem, R. C., 1996. A wind tunnel for controlled-environment studies of ascospore release by *Venturia inaequalis*. *Phytopathology*. 86, 596-601.
- Galagan, J. E., Calvo, S. E., Borkovich, K. A., Selker, E. U., Read, N. D., Jaffe, D., FitzHugh, W., Ma, L.-J., Smirnov, S., Purcell, S., 2003. The genome sequence of the filamentous fungus *Neurospora crassa*. *Nature*. 422, 859-868. 10.1038/nature01554.
- Goswami, R. S., Kistler, H. C., 2004. Heading for disaster: *Fusarium graminearum* on cereal crops. *Mol. Plant Pathol.* 5, 515-525. <http://dx.doi.org/10.1111/j.1364-3703.2004.00252.x>.
- Guenther, J. C., Trail, F., 2005. The development and differentiation of *Gibberella zeae* (anamorph: *Fusarium graminearum*) during colonization of wheat. *Mycologia*. 97, 229-237. <http://dx.doi.org/10.3852/mycologia.97.1.229>.
- Hartz, T., Johnstone, P., Nunez, J., 2005. Production environment and nitrogen fertility affect carrot cracking. *HortScience*. 40, 611-615.

- Inch, S., Fernando, W., Gilbert, J., 2005. Seasonal and daily variation in the airborne concentration of *Gibberella zeae* (Schw.) Petch spores in Manitoba. *Can. J. Plant Pathol.* 27, 357-363. <http://dx.doi.org/10.1080/07060660509507233>.
- Klittich, C., Leslie, J., 1988. Nitrate reduction mutants of *Fusarium moniliforme* (*Gibberella fujikuroi*). *Genetics*. 118, 417-423.
- Körstgens, V., Flemming, H. C., Wingender, J., Borchard, W., 2001. Uniaxial compression measurement device for investigation of the mechanical stability of biofilms. *J. Microbiol. Methods*. 46, 9-17. [http://dx.doi.org/10.1016/S0167-7012\(01\)00248-2](http://dx.doi.org/10.1016/S0167-7012(01)00248-2).
- Kuo, H.-C., Hui, S., Choi, J., Asiegbu, F. O., Valkonen, J. P. T., Lee, Y.-H., 2014. Secret lifestyles of *Neurospora crassa*. *Scientific Reports*. 4, 5135. 10.1038/srep05135 <http://www.nature.com/articles/srep05135#supplementary-information>.
- Lade, P. V., 1977. Elasto-plastic stress-strain theory for cohesionless soil with curved yield surfaces. *Int. J. Solids Struct.* 13, 1019-1035. [http://dx.doi.org/10.1016/0020-7683\(77\)90073-7](http://dx.doi.org/10.1016/0020-7683(77)90073-7).
- Latgé, J. P., 2007. The cell wall: a carbohydrate armour for the fungal cell. *Mol. Microbiol.* 66, 279-290. <http://dx.doi.org/10.1111/j.1365-2958.2007.05872.x>.
- Lenardon, M. D., Munro, C. A., Gow, N. A. R., 2010. Chitin synthesis and fungal pathogenesis. *Curr. Opin. Microbiol.* 13, 416-423. <http://dx.doi.org/10.1016/j.mib.2010.05.002>.
- Leslie, J. F., Summerell, B. A., Bullock, S., 2006. The *Fusarium* laboratory manual. Wiley Online Library, <http://dx.doi.org/10.1002/9780470278376>.
- Levitz, S. M., 2010. Innate recognition of fungal cell walls. *PLoS Pathog.* 6. <http://dx.doi.org/10.1371/journal.ppat.1000758>.
- Li, H., Diao, Y., Wang, J., Chen, C., Ni, J., Zhou, M., 2008. JS399-19, a new fungicide against wheat scab. *Crop Prot.* 27, 90-95. <http://dx.doi.org/10.1016/j.cropro.2007.04.010>.
- Lubarda, V., Lee, E., 1981. A correct definition of elastic and plastic deformation and its computational significance. *J. Appl. Mech.* 48, 35-40. <http://dx.doi.org/10.1115/1.3157589>.
- Martín-Udóroz, M., Madrid, M. P., Roncero, M. I. G., 2004. Role of chitin synthase genes in *Fusarium oxysporum*. *Microbiology*. 150, 3175-3187. <http://dx.doi.org/10.1099/mic.0.27236-0>.
- Martin, R. B., Ishida, J., 1989. The relative effects of collagen fiber orientation, porosity, density, and mineralization on bone strength. *J. Biomech.* 22, 419-426. [http://dx.doi.org/10.1016/0021-9290\(89\)90202-9](http://dx.doi.org/10.1016/0021-9290(89)90202-9).
- McMullen, M., Jones, R., Gallenberg, D., 1997. Scab of wheat and barley: a re-emerging disease of devastating impact. *Plant Dis.* 81, 1340-1348. <http://dx.doi.org/10.1094/pdis.1997.81.12.1340>.
- Meyers, M. A., Chen, P.-Y., Lin, A. Y.-M., Seki, Y., 2008. Biological materials: structure and mechanical properties. *Prog. Mater. Sci.* 53, 1-206. <http://dx.doi.org/10.1016/j.pmatsci.2007.05.002>.
- Morrison, T., Jung, H., Buxton, D., Hatfield, R., 1998. Cell-wall composition of maize internodes of varying maturity. *Crop Sci.* 38, 455-460. <http://dx.doi.org/10.2135/cropsci1998.0011183X003800020031x>.
- Munro, C., Gow, N., 2001. Chitin synthesis in human pathogenic fungi. *Med. Mycol.* 39, 41-53. <http://dx.doi.org/10.1080/mmy.39.1.41.53>.
- Paul, P., Lipps, P., De Wolf, E., Shaner, G., Buechley, G., Adhikari, T., Ali, S., Stein, J., Osborne, L., Madden, L., 2007. A distributed lag analysis of the relationship between *Gibberella zeae* inoculum density on wheat spikes and weather variables. *Phytopathology*. 97, 1608-1624. <http://dx.doi.org/10.1094/PHYTO-97-12-1608>.
- Paulitz, T., 1996. Diurnal release of ascospores by *Gibberella zeae* in inoculated wheat plots. *Plant Dis.* 80, 674-678. <http://dx.doi.org/10.1094/PHYTO-80-0674>.
- Paulitz, T., 1999. *Fusarium* head blight: a re-emerging disease. *Phytoprotection*. 80, 127-133. <http://dx.doi.org/10.7202/706188ar>.

- Prussin II, A. J., Li, Q., Malla, R., Ross, S. D., Schmale III, D. G., 2014a. Monitoring the long distance transport of *Fusarium graminearum* from field-scale sources of inoculum. *Plant Dis.* 98, 504-511. <http://dx.doi.org/10.1094/PDIS-06-13-0664-RE>.
- Prussin II, A. J., Marr, L. C., Schmale III, D. G., Stoll, R., Ross, S. D., 2015. Experimental validation of a long-distance transport model for plant pathogens: application to *Fusarium graminearum*. *Agr. Forest. Meteorol.* 203, 118-130. <http://dx.doi.org/10.1016/j.agrformet.2014.12.009>.
- Prussin II, A. J., Szanyi, N. A., Welling, P. I., Ross, S. D., Schmale III, D. G., 2014b. Estimating the production and release of ascospores from a field-scale source of *Fusarium graminearum* inoculum. *Plant Dis.* 98, 497-503. <http://dx.doi.org/10.1094/PDIS-04-13-0404-RE>.
- Reis, E., 1990. Effects of rain and relative humidity on the release of ascospores and on the infection of wheat heads by *Gibberella zeae*. *Fitopatol. Bras.* 15, 339-343.
- Ruiz-Herrera, J., 1991. Fungal cell wall: structure, synthesis, and assembly. CRC Press, Boca Raton, Florida <http://dx.doi.org/10.1201/b11873-4>.
- Schmale III, D. G., Arntsen, Q. A., Bergstrom, G. C., 2005a. The forcible discharge distance of ascospores of *Gibberella zeae*. *Can. J. Plant Pathol.* 27, 376-382. <http://dx.doi.org/10.1080/07060660509507235>.
- Schmale III, D. G., Bergstrom, G. C., 2003. Fusarium head blight. The Plant Health Instructor. doi: 10.1094/PHI-I-2003-0612-01. doi: 10.1094/PHI-I-2003-0612-01.
- Schmale III, D. G., Ross, S. D., 2015. Highways in the sky: scales of atmospheric transport of plant pathogens. *Annu. Rev. Phytopathol.* 53, 591-611. <http://dx.doi.org/10.1146/annurev-phyto-080614-115942>.
- Schmale III, D. G., Shah, D. A., Bergstrom, G. C., 2005b. Spatial patterns of viable spore deposition of *Gibberella zeae* in wheat fields. *Phytopathology.* 95, 472-479. <http://dx.doi.org/10.1094/phyto-95-0472>.
- Schmale III, D. G., Shields, E. J., Bergstrom, G. C., 2006. Night-time spore deposition of the fusarium head blight pathogen, *Gibberella zeae*, in rotational wheat fields. *Can. J. Plant Pathol.* 28, 100-108. <http://dx.doi.org/10.1080/07060660609507276>.
- Seifert, K. A., 1996. Notes on the typification of *Gibberella zeae*. *SYDOWIA-HORN.* 48, 83-89.
- Shaw, J. A., Mol, P. C., Bowers, B., Silverman, S. J., Valdivieso, M. H., Durán, A., Cabib, E., 1991. The function of chitin synthases 2 and 3 in the *Saccharomyces cerevisiae* cell cycle. *J. Cell Biol.* 114, 111-123. <http://dx.doi.org/10.1083/jcb.114.1.111>.
- Sikhakolli, U. R., López-Giráldez, F., Li, N., Common, R., Townsend, J. P., Trail, F., 2012. Transcriptome analyses during fruiting body formation in *Fusarium graminearum* and *Fusarium verticillioides* reflect species life history and ecology. *Fungal Genet. Biol.* 49, 663-673. <http://dx.doi.org/10.1016/j.fgb.2012.05.009>.
- Smith, A. E., Moxham, K. E., Middelberg, A. P. J., 2000a. Wall material properties of yeast cells. Part II. Analysis. *Chem. Eng. Sci.* 55, 2043-2053. [http://dx.doi.org/10.1016/S0009-2509\(99\)00501-1](http://dx.doi.org/10.1016/S0009-2509(99)00501-1).
- Smith, A. E., Zhang, Z., Thomas, C. R., 2000b. Wall material properties of yeast cells: Part 1. Cell measurements and compression experiments. *Chem. Eng. Sci.* 55, 2031-2041. [http://dx.doi.org/10.1016/S0009-2509\(99\)00500-X](http://dx.doi.org/10.1016/S0009-2509(99)00500-X).
- Snijders, C., 1990. Fusarium head blight and mycotoxin contamination of wheat, a review. *Neth. J. Plant Pathol.* 96, 187-198. <http://dx.doi.org/10.1007/BF01974256>.
- Stensvand, A., Gadoury, D. M., Amundsen, T., Semb, L., Seem, R. C., 1997. Ascospore release and infection of apple leaves by conidia and ascospores of *Venturia inaequalis* at low temperatures. *Phytopathology.* 87, 1046-1053. 10.1094/PHYTO.1997.87.10.1046.
- Sutton, J., 1982. Epidemiology of wheat head blight and maize ear rot caused by *Fusarium graminearum*. *Can. J. Plant Pathol.* 4, 195-209. <http://dx.doi.org/10.1080/07060668209501326>.

- Trail, F., 2007. Fungal cannons: explosive spore discharge in the Ascomycota. FEMS Microbiol. Lett. 276, 12-18. <http://dx.doi.org/10.1111/j.1574-6968.2007.00900.x>.
- Trail, F., Common, R., 2000. Perithecial development by *Gibberella zeae*: a light microscopy study. Mycologia. 92, 130-138. <http://dx.doi.org/10.2307/3761457>.
- Trail, F., Gaffoor, I., Vogel, S., 2005. Ejection mechanics and trajectory of the ascospores of *Gibberella zeae* (anamorph *Fusarium graminearum*). Fungal Genet. Biol. 42, 528-533. <http://dx.doi.org/10.1016/j.fgb.2005.03.008>.
- Trail, F., Seminara, A., 2014. The mechanism of ascus firing – merging biophysical and mycological viewpoints. Fungal Biol. Rev. 28, 70-76. <http://dx.doi.org/10.1016/j.fbr.2014.07.002>.
- Trail, F., Xu, H., Loranger, R., Gadoury, D., 2002. Physiological and environmental aspects of ascospore discharge in *Gibberella zeae* (anamorph *Fusarium graminearum*). Mycologia. 94, 181-189. <http://dx.doi.org/10.2307/3761794>.
- Tschanz, A. T., Horst, R., Nelson, P. E., 1976. The effect of environment on sexual reproduction of *Gibberella zeae*. Mycologia. <http://dx.doi.org/10.2307/3759003>, 327-340. <http://dx.doi.org/10.2307/3759003>.
- Tschanz, A. T., Horst, R. K., Nelson, P. E., 1975. Ecological aspects of ascospore discharge in *Gibberella zeae*. Phytopathology. 65, 597. <http://dx.doi.org/10.1094/Phyto-65-597>.
- Walker, L. A., Gow, N. A. R., Munro, C. A., 2010. Fungal echinocandin resistance. Fungal Genet. Biol. 47, 117-126. <http://dx.doi.org/10.1016/j.fgb.2009.09.003>.
- Windels, C. E., 2000. Economic and social impacts of fusarium head blight: changing farms and rural communities in the Northern Great Plains. Phytopathology. 90, 17-21. <http://dx.doi.org/10.1094/PHYTO.2000.90.1.17>.
- Zhao, L., Schaefer, D., Xu, H., Modi, S. J., LaCourse, W. R., Marten, M. R., 2005. Elastic properties of the cell wall of *Aspergillus nidulans* studied with atomic force microscopy. Biotechnol. Prog. 21, 292-299. <http://dx.doi.org/10.1021/bp0497233>.

7 Figures

Figure 1: Compression testing. Plan view of (A) load frame modified using 1.27-mm slotted-head, 0.32-mm pin, aluminum mounts; (B) failure surface and testing surface (enlarged view of orange box in (A)); (C) adhered perithecium on testing surface (enlarged view of red box in (B)). The perithecium was displaced at a rate of $1 \mu\text{m s}^{-1}$ toward the failure surface until a force of 50 N was applied.

Figure 2: Mean perithecium compression constant (MPCC) versus perithecial age and linear relationship between the two variables for strain FgVa, parent strain (PH-1) with fully developed perithecia, and mutant strain (4417) of PH-1, which does not develop asci. The dashed line in each panel provides a linear fit with FgVa ($y = (7.0 \times 10^{-3})x + (4.7 \times 10^{-2})$) having a significant linear relationship ($P < 0.01$ and $R^2 = 0.77$) between the MPCC and age of perithecia. The linear fits associated with PH-1 ($P = 0.31$; $R^2 = 0.17$; $y = (1.3 \times 10^{-3})x + (2.6 \times 10^{-2})$) and 4417 ($P = 0.54$; $R^2 = 0.07$; $y = (8.6 \times 10^{-4})x + (2.6 \times 10^{-2})$) had positive slopes though not significant. Older perithecia have a greater tolerance for compression failure and are more difficult to rupture.

Figure 3: Quantity of ascospores in perithecia of different ages (5 d to 12 d) for strain FgVa. Twelve perithecia were observed for each age. There is a positive, linear relationship ($P < 0.01$; $R^2 = 0.94$; $y = 877.09x - 585.69$) between the two variables indicating that older perithecia contain more ascospores.

Figure 1

FIGURES

Figure 2

Figure 3