Agricultural and Forest Meteorology 203 (2015) 118–130

Contents lists available at ScienceDirect

Agricultural and Forest Meteorology

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

Experimental validation of a long-distance transport model for plant pathogens: Application to *Fusarium graminearum*

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ARTICLE INFO

Article history:
Received 19 August 2014
Received in revised form
10 December 2014
Accepted 21 December 2014
Available online 16 January 2015

Keywords:
Atmospheric transport
Plant pathogenic fungi
Fusarium head blight
Gaussian plume

ABSTRACT

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

1. Introduction

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

Disease management for FHB has been a challenge for growers and farmers. Recent research has suggested that no-till practices have contributed to disease outbreaks by increasing the amount of potential inoculum sources (e.g., corn debris) on the soil surface (Dill-Macky and Jones, 2000; Keller et al., 2010, 2011). Additionally, fungicides have been found to have limited efficacy and must be

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http://dx.doi.org/10.1016/j.agrformet.2014.12.009
0168-1923/

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

A number of spore transport models have been developed based on an understanding of the atmospheric transport of plant pathogens (Aylor, 1986, 1999; Aylor and Flesch, 2001; Aylor and Sutton, 1992; Aylor et al., 1982). The atmospheric transport of plant pathogens can be described by the aerobiological processes of preconditioning, liberation, horizontal transport, deposition, impact, and infection (Isard et al., 2005). Models to predict the long distance transport of spores may assume a Gaussian distribution of spores (Aylor, 1999; Pasquill, 1976; Pasquill and Michael, 1977; Turner, 1970). The shape and width of the Gaussian distribution is dependent on atmospheric stability (Turner, 1970). Researchers have further developed these models to be specific for the long distance transport of plant pathogenic fungi (Aylor, 1986, 1999; Aylor and Sutton, 1992; Aylor et al., 1982). We consider long distance transport to be distances greater than or equal to 100 m from a source of inoculum. There are several factors in a Gaussian spore transport model necessary to predict the number of viable spores that will be deposited at any given location, including:

1. initial source strength
2. wind speed and direction
3. distance of healthy crops from the source
4. decrease in inoculum viability and spore loss due to solar radiation and deposition
5. dilution of spore plume due to turbulence

Lagrangian stochastic (LS) and Gaussian plume models are both popular choices for simulating the spread from a source on our scale of interest. Recently, an LS model was used to predict the transport of spores (sporangia) of the potato late blight pathogen, *Phytophthora infestans* up to 500 m from source fields (Aylor et al., 2011). The LS model was experimentally validated during two different field seasons with a series of aerial (unmanned aircraft) and ground-based (Rotorods) measurements (Aylor et al., 2011). Although LS models have been used to track the movement of plant pathogens (e.g., Aylor et al., 2011), they are complicated and require more computation time than a Gaussian transport model. Thus, Gaussian models may be more appropriate for farm use. Furthermore, a time averaged turbulent plume, of the kind produced by LS models, has been shown to be Gaussian over a sufficiently long period of time (Irwin et al., 2007), in particular, when concentrations are averaged over 30 min or longer, as is the case in our field experiments (Prussin II et al., 2014a).

Here, we extend the release-recapture concept of Aylor et al. (2011) to field studies of the long distance transport of *F. graminearum* assuming a Gaussian plume model for the dispersal of spores. Long distance transport models driven by a Gaussian distribution (1) can be calculated with limited meteorological data (wind speed, wind direction, rainfall rate, and solar radiation are the only variables needed), (2) are appropriate over the distances of interest (hundreds of meters) and expected to give reasonable results (Aylor, 1999), and (3) do not require numerical integration of differential equations, thus making them significantly less computationally intensive compared to LS models and therefore convenient to use. To verify the appropriateness of using a Gaussian dispersal model instead of an LS model to predict the long distance transport of a fungal plant pathogen, we created dispersal kernels predicted by both models for comparison.

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

2. Materials and methods

2.1. Field experiments

Field studies were conducted at Virginia Tech's Kentland Farm in Blacksburg, Virginia from 26 April to 25 May 2011 and 9 April to 14 May 2012, as previously described (Prussin II et al., 2014a).

2.1.1. Field inoculation

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

A plot area of 3716 m^2 (0.372 ha) of wheat was subdivided into 100 square plots (10 rows of 10 plots, 6.096 m (20 ft) × 6.096 m (20 ft)). Field inoculations were performed on 2 May 2011 (season 1) and 16 April 2012 (season 2) by releasing corn stalks from each