The Contribution of Within-Field Inoculum Sources of *Gibberella zeae* to Fusarium Head Blight in Winter Wheat and Barley

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ABSTRACT

Fusarium head blight (FHB) is one of the most economically important diseases of small grains and continues to impact crops when environmental conditions are favorable to *Gibberella zeae* (*Fusarium graminearum*), the causal agent of the disease. Corn residues are considered to be primary sources of inoculum for epidemics of FHB. Therefore, knowledge of the movement of *Gibberella zeae* from a local source of infested corn residue is critical to the management of FHB in wheat and barley. Previous research made significant progress in defining the spatial dissemination of inoculum sources of *G. zeae* within agricultural fields, but was unable to clearly distinguish between within-field and background sources. Using amplified fragment length polymorphism, released clones of *G. zeae* were tracked within wheat and barley fields. This strategy allowed the distinction between the contributions of released clones to FHB, compared to that of background inocula. Corn residue infested with clones of *G. zeae* was placed into small replicated plots in winter wheat fields in New York and Virginia in 2007 and 2008 and wheat spikes were collected at 0, 3, 6, and ≥24 m from the inoculum sources. Recovery of released clones decreased an average of 90% between 3 and 6 m from inoculum sources. Various amounts of corn residue infested with a single clone of *G. zeae* were placed into small replicated plots in winter wheat and barley fields in Virginia from 2008 to 2010. The use of minimal or conventional tillage and a moderately resistant cultivar of

wheat or barley may reduce the contribution of within-field inocula to FHB; however, environmental conditions play an important role in the effectiveness of these management strategies. With the increase of corn production due to incentives for ethanol-based fuel, overwintering sites for *G. zeae* on corn residue are likely to increase. Our work contributes to an increased understanding of the influence of overwintered corn residue to FHB which will also direct future research on how to reduce the inoculum potential from within-field sources.

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Attributions

Several colleagues have assisted with the completion of the research in chapters of this dissertation. The following is a brief description of their contributions.

Gary Bergstrom, Department of Plant Pathology and Plant-Microbe Biology, Cornell University

As a co-author of the publication represented by Chapter 2, Dr. Bergstrom provided partial funding for the research and expert advice and guidance throughout the research process and publication of the results.

Carl Griffey, Crop and Soil Environmental Sciences Department, Virginia Tech

As a co-author of the publication represented in Chapter 4, Dr. Griffey provided the photo for Figure 4 and editing advice.

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As a co-author of the publication represented in Chapter 4, Dr. Lin provided information for Table 2 and editing advice.

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Dr. Schmale served as the major adviser and provided funding and guidance during the implementation and completion of the research project and dissertation. He is listed as co-author of publications represented in Chapters 2, 3, and 4.

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As a co-author of the publication represented in Chapter 4, Dr. Stromberg provided content and editing advice.

Wade Thomason, Crop and Soil Environmental Science Department, Virginia Tech

As a co-author of the submitted publication represented in Chapter 3, Dr. Thomason provided field knowledge and advice as well as content and editing advice. As a co-author of the publication represented in Chapter 4, Dr. Thomason provided content and editing advice.

Katrina Waxman, Department of Plant Pathology and Plant-Microbe Biology, Cornell University

As a co-author of the publication represented in Chapter 2, Ms. Waxman provided technical support for our field experiments in New York and editing advice.

Chapter 1. Introduction

Fusarium head blight (FHB), also referred to as scab, continues to be one of the most challenging obstacles to producers of small grains. This fungal disease has plagued North America since the first recorded reports in the late nineteenth century (57) and has continued into the twenty-first century. FHB has been named the worst plant disease to affect the United States since the 1950s stem rust epidemic (70) with losses exceeding $3 billion since 1996 (36). Research published from experiments in Russia (33,71), Australia (10,53), China (65,74), and Brazil (17,18) indicates the importance of FHB in other parts of the world. Species of *Fusarium* including *Fusarium avenaceum*, *Fusarium culmorum*, and *Fusarium poae* can cause FHB; however, *Fusarium graminearum sensu stricto* (Schwabe) is still considered the predominant causal agent of FHB in North America (36,61). It is also known by the name of its sexual stage *Gibberella zeae* (Schwein.) Petch.

Economically important hosts of FHB include wheat (*Triticum aestivum*), durum wheat (*Triticum durum*), and barley (*Hordeum vulgare*). The term Fusarium (Gibberella) ear rot is used for *G. zeae* infection of corn (*Zea mays*). Wheat, barley, and corn residue are regarded as the three primary inoculum sources for FHB epidemics (44,54,61). *G. zeae* and its host will have a short-lived biotrophic relationship and conclude with a necrotrophic relationship (26). The fungus survives saprophytically on host residue and overwintered residue contributes a source of inoculum for infection of wheat and barley plants in the same field in which residue is present (22,61,67,69) and in more distant fields (25,31). *G. zeae* is an ascomycete that produces sexual spores called ascospores and asexual spores called macroconidia (32,54). Spore-containing structures called

perithecia form on residue remaining on the soil surface, and in the late spring and early summer release ascospores that can be deposited on wheat spikes (36). Macroconidia are produced in pink or orange spore masses called sporodochia (61,67).

Both ascospores and macroconidia of *G. zeae* contribute to FHB (32); though it is considered that airborne ascospores play the predominant role (54) in infection of wheat and barley florets from spike emergence through anthesis (61) and possibly through grain fill (12,13). Ascospore production has been observed on residue after two or more years (48). *G. zeae* is homothallic, and by definition is able to sexually reproduce without a partner (7). Heterothallic isolates are less common, but sexual recombination has been presented (7,72), but has not been demonstrated under field conditions (72).

Favorable environmental conditions such as frequent rainfall and high relative humidity enhance inoculum production on residue and resulting disease development (23,47). Extended periods (i.e., 48 to 72 hours) at greater than 90% relative humidity with temperatures between 15 and 30°C facilitate infection (20). Continued wet conditions after flowering may increase the potential for spores (14) to be windblown (31) or water-splashed (45) onto nearby spikes.

Resistance to FHB is the result of multiple traits in wheat and barley cultivars. Type I resistance is described as resistance to initial infection (4). Initial infection is determined by the percentage of spikes or spikelets (i.e., florets) infected. Type II resistance is described as resistance to spread from spikelet to spikelet within the spike (4). Resistant cultivars may have the capability to degrade the mycotoxin deoxynivalenol (DON) and this resistance has been designated as Type III resistance by Wang and Miller

(66). Type IV resistance is described as resistance to kernel infection and Type V resistance is tolerance to yield loss and quality regardless of FHB severity (38).

Symptoms of FHB typically appear near the middle of the wheat or barley spike and progress through the rest of the spike (9). In wheat, infested spikes will typically appear prematurely bleached (61,67). In barley, brown, chlorotic, or water-soaked lesions may be visible on outer florets (29). The infection process of *G. zeae* has been investigated using the green fluorescent protein gene (gfp) and Affymetrix GeneChips (28,41,55,59). Phases of infection include: i) a hyphal mat formed at the point of infection after spore germination, ii) mycelial growth to the crown, and iii) mycelial growth through internal crown tissues (59). Perithecia formation occurs near stomatal openings and silica cells in the presence of light (28). As FHB infection progresses, developing kernels may appear smaller than normal, shriveled, and white/pink in color and may contain mycotoxins produced by *G. zeae* (36).

Mycotoxins such as diacetoxyscirpenol, zearalenone, HT-2 toxin, T-2 toxin, nivalenol, and 3-ADON and 15-ADON acetylated forms of DON are commonly produced in *Fusarium*-infected wheat and barley (1,52). DON is considered to be the most economically important toxin produced by *G. zeae* (16) and has been shown to be a virulence factor in FHB (34). The United States Food and Drug Administration recommended tolerance levels for DON are 1 part per million in finished grain products for human consumption, and levels in grain or grain by-products for animal consumption are dependent upon the type of animal (2). Adverse health effects are possible in humans (50,51) and animals (49) if contaminated grain is consumed. Marketability of infected

wheat and barley grain decreases due to low test weight and unacceptable DON levels (3,36).

For the past two decades FHB has caused epidemics that have been devastating to many producers of wheat and barley (14,26,36,68). The severity of these epidemics has forced researchers to reevaluate the current understanding of the epidemiology and management strategies of FHB. Dill-Macky and Jones (22) found lower FHB incidence and severity with tillage treatments involving a moldboard plow than in those using a chisel plow or no tillage implement. They also found wheat planted after corn showed the highest FHB infection when compared to wheat planted after soybean (22). Nita et al. (42) evaluated FHB incidence, FHB severity, and DON concentration from field experiments in 2003 and 2004. The risk of FHB increased with greater concentrations of inoculum from within-field sources when environmental conditions were moderately favorable for disease. In both greenhouse and field experiments, Stein et al. (58) found that disease incidence and severity increased with an increasing number of *G. zeae* conidia applied. DON levels were also found to be positively related to inoculum concentration (58).

FHB management strategies include, but are not limited to, planting a moderately resistant small grains cultivar, fungicide, crop rotation, planting date coordination, biological control, and residue tillage. No completely resistant cultivar is available to small grains producers; however, breeding research is actively attempting to find genes to improve FHB resistance (11,73) and improved moderately resistant cultivars have recently been introduced (8,27,37). Fungicides are available and can minimize FHB effects (46), but are disputed as an economically beneficial choice for management (36).

A multi-state disease forecasting model is available for wheat producers to aid in the decision for fungicide application (20). No disease forecasting model is currently available for barley producers. Wheat and barley planted after non-host crops may reduce FHB infection (22). Staggering the planting dates of cultivars may avoid favorable conditions at the time of flowering for one cultivar but not another (60). Biological control agents have been identified and observed to reduce FHB (15,43) and with more research, may be more widely used.

Reduced tillage practices have been used for the past twenty-five years (36). The use of tillage in crop production is important for seedbed preparation, incorporation of nutrients, weed control, and pathogen management, but may also cause environmental problems including soil erosion and moisture loss (30). Corn residue remaining in a no-tillage or a minimal tillage system may provide overwintering sites for *G. zeae*. With the increase of corn production due to incentives for ethanol-based fuel, overwintering sites for *G. zeae* on corn residue are likely to increase (21). In regions of the U.S. where predominantly winter wheat is planted, FHB management decisions must be made prior to fall planting, and therefore, also prior to forecasting for environmental conditions favorable for FHB. Although no-tillage, minimal tillage, and conventional tillage practices are used in Virginia, there is an increase in no-tillage small grain production with recommended goals of 75 to 90% residue coverage (63). McMullen et al. (35) reported lower field FHB severity and DON levels when multiple management strategies were implemented rather than a single strategy. For winter wheat, spring wheat, and durum wheat grown in North Dakota, the planting of a moderately resistant cultivar reduced FHB levels when combined with another management strategy (e.g., fungicide or

crop rotation) (35). Miller et al. (40) reported tillage practices were less likely to reduce FHB levels than planting a moderately resistant cultivar. Regardless of management strategy utilized by wheat and barley growers, environmental conditions conducive for FHB may still negatively impact disease levels (25).

The reduction of *G. zeae*-infested residue within wheat and barley fields prior to planting may reduce the impact of future FHB epidemics (22,62); however, with the move away from conventional tillage practices (21), research is needed to better understand the influence of the remaining corn residue if alternative solutions are to be found. Dissemination from sources of *G. zeae* inoculum has been reported using a released clone with a unique phenotype (19,24,56). de Luna et al. (19) observed a 50% decline in concentration of ascospores within 18 m and 90% within 60 m of an inoculated source. Fernando et al. (24) observed a 50% decline in FHB infection within 10 m and 90% within 5 to 22 m of inoculated sources. Stack (56) observed a 50% decline in FHB infection within 2 to 3 m of inoculated sources. Previous research has not unambiguously distinguished released inocula from background (i.e., atmospheric) sources of *G. zeae*.

Amplified fragment length polymorphism (AFLP) (64) has been used to study populations of other plant pathogens (5,6) and the technique is expected to persist for years to come due to its adaptability (39). Current literature has not addressed the use of molecular techniques to study dissemination from different amounts of within-field inoculum sources.

Knowledge of the contribution of within-field inocula compared to that of background (i.e., atmospheric) inocula and dissemination of *G. zeae* from within-field inocula is critical to FHB management strategies. McMullen et al. (36) and Dill-Macky

and Jones (22) discuss the importance of within-field inoculum sources to FHB and management of these sources as a way to reduce FHB. With the addition to the literature of research by Maldonado-Ramirez et al. (31), the importance of atmospheric sources of *G. zeae* was recognized and FHB management strategies were reevaluated. A better understanding of the contributions of both within-field and atmospheric inoculum sources to FHB are necessary for future management strategies.

Research Objectives

1. Determine the contribution of within-field, clonal inoculum sources of *G. zeae* to FHB in wheat,

2. Evaluate the influence of amount of corn residues and disease resistance on FHB in Virginia wheat and barley,

3. Survey wheat and barley fields in Virginia for potential inoculum sources (e.g. crop residues) of *G. zeae*, and

4. Develop a tool to communicate current research in FHB management to Virginia extension agents, and ultimately to Virginia growers.

Literature Cited

1. Ambramson, D., Clear, R.M., and Nowicki, T.W. 1987. *Fusarium* species and trichothecene mycotoxins in suspect samples of 1985 Manitoba wheat. Can. J. Plant Sci. 67:611-619.
2. Anonymous. 2010. Guidance for Industry and FDA: Advisory Levels for Deoxynivalenol (DON) in Finished Wheat Products for Human Consumption and Grains and Grain By-Products used for Animal Feed. U.S. Department of Health and Human Services. Food and Drug Administration. Accessed online (July 16, 2010).
3. Bai, G., and Shaner, G. 1994. Scab of wheat: Prospects for control. Plant Dis. 78:760-766.
4. Bai, G., and Shaner, G. 2004. Management and resistance in wheat and barley to Fusarium head blight. Annu. Rev. Phytopathol. 42:135-161.
5. Bayon, C., Pei, M.H., Ruiz, C., Hunter, T., Karp, A., and Tubby, I. 2009. Genetic structure and population dynamics of a heteroecious plant pathogen *Melampsora larici-epitea* in short-rotation coppice willow plantations. Mol. Ecol. 18:3006-3019.
6. Bennett, R.S., Milgroom, M.G., Sainudiin, R., Cunfer, B.M., and Bergstrom, G.C. 2007. Relative contribution of seed-transmitted inoculum to foliar populations of *Phaeosphaeria nodorum*. Phytopathology 97:584-591.
7. Bowden, R.L., and Leslie, J.F. 1999. Sexual recombination in *Gibberella zeae.* Phytopathology 89:182-188.

8. Brooks, W.S., Vaughn, M.E., Griffey, C.A., Thomason, W.E., Paling, J.J., Pitman, R.M., Dunaway, D.W., Corbin, R.A., Kenner, J.C., Hokanson, E.G., Behl, H.D., Beahm, B.R., Liu, S.Y., Gundrum, P.G., Price, A.M., Brann, D.E., Whitt, D.L., Custis, J.T., Starner, D.E., Gulick, S.A., Ashburn, S.R., Jones Jr., E.H., Marshall, D.S., Fountain, M.O., Tuong, T.D., Livingston, D.P., Premakumar, R., Kurantz, M.J., Taylor, F., Moreau, R.A., and Hicks, K.B. 2011. Registration of 'Dan' winter hulless barley. J. of Plant Reg. 5:1-4.
9. Bushnell, W.R. 2003. Histology and physiology of Fusarium head blight. Pages 44-83 in: Fusarium Head Blight of Wheat and Barley. K.J. Leonard and W.R. Bushnell, eds. American Phytopathological Society, St. Paul, MN.
10. Chakraborty, S., Obanor, F., Westecott, R., and Abeywickrama, K. 2010. Wheat crown rot pathogens *Fusarium graminearum* and *F. pseudograminearum* lack specialization. Phytopathology. 100:1057-1065.
11. Chen, X., Faris, J., Hu, J., Stack, R., Adhikari, T., Elias, E., Kianian, S., and Cai, X. 2007. Saturation and comparative mapping of a major Fusarium head blight resistance QTL in tetraploid wheat. Mol. Breeding 19:113-124.
12. Cowger, C., and Arrellano, C. 2010. Plump kernels with high deoxynivalenol linked to late *Gibberella zeae* infection and marginal disease conditions in winter wheat. Phytopathology 100:719-728.
13. Cowger, C., Patton-Özkurt, J., Brown-Guedira, G., and Perugini, L. 2009. Post-anthesis moisture increased Fusarium head blight and deoxynivalenol levels in North Carolina winter wheat. Phytopathology 99:320-327.

14. Cowger, C., and Sutton, A.L. 2005. The southeastern U.S. Fusarium head blight epidemic of 2003. Published Online. Plant Health Progress DOI:10.1094/PHP-2005-1026-01-RS.

15. Crane, J.M., Gibson, D.M., and Bergstrom, G.C. 2010. Ecology of *Bacillus Amyloliquefaciens* on wheat florets in relation to biological control of FHB/DON. Page 77 in: Proc. Natl. Fusarium Head Blight Forum, Milwaukee, WI.

16. Culler, M.D., Miller-Garvin, J.E., and Dill-Macky, R. 2007. Effect of extended irrigation and host resistance on deoxynivalenol accumulation in *Fusarium*-infected wheat. Plant Dis. 91:1464-1472.

17. Del Ponte, E.M., Fernandes, J.M.C., and Bergstrom, G.C. 2007. Influence of growth stage on Fusarium head blight and deoxynivalenol production in wheat. J. Phytopathology 155:577-581.

18. 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 Brazil. J. Phytopathol. 157:675-681.

19. de Luna, L., Bujold, I, Carisse, O, and Paulitz, T.C. 2002. Ascospore gradients of *Gibberella zeae* from overwintered inoculum in wheat fields. Can. J. Plant Pathol. 24:457-464.

20. 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.

21. Dill-Macky, R. 2008. Cultural control practices for Fusarium head blight: Problems and solutions. 3rd Int. Fusarium Symposium. Szeged, Hungary.

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

23. 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. Can. J. Bot. 78:497-505.

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

25. Francl, L., Shaner, G., Bergstrom, G., Gilbert, J., Pedersen, W., Dill-Macky, R., Sweets, L., Corwin, B., Jin, Y., Gallenberg, D., and Wiersma, J. 1999. Daily inoculum levels of *Gibberella zeae* on wheat spikes. Plant Dis. 83:662-666.

26. Goswami, R.S., and Kistler, H.C. 2004. Heading for disaster: *Fusarium graminearum* on cereal crops. Mol. Plant Pathol. 5:515-525.

27. Griffey, C.A., Thomason, W.E., Pitman, R.M., Beahm, B.R., Gundrum, P.G., Liu, S.Y., Chen, J., Paling, J.J., Dunaway, D.W., Brooks, W.S., Vaughn, M.E., Seago, J.E., Will, B.C., Hokanson, E.G., Behl, H.D., Corbin, R.A., Lewis, T.R., Hall, M.D., Custis, J.T., Starner, D.E., Gulick, S.A., Ashburn, S.R., Whitt, D.L., Bockelman, H.E., Murphy, J.P., Navarro, R.A., Souza, E.J., Brown-Guedira, G.L., Kolmer, J.A., Long, D.L., Jin, Y., Chen, X., and Cambron, S.E. 2011. Registration of 'SW049029104' wheat. J. of Plant Reg. 5:91-97.

28. Guenther, J.C., and Trail, F. 2005. The development and differentiation of *Gibberella zeae* (anamorph: *Fusarium graminearum*) during colonization of wheat. Mycologia 97:229-237.

29. Lewandowski, S. and Bushnell, W.R. 2001. Development of *Fusarium graminearum* on floret surfaces of field-grown barley. Page 128 in: Proc. 2001 Natl. Fusarium Head Blight Forum, Erlanger, KY.

30. Lobb, D.A., Huffman, E., and Reicosky, D.C. 2007. Importance of information on tillage practices in the modeling of environmental processes and in the use of environmental indicators. Journal of Env. Manag. 82:377-387.

31. Maldonado-Ramirez, S.L., Schmale, 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. Agric. For. Meteorol. 132:20-27.

32. Markell, S.G., and Francl, L.J. 2003. Fusarium head blight inoculum: Species prevalence and *Gibberella zeae* spore type. Plant Dis. 87: 814-820.

33. Martynov, S.P. and Dobrotvorskaya, T.V. 2006. Geneological analysis of resistance to Fusarium head blight in Russian and Ukrainian cultivars of common wheat *Triticum aestivum* L. Rus. J. of Genetics. 42:905-914.

34. McCormick, S. 2003. The role of DON in pathogenicity. Pages 165-183 in: Fusarium Head Blight of Wheat and Barley. K.J. Leonard and W.R. Bushnell, eds. American Phytopathological Society, St. Paul, MN.

35. McMullen, M.P., Halley, S., Schatz, B., Meyer, S., Jordahl, J., and Ransom, J. 2008. Integrated strategies for Fusarium head blight management in the United States. 3rd Int. FHB Symposium. Szeged, Hungary.

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

37. Mergoum, M., Simsek, S., Frohberg, R., Rasmussen, J., Friesen, T., and Adhikari, T. 2011. 'Barlow': A high-quality and high-yielding hard red spring wheat cultivar adapted to the north central plains of the USA. J. of Plant Reg. 5:62-67.

38. Mesterházy, Á., Bartók, T., Mirocha, C.G., and Komoróczy, R. 1999. Nature of wheat resistance to Fusarium head blight and the role of deoxynivalenol for breeding. Plant Breeding 118:97-110.

39. Meudt, H.M., and Clark, A.C. 2007. Almost forgotten or latest practice? AFLP applications, analyses, and advances. Trends in Plant Sci. 12:106-117.

40. Miller, J.D., Culley, J., Fraser, K., Hubbard, S., Meloche, F. Ouellet, T., Seaman, W.L., Seifert, K.A., Turkington, K., and Voldeng, H. 1998. Effect of tillage practice on Fusarium head blight of wheat. Can. J. Plant Pathol. 20:95-103.

41. Miller, S.S., Chabot, D.M.P., Ouellet, T., Harris, L., and Fedak, G. 2004. Use of a *Fusarium graminearum* strain transformed with green fluorescent protein to study infection in wheat (*Triticum aestivum*). Can. J. Plant Pathol. 26:453-463.

42. Nita, M., De Wolf, E., Madden, L., Paul, P., Shaner, G., Adhikari, T., Ali, S., Stein, J., and Osborne, L. 2006. Effect of corn residue level on disease intensity of Fusarium head blight (FHB) and on deoxynivalenol (DON) concentration: A mulit-state field study. (Abstr.) Phytopathology 96:S85.

43. Palazzini, J.M., Ramirez, M.L., Alberione, E.J., Torres, A.M., and Chulze, S.N. 2009. Osmotic stress adaptation, compatible solutes accumulation, and biocontrol efficacy of two potential biocontrol agents on Fusarium head blight in wheat. Biol. Control 51:370-376.

44. Parry, D.W., Jenkinson, P., and McLeod, L. 1995. *Fusarium* ear blight (scab) in small grain cereals-a review. Plant Pathol. 44:207-238.

45. Paul , P.A., El-Allaf, S.M., Lipps, P.E., and Madden, L.V. 2004. Rain splash dispersal of *Gibberella zeae* within wheat canopies in Ohio. Phytopathology 94:1342-1349.

46. Paul, P.A., McMullen, M.P., Hershman, D.E., and Madden, L.V. 2010. Meta-analysis of the effects of triazole-based fungicides on wheat yield and test weight as influenced by Fusarium head blight intensity. Phytopathology 100:160-171.

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

48. Pereyra, S.A., Dill-Macky, R., and Sims, A.L. 2004. Survival and inoculum production of *Gibberella zeae* in wheat residue. Plant Dis. 88:724-730.

49. Pestka, J.J. 2007. Deoxynivalenol: Toxicity, mechanisms and animal health risks. Anim. Feed Sci. and Tech. 137:283-298.

50. Pestka, J.J. 2010. Deoxynivalenol: mechanisms of action, human exposure, and toxilogical relevance. Arch Toxicol. 84:663-679.

51. Pestka, J.J, and Smolinski, A.T. 2005. Deoxynivalenol: Toxicology and potential effects on humans. J. Toxicol. Environ. Health B Crit. Rev. 8:39-69.

52. Salas, B., Steffanson, B.J., Casper, H.H., Tacke, B., Prom, L.K., Fetch, T.G., Jr., and Schwarz, P.B. 1999. *Fusarium* species pathogenic to barley and their associated mycotoxins. Plant Dis. 83:667-674.

53. Scott, J.B. and Chakraborty, S. 2010. Genotypic diversity in *Fusarium pseudograminearum* populations in Australian wheat fields. Plant Pathol. 59:338-347.

54. Shaner, G.E. 2003. Epidemiology of Fusarium head blight of small grain cereals in North America. Pages 84-119 in: Fusarium Head Blight of Wheat and Barley. K.J. Leonard and W.R. Bushnell, eds. American Phytopathological Society, St. Paul, MN.

55. Skadsen, R.W., and Hohn, T.M. 2004. Use of *Fusarium graminearum* transformed with gfp to follow infection patterns in barley and *Arabidopsis*. Phys. and Mol. Plant Pathol. 64:45-53.

56. Stack, R.W., 1997. Gradients of Fusarium head blight in wheat along transects away from a concentrated source of *Gibberella zeae* ascospore inoculum. Page 60 in: Proc. Natl. Fusarium Head Blight Forum, St. Paul, MN.

57. Stack, R.W. 2003. History of Fusarium head blight with emphasis on North America. Pages 1-34 in: Fusarium Head Blight of Wheat and Barley. K.J. Leonard and W.R. Bushnell, eds. The American Phytopathological Society Press, St. Paul, MN.

58. Stein, J.M., Osborne, L.E., Bondalapati, K.D., Glover, K.D., and Nelson, C.A. 2009. Fusarium head blight severity and deoxynivalenol concentration in wheat in response to *Gibberella zeae* inoculum concentration. Phytopathology 99:759-764.

59. Stephens, A.E., Gardiner, D.M., White, R.G., Munn, A.L., and Manners, J.M. 2008. Phases of infection and gene expression of *Fusarium graminearum* during crown rot disease of wheat. Mol. Plant-Microbe Interact. 21:1571-1581.

60. Subedi, K.D., Ma, B.L., and Xue, A.G. 2007. Planting date and nitrogen effects on Fusarium head blight and leaf spotting diseases in spring wheat. Agron. J. 99:113-121.

61. Sutton, J.C. 1982. Epidemiology of wheat head blight and maize ear rot caused by *Fusarium graminearum*. Can. J. Pathol. 4:195-209.

62. Teich, A.H., and Hamilton, J.R. 1985. Effect of cultural practices, soil phosphorus, potassium, and pH on the incidence of Fusarium head blight and deoxynivalenol levels in wheat. Appl. and Env. Micro. 49:1429-1431.

63. Thomason, W.E., Alley, M.M., Stromberg, E.L., Hagood, E.S., Herbert, A. 2009. No-tillage small grain production in Virginia. 424-005. Virginia Cooperative Extension.

64. Vos, P., Hogers, R., Bleeker, M., Reijans, M., van de Lee, T., Hornes, M., Frijters, A., Pot, J., Peleman, J., Kuiper, M., and Zabeau, M. 1995. AFLP: A new technique for DNA fingerprinting. Nucleic Acids Res. 23:4407-4414.

65. Wang, X., Cui, Y., Fan, F., Song, Y., Ren, J., Meng, Q., Xu, W., and Jiang, L. 2010. Phylogenetic, carbendazim sensitivity and mycotoxin genotype analyses of *Fusarium graminearum* complex species isolated from wheat Fusarium head blight in China. J. Phytopathol. 158:576-578.

66. Wang, Y.Z., and Miller, J.D. 1988. Effects of *Fusarium graminearum* metabolites on wheat tissue in relation to Fusarium head blight resistance. J. Phytopathol. 122:118-125.

67. Wiese, M.V. 1987. Compendium of wheat diseases. American Phytopathological Society, St. Paul, MN.

68. 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.

69. Windels, C.E., and Kommedahl, T. 1984. Late-season colonization and survival of *Fusarium graminearum* group II in cornstalks in Minnesota. Plant Dis. 68:791-793.

70. Wood, M., Comis, D., Harden, D., McGraw, L., and Stelljes, K.B. 1999. Fighting Fusarium. Agricultural Research. June issue. United States Department of Agriculture, Agricultural Research Service, Beltsville, MD.

71. Yli-Mattila, T., Gagkaeva, T., Ward, T., Aoki, T., Kistler, H.C., and O'Donnell, K. 2009. A novel Asian clade within the *Fusarium graminearum* species complex includes a newly discovered cereal head blight pathogen from the Russian Far East. Mycologia. 101-841-852.

72. 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.

73. Zhang, X., Shen, X., Hao, Y., Cai, J., Ohm, H., and Kong, L. 2011. A genetic map of *Lophopyrum ponticum* chromosome 7E, harboring resistance genes to Fusarium head blight and leaf rust. Theor. Appl. Genet. 122:263-270.

74. Zhang, Z., Zhang, H., van der Lee, T., Chen, W., Arens, P., Xu, J., Xu, J., Yang, L., Yu, D., Waalwijk, C., and Feng, J. 2010. Geographic substructure of *Fusarium asiaticum* isolates collected from barley in China. Eur. J. Plant Pathol. 127:239-248.

Chapter 2. Local Distance of Wheat Spike Infection by Released Clones of *Gibberella zeae* Disseminated from Infested Corn Residue

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Local Distance of Wheat Spike Infection by Released Clones of *Gibberella zeae* Disseminated from Infested Corn Residue

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ABSTRACT

Knowledge of the movement of *Gibberella zeae* (*Fusarium graminearum*) from a local source of inoculum in infested cereal debris is critical to the management of Fusarium head blight (FHB) of wheat. Previous spatial dissemination and infection studies were unable to completely distinguish the contributions of released inocula from those of background inocula. Clones of *G. zeae* were released and recaptured in five wheat fields in New York and Virginia in 2007 and 2008. Amplified fragment length polymorphisms were used to track and unambiguously identify the released clones in heterogeneous populations of the fungus recovered from infected wheat spikes collected at 0, 3, 6, and ≥24 m from small area sources of infested corn residues. The percent recovery of the released clones decreased significantly at fairly short distances from the inoculum sources. Isolates of *G. zeae* recovered at 0, 3, 6, and ≥24 m from the center of source areas shared 65, 19, 13, and 5% of the genotypes of the released clones, respectively. More importantly, the incidence of spike infection attributable to released clones averaged 15, 2, 1, and <1% at 0, 3, 6, and ≥24 m from source areas, respectively. Spike infection attributable to released clones decreased an average of 90% between 3 and 6 m from area sources of inoculum, and the spike infection potential of inocula dispersed at this range did not differ significantly from background sources. Our data suggest FHB field experiments including a cereal debris variable should incorporate debris-free

borders and interplots of at least 3 m and preferably 6 m to avoid significant interplot interference from spores originating from within-field debris.

INTRODUCTION

Fusarium head blight (FHB) or scab is a devastating disease of wheat and barley in the United States, with losses exceeding $3 billion since 1996 (23). Grain resulting from FHB infection is often contaminated with the mycotoxin deoxynivalenol (DON) (32,34). DON is an important inhibitor of protein synthesis (26,27) and may render grain unfit for human or domestic animal consumption (32). The disease and associated DON contamination continue to cause economic hardships on farms and in rural communities where small grains are grown (38). The principal causal agent of FHB in North America is the fungus *Gibberella zeae* (Schwein.) Petch (anamorph: *Fusarium graminearum*) (23,34). Frequent rainfall and high relative humidity (12,25) from spike emergence through anthesis favor inoculum production on cereal debris and resulting disease development (34). Overwintered cereal debris is a significant reservoir for *G. zeae*, and contributes a source of inoculum for infection of wheat and barley plants in the same field in which debris is present (11,34,37,39) and in more distant fields (21,30). Future strategies for managing FHB in individual wheat and barley fields should be guided by a better understanding of the movement of *G. zeae* from within-field sources of inoculum.

Both ascospores and macroconidia of *G. zeae* contribute to FHB (22), though it is generally considered that airborne ascospores play the predominant role in infection of wheat and barley florets at anthesis (28,31). Rain splash may disperse macroconidia or

ascospores of *G. zeae* across meter distances within wheat canopies (24), whereas airborne ascospores may be dispersed over kilometer distances from their source (21,29,30). The role of local and more distant sources of inoculum to FHB infection has not been quantified definitively.

The local dissemination of *G. zeae* from sources of inoculum has been investigated. de Luna et al. (10) reported a 50% decline in ascospore concentration within 18 m of an inoculum source and 90% within 60 m. Fernando et al. (13) reported a 50% reduction in FHB within 1 to 10 m from the center of an inoculated plot and a 90% reduction within 5 to 22 m in some locations. Stack (33) reported a 50% decline in FHB within 2-3 m of a small-area source of inoculum and within 20 to 50 m of a large-area source of inoculum. In the spatial dissemination studies performed to date, it has not been possible to completely distinguish the contributions of released inocula from that of background inocula.

The extent to which cereal debris management by crop rotation (i.e., avoidance), tillage, or other treatment contributes to integrated management of FHB must be assessed through field experimentation. Design and interpretation of field experiments involving a cereal debris (inoculum source of *G. zeae*) management variable must consider and minimize the effects of interplot interference from spores produced on local cereal debris. Agronomic researchers typically establish wheat field plots using small experimental drills (6 ft = 1.8 m wide) or small commercial drills (10 ft = 3.0 m wide). The results of Fernando et al. (13) suggested that the contribution of spores to wheat infection from a concentrated area source of inoculated cereal debris is diminished to background levels within a few to several meters of the local source. In order to determine whether local

distance of wheat spike infection is reduced to background levels within one to two commercial drill widths (i.e., 3 to 6 m) of infested corn residue, clones of *G. zeae* were released and recaptured in five experimental wheat fields in New York and Virginia in 2007 and 2008. A genotyping technique known as amplified fragment length polymorphism (AFLPs) (35) was used to track and unambiguously identify the released clones in heterogeneous populations of the fungus recovered from infected wheat plants collected 0, 3, 6, and ≥24 m from small-area sources of inoculum consisting of infested corn residues. Abstracts on portions of this work have been published (18,19).

MATERIALS AND METHODS

Experimental fields. Experiments were conducted in commercial wheat fields in Aurora, NY (2007 and 2008), LeRoy, NY (2008), and New Kent, VA (2007 and 2008). All fields lacked previous-season residues of corn or small grains known to be inoculum sources of *G. zeae* (11,23). Any corn residue present within the fields would have been from two or more seasons prior to the experiments. The Aurora, NY fields were planted with the soft white winter wheat cv. Caledonia following harvest of soybean. The LeRoy, NY field was planted with the soft red winter wheat cv. Pioneer 25R57 following harvest of pea. New Kent, VA (2007 and 2008) fields were planted with the soft red winter wheat cv. Pioneer 26R15 following harvest of soybean.

Inocula preparation. Four single-spored strains of *G. zeae* (two recovered from diseased wheat in Aurora, NY, and two recovered from diseased wheat in Riner, VA) were used in the experiments. These strains represented two of the predominant

trichothecene mycotoxin genotypes of the fungus: 3-acetyl deoxynivalenol (3-ADON) and 15-acetyl deoxynivalenol (15-ADON) genotypes (16). Strains Gz_NY_TS59_3-ADON (hereafter referred to as GZNY3) and Gz_NY_T1S1_15-ADON (hereafter referred to as GZNY15) were used to inoculate the New York fields, and Gz_VA_GPS13N4_3-ADON (hereafter referred to as GZVA3) and Gz_VA_GPS8N12_15-ADON (hereafter referred to as GZVA15) were used to inoculate the Virginia fields. Cultures of each strain were grown for 2 weeks on quarter-strength potato dextrose agar (PDA) medium. Autoclaved corn stalk pieces (7.6 to 15.2 cm in length) and corn kernels were inoculated with individual strains in 4-liter plastic containers. Pre-inoculation, tissue dry weights per plot were 300 g stalk pieces and 500 g of kernels. Containers were stored at ambient room temperature for approximately 6 weeks to allow colonization of corn kernels or stalk pieces.

Field inoculations. In 2007, field inoculations were performed 12 May (Aurora, NY) and 19 April (New Kent, VA). In 2008, field inoculations were performed 29 April (Aurora, NY), 25 April (LeRoy, NY), and 22 March (New Kent, VA). Corn stalks or corn kernels containing individual strains of *G. zeae* were placed within replicated circular plots 1 m in diameter with an area of 0.79 m^2. Plots were surrounded by wire cages to prevent animal tampering and wind dispersal of debris. In 2007, each of the New York and Virginia fields contained a total of 10 plots: 1 control plot of noninoculated corn kernels, 1 control plot of noninoculated corn stalk pieces, 2 plots of corn stalk pieces inoculated with GZNY3 or GZVA3, 2 plots of corn kernels inoculated with GZNY3 or GZVA3, 2 plots of corn stalk pieces inoculated with GZNY15 or

GZVA15, and 2 plots of corn kernels inoculated with a GZNY15 or GZVA15. Plots were separated by ≥24 m. In 2008, all of the plots contained corn stalk pieces. The two New York fields contained a total of six plots: two control plots of non-inoculated corn stalk pieces, two plots of corn stalk pieces inoculated with GZNY3, and two plots of corn stalk pieces inoculated with GZNY15. The Virginia field contained a total of five plots: one control plot of non-inoculated corn stalk pieces, two plots of corn stalk pieces inoculated with GZVA3, and two plots of corn stalk pieces inoculated with GZVA15. All fields maintained a complete wheat canopy throughout the study. No chemical or mechanical disturbance of the field occurred.

Collection of wheat spikes and isolation of *G. zeae*. In 2007, wheat spikes were collected 2 July (Aurora, NY) and 22 May (New Kent, VA). In 2008, wheat spikes were collected 2 July (Aurora, NY), 1 July (LeRoy, NY), and 17 May (New Kent, VA). Collection occurred approximately 4 weeks after anthesis (Zadoks growth stage 85) in New York and 2 weeks after anthesis (Zadoks growth stage 77) in Virginia. Spikes were collected at random at 0 (directly above the source), 3, 6, and ≥24 m from the sources. A string was tied to a stake in the center of the plot and was used to collect wheat spikes at radii of 3 and 6 m circling the source. Spikes were also collected from noninoculated control plots located ≥24 m from the released sources. A total of 100 spikes was assessed for FHB infection (2,23,34) for each sampling distance per plot (e.g., 100 spikes at 0 m, 100 spikes at 3 m, and 100 spikes at 6 m) for the Aurora, NY fields, 75 spikes for each sampling distance per plot for the LeRoy, NY fields, and 55 spikes for each sampling distance per plot for the New Kent, VA fields. Following FHB assessments,

the spikes were surface disinfested in a 20% bleach solution for 1 min and rinsed with distilled water for 1 min. Disinfested spikes were plated onto a *Fusarium*-selective medium (FSM) (6) containing neomycin at 0.35 g/liter as described by Schmale et al. (30). Plates containing the disinfested spikes were incubated for 5 to 7 days in the laboratory at ambient room temperature. One colony of a *Fusarium* sp. per collected spike was subcultured to petri plates containing quarter-strength PDA. Colonies producing characteristic red or pink mycelia and containing only macroconidia characteristic of *F. graminearum* (20) were single-spored onto additional plates of quarter-strength PDA. Single-spored cultures were placed in an aqueous suspension of 20% glycerol for cryogenic storage at -80C.

DNA extraction and AFLP methodology. Each single-spored isolate of *G. zeae* was grown in 250-ml flasks of quarter-strength potato dextrose (PD) broth on a shaker operating at 100 rpm for 5 days at 20°C. Mycelia were harvested from individual flasks and lyophilized for at least 12 h. Lyophilized mycelia (0.1 to 0.3 g) were homogenized in microcentrifuge tubes containing 0.5 mm zirconia/silica beads on a mini-beadbeater (Model no. 3110BX, BioSpec Products, Inc., Bartlesville, OK) for 1.5 min at 2,500 rpm. DNA was extracted from the homogenized mycelium using Qiagen's BioSprint 15 workstation (Qiagen, Inc. USA, Valencia, CA) and the Biosprint 15 DNA Plant Kit (Qiagen, Inc. USA, Valencia, CA) following the manufacturer's protocols.

AFLPs (35) were used to genotype all of the recovered isolates of *G.zeae* from field populations. Two primer pair combinations with two selective nucleotides on each primer were used: *EcoR1*+AA labeled with HEX, and *EcoR1*+CC labeled with FAM.

Digestions, ligations, pre-amplifications, and selective amplifications were conducted following standard protocols (29). AFLPs were viewed and analyzed on an Applied Biosystems Genetic Analyzer 3130xl using the Foundation Data Collection Software, Version 3.0. Applied Biosystems Hi-Di formamide (Model no. 4311320, Applied Biosystems, Inc., Foster City, CA) and GeneScan 500Liz size standard (Model no. 4322682, Applied Biosystems, Inc.) were added to each of the sample tubes prior to each run. GeneMarker (version 1.7; SoftGenetics, LLC, State College, PA) was used to visualize electropherograms, select markers and alleles, and compare and contrast genotypes of the isolates. Polymorphic bands (alleles) were scored from 100 to 500 bp at a peak detection threshold intensity greater than 250. Nine unique alleles representative of GZNY3 and GZNY15 and nine unique alleles representative of GZVA3 and GZVA15 were selected to represent the genotypes of the released strains (Table 1). The selection of these alleles was determined by censoring initial field populations for alleles with a frequency lower than 10% or greater than 90%. The presence or absence of these alleles was recorded in a binary format (allele present =1 or absent = 0) for all of the isolates. Isolates were considered to be the released clones when the profiles of the 9 unique alleles were identical (Table 1).

Statistical Methods. Analysis of variance was used to test for differences in the percentage of spikes infected with released clones collected at 0, 3, 6, and ≥24 m from the source areas and the difference between the percentage of spikes infected with released clones in plots containing 3-ADON and 15-ADON genotypes. Tukey's Studentized Range Test and Scheffe's Test were used to test for differences in the

percentage of spikes infected with released clones collected between 0 and 3 m, 0 and 6 m, and 0 and ≥24 m, 3 and 6 m, 3 and ≥24 m, and 6 and ≥24 m. Analyses were performed using PROC GLM in SAS Systems for Windows (version 9.2; SAS Institute, Cary, NC). Significance was evaluated at $P < 0.05$ for all tests.

RESULTS AND DISCUSSION

Released clones were distinguished unambiguously from background isolates of *G. zeae* by AFLPs, an approach that has been employed successfully to monitor other plant pathogens within heterogeneous populations (1,3,4,29,40). Isolates of *G. zeae* recovered at 0, 3, 6, and ≥24 m from the center of source areas shared 65% (314/483), 19% (35/188), 13% (26/196), and 5% (3/63) of the genotypes of released clones, respectively. More importantly, the incidence of spike infection attributable to released clones averaged 15% (314/2160), 2% (35/2160), 1% (26/2160), and <1% (3/715) at these distances (Table 2, Fig. 1). The percentage of spikes infected with the released clones varied significantly across sampling distances ($P < 0.001$). Pairwise comparisons between 0 and 3 m, 0 and 6 m, and 0 and ≥24 m were significantly different ($P < 0.05$ for both Tukey's and Scheffe's tests), when controlling for all five fields in both years of experimentation.

Asymptomatic spikes (i.e., spikes without any visible symptoms of FHB) within individual plots ranged from 86 to 100% for Virginia. All of the wheat spikes in the New York plots were asymptomatic yet many spikes were infected, as revealed by plating of surface-disinfested spikes (Table 2). Incidence of FHB symptoms was only slightly higher above clonal source plots (92/1320 = 7%) than control plots (5/110 = 5%) in New

Kent, VA in 2007 where moisture conditions were conducive for infection at anthesis. Despite the very low incidence of symptoms on wheat spikes from the other four experimental sites (where low moisture at anthesis did not favor infection), the fungus was recovered from a significant percentage of asymptomatic spikes. These spikes likely were infected post flowering and there may not have been enough time for symptoms to develop prior to spike collection. Thus, our data support the idea that FHB symptoms alone may underestimate the amount of spike infection and resulting DON contamination (7-9).

The percentage of spikes infected by background inocula (i.e., isolates other than released clones) did not differ significantly ($P = 0.83$) by sampling location (including clonal source and control plots) in any of the five wheat fields. Background inocula accounted for an average of 35% of spike infection directly above concentrated (corn stalk pieces bearing abundant perithecia) clonal inoculum sources. Because our experimental fields contained little or no residues of corn or small grains from previous-year crops, the recovered background populations of *G. zeae* likely originated from more distant sources outside of our fields (14,21,29,30).

Epidemic populations of *G. zeae* from wheat in the United States contain both 3-ADON and 15-ADON genotypes (15,36), but recent observations suggest that the 3-ADON genotype may be increasing in frequency in the Midwest (15) and Canada (36). Certain strains of the 3-ADON genotype may be more aggressive than those of the 15-ADON genotype (36). In our study, the percentage of spikes infected with the released clones did not differ significantly ($P = 0.96$) between plots containing 3-ADON and 15-ADON genotypes.

Our experimental design was spatially similar to those of Fernando et al. (13) and Stack (33) (i.e., documenting infection at distances from 1-m diameter or 1-m^2 infested plots). Fernando et al. (13) reported that FHB declined by 50% at distances 1 to 10 m from the inoculum sources and by 90% at distances of 10 to 25 m, whereas Stack (33) observed a 50% decline in FHB within 2 to 3 m of the small area sources. Stack (33 and *personal communication*) also observed a 50% decline in spike infection within 20 to 50 m of a large-area (30-m^2) source of concentrated inoculum. Infection gradients from small area sources may be steeper than gradients from large area sources (17). In our experiments, spike infection attributable to released clones decreased an average of 90% between 3 and 6 m from area sources of inoculum (Table 2), suggesting a steeper local spike infection gradient than reported by previous investigators (13,33).

Studies with inoculated substrates may tend to overestimate the local contribution of natural, within-field inoculum sources compared to background sources. Release of naturally overwintered corn residues in wheat fields resulted in significantly lower levels of FHB and DON accumulation than did concentrated inoculum sources in five of six experiments in New York (5). Our data suggest that small-plot field experiments on FHB management, including a cereal debris variable, should incorporate cereal debris-free borders and interplots of at least 3 m and preferably 6 m to avoid significant interplot interference from spores originating from within-field cereal debris. Future research may identify a need for even larger cereal debris-free borders and interplots to separate research plots conducted on an agricultural field scale.

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LITERATURE CITED

1. Akinsanmi, O.A., Backhouse, D., Simpfendorfer, S., and Chakraborty, S. 2006. Genetic diversity of Australian *Fusarium graminearum* and *F. pseudograminearum*. Plant Pathol. 55:494-504.
2. Bai, G., and Shaner, G. 1994. Scab of wheat: Prospects for control. Plant Dis. 78:760-766.
3. Bayon, C., Pei, M.H., Ruiz, C., Hunter, T., Karp, A., and Tubby, I. 2009. Genetic structure and population dynamics of a heteroecious plant pathogen *Melampsora larici-epitea* in short-rotation coppice willow plantations. Mol. Ecol. 18:3006-3019.
4. Bennett, R.S., Milgroom, M.G., Sainudiin, R., Cunfer, B.M., and Bergstrom, G.C. 2007. Relative contribution of seed-transmitted inoculum to foliar populations of *Phaeosphaeria nodorum*. Phytopathology 97:584-591.
5. Bergstrom, G.C., and Waxman, K.D. 2008. Microplots in commercial wheat fields for quantifying the local contribution of Gibberella zeae from natural corn debris to Fusarium head blight and deoxynivalenol accumulation. Pages 6-8 in: Proc. 2008 Natl. Fusarium Head Blight Forum, Indianapolis, IN.
6. Burgess, L.W., Summerell, B.A., Bullock, S., Gott, K.P., and Backhouse, D. 1994. Laboratory Manual for Fusarium Research, 3rd ed. University of Sydney, Australia.
7. Cowger, C., Patton-Özkurt, J., Brown-Guedira, G., and Perugini, L. 2009. Post-anthesis moisture increased Fusarium head blight and deoxynivalenol levels in North Carolina winter wheat. Phytopathology 99:320-327.

8. Cowger, C., and Sutton, A.L. 2005. The southeastern U.S. Fusarium head blight epidemic of 2003. Published Online. Plant Health Progress DOI:10.1094/PHP-2005-1026-01-RS.
9. Del Ponte, E.M., Fernandes, J.M.C., and Bergstrom, G.C. 2007. Influence of growth stage on Fusarium head blight and deoxynivalenol production in wheat. J. Phytopathol. 155:577-581.
10. de Luna, L., Bujold, I., Carisse, O., and Paulitz, T.C. 2002. Ascospore gradients of *Gibberella zeae* from overwintered inoculum in wheat fields. Can. J. Plant Pathol. 24:457-464.
11. Dill-Macky, R., and Jones, R.K. 2000. The effect of previous crop residues and tillage on Fusarium head blight of wheat. Plant Dis. 84:71-76.
12. 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. Can. J. Bot. 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. Francl, L., Shaner, G., Bergstrom, G, Gilbert, J., Pedersen, W., Dill-Macky, R., Sweets, L., Corwin, B., Jin, Y., Gallenberg, D., and Wiersma, J. 1999. Daily inoculum levels of *Gibberella zeae* on wheat spikes. Plant Dis. 83:662-666.
15. Gale, L.R., Ward, T.J., Balmas, V., and Kistler, H.C. 2007. Population subdivision of *Fusarium graminearum* sensu stricto in the upper midwestern United States. Phytopathology 97:1434-1439.

16. Goswami, R.S., and Kistler, H.C. 2004. Heading for disaster: *Fusarium graminearum* on cereal crops. Mol. Plant Pathol. 5:515-525.

17. Gregory, P.H. 1968. Interpreting plant disease dispersal gradients. Annu. Rev. Phytopathol. 6: 189-212.

18. Keller, M.D., Duttweiler, K.D., Schmale, D.G., and Bergstrom, G.C. 2007. Contribution of within-field inoculum sources to Fusarium head blight in wheat. Page 98 in: Proc. 2007 Natl. Fusarium Head Blight Forum, Kansas City, MO.

19. Keller, M.D., Schmale, D.G., Waxman, K.D., and Bergstrom, G.C. 2008. Released clones and background inocula of *Gibberella zeae* contributed to Fusarium head blight in winter cereals in New York and Virginia. Page 37 in: Proc. 2008 Natl. Fusarium Head Blight Forum, Indianapolis, IN.

20. Leslie, J.F. and Summerell, B.A. 2006. The Fusarium Laboratory Manual. Blackwell Publishing.

21. Maldonado-Ramirez, S.L., Schmale, 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. Agric. For. Meteorol. 132:20-27.

22. Markell, S.G., and Francl, L.J. 2003. Fusarium head blight inoculum: Species prevalence and *Gibberella zeae* spore type. Plant Dis. 87: 814-820.

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

24. Paul , P.A., El-Allaf, S.M., Lipps, P.E., and Madden, L.V. 2004. Rain splash dispersal of *Gibberella zeae* within wheat canopies in Ohio. Phytopathology 94:1342-1349.

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

26. Pestka, J.J. 2007. Deoxynivalenol: Toxicity, mechanisms and animal health risks. Anim. Feed Sci. and Tech. 137:283-298.

27. Pestka, J.J, and Smolinski, A.T. 2005. Deoxynivalenol: Toxicology and potential effects on humans. J. Toxicol. Environ. Health B Crit. Rev. 8:39-69.

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

29. Schmale, D.G., III, 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.

30. Schmale, D.G., III, Shields, E.J., and Bergstrom, G.C. 2006. Night-time spore deposition of the Fusarium head blight pathogen, *Gibberella zeae*. Can. J. Plant Pathol. 28:100-108.

31. Shaner, G.E. 2003. Epidemiology of Fusarium head blight of small grain cereals in North America. Pages 84-119 in: Fusarium Head Blight of Wheat and Barley. Leonard, K.J. and W. Bushnell, eds. American Phytopathological Society, St. Paul, MN.

32. Snijders, C.H.A. 1990. Fusarium head blight and mycotoxin contamination of wheat, a review. Neth. J. Plant Pathol. 96:187-198.

33. Stack, R.W. 1997. Gradients of Fusarium head blight in wheat along transects away from a concentrated source of *Gibberella zeae* ascospore inoculum. Page 60 in Proc. National Fusarium Head Blight Forum, 10-12 November 1997. St. Paul, MN.

34. Sutton, J.C. 1982. Epidemiology of wheat head blight and maize ear rot caused by *Fusarium graminearum*. Can. J. Pathol. 4:195-209.

35. Vos, P., Hogers, R., Bleeker, M., Reijans, M., van de Lee, T., Hornes, M., Frijters, A., Pot, J., Peleman, J., Kuiper, M., and Zabeau, M. 1995. AFLP: A new technique for DNA fingerprinting. Nucleic Acids Res. 23:4407-4414.

36. Ward, T.J., Clear, R.M., Rooney, A.P., O'Donnell, K., Gaba, D., Patrick, S., Starkey, D.E., Gilbert, J., Geiser, D.M., and Nowicki, T.W. 2008. An adaptive evolutionary shift in Fusarium head blight pathogen populations is driving the rapid spread of more toxigenic *Fusarium graminearum* in North America. Fungal Genet. Biol. 45:473-484.

37. Wiese, M.V. 1987. Compendium of Wheat Diseases. 2nd ed. American Phytopathological Society, St. Paul, MN.

38. 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.

39. Windels, C.E., and Kommedahl, T. 1984. Late-season colonization and survival of *Fusarium graminearum* group II in cornstalks in Minnesota. Plant Dis. 68:791-793.

40. 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. Nine alleles were used to identify the released clones of *Gibberella zeae* in heterogeneous populations of the fungus collected from infected plants in Virginia and New York

State	Allele[a]	Primers and fluorescent labels[b]
VA	102.1	FAM-Eco-aa+MSE-at
VA	106.4	HEX-Eco-cc+MSE-cg
VA	142.6	FAM-Eco-aa+MSE-at
VA	192.4	FAM-Eco-aa+MSE-at
VA	196.2	FAM-Eco-aa+MSE-at
VA	262.7	FAM-Eco-aa+MSE-at
VA	328.8	FAM-Eco-aa+MSE-at
VA	355.6	FAM-Eco-aa+MSE-at
VA	383.9	FAM-Eco-aa+MSE-at
NY	106.3	HEX-Eco-cc+MSE-cg
NY	106.9	HEX-Eco-cc+MSE-cg
NY	126.8	HEX-Eco-cc+MSE-cg
NY	157.2	HEX-Eco-cc+MSE-cg
NY	168.7	FAM-Eco-aa+MSE-at
NY	199.9	HEX-Eco-aa+MSE-cg
NY	245.6	FAM-Eco-aa+MSE-at
NY	271.6	FAM-Eco-aa+MSE-at
NY	368.3	HEX-Eco-aa+MSE-cg

[a]Polymorphic bands (alleles) were scored from 100 to 500 bp at a peak detection threshold intensity greater than 250.

[b]Fluorescent labels FAM and HEX were incorporated during the final selective polymerase chain reaction amplification.

Table 2. Recovery of released clones of *Gibberella zeae* from wheat plants at different distances from inoculum sources[a]

Field location, state, and year	No. of plots[b]	Clone Released[c]	Distance (m) from center of released source[d]	Spikes infected by *G. zeae* (%)[e]	Spikes infected by clone (%)[f]	Spikes infected by background (%)[g]	Clone recovered (%)[h]
Aurora, NY, 2007	4	GZNY3	0	4	1	3	14
Aurora, NY, 2007	4	GZNY3	3	2	0	2	0
Aurora, NY, 2007	4	GZNY3	6	3	0	2	10
Aurora, NY, 2007	4	GZNY15	0	2	1	1	33
Aurora, NY, 2007	4	GZNY15	3	3	0	3	0
Aurora, NY, 2007	4	GZNY15	6	2	0	2	0
Aurora, NY, 2007	2	Control	30	5	1	5	10
Aurora, NY, 2008	2	GZNY3	0	14	12	2	86
Aurora, NY, 2008	2	GZNY3	3	4	1	3	25
Aurora, NY, 2008	2	GZNY3	6	8	0	8	0
Aurora, NY, 2008	2	GZNY15	0	31	28	4	89
Aurora, NY, 2008	2	GZNY15	3	8	3	5	33
Aurora, NY, 2008	2	GZNY15	6	7	2	5	23
Aurora, NY, 2008	2	Control	30	11	1	11	5
LeRoy, NY, 2008	2	GZNY3	0	35	32	3	92
LeRoy, NY, 2008	2	GZNY3	3	1	1	0	100
LeRoy, NY, 2008	2	GZNY3	6	3	2	1	60
LeRoy, NY, 2008	2	GZNY15	0	24	15	9	61
LeRoy, NY, 2008	2	GZNY15	3	7	4	3	60
LeRoy, NY, 2008	2	GZNY15	6	2	1	1	33
LeRoy, NY, 2008	2	Control	30	1	0	1	0
New Kent, VA, 2007	4	GZVA3	0	35	18	17	51
New Kent, VA, 2007	4	GZVA3	3	25	1	24	5
New Kent, VA, 2007	4	GZVA3	6	24	2	22	8
New Kent, VA, 2007	4	GZVA15	0	35	15	19	45
New Kent, VA, 2007	4	GZVA15	3	23	1	23	2
New Kent, VA, 2007	4	GZVA15	6	27	1	27	2
New Kent, VA, 2007	2	Control	24	27	1	26	3
New Kent, VA, 2008	2	GZVA3	0	67	39	28	58
New Kent, VA, 2008	2	GZVA3	3	10	4	6	36
New Kent, VA, 2008	2	GZVA3	6	22	10	13	42
New Kent, VA, 2008	2	GZVA15	0	53	41	12	78
New Kent, VA, 2008	2	GZVA15	3	16	11	5	67
New Kent, VA, 2008	2	GZVA15	6	5	3	3	50

New Kent, VA, 2008	1	Control	24	0	0	0	0
Aggregate Data	28	All clones	0	22	15	8	65
	28	All clones	3	9	2	7	19
	28	All clones	6	9	1	8	13
	9	Control	≥24	9	0	8	5

[a] Percentages were calculated from the total number of spikes sampled or isolates collected at 0 (above the source), at 3, 6, and ≥24 m from the center of the released sources.

[b]Number of 1-m diameter plots per sampling areas represented containing clonal inocula (source, 0 m) or no inocula (control, ≥24 m) separated by 24 m (Virginia) or 30 m (New York) within commercial wheat fields or number of circular sampling areas at 3 and 6 m from centers of source plots.

[c]Two clones of *G. zeae* were released in New York (GZNY3 and GZNY15) and two clones were released in Virginia (GZVA3 and GZVA15). Corn kernels and corn stalk pieces were infested with *G. zeae* in 2007; in 2008, only corn stalk pieces were infested. Control plots contained noninoculated corn stalk pieces or kernels in 2007 but only noninoculated stalk pieces in 2008.

[d]Spikes were collected at 0 (above the source), at 3, 6, and ≥24 m from the center of the released sources.

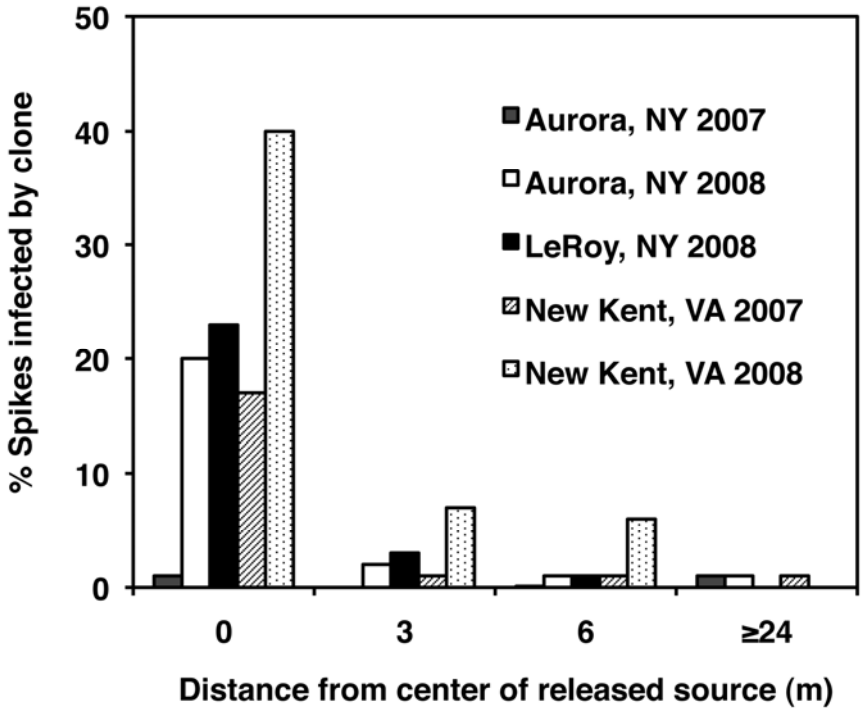
[e] Number of infected spikes divided by the total number of spikes collected at 0, 3, 6, and ≥24 m from the center of the released sources. Isolates of *G. zeae* were recovered from 55 wheat spikes per sampling-distance in Virginia, 100 in Aurora, NY, and 75 in LeRoy, NY. Percentages rounded to integer values.

[f]Released clones of *G. zeae* recovered from total number of wheat spikes collected. Percentages rounded to integer values.

[g]Background isolates of *G. zeae* recovered from total number of wheat spikes collected. Percentages rounded to integer values.

[h]Released clones of *G. zeae* divided by the total number of isolates recovered from spikes collected at 0, 3, 6, and ≥24 m from the center of the released sources. Percentages rounded to integer values.

Fig. 1. Percent wheat spikes infected by locally released clones of *Gibberella zeae* at distances from inoculum sources for New York and Virginia in 2007 and 2008. Percentages were calculated from the total number of isolates collected at 0 (above the source), at 3, 6, and ≥24 m from the center of the released sources.



Chapter 3. The Dissemination of a Released Clone of *Gibberella zeae* is Influenced by the Amount of Corn Residue

The following chapter was formatted to facilitate publication in *Plant Disease*. This work was submitted by Keller, Thomason, and Schmale on March 18, 2011.

The Dissemination of a Released Clone of *Gibberella zeae* is Influenced by the Amount of Corn Residue

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ABSTRACT

Keller, M.D., Thomason, W.E., and Schmale, D.G., III. 2011. The dissemination of a released clone of *Gibberella zeae* is influenced by the amount of corn residue. Plant Dis. XX: XXX-XXX.

Corn residue is a significant source of inoculum for epidemics of Fusarium head blight (FHB) in wheat and barley, but little is known about the influence of different amounts of corn residue on FHB. We monitored the dissemination of a released clone of *Gibberella zeae* (*Fusarium graminearum*), causal agent of FHB, from small 0.84-m diameter research plots containing 45, 200, and 410 g of infested corn stalk pieces in winter wheat and barley fields in Virginia over three years (2008-2010). The fungus was recaptured through the collection of wheat and barley spikes at 0 and 3 m from the source and the released clone was identified in heterogeneous background populations using amplified fragment length polymorphisms. Results showed that the dissemination of the released clone was generally greater with increasing amounts of corn stalk residue. Plots containing larger inoculum amounts (410 g) resulted in an average overall dissemination gradient of FHB from 0 to 3 m of 84% and 79% for barley in moderate and low epidemic years, respectively, and 28% and 80% for wheat in high and low to moderate epidemic years, respectively. The use of greater tillage intensity (minimal or conventional) and a moderately resistant cultivar of wheat or barley may reduce the impact of within-field inoculum sources for FHB.

INTRODUCTION

Fusarium head blight (FHB) has been recognized in North America for over one hundred years (35). It is one of the most economically important diseases of wheat and barley (41) and continues to impact areas when environmental conditions are favorable to the pathogen. FHB is primarily caused by *Gibberella zeae* (Schwein.) Petch (anamorph: *Fusarium graminearum sensu stricto* Schwabe) in the United States (24,38). A mycotoxin known as deoxynivalenol (DON) may be produced by the fungus in the infected grain. The tolerance levels recommended for DON by the United States Food and Drug Administration are 1 ppm in finished food products for human consumption and levels in grain or grain by-products for animal consumption are dependent upon the type of animal (1). Adverse health effects are possible for humans (31,32) and domestic animals (30) if contaminated grain is consumed.

G. zeae survives saprophytically on wheat, barley, and corn residues, all three of which are regarded as primary inoculum sources for FHB epidemics (28,35,38). Perithecia form on corn residue, and in the late spring and early summer release ascospores that can be deposited on wheat and barley spikes. If environmental conditions are favorable (i.e., high relative humidity and frequent rainfall) (12,24,29), infection can occur from the time of anthesis and possibly through grain fill (6,7). Infected seed may appear smaller than normal, white/pink, wrinkled, and contain high levels of DON which may affect marketability of infected grain (24).

Previous research has investigated the role of corn residue remaining on the soil surface prior to the planting of wheat or barley and its impact on FHB infection. Dill-Macky and Jones (11) found lower FHB incidence and severity with tillage treatments

involving a moldboard plow to minimize residue than in those using a chisel plow or no tillage implement. They also found wheat planted after corn showed the highest FHB infection when compared to wheat planted after soybean (11).

Nita et al. (26) evaluated FHB disease incidence, FHB severity, and DON concentration from field experiments in 2003 and 2004. The risk of FHB increased with greater concentrations of inoculum from within-field sources when environmental conditions were moderately favorable for disease. In both greenhouse and field experiments, Stein et al. (37) found that disease incidence and severity increased with an increasing number of *G. zeae* conidia applied. DON levels were also found to be positively related to inoculum concentration. The mathematical functions provided accurate FHB predictions in moderate epidemic seasons (37).

Reduced tillage practices have been used to minimize soil erosion and moisture loss for the past twenty-five years (24). Corn residue remaining in a no-till or minimal till system may provide overwintering sites for *G. zeae*. With the increase of corn production due to incentives for ethanol-based fuel and as an alternative crop to small grains in FHB prone regions (10), overwintering sites for *G. zeae* on corn residue are likely to increase. Conservation tillage practices are prevalent in Virginia (39) with many counties predominantly using conservation and minimal tillage to manage corn residue.

The reduction of *G. zeae*-infested residue within wheat and barley fields prior to planting may reduce the impact of future FHB epidemics; however, with the move away from conventional tillage practices (10), research is needed to better understand the influence of the remaining corn residue. Dissemination from sources of *G. zeae* inoculum has been reported using a released clone with a unique phenotype (13,36) and released

clones with unique genotypes (20). Research to date has reported the influence of local inoculum sources on FHB infection within several meters of the source; however, the potential impact of different amounts of corn residue on dissemination of *G. zeae* from local inoculum sources has not been examined in detail. We hypothesized that (i), the dissemination of a released clone of *G. zeae* is greater with increasing amounts of local corn stalk residue and (ii), recovery of the clone from plots containing a moderately resistant cultivar is lower than those containing a susceptible cultivar. To test these hypotheses, corn stalk pieces (45, 200, and 410 g) colonized with a single clone of *G. zeae* were released in small 0.84-m diameter replicated plots in winter wheat and barley fields in Virginia over three years (2008-2010). Amplified fragment length polymorphism (AFLP) (40) was used to identify the released clone in heterogeneous background populations of the fungus at the source plots (0 m) and at 3 m from the source plots. Assessments were conducted in 31 commercial wheat and barley fields to estimate corn stalk residue coverage in Virginia. An abstract on a portion of this work has been published (19).

MATERIALS AND METHODS

Experimental fields. Experiments were conducted in two barley and two wheat fields. Barley experiments were conducted in a commercial field in New Kent County, VA (2008) hereafter referred to as 'BR08' and in an experimental field in Blacksburg, VA (2009) hereafter referred to as 'BR09'. In both years, the barley cv. Nomini (moderately resistant to FHB) was planted following corn harvest in a conventional tillage system. Minimal corn residue was present at the time of planting. Wheat was planted in two

experimental fields at the Kentland Research Farm of Virginia Tech in Blacksburg, VA (2009 and 2010) and are hereafter referred to as 'WH09' and 'WH10', respectively. Each wheat field contained the cvs. Tribute, Vigoro 9510, Southern States (SS) 560, and Pioneer Brand 26R12 of soft red winter wheat in strip plots averaging 34 x 79 m. Tribute and Vigoro 9510 are moderately resistant and SS560 and Pioneer 26R12 are susceptible to FHB. Three-meter fallow strips divided the different cultivars. Both wheat fields followed the harvest of corn and were prepared with conventional tillage methods to minimize corn residue at the time of planting.

Field inoculations. One single-spored *G. zeae* strain (Gz_VA_GPS13N4_3-ADON, hereafter referred to as GZVA3) was used to inoculate plots at all four field locations (BR08, BR09, WH09, WH10). GZVA3 was recovered from diseased wheat in Riner, VA (20). The strain represented the trichothecene mycotoxin 3-acetyl deoxynivalenol (3-ADON) genotype (15,16). Mature corn stalks were collected in the fall of each year and dried in an oven at approximately 65°C for storage prior to inoculation. Corn stalks were cut into pieces measuring approximately 10 to 15 cm and were separated into groups of 5, 25, and 50 pieces with dry weights (prior to autoclaving) of 45, 200, and 410 g, respectively. Corn stalk pieces were stored at room temperature until ready for autoclaving and inoculation. Autoclaved corn stalk pieces were inoculated with GZVA3 and stored in 4-liter plastic containers at ambient room temperature for approximately 10 weeks. Inoculations of BR08 and BR09 were performed on 22 March (New Kent County, VA) and on 3 April (Blacksburg, VA), respectively. Inoculations of WH09 and WH10 were performed on 18 April (Blacksburg, VA) and on 12 April (Blacksburg, VA),

respectively. Corn stalk pieces were uniformly distributed in replicated 0.84-m diameter circular plots (Fig. 1).

BR08 and BR09 each contained a total of 7 plots, 6 of which had corn stalk pieces inoculated with GZVA3: 2 plots contained 45 g, 2 plots contained 200 g, and 2 plots contained 410 g. One control plot contained 200 g of noninoculated autoclaved corn stalk pieces. All plots were separated from each other by ≥18 m. In WH09 and WH10, each of the four cultivar strip plots contained a total of 5 plots, 4 of which had corn stalk pieces inoculated with GZVA3: 2 plots containing 45 g and 2 plots containing 410 g. One control plot contained 200 g of noninoculated autoclaved corn stalk pieces. No chemical or mechanical disturbance of the fields occurred during the study. No additional inoculum was applied, nor was misting used to enhance infection.

Isolation of *G. zeae*. BR08 spikes were collected 6 May and BR09 spikes were collected 19 May. WH09 spikes were collected 29 May and WH10 spikes were collected 4 June. Collection occurred between Zadoks growth stage 77 and 83 for all fields. Barley and wheat spikes were collected at random directly above each plot (0 m), at 3 m, and at distances ≥18 m (control plots). A string was secured to a stake in the center of each plot and was used to collect spikes at radii of 3 m circling the plots. A total of 55 spikes was assessed for FHB infection (i.e., premature bleaching) (2,24) for each plot. The spikes were then surface-disinfested in a 20% bleach solution for one min and rinsed with distilled water for one min. After disinfestation, spikes were plated onto *Fusarium*-selective medium (FSM) (5) containing increased neomycin (34) at 0.35 g/liter. Spikes were incubated for 5 to 7 days at ambient room temperature. One colony of *Fusarium* sp.

was subcultured from each collected spike and was plated onto quarter-strength potato dextrose agar (PDA). Single spores of these colonies were plated onto additional plates of quarter-strength PDA after identification of the characteristic red/pink mycelia and macroconidia characteristic of *F. graminearum* (21).

DNA extraction and AFLP analysis. DNA extractions and AFLPs were performed following standard protocols (20). Nine alleles used by Keller et al. (20) were selected to represent the genotype of the released strain GZVA3. Alleles with a frequency lower than 10% and higher than 90% within initial populations were excluded from the alleles chosen. Presence/absence of these alleles was scored based on a binary format (i.e., allele present = 1, absent = 0) for all recovered isolates. When profiles of the 9 alleles were identical, isolates were considered to be the released clone of *G. zeae*.

Estimation of corn residue in Virginia wheat and barley fields. Virginia county extension agents from 10 counties participated in assessing corn residue coverage prior to wheat or barley planting. Each county agent was sent residue assessment materials to assess field location, tillage practice used, tillage implement, previous corn yield, and purpose for corn (i.e., silage or grain). To estimate corn residue coverage, a line-transect method was used (18,27). A 15.2 m (50 ft) rope containing 100 black marks spaced 15.2 cm apart was placed diagonal to crop rows in four different locations within each field assessed. Stakes at each end of the rope were used as anchors to minimize movement of the rope's location. Every mark was visually checked for corn residue in contact with the black mark's location on the rope and recorded (present = 1 and absent = 0). Percent

coverage was equal to the percentage of marks counted as "1" when compared to the total number of marks counted. This was repeated for the other three locations in each field. The four reported percentages from each field were averaged and multiplied by 0.6 (33) to minimize the counting of leaf debris as corn residue coverage.

Percent coverage calculation of plots. Experimental plots containing 45, 200, and 410 g of *G. zeae*-infested corn residue contained 5, 25, and 50 corn stalk pieces, respectively. The surface area of the stalk pieces was calculated for each of the three amounts of corn residue and was divided by the total area (0.55 m^2) of a 0.84-m diameter plot to determine percent corn residue coverage.

Weather data. Rainfall, humidity, and temperature for BR09, WH09, and WH10 were recorded at 30 minute intervals by a Vantage Pro2 weather station (Model #06152, Davis Instruments, Corp., Hayward, California) located at the Kentland Research Farm of Virginia Tech, Blacksburg, VA. Data were analyzed using WeatherLink Software (version 5.8.1; Davis Instruments, Corp.). No weather data were recorded for BR08.

Statistical analysis. All statistical analyses were performed using Statistical Analysis System (version 9.2; SAS Institute, Cary, NC). The FHB incidence of collected spikes and the recovery of the clone were analyzed using differences of least squares means (Proc. GENMOD). Both incidence and recovery analyses were performed accounting for amount of corn residue (45, 200, and 410 g) and distance from source plots (0 and 3 m).

BR08, BR09, WH09, and WH10 were analyzed independently of each other. Significance was evaluated at $P < 0.05$.

RESULTS

Differences in FHB incidence of spikes (Table 1; Table 2) varied among the years; therefore, each of the four field experiments was treated separately when calculating statistical significance in dissemination of clone recovery to 3 m from plots containing different amounts of corn residue (Table 3; Fig. 2).

Barley trials. BR08 spikes infected by *G. zeae* (i.e., incidence) were significantly different between plots containing 45 and 200 g ($P = 0.02$), 200 and 410 g ($P = 0.001$), and 45 and 410 g ($P = <0.0001$; Table 3) of *G. zeae*-infested corn residue. Moderate epidemic conditions were observed with spikes infected by *G. zeae* ranging from 20 to 56% in inoculated plots and 11% in the control plot (Table 1). Spikes infected by *G. zeae* were significantly different between 0 and 3 m for plots containing 200 ($P = 0.001$) and 410 g ($P = <0.0001$) but not for plots containing 45 g ($P = 0.73$). Spikes with incidence attributable to the clone were significantly different between plots containing 45 and 410 g ($P = 0.002$; Table 3), but not between plots containing 45 and 200 g ($P = 0.08$) or 200 and 410 g ($P = 0.13$). The steepest dissemination gradient of clone recovery from 0 to 3 m (Fig. 2) was observed in plots containing 200 g (95%; $P = 0.001$) and a more gradual but significant gradient from plots containing 45 (85%; $P = 0.01$; Table 3) and 410 g (84%; $P = <0.0001$; Table 3).

Fewer infected spikes and fewer infections attributable to the clone were observed for BR09 (Table 1). From the time of flowering to spike collection for BR09, the temperature range, total rainfall, and average relative humidity were 1.3 to 26.4°C, 116 mm, and 89%, respectively. Low epidemic conditions were observed with spikes infected by *G. zeae* ranging from 5 to 6% in inoculated plots and 9% in the control plot (Table 1). BR09 spikes infected by *G. zeae* were not significantly different between plots containing 45 and 200 g ($P = 1.00$), 200 and 410 g ($P = 0.78$), or 45 and 410 g ($P = 0.78$). No significant difference was observed for spikes infected with *G. zeae* between 0 and 3 m for plots containing 45, 200, or 410 g. Spikes with incidence attributable to the clone were significantly different between plots containing 200 and 410 g ($P = 0.01$), but not for plots containing 45 and 200 g ($P = 0.32$) and 45 and 410 g ($P = 0.05$; Table 3). The steepest dissemination gradient from 0 to 3 in recovery of the clone (Fig. 2) was observed in plots containing 45 g (100%; $P = <0.0001$; Table 3) and a more gradual gradient in plots containing 410 g (79%; $P = 0.01$; Table 3). There was no dissemination in clone recovery between 0 and 3 m for plots containing 200 g.

Wheat trials. From the time of flowering to WH09 spike collection, the temperature range, total rainfall, and average relative humidity were 1.3 to 26.8°C, 101 mm, and 79%, respectively. Frequent rainfall observed at flowering may have contributed to high epidemic conditions with spike infection by *G. zeae* ranging from 28 to 100% in susceptible inoculated plots, 70 to 94% in moderately resistant inoculated plots, and control plots from 8 to 23% in control plots (Table 2). Differences in spike infection were significant between plots containing 45 and 410 g in both susceptible and moderately

resistant cultivars (Table 3) and between 0 and 3 m for plots containing 45 and 410 g in all cultivars (*data not shown*). However, no significant differences were observed in spike incidence attributable to the clone for the four cultivars between plots containing 45 and 410 g (Table 3), although greater differences were observed between the susceptible cultivars than the moderately resistant cultivars (Table 2). The dissemination gradient from 0 to 3 m of spike infection attributable to the clone (Fig. 2) for cvs. Tribute, Vigoro 9510, SS560, and Pioneer 26R12 was 84, 81, 62, and 81% for plots containing 45 g and 0 (an increase of 17% was observed), 44, 38, and 30% for plots containing 410 g, respectively. The dissemination gradient from 0 to 3 m in spike infection attributable to the clone in plots containing 45 g was significant for all cultivars with the exception of SS560 ($P = 0.10$; Table 3) and no significance was observed for any plots containing 410 g (Table 3).

From the time of flowering to WH10 spike collection, the temperature range, total rainfall, and average relative humidity were -1.3 to 30.3°C, 58 mm, and 83%, respectively. Low to moderate epidemic conditions were observed with spike infection by *G. zeae* ranging from 3 to 46% in susceptible inoculated plots, 4 to 40% in moderately resistant inoculated plots, and 0 to 3% in control plots (Table 2). Significant differences in spike infection by *G. zeae* were observed for cvs. Tribute, Vigoro 9510, and Pioneer 26R12 between plots containing 45 and 410 g (Table 3). Differences in spike infection at 0 m was significantly greater than at 3 m for plots containing 45 g in cv. Tribute ($P = <0.0001$) and for plots containing 410 g for cvs. Tribute ($P = 0.004$), Vigoro 9510 ($P = <0.0001$), and Pioneer 26R12 ($P = <0.0001$) (*data not shown*). A higher percentage of spike infection attributable to the clone was observed for plots containing 410 g for

cultivars when compared to plots containing 45 g (Table 2). Significant differences in spike infection attributable to the clone in plots containing 45 and 410 g were observed for the susceptible cultivars, but not for the moderately resistant cultivars (Table 3). The moderately resistant cultivars had less recovery of the clone in 410 g plots compared to the 410 g plots of the susceptible cultivars (Table 2). The dissemination gradient from 0 to 3 m in spike infection attributable to the clone (Fig. 2) for cvs. Tribute, Vigoro 9510, SS560, and Pioneer 26R12 was 100, 0 (no clones recovered), 100, and 93% for plots containing 45 g and 76, 80, 83, and 82% for plots containing 410 g, respectively. The dissemination gradient from 0 to 3 m in spike infection attributable to the clone in plots containing 45 and 410 g was significant for all cultivars with the exception of the plots containing 45 g for cv. Vigoro 9510 ($P = 1.00$; Table 3).

Virginia corn residue assessment. Corn residue coverage was assessed in commercial fields to extend the experimental field results to a real world situation. Depending on the field assessed, different tillage practices (i.e., conservative, minimal, and conventional) were used affecting the corn residue coverage reported. The number of fields reported to have used conservation, minimal, and conventional tillage was 48% (15/31), 35% (11/31), and 16% (5/31), respectively (Table 4). Ranges of average corn residue coverage for conservation, minimal, and conventional tillage fields were 62 to 91%, 7 to 90%, and 5 to 26%, respectively (Table 4). When estimating only 60% as stalk residue coverage (33), averages were 37 to 55%, 4 to 54%, and 3 to 16%, respectively (Table 4). The surface area of corn stalk pieces in experimental plots containing 45, 200, and 410 g was 0.04, 0.21, and 0.41 m^2, respectively. Plots were calculated to represent 8, 37, and 75%

corn residue coverage based on surface area of corn stalk pieces and stalk residue coverage of 5, 22, and 45%, respectively.

DISCUSSION

The data generally supported our hypothesis that the dissemination of a released clone of *G. zeae* is greater with increasing amounts of local infested corn stalk residue. For WH09 and WH10, steeper dissemination gradients were observed in nearly all of the plots (with the exception of cv. Vigoro 9510 in WH10) containing 45 g compared to those with 410 g suggesting greater contributions from larger amounts of corn residue. This trend was also true in BR09; the dissemination gradient from 0 to 3 m in spike infection attributable to the released clone was 79% for 410 g and 100% for 45 g. During a severe epidemic year for FHB, larger amounts of corn residue may produce more inoculum than smaller amounts of corn residue. However, it may be difficult to predict the impact of local corn stalk residue during years highly favorable for FHB infection (26). We observed a steeper dissemination gradient than previously reported by Fernando et al. (13) and Stack (36), but a similar gradient reported by Keller et al. (20) within 3 m. Plots containing larger inoculum concentrations (410 g) resulted in an average overall FHB dissemination to 3 m of 84% and 79% for barley in a moderate and low epidemic years respectively, and 28% and 80% for wheat in high and low to moderate epidemic years, respectively. Thus in a low to moderate epidemic year, the use of minimal or conventional tillage may help reduce within-field inoculum potential for FHB.

FHB severity varied across the wheat and barley field experiments. It has been shown that the severity of FHB epidemics may be linked to environmental conditions that

are favorable for infection and resulting disease progression (14). Similar temperature ranges were recorded for BR09, WH09, and WH10. Although a larger amount of total rainfall was recorded for BR09 (116 mm), the rainfall events occurred sporadically between the time of flowering and spike collection. For WH09, rainfall was sporadic throughout flowering but became more frequent in the days just prior to spike collection. The timing and duration of rainfall is linked to *G. zeae* inoculum production and spike infection (14,29). Regional, background sources of inoculum may contribute to FHB epidemics regardless of local management practices unless performed over widespread production areas (22).

The dissemination gradient from 0 to 3 m in the recovery of the released clone from wheat plots containing a moderately resistant cultivar was generally lower than in those containing a susceptible cultivar. Significant differences in spike infection attributable to the released clone in plots containing 45 and 410 g were observed in WH10 for the susceptible cultivars, but not for the moderately resistant cultivars. The moderately resistant cultivars had less recovery of the clone in 410 g plots compared to the 410 g plots of the susceptible cultivars.

For WH09 and WH10, both moderately resistant cultivars and susceptible cultivars were chosen based on similar flowering dates (17) to minimize differences in exposure to the released inoculum. Though some of the spikes in our research plots did not show visible symptoms of FHB, many of these spikes produced colonies of *G. zeae* when surface disinfested and plated onto FSM plates. This supports previous research reporting possible post flowering infection (7,8,20).

Cultivar selection and tillage strategy for winter wheat must be made prior to fall planting, well in advance of accurate and reliable disease forecasting information for FHB (9). Conservation tillage practices are common in Virginia (39) and will continue to sustain potential inoculum sources of *G. zeae* as long as corn residues remain on the soil surface (10,24). The Virginia residue survey showed that 42% (13/31) of the fields had residue coverage comparable to the residue coverage of experimental plots containing 410 g of inoculated corn stalks. Thus, nearly half of the fields in Virginia would be expected to have a high inoculum potential should environmental conditions be favorable for regional FHB epidemics.

The continued use of conservation tillage practices in the U.S. will likely contribute to the continued availability of regional, atmospheric populations of *G. zeae*. Consequently, integrated strategies to manage FHB may need to be employed across wheat and barley production regions (10,23). McMullen et al. (23) reported lower field severity and DON levels when multiple FHB management strategies were implemented rather than a single strategy. For hard red winter wheat, hard red spring wheat, durum wheat, hard red winter wheat, and soft red winter wheat grown in North Dakota, the planting of a moderately resistant cultivar reduced FHB levels when combined with another management strategy (fungicide or crop rotation) (23). Miller et al. (25) reported tillage practices to be less likely to reduce FHB levels than the planting of a moderately resistant cultivar. Regardless of the management strategy utilized by wheat and barley growers, environmental conditions conducive for FHB may still increase disease levels (14). Environmental conditions in the presence of high inoculum may overcome the selection and deployment of resistant cultivars to manage FHB. The use of minimal or

conventional tillage may reduce *G. zeae* inoculum potential, but in years highly favorable to FHB, even small amounts of corn residue may impact the dissemination of *G. zeae* inoculum.

Ongoing research with different amounts of naturally overwintered and naturally infested corn residue in field experiments (3,4) will help determine if similar trends of local spike infection gradients are seen as those reported here.

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LITERATURE CITED

1. Anonymous. 2010. Guidance for industry and FDA: Advisory levels for deoxynivalenol (DON) in finished wheat products for human consumption and grains and grain by-products used for animal feed. U.S. Department of Health and Human Services. Food and Drug Administration. Accessed online (July 16, 2010).
2. Bai, G., and Shaner, G. 1994. Scab of wheat: Prospects for control. Plant Dis. 78:760-766.
3. Bergstrom, G.C., and Waxman, K.D. 2008. Microplots in commercial wheat fields for quantifying the local contribution of *Gibberella zeae* from natural corn debris to Fusarium head blight and deoxynivalenol accumulation. Pages 6-8 in: Proc. 2008 Natl. Fusarium Head Blight Forum, Indianapolis, IN.
4. Bergstrom, G.C., Waxman, K.D., Schmale, D.G. III, Bradley, C.A., Sweets, L.E., Wegulo, S.N., and Keller, M.D. 2010. Effects of within-field corn debris in microplots on FHB and DON in eleven U.S. wheat environments in 2010. Pages 69-70 in: Proc. 2010 Natl. Fusarium Head Blight Forum, Milwaukee, WI.
5. Burgess, L.W., Summerell, B.A., Bullock, S., Gott, K.P., and Backhouse, D. 1994. Laboratory Manual for Fusarium Research, 3rd ed. University of Sydney, Australia.
6. Cowger, C., and Arrellano, C. 2010. Plump kernels with high deoxynivalenol linked to late *Gibberella zeae* infection and marginal disease conditions in winter wheat. Phytopathology 100:719-728.

7. Cowger, C., Patton-Özkurt, J., Brown-Guedira, G., Perugini, L. 2009. Post-anthesis moisture increased Fusarium head blight and deoxynivalenol levels in North Carolina winter wheat. Phytopathology. 99:320-327.
8. Del Ponte, E.M., Fernandes, J.M.C., and Bergstrom, G.C. 2007. Influence of growth stage on Fusarium head blight and deoxynivalenol production in wheat. J. Phytopathol. 155:577-581.
9. 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.
10. Dill-Macky, R. 2008. Cultural control practices for Fusarium head blight: Problems and solutions. 3rd Int. FHB Symposium. Szeged, Hungary.
11. Dill-Macky, R., and Jones, R.K. 2000. The effect of previous crop residues and tillage on Fusarium head blight of wheat. Plant Dis. 84:71-76.
12. 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. Can. J. Bot. 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. Francl, L., Shaner, G., Bergstrom, G., Gilbert, J., Pedersen, W., Dill-Macky, R., Sweets, L., Corwin, B., Jin, Y., Gallenberg, D., and Wiersma, J. 1999. Daily inoculum levels of *Gibberella zeae* on wheat spikes. Plant Dis. 83:662-666.

15. Gale, L.R., Ward, T.J., Balmas, V., and Kistler, H.C. 2007. Population subdivision of *Fusarium graminearum* sensu stricto in the upper midwestern United States. Phytopathology 97:1434-1439.
16. Goswami, R.S., and Kistler, H.C. 2004. Heading for disaster: *Fusarium graminearum* on cereal crops. Mol. Plant Pathol. 5:515-525.
17. Haran, M., Bhat, K.S., Molineros, J., and DeWolf, E. 2009. Estimating the risk of a crop epidemic from coincident spacio-temporal processes. J. of Agricul., Bio, and Env. Stat. 15:158-175.
18. Hickman, J.S. and Schoenberger, D.L. 1989. Corn residue: Estimating corn residue. 5-89-45M; 5-92-5M. Manhattan, KS: Kansas State University Cooperative Extension Service.
19. Keller, M.D., Thomason, W.E., and Schmale, D.G. III. 2010. The recovery of released clones of *Gibberella zeae* from winter wheat and barley is influenced by the amount of local corn stalk residue. Page 84 in: Proc. 2010 Natl. Fusarium Head Blight Forum, Milwaukee, WI.
20. 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 Dis. 94:1151-1155.
21. Leslie, J.F. and Summerell, B.A. 2006. The Fusarium Laboratory Manual. Blackwell Publishing.
22. Maldonado-Ramirez, S.L., Schmale, 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. Agric. For. Meteorol. 132:20-27.

23. 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. 3rd Int. FHB Symposium. Szeged, Hungary.
24. McMullen, M.P., Jones, R., and Gallenberg, D. 1997. Scab of wheat and barley: A re-emerging disease of devastating impact. Plant Dis. 81:1340-1348.
25. Miller, J.D., Culley, J., Fraser, K., Hubbard, S., Meloche, F., Ouellet, T., Seaman, W.L., Seifert, K.A., Turkington, K., and Voldeng, H. 1998. Effect of tillage practice on Fusarium head blight of wheat. Can. J. Plant Pathol. 20:95-103.
26. Nita, M., De Wolf, E., Madden, L., Paul, P., Shaner, G., Adhikari, T., Ali, S., Stein, J., and Osborne, L. 2006. Effect of corn residue level on disease intensity of Fusarium head blight (FHB) and on deoxynivalenol (DON) concentration: A multi-state field study. (Abstr.) Phytopathology 96:S85.
27. NRCS. Undated. Estimating crop residue cover. Agronomy Tech Note #MN-19. St. Paul, MN: National Resources Conservation Service.
28. Parry, D.W., Jenkinson, P., and McLeod, L. 1995. *Fusarium* ear blight (scab) in small grain cereals-a review. Plant Pathol. 44:207-238.
29. Paulitz, T.C. 1996. Diurnal release of ascospores by *Gibberella zeae* in inoculated wheat plots. Plant Dis. 80:674-678.
30. Pestka, J.J. 2007. Deoxynivalenol: Toxicity, mechanisms and animal health risks. Anim. Feed Sci. and Tech. 137:283-298.

31. Pestka, J.J. 2010. Deoxynivalenol: mechanisms of action, human exposure, and toxicological relevance. Arch Toxicol. 84:663-679.

32. Pestka, J.J, and Smolinski, A.T. 2005. Deoxynivalenol: Toxicology and potential effects on humans. J. Toxicol. Environ. Health B Crit. Rev. 8:39-69.

33. Pordesimo, L.O., Edens, W.C., and Sokhansanj, S. 2004. Distribution of aboveground biomass in corn stover. Biomass and Bioenergy 26:337-343.

34. Schmale, D.G., III, 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.

35. Shaner, G. 2003. Epidemiology of Fusarium head blight of small grain cereals in North America. Pages 84-119 in: Fusarium Head Blight of Wheat and Barley. K.J. Leonard and W.R. Bushnell, eds. The American Phytopathological Society Press, St. Paul, MN.

36. Stack, R.W. 1997. Gradients of Fusarium head blight in wheat along transects away from a concentrated source of *Gibberella zeae* ascospore inoculum. Page 60 in Proc. Natl. Fusarium Head Blight Forum, 10-12 November 1997. St. Paul, MN.

37. Stein, J.M., Osborne, L.E., Bondalapati, K.D., Glover, K.D., and Nelson, C.A. 2009. Fusarium head blight severity and deoxynivalenol concentration in wheat in response to *Gibberella zeae* inoculum concentration. Phytopathology. 99:759-764.

38. Sutton, J.C. 1982. Epidemiology of wheat head blight and maize ear rot caused by *Fusarium graminearum*. Can. J. Pathol. 4:195-209.

39. Thomason, W.E., Alley, M.M., Stromberg, E.L., Hagood, E.S., Herbert, A. 2009. No-tillage small grain production in Virginia. 424-005. Virginia Cooperative Extension.

40. Vos, P., Hogers, R., Bleeker, M., Reijans, M., van de Lee, T., Hornes, M., Frijters, A., Pot, J., Peleman, J., Kuiper, M., and Zabeau, M. 1995. AFLP: A new technique for DNA fingerprinting. Nucleic Acids Res. 23:4407-4414.

41. 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.

Table 1. Recovery of the released clone of *Gibberella zeae* from barley spikes collected at 0, (above the source), 3, and ≥18 m from the center of the released inoculum sources[a]

Field/ year[b]	Inoc. conc[c]	No. of plots[d]	Distance (m) from center of released source[e]	Spikes infected by *G. zeae* (%)[f]	Spikes infected by clone (%)[g]	Spikes infected by background isolates (%)[h]	Dissemination from source (%)[i]
BR08	45	2	0	20	13	7	--
BR08	45	2	3	18	2	16	85
BR08	200	2	0	35	22	13	--
BR08	200	2	3	15	1	14	95
BR08	410	2	0	56	31	25	--
BR08	410	2	3	24	5	19	84
BR08	Control	1	≥18	11	2	9	--
BR09	45	2	0	6	5	1	--
BR09	45	2	3	3	0	3	100
BR09	200	2	0	6	3	4	--
BR09	200	2	3	5	3	3	0
BR09	410	2	0	5	14	0	--
BR09	410	2	3	5	3	3	79
BR09	Control	1	≥18	9	2	7	--

[a] Percentages were calculated from the total number of barley spikes collected at 0 (above the source), at 3, and ≥18 m from the center of the released sources.

[b] The barley cv. Nomini was planted in fields in New Kent County, Virginia in 2008 and in Blacksburg, Virginia in 2009.

[c] Plots were inoculated with *G. zeae*-infested corn stalk pieces with concentrations of 45, 200, and 410 g (dry weight after autoclaving). Control plots contained 200 g noninoculated corn stalk pieces.

[d] Number of 0.84-m diameter plots containing clonal inocula (source, 0 m) or no inocula (control, ≥18 m) separated by ≥18 m within commercial fields or number of circular sampling areas at 3 m from centers of source plots.

[e] Spikes were collected at 0 (above the source), at 3, and ≥18 m from the center of the released sources.

[f] Number of infected spikes divided by the total number of spikes collected at 0, 3, and ≥18 m from the center of the released sources. Isolates of *G. zeae* were recovered from 110 barley spikes per sampling distance. Percentages rounded to integer values.

[g]The released clone of *G. zeae* recovered from total number of spikes collected. Percentages rounded to integer values.

[h]Background isolates of *G. zeae* recovered from total number of spikes collected. Percentages rounded to integer values.

[i]Dissemination gradient of recovery of the released clone of *G. zeae* from 0 (above the source) to 3 m from the source plots. Dissemination from source plots to the control plots was not calculated due to the unknown origin of the plot most influencing the recovered *G. zeae* clone. Percentages rounded to integer values.

Table 2. Recovery of the released clone of *Gibberella zeae* from wheat spikes collected at 0 (above the source), 3, and ≥18 m from the center of the released inoculum sources[a]

Field/ Year[b]	Cultivar[c]	Inoc. conc.[d]	No. of plots[e]	Distance (m) from center of released source[f]	Spikes infected by *G. zeae* (%)[g]	Spikes infected by clone (%)[h]	% Spikes infected by background isolates[i]	Dissem-ination from source (%)[j]
WH09	Tribute	45	2	0	70	19	51	--
WH09	Tribute	45	2	3	25	3	23	84
WH09	Tribute	410	2	0	94	19	75	--
WH09	Tribute	410	2	3	23	23	0	0[k]
WH09	Tribute	Control	1	≥18	8	8	0	--
WH09	Vigoro 9510	45	2	0	79	21	58	--
WH09	Vigoro 9510	45	2	3	46	4	43	81
WH09	Vigoro 9510	410	2	0	94	18	76	--
WH09	Vigoro 9510	410	2	3	75	10	65	44
WH09	Vigoro 9510	Control	1	≥18	23	3	20	--
WH09	SS560	45	2	0	28	13	15	--
WH09	SS560	45	2	3	8	5	3	62
WH09	SS560	410	2	0	88	21	66	--
WH09	SS560	410	2	3	30	13	18	38
WH09	SS560	Control	1	≥18	8	0	8	--
WH09	Pioneer 26R12	45	2	0	71	16	55	--
WH09	Pioneer 26R12	45	2	3	33	3	30	81
WH09	Pioneer 26R12	410	2	0	100	23	78	--
WH09	Pioneer 26R12	410	2	3	75	16	59	30
WH09	Pioneer 26R12	Control	1	≥18	23	0	23	--
WH10	Tribute	45	2	0	5	13	0	--
WH10	Tribute	45	2	3	0	0	0	100
WH10	Tribute	410	2	0	25	25	0	--
WH10	Tribute	410	2	3	8	6	1	76
WH10	Tribute	Control	1	≥18	0	3	0	--
WH10	Vigoro 9510	45	2	0	4	0	4	--
WH10	Vigoro 9510	45	2	3	3	0	3	0
WH10	Vigoro 9510	410	2	0	40	20	20	--
WH10	Vigoro 9510	410	2	3	5	4	1	80
WH10	Vigoro 9510	Control	1	≥18	0	0	0	--
WH10	SS560	45	2	0	3	4	0	--
WH10	SS560	45	2	3	0	0	0	100
WH10	SS560	410	2	0	8	35	0	--
WH10	SS560	410	2	3	0	6	0	83

WH10	SS560	Control	1	≥18	3	0	3	--
WH10	Pioneer 26R12	45	2	0	3	14	0	--
WH10	Pioneer 26R12	45	2	3	10	1	9	93
WH10	Pioneer 26R12	410	2	0	46	45	1	--
WH10	Pioneer 26R12	410	2	3	16	8	9	82
WH10	Pioneer 26R12	Control	1	≥18	3	0	3	--

[a] Percentages were calculated from the total number of wheat spikes collected at 0 (above the source), at 3, and ≥18 m from the center of the released sources.

[b] Wheat was planted in commercial fields in 2009 and 2010 in Blacksburg, Virginia.

[c]The moderately resistant cvs. Tribute and Vigoro 9510 and the susceptible cvs. SS560 and Pioneer 26R12 were planted.

[d] Plots were inoculated with *G. zeae*-infested corn stalk pieces with concentrations of 45 and 410 g (dry weight after autoclaving). Control plots contained 200 g noninoculated corn stalk pieces.

[e] Number of 0.84-m diameter plots containing clonal inocula (source, 0 m) or no inocula (control, ≥18 m) separated by ≥18 m within commercial fields or number of circular sampling areas at 3 m from centers of source plots.

[f] Spikes were collected at 0 (above the source) and at 3 from the center of the released sources. Spikes were also sampled from control plots at distances ≤18 m.

[g] Number of infected spikes divided by the total number of spikes collected at 0 and 3 m from the center of the released sources. Isolates of *G. zeae* were recovered from 80 wheat spikes in 2009 and 2010. Percentages rounded to integer values.

[h] The released clone of *G. zeae* recovered from total number of wheat spikes collected. Percentages rounded to integer values.

[i] Background isolates of *G. zeae* recovered from total number of wheat spikes collected. Percentages rounded to integer values.

[j] Dissemination gradient of recovery of the released clone of *G. zeae* from 0 (above the source) to 3 m from the source plots. Dissemination from source plots to the control plots was not calculated due to the unknown origin of the plot most influencing the recovered *G. zeae* clone. Percentages rounded to integer values.

[k] No dissemination gradient was seen at this field plot location. An increase of 17% occurred from the source to 3 m. This was the only instance this trend was observed.

Table 3. Probabilities from a statistical analysis comparing 45 and 410 g of inoculated corn stalk pieces in wheat and barley research plots from 2008-2010[a]

Cultivar[b]	Field/ Year	Spikes infected by *G. zeae*[c]	Spikes infected by clone at 0 m: 45 versus 410 g plots[d]	Spikes infected by clone at 45 g plots: 0 versus 3 m[e]	Spikes infected by clone at 410 g plots: 0 versus 3 m[f]
Nomini	BR08	<0.0001	0.002	0.01	<0.0001
Nomini	BR09	0.78	0.05	<0.0001	0.01
Tribute	WH09	0.0004	1.000	0.004	0.56
Vigoro 9510	WH09	0.01	0.55	0.003	0.17
SS560	WH09	<0.0001	0.14	0.10	0.14
Pioneer 26R12	WH09	<0.0001	0.32	0.01	0.32
Tribute	WH10	0.001	0.05	<0.0001	0.002
Vigoro 9510	WH10	<0.0001	1.000	1.000	0.004
SS560	WH10	0.17	<0.0001	<0.0001	<0.0001
Pioneer 26R12	WH10	<0.0001	<0.0001	0.02	<0.0001

[a] The GENMOD procedure was used to determine significance of differences between least-squares means for each variable shown.

[b] The moderately resistant barley cv. Nomini was planted in 2008 and 2009. The moderately resistant wheat cvs. Tribute and Vigoro 9510 and the susceptible wheat cvs. SS560 and Pioneer 26R12 were planted in 2009 and 2010.

[c] Number of infected spikes divided by the total number of spikes collected at 0 m in plots containing 45 and 410 g of *G. zeae*-infested corn residue.

[d] Incidence of spike infection attributable to the released clone within 45 and 410 g plots.

[e] Incidence of spike infection attributable to the released clone within 45 g plots when accounting for distances of 0 and 3 m.

[f]Incidence of spike infection attributable to the released clone within 410 g plots when accounting for distances of 0 and 3 m.

Table 4. Management practices and residue assessment of 31 commercial wheat and barley fields in 10 Virginia counties in 2009[a]

Tillage type[b]	VA county[c]	Implement used after corn harvest[d]	Corn purpose	Yield after corn harvest (bu/A)[e]	Reported residue coverage (%)[f]	Stalk residue coverage (%)[g]
Conservation	Northumberland	Bush-hog	Grain	180	91±2	55
Conservation	Essex	Stalk shredder	Grain	170	90±2	54
Conservation	Essex	John Deere StalkMaster head	Grain	155	86±2	52
Conservation	Gloucester	None	Grain	140	85±3	51
Conservation	Middlesex	Stalk chopper	Grain	125	85±1	51
Conservation	Westmoreland	Stalk shredder	Grain	165	80±1	48
Conservation	Virginia Beach	Stalk Mower	Grain	200	79±3	47
Conservation	Northumberland	Bush-hog	Grain	170	78±2	47
Conservation	Virginia Beach	Stalk Mower	Grain	185	77±4	46
Conservation	Westmoreland	Bush-hog	Grain	160	75±2	45
Conservation	Caroline	No-till corn planter/Drill	Grain	155	73±1	44
Conservation	Caroline	Unknown	Grain	170	72±2	43
Conservation	Virginia Beach	Stalk Mower	Grain	200	65±3	39
Conservation	Caroline	No-till corn planter/Drill	Grain	160	63±8	38
Conservation	Virginia Beach	Stalk Mower	Grain	204	62±2	37
Minimal	Northumberland	Turbo-till	Grain	180	90±2	54
Minimal	Middlesex	Turbo-till-one pass	Grain	160	87±1	52
Minimal	Northumberland	Turbo-till	Grain	190	85±2	51
Minimal	Essex	Bush-hog and turbo-till	Grain	200	66±3	40
Minimal	Montgomery	Deep-ripped-one pass	Silage	Unknown	27±2	16
Minimal	Chesapeake	Disk and roller	Grain	150	24±5	14
Minimal	Montgomery	Deep-ripped-one pass	Silage	Unknown	22±2	13
Minimal	Montgomery	Deep-ripped-one pass	Silage	Unknown	12±1	7
Minimal	Montgomery	Deep ripped-one pass	Silage	Unknown	10±2	6
Minimal	Montgomery	Deep-ripped-one pass	Silage	Unknown	7±1	4
Minimal	Chesapeake	Disk and roller	Grain	140	7±1	4
Conventional	Chesapeake	Disk-2 passes, roller	Grain	170	26±1	16
Conventional	Culpepper	Unknown	Silage	Unknown	22±1	13
Conventional	Chesapeake	Disk-2 passes, roller	Grain	175	8±1	5
Conventional	Culpepper	Unknown	Silage	Unknown	9±1	5
Conventional	Chesapeake	Stalk mower, disk-3 passes, roller	Grain	195	5±1	3

[a]Percentage of corn residue coverage from fields where a small grain was planted after corn harvest.

[b]Classification of tillage usage used before planting of small grain: conservative (i.e., no-till), minimal, and conventional.

[c]Virginia Cooperative Extension agents from 11 counties participated in assessing fields for corn residue coverage.

[d] Descriptions from extension agents about tillage implement usage in fields prior to planting of small grain.

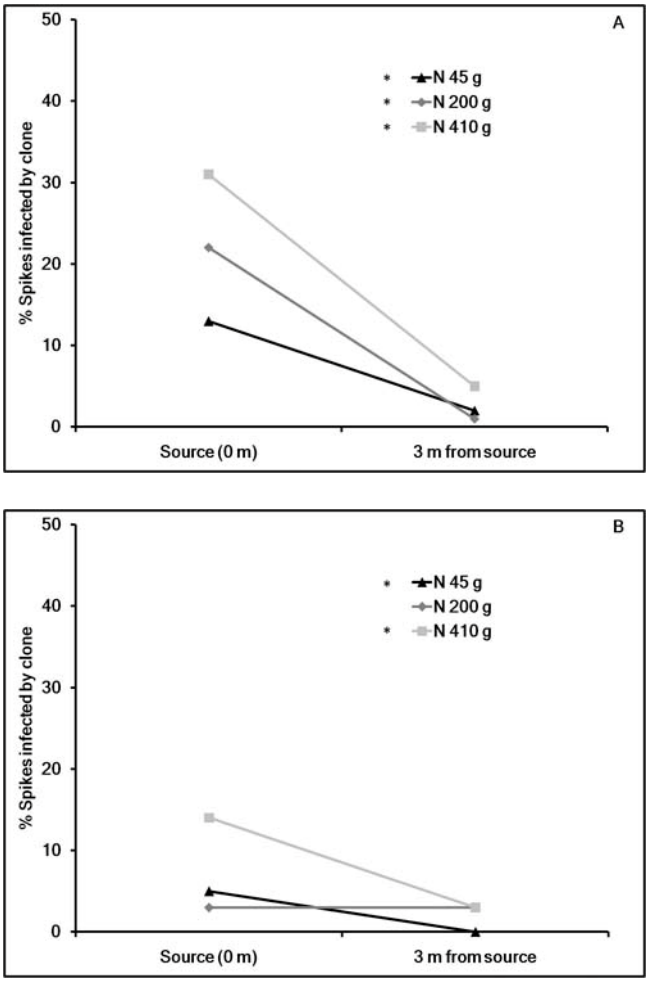
[e]Estimation of bushels per acre of corn harvested from fields.

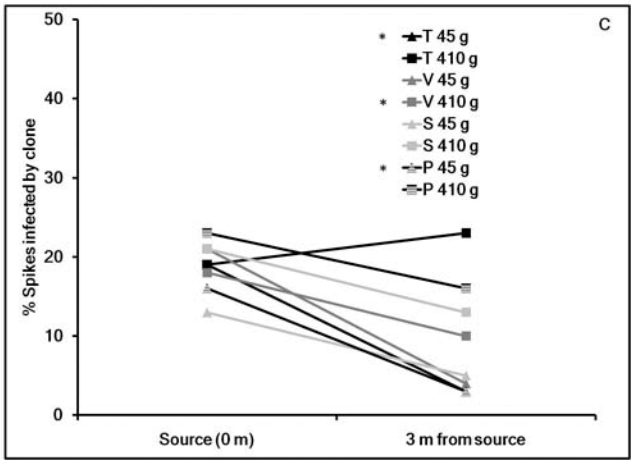
[f]Average corn residue coverage and standard error reported from 4 locations within each field assessed. Both stalk and leaf matter remaining after harvest included in reported value. Percentages rounded to integer values.

[g]Corn residue coverage multiplied by estimated stalk cover (60%) to eliminate possible leaf matter left after harvest. Percentages rounded to integer values.

Fig. 1. Experimental 0.84-m diameter wheat plots containing 45 g (5 stalks, left) and 410 g (50 stalks, right) of corn stalk pieces colonized with a single clone of *Gibberella zeae*.

Fig. 2. Effect of inoculum concentration on the dissemination from 0 (above the source) to 3 m of the released clone of *Gibberella zeae* in BR08 (A), BR09 (B), WH09 (C), and WH10 (D) replicated field plots. The results for the moderately resistant cv. Nomini are shown in A and B. The results from two moderately resistant cvs. Tribute (T) and Vigoro 9510 (V) and two susceptible cvs. SS560 (S) and Pioneer 26R12 (P) are shown in C and D. * = difference in dissemination significant at $P < 0.05$.

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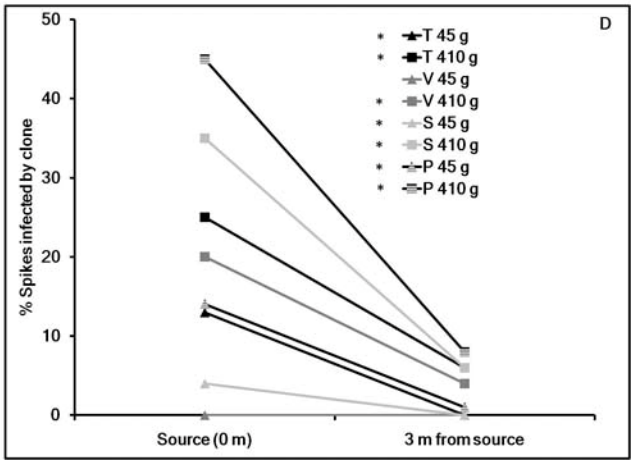
P 410 g
Source (0 m)
3 m from source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* S 45 g
* S 410 g
* P 45 g
* P 410 g
Source (0 m)
3 m from source

Chapter 4. Managing Fusarium Head Blight in Virginia Small Grains

The following chapter was formatted to facilitate publication for Virginia Cooperative Extension. This work was originally published by Keller, Griffey, Lin, Scruggs, Stromberg, Thomason, and Schmale as publication 3102-1535 (2011) (http://pubs.ext.vt.edu/3102/3102-1535/3102-1535.html).

MANAGING FUSARIUM HEAD BLIGHT IN VIRGINIA SMALL GRAINS (PUBLICATION 3102-1535)

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INTRODUCTION

Fusarium head blight (FHB), or scab, continues to impact small grain crops grown in Virginia. Caused primarily by the fungus *Fusarium graminearum* (also known as *Gibberella zeae*), this disease can negatively impact yield and grain quality. Grain may also contain toxins (mycotoxins) produced by the fungus and reduce the price received for grain at local mills and elevators. Corn and small grain residues remaining in the field prior to small grain planting are known to provide a place for the fungus to overwinter and proliferate during favorable environmental conditions.

SYMPTOMS

Symptoms of premature whitening or bleaching on heads **(Figure 1)** may be seen within days following infection. Heads are considered most susceptible when anthers are exposed during flowering **(Figure 2)**, although late infections can occur. Spore masses of the fungus may appear pink to orange and may be visible on infected heads **(Figure 3, 4)**. Often one-third to one-half of the head is affected, and in some cases the entire head may be colonized with the fungus. The bleached areas of the head may be sterile and contain shriveled and discolored kernels **(Figure 5)**. In barley, heads may appear to have a bleached or a brown, water-soaked appearance. Small, blue-black spore containers (perithecia) may be visible on crop residues remaining in the field and may be visible on infected heads closer to harvest.

SURVIVAL AND MOVEMENT OF SPORES

Crop residues may remain on the soil surface following a previous season's

harvest of small grains and corn. These residues can provide an overwintering media for the fungus and allow infestation to occur on small grains planted into these fields **(Figure 6)**. When optimal weather conditions including average temperatures of 75 to 85°F, extended periods of high humidity, and frequent rainfall occur, infection of flowering small grains is likely. Spores are produced and discharged from spore containers called perithecia found on crop residue. Discharged spores may be windblown or rain-splashed onto heads of small grains. Long-distance transport of these spores is possible, thus creating the potential for infection of fields statewide. Heads are susceptible from flowering through development of kernels. When late infection occurs, symptoms may not be visible due to grain maturation.

MYCOTOXINS

The mycotoxins most often found in Virginia small grains are deoxynivalenol (also referred to as DON or vomitoxin) and zearalenone. The presence of DON in grain can cause symptoms of vomiting and feed refusal in non-ruminant animals. When DON is found in harvested grain, producers will typically be offered lower prices or have grain refused when attempting to sell contaminated grain at elevators or mills. Advisory levels have been established by The Food and Drug Administration (FDA) for DON in food and feed **(Table 1)**. The presence of zearalenone in grain can cause reproductive problems in animals.

FHB-infected grain can be tested for mycotoxins for a fee by contacting The Virginia-Maryland Regional College of Veterinary Medicine Toxicology Lab, College of Veterinary Medicine, Virginia Tech, Blacksburg, VA, 24061 at 540-231-4835.

For more information about Virginia mycotoxins and FDA guidelines visit the following websites:

http://pubs.ext.vt.edu/news/livestock/2009/07/APS_07-10-09_10.html
www.gipsa.usda.gov/GIPSA/documents/GIPSA_Documents/b-vomitox.pdf

EFFECTS ON YIELD AND GRAIN QUALITY

In addition to a decrease in feed consumption and/or feed refusal in livestock, *Fusarium*-damaged grain can contain kernels that are shriveled and have a low test weight causing low yields and limited marketability. The amount of kernel damage by FHB is based on the extent of infection.

For a rough estimate of yield loss:

randomly select 100 heads from across the field; sort heads into piles for healthy heads, partially infected heads, and completely infected heads; and add number of completely infected heads to 1/2 the number of partially infected heads to calculate estimated yield loss percent (Method by Kansas State University),

www.ksre.ksu.edu/path-ext/factSheets/Wheat/Wheat%20Scab.asp

Diseased grain with high levels of mycotoxins can be mixed with healthy grain to reduce the overall mycotoxin contamination level, but often at a reduced price. In environmentally favorable years for FHB, late infection may cause grain to appear healthy, but may contain high DON levels. Milling quality is reduced by infected kernels lowering flour yield and impacting baking traits.

MANAGEMENT OF FHB

FHB epidemics occur sporadically and management is best accomplished when multiple strategies from those listed below are implemented. When environmental conditions are favorable for disease, the use of one strategy alone may prove ineffective against FHB.

RESISTANT CULTIVARS

To date, there is no cultivar of wheat or barley completely resistant to FHB. However, many cultivars with varying levels of resistance are available to Virginia growers. Resistance to disease spread within the infected head and to degradation of mycotoxins varies with each cultivar. To get current information regarding available Virginia cultivars and their trial performance ratings visit the following website:

http://pubs.ext.vt.edu/3007/3007-1455/3007-1455.html

TILLAGE OF CROP RESIDUE

No-till cropping systems are widespread in Virginia and large amounts of corn and small grains residue may remain on the soil surface. These residues can provide an overwintering medium for *Fusarium* species causing FHB and improve chances for FHB infection in the subsequent small grains crop. In no-till or minimal tillage cropping practices, reducing the size and spreading the residues may allow faster decomposition and reduce potential for the fungus to overwinter and produce spores. However, the use of tillage implements may have disadvantages such as moisture loss and erosion. Tillage practices that minimize and bury residue may reduce FHB in environmentally favorable

years.

CROP ROTATION

Small grains crops planted following corn or a small grain may have an increased chance of FHB infection in environmentally favorable years. Small grains rotated with soybean or another non-host crop have been shown to reduce FHB infection and mycotoxin contamination.

FUNGICIDE

Fungicides, with correct application, have the potential to reduce FHB by 50% to 60%. Fungicides are typically applied at early heading for barley and at early flowering for wheat. Proper coverage of the head is necessary and more information can be found at the link below. Fungicide trials are conducted in Virginia and recommendations are available based on these results. A multi-state FHB forecasting model is available to assist with fungicide application decisions. This model is a collaboration of multiple institutions and was developed to predict the risk of an epidemic based on weather patterns prior to the flowering of wheat. Users can select their wheat growing region, date of crop assessment, type of wheat (spring vs. winter) and a percentage of risk will be calculated. Commentary from a Virginia Cooperative Extension Agent may be available for the region chosen. Although this model is not equipped for assessments of barley at this time, barley-specific models may be available in the future.

Fungicide Application Technique:

http://www.ag.ndsu.edu/scabsmart/best%20application.html

Multi-state model: *http://www.wheatscab.psu.edu*

SEED TREATMENT

Kernels (seeds) colonized with the fungus may reduce stands due to poor germination. Planting certified seed or treated seed may reduce seedling blight caused by seeds colonized with the fungus. Seed with a test weight of at least 58 pounds per bushel and 90% germination is recommended. If replanting of saved seed is necessary, seed should be treated before planting if harvested from an FHB-infected field. Replanting of saved seed may illegally violate a cultivar patent. Check for patents on cultivars from which seed is saved.

PLANTING DATE

FHB infection depends on the amount of rainfall before and during flowering of small grains. Staggering planting dates or planting multiple cultivars will allow for different flowering dates and thus different timing for FHB susceptibility.

HARVEST CONSIDERATIONS

Increasing the fan speed of combines can remove light-weight FHB kernels with the chaff. It should be noted that these diseased kernels may germinate and provide a source of FHB. Kernels affected by late FHB infections may not exhibit shriveling and low test weight and therefore may not be removed. Fan speed may not remove diseased barley and oat kernels as easily as diseased wheat kernels.

QUALITY STANDARDS OF GRAIN MILLS AND ELEVATORS

The Federal Grain Inspection Service (FGIS) has been an agency of the U.S. Department of Agriculture since 1974. In 1994, a reorganization of departments led to the merging of FGIS and the Packers and Stockyards Administration to form the Grain Inspection, Packers and Stockyards Administration (GIPSA). FGIS now acts as a program within GIPSA. The national inspection of grain is conducted by federal, state, and private laboratories under the direct supervision of FGIS.

The FGIS grade is determined by test weight, heat damage, total damaged kernels, foreign material, shrunken or broken kernels, and total defects. Numerical grades U.S. No. 1 through U.S. No. 5 are assigned with U.S. No. 1 representing the highest quality. The moisture and mycotoxin levels of the grain do not affect the FGIS grade, but are still used in the determination of quality. Falling number (FN) is used to determine amount of kernel sprouting. FN of 300 seconds or higher usually indicates minimal sprouting damage.

Two special grade requirements might require observations of garlicky and smutty grain. If more than two green garlic bulblets or an equivalent quantity of dry or partially dry bulblets are found, the grain is considered garlicky. Smutty grain may contain smut balls, portions of smut balls, or spores of smut in a 250 g portion.

Sour, musty, or other commercially objectionable foreign odors (COFOs) from the grain may result in price reductions or rejection. Sour odors are described as rancid and sharp and are generally caused by insect waste and/or fermenting grain.

Musty odors are generally earthy and moldy while COFOs include any other odors not typically found in grain such as odors of fertilizer, smoke, skunk, or decaying animal

or plant material.

Heating of grain is common in grain that is spoiling and will not only have a high temperature, but may also have a sour odor. Heating can be caused by insect infestation or by microorganisms. Sound grain can be warm due to storage in bins or other containers in hot weather.

Optional assessments may include insect infestation, pesticide residue, and heavy metals. Grain is also tested for mycotoxins such as DON, zearalenone, and others (see Mycotoxins section for more information). Thin-layer chromatography (thins) may be used to detect the presence of toxins within grain. Examples of quality testing for Virginia grain are shown **(Table 2 & Table 3)**. Quality of grain is determined by standardized testing discussed on the GIPSA website:

http://archive.gipsa.usda.gov/reference-library/standards/810wheat.pdf
http://archive.gipsa.usda.gov/reference-library/standards/810barley97.pdf

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Table 1. FDA Advisory Levels for DON.

FDA Advisory Levels for DON (parts per million)	End-Use Description
1 ppm	Finished grain products for human consumption
	Grain and grain by-products destined for swine and other animal species (except cattle and chickens); not to exceed 20% of the diet for swine, and not to exceed 40% for other animal species.
10 ppm	Grain and grain by-products for ruminating beef and feedlot cattle older than 4 months and for chickens; not to exceed 50% of the diet.

Table 2. Quality Standards of The Mennel Milling Company, Roanoke, Virginia.

(Information courtesy of C.J. Lin, The Mennel Milling Co.)

Quality Standards for Wheat	Requirements*
FGIS Grade	No. 2 or better
Moisture	13.5% or below
Vomitoxin (DON)	2 ppm or below
Falling Number (FN)	>250 seconds
Smut Ball	None
Garlic Ball	None
Infestation	None
Pesticide Residue	None

*Any wheat exceeding the above limits will be subject to rejection or market discount.

Table 3. Quality Standards of Osage Bio Energy, Hopewell, Virginia.

(Information courtesy of Bill Scruggs, Osage Bio Energy)

Quality Standards for Barley	Recommended Requirements*
FGIS Grade	No. 2 or better
Test weight	46 lbs.
Moisture	13% or below
Vomotoxin (DON)	2 ppm or below
Thins	15%
Total Damage	4%
Heat Damage	0.3%
Garlic	Less than 3 bulbs
Sound	94%
Foreign material	2%
Infestation	None
Musty Odor	None
Sour Odor	None
COFO	None
Smutty	None
Heating	None
Damage/Stained material	None

*Barley not meeting the recommended requirements will be subject to market discount or rejection due to the feed requirements on Barley Protein Meal (BPM), the feed co-product from ethanol production.

Figure 1. Premature bleaching of wheat heads caused by FHB. (photo by Melissa Keller)

Figure 2. Anthers emerging from wheat head during flowering. (photo by Melissa Keller)

Figure 3. Orange spore masses on wheat head. (photo by Melissa Keller)

Figure 4. Orange spore masses on barley head. (Used with Permission of Carl Griffey)

Figure 5. Healthy kernels (left) and diseased kernels (right) caused by FHB. (photo by Melissa Keller)

Figure 6. FHB disease cycle. (photos by Melissa Keller)

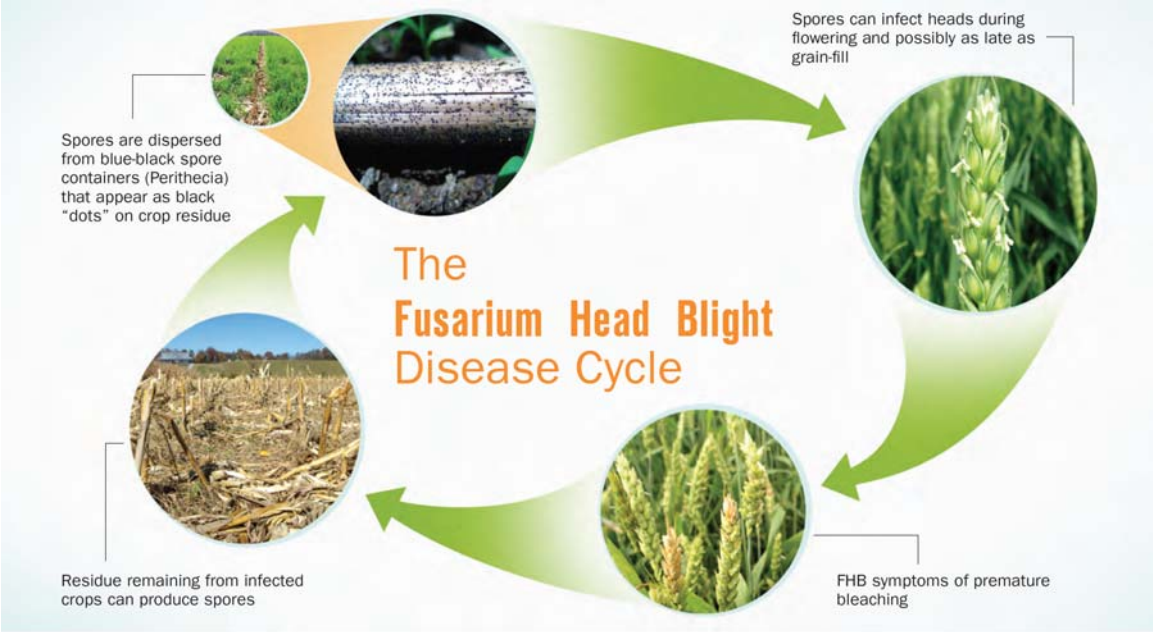

Figure 6. FHB disease cycle. *(photos by Melissa Keller)*

Chapter 5. Conclusions and Future Direction

Fusarium head blight (FHB) will continue to cause losses to small grains producers until resistant cultivars are available or better management strategies are identified. The continued use of conservation tillage in the United States (9,18), and in the state of Virginia (21), will contribute to atmospheric populations of *Gibberella zeae* and thus to FHB infection of small grains. Maintaining crop residue on the soil surface in conservation tillage systems is crucial to prevention of soil erosion and moisture loss to maintain nutrients for subsequent crops. Production costs due to fuel, labor, and equipment can be minimized with conservation tillage. One of the negative effects of maintaining corn, wheat, and barley residue is its potential to harbor plant pathogens. FHB levels are known to increase when corn residue is present in wheat fields (10).

However, despite persistent *G. zeae* proliferation on corn residue in no-tillage and minimal tillage systems, small grains producers are choosing to maintain corn residue in increasing numbers (9,18). Our research found that 42% (13/31) of Virginia small grain fields contained corn residue coverage comparable to our larger inoculum source plots (410 g), and thus the potential for higher FHB levels. Overwintering sites for *G. zeae* on corn residue are likely to intensify with the increase of corn production due to incentives for ethanol-based fuel. The decision to maintain conservation tillage practices in Virginia and in the United States must be respected rather than challenged by FHB researchers. FHB epidemics are sporadic and the environmental and economical benefits of conservation tillage are consistent. However, future FHB epidemics are imminent; therefore researchers must find alternate ways to reduce *G. zeae* populations. Research

efforts are now focused on reducing the pathogen populations on residues and residue decomposition rates (9) and the use of integrated management strategies (17).

FHB management research will benefit from progress in the following two areas: i) an update of FHB risk prediction models, and ii) to determine successful integrated FHB management strategies. The 2011 FHB risk assessment model has undergone necessary improvements such as allowing prediction information as early as March and the addition of more wheat growing states from the southern United States region (8). Commentary has been solicited from extension agents in many of the states; however, others are lacking in participation. Because ground assessments of symptoms and fungicide treatment timing may be specific to each state, updated extension agent commentary posted to the model is needed. A grower's access to the predictions given by the FHB model with complementary fungicide suggestions by agents within their state is a critical first-line-of-defense in the FHB management challenge.

Currently, the FHB risk assessment model uses temperature, relative humidity, and rainfall reported within a region to generate predictions of epidemic severity (7). The model does not take into consideration the potential amount of *G. zeae* inoculum within the regions. With better understanding of the contribution of both within-field and atmospheric inoculum sources, it may be necessary for the model to take into account residue tillage practices within that region to get an accurate FHB risk assessment. We found moderate epidemic years to be the most predictable when considering the influence from different inoculum amounts. Atmospheric inoculum sources have been investigated (15) and quantification of within-field sources has been addressed (13). The risk model also assumes FHB susceptibility to occur at anthesis and early stages of development;

however research has recently shown late infection can possibly occur as late as grain-fill (5,6). It has also been suggested the period of FHB susceptibility is dependent upon cultivar resistance level based on preliminary data of deoxynivalenol (DON) accumulation and symptom expression (4) and improvements to risk prediction based on infection timing should be addressed (14). We found in low to moderate epidemic years moderately resistant cultivars were effective in reducing FHB levels when large amounts of corn residue were present. Tillage of crop residue in low and moderate epidemic years may also reduce within-field inoculum sources more than in high epidemic years. The current risk assessment model does not include an option to predict FHB potential on barley or provide management suggestions. Inclusion of barley within the current risk model or a separate barley risk model is needed to support barley growers within the United States. South Dakota State University has stated this need and has presented preliminary data (3).

Determining successful integrated FHB management strategies has been a challenge for many years. Small grains production continues to be affected by FHB epidemics. Successful FHB management requires integrating multiple strategies rather than the use of a single strategy (17). In winter wheat, spring wheat, and durum wheat grown in North Dakota, the planting of a moderately resistant cultivar reduced FHB levels when combined with either fungicide or crop rotation (17). Preliminary reports from Wisconsin field studies concluded the use of a moderately resistant cultivar and a fungicide may effectively reduce FHB (11), but these strategies were not as clear in New York field experiments (22). Although moderately resistant cultivars are available and proven to be effective in reducing FHB, harvested crops may have reduced yields

compared to those of available susceptible cultivars (11). This may impact small grains producer's decisions for FHB management strategies (19).

A survey was conducted in North Dakota and Minnesota to determine the most widely used FHB management strategies among growers (16). It was found that the use of moderately resistant cultivars was the most common single strategy. For those using integrated management practices, the use of three strategies, cultivar resistance, fungicide, and crop rotation, was the most widely implemented. Another interesting conclusion of the survey was the high percentage of growers (69%) reporting their use of university extension resources to make FHB management decisions (16).

When conducting field experiments with integrated management strategies, to avoid exaggeration of FHB incidence, FHB severity, and DON accumulation, infection caused by natural conditions may be more accurate. The use of artificial *G. zeae* inoculation in field trials has been questioned due to the possibility of representing conditions that would not normally be seen in grower's fields (23). Small plot field research involving natural corn residue is ongoing (1,2). Preliminary results have been found indicating the need for regional inoculum reduction to lower FHB and DON levels (2). Additional research on the effects of naturally infested corn residue is critical to the continued improvement of FHB integrated management strategies.

Our research has contributed to the need for quantification of both within-field and atmospheric *G. zeae* inoculum sources. We have shown both sources to be influencing Fusarium head blight (FHB) levels in all field experiments in all years; therefore both within-field and atmospheric sources must be considered when making FHB management decisions. We have also shown dissemination of *G. zeae* infection

from our source plots to be steeper than those reported in previous literature (12,20). Ultimately, weather conditions (specifically the timing and duration of rainfall) determine the impact of within-field inoculum sources. The continued use of no-tillage and minimal tillage will continue to contribute to both within-field and atmospheric inoculum sources. The identification of successful integrated FHB management strategies are needed until a resistant cultivar is commercially released to small grains producers.

Literature Cited

1. Bergstrom, G.C., and Waxman, K.D. 2008. Microplots in commercial wheat fields for quantifying the local contribution of *Gibberella zeae* from natural corn debris to Fusarium head blight and deoxynivalenol accumulation. Pages 6-8 in: Proc. 2008 Natl. Fusarium Head Blight Forum, Indianapolis, IN.
2. Bergstrom, G.C., Waxman, K.D., Schmale, G.C. III, Bradley, C.A., Sweets, L.E., Wegulo, S.N., and Keller, M.D. 2010. Effects of within-field corn debris in microplots on FHB and DON in eleven U.S. wheat environments in 2010. Pages 69-70 in: Proc. 2010 Natl. Fusarium Head Blight Forum, Milwaukee, WI.
3. Bondalapati, K.D., and Stein, J.M. 2010. Validation of barley DON risk prediction model. Pages 71-73 in: Proc. 2010 Natl. Fusarium Head Blight Forum, Milwaukee, WI.
4. Cowger, C. 2010. Defining the window of scab susceptibility in mid-atlantic winter wheat. Page 76 in: Proc. 2010 Natl. Fusarium Head Blight Forum, Milwaukee, WI.
5. Cowger, C., and Arrellano, C. 2010. Plump kernels with high deoxynivalenol linked to late *Gibberella zeae* infection and marginal disease conditions in winter wheat. Phytopathology 100:719-728.
6. Cowger, C., Patton-Özkurt, J., Brown-Guedira, G., and Perugini, L. 2009. Post-anthesis moisture increased Fusarium head blight and deoxynivalenol levels in North Carolina winter wheat. Phytopathology 99:320-327.

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. DeWolf, E., Shah, D., Paul, P., Madden, L., Willyerd, K., Knight, P., and Miller, D. 2010. Advances in the development and application of prediction models for FHB and DON. Page 79 in: Proc. 2010 Natl. Fusarium Head Blight Forum, Milwaukee, WI.
9. Dill-Macky, R. 2008. Cultural control practices for Fusarium head blight: Problems and solutions. 3rd Int. FHB Symposium. Szeged, Hungary.
10. Dill-Macky, R. and Jones, R.K. 2000. The effect of previous crop residues and tillage of Fusarium head blight of wheat. Plant Dis. 84:71-76.
11. Esker, P., Koval, N., Lackermann, K., Conley, S., Gaska, J., and Martinka, M. 2010. Integrated management for Fusarium head blight in Wisconsin. Page 80 in: Proc. 2010 Natl. Fusarium Head Blight Forum, Milwaukee, WI.
12. 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.
13. 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 Dis. 94:1151-1155.
14. Madden, L.V., Kriss, A.B., Paul, P.A., and Xu, X. 2010. Data mining of weather and climatic data to improve risk prediction of Fusarium head blight. Page 85 in: Proc. 2010 Natl. Fusarium Head Blight Forum, Milwaukee, WI.

15. Maldonado-Ramirez, S.L., Schmale, 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. Agric. For. Meteorol. 132:20-27.
16. McKee, G., Ransom, J., and McMullen, M. 2010. Determinants of adoption of scab management techniques. Pages 86-89 in: Proc. 2010 Natl. Fusarium Head Blight Forum, Milwaukee, WI.
17. McMullen, M.P., Halley, S., Schatz, B., Meyer, S., Jordahl, J., and Ransom, J. 2008. Integrated strategies for Fusarium head blight management in the United States. 3rd Int. FHB Symposium. Szeged, Hungary.
18. McMullen, M.P., Jones, R., and Gallenberg, D. 1997. Scab of wheat and barley: A re-emerging disease of devastating impact. Plant Dis. 81:1340-1348.
19. Ransom, J.K., Pederson, J., and Halley, S. 2010. Influence of row spacing, seeding rate, fungicide, and variety on yield and FHB development in spring wheat, durum, and barley. Page 93 in: Proc. 2010 Natl. Fusarium Head Blight Forum, Milwaukee, WI.
20. Stack, R.W. 1997. Gradients of Fusarium head blight in wheat along transects away from a concentrated source of *Gibberella zeae* ascospore inoculum. Page 60 in Proc. Natl. Fusarium Head Blight Forum, 10-12 November 1997. St. Paul, MN.
21. Thomason, W.E., Alley, M.M., Stromberg, E.L., Hagood, E.S., Herbert, A. 2009. No-tillage small grain production in Virginia. 424-005. Virginia Cooperative Extension.

22. Waxman, K.D., and Bergstrom, G.C. 2010. Evaluation of integrated methods for managing FHB and DON in winter wheat in New York in 2010. Pages 104-105 in: Proc. 2010 Natl. Fusarium Head Blight Forum, Milwaukee, WI.
23. Willyerd, K., Madden, L., McMullen, M., Wegulo, S., Bockus, B., Sweets, L., Bradley, C., Wise, K., Hershman, D., Bergstrom, G., Grybauskas, A., Osborne, L., Esker, P., and Paul, P. 2010. Inoculated field trials for evaluating FHB/DON integrated management strategies. Pages 109-110 in: Proc. 2010 Natl. Fusarium Head Blight Forum, Milwaukee, WI.

APPENDIX A

Cultivar Susceptibility

Virginia Soft Red Winter Wheat Cultivars Tested for Susceptibility to *Gibberella zeae* strain Gz_VA_GPS13N4_3-ADON in Growth Chamber

Protocol: Two Virginia soft red winter wheat cultivars moderately resistant to Fusarium head blight (FHB) (Tribute and Vigoro 9510) and two susceptible cultivars (Southern States 560 (SS560) and Pioneer 26R12) were tested for susceptibility to a *Gibberella zeae* strain Gz_VA_GPS13N4_3-ADON collected from Riner, VA in 2006. Pots containing seeds of each cultivar were placed into a growth chamber (Conviron, Pembina, ND) at temperatures from 18°C to 24°C depending upon the time of day. During vernalization temperature was reduced to 4°C. Inoculation of *G. zeae* was performed using both spray and point inoculation methods (1) at flowering. Spore concentration of 5×10^5 was calculated using a hemocytometer and dilution plates of 1:10, 1:100, and 1:1000 were used to test viability of spores. Wheat plants of each cultivar were either inoculated with spray inoculation or point inoculation and within two weeks were assessed for visible symptoms of FHB. All cultivars had visible symptoms of FHB (2) caused by the Gz_VA_GPS13N4_3-ADON strain.

Literature Cited

1. Miedaner, T., Moldovan, M., Ittu, M. 2003. Comparison of spray and point inoculation to assess resistance to Fusarium head blight in a multienvironment wheat trial. Phytopathology 93:1068-1072.
2. Sutton, J.C. 1982. Epidemiology of wheat head blight and maize ear rot caused by *Fusarium graminearum*. Can. J. Pathol. 4:195-209.

Fig. 1. Winter wheat cultivars Tribute, Vigoro 9510, SS560, and Pioneer 26R12 were placed in growth chamber and inoculated to confirm susceptibility to *Gibberella zeae* strain Gz_VA_GPS13N4_3-ADON before using cultivars for subsequent field experiments.

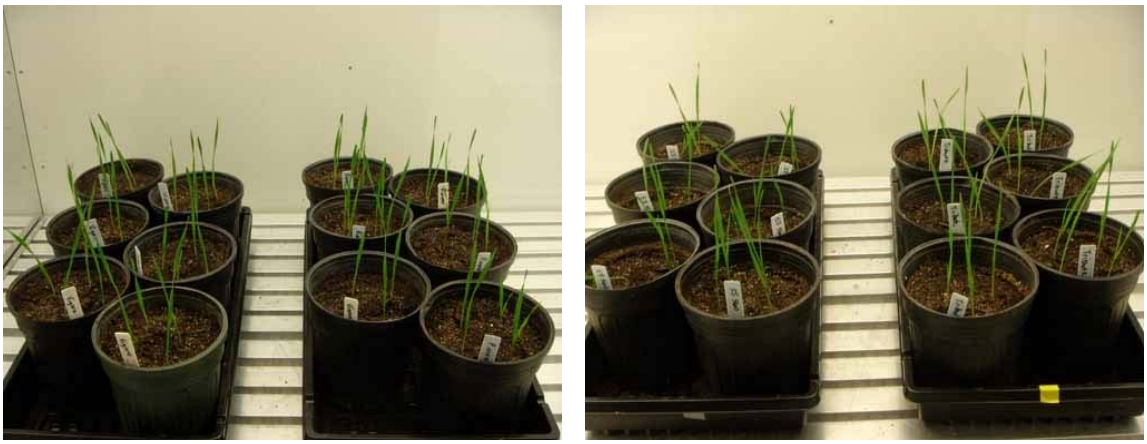

Fig. 2. FHB symptoms of premature bleaching observed on winter wheat cultivars A) Tribute, B) Vigoro 9510, C) SS560, and D) Pioneer 26R12 following inoculation with *Gibberella zeae* strain Gz_VA_GPS13N4_3-ADON.

APPENDIX B

Wheat Planting

Fig. 1. Four winter wheat cultivars (Tribute, Vigoro 9510, Pioneer 26R12, and Southern States (SS) 560) were planted at Virginia Tech's Kentland Research Farm in 2009 and 2010. Corn fields (A) were harvested, deep-ripped and disked three times to minimize corn residue (B) before planting (C), and wheat planter was cleaned before addition of each cultivar's seed (D) to avoid mixing of cultivars within plots.

Appendix C

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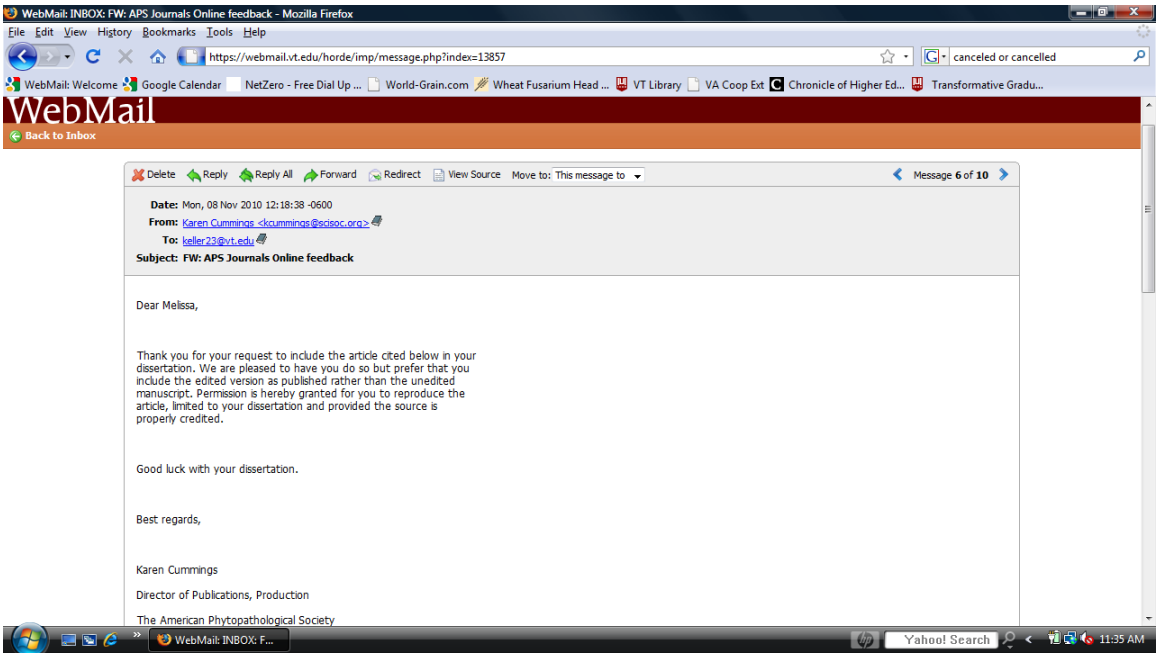
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Appendix D

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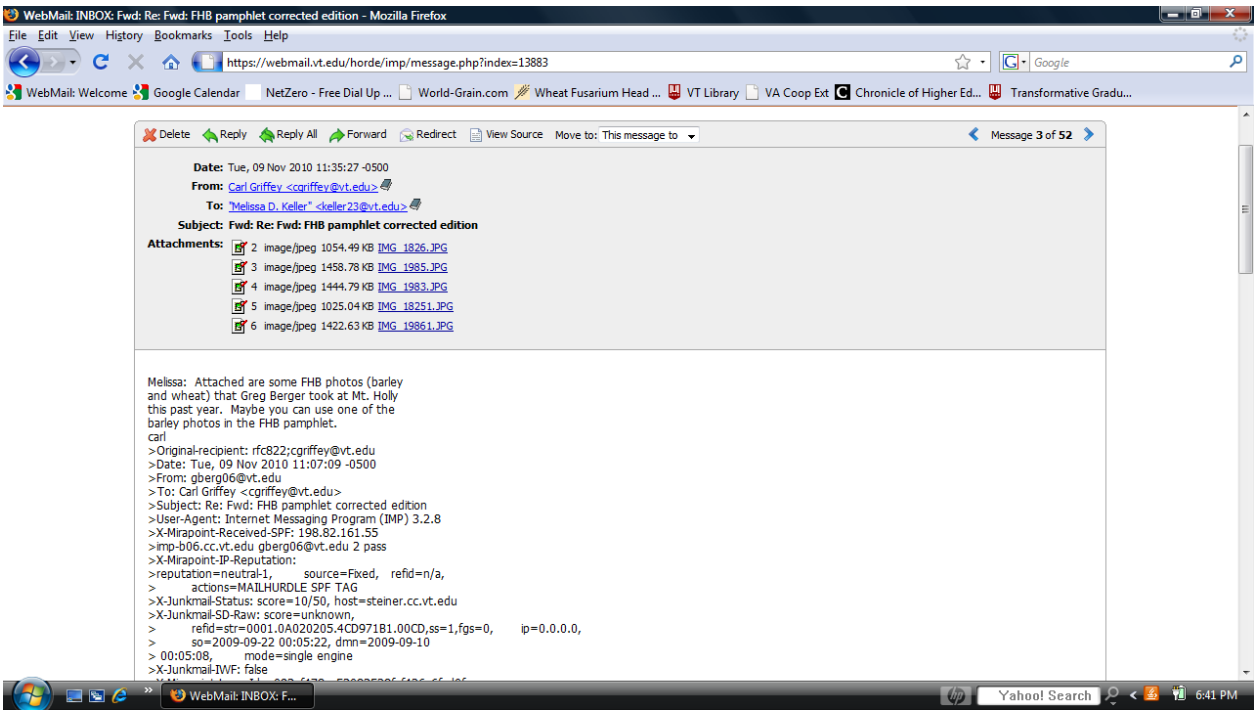
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Attachments:
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3 image/jpeg 1458.78 KB IMG_1985.JPG
4 image/jpeg 1444.79 KB IMG_1983.JPG
5 image/jpeg 1025.04 KB IMG_18251.JPG
6 image/jpeg 1422.63 KB IMG_19861.JPG
Melissa: Attached are some FHB photos (barley
and wheat) that Greg Berger took at Mt. Holly
this past year. Maybe you can use one of the
barley photos in the FHB pamphlet.
carl
>Original-recipient: rfc822;cgriffey@vt.edu
>Date: Tue, 09 Nov 2010 11:07:09 -0500
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