Monitoring and Predicting the Long Distance Transport of *Fusarium graminearum*,
Causal Agent of Fusarium Head Blight in Wheat and Barley

Aaron Justin Prussin, II

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David G. Schmale III, Co-Chair
Shane D. Ross, Co-Chair
Jacob N. Barney
Antonius B. Baudoin
Linsey C. Marr

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ABSTRACT

Fusarium head blight (FHB), caused by *Fusarium graminearum*, is a serious disease of wheat and barley that has caused several billion dollars in crop losses over the last decade in the United States. Spores of *F. graminearum* are released from corn and small grain residues left-over from the previous growing season and are transported long distances in the atmosphere before being deposited. Current risk assessment tools consider environmental conditions favorable for disease development, but do not include spore transport. Long distance transport models have been proposed for a number of plant pathogens, but many of these models have not been experimentally validated. In order to predict the atmospheric transport of *F. graminearum*, the potential source strength (Q_{pot}) of inoculum must be known. We conducted a series of laboratory and field experiments to estimate Q_{pot} from a field-scale source of inoculum of *F. graminearum*. Perithecia were generated on artificial (carrot agar) and natural (corn stalk) substrates. Artificial substrate (carrot agar) produced 15±0.4 perithecia cm^{-2}, and natural substrate (corn stalk) produced 44±2 perithecia cm^{-2}. Individual perithecia were excised from both substrate types and allowed to release ascospores every 24 hours. Perithecia generated from artificial (carrot agar) and natural (corn stalk) substrates released a mean of 104±5 and 276±16 ascospores, respectively. A volumetric spore trap was placed inside a 3,716 m^2 clonal source of inoculum in 2011 and 2012. Results indicated that ascospores were

released under field conditions predominantly (>90%) during the night (1900 to 0700 hours). Estimates of Q_{pot} for our field-scale sources of inoculum were approximately 4 billion ascospores per 3,716 m^2. Release-recapture studies were conducted from a clonal field-scale source of *F. graminearum* in 2011 and 2012. Microsatellites were used to identify the released clone of *F. graminearum* at distances up to 1 km from the source. Dispersal kernels for field observations were compared to results predicted by a Gaussian dispersal-based spore transport model. In 2011 and 2012, dispersal kernel shape coefficients were similar for both results observed in the field and predicted by the model, with both being dictated by a power law function, indicating that turbulence was the dominant transport factor on the scale we studied (~ 1 km). Model predictions had a stronger correlation with the number of spores being released when using a time varying q_0 emission rate (r= 0.92 in 2011 and r= 0.84 in 2012) than an identical daily pattern q_0 emission rate (r= 0.35 in 2011 and r= 0.32 in 2012). The actual numbers of spores deposited were 3 and 2000 times lower than predicted if Q_{pot} were equal to the actual number of spores released in 2011 and 2012, respectively. Future work should address estimating the actual number of spore released from an inoculated field during any given season, to improve prediction accuracy of the model. This work should assist in improving current risk assessment tools for FHB and contribute to the development of early warning systems for the spread of *F. graminearum*.

To JBP for her love and support

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Attributions

Below is a brief description of the contribution of several colleagues' assistance to the completion of the research chapters of this dissertation.

Qing Li, Department of Statistics, Virginia Tech

As a co-author in Chapter 3, Ms. Li provided statistical analyses and advice, as well as editing advice.

Rimy Malla, Department of Statistics, Virginia Tech

As a co-author in Chapter 3, Ms. Malla provided statistical analyses and advice, as well as editing advice.

Linsey C. Marr, Department of Civil and Environmental Engineering, Virginia Tech

Dr. Marr served as a committee member and was listed as a co-author in Chapter 4. Dr. Marr provided both advice for examining model performance as well as editing advice.

Shane D. Ross, Department of Engineering, Science, and Mechanics, Virginia Tech.

Dr. Ross served as a major co-advisor and provided funding and guidance through the completion of this dissertation. He is listed as a co-author in Chapters 2, 3, and 4.

David G. Schmale, III, Department of Plant Pathology, Physiology, and Weed Science, Virginia Tech.

Dr. Schmale served as a major co-advisor and provided funding and guidance through the completion of this dissertation. He is listed as a co-author in Chapters 2, 3, and 4.

Nicole A. Szanyi, Department of Plant Pathology, Physiology, and Weed Science, Virginia Tech.

As a co-author in Chapter 2, Ms. Szanyi provided technical support in the field and laboratory, as well as editing advice.

Patricia I. Welling, Department of Plant Pathology, Physiology, and Weed Science, Virginia Tech.

As a co-author in Chapter 2, Ms. Welling provided technical support in the field and laboratory, as well as editing advice.

Chapter 1. Introduction

Many plant pathogens are transported from inoculum sources on the ground to healthy crops through the atmosphere (1-4). *Phakopsora pachyrhizi*, causal agent of Asian soybean rust (19), and *Puccinia graminis* f. sp. *tritici*, causal agent of wheat stem rust (42), are two pathogens of recent concern, due to their potential economic damage, that use the atmosphere to transport their spores (19, 21, 27, 39, 40, 42).

The movement of microbes in the atmosphere is broadly governed by five main processes: inoculum development, liberation (take off and ascent of microbes), passive horizontal transport of microbes (movement from initial to final location), deposition (descent and landing of microbes), and impact (16). Aerobiological processes are complex steps that are inter-connected with each other. Liberation can depend on many factors such as spore maturity, weather conditions, structure of the canopy, and the ability to get into the turbulent airflow (16). The distances microbes can be transported in the atmosphere depend on both the turbulent airflow and the survival of spores. Factors that affect the number of viable spores that remain airborne include UV radiation and washout due to deposition. Deposition can either be 'dry' which is when microbes land due to natural airflow or 'wet' which is when microbes are washed out due to moisture.

Fusarium graminearum is a fungal plant pathogen that utilizes the atmosphere for spore transport (23, 32, 37). This fungus is responsible for Fusarium head blight (FHB) of wheat and barley, which has resulted in more than $3 billion in crop losses in the United States recently (24, 31, 34, 50). *F. graminearum* produces deoxynivalenol (DON), a mycotoxin, that may contaminate food and feed and threaten the health of both humans and livestock (41, 43).

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Airborne pathogens pose a challenge for growers and farmers when trying to manage disease. *Puccinia graminis* f. sp. *tritici* has historically been managed using resistant cultivars, however, recently the fungus has overcome the *Sr31* resistance gene and now threatens the world wheat supply (25, 39, 49). *Fusarium graminearum* is another pathogen in which disease control is difficult. Recently, research has suggested that the cultural move to reduced tillage for soil conservation has been a contributing factor for disease outbreak due to infected crop residues being left at the surface (9, 17, 18). Additionally, fungicides have been found to have only a small effect on controlling FHB, and are only somewhat effective when used in certain environmental conditions (5). Being able to predict the natural spread of the pathogen and have early detection is effective in the implementation of control methods (15). Recently, a risk assessment tool was developed for FHB (7, 8). The main considerations of the current FHB risk assessment tool are environmental factors such as precipitation, relative humidity, and temperature to determine the relative risk of disease. Although, environmental factors are the main component of disease development of FHB on wheat and barley, the current risk assessment tool does not include the ability to predict the movement of *F. graminearum* spores from known infected regions. The current FHB risk assessment tool can be improved with the inclusion of spore transport from infected regions to healthy regions.

Transport models of spore movement for plant pathogenic fungi have been developed that are derived from the principal steps of atmospheric transport (1-4). The atmospheric transport of plant pathogens can be described by the aerobiological processes of inoculum development, liberation, horizontal transport, deposition, and impact (16). Models to predict the long distance transport of fungal spores assume a

Gaussian distribution of spores (28, 29, 47). The shape and width of the Gaussian distribution is dependent on the atmospheric stability, which is dictated by solar radiation and wind speed (47). Aylor et al. (1-4) have further developed these models to be specific for the long distance transport of fungal spores. Long distance transport can be considered to be anything further than 100 m from a source of inoculum (1). There are several factors in Gaussian spore transport models necessary to predict the number of viable spores that will be deposited at any given location, including:

1. Initial source strength

2. Wind speed and direction

3. Distance of healthy crops from the source

4. Spore loss due to solar radiation and deposition

5. Dilution of spore plume due to turbulence

Although, the principals of these transport models are well known, they have not been experimentally validated for *F. graminearum*. In order to experimentally validate these transport models, each of the principal steps of the aerobiological process model need to be fully understood for *F. graminearum*, and spore transport needs to be tracked from release in field to deposition at a new location.

Despite over two decades of research on FHB, we do not yet have a complete understanding of the aerobiological process model for *F. graminearum*. This is due in part to the difficulty of capturing samples in the atmosphere because of the lack of appropriate tools to study particles in the atmosphere. Additionally, being able to track the long distance movement of spores is also an issue because in order to track long

distance movement one needs a reliable and robust method for knowing the initial source of the pathogen.

Previous work has addressed understanding preconditioning and liberation of *F. graminearum* spores, and a simple disease cycle was created (34). The fungus overwinters in infected debris of corn and small grains until conditions become favorable for sporulation. In the spring when temperatures begin to rise and humidity increases, sexual structures known as perithecia will form on the crop debris (10). The perithecia contain ascospores that are forcibly discharge into the atmosphere (33, 45). Although, the number of ascospores contained within perithecia has not been examined, this becomes important when trying to estimate the potential strength of an inoculum source. Studies have indicated that the spores get discharged with an acceleration of 870,000g, the highest acceleration reported for any biological system (45). It has also been shown that *F. graminearum* spores are discharged only millimeter distances, however this may be far enough to get into the turbulent (mixed) layer in the atmosphere given correct environmental conditions (33, 45). The lower atmosphere contains two layers: laminar and turbulent (14). In order for spores to be transported in the atmosphere they must get into the turbulent layer.

Studies have suggested that there is a diurnal release pattern for spores in the field; however these reports have been conflicting. Multiple studies have suggested that ascospores are released predominantly at night (10 PM to 8 AM) due to an increase in relative humidity (12, 30, 46), while Maldonado-Ramirez (22) provided evidence for a predominantly daytime release. After the ascospores have been discharged they may be transported over some distance through the atmosphere and deposited on a healthy crop.

The disease cycle will then continue the next year. The latter two steps of *F. graminearum* aerobiology, horizontal transport and deposition, are poorly understood.

One of the reasons horizontal transport of *F. graminearum* is poorly understood is due to the lack of appropriate tools to collect and study microbes in the atmosphere. Maldonado-Ramirez et al. (23) used remote-controlled unmanned aerial vehicles (UAVs) to collect *F. graminearum* samples in the planetary boundary layer (60 m above ground level) of the atmosphere. Maldonado-Ramirez et al. (23) found that viable *F. graminearum* spores were abundant 60m above the ground regardless of day or time of day over a 2 month sampling period. Additionally, the atmospheric population of *F. graminearum* was found to be genetically diverse (37). Recently, a new UAV platform was developed for collecting and studying plant pathogens in the atmosphere (44). This lead to the discovery that the atmosphere is well mixed with *Fusarium* species (20). Although, this work has had increased our knowledge about *F. graminearum*, researchers did not know the initial sources of inoculum or where the collected fungal spores were coming from.

Recently, there have been advances in release-recovery experiments of *F. graminearum* over short distances (< 30 m) using a genotyping method known as amplified fragment length polymorphism (AFLP) (17, 18). In these studies, small amounts of inoculum (45-410 g) were placed in 0.55 m^2 plots and diseased wheat heads were collected short distances (< 30 m) from the source of inoculum and analyzed for the released clone of *F. graminearum*. Though these studies were among the first in this pathosystem to track the fungus from known inoculum sources, the size of the inoculum sources and the scale of the study limited collections of released clone to within distances

of 30 m from the source. Fernando et al. (13) used an isolate of *F. graminearum* exhibiting a yellow phenotype on standard culture medium to help distinguish the released clone from natural (background) populations of the fungus. Both of these techniques (AFLPs and the release of a yellow phenotype) have some limitations. AFLPs require a considerable amount of time and resources (the genomic DNA from each isolate is subjected to digestion and ligation reactions with different enzymes followed by multiple PCRs), and the physical location of and selection on AFLP markers is often unknown. The release and recapture of a yellow phenotype of *F. graminearum* is limited since yellow phenotypes of the fungus may be present in natural populations of the fungus. Recently, researchers have developed a method to overcome these limitations by using a barcoded spore approach to identify a released microbe from background populations in release-recapture studies (6, 11). The researchers inserted a small DNA sequence into a neutral region of *Bacillus thuringiensis* subsp. *Kurstaki*, a biopesticide agent, to distinguish what was released from wild type strains. Although this method allows for unambiguous discrimination of a released spore, there are challenges and regulations, both with the government and public perception, associated when releasing genetically modified plant pathogenic agents. An alternative strategy to identify a specific fungal individual from a background population is to use a series of microsatellites—short conserved sequences of DNA scattered across the genome (48). The use of microsatellites to identify a released clone of *F. graminearum* within a heterogeneous natural population could provide a rapid, cost efficient, and robust method to track the long distance movement of *F. graminearum* from a known inoculum source.

Once spores have been released and horizontally transported, they must fall back to the earth's surface. Spore deposition can be either 'wet' or 'dry'. Wet deposition occurs when the spore column is washed out of the atmosphere due to a rain or snow event; while dry deposition occurs by the natural settling of the spores back to the ground. Recent work has suggested that *F. graminearum* spores are predominantly deposited at night and explained that rates might be higher at night due to an inversion layer being formed from a rapid cooling of earth's surface compared to the atmosphere (26, 35, 36, 38). Predominant nighttime spore deposition could also be correlated to when spores are released as found by some researchers (12, 30, 46). However, as with the majority of the work examining horizontal transport of *F. graminearum* spores, the researchers looking at deposition did not have knowledge of where the spores were coming from.

In order to experimentally validate current spore transport models for *F. graminearum*, each of the aerobiological processes must be studied from an artificially inoculated field and spore transport needs to be tracked from this known source of inoculum (1-4). This knowledge will allow for the development of spore dispersal kernels for *F. graminearum*. Additionally, research must focus on estimating the inoculated field's potential source strength, as this is one of the largest unknown components of the current transport models (2). If spore transport models can be experimentally validated for *F. graminearum*, they should be incorporated into the current FHB risk assessment tool (7, 8). FHB risk assessment models will be more powerful with the incorporation of spore transport models as they would include disease

risk due to favorable environmental factors, in addition to the potential of spores to be transported from neighboring field.

Research Objectives

1. Determine the potential source strength of a field-scale source of inoculum of *F. graminearum* inoculum.

2. Develop a method to track the long distance transport of *F. graminearum* ascospores from a clonal field-scale source of inoculum.

3. Determine the dispersal kernel for spores of *F. graminearum* transported from a clonal field-scale source of inoculum.

4. Refine and validate mathematical models of spore transport based on experimental results from studies with *F. graminearum*.

Literature Cited

1.   Aylor, D. E. 1999. Biophysical scaling and the passive dispersal of fungus spores: relationship to integrated pest management strategies. Agricultural and Forest Meteorology 97:275-292.

2.   Aylor, D. E. 1986. A framework for examining inter-regional aerial transport of fungal spores. Agricultural and Forest Meteorology 38:263-288.

3.   Aylor, D. E., and Sutton, T. B. 1992. Release of *Venturia inaequalis* ascospores during unsteady rain: Relationship to spore transport and deposition. Phytopathology 82:532-540.

4.   Aylor, D. E., Taylor, G. S., and Raynor, G. S. 1982. Long-range transport of Tobacco blue mold spores. Agricultural Meteorology 27:217-232.

5.   Bai, G., and Shaner, G. 2004. Management and resistance in wheat and barley to Fusarium head blight 1. Annual Review of Phytopathology 42:135-161.

6.   Buckley, P., Rivers, B., Katoski, S., Kim, M. H., Kragl, F. J., Broomall, S., Krepps, M., Skowronski, E. W., Rosenzweig, C. N., and Paikoff, S. 2012. Genetic barcodes for improved environmental tracking of an anthrax simulant. Applied and Environmental Microbiology 78 (23): 8272-8280.

7.   De Wolf, E. D., Madden, L. V., and Lipps, P. E. 2003. Risk assessment models for wheat Fusarium head blight epidemics based on within-season weather data. Phytopathology 93:428-435.

8.   Del Ponte, E. M., Fernandes, J. M. C., Pavan, W., and Baethgen, W. E. 2009. A Model-based Assessment of the impacts of climate variability on Fusarium head blight seasonal risk in southern Brazil. Journal of Phytopathology 157:675-681.

9.   Dill-Macky, R., and Jones, R. 2000. The effect of previous crop residues and tillage on Fusarium head blight of wheat. Plant Disease 84:71-76.

10. Dufault, N., De Wolf, E., Lipps, P., and Madden, L. 2006. Role of temperature and moisture in the production and maturation of Gibberella zeae perithecia. Plant Disease 90:637-644.

11. Emanuel, P. A., Buckley, P. E., Sutton, T. A., Edmonds, J. M., Bailey, A. M., Rivers, B. A., Kim, M. H., Ginley, W. J., Keiser, C. C., and Doherty, R. W. 2012. Detection and tracking of a novel genetically-tagged biological simulant in the environment. Applied and Environmental Microbiology 78(23): 8281-8288.

12. Fernando, W. G., Miller, J. D., Seaman, W. L., Seifert, K., and Paulitz, T. C. 2000. Daily and seasonal dynamics of airborne spores of *Fusarium graminearum*

and other *Fusarium* species sampled over wheat plots. Canadian Journal of Botany 78:497-505.

13.   Fernando, W. G. D., Paulitz, T. C., Seaman, W. L., Dutilleul, P., and Miller, J. D. 1997. Head blight gradients caused by *Gibberella zeae* from area sources of inoculum in wheat field plots. Phytopathology 87:414-421.

14.   Garratt, J. R. 1994. The atmospheric boundary layer. Cambridge University Press.

15.   Gregory, P. H. 1961. The microbiology of the atmosphere. Leonard Hill.

16.   Isard, S. A., Gage, S. H., Comtois, P., and Russo, J. M. 2005. Principles of the atmospheric pathway for invasive species applied to Soybean rust. Bioscience 55:851-861.

17.   Keller, M. D., Thomason, W. E., and Schmale III, D. G. 2011. The spread of a released clone of *Gibberella zeae* from different amounts of infested corn residue. Plant Disease 95:1458-1464.

18.   Keller, M. D., Waxman, K. D., Bergstrom, G. C., and Schmale III, D. G. 2010. Local distance of wheat spike infection by released clones of *Gibberella zeae* disseminated from infested corn residue. Plant Disease 94:1151-1155.

19.   Krupa, S., Bowersox, V., Claybrooke, R., Barnes, C. W., Szabo, L., Harlin, K., and Kurle, J. 2006. Introduction of Asian soybean rust urediniospores into the midwestern United States—A case study. Plant Disease 90:1254-1259.

20.   Lin, B., Bozorgmagham, A., Ross, S. D., and Schmale III, D. G. 2012. Small fluctuations in the recovery of *Fusaria* across consecutive sampling intervals with unmanned aircraft 100 m above ground level. Aerobiologia:1-10.

21.   Livingston, M., Johansson, R., Daberkow, S., Roberts, M., Ash, M., and Breneman, V. 2004. Economic and policy implications of wind-borne entry of Asian soybean rust into the United States. US Department of Agriculture, Economic Research Service.

22.   Maldonado-Ramirez, S. L. 2001. Aerobiology of the wheat scab fungus, *Gibberella zeae*: discharge, atmospheric dispersal, and deposition of ascospores. Ph. D. thesis, Cornell University, Ithaca, NY.

23.   Maldonado-Ramirez, S. L., Schmale III, D. G., Shields, E. J., and Bergstrom, G. C. 2005. The relative abundance of viable spores of *Gibberella zeae* in the planetary boundary layer suggests the role of long-distance transport in regional epidemics of Fusarium head blight. Agricultural and Forest Meteorology 132:20-27.

24. McMullen, M., Jones, R., and Gallenberg, D. 1997. Scab of wheat and barley: A re-emerging disease of devastating impact. Plant Disease 81:1340-1348.

25. Mundt, C. 2002. Use of multiline cultivars and cultivar mixtures for disease management. Annual Review of Phytopathology 40:381-410.

26. Oke, T. R. 1987. Boundary layer climates. 2 ed. Cambridge University Press, Cambridge, UK.

27. Pan, Z., Yang, X., Pivonia, S., Xue, L., Pasken, R., and Roads, J. 2006. Long-term prediction of Soybean rust entry into the continental United States. Plant Disease 90:840-846.

28. Pasquill, F. 1976. Atmospheric dispersion parameters in Gaussian plume modeling. Part 2: Possible requirements for change in the Turner workbook values. Environmental Protection Agency.

29. Pasquill, F., and Michael, P. 1977. Atmospheric diffusion. Physics Today 30:55.

30. Paulitz, T. C. 1996. Diurnal release of ascospores by *Gibberella zeae* in inoculated wheat plots. Plant Disease 80:674-678.

31.   Paulitz, T. C. 1999. Fusarium head blight: a re-emerging disease. Phytoprotection (Québec) 80.

32.   Schmale, D., Ross, S., Fetters, T., Tallapragada, P., Wood-Jones, A., and Dingus, B. 2012. Isolates of *Fusarium graminearum* collected 40–320 meters above ground level cause Fusarium head blight in wheat and produce trichothecene mycotoxins. Aerobiologia 28:1-11.

33.   Schmale, D. G., Arntsen, Q. A., and Bergstrom, G. C. 2005. The forcible discharge distance of ascospores of *Gibberelia zeae*. Canadian Journal of Plant Patholology. 27:376-382.

34.   Schmale III, D. G., and Bergstrom, G. C. 2003. Fusarium head blight in wheat. The Plant Health Instructor. DOI: 10.1094/PHI-I-2003-0612-01.

35.   Schmale III, D. G., and Bergstrom, G. C. 2004. Spore deposition of the ear rot pathogen, *Gibberella zeae*, inside corn canopies. Canadian Journal of Plant Pathology 26:591-595.

36.   Schmale III, D. G., Bergstrom, G. C., and Shields, E. J. 2006. Night-time spore deposition of the Fusarium head blight pathogen, *Gibberella zeae*, in rotational wheat fields. Canadian Journal of Plant Pathology 28:100-108.

37. Schmale III, D. G., Leslie, J. F., Zeller, K. A., Saleh, A. A., Shields, E. J., and Bergstrom, G. C. 2006. Genetic structure of atmospheric populations of *Gibberella zeae*. Phytopathology 96:1021-1026.

38. Schmale III, D. G., Shah, D. A., and Bergstrom, G. C. 2005. Spatial patterns of viable spore deposition of *Gibberella zeae* in wheat fields. Phytopathology 95:472-479.

39. Singh, R. P., Hodson, D. P., Huerta-Espino, J., Jin, Y., Njau, P., Wanyera, R., Herrera-Foessel, S. A., and Ward, R. W. 2008. Will stem rust destroy the world's wheat crop? Advances in Agronomy 98:271-309.

40. Singh, R. P., Hodson, D. P., Jin, Y., Huerta-Espino, J., Kinyua, M. G., Wanyera, R., Njau, P., and Ward, R. W. 2006. Current status, likely migration and strategies to mitigate the threat to wheat production from race Ug99 (TTKS) of stem rust pathogen. CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources 1.

41. Snijders, C. H. A. 1990. Fusarium head blight and mycotoxin contamination of wheat, a review. European Journal of Plant Pathology 96:187-198.

42. Stokstad, E. 2007. Deadly wheat fungus threatens world's breadbaskets. Science 315:1786-1787.

43.   Sutton, J. 1982. Epidemiology of wheat head blight and maize ear rot caused by *Fusarium graminearum*. Canadian Journal of Plant Pathology 4:195-209.

44.   Techy, L., Schmale III, D. G., and Woolsey, C. A. 2010. Coordinated aerobiological sampling of a plant pathogen in the lower atmosphere using two autonomous unmanned aerial vehicles. Journal of Field Robotics 27:335-343.

45.   Trail, F., Gaffoor, I., and Vogel, S. 2005. Ejection mechanics and trajectory of the ascospores of *Gibberella zeae* (anamorph *Fuarium graminearum*). Fungal Genetics and Biology 42:528-533.

46.   Tschanz, A., Horst, K., and Nelson, P. E. 1975. Ecological aspects of ascospore discharge in *Gibberella zeae*. Phytopathology 65:597-599.

47.   Turner, D. B. 1970. Workbook of atmospheric dispersion estimates. US Department of Health, Education, and Welfare, National Center for Air Pollution Control.

48.   Vogelgsang, S., Widmer, F., Jenny, E., and Enkerli, J. 2009. Characterisation of novel *Fusarium graminearum* microsatellite markers in different *Fusarium* species from various countries. European Journal of Plant Pathology 123:477-482.

49. Wanyera, R., Kinyua, M., Jin, Y., and Singh, R. 2006. The spread of Stem rust caused by *Puccinia graminis* f. sp. *tritici*, with virulence on Sr31 in wheat in Eastern Africa. Plant Disease 90:113-113.

50. 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.

Chapter 2: Estimating Potential Source Strength (Q_0) of a Field-Scale Source of

***Fusarium graminearum* Inoculum**

Aaron J. Prussin, II[1], Nicole A. Szanyi[1], Patricia I. Welling[1], Shane D. Ross[2], and

David G. Schmale, III[1*]

[1]Department of Plant Pathology, Physiology, and Weed Science, Virginia Tech,

Blacksburg, VA 24061-0390

[2]Department of Engineering Science and Mechanics, Virginia Tech, Blacksburg, VA

24061-0219

*Corresponding Author: David G. Schmale, III; PH: (540)-231-6943; E-mail:

dschmale@vt.edu

Fusarium head blight (FHB) is a devastating disease of wheat and barley caused by the fungus *Fusarium graminearum*. The fungus produces spores that may be transported over long distances in the atmosphere. In order to predict the atmospheric transport of *F. graminearum*, the initial source strength (Q_0) of inoculum must be known. We conducted a series of laboratory and field experiments to estimate Q_0 from a field-scale source of inoculum of *F. graminearum*. Perithecia were generated on artificial (carrot agar) and natural (corn stalk) substrates. The artificial substrate (carrot agar) produced 15±0.4 perithecia cm^{-2}, and natural substrate (corn stalk) produced 44±2 perithecia cm^{-2}. Individual perithecia were excised from both substrate types and allowed to release ascospores every 24 hours. Perithecia generated from carrot agar and corn stalks substrates released a mean of 104±5 and 276±16 ascospores, respectively. A volumetric spore trap was placed inside a 3,716 m^2 clonal source of inoculum in 2011 and 2012. Ascospores were released under field conditions predominantly (>90%) during the night (1900 to 0700 hours). Estimates of Q_0 for our field-scale sources of inoculum were approximately 4 billion ascospores. Mathematical models can use these estimates of Q_0 in the future to assist in predicting the long-distance transport of *F. graminearum*.

Plant pathogens can be transported to healthy crops through a variety of mechanisms. Soil-borne pathogens such as *Pythium* spp., rely on water to transport zoospores (42), some studies have speculated that fungi that cause Fusarium wilt rely on an interaction with root-knot nematodes (*Meloidogyne* spp.) (19). There are many

pathogens that use the atmosphere to be transported to healthy crops. *Phakopsora pachyrhizi*, causal agent of Asian soybean rust (4, 18) and *Puccinia graminis* f. sp. *tritici*, causal agent of wheat stem rust (36) are two devastating pathogens that may be transported through the atmosphere. *Fusarium graminearum*, causal agent of Fusarium head blight (FHB) in wheat and barley, is another important fungal plant pathogen that is transported through the atmosphere (27, 33, 37). This pathogen of economic concern has caused over $3 billion in crop losses over the past two decades in the United States (22, 25, 30), in large part due to the production of deoxynivalenol, a mycotoxin that contaminates grain and renders it unfit for consumption (35, 37). Inoculum development, liberation, horizontal transport, deposition, and impact are the aerobiological processes that govern atmospheric transport of plant pathogens, such as *F. graminearum* (14).

Ascospores of *F. graminearum* are liberated from fruiting bodies known as perithecia. Warm temperatures and water availability in the crop residue promote the development of mature perithecia (11). Mature perithecia produce multiple asci, and each ascus contains eight ascospores (39). Ascospore release from mature perithecia is triggered in part by an increase in relative humidity (37, 40). Ascospores are typically released distances of about 5 mm, at a speed of 34.5 m s^{-1}, and acceleration of 860,000 g (28, 39). There have been conflicting reports documenting the timing of ascospore release. Some studies have shown that ascospores are released predominantly at night due to an increase in relative humidity (12, 24, 41). However, studies by Maldonado-Ramirez (20) suggested that ascospores are released primarily during daylight hours. Viable spores of *F. graminearum* have been recovered from the lower atmosphere during all times of the day and night (21, 27, 33). Schmale et al. (31, 32) reported that spores of

F. graminearum were deposited predominantly during the night, suggesting that deposition rates might be higher at night due to an inversion layer being formed from a rapid cooling of earth's surface compared to the atmosphere (23).

FHB is managed in part by the use of risk assessment models that guide the appropriate timing of fungicide application (8, 9). These models leverage knowledge of environmental factors and disease reports from local extension agents. These models, however, fail to address the atmospheric transport of *F. graminearum* spores from potential inoculum sources.

Aylor et al. (1-4), pioneered the development of mathematical models to predict the atmospheric movement of plant pathogenic fungi. The accuracy and reliability of these models is tightly linked to uncertainties regarding spore release dynamics and the initial inoculum source strength (Q_0) (2). Uncertainties in source strength and release dynamics can affect the magnitude of the dispersal kernels, probability density functions that describe the average spatial distribution of spores from an inoculated field, for fungal spores 100 to 1000 times (2). Consequently, there is a need to develop a reliable estimate of Q_0 to increase the accuracy and precision of these models.

We conducted a series of laboratory and field experiments to estimate Q_0 from a field-scale source of inoculum of *F. graminearum*. We hypothesized that the substrate used would affect perithecia production potential, in addition to the number of ascospores released from a perithecium. We also hypothesized that 10's to 100's of ascospores would be released from a perithecium. Finally, we hypothesized that night-time spore release would predominant. In order to test these hypotheses, perithecia were generated on artificial (carrot agar) and natural (corn stalk) substrates. Individual perithecia were

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excised from both substrate types and allowed to release ascospores every 24 hours. A volumetric spore trap was placed inside a 3,716 m^2 clonal source of inoculum in 2011 and 2012. Results from these experiments were used to estimate Q_0 for a field-scale source of inoculum. The specific objective of this study was to develop an accurate estimate of Q_0 for a field-scale source of inoculum of *F. graminearum*. Mathematical models can leverage estimates of Q_0 in the future to assist in predicting the long-distance transport of *F. graminearum*.

MATERIALS AND METHODS

Field experiments.

Field studies were conducted at Virginia Tech's Kentland Farm in Blacksburg, Virginia during the spring of 2011 and 2012. The Kentland Farm is composed of about 810 hectares of farmland. Two hectares of winter wheat (no seed treatment Southern States variety SS560) were planted in October 2010 for the 2011 field campaign and October 2011 for the 2012 field campaign. The winter wheat field was not treated with any fungicides.

Inoculum was prepared as described previously (26). Briefly, fifty 18.9-liter buckets were filled with approximately 15-cm cut, dried, and autoclaved corn stalk pieces. The corn stalks were inoculated with Fg_Va_GPS13N4_3ADON (hereafter referred to as FGVA4) and the fungus was allowed to colonize the corn stalks for approximately 10 weeks at room temperature.

A plot area of 3,716 m^2 (0.372 ha) of wheat was subdivided into 100 square plots (10 rows of 10 plots, 6.1 m x 6.1 m). Field inoculations were performed on 2 May 2011 and 16 April 2012 by placing corn stalks from each of the 50 buckets into 50 of the

subplots in a checkerboard pattern (stalks from one bucket were used for each of the subplots).

Production of perithecia on different substrates.

Perithecia were generated on artificial (carrot agar, CA) and natural (corn stalk) substrates (16, 29, 38, 39). Cultures of FGVA4 and Fg_Va_N10_15ADON (hereafter referred to as FGVA10) were grown on petri dishes (19.63 cm^2) containing CA, with fresh organic carrot concentrations ranging from 0-400 g carrots L^{-1} medium. Eight cultures for both FGVA4 and FGVA10 were grown at 0, 100, 200, 225, 250, 275, 300, and 400 g carrots L^{-1} media. The cultures were incubated at room temperature until aerial mycelium covered the petri dishes (approximately 5-7 days). A 2.5% Tween 60 (Product No. 278622500; Acros Organics; Fair Lawn, New Jersey) solution was then added to the cultures and mycelium was flattened using a sterile rod. The cultures were put under a 12-12 hour light-dark cycle at ambient room temperature to encourage perithecia development. Perithecia were observed approximately three days after the addition of the 2.5% Tween 60 solution. The total number of perithecia formed on each culture plate was quantified nine days after the addition of the Tween solution. The average number of perithecia cm^{-2} on the CA surface was calculated for both FGVA4 and FGVA10 isolates at all eight nutrient concentrations.

Perithecia were also quantified from the inoculated corn stalks incubated in the field under natural conditions. Three corn stalks from 49 of the 50 (samples from one subplot were lost) inoculated subplots in 2012 were randomly collected (17 May), giving a total sample size of 147 corn stalks. The corn stalks were brought back to the lab and

the total number of perithecia in six random 0.25 cm^2 sections were counted. Six 0.25 cm^2 areas were painted onto mesh window screening containing a screen size of 2.25 mm^2. The window screening was then wrapped around the corn stalks allowing for the unbiased selection of the six 0.25 cm^2 areas to count.

Quantification of ascospores from perithecia generated on artificial and natural substrates.

Ascospores were quantified from perithecia generated on artificial (carrot agar, CA) and natural (corn stalk) substrates. For perithecia from artificial substrate, cultures of FGVA4 were grown on sterile circles of filter paper (0.25 cm in diameter) scattered on plates of CA containing 400 g carrots L^{-1} medium. Perithecia production was encouraged using a 2.5% Tween 60 solution as described above. The cultures were checked every day for mature perithecia, characterized as being spherical bodies, bluish-black in color (30). As soon as mature perithecia appeared, as early as 3 days, 80 pieces of filter paper each containing an individual perithecium were transferred and placed on the lids of 200 μL microcentrifuge tubes (Product No. 1402-1880; USA Scientific; Ocala, Florida), with the ostiole of the perithecium facing down. The moisture on the filter paper from the CA held the filter paper containing the perithecia in place on the lid. These discharge chambers were left at room temperature to allow the perithecia to release ascospores into the tubes for 24 hours, after which each lid was transferred to a new discharge chamber. The chambers that had been exposed to ascospore discharge from the perithecia were centrifuged at 2000 g for approximately 5 seconds, and the ascospores were re-suspended in 12 μL of sterile water and quantified using a hemacytometer (Product No. 3100;

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Hausser Scientific; Horsham, Pennsylvania) and light microscope. Ascospores were quantified for a total of 10 discharge days.

For perithecia from natural substrate, corn stalks inoculated with FGVA4 were collected from the field 8 days following their release, corresponding to the first day perithecia appeared. Sixty perithecia were removed off the corn stalks by using a scalpel to cut off a portion of the corn stalk containing a single perithecium and placed in discharge chambers by using superglue to affix the corn stalk to the PCR tube lid with the ostiole of the perithecium facing down. Ascospores were quantified from perithecia for a total of 10 discharge days.

Since each mature ascus contains eight ascospores (38), the number of asci in a perithecium was calculated by dividing the total number of ascospores by eight.

Timing of ascospore release from a field-scale source of inoculum under field conditions.

A volumetric spore sampler (Quest Developments, Brits, South Africa) was placed in the center of a 3,716 m^2 wheat field artificially inoculated with FGVA4 colonized corn stalks in 2011 and 2012, as described above (26). The sampler collected a continuous sample of ascospores on a rotating disc coated with silicone grease. Samples were collected between 1700 hours 19 May to 0800 hours 3 June 2011 and 1800 hours 26 April to 1100 hours 14 May 2012, corresponding with when inoculum was present. To assess the background concentration of ascospores, samples were collected with the Quest sampler between 1700 hours 9 April and 0400 hours 16 April 2012, corresponding to the week before inoculum was released in the field. Samples collected by the Quest sampler were

brought back to the lab and stained with Calberla's Stain (Multidata Inc., Saint Louis Park, Minnesota) to allow the *F. graminearum* ascospores to be visualized. Ascospores of *F. graminearum* were identified based on morphological characteristics of spores containing 3-4 septa and having rounded edges, as previously described (30). The total number of *F. graminearum* ascospores captured by the Quest sampler was quantified hourly for each period of sampling in both years (ascospores released hr^{-1}.) Due to the large sample size and very high number of ascospores being released from our inoculated source, a method had to be developed to allow for the efficient collection of data. The total number of ascospores in one field of view at 400x was quantified at the two edges of each hourly interval (i.e. 0600- 0615 hours and 0645- 0700 hours). The average of the two counts was taken and extrapolated to get an estimate of the total number of ascospores released during the one-hour period. To validate this counting method, six time points were chosen where all of the ascospores were counted across the entire 1-hour zone and compared to the counting method described above. Two time points at random were chosen for high, medium, and low counts which corresponded to less than 10, between 10 and 40, and greater than 40 ascospores in one field of view at 400x, respectively. The r^2 value between the described counting method and counting everything across each hour time interval was 0.97.

Estimation of source strength of a field-scale source of inoculum.

The potential source strength, Q_0, or number of ascospores potentially released, was estimated using natural (corn stalks) and artificial (CA) substrate measurements. The total number of corn stalks (5800) released across our 3,716 m^2 source was estimated from

counts of corn stalks in six of the buckets. The above-ground surface area of these stalks (the area not in contact with the soil surface) was calculated by subtracting the surface area of the corn stalk in contact with the ground (perithecia forming from this surface would not be able to contribute to ascospores in the atmosphere) from total corn stalk surface area (calculated by measuring length and diameter of corn stalk.) The surface area of the corn stalk in contact with the ground was calculated by laying 147 corn stalks in a bed of flour. Each stalk was removed from the flour, and the surface area covered in flour was calculated and subtracted from the total surface of the stalk. Potential source strength (Q_0) was then estimated using the following equation:

$$Q_0 = N_B * CS_B * SA_{CS} * S_P$$

(1)

where, N_B is the number of 18.9 L buckets released, CS_B is the number of corn stalks per 18.9 L bucket, SA_{CS} is the exposed surface area per corn stalk, and S_P is the number of ascospores released per perithecium.

The resulting number gives an estimate of the total number of ascospores potentially released from a field-scale source of inoculum containing 5800 stalks.

Statistical analyses.

Analysis of variance (ANOVA) was used to test for significant differences in number of perithecia formed for different carrot concentrations (0 - 400 g carrots/ L medium) and between two *F. graminearum* isolates (FGVA4 and FGVA10). ANOVA was used to determine significant differences in ascospore release between day (0700-1900) and night (1900-0700), in 2011 and 2012. Additionally, a one-way ANOVA was used to determine

significant differences between day (0700-1900) and night (1900-0700) with respect to the number of ascospores released. Finally, ANOVA was used to test for plot uniformity, when controlling for the sub-plot a corn stalk came from in the field-scale source of inoculum and testing for significant differences in total surface area, surface area in the ground, and number of perithecia cm^{-2} formed. Statistical analyses were performed using JMP for Windows (Release 10, SAS Institute Inc., Cary, NC). The mean separation test, Tukey's-Kramer HSD test, was used to conduct pairwise comparisons between each of the variables. For all of the tests significance was evaluated at $P < 0.05$.

RESULTS

Production of perithecia on different substrates.

For the production of perithecia on CA, an increasing number of perithecia cm^{-2} were observed as the concentration of carrots increased from 0 to 400 g L^{-1} medium (**Figure 1**). No perithecia appeared for either FGVA4 or FGVA10 isolates on plates with nutrient concentrations of 0 g carrots L^{-1} medium or 100 g carrots L^{-1} medium. The differences in the number of perithecia cm^{-2} as the concentration of carrots increased were significant for both FGVA4 ($P < 0.001$) and FGVA10 ($P < 0.001$). CA that contained 400 g of carrots per liter induced an average of 14.7 ± 0.36 perithecia cm^{-2} for FGVA4 and 10.5 ± 0.24 perithecia cm^{-2} for FGVA10. The number of perithecia cm^{-2} formed did not differ ($P = 0.243$) between the two isolates studied (FGVA4 and FGVA10).

Corn stalks inoculated with FGVA4 produced an average of 44 ± 2.1 perithecia cm^{-2} (**Figure 1**). Approximately three times more perithecia appeared on corn stalks per

unit area than when the fungus was grown on CA at a concentration of 400 g carrots L^{-1} medium (**Figure 1**).

Quantification of ascospores from perithecia generated on artificial and natural substrates.

The total number of ascospores and duration of ascospore release were quantified from perithecia generated on CA (400 g carrots L^{-1} medium) and corn stalks. Over a 10-day period immediately following mature perithecia development, a mean of 104±5 ascospores and 276±16 ascospores were released from perithecia generated on CA (n=71) and corn stalks (n=40), respectively (**Figure 2**; **Table 1**). The total number of ascospores contained within perithecia from CA and those from corn stalks were significantly different (P <0.001). The number of asci contained within each perithecium was estimated to be a mean of 13±0.6 and 34±2 for perithecia from CA and corn stalks, respectively (**Table 1**).

Trends in daily ascospore release were also examined for the perithecia generated on the two different types of substrates (CA and corn stalks). A similar trend was observed for perithecia from both types of substrates; the number of ascospores released increased for approximately four or five days before decreasing over several days until ascospores were no longer released (**Figure 2, Table 1**). The average duration of the active period of ascospore release from perithecia was approximately seven and six days after mature perithecia first developed on CA and corn stalks, respectively (**Figure 2**; **Table 1**).

Timing of ascospore release from a field-scale source of inoculum under field conditions. The timing of ascospore release was studied hourly between 1700 hours 19 May to 0800 hours 3 June 2011 and 1800 hours 26 April to 1100 hours 14 May 2012 for an artificially inoculated field with FGVA4 corn stalks (**Figure 3**). The majority, 98% (616,594/627,165) and 92% (264,418/288,508), of the ascospores were captured by the volumetric spore sampler between 1900 hours and 0700 hours in 2011 and 2012, respectively. In 2011, the plurality of ascospores were released between 0100 hours and 0200 hours, 28% (175,637/627,165) of ascospores were released during this hour. In 2012, the plurality of ascospores were released between 2200 hours and 2300 hours; 17% (48,180/288,508) of ascospores were released during this hour. Ascospore release was significantly different between day (0700-1900) and night (1900-0700) for 2011 ($P = 0.035$) and 2012 ($P = 0.003$). Additionally, the number of ascospores released from the inoculated field varied significantly among hours of the day or night in both 2011 ($P = 0.003$) and 2012 ($P < 0.001$). Ascospore release varied among sampling days (**Figure 4; Figure 5**). Four and seven peak ascospore release nights (1900-0700) were observed in 2011 and 2012, respectively. A peak release night was declared if more ascospores were released than the average number of ascospores released during the night (1900-0700) during the entire sampling period in 2011 (average number of spores released each night was 41,106) and 2012 (average number of spores released each night was 14,690.) Significant differences were observed between number of ascospores released and sampling day in both 2011 ($P < 0.001$) and 2012 ($P < 0.001$). Relative humidity was not correlated with spore released (correlation coefficient of r=0.17 for 2011 and r=0.11 for 2012) and should be further investigated.

In 2012, the timing of ascospore release was measured before inoculum was released in the field. The number of ascospores caught did not vary with time of day ($P = 0.958$), suggesting that the majority of ascospores being collected when inoculum was present in the field were coming from the inoculated field, rather than background source(s). Finally, the average hourly rate (day and night combined) of ascospore release was 17.4 times greater after inoculum was released in the field.

Estimation of source strength of a field-scale source of inoculum.

To estimate the potential source strength of our field-scale source of inoculum, 147 corn stalks were randomly collected across the field and further analyzed in the lab. The average number of corn stalks contained within each 5-gallon bucket placed in the inoculated field was 116 ± 7 corn stalks. The average total surface area of one corn stalk used to inoculate the 3,716 m^2 plot was 73.4 ± 2.2 cm^2; however since 20.2 ± 0.75 cm^2 was estimated to be the average surface area of the corn stalk exposed to the ground (and thus not contribute to the release of ascospores into the atmosphere), the total surface area of the corn stalk exposed to the atmosphere was calculated to be an average of 53.2 ± 1.8 cm^2. Using **eq. (1),** the number of ascospores released from the inoculated field was estimated to be 3.7×10^9 ascospores based on estimates from corn stalks and 4.8×10^8 ascospores based on estimates from carrot agar (**Table 1**).

There were no significant differences between subplots with respect to perithecia cm^{-2} ($P = 0.417$), total surface area ($P = 0.167$), or exposed surface area ($P = 0.151$) of the corn stalk, indicating a uniform source of potential inoculum.

DISCUSSION

Differences in source strength (Q_0) have the potential to affect the magnitude of the dispersal kernels for fungal spores (1-4). Consequently, there is a need to develop a reliable estimate of Q_0 to increase the accuracy and precision of these models. In this study, we estimated Q_0 from a 3,716 m^2 field-scale source of inoculum of a clonal isolate of *F. graminearum* released at Virginia Tech's Kentland Farm in Blacksburg, VA. Perithecia were generated on artificial (carrot agar) and natural (corn stalk) substrates, and were excised from both substrate types and allowed to release ascospores every 24 hours. A volumetric spore trap was placed inside a 3,716 m^2 clonal source of inoculum over two years of field experimentation. Results from these experiments were used to estimate Q_0 for a field-scale source of inoculum. Estimates of Q_0 for our field-scale sources of inoculum were approximately 4 billion ascospores per 3,716 m^2. Mathematical models can use these estimates of Q_0 in the future to assist in predicting the long-distance transport of *F. graminearum.*

Carrot agar produced 15 ± 0.4 perithecia cm^{-2}, and corn stalks produced 44 ± 2 perithecia cm^{-2}. Multiple studies have reported the effect of temperature and relative humidity on perithecia development (6, 10, 11, 15, 37), yet the production of perithecia under different nutrient regimes requires further attention. Hall (13) examined the effect of carbon:nitrogen ratios on the production of perithecia of *Sordaria fimicola* (Roberge) Ces. And deNot, and found there was an optimal ratio to maximize perithecia development. Corn stalks have a much higher carbon:nitrogen ratio (60:1) than carrots (27:1) (17, 34). The difference in the carbon:nitrogen ratios between corn stalks and carrots could explain at least in part the large difference in number of perithecia formed

between corn stalks and carrot agar. Future work might examine the effect of carbon:nitrogen ratios on perithecia development for *F. graminearum*. It is important to note that we only considered the area density of perithecia projected onto the surface of a cylinder approximately matching the radius and height of each corn stalk. The detailed folded geometry of a corn stalk was not considered. The ratio of surface area and projected area used for the perithecia density calculations are approximately equal for carrot agar. However, if the ratio of surface area and projected area is close to three for corn stalks, it is possible the perithecia area density is closer to the results obtained on carrot agar at a concentration of 400 g carrots L^{-1} medium.

Perithecia generated from carrot agar and corn stalks released a mean of 104±5 and 276±16 ascospores, respectively. A possible explanation for the difference in number of ascospores between these substrates could also be due to the carbon:nitrogen ratios in the different substrates as discussed earlier. It is possible perithecia from carrot agar might not be able to fully develop the same number of mature asci as perithecia from corn stalks (5). It is important to note that we are only able to report the number of released ascospores from each perithecium. It is possible there were more ascospores that were never released from the perithecia. Previous research suggested that if environmental conditions for ascospores release are not optimal, ascospores get released from a perithecium as a cirrhus, due to the accumulation of mannitol (39, 40). One report suggested that cirrhi from individual perithecia contained as many as 1000-5000 ascospores (7). Thus, we are probably underestimating the true number of ascospores contained in a perithecium and the number of asci contained within a perithecium as our calculation was based on the number of released ascospores and knowing that each

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mature ascus contains eight ascospores (38). Due to the differences observed in both the number of perithecia formed and number of ascospores released when two different substrates were used (carrot agar and corn stalks), it is also possible that ascospore release dynamics could be different for perithecia from natural substrates, such as corn stalks, than for perithecia from artificial substrates, such as carrot agar (28, 39). This is beyond the scope of this work, but should be further investigated, as previous studies have used perithecia from carrot agar to examine release dynamics of ascospores from perithecia (29, 40).

Perithecia from both carrot agar and corn stalks showed the same general trend of daily release dynamics. The number of ascospores released each day increased for a few days before decreasing. Ascospores were no longer released approximately one week after the formation of mature perithecia. It is possible that all ascospores were released during this one week period; however another possibility for the decrease and eventual cease of ascospore release is due to the formation of cirrhi (7).

Ascospores of *F. graminearum* were released under field conditions predominantly during the night (1900 to 0700 hours). Our results are consistent with several previous studies (12, 24, 41), but contradict one study (20). There is evidence that ascospores are released due to a drying-wetting cycle of perithecia, where the perithecia dry out during the day when the humidity is low, but then are released during the evening when relative humidity increases (24, 41). Previous studies found that ascospore release is diminished during constant rainfall or high relative humidity (12, 24). In 2012, the time period that ascospore release was highest was between 2200 hours to 2300 hours, while in 2011, 0100 hours to 0200 hours showed the highest amount of

ascospore release. Possible explanations for this small difference (3 hours) in peak release interval include differences in environmental conditions and timing of the experiments (the samples were collected approximately one month earlier in 2012 than in 2011). We observed major release events; 41% (256,905/627,165) and 28% (80,190/288,508) of all of the ascospores captured by the volumetric spore sampler during the field sampling period were captured during single days in 2011 (20 May) and 2012 (9 May), respectively. This demonstrates that ascospores are not consistently released at the same rate each day and environmental conditions must be appropriate to trigger ascospore releases (24, 28, 39-41). Understanding the timing of release of *F. graminearum* ascospores is important when trying to validate long distance transport models, as one of the components of the models is spore release (1-4). Transport models must be calibrated to account for variability in release patterns of *F. graminearum* ascospores.

Estimates of Q_0 for our field-scale sources of inoculum were approximately 4 billion ascospores for both 2011 and 2012, when using variables (number of perithecia cm^{-2} and number of ascospores discharged) collected from corn stalks. It is important to acknowledge that this is only an estimate, since field and laboratory observations may differ. If variables (number of perithecia cm^{-2} and number of ascospores discharged) collected from an artificial substrate (carrot agar) in the laboratory are used, Q_0 is only estimated to be approximately 500 million ascospores, almost an order of magnitude lower than using estimates from a natural substrate (corn stalk). One difference between laboratory and field conditions is relative humidity does not vary as much in the laboratory as it does in the field. Previous research has indicated that the change in

relative humidity is an important factor in triggering ascospore release (24, 41) Additionally, it is important to address that under a laboratory setting we are assuming all the perithecia have formed and are ready to discharge their ascospores at the same time. However, in the field perithecia formation and ascospore release may occur in 'waves' as the conditions become favorable. The large difference seen in the estimation of Q_0 when using a natural substrate (corn stalks) versus artificial substrate (carrot agar), suggests that researchers must use caution when translating laboratory measurements to the field.

Initial source strength is one of the most difficult components to estimate in transport models for plant pathogenic fungi (1-4). The work presented here provides an experimental means to estimate the potential source strength, Q_0, of a 3,716 m^2 field-scale source of *F. graminearum* inoculum. Future work may include the validation of current spore transport models by combining the knowledge of initial source strength, spore release patterns, and field results for spore transport and deposition (1-4, 26). After validation of spore transport models, source strength estimates could be integrated into current FHB risk assessment tools, which currently only consider environmental factors for disease development to have a more complete risk assessment tool that takes into account both spore transport and environmental factors (8, 9).

LITERATURE CITED

1.   Aylor, D. E. 1999. Biophysical scaling and the passive dispersal of fungus spores: relationship to integrated pest management strategies. Agricultural and Forest Meteorology 97:275-292.

2.   Aylor, D. E. 1986. A framework for examining inter-regional aerial transport of fungal spores. Agricultural and Forest Meteorology 38:263-288.

3.   Aylor, D. E., and Sutton, T. B. 1992. Release of *Venturia inaequalis* ascospores during unsteady rain: Relationship to spore transport and deposition. Phytopathology 82:532-540.

4.   Aylor, D. E., Taylor, G. S., and Raynor, G. S. 1982. Long-range transport of Tobacco blue mold spores. Agricultural Meteorology 27:217-232.

5.   Barnett, H., and Lilly, V. G. 1947. The effects of biotin upon the formation and development of perithecia, asci and ascospores by *Sordaria fimicola* Ces. and de North American. Journal of Botany: 196-204.

6.   Beyer, M., Röding, S., Ludewig, A., and Verreet, J. A. 2004. Germination and survival of *Fusarium graminearum* macroconidia as affected by environmental factors. Journal of Phytopathology 152:92-97.

7. Bowden, R. L., and Leslie, J. F. 1999. Sexual recombination in *Gibberella zeae*. Phytopathology 89:182-188.

8. De Wolf, E. D., Madden, L. V., and Lipps, P. E. 2003. Risk assessment models for wheat Fusarium head blight epidemics based on within-season weather data. Phytopathology 93:428-435.

9. Del Ponte, E. M., Fernandes, J. M. C., Pavan, W., and Baethgen, W. E. 2009. A Model-based assessment of the impacts of climate variability on Fusarium head blight seasonal risk in southern Brazil. Journal of Phytopathology 157:675-681.

10. Doohan, F., Brennan, J., and Cooke, B. 2003. Influence of climatic factors on *Fusarium* species pathogenic to cereals. European Journal of Plant Pathology 109:755-768.

11. Dufault, N., De Wolf, E., Lipps, P., and Madden, L. 2006. Role of temperature and moisture in the production and maturation of *Gibberella zeae* perithecia. Plant Disease 90:637-644.

12. Fernando, W. G., Miller, J. D., Seaman, W. L., Seifert, K., and Paulitz, T. C. 2000. Daily and seasonal dynamics of airborne spores of *Fusarium graminearum* and other *Fusarium* species sampled over wheat plots. Canadian Journal of Botany 78:497-505.

13. Hall, R. 1971. Effect of carbon-nitrogen ratios on production of perithecia by *Sordaria fimicola*. Canadian Journal of Microbiology 17:132-134.

14. Isard, S. A., Gage, S. H., Comtois, P., and Russo, J. M. 2005. Principles of the atmospheric pathway for invasive species applied to Soybean rust. Bioscience 55:851-861.

15. Khonga, E., and Sutton, J. 1988. Inoculum production and survival of *Gibberella zeae* in maize and wheat residues. Canadian Journal of Plant Pathology 10:232-239.

16. Klittich, C., and Leslie, J. 1988. Nitrate reduction mutants of *Fusarium moniliforme* (*Gibberella fujikuro*i). Genetics 118:417-423.

17. Kourik, R. 2005. Designing and maintaining your edible landscape naturally. Chelsea Green Publishing.

18. Krupa, S., Bowersox, V., Claybrooke, R., Barnes, C. W., Szabo, L., Harlin, K., and Kurle, J. 2006. Introduction of Asian soybean rust urediniospores into the midwestern United States—A case study. Plant Disease 90:1254-1259.

19.   Mai, W., and Abawi, G. 1987. Interactions among root-knot nematodes and Fusarium wilt fungi on host plants. Annual Review of Phytopathology 25:317-338.

20.   Maldonado-Ramirez, S. L. 2001. Aerobiology of the wheat scab fungus, *Gibberella zeae*: discharge, atmospheric dispersal, and deposition of ascospores. Ph. D. thesis, Cornell University, Ithaca, NY.

21.   Maldonado-Ramirez, S. L., Schmale III, D. G., Shields, E. J., and Bergstrom, G. C. 2005. The relative abundance of viable spores of *Gibberella zeae* in the planetary boundary layer suggests the role of long-distance transport in regional epidemics of Fusarium head blight. Agricultural and Forest Meteorology 132:20-27.

22.   McMullen, M., Jones, R., and Gallenberg, D. 1997. Scab of wheat and barley: A re-emerging disease of devastating impact. Plant Disease 81:1340-1348.

23.   Oke, T. R. 1987. Boundary layer climates. 2 ed. Cambridge University Press, Cambridge, UK.

24.   Paulitz, T. C. 1996. Diurnal release of ascospores by *Gibberella zeae* in inoculated wheat plots. Plant Disease 80:674-678.

25.   Paulitz, T. C. 1999. Fusarium head blight: a re-emerging disease. Phytoprotection (Québec) 80.

26.   Prussin II, A. J., Li, Q., Malla, R., Ross, S. D., and Schmale, III, D. G. 2013. Monitoring the long distance transport of *Fusarium graminearum* from field-scale sources of inoculum. Plant Disease *Submitted*.

27.   Schmale, D., Ross, S., Fetters, T., Tallapragada, P., Wood-Jones, A., and Dingus, B. 2012. Isolates of Fusarium graminearum collected 40–320 meters above ground level cause Fusarium head blight in wheat and produce trichothecene mycotoxins. Aerobiologia 28:1-11.

28.   Schmale, D. G., Arntsen, Q. A., and Bergstrom, G. C. 2005. The forcible discharge distance of ascospores of *Gibberelia zeae*. Canadian Journal of Plant Patholology 27:376-382.

29.   Schmale III, D. G., Arntsen, Q. A., and Bergstrom, G. C. 2005. The forcible discharge distance of ascospores of *Gibberella zeae*. Canadian Journal of Plant Pathology 27:376-382.

30.   Schmale III, D. G., and Bergstrom, G. C. 2003. Fusarium head blight in wheat. The Plant Health Instructor. DOI: 10.1094/PHI-I-2003-0612-01.

31. Schmale III, D. G., and Bergstrom, G. C. 2004. Spore deposition of the ear rot pathogen, *Gibberella zeae*, inside corn canopies. Canadian Journal of Plant Pathology 26:591-595.

32. Schmale III, D. G., Bergstrom, G. C., and Shields, E. J. 2006. Night-time spore deposition of the Fusarium head blight pathogen, *Gibberella zeae*, in rotational wheat fields. Canadian Journal of Plant Pathology 28:100-108.

33. Schmale III, D. G., Leslie, J. F., Zeller, K. A., Saleh, A. A., Shields, E. J., and Bergstrom, G. C. 2006. Genetic structure of atmospheric populations of *Gibberella zeae*. Phytopathology 96:1021-1026.

34. Shouru, S., Qiusheng, Y., Xiaoyu, D., and Jiqing, W. 2008. Analysis of mineral element contents and physical and chemical properties of corn stalk substrate. Transactions of the Chinese Society of Agricultural Engineering 24:41-44.

35. Snijders, C. H. A. 1990. Fusarium head blight and mycotoxin contamination of wheat, a review. European Journal of Plant Pathology 96:187-198.

36. Stokstad, E. 2007. Deadly wheat fungus threatens world's breadbaskets. Science 315:1786-1787.

37.   Sutton, J. C. 1982. Epidemiology of wheat head blight and maize ear rot caused by *Fusarium graminearum*. Canadian Journal of Plant Pathology 4:195-209.

38.   Trail, F., and Common, R. 2000. Perithecial development by *Gibberella zeae*: a light microscopy study. Mycologia:130-138.

39.   Trail, F., Gaffoor, I., and Vogel, S. 2005. Ejection mechanics and trajectory of the ascospores of *Gibberella zeae* (anamorph *Fuarium graminearum*). Fungal Genetics and Biology 42:528-533.

40.   Trail, F., Xu, H., Loranger, R., and Gadoury, D. 2002. Physiological and environmental aspects of ascospore discharge in *Gibberella zeae* (anamorph *Fusarium graminearum*). Mycologia 94:181-189.

41.   Tschanz, A., Horst, K., and Nelson, P. E. 1975. Ecological aspects of ascospore discharge in *Gibberella zeae*. Phytopathology 65:597-599.

42.   Weller, D. M. 1988. Biological control of soilborne plant pathogens in the rhizosphere with bacteria. Annual Review of Phytopathology 26:379-407.

Table 1. Differences in ascospore release of a single strain of *F. graminearum* (FGVA4) from perithecia generated on artificial (carrot agar) and natural (corn stalks) substrates. Results presented are means plus/minus standard errors.

	Carrot Agar[A,B]	Corn Stalks[C]
perithecia cm^{-2}	15 ± 0.4	44 ± 2.1
Total number of ascospores released/perithecium	104 ± 5	276 ± 16
Total number asci/perithecium[D]	13 ± 0.6	34 ± 2.0
Duration of ascospore release[E]	7.0 ± 0.2	5.9 ± 0.2
Peak ascospore release day[F]	4.2 ± 0.2	4.8 ± 0.2
Estimation of Q_0 for inoculated field[G]	4.8x10^{8} [H]	3.7x10^{9}

[A] Carrot agar concentration was 400 g carrots L^{-1} medium.

[B] Sample size of n= 71.

[C] Sample size of n=40.

[D] Estimated by dividing total number of ascospores by eight.

[E] Number of days the perithecia released ascospores after formation of mature perithecia.

[F] Number of days after formation of mature perithecia that ascospore release was the highest.

[G] Potential number of ascospores released from 3,716 m^{2} inoculated field. Values were calculated using **eq. 1**.

[H] Assumed same surface area present as corn stalks released in the field.
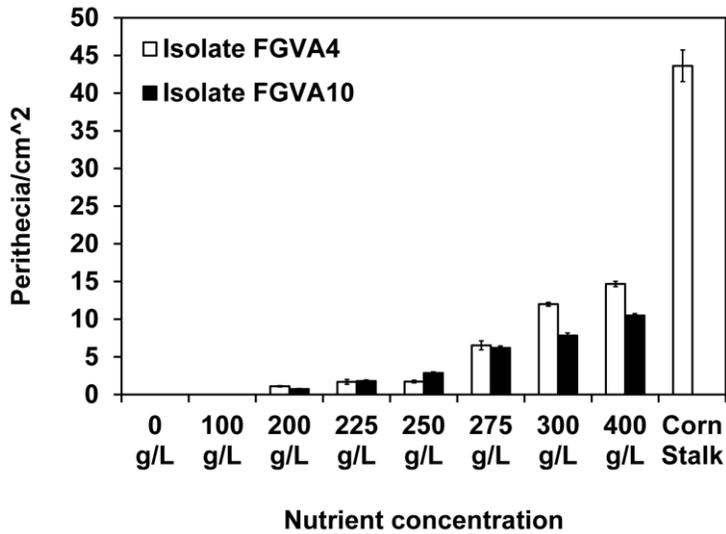
Figure 1. Production of perithecia on artificial (carrot agar, CA) and natural (corn stalk) substrates. CA contained different amounts of carrots ranging from 0 g carrots L^{-1} medium to 400 g carrots L^{-1} medium. CA plates were inoculated with two different *F. graminearum* isolates naturally recovered in Virginia, FGVA4 and FGVA10. A general trend was observed of an increase in the number of perithecia cm^{-2} as nutrient availability increased for both isolates studied. A sample size of eight was used for each isolate at each CA concentration. Corn stalks inoculated with FGVA4 were recovered from a 3,716 m^2 field-scale source and the number of perithecia cm^{-2} was quantified (n = 147). Approximately three times more perithecia formed on corn stalks than on the highest CA nutrient plate (400 g carrots L^{-1} medium), suggesting corn stalks are more conducive for perithecia production than CA.
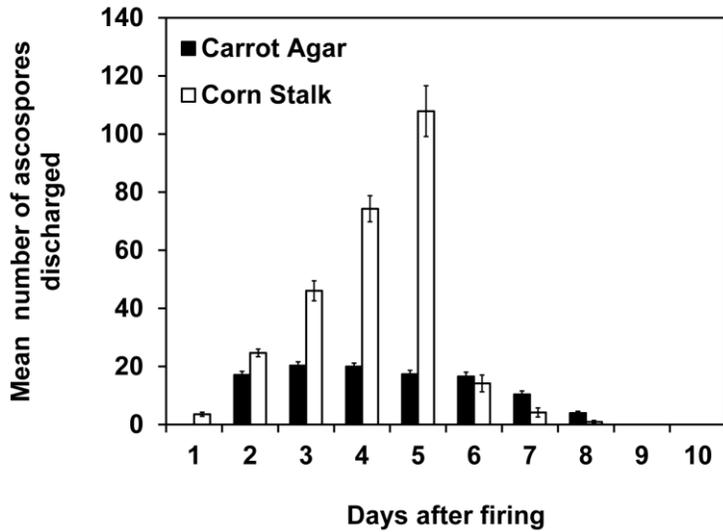
Figure 2. The number of ascospores released from perithecia generated from carrot agar (CA) and corn stalks. CA and corn stalks were both inoculated with FGVA4 and checked daily for the formation of mature perithecia. As soon as mature perithecia were present, perithecia were extracted from the substrates and the number of ascospores released each day was studied. Sample sizes of 71 and 40 perithecia were used for CA and corn stalks, respectively. Perithecia from corn stalks and CA released an average of 276 ± 16 and 104 ± 5 ascospores over a 10-day period, respectively.
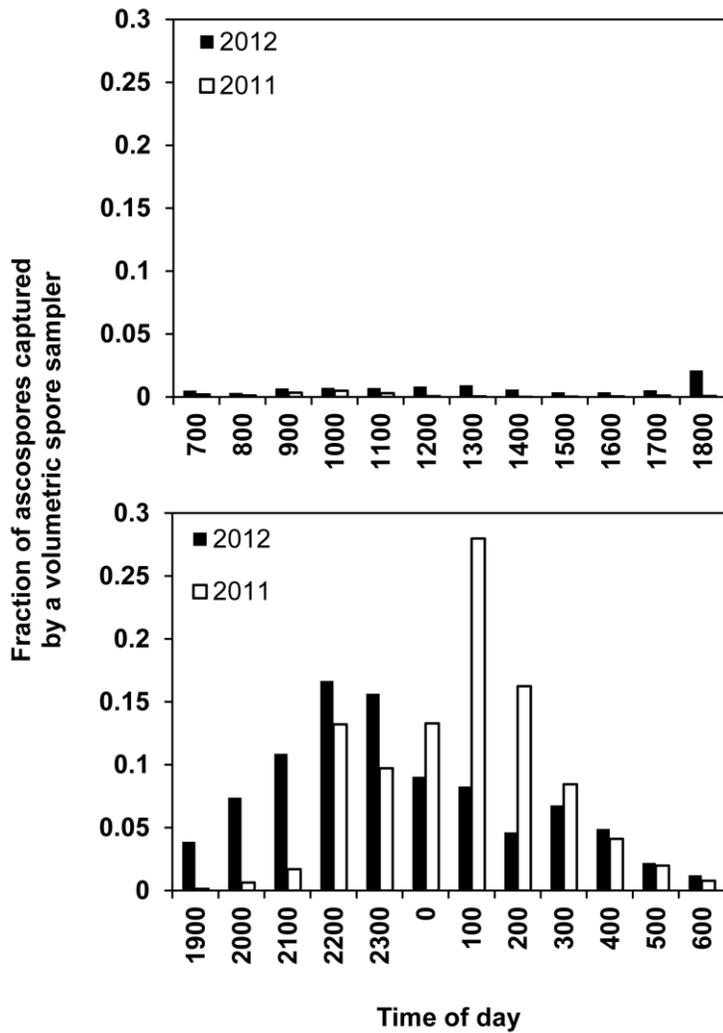
Figure 3. Fraction of ascospores released and captured by a Quest volumetric spore sampler placed in the center of a 3,716 m^2 wheat field inoculated with FGVA4, during the day (0700-1900; top) and night (1900-0700; bottom). The volumetric spore sampler was continuously sampling between 1700 hours 19 May to 0800 hours 3 June, 2011 and 1800 hours 26 April to 1100 hours 14 May, 2012. These dates correspond to when corn stalks inoculated with FGVA4 were present in the 3,716 m^2 wheat field. The majority, 98% (616,594/627,165) and 92% (264,418/288,508), of the ascospores were captured by

the volumetric spore sampler between 1900 hours and 0700 hours in 2011 and 2012, respectively.
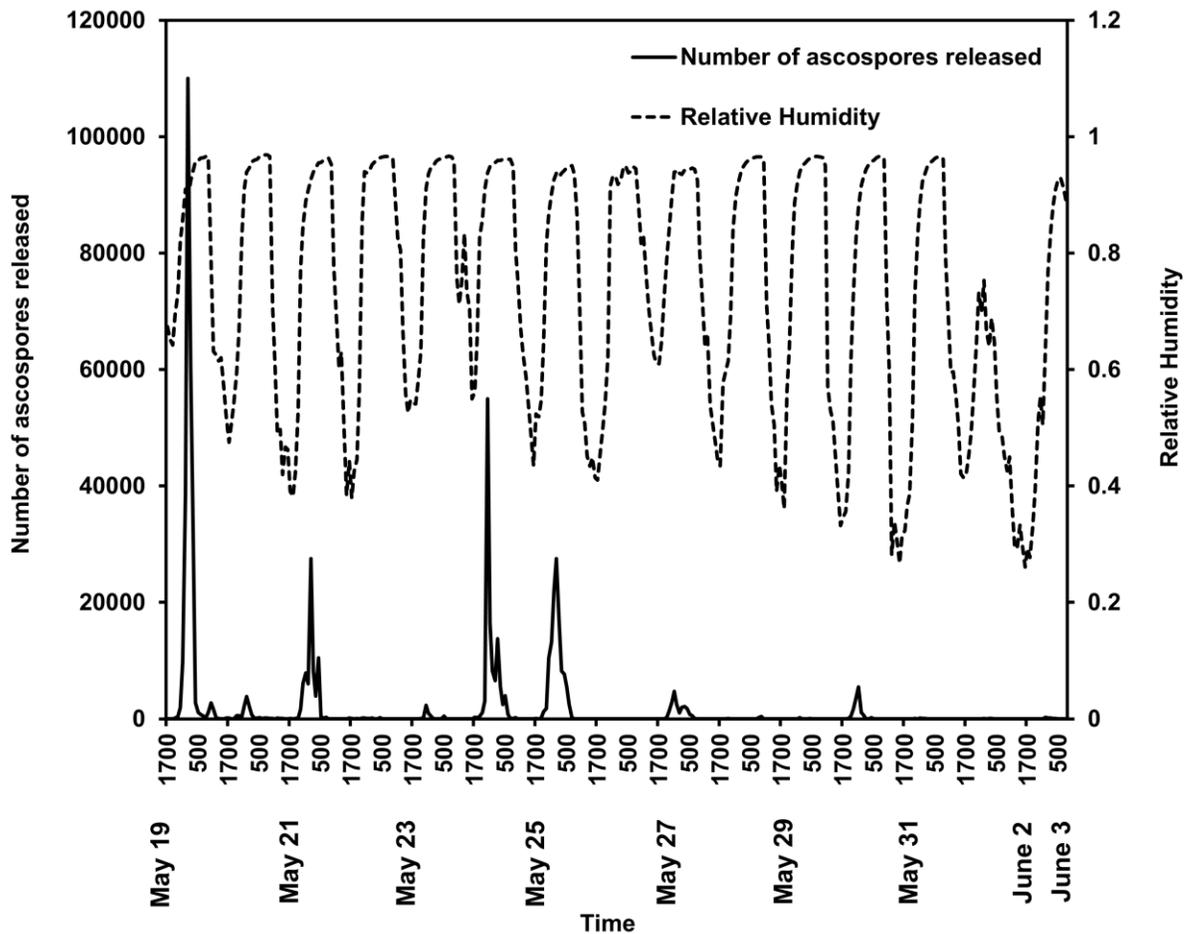
Figure 4. Daily ascospore release pattern for a field-scale source of *F. graminearum* in 2011 (solid line). Ascospores were captured using a Quest volumetric spore sampler placed in the center of a 3,716 m^2 wheat field inoculated with FGVA4. In 2011, ascospores were continuously sampled between 1700 hours 19 May to 0800 hours 3 June corresponding to when corn stalks inoculated with FGVA4 were present in the 3,716 m^2 wheat field. Major release events are observed when inoculum is present in the field. Daily patterns of relative humidity are also given for reference (dashed line). Relative humidity was not correlated with spore release (r=0.17) and should be further investigated. Rainfall and spore release will be discussed in chapter 4.
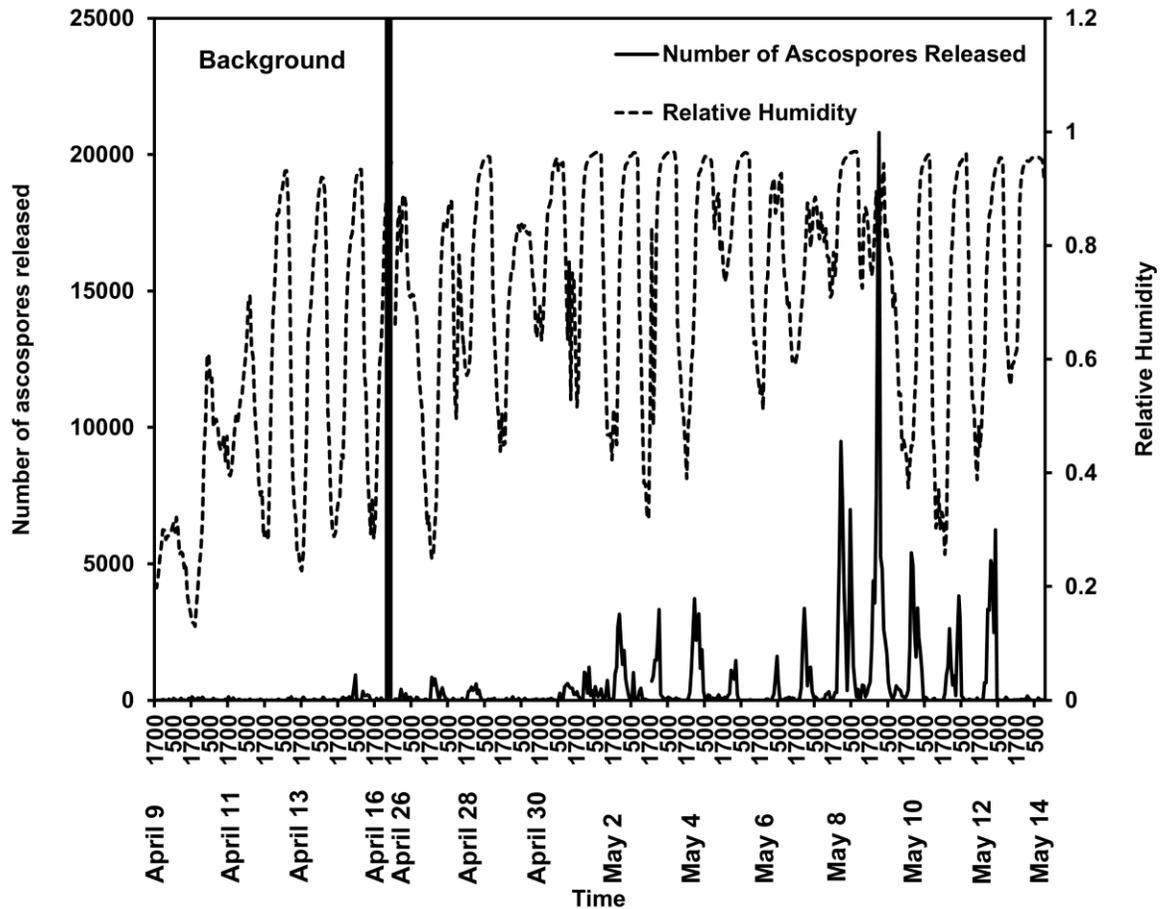
Figure 5. Daily ascospore release pattern for a field-scale source of *F. graminearum* in 2012 (solid line). Ascospores were captured using a Quest volumetric spore sampler placed in the center of a 3,716 m^2 wheat field inoculated with FGVA4. Samples were collected from 1700 hours 9 April to 0400 hours 16 April corresponding to the week before corn stalks inoculated with FGVA4 were released in the 3,716 m^2 wheat field to assess the background source potential. After inoculated corn stalks were released in the 3,716 m^2 wheat field and mature perithecia formed, samples were collected with the volumetric spore sampler between 1800 hours 26 April to 1100 hours 14 May. Major release events are observed when inoculum is present in the field. Daily patterns of relative humidity are also given for reference (dashed line). Relative humidity was not

correlated with spore release (r=0.11) and should be further investigated. Rainfall and spore release will be discussed in chapter 4.

Chapter 3: Monitoring the Long Distance Transport of *Fusarium graminearum*

from Field-Scale Sources of Inoculum

Aaron J. Prussin, II[1], Qing Li[2], Rimy Malla[2], Shane D. Ross[3], and David G. Schmale III[1*]

[1]Department of Plant Pathology, Physiology, and Weed Science, Virginia Tech, Blacksburg, VA 24061-0390

[2]Department of Statistics, Virginia Tech, Blacksburg, VA 24061-0219

[3]Department of Engineering Science and Mechanics, Virginia Tech, Blacksburg, VA 24061-0219

*Corresponding Author: David G. Schmale, III; PH: (540)-231-6943; E-mail:

dschmale@vt.edu

The fungus *Fusarium graminearum* causes Fusarium head blight (FHB) of small grains. Little is known about how spores of the fungus are transported from field-scale sources of inoculum. We used a unique method to track the movement of a clonal isolate of *F. graminearum* over two field seasons. Ground-based collection devices were placed at distances of 0 m (in the source), 100 , 250 , 500 , 750 , and 1000 m from the center of 3,716 m^2 clonal sources of inoculum. Three polymorphic microsatellites were used to identify the released clone from 1,027 isolates (790 in 2011, and 237 in 2012) of the fungus. Recovery of the released clone decreased at greater distances from the source. The majority (87%, 152/175 in 2011; 77%, 74/96 in 2012) of the released clone was recaptured during the night (1900-0700). The released clone was recovered up to 750 m from the source. Background populations of the fungus collected prior to and during the release of the clone in 2011 had similar microsatellite allele frequencies. This work offers a means to experimentally determine the dispersal kernel of a plant pathogen, and could be integrated into management strategies for FHB.

Many plant pathogens use the atmosphere to move over long distances (2, 4), including *Peronospora tabacina*, causal agent of tobacco blue mold, (1) *Phakopsora pachyrhizi*, causal agent of Asian soybean rust (1, 16), and *Puccinia graminis* f. sp. *tritici*, causal agent of wheat stem rust (31). The atmospheric transport of plant pathogens is broadly characterized by the aerobiological processes of inoculum development, liberation, horizontal transport, deposition, and impact (13). A detailed understanding of these processes can help in the development of successful plant disease management

strategies, yet few technologies are presently available to adequately examine each of these processes in detail.

One of the greatest challenges in understanding the atmospheric transport of plant pathogens is the ability to track their movement from a known source (e.g., an infected field) to a final destination (e.g., a susceptible crop). In this work, we showcase a unique method to release and re-capture an important plant pathogen within distances of 1000 m from a field-scale source of inoculum. Our method was developed for the plant pathogen *Fusarium graminearum* (synonym *Gibberella zeae*). This fungus causes Fusarium head blight (FHB) of wheat and barley, which has resulted in more than $3 billion in crop losses in the United States over the past two decades (20, 22, 24). The fungus produces a mycotoxin known as deoxynivalenol (DON) that may contaminate food and feed and threaten the health of humans and livestock (30, 32).

Recently, there have been advances in release-recovery experiments of *F. graminearum* over short distances (< 30 m) using a genotyping method known as amplified fragment length polymorphism (AFLP) (14, 15). In these studies, small amounts of inoculum (45-410 g) were released from 0.55 m^2 plots and diseased wheat heads were collected short distances (< 30 m) from the source of inoculum and analyzed for the released clone of *F. graminearum*. Though these studies were among the first in this pathosystem to track the fungus from known inoculum sources, the size of the inoculum sources and the scale of the study limited collections of released clone to within distances of 30 m from the source. Fernando et al. (10) used an isolate of *F. graminearum* exhibiting a yellow phenotype on standard culture medium to help distinguish the released clone from natural (background) populations of the fungus. Both of these

techniques (AFLPs and the release of a yellow phenotype) have some limitations. AFLPs require a considerable amount of time and resources (the genomic DNA from each isolate is subjected to digestion and ligation reactions with different enzymes followed by multiple PCRs), and the physical location of and selection on AFLP markers is often unknown. The release and recapture of a yellow phenotype of *F. graminearum* is limited since yellow phenotypes of the fungus may be present in natural populations of the fungus. Recently, researchers have used a barcoded spore approach to identify a released microbe from background populations (5, 9). In these studies, the researchers inserted a small DNA sequence into a neutral region of *Bacillus thuringiensis* subsp. *Kurstaki*, a biopesticide agent, to distinguish what was released from wild type strains. Although this method allows for unambiguous discrimination of a released agent, such a method would likely not be appropriate in an agricultural setting since biosecurity regulations and political challenges preclude the release of genetically modified plant pathogens into commercial crop fields. An alternative strategy to identify a specific fungal individual from a background population is to use a series of microsatellites—short conserved sequences of DNA scattered across the genome (33). The use of microsatellites to identify a released clone of *F. graminearum* within a heterogeneous natural population could provide a rapid, cost-efficient, and robust method to track the long distance movement of *F. graminearum* from a known inoculum source.

The specific objective of this study was to develop a robust microsatellite method to track the long distance movement (up to 1000 m) of a released plant pathogen from a field-scale source of clonal inoculum. Though Schmale et al. (29) speculated that spores of *F. graminearum* may be transported tens to hundreds of kilometers in the atmosphere,

to our knowledge, detailed studies on the long distance transport (on the scale of ~1000 m) of *F. graminearum* from known sources of inoculum have not yet been conducted. We hypothesized that the recovery of the released clone would decrease at increasing distances downwind from a large area source of inoculum. To test this hypothesis, a series of ground-based collection devices were placed at distances of 0 m (in the source), 100 m, 250 m, and 500 m from the center of a 3,716 m^2 (0.372 ha) clonal (one strain) source of inoculum in 2011, and 0 m (in the source), 100 m, 250 m, 500 m, 750 m, and 1000 m, from the center of a 3,716 m^2 clonal source of inoculum in 2012. Three polymorphic microsatellites located on three different chromosomes (chromosomes 1, 2, and 4) were used to identify the released clone from natural (background) populations of the fungus. This work highlights a simple, cost-effective method to track the long distance movement of a plant pathogen in the atmosphere. Methods such as this may help forecast the spread of other high-risk plant pathogens (e.g., soybean rust and wheat stem rust) in the future and help target the early application of appropriate fungicides. It is also important to be able to track the long distance movement of plant pathogens in order to create and validate mathematical models to predict the movement of high-risk plant pathogens from infected crops to healthy crops. An abstract on a portion of this work has been published (23).

MATERIALS AND METHODS

Experimental fields. Field studies were conducted at Virginia Tech's Kentland Farm in Whitethorne, Virginia from 26 April to 25 May 2011 and 9 April to 14 May 2012. The Kentland Farm is composed of about 810 hectares of farmland. Two hectares of winter

wheat (untreated Southern States variety SS560) were planted in October 2010 for the 2011 field campaign and October 2011 for the 2012 field campaign. The winter wheat fields were not treated with any fungicides. One month prior to the start of the field inoculations nitrogen was applied at a rate of 22.7 kg/4047 m^2 to the wheat plots.

Preparation of clonal inoculum source. Mature, green corn stalks were collected in August 2010 and 2011 from corn fields at Virginia Tech's Kentland Farm in Blacksburg, VA and dried in a glass house for 6 months. The dried corn stalks were then cut into ~15 cm pieces and placed into 50 individual 18.9 L steel buckets (Product No. 652560; Global Industrial; Port Washington, NY). Each of 50 buckets was filled approximately 2/3 full with cut corn stalks and autoclaved for 120 min. After the initial autoclaving step, the corn stalks were soaked in deionized water overnight, the water was then removed, and the corn stalks were autoclaved again for 120 min. The autoclaved corn stalks were then inoculated with colonized agar pieces of *F. gramineaerum* isolate Fg_Va_GPS13N4_3ADON (hereafter referred to as FGVA4) from five 100 mm diameter petri dishes that had been cultured on ¼-strength PDA for 12 days. The buckets containing the inoculated corn stalks were stored at ambient room temperature for approximately 10 weeks to allow the fungus to colonize the corn stalks.

A plot area of 3,716 m^2 (0.372 ha) of wheat (described above) was subdivided into 100 square plots (10 rows of 10 plots, 6.1 m x 6.1 m). Field inoculations were performed on 2 May 2011 (season 1) and 16 April 2012 (season 2) by releasing corn stalks from each of the 50 buckets into 50 of the subplots in a checkerboard pattern (stalks from one bucket were used for each of the subplots). The field inoculation was

carried out when the wheat heads began to boot (Zadoks growth stage 4). Perithecia were first observed within 10 days after the inoculated corn stalks were released in the field in both years.

Collection of the released clone at different distances from the source. A series of ground-based collection devices were placed at distances of 0 (in the source), 100 , 250 , and 500 m from the center of the inoculated field in 2011, and 0 (in the source), 100 , 250 , 500 , 750 , and 1000 m, from the center of the inoculated field 2012. Petri plates (100 mm in diameter; surface area = 78.5 cm^2) containing a Fusarium selective medium (FSM) were placed on the top of a 1 m wooden stake parallel to the ground to collect viable spores of *Fusarium*. The FSM consisted of agar (15 g L^{-1}) (Product no. BP26411; Fisher Scientific; Fair Lawn, NJ), Bacto peptone (15 g L^{-1}) (Product no. 211820; BD; Sparks, MD), potassium phosphate monobasic (1 g L^{-1}) (Product no. P380-500; Fisher Scientific; Fair Lawn, NJ), magnesium sulfate (0.5 g L^{-1}) (Product no. M63-500; Fisher Scientific; Fair Lawn, NJ), Terraclor fungicide (1 g/L) (Product no. 509428; Crompton; Middlebury, CT), streptomycin (1 g L^{-1}) (Product no. BP910-50; Fisher Scientific; Fair Lawn, NJ), and neomycin (350 mg L^{-1}) (Product no. N-1876; Sigma; St. Louis, MO) (28). In 2011, three sampling devices were placed in the field scale source, 12 were placed in a circle 100 m from the center of the source, and five were placed at 250 m and 500 m from the source center in the prevalent downwind direction (**Figure 1, 2**). Three additional sampling devices were placed in the prevalent upwind direction (two were placed 250 m from the source center and one was place 500 m from the source center). In 2012, three sampling devices were placed in the field scale source, 12 were placed in a

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circle 100 m from the center of the source, five were placed at 250 m and 500 m from the source center in the prevalent downwind direction, and six were placed at 750 m and 1000 m from the source center in the prevalent downwind direction (**Figure 1**). Three additional sampling devices were placed in the prevalent upwind direction (two were placed 250 m from the source center and one was placed 500 m from the source center). Wind data were collected from the Virginia Agricultural Experimental Station Mesonet weather station located at the Kentland Farm, approximately 250 m northwest from the center of our source of inoculum in 2011, and approximately 350 m northwest from the center of our source of inoculum in 2012. Wind speed and direction was recorded in 15-minute intervals, and was used to illustrate the fraction of time the wind was coming from a particular direction (**Figure 2**).

In 2011, samples were collected continuously over four time intervals each day: 0700-1100, 1100-1500, 1500-1900, and 1900-0700. Sampling was also conducted immediately prior to the release of the clone into the field from 26 April to 2 May 2011 (the field was inoculated 2 May 2011) to assess the presence of our released clone in background populations of *F. graminearum*. Field sampling for the released clone started immediately following the observation of perithecia on the inoculated corn stalks. Field samples were collected for 14 consecutive days (12 May through 25 May 2011).

In 2012, samples were collected continuously over two time intervals each day: 0700-1100 and 1900-0700. Sampling was also conducted prior to the release of the clone into the field from 9 April to 12 April 2012 (the field was inoculated 16 April 2012) to assess the presence of our released clone in background populations of *F. graminearum*. Field sampling for the released clone started immediately following the observation of

perithecia on the inoculated corn stalks. Field samples were collected for 19 consecutive days (26 April through 14 May 2012).

Fungal isolation and DNA extraction. After each sampling period, exposed petri plates were immediately removed from the field, covered, and placed in small plastic boxes for transport to the laboratory. The plates were incubated for 7-10 days in the laboratory at ambient room temperature and the number of *Fusarium* colonies (distinct white, fluffy colonies approximately 15 mm in diameter) collected at each location during each sampling period was recorded. In 2011, up to five *Fusarium* colonies from each plate were randomly selected and sub-cultured to petri plates containing 1/4-strength PDA,. In 2012, the number of *Fusarium* samples further sub-cultured and studied was increased to ten colonies per plate. Colonies producing red, pink, or yellow mycelia characteristic of *F. graminearum* and containing only macroconidia on ¼-strength PDA, were single-spored onto additional plates. Single-spored cultures were placed in 20% glycerol and stored at -80°C. Single-spored isolates identified as *F. graminearum* were grown in 100 ml of 1/4-strength PD broth on a shaker at 100 rpm for 5-7 days at ambient room temperature. Harvesting of mycelia and extraction of DNA were conducted following previously published methods (14, 15).

Identification of the released clone from field collections. Three microsatellites (FusSSR22, FusSSR23, and FusSSR27) were used to genotype all of the singled-spored isolates of *F. graminearum* from the field collections (**Table 1**, (33)). The forward primers were labeled with fluorescent dyes (FusSSR22 was labeled with Ned, FusSSR23

was labeled with Ned, and FusSSR27 was labeled with Fam) and the PCR amplicons were visualized and accurately sized on an Applied Biosystems Genetic Analyzer 3130xl (**Figure 3**). PCR was performed in 25 μl volumes containing approximately 50 ng of template DNA, Taq 2X Master Mix (Product no. M0270S; New England BioLabs, Ipswich, MA), and 0.2 μM forward (fluorescently labeled) and reverse primers (Integrated DNA Technologies, Coralville, IA). Cycling conditions consisted of 1 min initial denaturation at 95°C, followed by 36 cycles of 30 s denaturation at 95°C, 30 s annealing at 65°C, and 30 s extension at 72°C. PCR was completed with a final extension for 10 min at 72°C. Microsatellites were sized and scored on an Applied Biosystems Genetic Analyzer 3130xl equipped with a 36 cm capillary and POP-7 polymer. Before each run, the PCR product was diluted 5-fold in sterile DI water and 2 μl of the diluted PCR product, 9.8 μl of Hi-Di formamide (Model no. 4311320; Applied Biosystems, Inc., Foster City, CA) and 0.2 μl of GeneScan 500Liz size standard (Model no. 4322682; Applied Biosystems, Inc., Foster City, CA) were added to each tube. Genemarker Software (version 1.7; SoftGenetics, State College, PA, USA) was used to analyze the data. Isolates with the same product sizes as FGVA4 for all three microsatellites were identified as the released clone. Microsatellites for FusSSR22, FusSSR23, and FusSSR27 are located on chromosomes 4, 2 and 1, respectively. Microsatellite locations on chromosomes were determined by performing BLAST queries against the genome of *F. graminearum* (6).

Assay to determine wheat seed infected with clone. To assess whether FGVA4 contributed to infection in our inoculated wheat subplots, a seed infected with clone assay

(SIC) was performed. Two wheat heads were collected randomly from 49 of the 50 inoculated sub-plots in 2011 (one of the infected plots had all the wheat removed in order to accommodate a spore release experiment, and consequently heads were not collected from this plot). In 2012, five wheat heads were collected randomly from all 50 of the inoculated sub-plots. Wheat kernels (seeds) were removed and cultured on FSM for 5-7 days, and a single isolate of *F. graminearum* from each head was purified and genotyped as described in the previous sections.

Statistical analyses. Data were analyzed for each year (2011 and 2012), and for both years combined (2011 and 2012). Cochran-Mantel-Haenzel tests (which include the General Association and Correlation tests) were calculated to examine associations between the counts of the released clone and the distance from the source. The Kruskal–Wallis one-way analysis of variance was conducted to examine significant differences between distance (0 m (in the source), 100 m, 250 m and 500 m in 2011; 0 m (in the source), 100 m, 250 m, 500 m, 750 m, and 1000 m in 2012; all the distances in 2011 and 2012 combined) and time (collections during the night, day, and day and night combined). The null hypothesis for this test is that the medians of the samples from all the sampling distances are the same for all collection periods, and the alternative hypothesis is that at least one median is significantly different. The percent recovery of released clone [(released clone count/total clone count)*100] was natural log transformed. Regression analyses were performed to quantify the relationship between the percent recovery of the released clone and distance from source. All of the statistical analyses were performed using JMP System for Windows (Release 9, SAS Institute Inc.,

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Cary, NC), R, and SAS (Release 9.2, SAS Institute Inc., Cary, NC). Box plots were generated to illustrate the range of observations for the percent recovery of released clone for all sampling locations during the night, day, and day and night combined for each year, and for both years combined.

RESULTS

Field collections. In 2011, 12,409 viable *Fusarium* colonies were recovered over 14 consecutive days at distances up to 500 m from the center of the inoculum source; 77.4% (9,607/12,409) of the colonies were collected at night (1900-0700), and 22.6% (2,802/12,409) of the colonies were collected during the day (0700-1900). Of the 12,409 colonies recovered, 2,358 colonies were sub-cultured on ¼-strength PDA for tentative identification of *F. graminearum*. 1,055 colonies were tentatively identified as *F. graminearum* based on morphological features, and 790 isolates were confirmed to be *F. graminearum* based on microsatellites.

In 2012, 6,283 viable *Fusarium* colonies were recovered over 19 consecutive days at distances up to 1000 m from the center of the inoculum source; 63.3% (3,976/6,283) of the colonies were collected at night (1900-0700), and 36.7% (2,307/6,283) of the colonies were collected during the day (0700-1100). Of the 6,283 colonies recovered, 3,551 colonies were sub-cultured on ¼-strength PDA for tentative identification of *F. graminearum*. Three hundred and eighty-two colonies were tentatively identified as *F. graminearum*, and 237 isolates of *F. graminearum* were analyzed for the released clone using microsatellites.

Validation of microsatellite analysis using DNA from control strains. Prior to analyzing our field collections for these microsatellites, we obtained 12 *F. graminearum* genomic DNA samples from S. Vogelgsang (33) to serve as positive controls for each of the selected microsatellites. All 12 control samples produced amplicon sizes identical to those produced by Vogelgsang et al. (33) (e.g., **Figure 3**).

Sampling prior to the release of the clone. In 2011, to assess potential natural (background) populations of our FGVA4 prior to its release, field samples were collected from 26 April to 2 May 2011 (the field was inoculated on 2 May 2011). A total of 1,985 viable *Fusarium* colonies were recovered during this interval, and 1,037 colonies were sub-cultured on ¼-strength PDA for tentative identification of *F. graminearum*. Of these, 283 were tentatively identified as *F. graminearum*, and 141 isolates were analyzed for the released clone using microsatellites. Three of these isolates (2.1%, 3/141) produced amplicon sizes identical to the released clone at all three microsatellites and thus could not be distinguished from the released clone based on these three microsatellites. Results from the microsatellite analysis for the 141 isolates analyzed for the background populations collected prior to the released clone yielded 7 unique amplicons for FusSSR22, 26 unique amplicons for FusSSR23, and 27 unique amplicons for FusSSR27 (**Figure 4, Table 2, Table 3**). The allele frequencies for amplicon sizes for the released clone for FusSSR22 (200 bp), FusSSR23 (167 bp), and FusSSR27 (183 bp), were 0.614, 0.035, and 0.156 respectively (**Table 2**). Based on the allele frequencies calculated from the 141 background isolates collected prior to the release of the clone, 0.34% (0.614 *

0.035 * 0.156) of the isolates identified as the released clone would be expected to be false positives.

In 2012, to assess potential natural (background) populations of our released clone (FGVA4) prior to the release of the clone, field samples were collected from 9 April to 12 April 2012 (the field was inoculated on 16 April 2012). Eighty-nine viable *Fusarium* colonies were recovered during this interval, and 89 colonies were sub-cultured on ¼-strength PDA for tentative identification of *F. graminearum*. Of these, 7 were tentatively identified as *F. graminearum*, and were analyzed for the released clone using microsatellites. None of these isolates (0%, 0/7) produced amplicon sizes identical to the released clone at all three microsatellites.

Sampling after the release of the clone. In 2011, of the 790 isolates analyzed for the three microsatellites, 22.1% (175/790) of these isolates produced amplicons that were identical to the released clone at all three microsatellites analyzed and thus were determined to be the released clone. The remaining 77.9 % (615/790) did not produce amplicons that were identical to the released clone at one or more microsatellites and, thus, were were likely to represent background populations from other sources (14, 15).

In 2012, of the 237 isolates analyzed for the three microsatellites, 40.5% (96/237) of these isolates produced amplicons that were identical to the released clone at all three microsatellites analyzed and were determined to be the released clone. The remaining 59.5% (141/237) did not produce amplicons that were identical to the released clone at one or more microsatellites.

Comparisons between background populations collected prior to and during the release of the clone. To examine potential differences in allele frequencies between the background populations collected prior to and during the release of the clone, we randomly selected 141 background isolates that were collected during the release of the clone for further analysis. This was the same number of isolates analyzed from the background populations collected prior to the release of the clone, and thus allowed us to compare two identical sample sizes. These isolates were selected at random from background populations collected at different sampling distances during different sampling days (**Table 3**). An analysis for the natural population after the release of FGVA4 of the polymorphisms for each microsatellite showed that 11 amplicon sizes were observed for FusSSR22, 32 sizes for FusSSR23, and 30 sizes for FusSSR27 (**Figure 4, Table 2, Table 3**). The allele frequencies for amplicon sizes for the released clone FusSSR22 (200 bp), FusSSR23 (167 bp), and FusSSR27 (183 bp) were 0.525, 0.071, and 0.227 respectively (**Table 2**). Based on the allele frequencies calculated from the 141 background isolates collected during the release of the clone, 0.85% (0.525 * 0.071 * 0.227) of the isolates identified as the released clone would be expected to be false positives.

Day and night collection of *F. graminearum*. In 2011, most of the *F. graminearum* isolates analyzed for microsatellites (clonal populations) were collected during the night (70.8% (559/790), 1900-0700) and early morning (24.5% (194/790), 0700-1100) hours (**Figure 5**). The remainder of the isolates of the fungus analyzed for microsatellites were collected during the day; 3% (21/792) for 1100-1500 and 2% (16/790) for 1500-1900.

Based on data collected in 2011, samples were only collected during the night (1900-0700) and early morning (0700-1100) hours for the 2012 field season. In 2012, 81% (191/237) of the *F. graminearum* isolates analyzed for microsatellites (clonal populations) were collected during the night (1900-0700) and 19% (46/237) were collected during the early morning (0700-1100).

Long distance transport of released clone. The majority of the released clone was recaptured during the night (1900-0700) and early morning (0700-1100) sampling, with a decrease of the released clone at increasing distances from the source. Box plots presented in Figure 6 show the range and spread of observations for the percent recovery of released clone for all sampling locations during the night, day, and day and night combined in each of the years, and in both years combined. These plots show a general trend of decreasing recovery of the released clone with increasing distances from the source of inoculum.

In 2011, there was a 60.6% (43/71), 36.2% (83/229), 15.1% (22/146), and 3.5% (4/113) recovery of the released clone at distances of 0 m (in the source), 100 m, 250 m, and 500 m, respectively from the source during the night (1900-0700). There was a 34.6% (9/26), 7.9% (6/76), 4.7% (3/64), and 7.7% (5/65) recovery of the released clone at deposition distances 0 m (in the source), 100 m, 250 m, and 500 m from the source, respectively during the day (0700-1900) (**Figure 5**).

In 2012, there was a 87.5% (63/72), 18.2% (10/55), 0% (0/9), 0% (0/23), 6.7% (1/15), and 0% (0/17) recovery of the released clone at distances of 0 m (in the source), 100 m, 250 m, 500 m, 750 m, and 1000 m, respectively from the source during the night

(1900-0700). There was a 92.3% (12/13), 56.3% (9/16), 0% (0/3), 20% (1/5), 0% (0/5), and 0% (0/4) recovery of the released clone at distances of 0 m (in the source), 100 m, 250 m, 500 m, 750 m, and 1000 m, respectively from the source during the day (0700-1100) (**Figure 5**). As in the case of 2011, there was a decreasing trend of the recovery of the released clone with the increasing distances from the source for 2012 data. But the decreasing rate seems to be higher compared to the 2011 data. By combining the data for 2011 and 2012, there was a 74.1% (106/143), 32.7% (93/284), 14.2% (22/155), 2.9% (4/136), 6.7% (1/15), and 0% (0/17) recovery of the released clone at distances of 0 m (in the source), 100 m, 250 m, 500 m, 750 m, and 1000 m, respectively from the source during the night (1900-0700). There was a 53.8% (21/39), 16.3% (15/92), 4.5% (3/67), 8.6% (6/70), 0% (0/5), and 0% (0/4) recovery of the released clone at distances of 0 m (in the source), 100 m, 250 m, 500 m, 750 m, and 1000 m, respectively from the source during the day (0700-1100) (**Figure 5**).

General Association tests for all collections in 2011 were significant (night ($P <$ 0.0001), day ($P = 0.0001$), and day and night combined ($P < 0.0001$)), demonstrating an association between the collection of the released clone and sampling distance from the source. The Correlation tests for all collections were also significant (night ($P < 0.0001$), day ($P = 0.0361$), and day and night combined ($P < 0.0001$), supporting the alternative hypothesis of a linear association. General Association tests for all collections in 2012 were also significant (night ($P < 0.0001$), day ($P = 0.0004$), and day and night combined ($P < 0.0001$)). The Correlation tests for all collections in 2012 were also significant (night ($P < 0.0001$), day ($P < 0.0001$), and day and night combined ($P < 0.0001$).

The Kruskal-Wallis test showed a significant difference ($P = 0.0025$) at different distance levels for percent recovery of released clone during the night in 2011. Nonparametric multiple comparisons of the percent recovery of the released clone at different distance levels during the night were significant for all but three of the comparisons at 0 m (in the source) and 100 m, $P = 0.1293$; 0 m (in the source) and 250 m, $P = 0.0518$; 100 m and 250 m, $P = 0.1219$. In contrast, the Kruskal-Wallis test performed on the different distance levels for the percent recovery of released clone during the day was not significant ($P = 0.2601$). The Kruskal–Wallis test also showed a significant difference ($P = 0.0024$) for the different levels of distance for day and night combined. Nonparametric multiple comparisons showed that the percent recovery of released clone at 100 m, 250 m and 500 m were significantly different from 0 m (in the source) ($P < 0.05$).

The Kruskal-Wallis test performed at different levels of distance for the night data in 2012 was significant ($P = 0.0005$). Nonparametric multiple comparisons showed that the percent recovery of released clone were significantly different for all the distances compared to the distances 0 m (in the source) and 100 m ($P < 0.05$), with the exception of the comparison of 100 m and 750 m ($P > 0.05$). The tests failed to reject the null hypothesis of same median for all the remaining comparisons. The recovery rate at 250 m, 500 m and 1000 m was 0 for the data collected at night in 2012, therefore there was no test performed for the comparisons of 250 and 500 m; 250 and 1000 m; and 500 and 1000 m. The Kruskal-Wallis test performed at different levels of distance for the day data in 2012 was significant ($P = 0.0008$). Nonparametric multiple comparisons showed that the percent recovery of released clone were significantly different for all the distances

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compared to the distances 0 m (in the source) and 100 m ($P < 0.05$) except for the comparisons of 0 m (in the source) and 100 m; 0 m (in the source) and 500 m; 100 m and 500 m ($P > 0.05$). The test failed to reject the null hypothesis of same median for all the remaining comparisons. The recovery rate at 250 m, 750 m and 1000 m was 0 for the data collected in day 2012, therefore no tests were performed for the comparisons of 250 and 750 m; 250 and 1000 m; and 750 and 1000 m. The Kruskal-Wallis test performed at different levels of distance for the day and night combined data in 2012 was significant ($P = 0.0003$). Nonparametric multiple comparisons showed that the percent recovery of released clone were significantly different for all the distances compared to the distances 0 m (in the source) and 100 m ($P < 0.05$), except for the comparisons of 0 m (in the source) and 500 m; and 100 m and 500 m ($P > 0.05$). The tests failed to reject the null hypothesis of same median for all the remaining comparisons. The recovery rate at 250 m and 750 m was 0 for the combined data collected in day and night 2012, therefore no tests were performed for the comparison of 250 m and 750 m.

The Kruskal-Wallis test performed on the different levels of distance for the 2011 and 2012 combined night data were significant ($P < 0.0001$). Nonparametric multiple comparisons showed that the percent recovery of released clone were significantly different for all the distances compared to the distances 0 m (in the source) and 100 m with $P < 0.05$. The tests for all the other remaining comparisons failed to reject the null hypothesis of same median. The Kruskal-Wallis test performed on the different levels of distance for the 2011 and 2012 combined day data were significant ($P = 0.0001$). Nonparametric multiple comparisons showed that the percent recovery of released clone were significantly different for all the distances compared to the distances 0 m (in the

source) and 100 m ($P < 0.05$) except for the comparisons between 0 m (in the source) and 100 m; and 100 m and 500 m ($P > 0.05$). Since the recovery rate at 750 m and 1000 m was 0 for the 2011 and 2012 combined day data, there was no test performed for the comparison of 750 and 1000 m. The Kruskal-Wallis test performed at different levels of distance for the 2011 and 2012 combined day and night data were significant ($P < 0.0001$). Nonparametric multiple comparisons showed that the percent recovery of released clone were significantly different for all the distances compared to the distances 0 m (in the source) and 100 m ($P < 0.05$). Regression analyses on log transformed data for the percent recovery of released clone showed significant linear relationships for collections during the night and for day and night combined in 2011 (**Figure 7**). For collections during the day and night combined (**Figure 7A**), the model explains 48% of the variation for the percent recovery of released clone, which indicates a strong negative correlation (r = -0.69, $P < 0.0001$) between the log percent recovery of released clone during the day and night over all distances. The fitted model was: Log (percent recovery of released clone) = 3.56 - 0.0040*Distance from source. For collections during the night (**Figure 7B**), the model explained 48% of the variation for the log percent recovery of released clone during the night over all distances, which indicates a strong negative correlation (r = -0.69, $P = 0.0003$) between the log percent recovery of released clone during the night and the distance. The fitted model was: Log (percent recovery of released clone) = 3.85 - 0.0041*Distance from source. For collections during the day (**Figure 7C**), the model explained 24% of the variation for the log percent recovery of the released clone during the day. The estimate was not-significant with $P = 0.089$ for the

linear term. The fitted model was: Log (percent recovery of released clone) = 3.15 - 0.0014*Distance from source.

Linear regression on the log percent recovery of released clone against distance showed that the slope estimates were not significant ($P > 0.05$) for collections during the day and for day and night combined in 2012 (**Figure 7**). For collections during the day and night combined (**Figure 7A**), the model explains 23% of the variation for the log transform of the percent recovery of released clone, which indicates a negative correlation (r = -0.48, $P = 0.071$) between the percent recovery of released clone during the day and night over all distances. The fitted model was: Log (Percent recovery of released clone) = 4.07 - 0.0019*Distance from source. For collections during the night (**Figure 7B**), the model explained 32% of the variation for the log percent recovery of released clone during the night over all distances, which indicates a negative correlation (r = -0.56, $P = 0.0708$) between the log percent recovery of released clone during the night and the distance. The fitted model was: Log (Percent recovery of released clone) = 3.92 - 0.0027*Distance from source. For collections during the day (**Figure 7C**), the model explained 1.3% of the variation for the log percent recovery of the released clone during the day, which indicates a positive correlation ($r = 0.11$, $P = 0.73$) between the log percent recovery of released clone during the day and the distance. The fitted model was Log (Percent recovery of released clone)= 4.32 + 0.00029* Distance from source. A possible reason for the positive correlation is that one sampler at 500 m collected one spore of *F. graminearum* which was the released clone, thus having a percent recovery of 100% (Figure 7C). This data point was a strong outlier and when removed, the model explained 12% of the variation for the log percent recovery of the released clone during

the day, which indicated a negative correlation (r = -0.34, P = 0.30) between the log percent recovery of released clone during the day and the distance. The fitted model when the outlier at 500 m was removed was Log (Percent recovery of released clone)= 4.51-0.0025*Distance from source.

Regression analyses on the log transformed data for the percent recovery of released clone showed significant linear relationships for collections during the day and the night in 2011 and 2012 combined (**Figure 7**). For collections during the day and night combined (**Figure 7A**), the model explained 35% of the variation for the percent recovery of released clone, which indicated a negative correlation (r = -0.59, P < 0.0001) between the log percent recovery of released clone during the day and night over all distances. The fitted model was: Log (Percent recovery of released clone) = 3.75 - 0.0034*Distance from source. For collections during the night (**Figure 7B**), the model explained 41% of the variation for the log percent recovery of released clone during the night over all distances, which indicates a strong negative correlation (r = -0.64, P < 0.0001) between the log percent recovery of released clone during the night and the distance. The fitted model was: Log (Percent recovery of released clone) = 3.84 - 0.0036*Distance from source. For collections during the day (**Figure 7C**), the model explained 21% of the variation for the log percent recovery of the released clone during the day, which indicates a negative correlation (r = -0.46, P =0.02) between the log percent recovery of released clone during the night and the distance. The fitted model was: Log (Percent recovery of released clone) = 3.96 − 0.0024*Distance from source.

Seed infected with clone assay (SIC). In 2011, two wheat heads from 49 subplots (one plot had the wheat removed to make room for a volumetric spore trap) were analyzed for infection with the released clone. There was an 84.7% (83/98) recovery of the released clone in the seed infected with clone assay. Each of the plots analyzed had at least one wheat head infected by the released clone. In 2012, five wheat heads for each of the 50 subplots were analyzed for being infected with the released clone. There was an 80% (141/177) recovery of the released clone in the seed infected with clone assay.

Wind direction. Figure 2 shows the fraction of the time the wind direction was coming from a particular 45 degree sector during the day, night, and day and night combined from 26 April to 26 May for 2009, 2010, and 2011 and 9 April to 14 May for 2012. Knowledge of the prevalent wind direction and the farm topography (hills, forest, river, etc.) guided the placement of the sampling devices.

DISCUSSION

In this study, we monitored the movement of *F. graminearum* from a 3,716 m^2 (0.372 ha) clonal field-scale source of inoculum released at Virginia Tech's Kentland Farm. The size of this inoculum source approximates a real-world cropping scenario. A series of ground-based collection devices were strategically placed at distances of 0 m (in the source), 100 m, 250 m, and 500 m from the center of the source in 2011; in 2012 we included two additional distances of 750 m and 1000 m. Three polymorphic microsatellites (short repeating sequences of DNA) were used to identify the released clone from 1,027 isolates of *F. graminearum* collected across 33 sampling dates. Our

work represents a unique approach to track the long distance movement of an important plant pathogen from a field-scale source of inoculum. We extend the previous work of Fernando (10) and Keller (14, 15) by using a new microsatellite approach that only requires a single PCR amplification step for each of the three primer pairs used, greatly increasing the speed and efficiency of genotyping samples collected from the field compared to Amplified Fragment Length Polymorphisms (AFLPs).

Comparisons between the 141 background isolates analyzed from collections prior to the release of the clone and the 141 isolates analyzed from collections during the release of the clone demonstrated that background populations were similar, at least in terms of the number of unique amplicons and allele frequencies for the three microsatellites. The number of unique amplicon sizes was comparable for both populations for all three microsatellites (7 and 11 unique amplicons for FusSSR22 prior to and during release, respectively; 26 and 32 unique amplicons for FusSSR23 prior to and during release, respectively; and 27 and 30 unique amplicons for FusSSR27 prior to and during release, respectively), and the allele frequencies were similar (0.614 and 0.525 for FusSSR22 prior to and during release, respectively; 0.035 and 0.071 for FusSSR23 prior to and during release, respectively; and 0.156 and 0.227 for FusSSR27 prior to and during release, respectively). These results support the hypothesis that background populations of *F. graminearum* collected from the atmosphere are well-mixed and not dominated by one isolate of *F. graminearum* (28, 29). Moreover, this analysis demonstrates that the possibility of having a false positive was less than 1% for both populations (0.34% for collections prior to the release of the clone and 0.85% during the

release of the clone). Thus, isolates producing amplicons identical to our released clone at all three microsatellites can be identified as the released clone.

The release and recapture studies were done in replicates over two field seasons during 2011 and 2012. In 2011, we attempted to recapture the released clone up to 500 m from the field scale source of inoculum. Since we were able to recover the clone at 500 m in 2011, we increased our sampling distances (750 m and 1000 m) and the number of samplers (40) during our second year of experimentation. In both years, we observed a similar trend of a decrease of the released clone being recaptured with an increased distance from the field scale source of inoculum. Additionally, both in 2011 and 2012 we recovered the majority of the released clone during the night (1900-0700). A major difference observed between 2011 and 2012 was the steep drop off of the dispersal kernel in 2012 compared to 2011. In 2012, there was a much higher recovery of the released clone (88%) than in 2011 (53%) at 0 m (in the source) from the field scale source of inoculum. However, when the samplers were located 100 m from the field scale source of inoculum, recovery of the released clone in 2012 (26%) was less than the recovery in 2011 (29%). Aylor et al. (3, 4) discusses the importance of rainfall in 'washing out' spores from a column of air. One possible explanation for the differences observed between 2011 and 2012 is that ascospore release and rainfall need to be coupled events to have a high recovery of the released clone at distances outside the source of inoculum. This idea will be further investigated when validating spore transport models (1, 2, 3, 4) using the experimental results reported here.

The released clone of *F. graminearum* was recovered up to 750 m from our field scale source; to our knowledge this is the first report demonstrating the transport of *F.*

graminearum spores at least 750 m from a known field-scale source. Although advances have been made in mathematically modeling the long distance transport of plant pathogens, some of these models have not been validated experimentally. Using the microsatellite method described within this paper provides a relatively simple technique to experimentally validate these models. Assuming a Gaussian plume of spores released from our source (2, 3, 4), the dispersal kernel should drop off quickly at greater downwind distances from the source (as a power law times an exponential). Sampling at even further distances, in the tail of the dispersal kernel, poses a challenge since there is expected to be a dilution in the concentration of spores at downwind distances from the source and the sampling area effectively becomes much larger. This could be one possible explanation for not capturing the released clone at 1000 m (spores of the released clone may have actually traveled 1000 m or more, but we did not recover them at our sampling sites). Experimentally determining the 'fat tail' of the dispersal kernel is important since slight changes in the long-distance dispersal tail can lead to large changes in predicted spread rates, since rare long-distance dispersal events described by the tails are the dominant factor in determining the rate of disease spread (12, 17).

Recovery of the released clone of *F. graminearum* decreased at increased distances from the field-scale source. General Association tests for all collections (night, day, and day and night combined, 2011 and 2012) were significant, demonstrating an association between the collection of the released clone and sampling distance from the source. A linear regression analysis applied to the log percent recovery of the released clone for combined day and night collections and all sampling distances from the source was significant (r = -0.69, *P* < 0.0001) in 2011, not significant in 2012 (r = -0.49, *P* =

0.071) and significant (r = -0.67 , $P < 0.0001$) for 2011 and 2012 combined. Keller et al.

(14, 15) observed a similar trend from small area sources of inoculum, but observed a much steeper gradient of recovery over much shorter distances. The furthest distance sampled from the released source was 30 m, where the recovery was ≤1 % for all scenarios. It is important to note that Keller et al. (14, 15) released small amounts of inoculum (<500 g) in 0.84 m diameter circular plots which could have contributed to a steep drop off of released clone recovered, compared to our experiment where there was a 0.372 ha source (estimated 78,000 g of corn stalks). Our aim was to collect viable spores deposited out of the atmosphere onto plates of selective medium, whereas Keller et al. (14, 15) attempted to collect the released clone from diseased wheat heads. Thus, subtle differences between the results we obtained and those of Keller et al. (14, 15) might be attributed to factors affecting the infection and recovery of the released clone in the different experimental systems. One of the major limitations with the current experimental design is the knowledge of source variability, as different microclimates across the field could drive different rates of spore release. Additionally, a large proportion of spores released are expected to be unable to escape the turbulent boundary layer and will thus not be able to travel over long distances (26).

The majority (87% for 2011; 77% for 2012) of the released clone was collected during the night (1900-0700). The Kruskal-Wallis test for 2011 showed a significant difference ($P = 0.0025$) at different distances from the source for percent recovery of released clone during the night, but not during the day ($P = 0.2601$). The Kruskal-Wallis tests performed on 2012 data were significant for night ($P = 0.0005$) and day ($P = 0.0008$), and the day and night combined ($P = 0.0003$). These results are consistent with

previous literature examining the release dynamics of spores from artificially inoculated plots. Fernando et al. (11) found that the highest aerial concentration of spores at a spore sampler located 1.5 m from an inoculated plot occurred between 2000-0800, with a low number of spores present between 1200-1600, suggesting a night-time release of ascospores from perithecia. Schmale et al. (27) studied the deposition of *F. graminearum* from unknown sources and found the majority of spores were deposited at night with peak deposition between 0400-0600. Previous research also showed that the majority (91%) of *F. graminearum* was deposited in corn canopies during the night (25). Our results are consistent with this finding; the majority (95%) of *F. graminearum* we collected through deposition occurred from 1900-1100. It is possible deposition rates are higher at night, due to a rapid cooling of earth's surface compared to the atmosphere and an inversion layer being formed due to a downward transfer of heat from the atmosphere to the surface (21). Differences in meteorological conditions during the day and night could help explain small variations in the recovery of the released clone during the day and night. There has been speculation that there is an uncoupling between the release and deposition of *F. graminearum* spores, with conditions for spore release being most favorable during the day and deposition occurring at night (18, 26, 27).

Data from background sampling prior to the release of the clone in the field during 2011 showed that our released clone appeared to be present in about two percent (3/141) of the isolates. In 2012, we only captured one *F. graminearum* isolate during our background sampling, and this isolate was not the released clone. Thus, the potential contribution of background sources of the clone (i.e., sources other than the source we released) is possible, but very small. It is important to note that the isolate we released in

this study was originally collected from an infected wheat head in Riner, VA, approximately 19 km from Kentland Farm. Vogelgsang et al. (33) proposed 15 different microsatellite markers for *F. graminearum*. In an attempt to resolve the three background isolates whose allelic sizes were identical to the released clone for the three microsatellites studied, we examined those three isolates for all 15 microsatellite markers proposed by Vogelgsang et al. (33) and compared their allelic sizes to the released clone (data not shown). Two of background isolates showed identical allelic sizes as the released clone at all 15 microsatellite markers, but one of the background isolates yielded a different product, suggesting that this third isolate was not the released clone. Thus, the inclusion of additional microsatellite markers (beyond the three included in this study) has the potential to further decrease the markedly low percentage of false positives.

The majority (85% for 2011; 80% for 2012)of wheat heads analyzed in the seed infected with clone (SIC) assay were infected with our released clone, confirming the ability of our clone to cause FHB in wheat. In 2011, there was, however, a higher recovery rate of the released clone in the SIC assay compared to collections on sampling plates in the source (0 m). One possible explanation for this difference is that the SIC assay reflects cumulative exposure of wheat heads to spores over a long interval of time (weeks), whereas the sampling plates were only exposed for a short interval of time (hours). We did not observe this trend in 2012, the recovery rate of the released clone for the sampling plates in the source (0 m) was slightly higher (88%) than for the SIC assay. It is possible that all of the wheat heads were infected with the released clone, but we only sampled and analyzed a single isolate from each infected head. Zeller et al. (34)

found that it is possible for multiple isolates of *F. graminearum* to infect a single wheat head and cause FHB.

Our work showcases a unique approach to track the long distance movement of plant pathogens in the atmosphere. This approach, however, is limited by the amount of time and labor that is required to process large populations of microorganisms, particularly when the released clone represents a relatively small proportion of the background population. One potential strategy to overcome these limitations would be to combine the release of yellow phenotype pioneered by Fernando et al. (10) with microsatellites; the yellow phenotype would provide an initial screen for quick sorting of tentative clones from background populations, and the microsatellites could then be used identify of the released clone within this sorted population characterized initially by the yellow culture phenotype.

Future work may include the use of a meteorological-based mathematical model to predict and validate the local (spores from a released source) and regional (spores from other sources) transport of *F. graminearum*. Such work may help improve management practices for FHB and contribute to the development of early warning systems for the spread of *F. graminearum* and other important plant pathogens. For example, a current online prediction tool for FHB (http://www.wheatscab.psu.edu/riskTool_2011.html) is available. The main considerations of the current FHB risk assessment tool (7, 8) are environmental factors such as temperature, precipitation, and relative humidity to determine the risk of an FHB outbreak. In addition to environmental factors, the risk assessment tool also considers local extension agents reports for disease. Although environmental factors are the main components of disease development of FHB on wheat

and barley, the current risk assessment tool does not include the ability to predict the movement of *F. graminearum* spores from potential source areas. Previous literature has shown that *F. graminearum* spores are ubiquitous in the planetary boundary layer of the atmosphere (19), and may be transported across broad geographical regions (29). Future work may include the development of mathematical models to predict the long distance transport of *F. graminearum*, and these models could leverage release-recapture experiments such as those described here to validate these models. Such models could provide a powerful prediction tool and allow producers of small grains to employ improved disease management practices for FHB, such as the early application of appropriate fungicides. Moreover, this work could find immediate application in the development of model-based early warning systems for the spread of other high risk plant pathogens such as *Peronospora tabacina*, causal agent of tobacco blue mold, (1) *Phakopsora pachyrhizi*, causal agent of Asian soybean rust (1, 16), and *Puccinia graminis* f. sp. *tritici*, causal agent of wheat stem rust (31).

LITERATURE CITED

1. Aylor, D. E., Taylor, G. S., and Raynor, G. S. 1982. Long-range transport of Tobacco blue mold spores. Agricultral Meteorology 27:217-232.

2. Aylor, D. E. 1986. A framework for examining inter-regional aerial transport of fungal spores. Agricultural and Forest Meteorology 38:263-288.

3. Aylor, D. E. and Sutton, T. B. 1992. Release of *Venturia inaequalis* ascospores during unsteady rain: Relationship to spore transport and deposition. Phytopathology 82:532-540.

4. Aylor, D. E. 1999. Biophysical scaling and the passive dispersal of fungus spores: Releationship to integrated pest management strategies. Agricultural and Forest Meteorology 97:275-292.

5. Buckley, P., B. Rivers, S. Katoski, M. H. Kim, F. J. Kragl, S. Broomall, M. Krepps, E. W. Skowronski, C. N. Rosenzweig, S. Paikoff, Palkoff, S., and Gibbons, H. S.. 2012. Genetic barcodes for improved environmental tracking of an anthrax simulant. Applied and Environmental Microbiology 78(23): 8272-8280.

6. Cuomo, C. A., Güldener, U., Xu, J., Trail, F., Turgeon, B. G., Di Pietro, A., Walton, J. D., Ma, L., Baker, S. E., Rep, M., Adam, G., Antoniw, J., Baldwin, T., Calvo, S., Chang, Y., DeCaprio, D., Gale, L. R., Gnerre, S., Goswami, R. S., Hammond-Kosack, K., Harris,

L. J., Hilburn, K., Kennell, J. C., Kroken, S., Magnuson, J. K., Mannhaupt, G., Mauceli, E., Mewes, H., Mitterbauer, R., Muehlbauer, G., Münsterkötter, M., Nelson, D., O'Donnell, K., Ouellet, T., Qi, W., Quesneville, H., Roncero, M. I. G., Seong, K., Tetko, I. V., Urban, M., Waalwijk, C., Ward, T. J., Yao, J., Birren, B. W., and Kistler, H. C. 2007. The *Fusarium graminearum* genome reveals a link between localized polymorphism and pathogen specialization. Science 317: 1400-1402.

7. De Wolf, E. D., Madden, L. V., and Lipps, P. E. 2003. Risk assessment models for wheat Fusarium head blight epidemics based on within-season weather data. Phytopathology 93:428-435.

8. Del Ponte, E. M., Fernandes, J. M. C., Pavan, W., and Baethgen, W. E. 2009. A model-based assessment of the impacts of climate variability on Fusarium head blight seasonal risk in southern Brazilian Journal of Phytopathology 157:675-681.

9. Emanuel, P. A., P. E. Buckley, T. A. Sutton, J. M. Edmonds, A. M. Bailey, B. A. Rivers, M. H. Kim, W. J. Ginley, C. C. Keiser, R. W. Doherty, Kragl, F. J., Narayanan, F. E., Katoski, S. E., Palkoff, S., Leppert, S. P., Strawbridge, J. B., VanReenen, D. R., Biberos, S. S., Moore, D., Phillips, D. W., Mingioni, L. R., Melles, O., Ondercin, D. G., Hirsh, B., Bieschke, K. M., Harris, C. L., Omberg, K. M., Rastogi, V. K., Van Cuyk, S., and Gibbons, H. S. 2012. Detection and tracking of a novel genetically-tagged biological simulant in the environment. Applied and Environmental Microbiology. 78(23): 8281-8288.

10. Fernando, W. G. D., Paulitz, T. C., Seaman, W. L., Dutilleul, P., and Miller, J. D. 1997. Head blight gradients caused by *Gibberella zeae* from area sources of inoculum in wheat field plots. Phytopathology 87:414-421.

11. Fernando, W. G. D., Miller, J. D., Seaman, W. L., Seifert, K., and Paulitz, T. C. 2000. Daily and seasonal dynamics of airborne spores of *Fusarium graminearum* and other *Fusarium* species sampled over wheat plots. Canadian Journal of Botany 78:497-505.

12. Higgins, S. I., Nathan, R., and Cain, M.L. 2003. Are long-distance dispersal events in plants usually caused by nonstandard means of dispersal? Ecology 84:1945–1956.

13. Isard, S. A., Gage, S. H., Comtois, P., and Russo, J. M. 2005. Principles of atmospheric pathway for invasive species applied to soybean rust. BioScience 55(10):851-861.

14. Keller, M. D., Waxman, K. D., Bergstrom, G. C., and Schmale, D. G., III. 2010. Local distance of wheat spike infection by released clones of *Gibberella zeae* disseminated from infested corn residue. Plant Disease 94:1151-1155.

15. Keller, M. D., Thomason, W. E., and Schmale, D. G., III. 2011. The spread of a released clone of *Gibberella zeae* from different amounts of infested corn residue. Plant Disease 95:1458-1464.

16. Krupa, S., Bowersox, V., Claybrooke, R., Barnes, C. W., Szabo, L., Harlin, K., and Kurle, J. 2006. Introduction of Asian soybean rust urediniospores into the midwestern United States-a case study. Plant Disease 90:1254-1259.

17. Lewis, M. A. 1997. Variability, patchiness and jump dispersal in the spread of an invading population, Chapter 3 In: Spatial Ecology: The Role of Space in Population Dynamics and Interspecific Interactions (D. Tilman and P. Kareiva Eds.), Princeton University Press, pp. 46–69.

18. Maldonado-Ramirez, S. L. 2001. Aerobiology of the wheat scab fungus, *Gibberella zeae*: discharge, atmospheric dispersal, and deposition of ascospores. Ph.D. thesis, Cornell University, Ithaca, NY.

19. Maldonado-Ramirez, S. L., Schmale, D. G., III, Shields, E. J., and Bergstrom, G. C. 2005. The relative abundance of viable spores of *Gibberella zeae* in the planetary boundary layer suggests the role of long-distance transport in regional epidemics of Fusarium head blight. Agricultural and Forest Meteorology 132:20-27.

20. McMullen, M., Jones, R., and Gallenberg, D. 1997. Scab of wheat and barley: a re-emerging disease of devastating impact. Plant Disease 81(12):1340-1348.

21. Oke, T. R. 1987. Boundary layer climates. 2 ed. Cambridge University Press, Cambridge, UK.

22. Paulitz, T. C. 1999. Fusarium head blight: a re-emerging disease. Phytoprotection 80(2):127-133.

23. Prussin, A. J., II, Ross, S. D., and Schmale, D. G., III. 2011. Tracking the long distance movement of *Fusarium graminearum*, causal agent of Fusarium head blight in wheat and barley. American Society of Microbiology-Virginia Branch Meeting, Blacksburg, Virginia.

24. Schmale, D. G., III, and Bergstrom, G. C. 2003. Fusarium head blight in wheat. The Plant Health Instructor. DOI:10.1094/PHI-I-2003-0612-01

25. Schmale, D. G., III, and Bergstrom, G. C. 2004. Spore deposition of the ear rot pathogen, *Gibberella zeae*, inside corn canopies. Canadian Journal of Plant Pathology 26:591-595.

26. Schmale, D. G., III, Arntsen, Q. A., and Bergstrom, G. C. 2005. The forcible discharge distance of ascospores of *Gibberella zeae*. Canadian Journal of Plant Pathology 27:376-382.

27. Schmale, D. G., III, Shields, E. J., and Bergstrom, G. C. 2006. Night-time spore deposition of the Fusarium head blight pathogen, *Gibberella zeae*, in rotational wheat fields. Canadian Journal of Plant Pathology 28:100-108.

28. Schmale, D. G., III, Leslie, J. G., Zeller, K. A., Saleh, A. A., Shields, E. J., and Bergstrom, G. C. 2006. Genetic structure of atmospheric populations of *Gibberella zeae*. Phytopathology 96:1021-1026.

29. Schmale, D.G., Ross, S.D., Fetters, T.L., Tallapragada, P., Wood-Jones, A.K, and Dingus, B. 2012. Isolates of *Fusarium graminearum* collected 40–320 meters above ground level cause Fusarium head blight in wheat and produce trichothecene mycotoxins. Aerobiologia 28: 1-11.

30. Snijders, C. H. A. 1990. Fusarium head blight and mycotoxin contamination of wheat, a review. Netherlands Journal of Plant Pathology 96:187-198.

31. Stokstad, E. 2007. Deadly wheat fungus threatens world's breadbaskets. Science, 315:1786-1787.

32. Sutton, J. C. 1982. Epidemiology of wheat head blight and maize ear rot caused by *Fusarium graminearum*. Canadian Journal of Pathology 4:195-209.

33. Vogelgsang, S., Widmer, F., Jenny, E., and Enkerli, J. 2009. Characterisation of novel *Fusarium graminearum* microsatellite markers in different *Fusarium* species from various countries. European Journal of Plant Pathology 123:477-482.

34. Zeller, K. A., Bowden, R. L., and Leslie, J. F. 2003. Diversity of epidemic populations of *Gibberella zeae* from small quadrats in Kansas and North Dakota. Phytopathology 93:874-880.

Table 1. Primers used for the identification of our released clone with three microsatellites.

Name	Sequence	Allelic size range (bp)[A]	No. Alleles[B]	Released Isolate (FGVA4) Allelic Size (bp)	T_{anneal} (°C)	Fluorescent Label
FusSSR22	[f]GAGGGCGATGGTTGAAGTGTAC	200-212	4	200	65	Ned
	[r]TGGGCATGAAACAAGAGAGAGAC					
FusSSR23	[f]GTTGACACAGAAGAATGGCAGG	165-221	19	167	65	Ned
	[r]CGCTAGGTACAAATTGCTGGG					
FusSSR27	[f]TCACCAAAAGTCTCCTCAGTCAAC	157-205	12	183	65	Fam
	[r]GTGGTCTCCGTAAACGAGCC					

[A,B] Reported by Vogelgsang et al. (33) based on 33 isolates, five of which were from the USA.

Table 2. Allele frequency of three microsatellites in background populations of *Fusarium graminearum* prior to the release of the clone (FGVA4) (26 April to 2 May, 2011) and during the release of the clone (12 May to 25 May, 2011) in the first field campaign in 2011. A total of 141 isolates of *F. graminearum* was analyzed for the background populations collected prior to the release of the clone, which represented all (141/141) of the background isolates analyzed for this interval. A total of 141 isolates (the same number from the background populations prior to the release) was selected at random from background populations collected during the release of the clone, which represented 23 percent (141/617) of the background isolates analyzed during this interval.

Prior to or During the Release of the Clone	Microsatellite	Amplicon Size of Released Clone (bp)	Number of Amplicons	Allele Frequency of the Released Clone	Sample Size
Prior to release	FusSSR22	200	7	0.614	140[a]
During release	FusSSR22	200	11	0.525	141
Prior to release	FusSSR23	167	26	0.035	141
During release	FusSSR23	167	32	0.071	141
Prior to release	FusSSR27	183	27	0.156	135[b]
During release	FusSSR27	183	30	0.227	141

[a] No amplicon was obtained for 1 isolate after three independent PCR reactions

[b] No amplicon was obtained for 6 isolates after three independent PCR reactions.

Table 3. Background isolates analyzed for three microsatellites (FusSSR22, FusSSR23, and FusSSR27) from collections prior to the release of the clone (26 April- 2 May, 2011) and during the release of the clone (12 May- 25 May, 2011) for the first field campaign in 2011. One hundred and forty one isolates from each of these populations were selected for the comparisons of background populations; isolates for the first set represented all of the isolates analyzed prior to the release of the clone, and isolates for the second set the second were chosen at random form the larger population of isolates across multiple sampling days and distances from the source.

Prior to or During the Release of the Clone	Sampling Date	Number of Isolates Analyzed at Each Sampling Distance				Total Background *Fusarium graminearum* Isolates Analyzed
		0 meters (in the source)	100 meters	250 meters	500 meters	
Prior to release	April 26, 2011	1	13	10	6	30
Prior to release	April 27, 2011	3	17	11	4	35
Prior to release	April 29, 2011	3	19	15	11	48
Prior to release	May 2, 2011	2	12	7	7	28
During release	May 12, 2011	1	8	2	3	14
During release	May 13, 2011	0	4	5	3	12
During release	May 14, 2011	1	7	2	3	13
During release	May 15, 2011	4	1	5	6	16
During release	May 16, 2011	0	6	2	7	15
During release	May 17, 2011	0	8	5	5	18
During release	May 18, 2011	3	3	4	2	12
During release	May 19, 2011	0	0	0	0	0
During release	May 20, 2011	0	5	10	2	17
During release	May 21, 2011	0	4	3	1	8
During release	May 22, 2011	1	3	5	5	14
During release	May 23, 2011	0	2	0	0	2
During release	May 24, 2011	0	0	0	0	0
During release	May 25, 2011	0	0	0	0	0

FIGURE 1. Collection locations at different distances from clonal 3,716 m^2 (0.372 ha) sources of inoculum (grey squares) of *Fusarium graminearum* at Virginia Tech's Kentland Farm in Blacksburg, VA for the 2011 (A) and 2012 (B) field campaigns. In 2011, collection sites 1-12 were 100 m from the center of the source, sites 13-17, 23, and 25 were 250 m from the center of the source, sites 18-22 and 24 were 500 m from the center of the source, and sites 26-28 were in the source (site 26 was in the center of the source). In 2012, collection sites 1-3 were in the source, sites 4-15 were 100 m from the center of the source, sites 16-20, 26, and 27 were 250 m from the center of the source, sites 21-25 and 28 were 500 m from the center of the source, sites 29-34 were 750 m from the center of the source, and sites 35-40 were 1000 m from the center of the source. Collection locations contained single 1 m wooden stakes with a single large (100 mm) plate of Fusarium selective medium placed on top during each sampling interval.
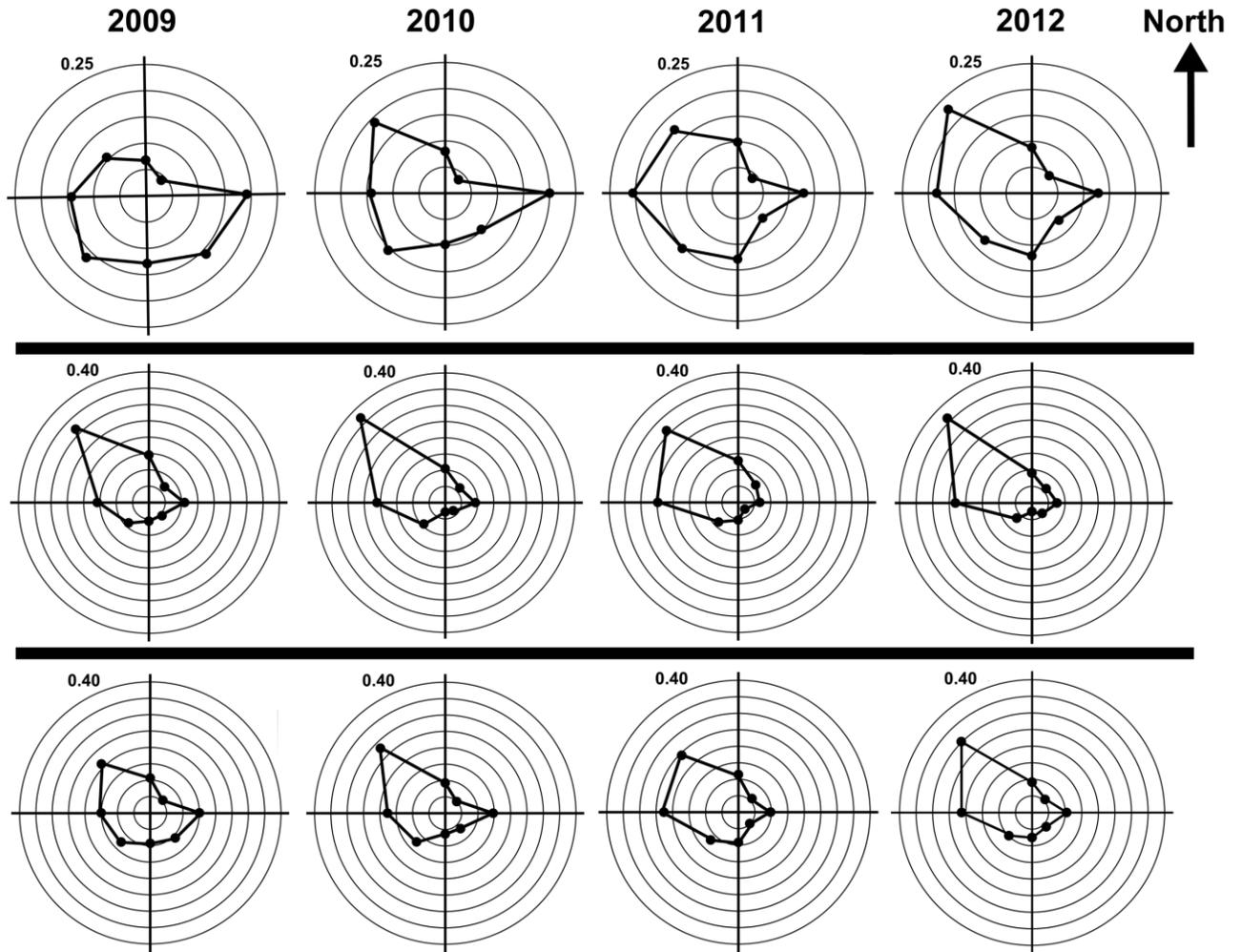
FIGURE 2. Fraction of the time the wind direction was coming from a particular 45 degree sector during the day (top; 0700-1900), night (middle; 1900-0700), and day and night combined (bottom; 0700-0700) from 26 April to 26 May for 2009, 2010, and 2011; and 9 April to 14 May for 2012. The scale is given by the concentric circles with radii spaced at 0.05 intervals. Knowledge of the historical average prevalent wind direction and the farm topography (hills, forest, river, etc.) guided the placement of the sampling devices.
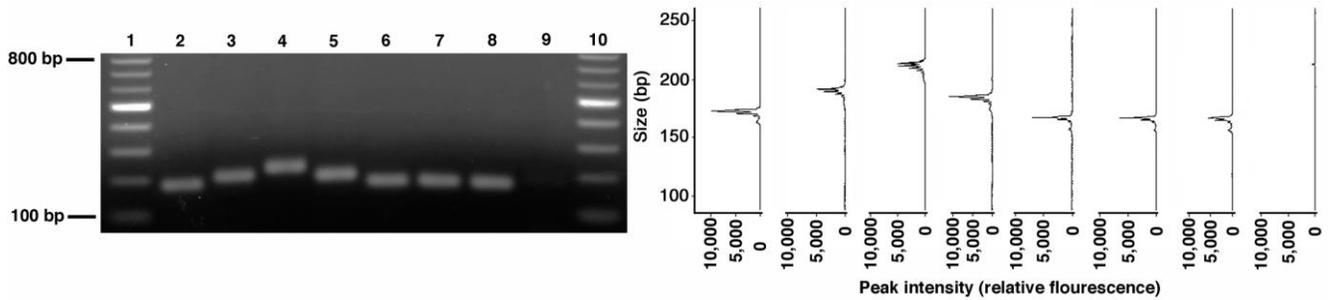
FIGURE 3. PCR products (left) and electropherograms (right) for eight different isolates of *F. graminearum* using the FusSSR23 primers. Lanes 1-10 represent the following in order from left to right: 100 bp mass ladder, isolates HUGR2, 11669, 4528, 6473 (33), the released clone FGVA4, an unknown isolate collected 500 m from the source, an unknown isolate collected 0 m (in the source) from the source, negative control (no template DNA), and 100 bp mass ladder. Electropherograms (right) show product sizes of 173 bp, 191 bp, 213 bp, 185 bp, 167 bp, 167 bp, and 167 bp, which correspond to the same isolates listed for lanes 2 through 8. The product sizes for isolates HUGR2, 11669, 4528, and 6473 are identical to those obtained by Vogelgsang et al. (33; S. Vogelgsang, personal communication), and the unknown isolates produced the same product size as the released isolate FGVA4.
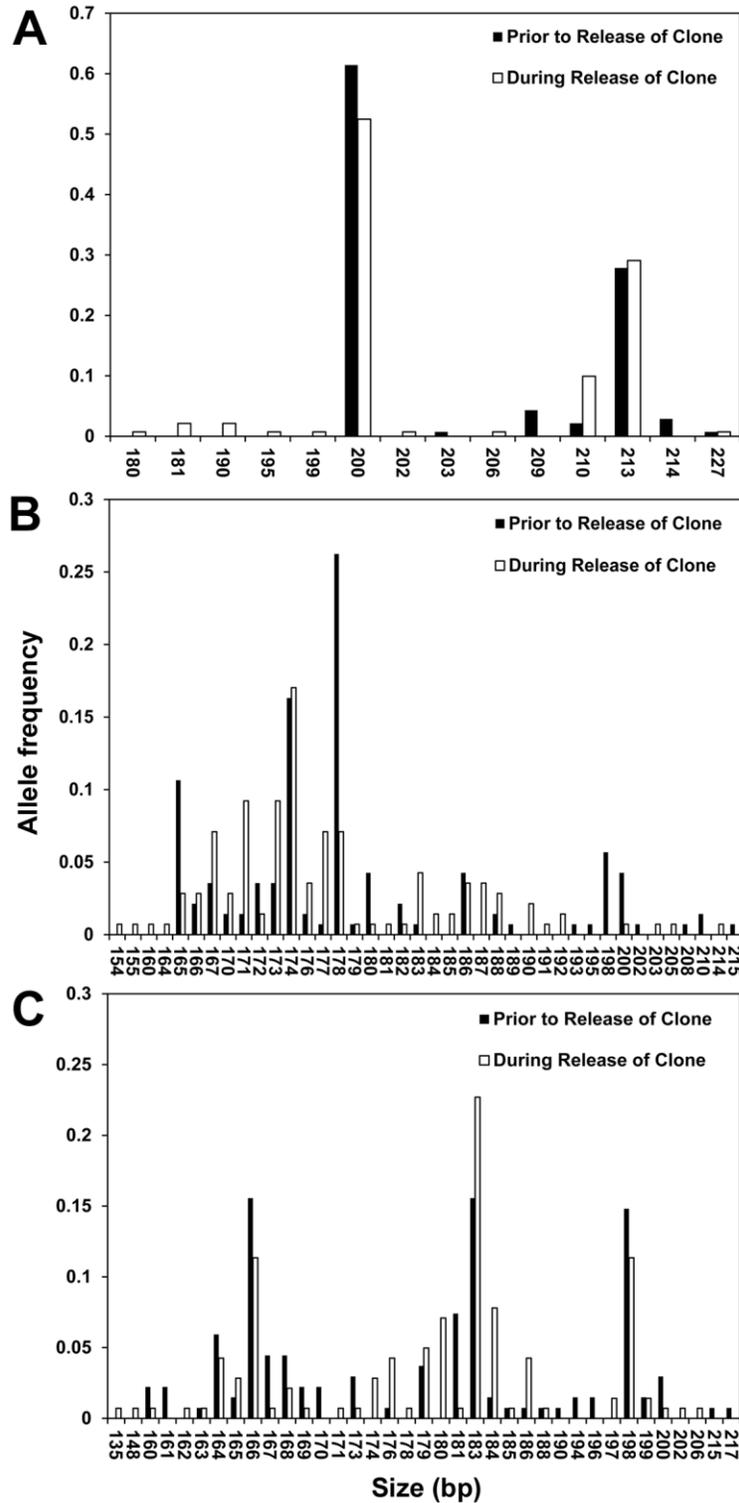
FIGURE 4. Allelic frequencies of background *Fusarium graminearum* populations for microsatellites FusSSR22 (A), FusSSR23 (B), and FusSSR27 (C) prior to (141 isolates)

and during the release of the clone (141 isolates). Background populations collected prior

to and during the release of the clone had similar allele frequencies for the three

microsatellites. The allelic sizes for the released clone are 200 bp, 167 bp, and 183 bp for

microsatellites FusSSR22, FusSSR23, and FusSSR27, respectively.
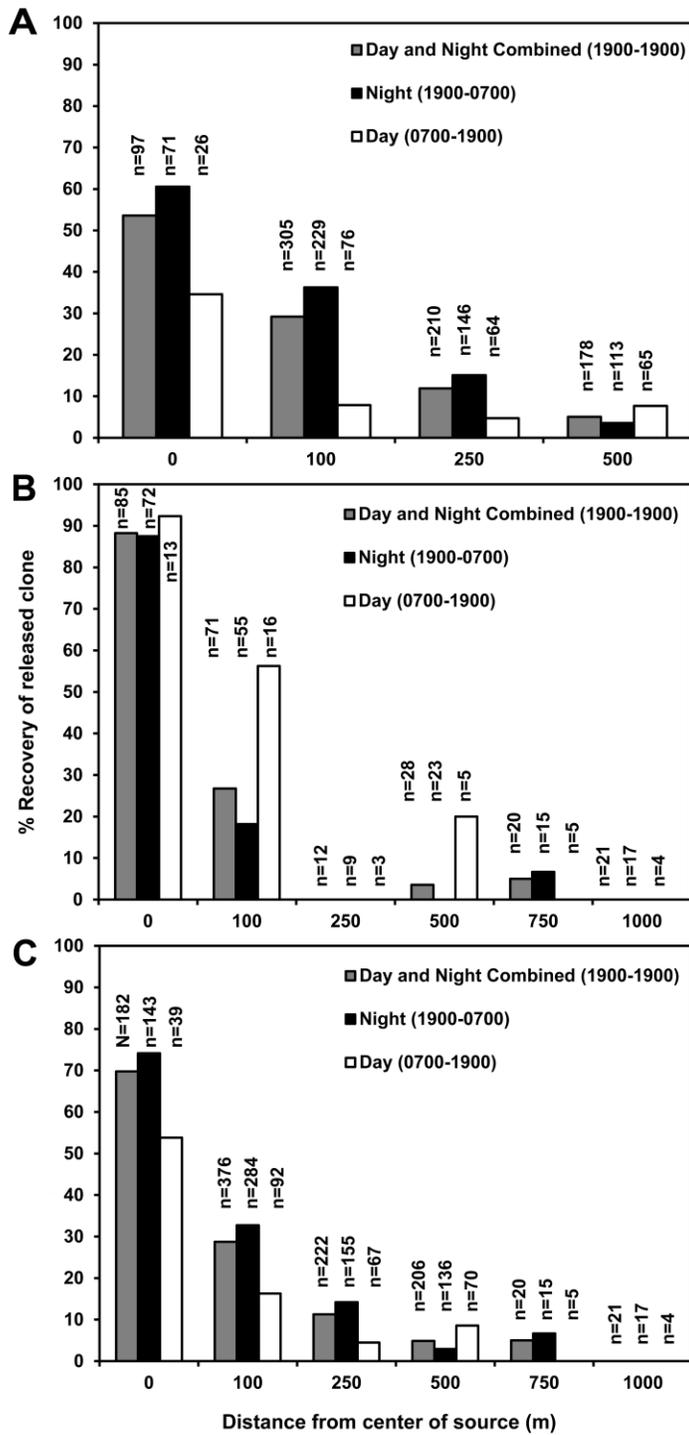
FIGURE 5. Recovery of the released clone (compared to background *F. graminearum* populations) during the night, day, and night and day combined at different distances from the center of a 3,716 m^2 clonal source of inoculum for 2011 (A), 2012 (B), and 2011

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and 2012 data combined (C). Samples were collected during the day (0700-1900) and night (1900-0700) 12-25 May, 2011 in the 2011 season. Samples were collected during the day (0700-1100) and night (1900-0700) 26 April-14 May, 2012 in the 2012 season. Sample sizes (n) listed above each of the bars in the figure represent the total isolates from which the percentage was calculated. The majority of the released clone was recaptured during the night (1900-0700), and the recovery of the released clone decreased at greater distances from the source. The distance of 0 m from the center of the source refers to the three samplers placed in the field-scale source of inoculum.
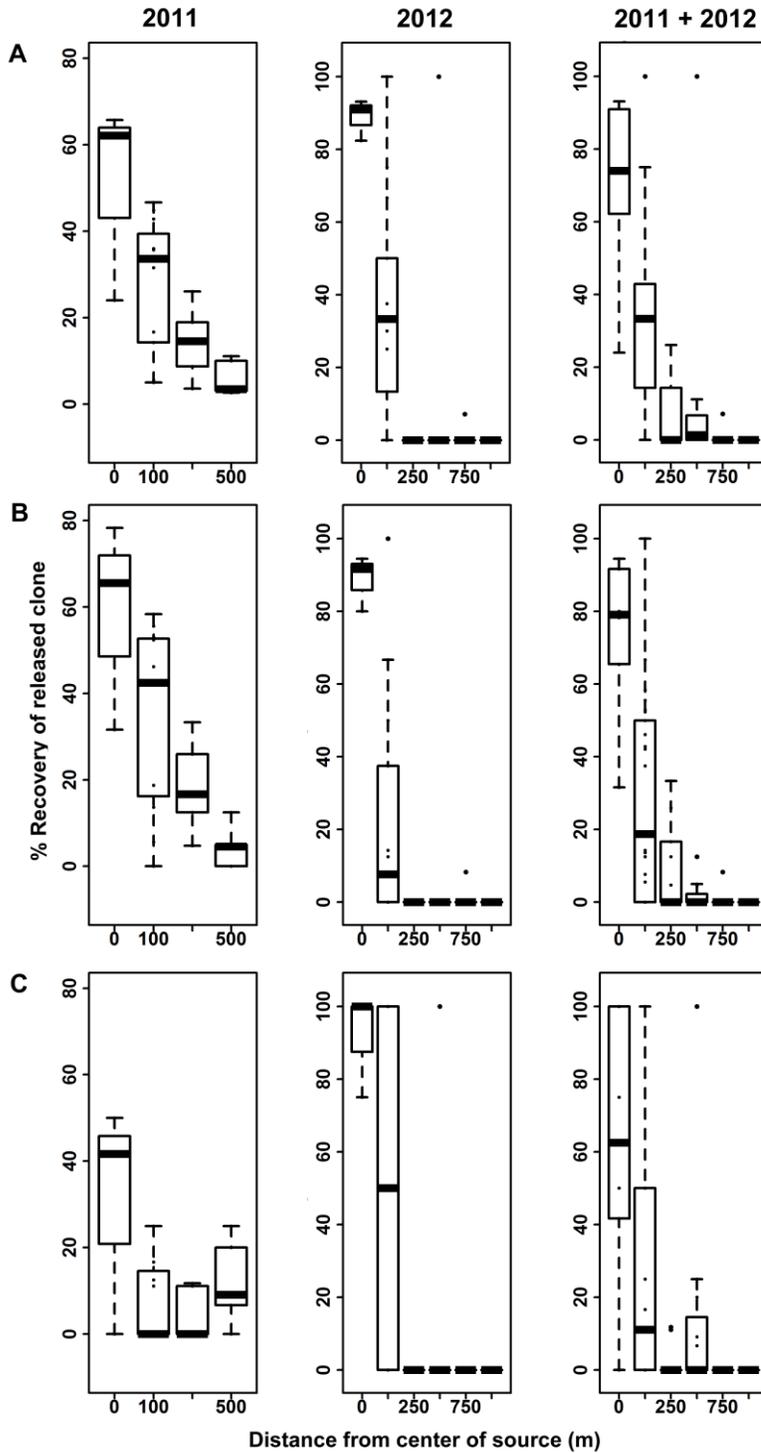
FIGURE 6. Box plots illustrating the range of observations for the percent recovery of released clone in 2011, 2012 and 2011 and 2012 combined for all sampling locations, during the day and night combined (A), night (B), and day (C). The median is used as the

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reference line. The recovery of the released clone decreased at greater distances from the source. The distance of 0 m from the center of the source refers to the three samplers placed in the field scale source of inoculum.
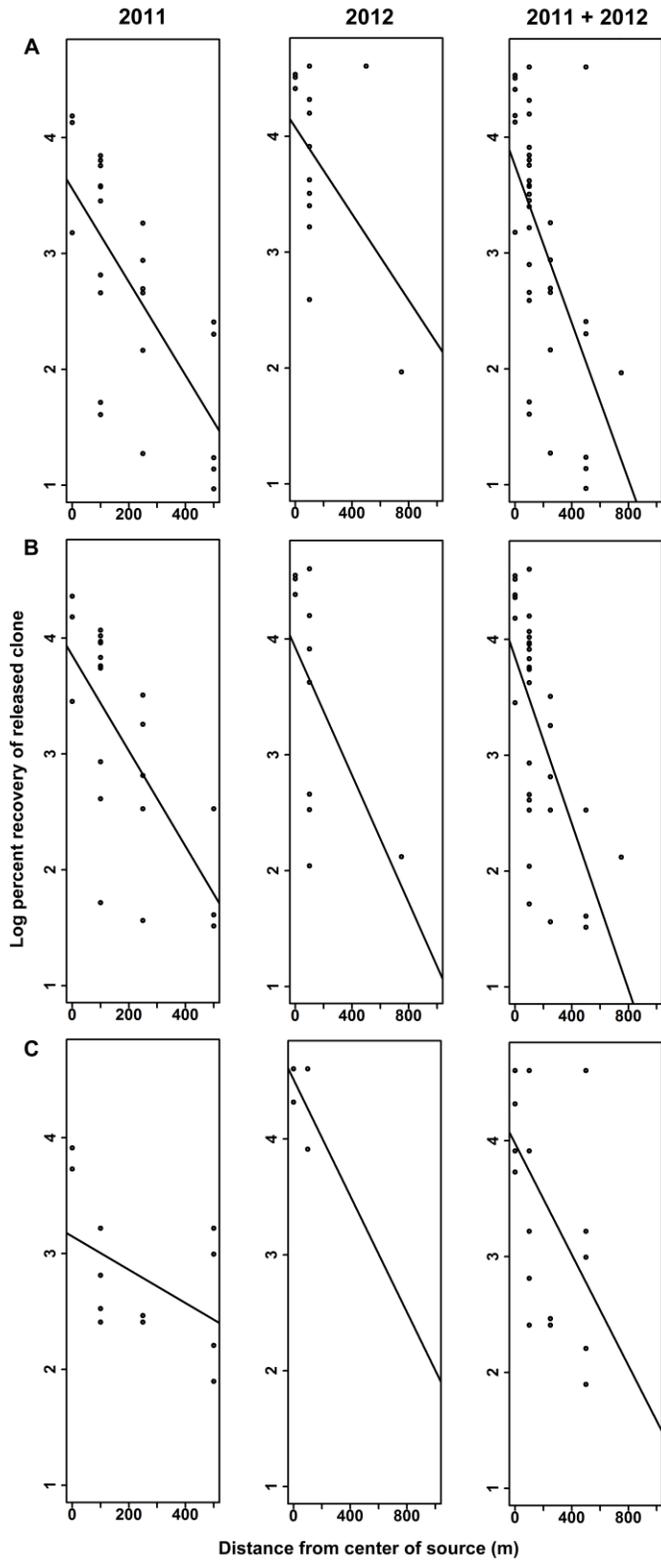
FIGURE 7. Regression plots of the log percent recovery of the released clone in 2011,

2012, and 2011 and 2012 combined at different distances from the source of inoculum

during the day and night combined (A), night (B), and day (C). Accompanying model statistics are reported in the text of the manuscript. The linear regression plot for the log percent recovery of the released clone during the day in 2012, shows a positive correlation, however when the outlier was removed at 500 m, a negative correlation was observed. The distance of 0 m from the center of the source refers to the three samplers placed in the field scale source of inoculum.

Chapter 4: Experimental Validation of a Long-Distance Transport Model for Plant Pathogens: Application to *Fusarium graminearum*

Aaron J. Prussin, II[1*], Linsey C. Marr[2], David G. Schmale, III[1], and Shane D. Ross[3]

[1]Department of Plant Pathology, Physiology, and Weed Science, Virginia Tech, Blacksburg, VA 24061-0390

[2]Department of Civil and Environmental Engineering, Virginia Tech, Blacksburg, VA 24061-0246

[3]Department of Engineering Science and Mechanics, Virginia Tech, Blacksburg, VA 24061-0219

*Corresponding Author: Aaron J. Prussin, II; PH: (540) 231-0733; E-mail:

aprussin@vt.edu

Fusarium graminearum, causal agent of Fusarium head blight (FHB) of wheat and barley, is a devastating plant pathogen that may be transported through the atmosphere over long distances. A Gaussian dispersal model has been developed to predict the long distance transport of plant pathogens, but this model has not yet been experimentally validated for *F. graminearum*. Here, we compare the results of two release-recapture studies (conducted in 2011 and 2012) of *F. graminearum* from known field-scale sources of inoculum to those predicted by a Gaussian dispersal spore transport model. Dispersal kernel shape coefficients were similar for both results observed in the field and predicted by the model, with both being dictated by a power law function, indicating that turbulence was the dominant factor on a kilometer scale. Model predictions had a stronger correlation with the number of spores being released when using a time varying q_0 emission rate (r= 0.92 in 2011 and r= 0.84 in 2012) than an identical daily pattern q_0 emission rate (r= 0.35 in 2011 and r= 0.32 in 2012). Temporal patterns of spore release and spore deposition in the field were not correlated (correlation coefficient of r= -0.12 for 2011 and r= 0.45 for 2012). The actual numbers of spores deposited from our known sources were monitored using microsatellites (short, repeated sequences of DNA), and were 3 and 2000 times lower than predicted if potential source strength, Q_{pot}, was equal to the actual number of spores released in 2011 and 2012, respectively. Differences between predicted and observed results in both years may have been due in part to variability in environmental conditions and spore release rates. This work provides a unique approach for validating a Gaussian spore transport model to predict the

spore transport of *F. graminearum* over kilometer distances, and could be applied to other airborne plant pathogens in the future.

1. Introduction

Many plant pathogens are transported from a host to healthy crops through the atmosphere (Aylor, 1986; Aylor, 1999; Aylor and Sutton, 1992; Aylor et al., 1982). *Phakopsora pachyrhizi*, causal agent of Asian soybean rust (Krupa et al., 2006), and *Puccinia graminis* f. sp. *tritici*, causal agent of wheat stem rust (Stokstad, 2007), are two pathogens of recent concern that disperse their spores via the atmosphere (Krupa et al., 2006; Livingston et al., 2004; Pan et al., 2006; Singh et al., 2008; Singh et al., 2006; Stokstad, 2007). *Fusarium graminearum* is another fungal plant pathogen that utilizes the atmosphere for spore transport (Maldonado-Ramirez et al., 2005; Schmale et al., 2012; Schmale III et al., 2006). This fungus is responsible for Fusarium head blight (FHB) of wheat and barley, which has resulted in more than $3 billion in crop losses in the United States over the past couple of decades (McMullen et al., 1997; Paulitz, 1999; Schmale III and Bergstrom, 2003). *Fusarium graminearum* produces deoxynivalenol (DON), a mycotoxin, that may contaminate food and feed and threaten the health of both humans and livestock (Snijders, 1990; Sutton, 1982).

Disease management for FHB has been a challenge for growers and farmers. Recent research has suggested that no-till practices have contributed to disease outbreaks by increasing the amount of potential inoculum sources (e.g., corn debris) on the soil

surface (Dill-Macky and Jones, 2000; Keller et al., 2011; Keller et al., 2010). Additionally, fungicides have been found to have limited efficacy and must applied at the appropriate times and during specific environmental conditions (Bai and Shaner, 2004). It is easier to control disease and apply appropriate measures if the pathogen is detected early and the natural spread of the pathogen can be predicted (Gregory, 1961). Risk assessment tools have been developed for FHB (De Wolf et al., 2003; Del Ponte et al., 2009). The main considerations of these tools are environmental factors such as precipitation, relative humidity, and temperature to determine the relative risk of FHB. In addition to tools being able to predict FHB, recently tools have been developed to predict the amount of DON that might be produced based on environmental conditions (Schaafsma and Hooker, 2007). Although environmental factors are important for disease development of FHB on wheat and barley and mycotoxin production, the current risk assessment tools do not include the ability to predict the movement of *F. graminearum* spores from known inoculum sources. FHB risk assessment tools have the potential to be improved with the inclusion of knowledge regarding spore transport from known inoculum sources.

A number of spore transport models have been developed based on an understanding of the atmospheric transport of plant pathogens (Aylor, 1986; Aylor, 1999; Aylor and Flesch, 2001; Aylor and Sutton, 1992; Aylor et al., 1982). The atmospheric transport of plant pathogens can be described by the aerobiological processes of inoculum development, liberation, horizontal transport, deposition, impact, and infection (Isard et al., 2005). Models to predict the long distance transport of spores may assume a Gaussian distribution of spores (Aylor, 1999; Pasquill, 1976; Pasquill and Michael, 1977;

Turner, 1970). The shape and width of the Gaussian distribution is dependent on the atmospheric stability, which is dictated by solar radiation and wind speed (Turner, 1970). Researchers have further developed these models to be specific for the long distance transport of plant pathogenic fungi (Aylor, 1986; Aylor, 1999; Aylor and Sutton, 1992; Aylor et al., 1982). Long distance transport is considered to be distances ≥ 100 m from a source of inoculum (Aylor, 1999.) There are several factors in a Gaussian spore transport model necessary to predict the number of viable spores that will be deposited at any given location, including:

1. Initial source strength

2. Wind speed and direction

3. Distance of healthy crops from the source

4. Spore loss due to solar radiation and deposition

5. Dilution of spore plume due to turbulence

Recently, a Lagrangian stochastic (LS) model was used to predict the transport of spores (sporangia) of the potato late blight pathogen, *Phytophthora infestans* up to 500 m from source fields (Aylor et al., 2011). The LS model was experimentally validated during two different field seasons with a series of aerial (unmanned aircraft) and ground-based (Rotorods) measurements (Aylor et al. 2011). Here, we extend the release-recapture concept of Aylor et al. (2011) to field studies of the long distance transport of *F. graminearum* assuming a Gaussian distribution of spores. Long distance transport models driven by a Gaussian distribution (1) are appropriate over the distances we are interested in (hundreds of meters) and expected to give reasonable results (Aylor, 1999) and (2) do

not require numerical integration of differential equations, thus making them significantly less computationally intensive compared to LS models and therefore convenient to use.

The specific objectives of this study were to (1) model the long distance transport of *F. graminearum* using a previously described Gaussian spore transport model and meteorological data collected at our sampling site, and (2) validate the long distance transport model with release-recapture studies in the field over two growing seasons (Aylor, 1986; Aylor, 1999; Aylor and Sutton, 1992; Aylor et al., 1982). *Fusarium graminearum* spore dispersal kernels were derived from results predicted by the transport model and observed in the field and compared to determine the accuracy of the transport model. We hypothesized that the shape of the dispersal kernel for model and field observations would follow a power law, due to turbulence being the dominant factor of long distance transport over our distances of interest, between 100 m and 1000 m (Aylor, 1999; Oboukhov, 1962). However, since the spore transport model is a simplification of a real-world cropping scenario and assumptions are made, we hypothesized that the spore transport model will overestimate the transport distance of spores and the number of spores that are deposited, as the model assumes a best case scenario in the environment for transport. Finally, we hypothesize that there will be greater accuracy in model predictions of spore deposition when time-resolved rather than time-averaged values of q_0 are used.

2. Materials and Methods

2.1 *Field Experiments*

Field studies were conducted at Virginia Tech's Kentland Farm in Blacksburg, Virginia from 26 April to 25 May 2011 and 9 April to 14 May 2012, as previously described (Prussin II et al., 2013a).

2.1.1 *Field Inoculation*

Two hectares of winter wheat (untreated Southern States variety SS560) were planted in October 2010 for the 2011 field campaign and October 2011 for the 2012 field campaign. Mature, green corn stalks were collected in August 2010 and 2011 from corn fields at Virginia Tech's Kentland Farm in Blacksburg, VA and dried in a glass house for 6 months. The dried corn stalks were then cut into ~15 cm pieces and placed into 50 individual 18.9 L steel buckets. Each of 50 buckets was filled approximately 2/3 full with cut corn stalks and autoclaved for 120 min. After the initial autoclaving step, the corn stalks were soaked in DI water overnight, the water was then removed, and the corn stalks were autoclaved again for 120 min. The autoclaved corn stalks were then inoculated with colonized agar pieces of *F. gramineaerum* isolate Fg_Va_GPS13N4_3ADON (hereafter referred to as FGVA4) from five 100 mm diameter petri dishes that had been cultured on ¼-strength PDA for 12 days. The buckets containing the inoculated corn stalks were stored at ambient room temperature for approximately 10 weeks to allow the fungus to colonize the corn stalks.

A plot area of 3,716 m^2 (0.372 ha) of wheat was subdivided into 100 square plots (10 rows of 10 plots, 6.096 m (20 ft) x 6.096 m (20 ft)). Field inoculations were performed on 2 May 2011 (season 1) and 16 April 2012 (season 2) by releasing corn

stalks from each of the 50 buckets into 50 of the subplots in a checkerboard pattern (stalks from one bucket were used for each of the subplots).

2.1.2 *Sample Collection and Identification*

As described previously, a series of ground-based collection devices were placed in the source and at distances of 100 m, 250 m, and 500 m from the center of the inoculated field in 2011, and in the source and at 100 m, 250 m, 500 m, 750 m, and 1000 m, from the center of the inoculated field in 2012 (**Figure 1**) (Prussin II et al., 2013a). The location of these devices was informed based on historical wind data as reported by Prussin et al. (2013a). The ground-based collection devices consisted of a Petri plate containing a Fusarium selective medium (FSM) placed atop a 1 m wooden stake to collect viable spores of *Fusarium*. The FSM was prepared as previously described (Schmale III et al., 2006). In 2011, spore deposition samples were continuously collected in the field during the day (0700-1900) and night (1900-0700) for 14 consecutive days (12 May through 25 May 2011), corresponding to the presence of perithecia on the corn stalks. In 2012, spore deposition samplers were continuously collected in the field during the day, specifically morning (0700-1100) and night (1900-0700) for 19 consecutive days (26 April through 14 May 2012), corresponding to the presence of perithecia on the corn stalks.

After each sampling period, exposed petri plates were immediately removed from the field, covered, and placed in small plastic boxes for transport to the laboratory. The plates were incubated for 7-10 days in the laboratory at ambient room temperature and the number of *Fusarium* colonies (distinct white, fluffy colonies approximately 15 mm in diameter) collected at each location during each sampling period was recorded. Up to

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five *Fusarium* colonies from each plate were randomly selected and sub-cultured to petri plates containing 1/4-strength PDA, in 2011. In 2012, the number of *Fusarium* samples further sub-cultured and studied was increased to ten colonies per plate. Colonies producing red, pink, or yellow mycelia characteristic of *F. graminearum* and containing only macroconidia on ¼-strength PDA, were single-spored onto additional plates. Single-spored cultures were placed in 20% glycerol and stored at -80°C. Single-spored isolates identified as *F. graminearum* were grown in 100 mL of 1/4-strength PD broth on a shaker at 100 rpm for 5-7 days at ambient room temperature. Harvesting of mycelia and extraction of DNA were conducted following previously published methods (Keller et al., 2011; Keller et al., 2010). Three microsatellites were used to genotype all *F. graminearum* isolates from the field collection (Vogelgsang et al., 2009). *F. graminearum* samples having identical allelic sizes as the released clone, FGVA4, at all three microsatellite locations were identified as being the released clone as previously described (Prussin II et al., 2013a) with a likely error rate of less than 1%.

Since only a subset of all the *Fusarium* colonies were sub-cultured and analyzed for the released clone, to estimate the total number of the released clone recaptured on each sampling plate, the fraction of sub-cultured isolates that were identified as the released clone was multiplied by the total number of *Fusarium* colonies.

2.1.3 *Meteorological Data*

Meteorological data (wind speed, wind direction, solar radiation, and rainfall rate) used in the transport model was obtained from the Virginia Agricultural Experimental Station Mesonet weather station located at Kentland Farm. The weather station was located approximately 250 m northwest from the center of our source of inoculum in 2011, and

approximately 350 m northwest from the center of our source of inoculum in 2012. Weather data was recorded at 15-minute intervals.

2.2 Model

We used a universal transport model for plant pathogens described by Aylor (1999) to compare model predictions to field observations, as shown below. Microsoft excel was used for all calculations.

2.2.1 Gaussian Plume Model

The following assumptions were made for our model: (1) a continuous release of *F. graminearum* ascospores from the source of inoculum, (2) a point source of inoculum, (3) the long distance transport of spores from a known source of inoculum followed a Gaussian plume distribution, and (4) the concentration at any given location in space is represented by the following equation (Aylor, 1999; Gifford Jr, 1968):

$$C(x, y, z) = \frac{q_0}{\pi \sigma_y \sigma_z U_c} \exp\left(\frac{-y^2}{2\sigma_y^2}\right) \exp\left(\frac{-z^2}{2\sigma_z^2}\right)$$

(1)

where distances *x, y,* and *z* correspond to the distance of the sampling location downwind from the center of the source of inoculum, distance away from the center of the spore plume, and the sampling height, respectively. Wind speed is represented by U_c (m s^{-1}) and spore release rate is given by q_0 (number spores released/sec.) Standard deviations of the spread of the plume in the *y* and *z* directions are given by σ_y and σ_z, respectively, which are functions of sampling distance downwind, *x* (meters), and can be calculated using the following equations (Turner, 1970):

$$\sigma_y(x) = \exp[I_y + J_y \ln x + K_y (\ln x)^2]$$

(2a)

$$\sigma_z(x) = \exp[I_z + J_z \ln x + K_z (\ln x)^2]$$

(2b)

where I_y, J_y, K_y, I_z, J_z, and K_z are constants that depend on the atmospheric stability class, as shown in **Table 1** (Gifford Jr, 1961; Gifford Jr, 1968; Pasquill, 1976; Turner, 1970). Atmospheric stability was calculated using Pasquill stability classes (Turner, 1970). There are six stability classes; Classes A, B, C, D, E, F corresponding to extremely unstable, moderately unstable, slightly unstable, neutral, slightly stable, and moderately stable, respectively. Atmospheric stability class was calculated as a function of solar radiation and wind speed as shown in **Table 2**.

We note that in general, U_c, q_0, σ_y, and σ_z vary in time, and the greatest uncertainty lies in estimating $q_0(t)$. Previous work has suggested the uncertainty in estimating $q_0(t)$ is between a factor of 100 and 1000 (Aylor, 1986). We will provide two assumptions to estimate $q_0(t)$, discussed below.

2.2.2 *Distance of Sampling Location from Center of Spore Plume*

Each sampling location had fixed (X,Y) coordinates, where X and Y are the eastward and northward distances (m), respectively, of the sampler with respect to the center of the source. For modeling, the relevant position is (x,y) (see **Figure 1C**). To calculate the x and y distances (m) used in the model the following equations were used:

$x = X\cos\theta + Y\sin\theta$

(3a)

$y = Y\cos\theta - X\sin\theta$

(3b)

where θ is the angle of the wind heading, as shown in **Figure 1C**. The values of X and Y were calculated using GPS waypoints for each sampling location in 2011 (**Figure 1A**) and 2012 (**Figure 1B**).

2.2.3 *Spore Loss*

Equation (**1**) assumes all spores remain airborne and viable during transport. There are two major processes that contribute to spore loss during long distance transport: solar radiation and deposition. The fraction of spores surviving exposure to solar radiation is given by the following expression (Aylor, 1999),

$$f_s = \exp\left(\frac{-Ix}{I_*t_*U_c}\right)$$

(4a)

where I is solar radiation (W m^{-2}) and I_*t_* is the dose of radiation to kill 1-1/e spores. There is a wide range of values I_*t_*, for fungal spores ranging from less than 1 MJ m^{-2} to over 35 MJ m^{-2} (Aylor, 1999). To our knowledge I_*t_* is unknown for *F. graminearum*. Therefore, the two extreme I_*t_* values were examined (1 MJ m^{-2} and 35 MJ m^{-2}), and we determined that on the scale we are modeling transport (<1 km), I_*t_* was negligible for spore loss. For our model, an I_*t_* of 20 MJ m^{-2} was assumed as this was in the mid-range of reported I_*t_* values (Aylor, 1999). Additionally, *Venturia inaequalis*, another ascomycete, has an I_*t_* value of 21 MJ m^{-2} (Aylor and Sanogo, 1997).

Deposition can occur either due to the natural settling of spores, known as dry deposition, or because of spore washout due to precipitation, known as wet deposition. The fraction of spores that are not removed from the atmosphere by either wet or dry deposition is given by the following equation (Aylor, 1999):

$$f_d = \exp\left(\frac{-(\Gamma_w + \Gamma_d)x}{U_c}\right)$$

(4b)

Γ_w and Γ_d are the removal coefficients (s^{-1}) for wet and dry deposition, respectively, and can be represented as (Aylor, 1999; Aylor and Sutton, 1992) :

$$\Gamma_w = 0.000272R^{0.7873}$$

(4c)

$$\Gamma_d = \sqrt{\frac{2}{\pi}}\left(\frac{v_s}{x}\right)\int_{x_0}^{x}\left[\frac{dx}{\sigma_z(x)}\right]$$

$$= \sqrt{\frac{2}{\pi}}\left(\frac{v_s}{x}\right)\left(\frac{\sqrt{\pi}\exp\left(\frac{(J_z-1)^2}{4K_z}-I_z\right)\left(1+\mathrm{erf}\left(\frac{2K_z\ln(x)+J_z-1}{2\sqrt{K_z}}\right)\right)}{2\sqrt{K_z}}\right)$$

(4d)

where R is the rain fall rate given in mm hr^{-1} and v_s is the settling velocity of *F. graminearum* ascospores. The settling velocity of *F. graminearum* ascospores is approximately 1.27 mm s^{-1} (Schmale III et al., 2005; Trail et al., 2005).

The number of spores remaining airborne and not lost due to solar irradiation at some distance downwind from the center of the source of inoculum, x, and atmospheric wind speed, U_c, can be represented as (Aylor, 1999):

$$q\left(\frac{x}{U_c}\right) = f_s f_d q_0$$

(4e)

where q_0 is the initial spore release rate at $x = 0$, the center of the inoculum source. The concentration of viable *F. graminearum* spores at any distance from the center of the

source of inoculum (taking into account spore loss due to deposition and solar radiation) can be determined by substituting $q(x/U_c)$ for q_0 in (1), giving:

$$C(x,y,z) = \frac{q(\frac{x}{U_c})}{\pi \sigma_y \sigma_{z U_c}} \exp(\frac{-y^2}{2\sigma_y^2}) \exp(\frac{-z^2}{2\sigma_z^2})$$

(4f)

2.2.4 Number of Spores Deposited

The number of *F. graminearum* spores deposited at each sampling location can be calculated by determining the deposition flux. Deposition velocity is given by (Aylor and Sutton, 1992):

$$v_x = v_d + v_w$$

(5a)

where v_d and v_w are the velocities for dry and wet deposition, respectively, and can be represented as (Aylor and Sutton, 1992):

$$v_d = v_s$$

(5b)

$$v_w = 1.25 \Gamma_w \sigma_z$$

(5c)

Finally, the total number of spores deposited on a horizontal dish of surface area S, over a sampling duration T, at any given location (x,y,z), is

$$D = C(x,y,z) v_x ST$$

(5d)

2.2.5 *Estimation of q_0*

One of the most difficult model parameters to estimate is the time dependent spore release rate for the source, $q_0(t)$ (Aylor, 1986). In previous work, Prussin et al. (2013b) quantified Q_{pot}, or the maximum potential number of *F. graminearum* ascospores released from our plot throughout the entire sampling period. In order to estimate q_0 we used a combination of spore capture data collected by a volumetric spore sampler, at ground level, and knowledge of Q_{pot}. The potential source strength, Q_{pot}, of our 3,716 m^2 artificially inoculated plot was estimated to be approximately 3.7 billion ascospores (Prussin II et al., 2013b). The relative number of spores released each hour was determined and combined with Q_{pot} to give an estimated number of spores being released from the 3,716 m^2 plot each hour. The relative number of spores being released was quantified from one volumetric spore sampler placed in the center of the inoculated field and spore release was assumed to be spatially uniform throughout the entire field. Since the model was completed with a time resolution of 15 minutes, we divided the number of spores being released each hour by 4 to get an input value for q_0 for each 15-minute interval. This $q_0(t)$ we refer to as time varying $q_0^{tv}(t)$. In 2011, we only had spore release data for the second week of field sampling (19 May 2011 to 25 May 2011) and therefore could not use $q_0^{tv}(t)$ for the first week of sampling (12 May 2011 to 19 May 2011). To get an estimate of $q_0(t)$ which could include the first week of sampling, an identical daily pattern $q_0^{idp}(t)$ was calculated for each hour interval in a day based on the average observed pattern (**Figure 2**) (e.g., spore release was assumed to be the same at 0100 everyday, the same at 0200 everyday, etc.) (Prussin II et al., 2013b). Identical daily pattern $q_0^{idp}(t)$ was used for the entire sampling periods in both in 2011 and 2012.

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Additionally, we compared the results of using either identical daily pattern $q_0^{idp}(t)$ or time varying $q_0^{tv}(t)$ in the model.

2.3 *Comparison of Model Results to Field Results*

Spore deposition observed in the field and predicted by the model was analyzed for both 2011 and 2012. In 2012 and the second week of 2011 (19 May 2011 to 25 May 2011), q_0 was estimated using time varying $q_0^{tv}(t)$. Additionally, for the entire sampling periods in 2011 and 2012 q_0 was estimated using the identical daily pattern $q_0^{idp}(t)$. Note that if we approximate the deposition as occurring exactly downwind (i.e., y=z=0), (**5d**) has the functional form,

$$D(x) = ax^b e^{-cx}$$

(6a)

Where

$$a = \frac{q_0^{ave} v_x ST}{\pi U_c e^{I_y + I_z}}$$

(6b)

$$b = -(J_y + J_z)$$

(6c)

$$c = \frac{I}{I_* t_* U_c} + \frac{\Gamma_w + \Gamma_d}{U_c}$$

(6d)

Thus, curve-fitting via non-linear regression analysis allows us to estimate *a, b,* and *c*. If other factors are known, this provides an independent means to approximate the average release rate q_0^{ave}. As the Gaussian point source model is a better approximation outside the inoculated field, samples that were in the source of inoculum were not included in the analyses.

The model was evaluated by plotting the measured number of spores deposited in the field at each location for each sampling period against the corresponding model prediction for that location and time. Additionally, model performance was evaluated using the mean bias (B_{MB}), mean normalized bias (B_{MNB}), mean absolute gross error (E_{MAGE}), and mean normalized gross error (E_{MNGE}). These values report whether the model tends to over-predict or under-predict the observations and how large the differences.

3. Results

3.1 *Comparison of Field Observations to Model Prediction*

The number of *F. graminearum* spores released from the inoculated field and deposited compared to the number of spores predicted to be deposited by the model is shown in **Figure 3**. In 2011, using the identical daily pattern $q_0^{idp}(t)$ in the model, the majority of the points fall above the diagonal line, indicating an over-prediction of the actual number of spores being deposited; however when time varying $q_0^{tv}(t)$ was used in the model, points fall closer to the diagonal line indicating better agreement between the model and results observed in the field. More data points were observed when using identical daily pattern $q_0^{idp}(t)$, because we were able to include the entire sampling period (12 May- 25 May 2011) in our analysis, rather than just the second week of sampling (19 May- 25 May 2011) which could only be used when using time varying $q_0^{tv}(t)$ in the model (there were no volumetric spore sampler data for 12 May-19 May 2011). In 2012, when either time varying $q_0^{tv}(t)$ or identical daily pattern $q_0^{idp}(t)$, was used in the model, all the data points fell above the horizontal line indicating an over-prediction of the model when compared to results observed in the field. Model performance was evaluated using the

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mean bias (B_{MB}), mean normalized bias (B_{MNB}), mean absolute gross error (E_{MAGE}), and mean normalized gross error (E_{MNGE}). The results of these analyzes are shown in **Table 3**. The E_{MNGE} results indicate that for 2011 the model over-predicted the number of spores that would be deposited on any given sampler by an average of about 3 times and 150 times when either time varying or identical daily patterns were used as the model input for $q_0(t)$, respectively. In 2012, the E_{MNGE} results indicated that the model over predicted the number of spores that would be deposited on any given sampler by about 2000 times and 630 times when either time varying or identical daily patterns were used as the model input for $q_0(t)$, respectively.

In 2011, a daily comparison was made between the number of spores released from the field that were captured by the volumetric spore sampler in the center of the field (**Figure 4A**), the number of spores released from the field that were recaptured at the various deposition locations (**Figure 4B**), the model prediction assuming a time varying $q_0^{tv}(t)$ input (**Figure 4C**), the model prediction assuming an identical daily pattern $q_0^{idp}(t)$ input (**Figure 4D**), and rainfall (**Figure 4E**). There was no data for spore release between May 12 (day)- May 19 (day), which is the reason there are no results for the model assuming an time varying release $q_0^{tv}(t)$ input during the same time period. There was no discernible agreement between the spore release pattern and the field deposition pattern (r= -0.12) or the model assuming an identical daily pattern $q_0^{idp}(t)$ input (r= 0.35). However, agreement was observed between spore release pattern and the model assuming time varying release $q_0^{tv}(t)$ input (r=0.92). Interestingly, there was no discernible agreement between the patterns of field deposition and rainfall (r= -0.12). On the scale studied (<1 km), rainfall does not appear to have a significant influence on spore

deposition. This result is consistent with the model (assuming a time varying release $q_0^{tv}(t)$ input) predicting no relationship between spore deposition and rainfall (r= -0.15).

In 2012, a daily comparison was made between the number of spores released from the field that were captured by the volumetric spore sampler in the center of the field (**Figure 5A**), the number of spores released from the field that were recaptured at the various deposition locations (**Figure 5B**), the model prediction assuming a time varying $q_0^{tv}(t)$ input (**Figure 5C**), the model prediction assuming an identical daily pattern $q_0^{idp}(t)$ input (**Figure 5D**) and rainfall (**Figure 5E**). There was no discernible agreement between the spore release pattern and the field deposition pattern (r=0.45) or the model assuming an identical daily pattern $q_0^{idp}(t)$ input (r= 0.31). However, agreement was observed between spore release pattern and the model assuming time varying release $q_0^{tv}(t)$ input (r=0.84). As in 2011, during the 2012 field campaign rainfall does not appear to have a significant influence on spore deposition in the field (r= -0.17), which is consistent with the model (assuming time varying release $q_0^{tv}(t)$ input) predicting no relationship between spore deposition and rainfall (r= -0.10).

3.2 *Dispersal Kernels*

Separate from the time-resolved deposition modeling, overall dispersal kernels were estimated for the entire sampling period (~2 weeks) from results observed in the field and predicted by the model. These are shown in **Figure 6**, where the total deposition shown is normalized by the value at 100 m, i.e., D(100 m)=1. Dispersal kernels were created by fitting the data of deposition data to the functional form given by **Eq. 6**. Coefficients for *b* and *c* are given in **Table 4**. For 2011 using the identical daily pattern $q_0^{idp}(t)$ in the model, 2011 using time varying $q_0^{tv}(t)$ in the model, 2012 using time varying $q_0^{tv}(t)$ in the

model, and 2012 using the identical daily spore release pattern for $q_0^{idp}(t)$ in the model, coefficient b is the dominate factor in dictating the shape of the dispersal kernel on the scale studied. A dominating b coefficient indicates a dispersal kernel is governed by a power function and thus turbulent diffusion is the main factor in transport; while a dominating c coefficient indicates the dispersal kernel is governed by an exponential function and thus spore loss from solar irradiation and deposition (wet and dry) are the dominating factors in transport (Aylor, 1999). The results we obtained indicate that on the scale we are studying (< 1 km), turbulence is the dominating component of spore transport and spore loss from deposition and solar irradiation is negligible.

In 2011, the coefficient for the b variable was smaller for the results observed in the field than predicted by the model for both time varying $q_0^{tv}(t)$ and identical daily pattern $q_0^{idp}(t)$ inputs. This suggests that the model over-predicted the 'fat-tail' of the dispersal kernel and the distance the spores would be transported. However, in 2012, the coefficient for the b variable was slightly larger for the results observed in the field than predicted by the model, suggesting the model under-predicted the 'fat-tail' of the dispersal kernel and the distance spores can be transported.

An estimate of the average spore release rate, q_0^{ave}, and estimated source strength, Q_{est}, were obtained from the curve-fitted value of a for the dispersal kernel. Using eq. (**6b**), we considered the average wind speed, I_y, I_z, downward flux, sampling surface area, and total sampling time (T_{tot}) during the sampling period, and solved for the average spore release rate, q_0^{ave}. To obtain the estimated source strength, we used $Q_{est} = q_0^{ave} T_{tot}$ as calculated for 2011 and 2012 (**Table 5**). In 2011, the average q_0^{ave} was calculated to be 2820 spores s^{-1}, which yielded a Q_{est} of approximately 3.2 billion ascospores released

from the inoculated field during the sampling period. In 2012, the average q_0^{ave} was calculated to be 1.35 spores s^{-1}, which yielded a Q_{est} of approximately 2.1 million ascospores. The potential source strength of the field was estimated to be 3.7 billion ascospores (Prussin II et al., 2013b). Thus, approximately 86% of the total number of ascospores potentially present were released during our sampling period in 2011; while only 0.06% of the total number of ascospores potentially present were released in during our sampling period in 2012.

4. Discussion and Conclusions

In this study, we compared results predicted by a long distance transport model to results observed in release-recapture field studies conducted over two years for *F. graminearum*. We used a previously described transport model for plant pathogenic fungi in our study with parameters unique to *F. graminearum* (Aylor, 1986; Aylor, 1999; Aylor and Sutton, 1992; Aylor et al., 1982). This work provides a unique approach for validating a Gaussian spore transport model to predict *F. graminearum* spore transport over long distances. This work supports the idea that such a model could be used to enhance FHB risk assessment tools (De Wolf et al., 2003; Del Ponte et al., 2009; Schaafsma and Hooker, 2007). Additionally, this work could be extended to other airborne plant pathogens in the future.

Shape coefficients, *b* and *c*, were used to predict the shape of the dispersal kernels and were similar for both the results predicted by the transport model and observed in the field (**Table 4**). This result allows us to conclude that the model was reasonably accurate (in a long term average statistical sense) at predicting long distance transport of spores. The dispersal kernel provides the probability density function describing the average

spatial distribution of spores from an inoculated field, and this is an important input for regional and national scale epidemiological models to forecast future disease spread. However, it has been shown that small changes in the 'fat tails' of the dispersal kernel, which are caused by small changes in the b exponent, can cause order-of-magnitude changes in predicted spread rates (Higgins et al., 2003; Lewis, 1997), since rare, very long distance (> 1 km) dispersal events described by the tails of the kernel end up being the dominant factor in predicting the rate of spread.

In both 2011 and 2012, the shape of the dispersal kernel was strongly governed by a power law (dominate b coefficient) for both the field results and model predictions. When the shape of the disease kernel is governed by a power law, it indicates that atmospheric turbulence is the dominant factor as shown in **eq. (6c)** (Aylor, 1999). When spore dispersal is dictated by a power law, spores may be transported much greater distances than those whose dispersal is dictated by an exponential component. The reason for this phenomenon is that a power law causes a 'fat-tail' in the dispersal kernel and does not approach zero as quickly as an exponential law. Dispersal kernels that follow an exponential shape converge to zero with distance more rapidly, as spores do not survive transport due to solar irradiation and are thus not viable, or the entire spore column can be washed out either from a major rainfall event or passive deposition if transport is being studied on a large enough scale as shown in **eq. (6d)** (Aylor, 1986; Aylor, 1999; Rotem et al., 1985). It is possible, that our study was not conducted on a large enough scale for solar irradiation and deposition to be a factor in transport, due to the short transport times. In 2011, the dispersal kernel and distance of spore transport was slightly over-predicted by the model when compared to results observed in the field

(i.e. observed results had a smaller *b* coefficient); however in 2012 the dispersal kernel and distance of spore transport was slightly under-predicted by the model when compared to results observed in the field (i.e. observed results had a larger *b* coefficient); however the magnitude of differences in both years was small.

In order to estimate the total number of spores deposited at any given location away from a source of inoculum, one needs to know the potential source strength, Q_{pot}, and more importantly, the time dependent release rate, $q_0(t)$. Experimentally, prior to the sampling period, we were able to estimate the Q_{pot} as 3.7 billion ascospores from our inoculated fields in both 2011 and 2012 (Prussin II et al., 2013b). Additionally, we had spore release data during the sampling periods indicating the relative number of spores being released from the center of the plot on hourly intervals. This information was used to develop two different estimates for $q_0(t)$. The first estimate was time varying $q_0^{tv}(t)$, in which $q_0(t)$ was varied for each time point based on individual hourly spore release data. The second estimate was the identical daily pattern $q_0^{idp}(t)$, in which $q_0(t)$ was assumed to be the same for each hour interval of each day based on the average daily spore release patterns. The results comparing the number of spores deposited at various locations predicted by the model to those observed in the field indicated a strong over-prediction by the model in 2011 assuming identical daily pattern $q_0^{idp}(t)$ (over-predicted approximately 150 times), and in 2012 assuming either identical daily pattern $q_0^{idp}(t)$ or time varying $q_0^{tv}(t)$ (over-predicted approximately 630 and 2000 times, respectively). However, in 2011 when time varying $q_0^{tv}(t)$ was used, the model only slightly over-predicted spore deposition (over-predicted approximately 3 times). These results indicated that using time varying $q_0^{tv}(t)$ is the best estimate. One possible explanation for

this is that spore release from a field is patchy (heterogeneous) in time with a few 'major' release events (Fernando et al., 2000; Paulitz, 1996). This could partially be due to *F. graminearum* perithecia formation and spore release occurring in 'waves' as field conditions become favorable. The time varying $q_0^{tv}(t)$ takes into account these 'major' release events, while the identical daily pattern $q_0^{idp}(t)$ assumes that an identical number of spores are being released every single day under the same average daily pattern. Transport models in the future should prioritize time varying $q_0^{tv}(t)$ when attempting to predict spore transport and deposition since it captures the intermittent nature of the release pattern.

Temporal patterns of spore release and spore deposition in the field were not correlated (correlation coefficient of r= -0.12 for 2011 and r= 0.45 for 2012) (**Figure 4; Figure 5**). It is important to address that spore release data were collected by placing a single volumetric spore sampler (approximately 0.5 m high) in the center of the inoculated field, thus we were only able to monitor spore release for one location in the field. It is possible that spore release is heterogeneous across a field, and consequently there were different release patterns across the fields that were not observed due to varying environmental conditions and the formation of microclimates across the wheat field (Cutforth and McConkey, 1997; Fernando et al., 2000; Paulitz, 1996). This is one possibility for the disagreement between the deposition results observed in the field and spore release patterns. To validate the ability of an LS model to predict the transport of sporangia (spores) of *Phytophthora infestans*, Aylor et al. (2011) used a network of multiple Rotorod towers located 3 m directly above inoculated potato fields to quantify the number of sporangia being released. Future work examining *F. graminearum* spore

release patterns from a wheat field might include a network of volumetric spore samplers placed across a field to determine if spatial heterogeneity exists.

The greatest unknown input of the current spore transport model remains to be Q and $q_0(t)$ (Aylor, 1986). The release rate $q_0(t)$ affects both the spatiotemporal distribution of spore deposition as well as its average (reflected in the dispersal kernel). Thus, we would expect improved accuracy with better a priori estimates of Q and q_0. Prussin II et al. (2013b) estimated Q, and found differences between laboratory estimates and field estimates, which would impact model predictions. Thus, when estimating Q and q_0 it is important to do so under field conditions.

Using our field results, we were able to estimate the overall dispersal kernel for the entire sampling period, and from this, generate an a posteriori estimate for the source strength Q of approximately 3.2 billion spores and 2.1 million spores for 2011 and 2012, respectively, which is approximately 86% and 0.06% of the potential source strength Q_{pot}, respectively. This is notable as it provides a new way to estimate actual source strength, independent of other a priori experimental estimates of potential source strength (Prussin II et al., 2013b). The potential source strength, Q_{pot}, was assumed to be the same for both field seasons. The difference in the fraction of spores released is significant (86% in 2011 and 0.06% in 2012.) However, this difference is consistent with earlier estimates suggesting uncertainty in the actual source strength is around three orders of magnitude (Aylor, 1986). These results do not necessarily indicate that the potential source was weaker in 2012, as it is possible that there was a delay in spore release from the perithecia and there were no 'major' spore release events during our sampling period. Previous research has suggested that both *F. graminearum* perithecia formation and spore

release is highly dependent on environmental conditions (Dufault et al., 2006; Fernando et al., 2000; Trail et al., 2002; Tschanz et al., 1975; Xu, 2003). The 2012 field campaign was started approximately a month earlier than in 2011 due to a mild winter and earlier growth of the wheat crop. In 2012, it is possible that environmental conditions were not as conducive for spore release from perithecia as in 2011. Therefore, in 2012 during our sampling period fewer spores were being released from the ground and fewer spores were able to get out of the crop canopy and be transported long distances, than in 2011. This speculation could be assessed with future studies capturing spores with the volumetric spore sampler for a longer time period (e.g., before and after the ~ 2 week time window considered here) to see if delays occur in spore release or if the potential source is significantly different between seasons, even when the same amount of inoculum is released. Addition studies should also address the effect of temperature and the combination of different environmental factors on spore release from perithecia.

Another explanation for the differences observed between 2011 and 2012 is that it is possible just as many spores were being discharged from perithecia in 2012 and 2011, however, the number of spores escaping the crop canopy, and thus contributing to long distance transport, was different during the two seasons. *Fusarium graminearum* spores are first released from perithecia on corn stalks lying on the ground; however the height of the wheat canopy is approximately 1 meter. In order for spores to be released from the crop canopy, they must go from the laminar boundary layer to the turbulent layer and this vertical flux of spores is known to be significantly correlated with atmospheric turbulence (Aylor and Flesch, 2001). Many environmental factors affect turbulence in a wheat field (Lawson and Uk, 1979; Raupach and Thom, 1981). The

percentage of spores that escape a crop canopy can be calculated as a function of the height at which spores are releases inside the canopy, the settling velocity v_s and the friction velocity u_* (Aylor, 1999; Aylor and Flesch, 2001). The percentage of spores that escape the crop canopy and can be transported long distances increases with an increasing u_*. Aylor and Flesch (2001) computed u_* using a 3D sonic anemometer to obtain horizontal wind profiles, when studying the release of fungal spores from a grass canopy. We did not have measurements with a 3D sonic anemometer in our studies and did not have access to turbulence data in the field. Future work should examine potential differences between growing seasons that might affect turbulence in the field and the percentage of *F. graminearum* spores that can escape the crop canopy.

The long distance spore transport model we examined herein was able to correctly predict the power law characteristics of spore transport dispersal kernels when compared to results observed in a field study, indicating an appropriate model to predict spore transport from a known source of inoculum. Given the limitations in our ability to provide an accurate time-resolved release rate $q_0(t)$ as an input, our model provides some statistical agreement, while not providing time-resolved agreement. In order to be able to predict the actual number of spores that may be deposited at any location away from a known source of inoculum, a more accurate input for $q_0(t)$ needs to be used. Future work should examine how to improve the estimate for $q_0(t)$. Additionally, an increased understanding of all the environmental factors that trigger spore release of *F. graminearum* is needed. Future research should also investigate within-crop turbulent dynamics and the development of the fraction of spores that are released into the turbulent boundary layer compared to those that stay in the laminar boundary layer is

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needed. Finally, a comparison of a LS transport model (Aylor and Flesch, 2001) to a Gaussian transport model (Aylor, 1999) could be done for distances up to 1 km. The current long distance transport model can be used to predict long distance spore transport of *F. graminearum* and will be able to accurately predict the number of spores deposited with an improvement of $q_0(t)$ (Aylor, 1999).

Acknowledgements

Literature Cited

Aylor, D.E., 1986. A framework for examining inter-regional aerial transport of fungal spores. Agricultural and Forest Meteorology, 38(4): 263-288.

Aylor, D.E., 1999. Biophysical scaling and the passive dispersal of fungus spores: relationship to integrated pest management strategies. Agricultural and Forest Meteorology, 97(4): 275-292.

Aylor, D.E. and Flesch, T.K., 2001. Estimating spore release rates using a Lagrangian stochastic simulation model. Journal of Applied Meteorology, 40(7): 1196-1208.

Aylor, D.E. and Sanogo, S., 1997. Germinability of *Venturia inaequalis* conidia exposed to sunlight. Phytopathology, 87(6): 628-633.

Aylor, D.E., Schmale III, D.G., Shields, E.J., Newcomb, M. and Nappo, C.J., 2011. Tracking the potato late blight pathogen in the atmosphere using unmanned aerial vehicles and Lagrangian modeling. Agricultural and Forest Meteorology, 151(2): 251-260.

Aylor, D.E. and Sutton, T.B., 1992. Release of *Venturia inaequalis* ascospores during unsteady rain: Relationship to spore transport and deposition. Phytopathology, 82(5): 532-540.

Aylor, D.E., Taylor, G.S. and Raynor, G.S., 1982. Long-range transport of tobacco blue mold spores. Agricultural Meteorology, 27(3–4): 217-232.

Bai, G. and Shaner, G., 2004. Management and resistance in wheat and barley to Fusarium head blight 1. Annual Reviews of Phytopathology, 42: 135-161.

Cutforth, H. and McConkey, B., 1997. Stubble height effects on microclimate, yield and water use efficiency of spring wheat grown in a semiarid climate on the Canadian prairies. CanadianJournal of Plant Science, 77(3): 359-366.

De Wolf, E.D., Madden, L.V. and Lipps, P.E., 2003. Risk assessment models for wheat Fusarium head blight epidemics based on within-season weather data. Phytopathology, 93(4): 428-435.

Del Ponte, E.M., Fernandes, J.M.C., Pavan, W. and Baethgen, W.E., 2009. A model-based assessment of the impacts of climate variability on Fusarium head blight seasonal risk in southern Brazil. Journal of Phytopathology, 157(11-12): 675-681.

Dill-Macky, R. and Jones, R., 2000. The effect of previous crop residues and tillage on Fusarium head blight of wheat. Plant Disease, 84(1): 71-76.

Dufault, N., De Wolf, E., Lipps, P. and Madden, L., 2006. Role of temperature and moisture in the production and maturation of *Gibberella zeae* perithecia. Plant Disease, 90(5): 637-644.

Fernando, W.G., Miller, J.D., Seaman, W.L., Seifert, K. and Paulitz, T.C., 2000. Daily and seasonal dynamics of airborne spores of *Fusarium graminearum* and other *Fusarium* species sampled over wheat plots. Canadian Journal of Botany, 78(4): 497-505.

Gifford Jr, F.A., 1961. Use of routine meteorological observations for estimating atmospheric dispersion. Nuclear Safety, 2: 47-51.

Gifford Jr, F.A., 1968. An outline of theories of diffusion in the lower layers of the atmosphere, Environmental Science Services Administration, Oak Ridge, Tenn.).

Gregory, P.H., 1961. The microbiology of the atmosphere. Leonard Hill.

Higgins, S., Nathan, R. and Cain, M., 2003. Are long-distance dispersal events in plants usually caused by nonstandard means of dispersal? Ecology, 84(8): 1945-1956.

Isard, S.A., Gage, S.H., Comtois, P. and Russo, J.M., 2005. Principles of the atmospheric pathway for invasive species applied to Soybean rust. Bioscience, 55(10): 851-861.

Keller, M.D., Thomason, W.E. and Schmale III, D.G., 2011. The spread of a released clone of *Gibberella zeae* from different amounts of infested corn residue. Plant Disease, 95(11): 1458-1464.

Keller, M.D., Waxman, K.D., Bergstrom, G.C. and Schmale III, D.G., 2010. Local distance of wheat spike infection by released clones of *Gibberella zeae* disseminated from infested corn residue. Plant Disease, 94(9): 1151-1155.

Krupa, S. et al., 2006. Introduction of Asian soybean rust urediniospores into the midwestern United States—A case study. Plant Disease, 90(9): 1254-1259.

Lawson, T. and Uk, S., 1979. The influence of wind turbulence, crop characteristics and flying height on the dispersal of aerial sprays. Atmospheric Environment (1967), 13(5): 711-715.

Lewis, M.A., 1997. Variability, patchiness, and jump dispersal in the spread of an invading population. Spatial eEology: The Role of Space in Population Dynamics and Interspecific Interactions: 46-69.

Livingston, M. et al., 2004. Economic and policy implications of wind-borne entry of Asian soybean rust into the United States. US Department of Agriculture, Economic Research Service.

Maldonado-Ramirez, S.L., Schmale III, D.G., Shields, E.J. and Bergstrom, G.C., 2005. The relative abundance of viable spores of *Gibberella zeae* in the planetary boundary layer suggests the role of long-distance transport in regional epidemics of Fusarium head blight. Agricultural and Forest Meteorology, 132(1–2): 20-27.

McMullen, M., Jones, R. and Gallenberg, D., 1997. Scab of wheat and barley: A re-emerging disease of devastating impact. Plant Disease, 81(12): 1340-1348.

Oboukhov, A., 1962. Some specific features of atmospheric turbulence. Journal of Fluid Mechianics, 13(1): 77-81.

Pan, Z. et al., 2006. Long-term prediction of soybean rust entry into the continental United States. Plant Disease, 90(7): 840-846.

Pasquill, F., 1976. Atmospheric dispersion parameters in Gaussian plume modeling. Part 2: Possible Requirements for Change in the Turner Workbook Values. Environmental Protection Agency.

Pasquill, F. and Michael, P., 1977. Atmospheric diffusion. Physics Today, 30: 55.

Paulitz, T.C., 1996. Diurnal release of ascospores by *Gibberella zeae* in inoculated wheat plots. Plant Disease, 80(6): 674-678.

Paulitz, T.C., 1999. Fusarium head blight: a re-emerging disease. Phytoprotection (Québec), 80(2).

Prussin II, A.J., Li, Q., Malla, R., Ross, S.D. and Schmale, I., D. G., 2013a. Monitoring the long distance transport of *Fusarium graminearum* from field-scale sources of inoculum. Plant Disease, *Submitted*.

Prussin II, A.J., Szanyi, N.A., Welling, P.I., Ross, S.D. and Schmale III, S.D., 2013b. Estimating potential source strength of a field-scale source of *Fusarium graminearum* inoculum. Plant Disease *Submitted*.

Raupach, M. and Thom, A., 1981. Turbulence in and above plant canopies. Annual Reviews of Fluid Mechianics, 13(1): 97-129.

Rotem, J., Wooding, B. and Aylor, D., 1985. The role of solar radiation, especially ultraviolet, in the mortality of fungal spores. Phytopathology, 75(5): 510.

Schaafsma, A. and Hooker, D., 2007. Climatic models to predict occurrence of *Fusarium* toxins in wheat and maize. International Journal of Food Microbiology, 119(1): 116-125.

Schmale, D. et al., 2012. Isolates of *Fusarium graminearum* collected 40–320 meters above ground level cause Fusarium head blight in wheat and produce trichothecene mycotoxins. Aerobiologia, 28(1): 1-11.

Schmale III, D.G., Arntsen, Q.A. and Bergstrom, G.C., 2005. The forcible discharge distance of ascospores of *Gibberella zeae*. Canadian Journal of Plant Pathology, 27(3): 376-382.

Schmale III, D.G. and Bergstrom, G.C., 2003. Fusarium head blight in wheat. The Plant Health Instructor. DOI: 10.1094/PHI-I-2003-0612-01.

Schmale III, D.G. et al., 2006. Genetic structure of atmospheric populations of *Gibberella zeae*. Phytopathology, 96(9): 1021-1026.

Seinfeld, J.H. and Pandis, S.N., Atmospheric Chemistry and Physics - From Air Pollution to Climate Change (2nd Edition). John Wiley & Sons.

Singh, R.P. et al., 2008. Will stem rust destroy the world's wheat crop? Advances in Agronomy, 98: 271-309.

Singh, R.P. et al., 2006. Current status, likely migration and strategies to mitigate the threat to wheat production from race Ug99 (TTKS) of stem rust pathogen. CAB Reviews: Perspectives in Agriculture, Veterinary Science, Nutrition and Natural Resources, 1(054).

Snijders, C.H.A., 1990. Fusarium head blight and mycotoxin contamination of wheat, a review. European Journal of Plant Pathology, 96(4): 187-198.

Stokstad, E., 2007. Deadly wheat fungus threatens world's breadbaskets. Science, 315(5820): 1786-1787.

Sutton, J., 1982. Epidemiology of wheat head blight and maize ear rot caused by Fusarium graminearum. Canadian Journal of Plant Pathology, 4(2): 195-209.

Trail, F., Gaffoor, I. and Vogel, S., 2005. Ejection mechanics and trajectory of the ascospores of *Gibberella zeae* (anamorph *Fuarium graminearum*). Fungal Genetics and Biology, 42(6): 528-533.

Trail, F., Xu, H., Loranger, R. and Gadoury, D., 2002. Physiological and environmental aspects of ascospore discharge in *Gibberella zeae* (anamorph *Fusarium graminearum*). Mycologia, 94(2): 181-9.

Tschanz, A., Horst, K. and Nelson, P.E., 1975. Ecological aspects of ascospore discharge in *Gibberella zeae*. Phytopathology, 65: 597-599.

Turner, D.B., 1970. Workbook of atmospheric dispersion estimates. US Department of Health, Education, and Welfare, National Center for Air Pollution Control.

Vogelgsang, S., Widmer, F., Jenny, E. and Enkerli, J., 2009. Characterisation of novel Fusarium graminearum microsatellite markers in different Fusarium species from various countries. European Journal of Plant Pathology, 123(4): 477-482.

Xu, X., 2003. Effects of environmental conditions on the development of Fusarium ear blight. European Journal of Plant Pathology, 109(7): 683-689.

Table 1. Constants used to determine Gaussian plume spread parameters σ_y and σ_z.[A]

| Coefficient | Atmospheric Stability Class | | | | | |
	A	B	C	D	E	F
I_y	-1.104	-1.634	-2.054	-2.555	-2.754	-3.143
J_y	0.9878	1.0350	1.0231	1.0423	1.0106	1.0148
K_y	-0.0076	-0.0096	-0.0076	-0.0087	-0.0064	-0.0070
I_z	4.679	-1.999	-2.341	-3.186	-3.783	-4.490
J_z	-1.7172	0.8752	0.9477	1.1737	1.3010	1.4024
K_z	0.2770	0.0136	-0.0020	-0.0316	-0.0450	-0.0540

[A] Table adapted from Seinfeld and Pandis.

Table 2. Atmospheric stability class as a function of solar irradiation and wind speed.[A,B]

Wind speed (m s^{-1})	Daytime			Nighttime[F]
	Incoming solar radiation			
	Strong[C]	Moderate[D]	Slight[E]	
< 2	A	B	B	F
2-3	B	B	C	E
3-5	B	C	C	D
5-6	C	D	D	D
>6	C	D	D	D

[A] Table adapted from Turner (Turner, 1970)

[B] Atmospheric stability classes A, B, C, D, E, and F represent extremely unstable, moderately unstable, slightly unstable, neutral, slightly stable, and moderately stable conditions, respectively.

[C] Solar radiation > 700 W m^{-2}

[D] Solar radiation 350-700 W m^{-2}

[E] Solar radiation < 350 W m^{-2}

[F] No solar radiation present

Table 3. Statistical metrics to compare model performance to results observed in the field[A] in 2011[B] and 2012[C].

Metric	2011		2012	
	Time Varying Spore Release[D]	Identical Daily Spore Release Pattern[E]	Time Varying Spore Release[D]	Identical Daily Spore Release Pattern[E]
B_{MB}	75	1080	2000	778
B_{MNB}	261%	15200%	185000%	63000%
E_{MAGE}	98	1090	1970	778
E_{MNGE}	302%	15200%	182000%	63000%

[A] 3,716 m^2 plot was inoculated with a single clonal isolate of *Fusarium graminearum*.

[B] The 2011 field season consisted of 14 consecutive days of sampling (12 May- 25 May 2011).

[C] The 2012 field season consisted of 19 consecutive days of sampling (26 April- 14 May 2012).

[D] Model was performed using time varying spore release as an input for q_0.

[E] Model was performed using identical daily spore release pattern as an input for q_0.

Table 4. Coefficient values for the observed and predicted dispersal kernels of *F. graminearum* spores from a known source of inoculum in 2011 and 2012.[A]

q_0 Input	Year		b[E]	c[E]	R^2	Estimated[F] b	c
Identical Daily Spore Release Pattern[B]	2011	Observed	-1.91	0	0.99	-2.04	0.004
		Predicted	-1.40	0.0015	0.99	-	-
Identical Daily Spore Release Pattern[B]	2012	Observed	-1.28	0	0.99	-2.06	0.018
		Predicted	-1.71	0	0.99	-	-
Time Varying Spore Release[C]	2011	Observed[D]	-2.48	0	0.99	-2.06	0.004
		Predicted	-1.75	0	0.99	-	-
Time Varying Spore Release[C]	2012	Observed	-1.28	0	0.99	-2.06	0.018
		Predicted	-1.40	0.0007	0.99	-	-

[A] Dispersal kernels were fitted to the following function: $D=ax^b e^{-cx}$, where x is distance from the center of the source of inoculum. A continually releasing point source of inoculum was assumed.

[B] The identical daily spore release pattern was used for the q_0 input for the entire sampling period during each hour interval were used in the model (i.e. 0100 was the same for each day, 0200 was the same for each day, etc.)

[C] Spore release values depended on time and was correlated with spore release data collected by the volumetric spore sampler.

D Only included the second week of field data (19 May 2011-25 May 2011) and model

was only run for this time period.

E Calculated from dispersal kernel.

E b and c coefficients were estimated using **Eqs 6c** and **6d** with collected meteorological

data from the field. Coefficients were only estimated for the observed results in the field.

Table 5. Parameters used to estimate average q_0^{ave} and total source strength for 2011 and 2012.

Parameter	2011	2012
a^A (spores)	867887 spores	645 spores
S^B (m^2)	0.00567 m^2	0.00567 m^2
T_{tot}^C (s)	1123200 s	1533600 s
U_c^D (m s^{-1})	1.55 m s^{-1}	1.44 m s^{-1}
I_y^E	-2.33	-2.34
I_z^E	-2.88	-2.94
$f(x)^F$ (m s^{-1})	0.00127 m s^{-1}	0.00127 m s^{-1}
q_0^{aveG} (spores s^{-1})	2850 spores s^{-1}	1.35 spores s^{-1}
Q_{est}^H (spores)	3.2 x 10^9 spores	2.1 x 10^6 spores

[A] Calculated from dispersal kernel fit to **Eq. 6a** for field deposition data collected between 12 May- 25 May 2011 and 26 April- 14 May 2012.

[B] Surface area of sampling dish collecting deposition samples.

[C] Total sampling time for collecting deposition from 12 May- 25 May 2011 and 26 April- 14 May 2012.

[D] Average wind speed between 12 May- 25 May 2011 and 26 April- 14 May 2012.

[E] Average coefficient calculated from **Table 1** between 12 May- 25 May 2011 and 26 April- 14 May 2012.

[F] Average downward spore flux between 12 May- 25 May 2011 and 26 April- 14 May 2012.

[G] Average spore release rate from field calculated using **Eq. 6b**.

[H] Estimated total source strength during sampling period calculated from q_0^{ave} and T_{tot}.

Figure 1. Collection locations at different distances from clonal 3,716 m^2 (0.372 ha) sources of inoculum (grey squares) of *Fusarium graminearum* at Virginia Tech's Kentland Farm in Blacksburg, VA for the 2011 (A) and 2012 (B) field campaigns. In 2011, collection sites 1-12 were 100 m from the center of the source, sites 13-17, 23, and 25 were 250 m from the center of the source, sites 18-22 and 24 were 500 m from the center of the source, and sites 26-28 were in the source (site 26 was in the center of the source). In 2012, collection sites 1-3 were in the source, sites 4-15 were 100 m from the center of the source, sites 16-20, 26, and 27 were 250 m from the center of the source, sites 21-25 and 28 were 500 m from the center of the source, sites 29-34 were 750 m

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from the center of the source, and sites 35-40 were 1000 m from the center of the source. The location of these devices was informed based on historical wind data as reported by Prussin et al. (2013a). An example of the rotating matrix is shown (C). L is the location of a specific sampler, θ is the angle of the wind heading (with respect to directly east), and X and Y are distances from the center of the source of inoculum. In order to calculate x and y used in the model **Eq. 3** was used. Figure modified from Prussin et al. (2013a).
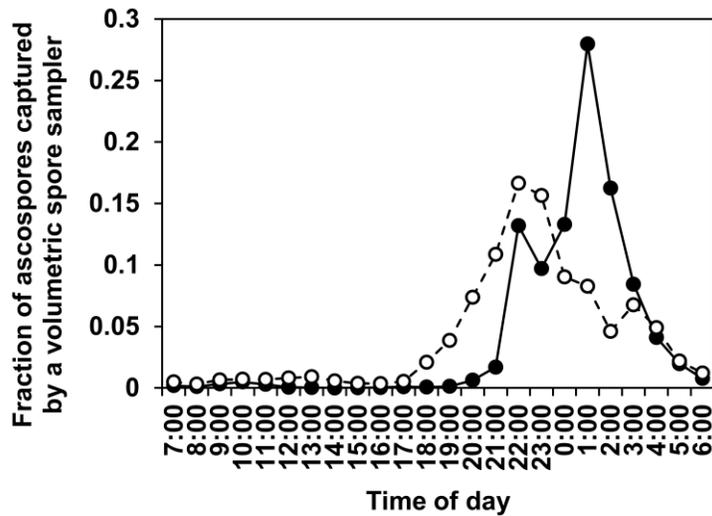
Figure 2. Fraction of ascospores captured by a volumetric spore sampler in the center of a 3,716 m^2 wheat field inoculated with FGVA4 during different times of the day in 2011 (closed circles) and 2012 (open circles). The volumetric spore sampler was continuously sampling between 19 May to 3 June, 2011 and 26 April to 14 May, 2012. These dates correspond to when corn stalks inoculated with FGVA4 were present in the 3,716 m^2 wheat field. Figure adapted from Prussin II et al. (2013b).
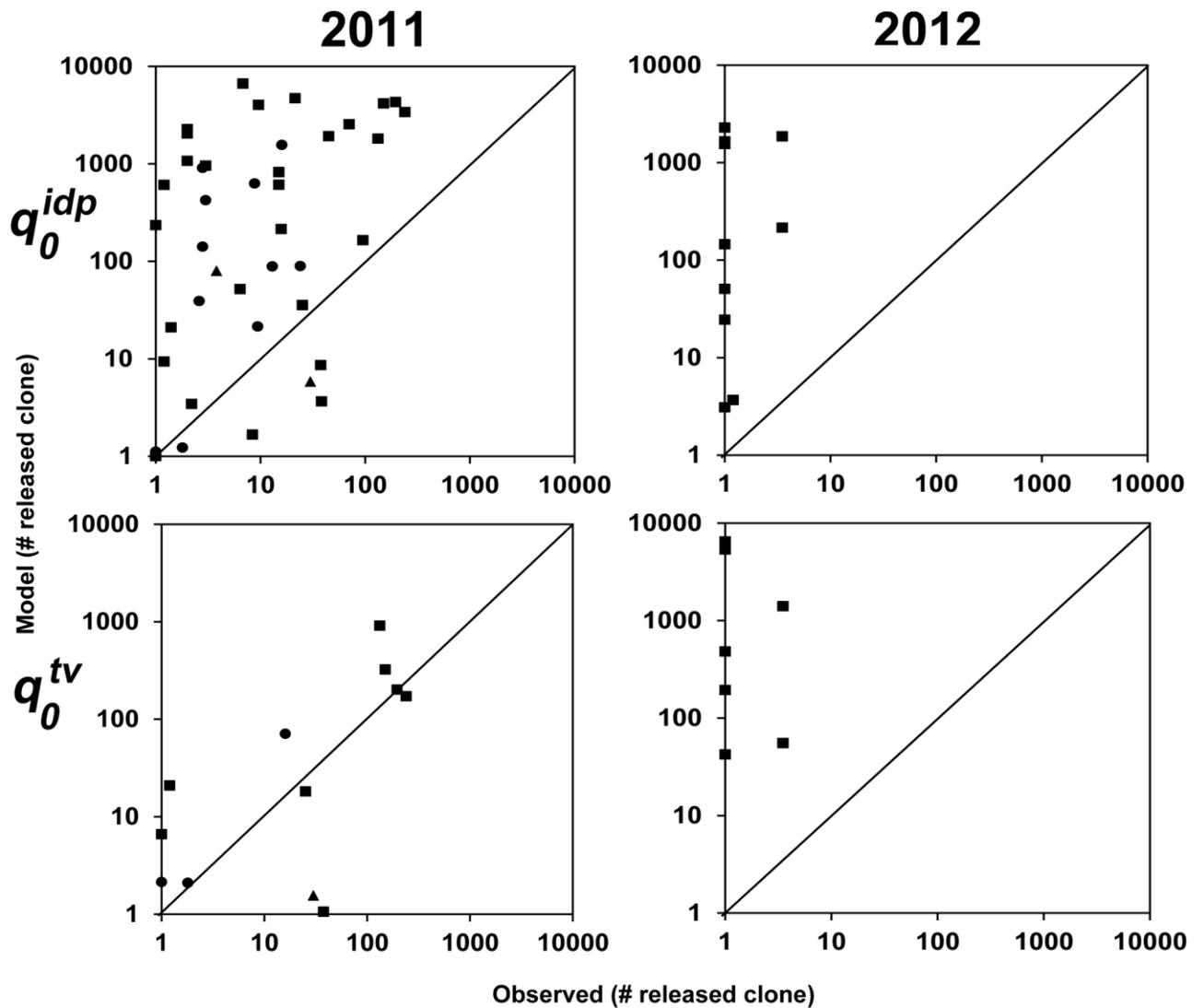
Figure 3. Comparison of the number of *F. graminearum* spores deposited that were observed in the field and predicted by the model in 2011 (left panel) and 2012 (right panel) assuming identical daily spore release pattern q_0^{idp} (top) and time varying spore release q_0^{tv} (bottom) for distances of 100 m (square), 250 m (circle), and 500 m (triangle) from the source of inoculum. Data points that fall on the diagonal line of each graph had agreement between the results predicted by the model and observed in the field. Data

points above the line were over-predicted by the model and those below the line were

under-predicted by the model.
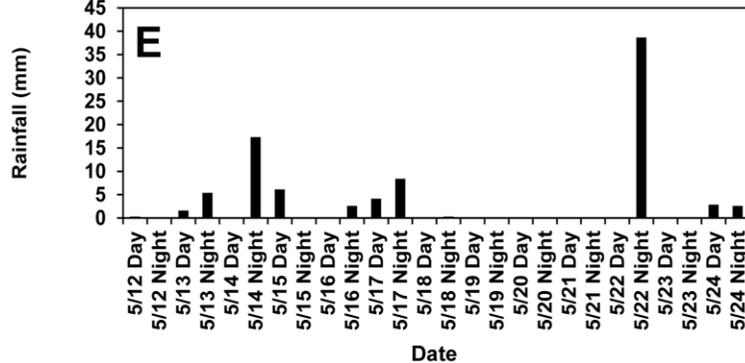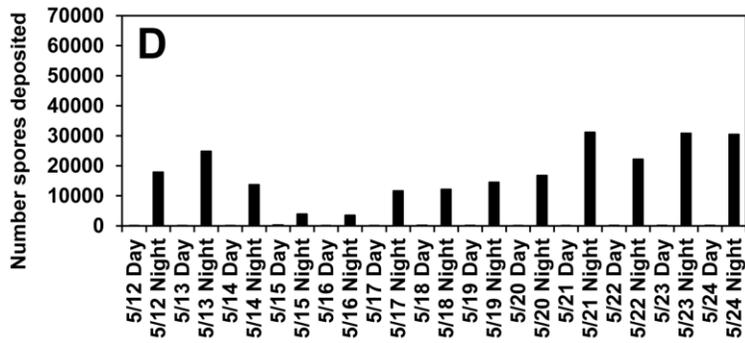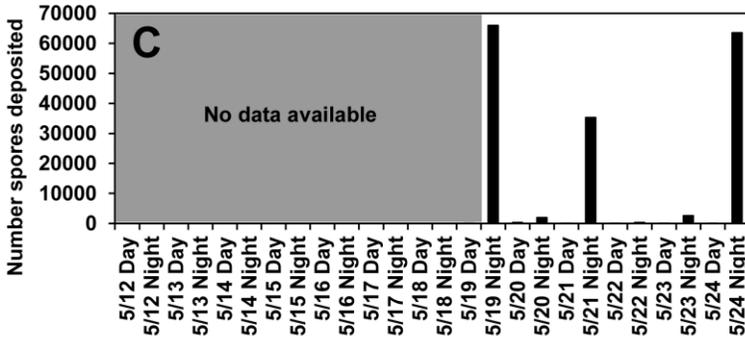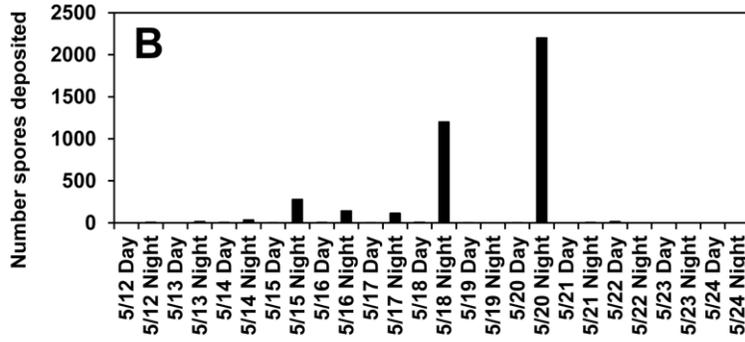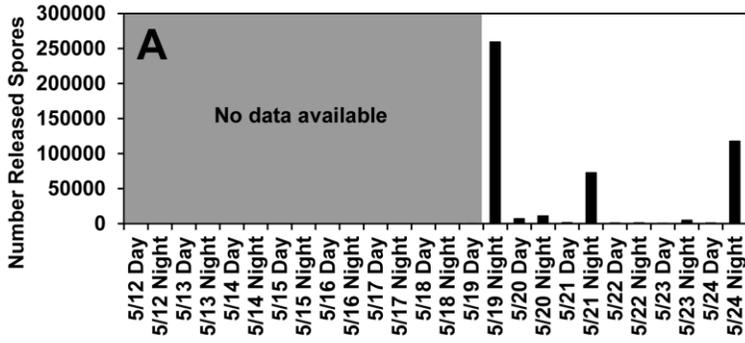
Figure 4. Comparison between the number of *F. graminearum* spores released and captured by the volumetric spore sampler in the center of the inoculated field (A), number of spores released from the source deposited in the field (B), number of spores predicted to be deposited using identical daily spore release pattern as the input for q_0 in the model (C), number of spores predicted to be deposited using time varying spore release as the input for q_0 in the model for 2011 (D), and total rainfall during each sampling period (E). Spore deposition samples in the field were collected from 12 May through 25 May 2011, corresponding to perithecia being present on inoculated corn stalks. Volumetric spore collection data was only available for the second week of field sampling (19 May - 25 May 2011) and thus the model using time varying spore release as the input for q_0 could only be run for the second week of sampling (19 May -25 May 2011). The model using identical daily spore release pattern as the input for q_0 was run for the entire sampling period (12 May -25 May 2011).
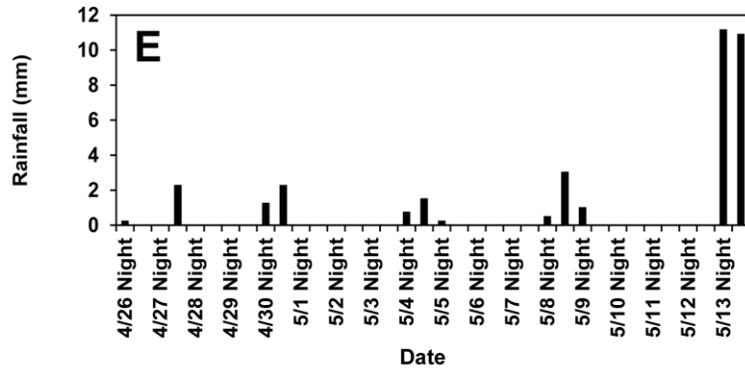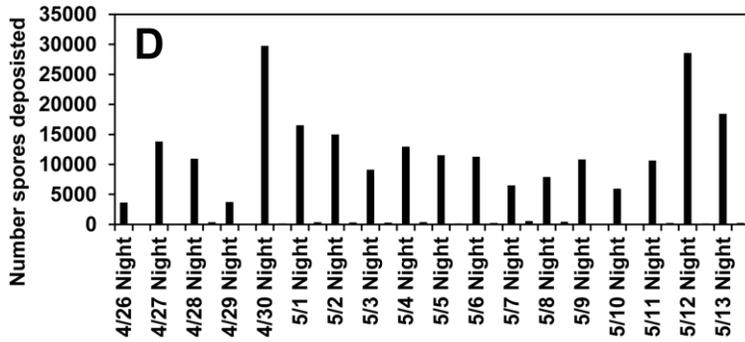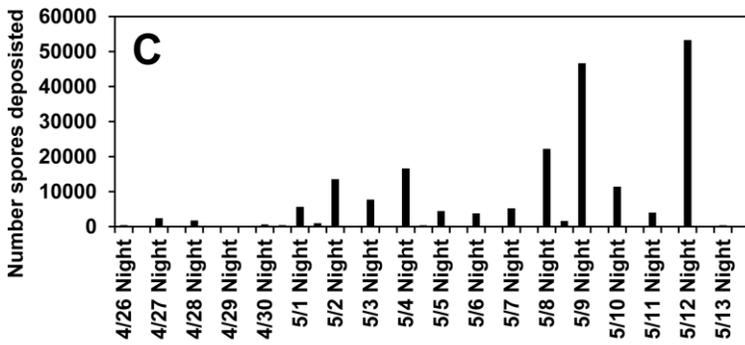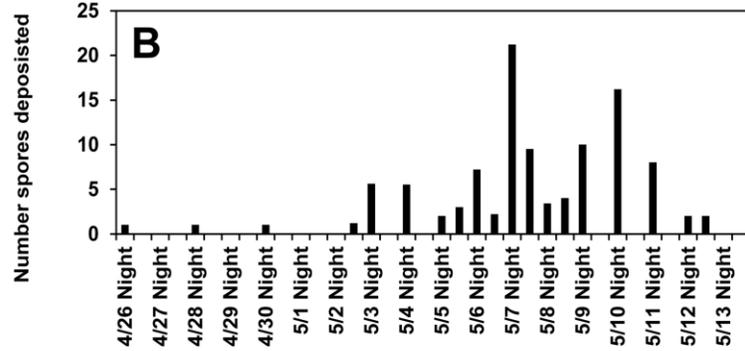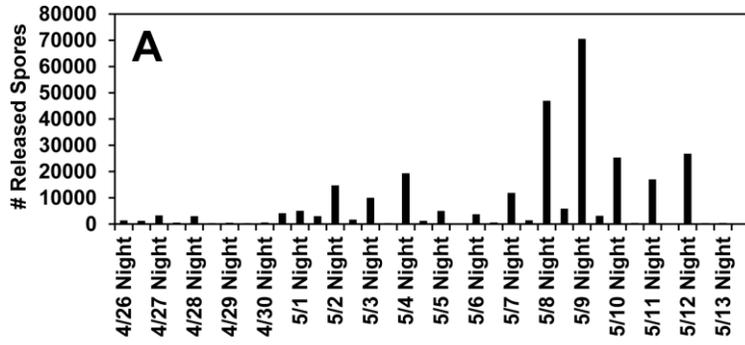
Figure 5. Comparison between the numbers of *F. graminearum* spores released and captured by the volumetric spore sampler in the center of the inoculated field (A), number of spores released from the source deposited in the field (B), number of spores predicted to be deposited using time varying spore release as the input for q_0 in the model for 2012 (C) number of spores predicted to be deposited using time varying spore release as the input for q_0 in the model for 2012 (D), and total rainfall during each sampling period (E). Spore deposition samples in the field were collected from 26 April through 14 May 2012, corresponding to perithecia being present on inoculated corn stalks. The model was run during this same time period.
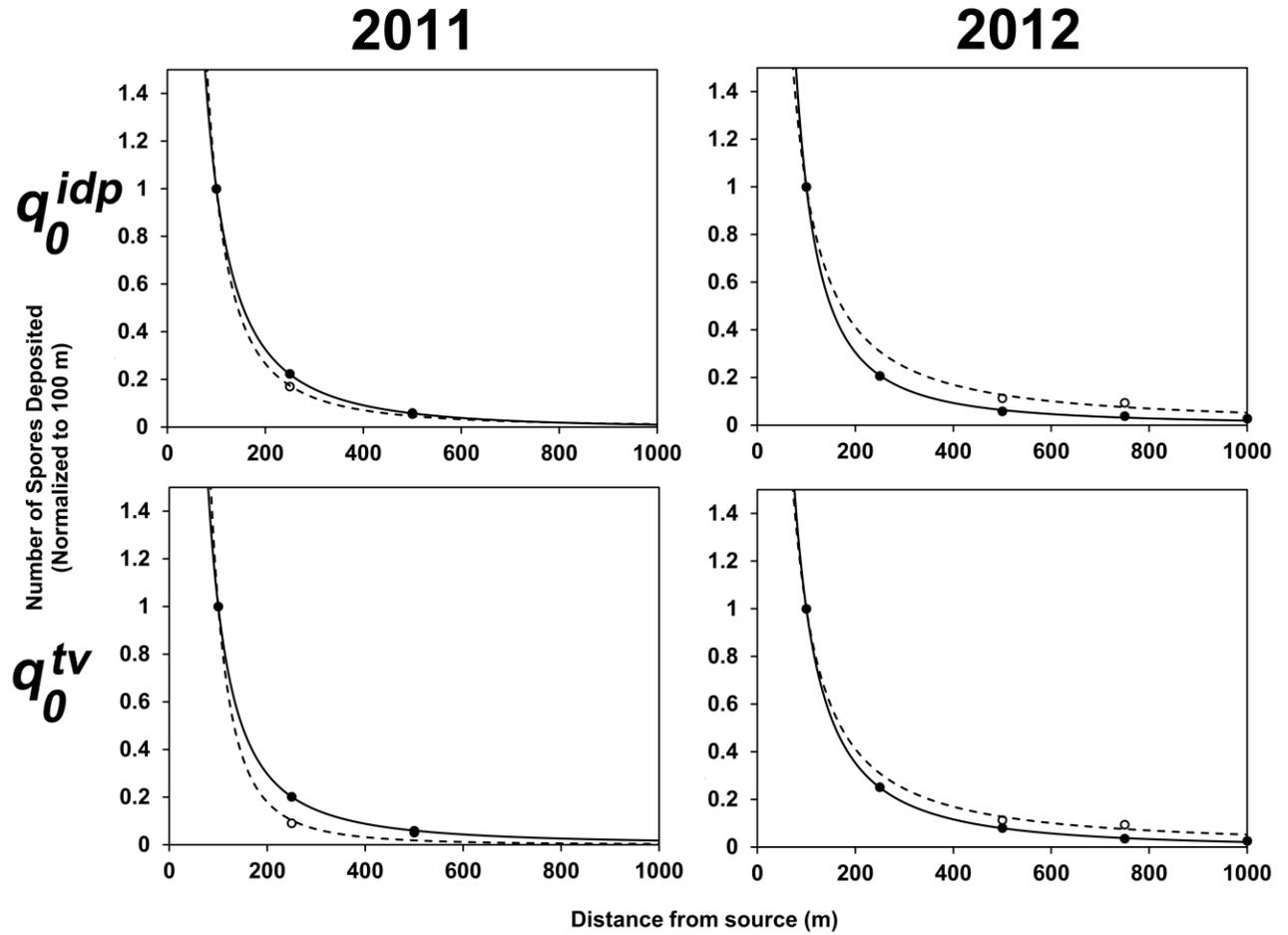
Figure 6. Dispersal kernels for *F. graminearum* spores released from a known source of inoculum predicted by the model (solid line) and observed in the field (dashed line) for 2011 (left panel) and 2012 (right panel) assuming identical daily spore release pattern q_0^{idp} (top) and time varying spore release q_0^{tv} (bottom). For the plots, total deposition, *D*, is normalized to samples collected 100 m from the source of inoculum (i.e. D(100 m)=1.) Solid circles are individual data points generated by the model at varying distances while open circles are data points collected in the field at varying distances. Dispersal kernels were created by fitting data to the nonlinear function: $D=ax^b e^{-cx}$, where *x* is distance from the center of the source of inoculum. Coefficients *a, b,* and *c* can be found in **Table 4**. Each dispersal kernel is dominated by the power law indicating dispersion is the

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dominating factor in spore transport on the scale of our experiments. In 2011 the transport model over-predicts the transport distance and 'fat' tail of the dispersal kernel, while in 2012 the transport model under-predicts the transport distance and 'fat' tail of the dispersal kernel. However, the model is considered to be a good prediction tool for spore transport, as the differences between the dispersal kernels predicted by the model and observed in the field are within an order of magnitude of each other.

Chapter 5. Conclusions and Future Directions

Fusarium head blight (FHB) caused by *Fusarium graminearum* continues to be one of the most difficult pathogens of wheat and barley for farmers to control as currently no resistant varieties are commercially available (5, 14, 25). Farmers rely on cultural practices such as tillage and crop rotations for FHB disease management (10, 16, 17, 21). Recently, an FHB disease risk assessment tool was developed which considers environmental factors such as, temperature, rainfall, and humidity, and the potential for disease development (8, 9). Farmers and growers can use this risk assessment tool to predict when FHB disease might develop and can apply control methods, such as fungicides at an appropriate time (11, 22). Although this risk assessment model is a valuable tool, there are some limitations associated with it. The current FHB risk assessment tool does not consider local sources of inoculum or *F. graminearum* spore transport and the potential for disease development. The FHB risk assessment tool can be improved by integrating a spore transport model that considers local sources of inoculum.

Universal spore transport models have been previously proposed for plant pathogens (1-4). In order to incorporate these spore transport models into the current FHB risk assessment tool they must be experimentally validated for *F. graminearum*. In order to experimentally validate them knowledge of the atmospheric transport processes of *F. graminearum* is necessary. Previous work has been conducted examining the three atmospheric transport steps, take-off and ascent, horizontal transport, and deposition, for *F. graminearum* (15, 19, 20, 23, 26-34). However, there was no knowledge of the source

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of the spores or the spore transport distance that were collected in studies examining horizontal transport and deposition (19, 20, 26-31). Additionally, to our knowledge there have been no studies tracking the complete atmospheric transport (take-off and ascent, horizontal transport, and deposition) of *F. graminearum* spores from a known field-scale source of inoculum. We experimentally validate the proposed spore transport model through tracking the atmospheric transport of *F. graminearum* spores from a known field-scale source of inoculum.

Historically, source strength has been one of the most difficult model inputs to estimate (2). We developed a method to estimate the potential source strength, Q_{pot}, of a field scale source of *F. graminearum* inoculum. Q_{pot} is the total number of *F. graminearum* spores that could potentially be released from the field and be transported long distances. However, in order to be transported long distances spores must first get out of the laminar boundary layer and into the turbulent boundary layer. The distance of the turbulent boundary layer above ground level depends on several meteorological components including solar radiation and wind speed (13). Additionally, crop canopies, such as a wheat field, can affect the distance of the turbulent boundary layer above ground level (6, 18, 24). Future work should address the fraction of spores from the Q_{pot} that are able to get out of the laminar boundary layer and into the turbulent boundary layer, as it is the latter spores that are being transported long distances. In order to determine the fraction of spores from the Q_{pot} that escape the laminar boundary layer and get into the turbulent boundary layer, the distance of the turbulent boundary layer above ground level in a wheat field will need to be calculated for a variety of environmental conditions. Previous research examining the discharge distance of *F. graminearum*

spores from perithecia can be incorporated to come up with an estimation of the fraction of spores getting into the turbulent boundary layer for any given environmental condition (27, 33).

Spore release rate, q_0, is another component related to source strength that describes how many spores are released from the source of inoculum per unit time. For our studies we estimated q_0 by placing a single volumetric spore sampler in the center of our inoculum source in 2011 and 2012 and quantified the number of spores being released on an hourly interval. We assumed that q_0 was constant and the same across the entire inoculated field as what was being observed at the location of our volumetric spore sampler; however q_0 is likely not the same across an entire field with regions of the inoculum source releasing spores at different times. The reason for different q_0 patterns across an inoculated field might be explained by the formation of microclimates and previous work has indicated that *F. graminearum* ascospore release is highly dependent on specific environmental conditions (34, 35). In order to get a better estimate of q_0, release dynamics and variability need to be studied across an entire source of inoculum. One possibility to address this would be to place a volumetric spore sampler in the center and at each corner of the inoculated field and compare patterns of q_0 at each sampling location. The spore transport model can then be reiterated to assume five point sources, with each of the locations of the volumetric spore samplers being a source of inoculum. This could provide a more accurate input for q_0 in the spore transport model.

Once *F. graminearum* spores have been released from perithecia and are in the turbulent boundary layer they are transported some distance before being deposited. We developed a unique method to track *F. graminearum* spores released and transport up to 1

km from a 3,716 m^2 artificially inoculated field and distinguish the released spores from background populations using microsatellites. To our knowledge this was the first study showing the long distance transport of *F. graminearum* from a known source of inoculum. Future work should focus on tracking spore transport even further distances from a known source of inoculum (up to 10 km) to prove very long transport events and validate the spore transport model on a larger scale. One of the main challenges associated with studying transport on this larger scale is dilution of spores in the spore plume. It is possible that *F. graminearum* spores will be transported very long distances (up to 10 km), but due to the large dilution of the spore plume recovery might be very difficult. Only a fraction of spores were sub-cultured from each plate and further analyzed for the released clone in our experimental set-up due to time and resources; however if one was to sample for transport on this larger scale it would be important to sub-culture and analyze every *Fusarium* colony collected for the released clone. As mentioned earlier, time and resources make analyzing every *Fusarium* colony collected in the field a challenge due to the multiple steps involved with processing a sample (sub-culture, single-spore, liquid culture, DNA extraction, and analysis with three microsatellites). A potential alternative to using microsatellites would be to release an isolate with a genetically modified tag that would allow a more streamline process of identifying samples as being from the released source or background population. Using a green fluorescent protein (GFP) tagged *F. graminearum* isolate would allow for a large sample size to be processed time efficiently. However, current government regulations do not allow for this type of study to be conducted in the United States. Although, recently researchers developed a DNA barcoding approach to track the atmospheric

transport of a bacterium, so it is possible government regulations could change in the future allowing GFP tagged *F. graminearum* in release-recapture studies (7, 12).

To validate the long distance spore transport model for *F. graminearum* stationary sampling sites were used in our experiments. Future experiments could use the model to predict spore transport and deposition locations in real-time. A mobile spore sampler could then be placed and moved to locations predicted to have the highest number of *F. graminearum* spores deposited that were released from the known source of inoculum. Additionally, forecasted meteorological data in conjuncture with spore transport model could be used to decide the sampling locations 24 hours prior to sampling (i.e. each day sampling locations would be moved and placed in the most probable locations for spore deposition.)

We were able to experimentally validate *F. graminearum* spore transport models through-release recapture studies. Now that the spore transport model has been validated, they should be incorporated into the current risk assessment tool for FHB (http://www.wheatscab.psu.edu/). The current FHB risk assessment tool would be improved with this incorporation, because it would include both the potential for disease development due to environmental factors as well as the number of spores that might be present at any given location. This would assist farmers and growers of wheat and barley in making more informed decisions for disease management practices.

Literature Cited

1.   Aylor, D. E. 1999. Biophysical scaling and the passive dispersal of fungus spores: relationship to integrated pest management strategies. Agricultural and Forest Meteorology 97:275-292.

2.   Aylor, D. E. 1986. A framework for examining inter-regional aerial transport of fungal spores. Agricultural and Forest Meteorology 38:263-288.

3.   Aylor, D. E., and Sutton, T. B. 1992. Release of *Venturia inaequalis* ascospores during unsteady rain: Relationship to spore transport and deposition. Phytopathology 82:532-540.

4.   Aylor, D. E., Taylor, G. S., and Raynor, G. S. 1982. Long-range transport of Tobacco blue mold spores. Agricultural Meteorology 27:217-232.

5.   Bai, G., and Shaner, G. 2004. Management and resistance in wheat and barley to Fusarium head blight 1. Annual Review of Phytopathology 42:135-161.

6.   Baines, G. 1972. Turbulence in a wheat crop. Agricultural Meteorology 10:93-105.

7.   Buckley, P., Rivers, B., Katoski, S., Kim, M. H., Kragl, F. J., Broomall, S., Krepps, M., Skowronski, E. W., Rosenzweig, C. N., and Paikoff, S. 2012. Genetic barcodes for improved environmental tracking of an anthrax simulant. Applied and Environmental Microbiology 78(23): 8272-8280.

8.   De Wolf, E. D., Madden, L. V., and Lipps, P. E. 2003. Risk assessment models for wheat Fusarium head blight epidemics based on within-season weather data. Phytopathology 93:428-435.

9.   Del Ponte, E. M., Fernandes, J. M. C., Pavan, W., and Baethgen, W. E. 2009. A model-based assessment of the impacts of climate variability on Fusarium head blight seasonal risk in southern Brazil. Journal of Phytopathology 157:675-681.

10.   Dill-Macky, R., and Jones, R. 2000. The effect of previous crop residues and tillage on Fusarium head blight of wheat. Plant Disease 84:71-76.

11.   Edwards, S., Pirgozliev, S., Hare, M., and Jenkinson, P. 2001. Quantification of trichothecene-producing *Fusarium* species in harvested grain by competitive PCR to determine efficacies of fungicides against Fusarium head blight of winter wheat. Applied and Environmental Microbiology 67:1575-1580.

12.   Emanuel, P. A., Buckley, P. E., Sutton, T. A., Edmonds, J. M., Bailey, A. M., Rivers, B. A., Kim, M. H., Ginley, W. J., Keiser, C. C., and Doherty, R. W. 2012. Detection and tracking of a novel genetically-tagged biological simulant in the environment. Applied and Environmental Microbiology 78(23): 8281-8288.

13.   Gregory, P. H. 1961. The microbiology of the atmosphere. Leonard Hill.

14.   Haber, S., Gilbert, J., Fox, S., and Robinson, S. 2012. Getting Fusarium head blight (FHB) resistance where it counts, in existing commercial cultivars. Page 60 in: 2012 National Fusarium Head Blight Forum.

15.   Isard, S. A., Gage, S. H., Comtois, P., and Russo, J. M. 2005. Principles of the atmospheric pathway for invasive species applied to Soybean rust. Bioscience 55:851-861.

16.   Keller, M. D., Thomason, W. E., and Schmale III, D. G. 2011. The spread of a released clone of *Gibberella zeae* from different amounts of infested corn residue. Plant Disease 95:1458-1464.

17.   Keller, M. D., Waxman, K. D., Bergstrom, G. C., and Schmale III, D. G. 2010. Local distance of wheat spike infection by released clones of *Gibberella zeae* disseminated from infested corn residue. Plant Disease 94:1151-1155.

18.   Lawson, T., and Uk, S. 1979. The influence of wind turbulence, crop characteristics and flying height on the dispersal of aerial sprays. Atmospheric Environment (1967) 13:711-715.

19.   Maldonado-Ramirez, S. L. 2001. Aerobiology of the wheat scab fungus, *Gibberella zeae*: discharge, atmospheric dispersal, and deposition of ascospores. Ph. D. thesis, Cornell University, Ithaca, NY.

20.   Maldonado-Ramirez, S. L., Schmale III, D. G., Shields, E. J., and Bergstrom, G. C. 2005. The relative abundance of viable spores of *Gibberella zeae* in the planetary boundary layer suggests the role of long-distance transport in regional epidemics of Fusarium head blight. Agricultural and Forest Meteorology 132:20-27.

21.   McMullen, M., Halley, S., Schatz, B., Meyer, S., Jordahl, J., and Ransom, J. 2008. Integrated strategies for Fusarium head blight management in the United States. Cereal Research Communications 36:563-568.

22.   Mesterhazy, A., Bartok, T., and Lamper, C. 2003. Influence of wheat cultivar, species of *Fusarium*, and isolate aggressiveness on the efficacy of fungicides for control of Fusarium head blight. Plant Disease 87:1107-1115.

23.   Paulitz, T. C. 1996. Diurnal release of ascospores by *Gibberella zeae* in inoculated wheat plots. Plant Disease 80:674-678.

24. Raupach, M., and Thom, A. 1981. Turbulence in and above plant canopies. Annual Reviews of Fluid Mechanics 13:97-129.

25. Schaafsma, A., Ilinic, L. T.-., Miller, J., and Hooker, D. 2001. Agronomic considerations for reducing deoxynivalenol in wheat grain. Canadian Journal of Plant Pathology 23:279-285.

26. Schmale, D., Ross, S., Fetters, T., Tallapragada, P., Wood-Jones, A., and Dingus, B. 2012. Isolates of Fusarium graminearum collected 40–320 meters above ground level cause Fusarium head blight in wheat and produce trichothecene mycotoxins. Aerobiologia 28:1-11.

27. Schmale, D. G., Arntsen, Q. A., and Bergstrom, G. C. 2005. The forcible discharge distance of ascospores of *Gibberelia zeae*. Canadian Journal of Plant Pathology 27:376-382.

28. Schmale III, D. G., and Bergstrom, G. C. 2004. Spore deposition of the ear rot pathogen, *Gibberella zeae*, inside corn canopies. Canadian Journal of Plant Pathology 26:591-595.

29. Schmale III, D. G., Bergstrom, G. C., and Shields, E. J. 2006. Night-time spore deposition of the Fusarium head blight pathogen, *Gibberella zeae*, in rotational wheat fields. Canadian Journal of Plant Pathology 28:100-108.

30. Schmale III, D. G., Leslie, J. F., Zeller, K. A., Saleh, A. A., Shields, E. J., and Bergstrom, G. C. 2006. Genetic structure of atmospheric populations of *Gibberella zeae*. Phytopathology 96:1021-1026.

31.   Schmale III, D. G., Shah, D. A., and Bergstrom, G. C. 2005. Spatial patterns of viable spore deposition of *Gibberella zeae* in wheat fields. Phytopathology 95:472-479.

32.   Trail, F., and Common, R. 2000. Perithecial development by *Gibberella zeae*: a light microscopy study. Mycologia:130-138.

33.   Trail, F., Gaffoor, I., and Vogel, S. 2005. Ejection mechanics and trajectory of the ascospores of *Gibberella zeae* (anamorph *Fuarium graminearum*). Fungal Genetics and Biology 42:528-533.

34.   Trail, F., Xu, H., Loranger, R., and Gadoury, D. 2002. Physiological and environmental aspects of ascospore discharge in *Gibberella zeae* (anamorph *Fusarium graminearum*). Mycologia 94:181-189.

35.   Tschanz, A., Horst, K., and Nelson, P. E. 1975. Ecological aspects of ascospore discharge in *Gibberella zeae*. Phytopathology 65:597-599.

APPENDIX A

Fraction of released spores from a source of inoculum recovered
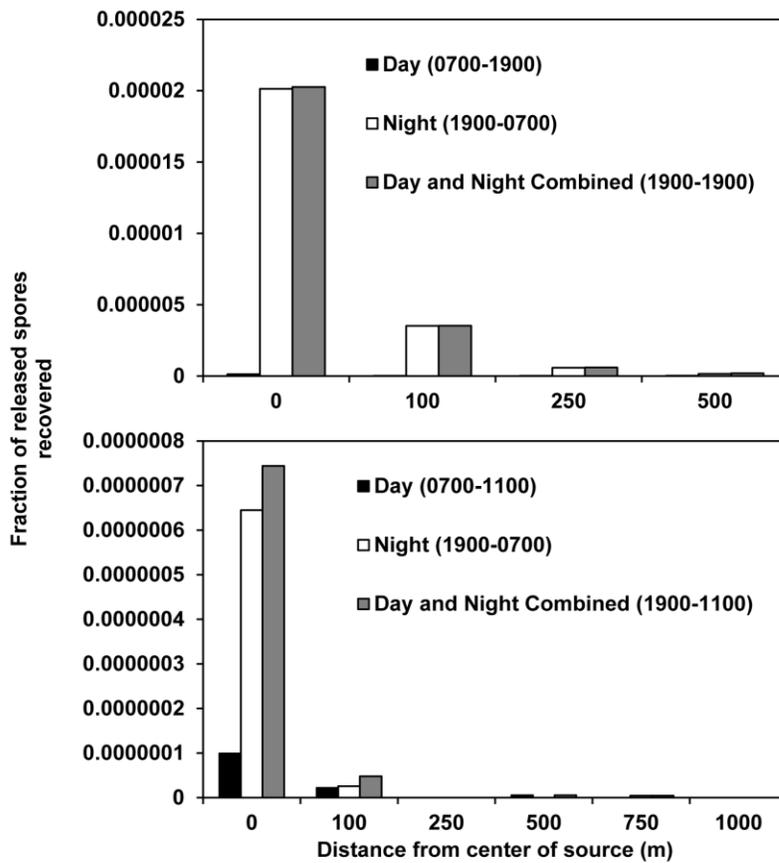
Figure 1. Fraction of released spores from a known source of inoculum recovered during the 2011 (top) and 2012 (bottom) field seasons. In 2011 spores we attempted to recover spores 0, 100, 250, and 500 m from the source of inoculum. In 2012, sampling also included locations 750 and 1000 m from the source of inoculum. The number of spores released was assumed to be 3.7 billion ascospores, based on data presented in chapter 2. The data was normalized based on the number of sampling locations at each distance. A general trend of a decrease in the number of spores recovered was observed as distance from the source of inoculum increased.