Inter-disciplinary approaches in food safety to expand mycotoxin detection, compare bacterial transfer rates, and forecast fungal inoculum under climate change

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Authorization to Submit Dissertation

This dissertation of Andrew L. Robinson, submitted for the degree of Doctor of Philosophy with a Major in Food Science and titled "Inter-disciplinary approaches in food safety to expand mycotoxin detection, compare bacterial transfer rates, and forecast fungal inoculum under climate change" has been reviewed in final form. Permission, as indicated by the signatures and dates below, is now granted to submit final copies to the College of Graduate Studies for approval.

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Dedication

This work is dedicated to my mother, Karen Donisthorpe Robinson (1957-2004), from whom I have inherited my appearance, creativity, and writing ability. I am grateful that she instilled into me a strong motivation to always learn more about the world she brought me into.

This work is also dedicated to my father, Robert Wayne Robinson, from whom I have inherited my temperament, analytical ingenuity, and autodidaticism. I am grateful that he understood motivation is not polished, not carefully directed, and not an object of control. His support has given me the confidence to fail so that I may learn and flourish through self-discovery.

Abstract

Food safety research is complex and interdisciplinary as it involves considerations from pre-production through final home preparation of a food product which passes a long chain of food processing where each stage has potential opportunities for the contamination. The aim of this dissertation was to develop novel methods which could generalize to future food safety issues regarding contamination. This goal was approached by (1) extending a protocol for detection of ochratoxin A into food matrices where phenolic compounds are present, (2) investigation into how bacterial transfer is affected by both hand washing and glove use, (3) interpreting sources of variability in a fungal inoculum source capable of producing deoxynivalenol, and (4) forecasting future shifts in fungal inoculum under climate change.

Ochratoxin A (OTA) is a fungal metabolite and putative carcinogen which can contaminate a variety of foods such as cereals, wine, and nuts. Commercial ELISA kits are known to give false-positive results for OTA concentrations when phenolic compounds are present. Pistachios represent a food matrix rich in phenolic compounds potentially contaminated with OTA, and polyvinylpolypyrrolidone (PVPP) was incorporated during extraction of OTA using a commercial ELISA protocol. HPLC methods were used to confirm that PVPP does not interact with OTA and the cross-reactivity of extracts also decreased with increasing PVPP application.

To assess bacterial transfer from hands to gloves and to compare bacterial transfer rates to food with different soap washing times and glove use, participants' hands were artificially contaminated with a ~10^9 CFU inoculum of *Enterobacter aerogenes* B199A. Different soap rubbing times (0, 3, and 20 s), glove use, and tomato dicing activities were followed. Different soap rubbing times did not significantly change the amount of bacteria recovered from participants' hands. Both glove use and adequate hand washing are necessary to reduce bacterial cross-contamination as increasing soap washing time decreased the incidence of bacterial contamination recovered from outside glove surfaces ($p < 0.05$) and dicing tomatoes with bare hands after 20 s of soap rubbing transferred significantly ($p < 0.01$) less bacteria to tomatoes compared with bare hands after 0 s of soap rubbing.

The plant pathogen *Fusarium culmorum* represents an inoculum source capable of contaminating grains with deoxynivalenol (DON) in the Inland Northwest (INW) region of the United States. A multilevel modelling approach utilizing varying intercepts for different sampling quadrats, fields, and iterations in the dataset was performed to characterize variability in isolation frequency of *F. culmorum* collected during a two-year soil survey. Differences in the isolation frequency of *F. culmorum* varied the most by sampled field followed by quadrat and iteration, respectively. Higher relative elevation within the sampling region of a field limited the amount of *F. culmorum* recovered.

Isolation records were extended to incorporate the soil dilution factor and used to construct a multilevel climate model. Varying intercepts and slopes were assigned to each unique agricultural field and a weather-based proxy for soil moisture, termed atmospheric water balance (AWB) was used as a predictor variable. Values of AWB derived from downscaled global climate models were used to forecast future shifts in the proportion of *F. culmorum* across all sampled fields. Population densities of *F. culmorum* are forecasted to remain constant during the winter and spring but decrease over the summer and fall under climate change, with the magnitude differing across fields.

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Statement of Contribution

This dissertation contains chapters which are published multi-authored articles. I, Andrew Robinson, am first author on all submitted articles as I was responsible for executing the corresponding experiments, drafting and writing of the manuscripts, and responding to comments during peer review.

Chapter 1: Introduction

Background

Diversity of food safety concerns

Food safety can be defined as the inverse of food risk, which is the probability of not suffering some hazard upon consumption of a specific food (Henson & Traill, 1993). Food safety research is complex and interdisciplinary as it involves considerations from pre-production through final home preparation of a specific food product. Recommendations for food safety management need to emphasize the broad input and coordination between all sectors of the food chain (M. C. Tirado et al., 2010).

Consumer's attitudes towards food safety influence the types of food they choose to purchase (Wilcock et al., 2004). The primary attitudes towards food safety by consumers are health issues, chemical issues, spoilage issues, regulatory practices, and deceptive practices the ratings of which differ across individuals (Brewer et al., 1994). Consumers can make rational decisions about food safety when they are aware of the associated health problems, have knowledge concerning those problems, and have judgement as to the level of risk involved in not changing their behavior (Axleson & Brinberg, 1989; Contento & Murphy, 1990).

However, there is a disparity between consumer knowledge of food safety and their self-reported food handling practices (McIntosh et al., 1994; Raab & Woodburn, 1997). This can be partly attributed to an optimistic bias effect, where individuals believe they are less at risk from a hazard than others (Miles et al., 1999). Consumers are given information about risk aimed at the general population and infer their own risk status as it is rare to obtain personal information about vulnerability to a specific hazard. This may result in a difference between an individual's perceived personal risk and actual risk status (Frewer et al., 1994).

In addition, many consumers believe that responsibility lies with food manufacturers or restaurants and are unaware that anywhere from 10% to 50% of foodborne illness arises in private homes (Cowden et al., 1995; Lindqvist et al., 2000; Olsen et al., 2000; Roberts, 1992; Tirado & Schmidt, 2000). Many consumers appear to lack a clear understanding of basic food safety terms (Fein et al., 1995)., and unfortunately food safety knowledge does not correlate with safe home food preparation practices (Albrecht, 1995; Altekruse et al., 1996; Williamson et al., 1992).

However, consumers are only the end-users in the long chain of food processing and preparation and each stage has potential opportunities for the contamination of food products. Transportation of food is a potential source of contamination, especially under poor sanitary conditions (Unnevehr, 2000). Vehicles can contaminate food with their exhaust in addition to being vectors for cross-contamination between different products. Food contamination can also occur during long distance shipping by chemicals used for disinfection (Nerín et al., 2007).

During processing foods can become contaminated by residues of disinfectants and cleaning agents on the surface of the food handling equipment (Nageli & Kupper, 2006; Villanueva et al., 2018). High temperatures during cooking can also potentially form toxic compounds such as acrylamide or furanes (Nerín et al., 2016). Food frying is a primary generator of many chemical contaminants during food preparation (Roccato et al., 2015). Microwaving foods can also lead to the production of chemical contaminants, primarily due to interactions with the packing material or wrapping film used during heating (Ehlert et al., 2008; Nerín et al., 2003). While food packing provides an important physical barrier, the packaging process makes use of additives such as stabilizers and plasticizers which can migrate into food products (Marsh & Bugusu, 2007). When metallic containers are used in packaging, corrosion becomes a potential source of food contamination (Buculei et al., 2012).

Foods also can become contaminated by the environment. Toxic heavy metals such as mercury and lead are present in industrial environments, which can be absorbed by plants at the base of the food chain (Peralta-Videa et al., 2009). Heavy metals can seep into soils and infect the raw materials used in food production (Krishna & Govil, 2006). Pesticides for plant protection, applied at a rate of 3 billion kilograms annually across the globe (Pimentel & Burgess, 2014), can also enter into the food chain causing a wide array of health problems (Abhilash & Singh, 2009).Water used in food processing can also become contaminated from industrial and municipal discharges, natural geological formations, runoff from both urban and rural areas, and the drinking water treatment process (Calderon, 2000).

Also, the environment itself is constantly changing through time. Global mean surface temperature has increased during the past half century (Trenberth, 2007). There is a direct influence of greater temperatures on precipitation, with effects ranging from increased

intensity and duration of drought to more devastating storm events (Trenberth, 2011). Forecasting the effect of climate change is difficult due to evaluating the feedbacks against observations. Clouds represent a significant problem as they both reflect sunlight and contribute a cooling effect while simultaneously contributing to warming by retaining heat as water vapor. An additional complication is that the climate system lags in response to greenhouse gas emissions or other forcings (Hansen et al., 2005), and the effect my lag 25 to 50 years behind the forcing events (Hansen et al., 1985).

It is unclear what affects climate change may have on food safety. High temperatures can be correlated with episodes of diarrheal disease, as in Peru where reports increased by 8% for each degree of temperature increase (Checkley et al., 2000). There are also associations between extreme weather events and monthly reports of outbreaks due to infections water-borne diseases (Fischlin et al., 2007). Water pollution can be exasperated by higher water temperatures, increased precipitation intensity, and longer durations of low flows (Kundzewicz et al., 2007). Ocean warming can facilitate the methylation of mercury and increase uptake in fish and mammal fats by 5% for each degree of increase in water temperature (Booth & Zeller, 2005). Agriculture can also accelerate desertification, as seen in the Aral Sea region after cotton monoculture, over irrigation, and pesticide abuse (Muntean et al., 2003). Many pesticides also have limited activity in dry conditions (Muriel et al., 2001).

Climate change also effects emerging parasitic diseases (Tirado et al., 2010). Cases of salmonellosis in several European countries increased by 5 to 50% for each degree of increase in weekly average temperature (Kovats et al., 2004). Increased large outbreaks of *Virbio paramaemolyticus* have been linked to the consumption of oysters harvested from northern regions with higher mean water temperatures (McLaughlin et al., 2005). Incidence of protozoan parasites such as cryptosporidiosis and giardiasis is affected by intense rainfall and changes in precipitation (Curriero et al., 2001).Global warming may also affect the transmission of trematodes, which use molluscs as intermediate hosts, and their geographical distribution and proliferation may be promoted in the future (Poulin & Mouritsen, 2006).

Neither the strongest nor the most intelligent species is the one that survives, it is the one which is most adaptable to change. In an ever-changing world consumers need to have both knowledge of a given food safety problem and a clear interpretable message of the

associated risks involved. Whenever risk is given in terms of some quantity of measure, it is important to understand how the calculation was made n order to make the most informed decisions.

Definitions of risk

In finance, a common metric for risk is simply the standard deviation of asset prices over some time interval. High standard deviations in the price of an asset are used to indicate its potential volatility in value, and the greater the risk the greater the potential rate of return. Using standard deviation defines risk as uncertainty and can be extended to other collections of random variables. For example, a specific variety of pepper may have a high standard deviation of Scoville heat units meaning that there is a great deal of uncertainty across peppers. A consumer cannot be sure of the resulting intensity of spiciness after consuming one, giving a high degree of uncertainty to their enjoyment of the resulting meal.

A more common definition of risk is the probability of an event occurring. What is important in these cases is how the probability was determined and what information the estimate was conditioned upon.

Risk assessment

Risk assessment consists of hazard identification, hazard characterization, exposure assessment, and risk characterization. The goal of a risk assessment is to identify uncertainty associated with the likelihood and severity of a given adverse effect occurring after exposure to a defined risk source. Risk assessment attempts to cover all possible hazards and distill the results in a communicable way to all involved parties so that they may make more informed decisions.

Hazard identification is performed in both in vitro experiments and in vivo studies using experimental animals. The goal is to systematically investigate all potential effects, but there is difficulty in extrapolating from the data to the population (Edler et al., 2002). The alternative approach to hazard identification is through epidemiological studies, but these do not provide as systematic of an assessment as they are typically focused on a specific effect of interest. After identification of hazards they need to be further characterized to better understand the nature of the observed effects through dose-response relationships (Dybing et al., 2002). The goal is to identify likely threshold doses where there would be no effect. A

threshold dose is used to estimate safety assurance, such as the no observed adverse effect level in experimental animals to a safe intake for humans.

The conclusions and advice given to all relevant parties depends on the quality and quantity of available exposure data. However, estimation of intake is a major source of uncertainty (Renwick et al., 2001). Combining intake data with data derived hazard characterization can be used to indicate whether a given intake is a possible health concern. When communicating risk it is important to identify the potential high risk groups, mention any possible combination effects, give advice on the relevant duration of exposure, inform of the risks associated with excessive exposure, and describe all the uncertainties that were involved in making the assessment (Renwick et al., 2003).

Types of Food Contaminants

Foods can become contaminated by a wide diversity of potential contaminants. Contaminants can be inert such as metals in the environment, living organisms such as bacteria in milk, or also substances left behind by living organisms such as mycotoxins in grains. Following an overview of different food contaminants, three specific contaminants will be discussed in greater detail. Ochratoxin A is a case study for a natural contaminant occurring in a vied variety of food products. Salmonella is a case study of a living contaminant which can be transferred in food handling environments. Lastly, deoxynivalenol is a case study for both a natural contaminant and the equally relevant living contaminant responsible for it’s incorporation into grains.

Types of environmental contaminants

Metals are one primary example of potential food contaminants originating in the environment. Mining can result in the release of arsenic and mercury into the environment (Bortey-Sam et al., 2015; Smedley & Kinniburgh, 2002). These metals can end up in grains around gold mining regions of China (Xiao et al., 2017) or in livestock organs nearby iron mines in Morocco (Nouri & Haddioui, 2016). Industrial regions pose a significant threat with cases such as cadmium in leaf and root vegetables nearby smelters in China (Li et al., 2016). Contamination by metals is a food safety concern as lead can reduce cognitive development (Szkup-Jabłońska et al., 2012), cadmium can cause renal tubular dysfunction (Nordberg, 2009), arsenic is associated with various carcinogenic effects (H.-J. Lin et al., 2013), and

mercury is linked to cardiovascular, reproductive, and developmental toxicity (Genchi et al., 2017).

The environment is also a reservoir for various persistent organic pollutants such as pesticides or byproducts of industrial and combustion processes. Notorious examples include polychlorinated biphenyls which are used as heat exchange fluids or paint additives, polychlorinated compounds which can be used as flame retardants, and perflourinated compounds used as lubricants (Thompson & Darwish, 2019). Other examples of environmental contaminants include antimicrobials fed to livestock (He et al., 2016), uncontrolled electronic waste processing (Wong et al., 2007), and plastics (Jeddi et al., 2015). A new environmental contaminant is nanoparticles which are used in paints and cosmetics (Kalman et al., 2015), with demonstrated cases of entering the food chain (Gatti et al., 2008). It is unclear what sore of pathways or effects these novel compounds may have on living organisms.

Types of natural contaminants

Natural contaminants represent compounds created by living organisms. One example is staphylococcal enterotoxin, commonly produced by *Staphlococcus aureus* in outbreaks of foodborne disease (Bergdoll et al., 1981). Large numbers of *S. aureus* need to be present in the food product in order to produce enough enterotoxin to cause illness. The relevant number of *S. aureus* is 10^6 CFU/g and the corresponding amount of enterotoxin to cause disease is around 200 nanograms for children and up to one microgram for adults (Evenson et al., 1988). The enterotoxin is not easily inactivated by heat (Humphreys et al., 1974). Most common symptoms are vomiting and diarrhea (Gourama et al., 1991). Human beings are a common reservoir for *S. aureus* in the nose and throat, hands and skin, or cuts and abrasions (Hazariwala et al., 2002).

Another common class of natural contaminants of foods are mycotoxins, which are metabolites produced by fungi during the colonization of a given food commodity. One of the most notorious mycotoxins are aflatoxins. The main fungi responsible for producing aflatoxins are *Aspergillus flavus A. parasiticus,* and *A. nomius* (Kurtzman et al., 1987). Aflatoxins can be found in nuts, cereals, oilseeds, and spices (Iqbal et al., 2014; Lancaster et al., 1961; Reddy et al., 2010). Contamination can occur both before and after harvesting, and

often through inappropriate storage conditions (Kumar et al., 2017). Aflatoxins target the liver, and toxicity is different across species. Aflatoxins are converted into an epoxide by cytochrome P450 enzymes (CYP) which is highly reactive and capable of binding to DNA, RNA, and proteins (Eaton & Gallagher, 1994). Toxicity varies across animal species and is related to both the rate of epoxide formation by CYP and the rate of detoxification by glutiathione S-transferase enzymes (GST). Mice have high activities of both CYP and GST, and exhibit the lowest sensitivity to aflatoxin (Hayes et al., 1992). Human CYP has an 8-fold lower activity than in mice, but increased toxicity is attributed to human GST having over 3000-fold less activity than in mice (Ramsdell & Eaton, 1990; Slone et al., 1995). Turkey is especially sensitive to AFB1 due to the very high activity of CYP and the inability of GST to detoxify the epoxide form of aflatoxin (Klein et al., 2000; Rawal et al., 2010).

Citrinin is a toxic secondary metabolite produced by various Aspergillus and Penicillium species. Citrinin is typically produced after harvest and can contaminate foods such as barley, beans, fruits, herbs, and spices (Milani, 2013)Patulin is a mycotoxin produced most commonly by *P. expnsum*, which is the causative agent of common brown soft rot on apples. Contamination is typically confined to the regions of rotten tissue, but it can be present in apple juice. However, it is metabolized by fermenting yeasts and does not occur in apple cider (Moss & Long, 2002). The mycotoxins ochratoxin A (OTA) and deoxynivalenol (DON) will be discussed in detail in following sections.

Types of living contaminants

Viruses are organisms at the edge of life replicating only within a host cell and existing as independent particles, or virions, within the environment (Rybicki, 1990). The two most common viral food contaminants are Hepatitis A and Norovirus. The Hepatatis A virus is the only human hepatitis virus known to be transferred through food and water (Cliver, 1994). The virus multiplies in the intestinal tract and symptoms typically appear 15 to 50 days after consumption of contaminated food, though the virus is shed in the feces of an infected individual for 7 to 10 days before symptoms appear (Cliver et al., 1992). Hepatitis A is more common in children than adults, though illness is typically more severe in adults than in children. Examples of outbreaks include a US Navy ship after a chef urinated in salad (Varnam & Evans, 1991), and in Wisconsin from a foodservice worker with poor hygiene

(MMWR, 1993). Norovirus causes illness which has been known to start with uncontrollable projectile vomiting (Halligan, 1992). In addition to being spread by the fecal-oral route Norovirus particles can also be detected in vomit (Greenberg et al., 1979),and the resulting aerosol formation is a factor in the spread of the disease (Nelson et al., 1992).

Viruses represent a food safety concern as the infectious dose is less than 10 viral particles for Hepatitis A and Norovirus (Halligan, 1992; Snyder & Poland, 1991). Globally Norovirus accounts for the largest number of cases of foodborne disease (Ahmed et al., 2014). Contamination occurs during the manual handling of food products and is aggravated with subsequent minimal processing (Koopmans & Duizer, 2004). Viral particles not only persist within the environment but are more resistant than bacteria to current methodologies for mitigating bacterial contamination during food processing, preservation, and storage (Baert et al., 2009).

Some common genera of bacterial food contaminants include *Listeria, Campylobacter. Listeria monocytogenes* is of concern for human health as it can cause serious invasive illness (Farber & Peterkin, 1991) with the elderly, infants, and immunocompromised being especially susceptible to listeriosis. *Listeria* is ubiquitous in the environment and can be primarily found in the soil and decaying vegetation. Interestingly, *L. monocytogenes* can also grow at refrigeration temperatures. *L. monocytogenes* is a facultative intracellular pathogen and can use actin within a host cell to become motile (Finlay & Cossart, 1997; Ireton & Cossart, 1997). Outbreaks of listeriosis have been caused by various refrigerated ready-to-eat foods such as dairy, vegetable, and meat products (Schuchat et al., 1991). *L. monocytogenes* can also persist in treated sewage (Luppi et al., 1988; MacGowan et al., 1994). Other environmental sources may serve as reservoirs for Listeria strains capable of infecting animals through contaminated feed (Donald et al., 1995; Fenlon, 1985).

Another potential bacterial contaminant of food is *Campylobacter*, which is fastidious and can be a commensal in ruminants, pigs, and avian species (Keener et al., 2004). The majority of human infections are from *C. jejuni* and *C. coli* and these organisms are themophilic (Acheson & Allos, 2001). Avain species are the most common Campylobacter host, likely due to their higher body temperature (Skirrow, 1977). Likewise, most illness is associated with consuming undercooked poultry products (Kapperud et al., 1993). Symptoms

are self-limiting and most cases do not require antibiotics. Sporadic outbreaks have been traced to raw milk and contaminated water (Altekruse et al., 1999).

Ochratoxin A

In 1965, researchers grew cultures of *Aspergillus ocraceus*, which was known to contaminate grains if stored above 16% moisture content, onto maize meal which were subsequently fed to ducklings, mice, and rats. The toxicity tests elucidated that a particular metabolite, ochratoxin A (OTA) was responsible for liver damage through fatty infiltration of parenchymal liver cells (der Merwe et al., 1965).

Definition and physiochemical properties

According to the International Union of Pure and Applied Chemistry OTA is (2S)-2-[[(3R)-5-chloro-8-hydroxy-3-methyl-1-oxo-3,4-dihydroisochromeme-7- carbonyl]-amino]-3-phenylpropanoic acid. OTA is a weak organic acid, slightly soluble in water, and soluble in polar organic solvents (el Khoury & Atoui, 2010). The heat stability of OTA makes it a food safety concern as it is only partially degraded during normal food processing conditions (Boudra et al., 1995; Müller, 1982; Trivedi et al., 1992). It is chlorinated, which is unusual for a biological metabolite, and implicated in causing nephrological toxicity (Gharbi et al., 1993).

Occurrence of OTA in food

Production of OTA has been observed in several species of fungi from two different fungal genera*:* *Aspergillus* and *Penicillium*. Multiple yellow *Aspergilli* from section *Circumdati* are OTA- producers, such as *A. ocraceous*, *A. westerdijkiae*, *A. sclerotiotium*, and *A. cretensis* (Gil-Serna et al., 2011). Black *Aspergilli* from section *Niger* can also potentially produce OTA (Abarca et al., 2004; Battilani et al., 2003; Dalcero et al., 2002; Magnoli et al., 2003), with *A. carbonarious* being a major OTA producer occurring on grapes (Horie, 1995; Leong et al., 2004) and coffee beans (Taniwaki et al., 2003). As for the genus *Penicillium*, *P. verrocusom* is an OTA producer found in stored grains (Geisen et al., 2004; Lund & Frisvad, 2003; Pitt, 1987), while *P. nordicum* is found in cured meat products (Larsen et al., 2001).

OTA producing fungi vary morphologically, with *Aspergillus* species resembling a device used to sprinkle holy water (Micheli, 1729), and *Penicillium* resembling a painter's brush (Link, 1809). Likewise, OTA producing fungi can also inhabit a variety of environments and potentially contaminate a wide array of food products. OTA can also be found in nuts, spices, coffee, cocoa, beer, wine, grape juice, and dried fruits (Aish et al., 2004; Al-Taher et al., 2013; Speijers & Van Egmond, 1993). Given the wide diversity of foods potentially contaminated by OTA there exists a need to expand current detection methods to new areas for routine monitoring.

OTA detection methods

Detecting mycotoxins is inherently difficult due since fungi typically develop in isolated pockets and are not evenly distributed storage containers for food commodities. Most of the error associated with mycotoxin assays can be attributed to how the original sample was collected (Bullerman et al., 1984; Lauren et al., 2006). Sensitive and reliable detection methods are needed since mycotoxins will be diluted in a large representative sample and potentially remain toxic at low concentrations. Failures in sampling and analysis can lead to unacceptable loads being accepted or satisfactory loads being unnecessarily rejected. Mycotoxins have a diverse array of chemical structures which leads to specialized techniques which do not readily transfer to different analytes or even different food matrices with the same analyte. In addition, the technical requirements for detection methods with high sensitivities typically requires specialist laboratories which creates challenges for routine inspections (Turner et al., 2009).

Sample preparation is the primary time factor in the analysis of a given mycotoxin, and also affects the choice of detection method. Hydrophilic mycotoxins such as fumonisins require the presence of water (Shephard, 1998), while hydrophobic mycotoxins such as aflatoxin require organic solvents (M Holcomb et al., 1992; Horwitz, 2010). The choice of extraction solvent is also dependent on the food matrix from which the mycotoxin of interest is to be extracted, as different chemicals can influence the recovery (Wilkes & Sutherland, 1998) Liquid-liquid extracts utilize the differences in solubility of a given mycotoxin between an aqueous phase and an organic phase, with the intention of extracting the mycotoxin into one phase and leaving the remainder of the food matrix in the other. This

procedure can work well for small scale preparations but is very specific to the problem at hand and requires long durations for analysis. Supercritical fluids, such as CO_2, can also be used for mycotoxin extraction due to the high solvating power (Young & Games, 1992). However, this technique is not suitable for routine analysis due to the high operating costs and specialized equipment (Holcomb et al., 1996).

A popular sample preparation procedure is solid phase extraction, wherein a mycotoxin present in a given solvent is loaded into small disposable cartridges with a stationary phase designed for specified bonding. The sample is transferred by reduced pressure through the column and rinsed to remove the bulk of other contaminates associated with a given food matrix. The mycotoxin is then eluted from the stationary phase using another solvent which is then further processed prior to detection. The stationary phases used are varied based on the problem at hand, ranging from silica gel, ion exchange materials, molecular imprinted polymers, and immunoabsorbents.(Giraudi et al., 2007; Jornet et al., 2000; Mateo et al., 2002; Vatinno et al., 2008; Visconti et al., 2000; Zambonin et al., 2001). Silica gel has been used to isolate OTA from sweet wine samples (Hernández et al., 2006). Through combinatorial synthesis a hexapeptide was developed which shoded good affinity to OTA (Giraudi et al., 2007), and the researchers further developed the hexapeptide into a stationary phase which performed as well as commercial immunoaffinity cartridges.

The researchers above referenced their results to immunoaffinity columns, which have been demonstrated great value in mycotoxin analysis (Mateo et al., 2002; Scudamore & MacDonald, 1998; Solfrizzo et al., 1998; Zimmerli & Dick, 1995).The excellent recovery is achieved through the specificity gained by using monoclonal or polyclonal antibodies designed to target a given mycotoxin. However, there are high costs associated with using the immunoaffinity columns as the mycotoxin elution step denatures the antibodies, making each column single use. Immunoaffinity columns have been used successfully to isolate OTA from wine (Visconti et al., 1999), coffee (Brera et al., 2005), pistachios (Lee et al., 2014), and even human plasma (Muñoz et al., 2006).

The traditional method for mycotoxin analysis was thin layer chromatography (Betina, 1985; L. Lin et al., 1998).This method offers a high throughput of samples which can be analyzed on the same plate, low operating cost, and straightforward identification

using UV-vis analysis. However, most modern analysis of mycotoxins utilizes high performance liquid chromatography (HPLC). Several mycotoxins, including OTA, have natural fluorescence and can be quantified directly using a fluorescence detector. Mycotoxins which do not fluoresce require a derivatiation step which adds additional complexity to the HPLC system (Chiavaro et al., 2001; Manuel Holcomb et al., 1993; Jiménez et al., 2000; Kussak et al., 1995; Neely et al., 1990), though there are some examples of OTA detection using derivatization (Jiao et al., 1992).

There is ample literature on different HPLC methods for OTA detection (80), and the primary utility of these methods is the high sensitivity resulting from a verified clean up procedure combined with fluorescence detection. The limit of detection for OTA in wine samples can typically be in the 0.01 ng/mL range (Brera et al., 2005; Visconti et al., 1999). However, such sensitive methods cannot be easily adopted for routine analysis due to the high operating costs, lengthy sample preparation, and the need for expensive specialized equipment.

Enzyme-linked immunosorbent assays (ELISA) represent an alternative to HPLC for mycotoxin quantification which has lower costs and easier application in fieldwork (Goryacheva et al., 2007; Morgan, 1989). Commercially available ELISA kits make use of a primary antibody specific to a given mycotoxin. The complex which is formed then interacts with a chromogenic substrate which yields a measurable result. Commercial kits for OTA detection utilize competitive ELISA for complication, which is a somewhat complex technique with an initially confusing result. The assay works by measuring the interference in an expected signal output. The amount of OTA in a given food sample competed with a reference amount when binding to a limited about of labeled antibodies. Signal output inversely correlated with the amount of OTA in a sample, since the higher the sample OTA concentration, the weaker the output signal. Competition ELISA is useful in that little sample processing may be required, the assay is more robust than simpler ELISA protocols to sample dilution and matrix effects, and there is less variability between duplicate samples.

However, antibodies still have limitations. Previously, it was discovered that phenolic compounds in the skins of pistachios were interfering with the antibodies present in a commercial ELISA kit for OTA detection (Lee et al., 2014). Antibodies designed to taget

OTA also had good binding affinity towards as gallic acid and catechin. Given the diversity of food products which can be potentially contaminated by OTA, along with the need for processing large amounts of samples to gain better measurements of OTA incidence in foods, current ELISA protocols need to be modified to expand their scope when evaluating different food commodities.

Salmonella

Characterization of Salmonella

Salmonella bacteria are one of the most common causes of food poisoning in many countries (Alakomi & Saarela, 2009). Species were originally named based on the source of isolation (Tindall et al., 2005), with *Salmonella enterica* being the species which can inhabit humans and other warm-blooded animals. *S. enterica* is a facultatively intracellular pathogen which prefers colonizing macrophages (Kaufmann et al., 2001). However, this intestinal bacterium is widespread in the environment and can be isolated from farm runoff (Liebana et al., 2003). Any other material subjected to fecal cross-contamination is also susceptible (Martinez-Urtaza et al., 2004). Meat can become contaminated by intestinal contents during butchering (Al-Saigh et al., 2004), and vegetables from contaminated water (Duffy et al., 2005).

A *Salmonella* infection leads to gasteroenteritidis which is typically self-limiting. However large outbreaks can sporadically occur in schools, hospitals, or restaurants (Guiney et al., 1995). Salmonellosis has been reported as the most common food-borne bacterial disease in the world (Forshell & Wierup, 2006; Herikstad et al., 2002), with actual incidences potentially being higher due to under-reporting. While heat treatment can kill *Salmonella* in a food source (Van Asselt & Zwietering, 2006), the concern of cross-contamination remains (Reij & Den Aantrekker, 2004).Therefore more research is needed to better understand how fecal contaminants can spread across surfaces within a food handling environment.

Research into bacterial transfer rates

Many food-borne disease outbreaks are associated with bacterial cross contamination (Roberts & others, 1990). However, it is difficult to derive epidemiological information about common bacterial contamination routes due to unknowns in the contamination source along with fragmented information across reports of outbreaks. Bacterial transfer is a general

term which involves wither a direct or indirect move from a contaminated item to another. Surface to food transfer in fluids is typically due to biofilm formation on the surfaces of pipelines or tanks. Standard cleaning and sanitation procedures cannot completely eliminate biofilms (Bremer et al., 2006; Chavant et al., 2004; Joseph et al., 2001). This type of transfer is of particular concern to the dairy industry in milk pipeline systems (Oliver et al., 2005).

Another important type of bacterial transfer is from surface to food by contact. This type of transfer is an important contributing factor to food-borne illnesses originating in a consumer's home (Beumer & Kusumaningrum, 2003; Bloomfield, 2003). Bacterial transfer across surfaces by contact can occur across a wide variety of situations within the food processing chain through contact with raw foods, equipment, cutting boards, dishcloths, and other food handling equipment (De Jesús et al., 2004; Haysom & Sharp, 2004; Hudson & Mott, 1993; Lunden et al., 2002). Most studies on bacterial transfer are involved with gaining a better understanding of transfer rates across surfaces by contact.

The design of the experiment influences the results and subsequent conclusions. For example, after assessing different decontamination treatments it was reported that more bacteria were recovered from plastic as opposed to wooden cutting boards (Ak et al., 1994). The recommendation was to use wooden cutting boards in transfer. Performing a similar experiment but instead measuring transfer rates, it was found that wood presented a greater risk for cross-contamination compared to plastic butting boards for *S. typhimurium* (Gough & Dodd, 1998). Studies can be performed under tight experimental control in a laboratory setting to gain information about specific processes and the influence of different factors (Kusumaningrum et al., 2003; Vorst et al., 2006). Other studies have been performed in real environments such as factories and kitchens to quantify the frequency of bacterial transfer events or identify transmission routes (De Jesús et al., 2004; Gorman et al., 2002). Data gathered from both types of studies can be utilized for exposure assessment and risk management strategies.

The swabbing method is frequently utilized in the food industry to evaluate the effectiveness of cleaning and disinfection procedures. However, the use of swabs in bacterial transfer studies suffers from low reproducibility due to the variability across the individuals performing the test (Yamaguchi et al., 2003). In addition, the recovery rates from swabbing

samples is low and only a small proportion of the total bacterial population on a surface may be enumerated (Angelotti et al., 1958; Bredholt et al., 1999). Bacteria collected by the swab can become trapped by the cotton fibers present in the tip and cannot be readily released (Salo et al., 2000).

One alternative to using swabs is collecting colonies with an agar surface which is stamped directly onto a surface of interest. The primary utility of such a method is the increased sensitivity in detecting low bacterial population levels (Foschino et al., 2003). However, the contact time and pressure needs to be optimized for a given situation prior to performing an experiment in order to obtain better recovery rates, and may underestimate counts when applied to an area with a high inoculum source. The workaround for high inoculum sources is to homogenize the agar stamp in broth prior to enumeration (Kusumaningrum et al., 2004). The agar stamp method is limited to flat surfaces, though it has shown utility in measuring bacterial transfer using fingertips and palms (Larson, 1985; Michaels et al., 2004).

The glove-juice method, wherein a glove is filled with broth and fitted onto a participant's hands and massaged for 1 min is a common enumeration technique when studying cross contamination scenarios involving contact with bare hands. This sampling procedure allows for a more accurate enumeration of both transient and residential bacteria from all surfaces of the hand (Koecher & Krenke, 2000). The glove juice method has been widely adopted to study rates of bacterial transfer through hand contact (Chen et al., 2001; Gustafson et al., 2000; Rebecca Montville et al., 2001).

Bacterial transfer is linked to bacterial attachment which in turn is affected by environmental factors. There is a higher rate of transfer after contact with contaminated foods with higher fat contents compared to foods with a higher lean content (Dickson, 1990; Luber et al., 2006; Vorst et al., 2006). Bacteria with more hydrophobic surfaces tend to adhere to hydrophobic materials such as rubber, PCV, and fatty meats which reduces the amount available to transfer (Chung et al., 1989). Bacteria with hydrophilic surfaces can adhere better to hydrophilic materials such as stainless steel, an example being S. aureus transferring at a greater rate from glove to broach compared to broach to glove (Knobben et al., 2007). The hydrophobic properties of both the donor and recipient can contribute significantly to the

rate of bacterial transfer. Rates of bacterial transfer are also related to the moisture content of a sample, providing an alternate explanation for higher bacterial transfer rates in lean tissues compared to fatty tissue (Dickson, 1990). Higher moisture also increases the rate of bacterial transfer from stainless steel (Moore et al., 2003), fabric (Sattar et al., 2001), and hands (Merry et al., 2001).

Bacterial attachment may depend on surface roughness, though the results may be related to differences in the efficacy of the recovery method (Flint et al., 2000). However, bacterial transfer is lower with the higher amount of surface roughness as bacteria colonize the recessed regions which do not come into contact with a transfer surface (Dawson et al., 2007; Midelet & Carpentier, 2002). Surface porosity can also influence bacterial transfer as aprons and sponges have shown lower transfer rates compared to stainless steel or knobs (Rusin et al., 2002; Scott & Bloomfield, 1990).

Factors intrinsic to the bacterium itself can also influence transfer rates. There is a strong negative correlation between inoculum size and transfer efficiency (Montville & Schaffner, 2003; Rusin et al., 2002). Bacterial transfer can also differ across species (Knobben et al., 2007; Midelet & Carpentier, 2002; Midelet et al., 2006). Increased contact time can also influence species level differences in transfer rates (Vermeltfoort et al., 2004).

Deoxynivalenol

In 1972, following unusually wet weather, states across the U.S Corn Belt reported that swine were either refusing to eat moldy grain or vomited after consuming small quantities. The culprit behind the moldy corn was *Fusarium graminearum* Schwabe, and the purified toxin was tentatively identified as 3, 7, 15-trihydroxy-12, 13-epoxytrichothec-9-en-8-one using IR and NMR spectra. It was given the trivial name of “vomitoxin” (Vesonder et al., 1973). Additional work confirmed the structure of vomitoxin after fermenting rice with an isolate of *F. graminearum* and the researchers replicated feed refusal in swine under controlled conditions (Vesonder et al., 1976).

Definition and physiochemical properties

Deoxynivalenol (DON), known colloquially as “vomitoxin”, is a trichothecene metabolite produced by several *Fusarium* species which are known to colonize agricultural crops. Trichothecenes represent a family of over 200 toxins with a common amphipathic

tricyclic 12, 13-epoxytrichothec-9-ene core structure. There are 4 groups (Type A, B, C, and D) which are classified based on the substitution pattern of the core structure. Type A trichothecenes include compounds with an ester function at C-8 such as T-2 toxin. Type B trichothecenes contain a carbonyl function at C-8 and include DON and nivalenol (NIV). Type C trichothecenes have a bonus epoxide group connecting C-7 and C-8, while Type D trichothecenes have an additional ring linking the C-4 and C-15 position (McCormick et al., 2011).

According to the International Union of Pure and Applied Chemistry DON is (3α, 7α)-3, 7, 15-trihydroxy-12, 13-epoxytrichothec-9-en-8-one. DON contains six oxygen atoms which reside in an epoxide, a cyclic ether, a carbonyl group, and three alcoholic hydroxyl groups. The epoxide and carbonyl group are mainly what give DON its toxic effects (McCormick et al., 2011; Nagy et al., 2005). DON was less toxic when administered to mice compared to NIV and fusarenon, leading the researchers to suggest that the C-4 hydroxy group may play an important role in mammalian toxicity of trichothecenes (Yoshizawa & Morooka, 1974). DON has a molecular formula of $H_{15}C_{20}O_6$, a molar mass of 296.32 g/mol, and is water soluble (Sobrova et al., 2010).

The primary toxic effect of trichothecenes is the inhibition of protein synthesis (Rocha et al., 2005). DON can disrupt cell signaling, differentiation, growth, and protein synthesis each of which can contribute to a wide range of toxic effects (Pestka & Smolinski, 2005). Depending on dosage level DON can by immunostimulatory or immunosuppressive to macrophages, T cells, and B cells (Pestka et al., 2004).DON elicited similar effects as anisomycin and emetine which are known translational inhibitors. The RNA-activated protein kinase R is involved in the upstream ribotoxic stress response and lack of the kinase was shown to reduce the induction of apoptosis by DON in a murine macrophage cell line (Zhou et al., 2003).

DON was also shown to activate JNK/p38 kinases which increased the induction of apoptosis in murine T-cells along with the inhibition of protein synthesis (Shifrin & Anderson, 1999). Other mitogen-activated protein kinases also play a role in the induction of apoptosis by DON following protein synthesis inhibition in murine macrophage and human leukemic cell lines (Yang et al., 2000). Exposing a human erthroleukemia cell line with DON

and NIV showed that NIV was approximately 4 times more toxic than DON and cytotoxicity was not induced through damage to the plasma membrane (Minervini et al., 2004).

DON administered orally to mice was shown to increase mRNA levels of the cytokines IL-1β and IL-6 in the spleen and Peyer's patches, TNF-α in the spleen and liver, and TNF-β in the kidneys and small intestine. These results demonstrated an immune response follows the inhibition of protein synthesis by DON in mice (Azcona-Olivera, et al., 1995a). Murine CD4+ cells exposed to DON had elevated levels of IL-2, IL-4 and IL-5 in the culture supernatants, suggesting that DON can induce interleukin secretion and mRNA transcript levels in CD4+ cells (Azcona-Olivera et al., 1995b).

Occurrence of DON in food

The worldwide contamination of cereal products with DON, along with hypotheses that *Fusarium* species are subdivided by the type of toxin they produce and distributed differently by region, has been known for a long time (Tanaka et al., 1988). A more recent analysis of 17,732 samples of feed and raw commodities sourced globally detected DON in 9,960 (56%) samples tested (Schatzmayr & Streit, 2013).

DON is present in most wheat grains across Austria, Germany, Slovakia (Berthiller et al., 2009), and in cereal products across the Czech Republic (Ornerova & Ehrenbergerov, 2007). DON is present in approximately half of cereal products across Serbia (Jajić, Jurić, & Abramović, 2008; Jajić, Jurić, Glamočić, et al., 2008). DON is present in most durum wheat grown in northern Tunisia (Ennouari et al., 2013). DON is present in wheat grains across Kenya (Muthomi et al., 2008). DON is present in wheat grains across Brazil, and now there is an equivalent amount of NIV being produced simultaneously (Del Ponte et al., 2012; Dos Santos et al., 2013). DON is commonly present in the Yangtze-Huaihe river basin region in China (Cui et al., 2013).

The most common cause of DON in wheat grains is a disease known as *Fusarium* Head Blight (FHB) which typically follows infection by *Fusarium graminearum*. Infection of a single floret can result in DON contamination in all the grains below as FHB progresses (Peiris et al., 2011; Savard et al., 2000). The yield loss due to shrunken "tombstone" kernels is correlated with DON content (C. Snijders & Perkowski, 1990). *Fusarium* damaged kernels which are red contain substantially more DON than white or shrunken kernels (Neuhof et al., 2008).

Levels of DON are generally highest in the bran and lowest in the flour (Trigo-Stockli et al., 1996). DON can be distributed throughout wheat grains and can remain in all fractions of wheat during the milling process (Hart & Braselton, 1983). Cleaning and milling cannot effectively remove DON in wheat grains, and DON can also remain in bread baked from naturally contaminated wheat flour (Abbas et al., 1985). Milling can lead to a fractionation of DON with increased levels in the bran and decreased levels in flours derived from the inner portions of the grain, leading researchers to suggest that fungal infection was greatest at or near the kernel surface (Young et al., 1984).

Despite cosmopolitan worldwide occurrence, DON is not present in grains grown in the Inland Pacific Northwest (INW) region of the United States. More broadly, hard and soft wheat grown in the northeast and midwestern states contain higher average DON concentrations at the processor level than the western states (Bianchini et al., 2015). However, it currently remains unknown if the INW will remain as one of the last regions capable of growing high-quality grains free of trichothecene mycotoxins in the future under climate change

Role of DON during the infection process of wheat

Infection of wheat crops by *Fusarium* species is optimal during anthesis when the floret is exposed. Pollen is a stimulant which increases the rate of germination and germ tube growth by *F. graminearum* macroconidia after arrival at the flower surface (Naik & Busch, 1978). How spores of most *Fusarium* species are able to reach the wheat canopy is poorly understood and largely attributed to "splash dispersal" following rainfall events (Fernando et al., 2011; Jenkinson & Parry, 1994a).

F. graminearum is self-fertle, meaning that a single spore or hyphal fragment with a nucleus can complete its entire life cycle. The telomorph, or sexual stage, is called *Gibberella zeae* which is characterized by production of blue colored fruiting bodies called perithecia. Inside the perithecia are 8 ascospores in a biseriate arrangement (Trail & Common, 2000). Ascospores are forcibly ejected from the ostiole at the perithecial apex with a pre-launch acceleration of approximately 870,000 *g* allowing them to reach the wheat canopy (Trail et al., 2005). The rate of ascospore discharge increases with relative humidity (Trail et al., 2002). The inoculum level of *G. zeae* on wheat spikes is typically greatest following rainfall events (Francl et al., 1999; Paulitz, 1996).

After colonization of the floret *F. graminearum* spreads throughout the spikelet and down into the rachis node (Brown et al., 2010). The infection front typically moves downward affecting all spikelets below the initial point of infection (Malbrán et al., 2012). After colonization of the wheat heads, *F. graminearum* can spread further into the stem and wheat culms (Guenther & Trail, 2005). An increase in levels of DON in wheat spikes follows the infection front as more and more spikelets are colonized (Savard et al., 2000). While all parts of the infected wheat plant contain DON following a systemic infection, the highest amounts are found in the rachis (Obanor & Chakraborty, 2014; Sinha & Savard, 1997). The rachis and internodes during anthesis contain high quantities of sucrose which contribute to the carbohydrate accumulation in kernels during grain filling (Chevalier & Rupp, 2001).

Plant cell walls thicken as a response to fungal infection. Most of the exoproteome of *F. graminearum* is implicated with carbohydrate metabolism through cell wall polysaccharide degradation (Phalip et al., 2005; Stephens et al., 2008). DON is produced in hyphae prior to penetration while in close contact with the inner surface of the lemma and palea or on the surface of the ovary and spreads into the host cell wall during penetration by the infection peg (Kang & Buchenauer, 2002). A mutant of *F. graminearum* unable to produce DON was not able to penetrate the rachis node which was heavily thickened as a response to infection, while the wild-type was able to penetrate the rachis node and little thickening was observed (Jansen et al., 2005).

Production of DON is not uniformly induced during the onset of infection but tissue specific and primarily localized in the center of the rachis node (Ilgen et al., 2009). Plant cell walls are also more acidic than their surroundings due to accumulation of hydrogen ions (Yu et al., 2000). Acidic pH is an inducer of DON production by *F. graminearum* (Merhej et al., 2010).Further evidence of DON's involvement with cell wall degradation can be seen when treating barley samples with cell wall degrading enzymes to increase the amount of DON extracted (Schwarz, 2008). In addition, after assessing the resistance of 22 wheat genotypes to *F. culmorum* infections the researchers concluded that FHB resistance is based on resistance to colonization and not establishment. In the resistant wheat line DON translocation from the chaff to the kernel was inhibited (Snijders & Krechting, 1992).

Several host stress responses also induce the production of DON. One method is an "oxidative burst" which results in the generation of reactive oxygen species such as hydrogen

peroxide. Hydrogen peroxide triggers the production of DON by *F. graminearum* (Kris Audenaert et al., 2010; Ponts et al., 2007). As DON itself also elicits hydrogen peroxide production by wheat, the resulting feedback loop may stimulate programmed cell death and assist in necrotrophic fungal growth (Desmond et al., 2008). Another response to infection is the activation of the polyamine biosynthetic pathway which precedes DON accumulation (Gardiner et al., 2010). L-arginine, guanine, putrescine, and agmatine are all potent inducers of DON production by *F. graminearum* (Gardiner, et al., 2009a). Amines and low pH act synergistically to increase production of DON by *F. graminearum* (Gardiner et al., 2009b).

F. graminearum can also spread systematically through a wheat plant when originating from the soil, and the water solubility of DON allows for contamination of wheat heads and kernels without the fungus being present in those tissues (Moretti et al., 2014). This phenomenon is shared by *F. culmorum* which typically infects wheat roots and crowns and constantly produces trichothecenes for the duration of infection (Beccari et al., 2011). While the highest concentrations of DON are typically found in stem bases, DON is able to translocate into the head and contaminate wheat kernels following infection by *F. culmorum* (Covarelli et al., 2012; Winter et al., 2013).

Environmental factors influencing the occurrence of FHB

Early split-plot experiments with controlled misting to maintain elevated humidity showed earlier and increased levels of isolation for the FHB pathogens *F. avenaceum*, *F. culmorum*, and *F. graminearum*. Increasing humidity also increased trichothecene contamination (Jones et al., 1997). Inoculating wheat ears with *F. culmorum* at different growth stages demonstrated that the most FHB symptoms and DON production occurred following inoculation around mid-anthesis. Extending the duration of wetness after inoculation using mist irrigation resulted in increased rates of infection and levels of DON (Lacey et al., 1999). These effects have been replicated, as a field experiment in North Carolina winter wheat showed that increasing the duration of post-anthesis mist increased the average amount of *Fusarium* damaged kernels, frequency of infection, and levels of DON (Cowger et al., 2009).

Further controlled-environment experiments elucidated temperature optima for infection at 27.5 °C and 26.5 °C for *F. graminearum* and *F. culmorum*, respectively. Increasing relative humidity from 65% to 100% increased the frequency of glumes infected

by *F. graminearum* but decreased the frequency of infection by *F. culmorum* (Rossi et al., 2001). The number of macroconidia produced by *F. graminearum* or *F. culmorum* on PDA is maximal at 30 °C, and rates of sporulation correlated with a corresponding field experiment. Since a temperature-dependent model accounted for 83% to 98% of the total variance in macroconidial production under field conditions, researchers emphasized temperature as an important meteorological factor in the development of FHB (Rossi, Pattori, et al., 2002). A further study comparing the FHB disease response by *F. graminearum* and *F. culmorum* in 8 wheat cultivars incubated at either 16 or 20 °C confirmed that disease symptoms increase with temperature (Brennan et al., 2005).

By altering the water activity of irradiated wheat grains and incubating cultures of *F. graminearum* and *F. culmorum* at either 15 or 25 °C for 40 days, researchers were able to create response surfaces for the effect of different environmental profiles on DON production. The range of water activities for DON production was narrower than the range required for growth, but for both species increasing the water activity increased DON production. Growth and DON production were optimal at 25 °C for both species, but F. *graminearum* was able to produce DON over a wider range of water activities compared to *F. culmorum* (Hope et al., 2005). A similar study using *F. graminearum* and irradiated wheat grains over a wider range of temperatures and water activities showed that DON was produced most rapidly when incubated at 25 °C and 0.995 a_w (Ramirez et al., 2006). Further experiments with multiple isolates showed strain-level differences though DON production by *F. graminearum* and *F. culmorum* was highest at 25 °C and 0.995 aw (Kokkonen et al., 2010). Expression of 6 transcription genes responsible for DON production was related to DON levels in incubation experiments of *F. culmorum* and *F. graminearum* on YES media at different water activities and temperatures (Schmidt-Heydt et al., 2011).

The amount of *Fusarium* macroconidia collected from a volumetric spore sampler which sampled air over winter wheat crops was related to meteorological conditions. Few conidia were sampled from air before rainfall, but the number progressively increased during rainfall and kept increasing afterwards in the presence of high humidity reaching a peak that then decreased rapidly when relative humidity dropped (Rossi et al., 2002). The amount of spores present in the air is relevant to FHB as an increased inoculum in the air is highly correlated with the frequency of *F. graminearum* infection and DON production during

flowering (Hellin et al., 2018). DON production by *F. graminearum* macroconidia and *G. zeae* ascospores increased with relative humidity, though the humidity requirements for germination were lower in ascospores compared to macroconidia (Beyer et al., 2005). A 3 year survey in northern Italy using a volumetric spore sampler found rain and vapor pressure deficit to be the climatic variables which most affected ascospore discharge (Manstretta & Rossi, 2015).

Modelling approaches to link FHB to climate

Modelling the risk of mycotoxin contamination in crops can be broadly divided into either an empirical approach or a mechanistic approach (Battilani, 2016). The empirical approach seeks to describe the relationship between various predictor variables and a specific event of interest. An example would be relating DON levels to weather data and selecting the set of variables which give the best description of the observed variability in DON contamination. Most models evaluating the effect of environmental models on FHB and DON contamination are empirical models (Prandini et al., 2009).

The mechanistic approach seeks to describe the relationship between various predictor variables at each step of the fungal infection cycle. An example for predicting the risk of DON and FHB included spores produced on inoculum sources, sporulation rate, spores landed on head surface, dispersal rate, infection rate, proportion of head tissue infected, disease symptoms, and mycotoxin content. These rates were influenced by multiple weather variables such as air temperature, relative humidity, and rainfall (Rossi et al., 2003). The whole process is difficult to interpret and poorly documented: little information on how rates were derived is given or how the model was validated.

A more recent mechanistic model to predict an infection index for FHB incorporated the dynamics of flowering, airborne inoculum density, and infection frequency. An infection event was based on a combination of rainfall and relative humidity over 2 day windows and infection frequency was a function of the corresponding mean daily temperature. Other rates and rules in the mechanistic model were influenced by mean temperature, solar radiation, relative humidity, and precipitation. Incorporating a correction factor with host susceptibility and daily spore cloud density the mechanistic model had a 93% predictive accuracy for disease severity and 69% predictive accuracy for disease incidence (Del Ponte et al., 2005).

The same mechanistic model was then used in combination with 50 years of historic data to investigate the potential seasonal variability in FHB epidemics. The risk of FHB was also suggested to be higher for later planting dates in the most recent decades and some locations were shown to become more favorable to FHB after the 1980s. This analytical approach allowed the researchers to suggest that an increased sea surface temperature in the Pacific which is linked to increased spring rainfall was associated with an increased frequency of FHB epidemics in southern Brazil (Del Ponte et al., 2009).

An early example of constructing an empirical model to identify important weather variables and their timing to predict DON content in mature grains involved a stepwise multiple regression procedure combining data from approximately 400 farm fields across southern Ontario from 1996 to 2000. The weather most influential for predicting DON was found to occur over 3 critical periods: 4 to 7 days before heading, 3 to 6 days after heading, and 7 to 10 days after heading. Important variables prior to heading were the number of days where rainfall exceeded 5 mm and the number of days where the temperature did not exceed 10 °C. Important variables after heading were the number of days where rainfall exceeded a defined threshold (3 or 5 mm), and the number of days where temperature exceeded a defined threshold (10 or 32 °C). This approach produced a model with 73% predictive accuracy (Hooker et al., 2002).

Another empirical model to predict epidemics of FHB involved a logistic regression model with nonparametric correlation analysis using data from 50 location-years across 4 states representing different wheat-production regions. Many predictor variables were intercorrelated meaning that not all could be used simultaneously in any model equation. The magnitude of correlations for weather variables were higher for time periods following anthesis rather than before anthesis. The variable most correlated with FHB epidemics was hours of the day where relative humidity exceeded 90% with a temperature between 15 and 30 °C for the 10 days following anthesis. The derived logistic regression models displayed an 84% prediction accuracy which was considered high by the researchers since the models only used data from 17 day windows (De Wolf et al., 2003).

A wide range of differing approaches have been used to construct empirical models to predict FHB or DON content. A neural network was used to predict DON content in wheat grains using data from 2 field experiments over 2002 to 2005. The winning neural network,

with 87% prediction accuracy, utilized preceding crop as a categorical variable with average April temperature, sum of April precipitation, average temperature 5 days prior to anthesis, and sum of precipitation 5 days prior to anthesis as continuous variables (Klem et al., 2007). FusaProg, a decision support system for Swiss wheat producers, is an empirical model which combines prevailing weather conditions with cropping factors, previous crops, and straw management into account. Weather variables used include average temperature, relative humidity, and total precipitation for specific dates. The online program was able to accurately predict DON content below or above a critical threshold of 0.5 ppm with 58% predictive accuracy using 23 cases (Musa et al., 2007). Boosted regression trees were able to classify FHB epidemics better than logistic regression models, and the strongest predictors of FHB epidemics were derived to be mean relative humidity and daily temperature. The predicted risk was substantially increased when mean relative humidity was above 70% which was a lower threshold than what was reported by previous empirical models (Shah et al., 2014).

Researchers have used a methodology called polynomial distributed lag regression analysis to investigate the effect of weather variables on the daily inoculum abundance of *G. zeae* on wheat spikes. The methodology is quite extensive and difficult to comprehend, but the researchers were able to assign the importance of weather variables on inoculum abundance and investigate differences going backwards in time. For temperature related variables a second-degree power was the highest order term, meaning that there was either an optimum or a minimum within the previous 8 days prior to measuring the inoculum abundance. For all variables using relative humidity and daily wetness duration a first-order term was used, meaning that the importance increased or decreased in the previous 8 days prior to measuring inoculum abundance. The effect of climate can be summarized as relative humidity having a positive association with inoculum abundance and the maginitude of the effect decreased linearly over time. This extensively in-depth analysis demonstrated that local weather conditions can be utilized to improve estimates of spore density on wheat spikes around anthesis (Paul et al., 2007).

A multiple regression approach was used to predict DON content in Dutch winter wheat. The model which incorporated climate data from 24 days both pre and post-heading performed better than a model which only incorporated data from 6 days around the heading

date. The predicted DON levels increased with temperature, precipitation, and humidity but decreased with the number of hours with a temperature above 25 °C (Franz et al., 2009). A logistic regression approach to identify climate variables in pre- and post-anthesis time windows derived models utilized derivatives of humidity and temperature, and suggested that relative humidity was a very important variable to use when characterizing moisture effects on FHB (Shah et al., 2013).

Empirical models are derived from the specific data that was used to produce them. In the case of DON content and FHB epidemics, the resulting published equations are only relevant to the region of study. Using a published model equation derived from one location may not be relevant for a different location. As such, empirical models should only be used within their interpolation region for making predictions (Baranyi et al., 1996). If there is no empirical model for a region of interest, it is necessary to both collect the relevant data and fit a region-specific model to predict different indices of FHB. However, previous literature provides a framework for what variables have been shown to perform well and comparing differences between empirical models leads to a better understanding of the general environmental phenomenon.

The comparison between DON content of wheat and FHB index using a meta-analysis of 126 field studies by comparing individual linear regressions showed that study location has a significant effect on the relationship between the two outputs of interest. Intercepts varied by location, and regression slopes between DON content and FHB index varied by wheat cultivar. Overall, there was still a positive relationship between DON content of wheat and FHP index (Paul et al., 2006).

A cross-validation study of a mechanistic and empirical model to predict DON in wheat at harvest using field data from the Netherlands and Italy demonstrated both approaches yield similar results. Both models could predict approximately 90% of wheat samples above a threshold of 1250 μg/kg correctly (Leggieri et al., 2013). An empirical model for growers using weather data from 10 days following anthesis was compared to a different empirical model for millers which used weather data from the entire cultivation period to wheat harvest. Both models performed poorly in predicting high concentrations of DON, but the researchers concluded that since both were able to predict lower concentrations

correctly 2 week weather forecast data can be reliably used to predict DON content in wheat (Fels-Klerx, 2014)

Climatic profile of the Inland Pacific Northwest

The Inland Pacific Northwest (INW) includes all of eastern Washington and northern Idaho, delineated between the Rocky Mountains on the east and Cascade Mountains on the west. Both mountain ranges contribute to a rain shadow from both directions, and produce a precipitation gradient within the Columbia Basin. The University of Idaho and Washington State University are located within the Palouse, a unique hilly region that is predominately associated with Whitman County, WA and Latah County, ID (Bowlick et al., 2015).

The Palouse region initially consisted of a perennial grass prairie dominated by bunchgrasses. Settlement by European-Americans began in the 1860s and by 1890 half the land in Whitman County was being farmed. Agricultural use increased from 1900 to 1930 after development of a railroad network but was still labor intensive. After 1930 approximately 90% of all Palouse wheat was being harvested by combines, and mechanization has continued through the present day (Black et al., 1998).

In the Palouse approximately 80% of annual precipitation occurs from October through May. The mild winters lead to a 150 day growing season across most of the region, though in the eastern region at higher elevation the growing season is only around 100 days. The climate combined with fertile soils allows the Palouse to be one of the most productive wheat growing regions, with yields averaging approximately 70 bushels per acre (Hall et al., 1999).

The variability in climatic conditions across the Palouse led researchers to develop a system of agroclimatic zones to delineate regions by soil depth, mean annual precipitation, and cumulative growing degree-days from January 1 through May 31. Soil depth was categorized as either shallow (less than 1 m), or deep (greater than 1 m). Deep soils require more than 1 winter's precipitation to recharge their water holding capacity unless annual precipitation is greater than 400 mm. Mean annual precipitation was categorized as very dry (less than 250 mm), dry (250 to 400 mm), and wet (greater than 400 mm). Water is the single most limiting factor for dryland wheat production, and deep soils in the wet class can typically product a crop 70% of the time. Cumulative growing degree-days were categorized as cold (less than 700), cool (700 to 1000), and warm (greater than 1000). The cold areas

have risk of frost every month and typically grow frost-tolerant varieties of wheat. The cool areas are optimum for growing wheat with cool temperatures and moderate evapotranspiration. The warm areas have high evaporative demand, a high probability of heat stress after the booting stage, and typically grow early-maturing varieties of wheat (Douglas et al., 1992).

Delineation of the INW by the above features produced 6 agroclimatic zones. Zone 1 is high elevation and mountainous terrain with only 20% of the area being cultivated. Zone 2 is the Palouse with 95% of the area being cultivated, and represents the optimum location for wheat production. Zone 3 contains lower elevation areas in the Palouse and Nez Perce with less than optimum precipitation for crop production, though 95% of the area is being cultivated. Zone 4 comprises regions of central Oregon with only 50% of the area being cultivated. Zone 5 is the grain-fallow region with 90% of the area being cultivated, and Zone 6 contains areas which cannot be cultivated without irrigation (Douglas et al., 1992).

Incidence of DON producing fungi across the INW

A survey of 132 irrigated and 35 dryland wheat fields across central Washington did not detect FHB in any of the dryland fields. FHB was found typically found in fields with center pivot irrigation, and the heavily infested region was primarily constrained within 16 meters of the center pivot. It was suggested that the increased humidity in the middle of center-pivot irrigated fields contributed to development of FHB, as FHB incidence was significantly lower in irrigated fields which used a wheel line or rill. The predominant species responsible for FHB were identified as *F. graminearum* and *F. culmorum* (C. Strausbaugh & Maloy, 1986).

A survey of fields across eastern Washington, Idaho, and Oregon following severe outbreaks of *Fusarium* root rot (FRR) identified *F. culmorum* as the predominant causal agent as it represented 93% of the isolates collected from diseased plants. Other species isolated included *F. pseudograminearum* and *F. avenaceum*. Soil sampling demonstrated that higher population densities of *F. culmorum* in soil correspond to higher FRR severity ratings within a wheat field. Field symptoms were replicated in the greenhouse and demonstrated that disease incidence increases with the amount of inoculum. It was suggested that a soil population of 100 *F. culmorum* propagules per gram soil (PPG) could cause yield reductions, and fields ranged from undetectable to 3208 PPG (R.J. Cook, 1968). A follow-up study of 80

wheat fields across eastern Washington, Idaho, and Oregon did not detect *F. culmorum* in 58 fields. Of the fields with *F. culmorum* 13 had populations less than 100 PPG, 7 were between 100 to 1000 PPG, and 2 were greater than 1000 PPG. Investigations into the ability of *F. culmorum* to colonize wheat straw led the researchers to suggest that stubble management is relatively insignificant in increasing or maintaining soil populations of *F. culmorum* (R.J. Cook & Bruehl, 1968a).

A survey of wheat and barley root lesions in fields across 13 counties in southern Idaho identified *F. culmorum* as the dominant pathogen in the survey area (Strausbaugh et al., 2004). F. culmorum was also the primary pathogen isolated from root lesions in the wetter location of Ririe, ID compared to the drier location of Arbon Valley, ID (Strausbaugh & Windes, 2006).

An extensive survey of winter wheat plants and soil from 288 dryland fields across Washington and Oregon during a dry and wet year reported the high degree of variability in the abundance of the FHB pathogens *F. pseudograminearum* and *F. culmorum* across time and location. *F. culmorum* was cosmopolitan in soil samples but only detected in wheat plants at half as many locations as *F. pseudograminearum*. Of 1318 *Fusarium* isolates identified from 5390 winter wheat tissue sections collected over 2 years, only 1 isolate of *F. graminearum* was reported. Species prevalence in wheat tissues was markedly different between consecutive years, with both *F. culmorum* and *F. graminearum* yielding higher overall isolation frequencies following a wet winter and spring with precipitation 30% above the 20 year mean in some locations in 1993 which contrasted a very dry summer and autumn with 67% less precipitation than the 20 year mean in 1994. Detection of *F. pseudograminearum* was weakly correlated with mean monthly temperature during July. The relative prevalence of different *Fusarium* species within a particular field varied across consecutive years (R W Smiley & Patterson, 1996).

A more recent survey of 210 wheat fields across Washington, Idaho, and Oregon aimed to collect winter wheat stubble and use a factor analysis approach to determine the effects of climate on the distribution of FRR pathogens and FRR disease severity. The mean frequency of isolation of *F. culmorum* and *F. pseudograminearum* was similar across both survey years, with F. pseudograminearum being higher in 2008 attributed to warmer and drier weather conditions. *F. culmorum* was more frequently isolated from wetter and cooler

locations while *F. pseudograminearum* was more frequently isolated in warmer locations. Isolation frequency of *F. pseudograminearum* and *F. culmorum* were positively and negatively correlated with temperature parameters, respectively. As one of the factors used in factor analysis based on precipitation and snowfall increased the probability of *F. culmorum* isolation increased from 60% to 80%. The probability of *F. culmorum* isolation was also found to decrease with increasing cropping intensity under environments with high precipitation and increase with cropping intensity under environments with low precipitation (Poole et al., 2013).

Most inoculum capable of causing FHB and FRR within the Pacific Northwest is soilborne (Paulitz et al., 2002). *F. culmorum* is able to survive in soil for 10 years, as evidenced by 100% survival in soil across 11 isolates studied (Windels et al., 1993). The long-term survival capability of *F. culmorum* was used to explain why inoculum levels within a field remain consistent despite crop rotations (R.J. Cook & Bruehl, 1968a).

However, there are species-specific differences which influence the survival of *F. culmorum* and *F. pseudograminearum* chlamydospores in soil. Chlamydospores of *F. culmorum* are able to survive in air dry soil at 9 °C with most of the initial inoculum remaining viable after 8 years while chlamydospores of *F. pseudograminearum* decreased over time under the same conditions and were completely eliminated after 5 years. These results suggested that the hardiness of *F. culmorum* chlamydospores allows the inoculum level to persist in soil and be consistently recovered through soil dilution plating (Sitton & Cook, 1981a). Experiments utilizing artificially infested soil with *F. culmorum* buried at either Lind or Pullman, WA showed that survival at Pullman was greater than at Lind over 3 years (Inglis & Cook, 1986b). The survival characteristics of *F. culmorum* chlamydospores may explain regional differences in species abundance.

Researchers inoculated 42 different *F. culmorum* isolates into winter rye across 2 fields in Germany and compared DON and NIV production between *in vivo* from the fields and *in vitro* from rye grain cultures. Of the 34 isolates which produced DON and the 7 which produced NIV, there was no correlation between the DON content of field-grown grain versus cultured grains. Maximum DON produced *in vivo* was 64.6 mg/kg, while maximum DON produced *in vitro* was 376.3 mg/kg. While DON production was not correlated to biomass, isolates with a higher aggressiveness rating did correlate (r=0.69) with mean DON

production in the field (Gang et al., 1998). It was also established that toxin production is a common feature among *F. culmorum* isolates.

Researchers inoculated 7 *F. culmorum* isolates from Norwegian cereals on rice grains and tricothecene production was quantified using GC-MS. Cytotoxicity of extracts was also assessed using swine kidney cells. While 5 *F. culmorum* isolates produced DON and 2 produced NIV, there was no correlation between DON production and cytotoxicity (Langseth et al., 1998). Another study utilized 34 DON-producing *F. culmorum* isolates on wheat grains and quantified toxin production using GC-MS. In addition, a 550-bp segment from the *TRI5* gene was amplified and sequenced. Sequencing results were correlated with in vitro toxin production, allowing the PCR assay to cluster *F. culmorum* isolates by high (>1 mg/kg) and low (<0.07 mg/kg) DON production (Bakan et al., 2002)

Researchers developed a PCR assay to indicate a DON or NIV chemotype using *F. culmorum, F.graminearum,* or *F. cerealis* isolates and targeting different sequences in the *TRI7* and *TRI13* genes which convert NIV to 4-acetyl-NIV and DON to NIV, respectively. Isolates with the NIV chemotype had functional copies of both genes, while isolates with the DON chemotype had both genes disrupted or deleted. Of the 53 *F. culmorum* isolates tested, 40 belonged to the DON chemotype and 13 belonged to the NIV chemotype (Chandler et al., 2003).

Using the above protocol, researchers determined the chemotypes of 153 *F. culmorum* isolates collected across 76 locations in England and Wales. Isolates from the same field did not all have the same chemotype. Grouped together, 90 isolates belonged to the DON chemotype and 63 isolates belonged to the NIV chemotype. Grouped by fields, 52 contained the DON chemotype and 24 contained the NIV chemotype. It was found that the NIV chemotype only predominated in the southwest of England and Wales while the DON chemotype predominated elsewhere, suggesting that chemotype varies by location within the UK (Jennings et al., 2004)

Also using the protocol of Chandler et al. (2003), researchers determined the chemotypes of 37 *F. culmorum* isolates from Europe and North America. Isolates which produced DON displayed higher mean aggressiveness after scoring inoculated wheat seedlings, and 30 *F. culmorum* isolates belonged to the DON chemotype and 7 belonged to the NIV chemotype (Tóth et al., 2004).

A different PCR assay for chemoytping 55 *F. culmorum* isolates from 8 European countries targeted the *tri3* and *tri7* genes so that 3-acetyl-DON and 15-acetyl-DON chemotypes could be further differentiated. Of the *F. culmorum* isolates tested, 7 belonged to the NIV chemotype and 35 belonged to the 3-acetyl-DON chemotype. Researchers also tested the protocol given by Bakan et al. (2002), and found that the primer set was indicative of the NIV chemotype rather than weak DON producers (Angela Quarta et al., 2006). These researchers also developed a multiplex-PCR assay targeting the *tri3, tri5*, and *tri7* genes and analyzed the same isolates to validate the methodology.

Researchers collected 29 *F. culmorum* isolates from Iraq and determined their chemotype using the protocol of Quarta et al. (2005). Only 5 isolates belonged to the NIV chemotype, and only originated from the mid-latitudes of Iraq. All the 24 isolates which belonged to the DON chemotype produced 3-acetyl-DON (Matny et al., 2016). A separate study collected 12 *F. culmorum* strains from Algeria and used the methods of both Chandler et al. (2003) and Quarta et al. (2005). All isolates belonged to the DON chemotype. The DON producing ability, assessed using MS media, was correlated with disease severity (r=0.88) and disease occurrence (r=0.70) in barley seedlings (Yekkour et al., 2015)

Reseachers analyzed 106 *F. culmorum* strains from Algeria and 85 *F. culmorum* isolates collected worldwide and determined population genetic structure using numerous PCR protocols. Globally, 160 of 191 *F. culmorum* isolates belonged to the 3-acetyl-DON chemotype and only 31 belonged to the NIV chemotype (Laraba et al., 2017).

Climate change and the INW

Forecasts for future climate trends can be inferred through analysis of historical trends. For example, data from 755 stations across the continental United States in the form of daily maximum and minimum temperatures from 1920 to 2013 showed that approximately half the highest maximum temperature records occurred during the 1930s. Linear models for trends however showed a significant increase in the extent of highest maximum temperature from 1950 to 2013. Climate models project an increase in the occurrence of highest temperature records through the 2050s (Abatzoglou & Barbero, 2014).

Observational data from 4 datasets for the Pacific Northwest from 1920 to 2012 was used to investigate the extent of historical changes. Over the timeframe analyzed mean annual temperature increased as did the increased temperature of the coldest night of the year

and growing season potential evapotranspiration. Spring precipitation showed a long term increase and from the 1970s potential evapotranspiration has increased (Abatzoglou et al., 2014).

Analysis of daily potential evapotranspiration records across 5 stations in eastern Washington from 1987 to 2014 showed an increase in the summer months of June to August. This increase in summertime potential evapotranspiration was attributed to an increase in solar irradiance over the same time period. The increase was not attributed to increases in temperature but rather decreases in cloudiness (Bond & Bumbaco, 2015).

A total of 41 global climate models were analyzed for their ability to reproduce historical data from the Pacific Northwest using a variety of statistics (Rupp et al., 2013). It is suggested to utilize as many different climate projections as possible to better understand and characterize uncertainty in future projections (Mote et al., 2011). The need for more specific location-based data for predicting wildfires led to the development of statistically downscaled climate models through multivariate adapted constructed analogs which have been validated for use in the western United States (Abatzoglou & Brown, 2012).

Future climate change forecasts for the Pacific Northwest based on simulations from 35 global climate models showed that mean annual temperature increases by 3 or 5 °C under RCP4.5 or RCP8.5, respectively by 2100. Annual precipitation is forecasted to increase by 4% overall, increase by 8% in the winter, and decrease by 4% in the summer by 2100 under RCP4.5. Increased summer warming and drying is a consistent feature across the global climate models tested. Variability in annual precipitation is projected to increase in the winter months of October through January, likely due to increases in extreme precipitation events as evidenced by an increase in the upper quartiles of precipitation totals (Rupp et al., 2017).

In 2015 widespread drought in Washington let to the largest area burned by wildfires on record. This was attributed to high summer temperatures following low winter snowfall. Researchers analyzed the environmental conditions of 2015 in comparison to historical data and future climate projections. Researchers suggested that 2015 is a model for typical future conditions in the Pacific Northwest by 2050 where droughts are likely to be caused by increased temperatures rather than precipitation deficits (Marlier et al., 2017).

Climate change and DON producing fungi

Researchers were able to utilize a weather-based model for FHB incidence in the United Kingdom with a wheat growth model to forecast anthesis dates to make future projections. The climate model utilized the amount of rainfall at anthesis and temperature over the preceding 6 weeks. The combination of a disease model and a crop model demonstrated that wheat anthesis dates will be earlier and FHB epidemics will be more severe by 2050, though the effect varies by location (Madgwick et al., 2011). Further research into those results demonstrated the importance of cropping factors when making forecasts for FHB incidence, as southern England has a high density of corn crops which may contribute a higher inoculum to that region. Researchers suggested that increased cultivation of corn in the future under climate change may also contribute to increased FHB epidemics in the future (West et al., 2012). Researchers utilized the HGCA risk assessment for Fusarium mycotoxins in wheat model, which has been validated for use in the UK, to forecast the future risk of FHB in wheat across Scotland. The model was combined with crop location data and a model for inoculum dispersal to demonstrate that the risk of FHB will decrease over time.(Skelsey & Newton, 2015).

Researchers combined observational data of DON concentrations in mature wheat from 717 fields across Norway, Sweden, Finland, and The Netherlands with agronomical and climactic factors to develop an empirical model. The final model with the best set of explanatory variables included relative humidity, temperature, and rainfall during wheat cultivation along with length of time between flowering and harvest. The model only had 50% predictive accuracy, though the researchers suggested that it could still be applied to climate change scenarios and possibly estimate the trend of climate change on DON content of wheat in northwestern Europe (Van der Fels-Klerx et al., 2012).

By quantifying fungal DNA in wheat heads across 4 European countries over 3 years and relating the results to standardized weather variables using canonical correspondence analysis, researchers were able to demonstrate that weather variables account for variability in species abundance, but not species prevalence *F. graminearum* was associated with warmer and more humid climates while *F. avenaceum* and *F. culmorum* were restricted to cooler and wetter climates across Europe (X. Xu et al., 2008). These results suggesting that climate may only influence the abundance of different species is akin to the old

microbiological adage “everything is everywhere, but the environment selects” by Dutch botanist Lourens Bass Becking in 1934.

The composition of *Fusarium* species in cereal grains across Northern Europe is expected to shift due to climate change. *F. graminearum* is expected to increase, *F. poae* is expected to become more prevalent during dry years, and *F, culmorum* is expected to remain common when harvest conditions are delayed due to humid conditions. *F. culmorum* is also expected to decrease with higher temperatures, with the exception of increases in spring draught (Parikka et al., 2012). Recent analysis of wheat samples from the Netherlands from 2000 to 2001 using multiplex PCR demonstrated that *F. graminearum* was the most abundant species, which contrasts reports from the 1980 to 1990 where *F. culmorum* was the predominant species (Waalwijk, Kastelein, de Vries, et al., 2003). From 2008 to 2012 there have been severe FHB epidemics and increased DON content in Norwegian cereals due to above normal precipitation during anthesis and grain maturation. As *F. graminearum* has become the predominant DON producing species, researchers attributed the increased DON content of Norwegian cereals to FHB epidemics caused by *F. graminearum* (Sundheim et al., 2013).

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Chapter 2: Polyvinylpolypyrrolidone reduces cross-reactions between antibodies and phenolic compounds in an enzyme-linked immunosorbent assay for the detection of ochratoxin A

Robinson, A. L., Lee, H. J., & Ryu, D. (2017). Polyvinylpolypyrrolidone reduces cross-reactions between antibodies and phenolic compounds in an enzyme-linked immunosorbent assay for the detection of ochratoxin A. *Food chemistry*, *214*, 47-52.

Abstract

Ochratoxin A (OTA) is a fungal metabolite and putative carcinogen which can contaminate a variety of foods such as cereals, wine, and nuts. Commercial ELISA kits are known to give false-positive results for OTA concentrations when phenolic compounds are present. Pistachios represent a food matrix rich in phenolic compounds potentially contaminated with OTA, and were used to model OTA cross-reactivity. Polyvinylpolypyrrolidone (PVPP) was incorporated during extraction of OTA using a commercial ELISA protocol. HPLC methods were used to confirm that PVPP does not interact with OTA and levels of gallic acid and catechin remaining in pistachio extracts decreased with increasing PVPP application. Cross-reactivity of extracts also decreased with increasing PVPP application, and color loss was used as an indicator of anthocyanin removal. Incorporating PVPP into ELISA protocols allows for the continued use of rapid immunological methods in food matrices containing phenolic compounds.

Introduction

Ochratoxin A (OTA) is a chlorinated fungal secondary metabolite which is produced most frequently by *Aspergillus ochraceus*, *A. carbonarius*, *Penicillium verrucosum*, and *P. nordicum* (Serra, Braga, & Venancio, 2005). OTA is a putative carcinogen based on its ability to induce nephropathies in rats such as hyperplasia, degeneration, and karyomegaly of renal tubular epithelial cells (IARC, 1993; NTP, 1989). The diversity of OTA-producing fungi is paralleled in the range of agricultural food commodities which can be contaminated with OTA, which are most commonly cereals, nuts, and coffee (Zaied, Abid, Bouaziz, Chouchane, Jomaa, & Bacha, 2010; Coronel, Marin, Cano-Sancho, Ramos, & Sanchis, 2012; Amezqueta et al., 2012). Robust analytical methods are required to quantify OTA concentrations in different food commodities in order to evaluate OTA risk assessment.

Analyzing food commodities using high performance liquid chromatography (HPLC) methods allows for a precise determination of OTA contamination. However, HPLC methods are lengthy and expensive as samples often need to undergo clean-up procedures before analysis (Zimmerli & Dick, 1995; Pittet, Tornare, Huggett, & Viani, 1996; Kieu & Ryu, 2014). Rapid methods, such as enzyme-linked immunosorbent assays (ELISA), offer a more cost-effective alternative to HPLC methods when analyzing a large amount of samples. ELISA kits for OTA quantification are easy to use, commercially available, and have been validated for use in multiple food matrices such as cereals, meats, and wine (Barna-Ventro et al., 1996; Visconti & Girolamo, 2006; Alcaide & Aguilar, 2008). ELISA kits showed good accuracy when compared to HPLC methods when testing 0 to 80 ng/g OTA in cereals, but the precision of ELISA methods decreased at lower OTA concentrations (Zheng, Hanneken, Houchins, King, Lee, & Richard, 2005; Flajs, Domijan, Ivic, Cvjetkovic, & Peraica, 2009). Moreover, ELISA methods are susceptible to overestimation or false-positives due to compounds which have structural similarities to the target analyte cross-reacting with antibodies during the assay.

Phenolic compounds in wine are known to interfere with OTA binding to antibodies (Visconti, Pascale, & Centornze, 1999). Analyzing pistachios for OTA using commercial ELISA kits resulted in significant overestimations of OTA content, which was not observed in kernels after the skins were removed (Lee, Meldrum, Rivera, & Ryu, 2014). Pistachios contain a wide variety of phenolic compounds, which are located primarily in their skins (Tomiano, Martorana, Arcoraci, Monteleone, Giovinazzo, & Saija, 2010). Multiple methods have been proposed to reduce the interference effects of phenolic compounds including additional sample clean-up steps, sample dilution, or blocking agents (Alcaide & Aguilar, 2008; Steinitz, 2000; Flajs, Domijan, Ivic, Cvjetkociv, & Peraica, 2009; Lee, Wang, Allan, & Kennedy, 2004). Additional sample processing steps increase the cost and duration of analysis while diluting samples may decrease OTA concentrations below the detection limit of the ELISA kit. Blocking agents reduce antibody binding to microwell surfaces but don’t address the cross-reactivity of phenolic compounds.

Reducing the cross-reactivity requires a compound which can facilitate the removal of phenolic compounds during OTA extraction. The insoluble phenol absorbent

polyvinylpyrrolidone (PVP) has been used to reduce phenolic compound interference during enzyme activity assays (Loomis & Battaile, 1966). When phenolic compounds present in plants were interfering with ELISA tests screening for Phytophthora disease, PVP was used during extraction in phosphate-buffered saline to minimize interference (Olson, 1995). Polyvinylpolypyrrolidone (PVPP) is an insoluble resin consisting of highly cross-linked PVP polymers that has been used as a fining reagent to clarify wine and beer since 1961 (Dahlstrom & Sfat, 1972). Multiple studies have investigated the effect of various fining reagents on the removal of OTA from wine, and PVPP did not significantly alter the OTA concentration during clarification (Fernandes, Ratola, Cerdeira, Alves, & Venacio, 2007; Castellari, Versari, Fabiani, Parpinello, & Galassi, 2001; Anh, Vural, & Bayram, 2001). The ability of PVPP to bind a wide range of phenolic compounds, remain insoluble in solution, and not interfere with OTA are all desirable characteristics for reducing cross-reactivity in commercial ELISA kits.

The aim of the current study was to determine the ability of PVPP to bind and remove phenolic compounds from a food matrix and reduce the cross-reactivity during OTA analysis using a commercial ELISA kit. PVPP binding characteristics were determined in model extraction solutions containing phenolic compounds and OTA. Pistachio skins were used as a model food matrix to evaluate the effect of PVPP application on removal of phenolic compounds, color loss, and cross-reactivity observed during ELISA.

Methods and Materials

Materials and Chemicals

Pistachios were purchased from a local supermarket chain (Moscow, ID, USA). Pistachio skins were collected by manually scraping kernels, ground into a fine powder using a coffee mill and stored at -20 °C until analysis. Purified standards of OTA, gallic acid, and catechin were purchased from Sigma-Aldrich (St. Louis, MO, USA). Working standards were prepared in 50% methanol, stored at -20 °C in amber glass vials, and brought to room temperature before use. HPLC grade methanol and acetonitrile were purchased from Fisher Scientific (Pittsburgh, PA, USA), and acetic acid (99.5%), sodium acetate (> 99%) and phosphate buffered saline (PBS) tablets were obtained from Sigma-Aldrich (St. Louis, MO,

USA). OchraTest WB immunoaffinity columns were purchased from VICAM (Watertown, MA, USA).

Model extraction

Solutions containing 5 mg/mL gallic acid were prepared in 0-70% methanol. Pre-weighed PVPP was added to give a final concentration of 10 mg/mL and the solutions were mixed using a wrist action shaker (Burrell Model 75, Pittsburgh, PA, USA) for 30 min. Two 1 mL aliquots of each sample were transferred to micro-centrifuge tubes before centrifugation (9391 ×g) at room temperature using an Eppendorf 5424 R centrifuge (Eppendorf, Hamburg, Germany). Supernatants were pooled and stored at 4 °C until analysis. For testing the effect of PVPP concentration, solutions containing 5 mg/mL of either gallic acid or catechin in 50% methanol (v/v) were spiked with OTA to give a final concentration of 10 ng/g. Pre-weighed PVPP was added to give final concentrations of 10, 25, 50, 100, or 150 mg/mL. Samples were shaken and centrifuged as described above.

Pistachio skin extraction

Ground pistachio skins (0.5 g) were weighed and placed into 30 mL centrifuge tubes. Either 50, 125, 250, 500, or 750 mg PVPP was added to each tube before the addition of 5 mL 50% methanol. Tubes were mixed using a wrist action shaker for 30 min before centrifugation (12,062 ×g) for 10 min at room temperature using a Sorvall RC-5 centrifuge (Thermo Scientific, Hudson, NH, USA). Supernatants were collected and stored at 4 °C until analysis.

HPLC analysis of OTA

For HPLC determination of the OTA, 1.2 g pistachio skins and 12 mL acetonitrile/water (80:20, v/v) were mixed in a 30 mL centrifuge tube for 30 min before centrifugation at 9391 ×g as described above. Two milliliters of the supernatant were diluted with 8 mL PBS before filtering through an IAC at a flow rate of about 2-3 mL/min. The column was washed with 10 mL of PBS followed by 10 mL of water and OTA was eluted with 3 mL of methanol into a vial at a flow rate of 2-3 mL/min. The eluate was evaporated to dryness under a gentle stream of nitrogen at 50 °C. The residue was re-dissolved in 500 µL 50% methanol (v/v) and 50 µL of the aliquot was injected into HPLC (Agilent 1260 Infinity HPLC system, Palo Alto, CA, USA) equipped with a quaternary pump, an autosampler, a

vacuum degasser, and a fluorescence detector. The chromatographic separation was performed on a reversed-phase Hypersil GOLD C18 column (3 × 100 mm, particle size 1.9 µm, Thermo Scientific, Hudson, NH, USA) with isocratic elution using 50% acetonitrile and 50% water containing 1% acetic acid at a flow rate of 0.4 mL/min. OTA was detected at 334 nm and 460 nm for excitation and emission, respectively.

OTA analysis using ELISA

A commercial OTA ELISA kit (Veratox®, Neogen Corporation, Lansing, MI, USA) was used to observe the cross-reactivity of pistachio skin extracts. Samples and standards were analyzed using the manufacturer's protocol, and the optical density was read at 620 nm using a microplate reader (Tecan Sunrise, Männedorf, Switzerland). Degree of interaction was calculated as the amount of OTA quantified using the ELISA protocol.

Analysis of phenolic compounds

The total phenolic compound contents of pistachio skins were determined by employing Folin-Ciocalteu colorimetric method (Arnous, Makris, & Kefalas, 2002). The total phenolic content was expressed as µg of gallic acid equivalents/g of fresh material, using calibration curves prepared with gallic acid as a standard.

Phenolic compound content was also measured using an Agilent 1260 Infinity HPLC system (Palo Alto, CA, USA) equipped with a Luna C18 column (4.6 × 250 mm, particle size 5 µm, Phenomenex, Torrance, CA, USA) and an UV-Vis detector. The mobile phase contained 1% acetic acid (solvent A) and acetonitrile (solvent B), with the linear gradient commencing at an A/B ratio of 92/8 and finishing at 73/27 over 40 min at a flow rate of 0.8 mL/min. Detection was performed at 280 nm. Injection volume was 20 µL for the analysis.

Color of pistachio skin extracts

To measure the effect of PVPP on clarifying pistachio skin extracts, a 1 mL aliquot of sample extract was loaded into a plastic dish and color was measured using a Minolta CR 400 colorimeter (Minolta, Osaka, Japan) mounted to a ring stand. Color values were recorded using the Lab color space, where L is the lightness dimension while a and b are color-opponent dimensions. To calculate the total color difference (ΔE), the following formula was used:

$$\Delta E = \sqrt{(((Lsample - Lstandard)2 + (asample - astandard)2 + (bsample-bstandard)2))}$$

Statistical analysis

The analytical results represent an average ± standard deviation of three determinations obtained for each parameter. Simple correlation coefficients and single factor ANOVA were used for determination of significant differences ($p < 0.05$). Statistical analysis was carried out using SPSS (IBM Corp., New York, NY, USA).

Results and Discussion

Effect of methanol concentration on PVPP binding efficiency

Previous studies showed that PVPP binding phenolic compounds was optimal when the methanol concentration was below 10% (Loomis & Battaile, 1966; Andersen & Sowers, 1968; Magalhaes et al., 2010). The first goal of this work was to verify if PVPP could remove phenolic compounds in 50% methanol used by the commercial ELISA protocol during the extraction of OTA. Treating 5 mg/mL gallic acid solutions with 10 mg/mL PVPP removed 46%, 41%, and 34% of the gallic acid in 10%, 30%, and 50% methanol, respectively (data not shown). This negative trend has been reported for flavonoids, as increasing the methanol concentration from 25% to 70% decreased the fraction of quercetin bound by PVPP from 85% to 50%, respectively (Doner, Becard, & Irwin, 1993). While phenolic compound removal would be more efficient at lower methanol concentrations, OTA is not as soluble in water as methanol (el Khoury & Atoui, 2010). The methanol concentration remained at 50% for all subsequent experiments so that the only deviation from the manufacturer's protocol was PVPP incorporation.

Selective removal of phenolic compounds by PVPP

Model extraction using 5 mg/mL of either gallic acid or catechin with 10 ng/g OTA were used to observe any PVPP interference with OTA concentrations while binding phenolic compounds. The chemical structures of compounds used in this study are presented in Figure 2.1. Gallic acid and catechin were chosen as they are the two of the most abundant phenolic compounds in pistachio skins (Tomiano et al., 2010). In addition, previous work demonstrated that gallic acid and catechin exhibited the highest degree of interaction in two commercial ELISA kits for OTA detection (Lee et al., 2014). Increasing the concentration of PVPP displayed a dose-dependent decrease in the amount of phenolic compounds remaining

without affecting the OTA concentration (Fig. 2.2). PVPP treatment significantly ($p < 0.05$) decreased the amount of gallic acid and catechin remaining. It appeared that during model extractions OTA remains in solution while phenolic compounds are interacting with PVPP, which may be attributed to the methanol concentration used during extraction. When wine was spiked with OTA and diluted in water before treatment using 10 mg/mL PVPP, 16% of the spiked OTA was lost (Bazin, Faucet-Marquis, Monje, El Khoury, Marty, & Pfohl-Leskowicz, 2013). The minimal interaction of PVPP with OTA concentrations in wine observed in previous studies (Fernandes et al., 2007; Castellari et al., 2001; Anh et al., 2001) could have been due to the low concentration of PVPP used (1.5 µg/mL to 1 mg/mL). Minimal amounts of fining reagents were used to reduce the loss of phenolic compounds which provide beneficial flavors.

PVPP was able to bind and remove catechin more effectively than gallic acid. This observation is in accordance with previous studies using PVPP binding assays (Magalhaes et al., 2010; Duran-Lara et al., 2015). The increased number of hydroxyl groups and additional benzene ring present on the catechin molecule compared to gallic acid may explain the difference in PVPP binding affinity. The main non-covalent interactions between a phenolic solute and PVPP are hydrophobic effects between the benzene ring of phenols and methylene groups of the polymer and hydrogen bonding between phenolic hydroxyl groups and the carbonyl group in the pyrrolidone ring (Molyneux & Vekavakayanondha, 1986). After testing various flavonoids in PVP binding assays, a trend emerged where PVP binding efficiency increased with the number of hydroxyl groups present on the molecule (Doner et al., 1993). Increasing the number of phenol groups also increases the PVP binding efficiency during dialysis (Molyneux & Frank, 1961). By calculating interaction energies, the lower gap energy between PVPP and catechin compared to gallic acid was used to explain the difference in observed binding affinities by PVPP (Duran-Lara et al., 2015).

Effect of phenolic compound removal on ELISA cross-reactivity

Previous work demonstrated that phenolic compounds in pistachio skins can cross-react with antibodies in commercially available ELISA kits for OTA detection (Lee et al., 2014). The pistachio skins contained 38919 ± 1428 µg/g total phenolic compounds as

determined by the Folin-Ciocalteu method. The gallic acid and catechin content of the pistachio skins were 306.4 and 993.8 μg/g, respectively.

Untreated pistachio skin extracts displayed a degree of interaction of approximately 16.74 ng/g. The degree of interaction represents the amount of OTA quantified by the ELISA kit, and was used to measure cross-reactivity as HPLC analysis verified no detectable OTA present in pistachio skins (data not shown). Increasing the PVPP concentration decreased the amount of phenolic compounds remaining, in addition to decreasing the cross-reactivity of the supernatants (Fig. 2.3). Concomitant to the model extract solutions, PVPP was able to bind and remove catechin more effectively than gallic acid. The gallic acid concentration of the supernatants was loosely correlated ($R^2 = 0.78$) to the resulting cross-reactivity, while the concentration of catechin was not ($R^2 = 0.32$). Gallic acid exhibited a significantly greater degree of interaction in ELISA kits compared to catechin (Lee et al., 2014). The degree of interaction was not significantly different between 50 and 100 mg/mL PVPP. At 150 mg/mL, PVPP was able to reduce the degree of interaction below the 2 ng/g OTA detection limit in two out of three replications and completely bound all gallic acid and catechin present in the pistachio skins.

Color loss of pistachio skin extracts as affected by PVPP concentration

The concentration of anthocyanins in pistachio skins influence the degree of red coloration (Bellomo & Fallico, 2007). Since the pistachio skins produced extracts with a dark red hue which faded with increasing PVPP application, color loss was measured as an indicator of PVPP effectiveness in removing anthocyanins. Color values of the extracts were recorded in the Lab color space and are presented in Table 2.1. All color dimensions were significantly affected by PVPP application ($p < 0.01$). The total color loss, ΔE, was linearly correlated to PVPP treatment ($R^2 = 0.90$). PVPP treatment clarified the extracts since the lightness dimension, L, increased while both a and b color dimensions approached zero with increasing PVPP treatment. Positive a and b color dimensions produce red, and a zero value translates to a neutral grey color in the Lab color space.

Conclusions

Overall, the simple and convenient addition of PVPP during the extraction step was able to significantly remove phenolic compounds from pistachio skins. PVPP demonstrated

no interaction with OTA during model extraction in 50% methanol. After separating phenolic compounds from a model food matrix which had been bound to PVPP using centrifugation, the resulting supernatants showed a dose-dependent decrease in cross-reactivity. Incorporating PVPP during OTA extraction using commercial ELISA kits is a feasible method to reduce the overestimation of OTA content in foods containing phenolic compounds.

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Table 2.1. Color properties of pistachio skin extracts as affected by polyvinylpolypyrrolidone (PVPP) concentration

Color Properties*	PVPP used during extraction (mg/ml)				
	0	**10**	**25**	**50**	**100**
L	25.1 ± 1.36	30.33 ± 1.66	29.35 ± 1.12	30.75 ± 0.47	33.47 ± 0.35
a	2.08 ± 0.64	1.27 ± 0.62	0.24 ± 0.49	-0.22 ± 0.18	-0.68 ± 0.03
b	3.86 ± 0.74	3.11 ± 1.07	1.33 ± 1.23	0.71 ± 0.22	-0.54 ± 0.47
ΔE	1.53 ± 0.62	4.61 ± 1.21	5.51 ± 0.58	6.89 ± 0.25	9.83 ± 0.36

**L*: Degree of whiteness (white +100 → 0 black); *a*: Degree of redness (red +100 → -80 green); *b*: Degree of yellowness (yellow +70 → -80 blue); *ΔE*: Total color difference =

$$\sqrt{\left((L_{\text{sample}} - L_{\text{standard}})^2 + (a_{\text{sample}} - a_{\text{standard}})^2 + (b_{\text{sample}} - b_{\text{standard}})^2\right)}$$

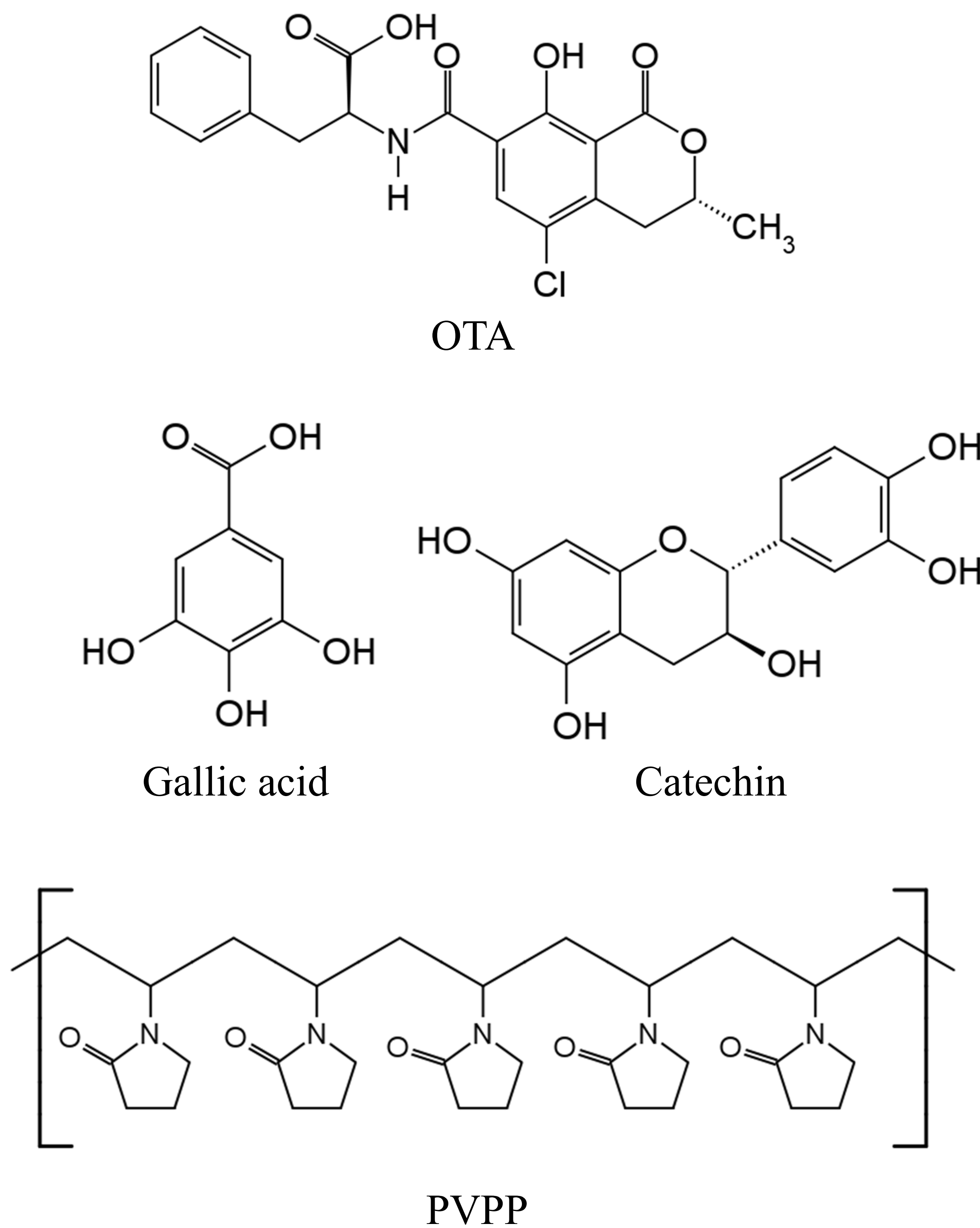

Figure 2.1 Chemical structure of ochratoxin A (OTA), gallic acid, catechin, and polyvinylpolypyrrolidone (PVPP)

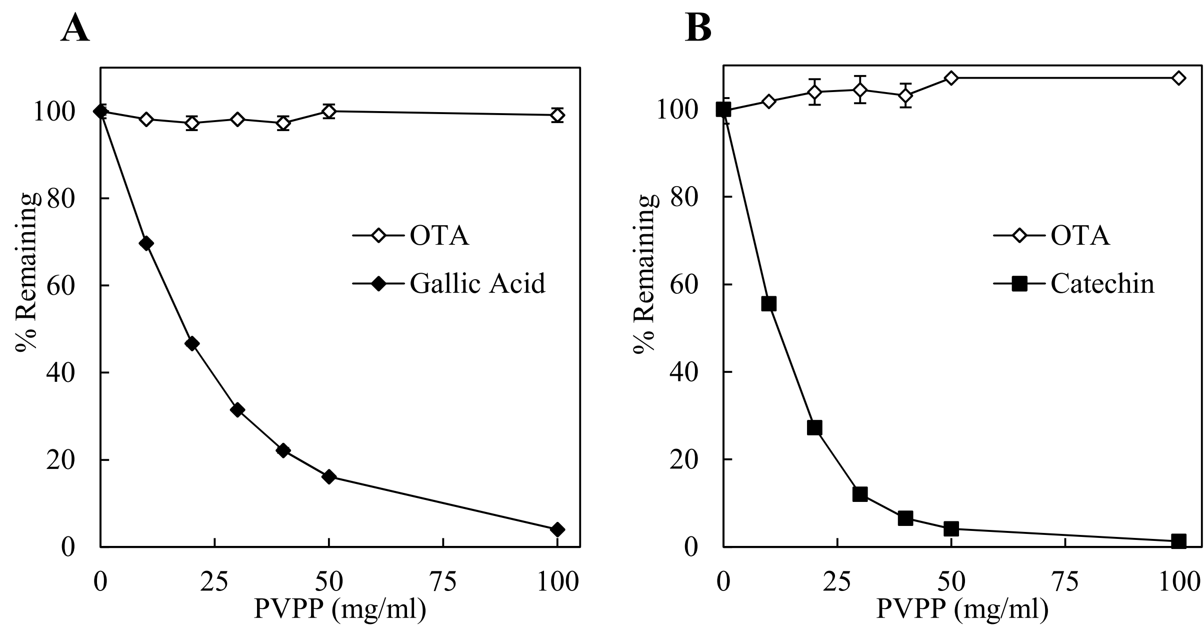

Figure 2.2 Effect of polyvinylpolypyrrolidone (PVPP) incorporation on the amount of gallic acid (A) and catechin (B) remaining in model extraction solvents spiked with 10 ng/g ochratoxin A (OTA).

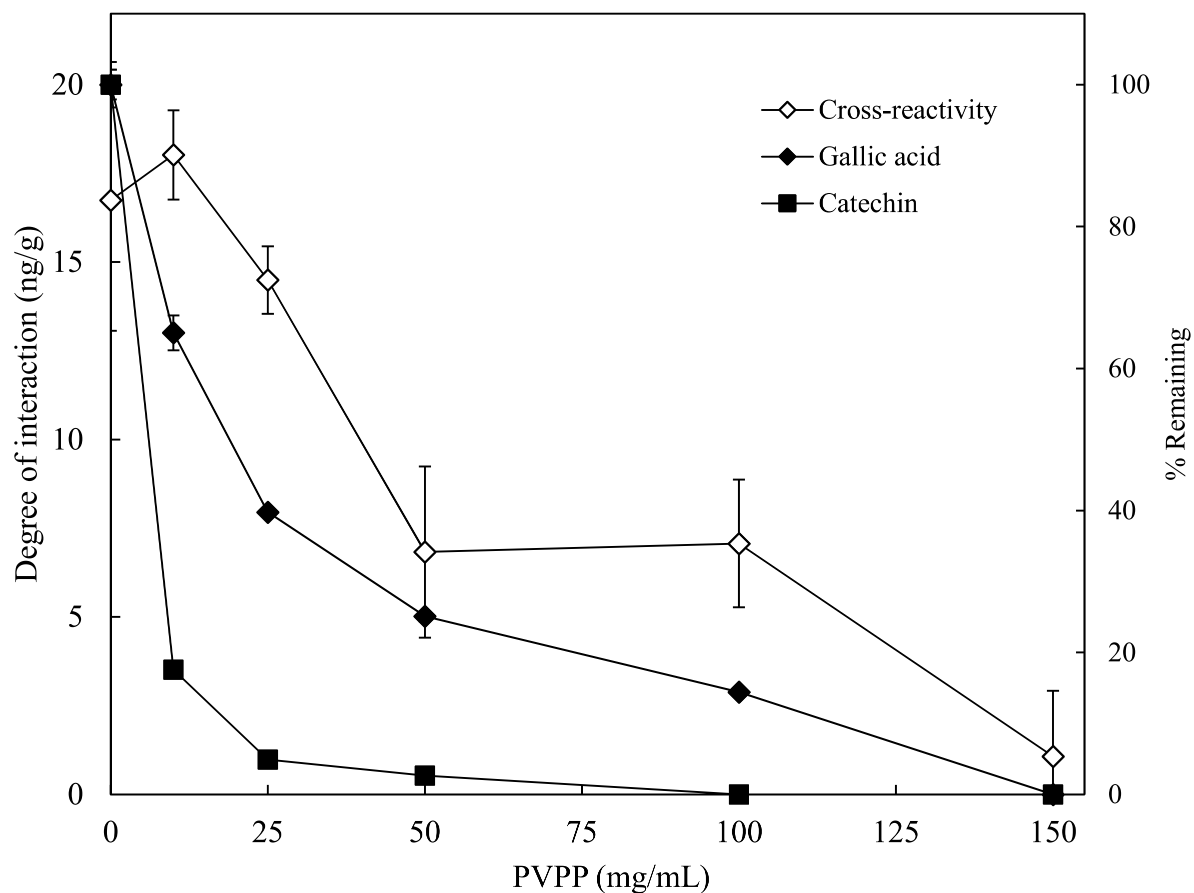

Figure 2.3 Effect of polyvinylpolypyrrolidone (PVPP) incorporation on the amount of gallic acid and catechin remaining in pistachio skin extracts, and the resulting ochratoxin A (OTA) cross-reactivity observed during ELISA.

Chapter 3: Adequate hand washing and glove use are necessary to reduce cross-contamination from hands with high bacterial loads

Robinson, A. L., Lee, H. J., Kwon, J., Todd, E., Rodriguez, F. P., & Ryu, D. (2016). Adequate hand washing and glove use are necessary to reduce cross-contamination from hands with high bacterial loads. *Journal of food protection*, *79*(2), 304-308.

Abstract

Hand washing and glove use are the main methods to reduce bacterial cross-contamination from hands to ready-to-eat food in a food service setting. However, bacterial transfer from hands to gloves is poorly understood, as is the efficacy of different durations of soap rubbing on bacterial reduction. To assess bacterial transfer from hands to gloves and to compare bacterial transfer rates to food with different soap washing times and glove use, participants' hands were artificially contaminated with a ~9 log10 CFU inoculum of Enterobacter aerogenes B199A. Different soap rubbing times (0, 3, and 20 s), glove use, and tomato dicing activities were followed. The bacterial counts in diced tomatoes and on participants' hands and gloves were analyzed. Different soap rubbing times did not significantly change the amount of bacteria recovered from participants' hands. Dicing tomatoes with bare hands after 20 s of soap rubbing transferred significantly ($p < 0.01$) less bacteria to tomatoes compared with bare hands after 0 s of soap rubbing. Wearing gloves greatly reduced the incidence of contaminated tomato samples when compared to bare hands. Increasing soap washing time decreased the incidence of bacterial contamination recovered from outside glove surfaces ($p < 0.05$). These results highlight that both glove use and adequate hand washing are necessary to reduce bacterial cross-contamination in food service environments.

Introduction

Human hands are perpetually inhabited by populations of residential and transient bacteria. Bacterial levels vary by location, with palms containing ~3.5 log_{10} CFU while the spaces underneath fingernails may contain ~5.4 log_{10} CFU (McGinley et al., 1998). The predominant genus of bacteria isolated from hands are *Staphylococci* which represent usual residential skin flora. In sampling the hands of food handlers, it is not uncommon to isolate bacteria such as *Staphylococcus aureus*, *Escherichia coli*, or *Bacillus subtilis* (Aycicek et al., 2004; Shojaei et al., 2006). Food service workers can harbor these bacteria

asymptomatically, and a greater frequency of foodborne outbreaks had been implicated with asymptomatic workers as opposed to sick workers (Todd et al., 2007).

The primary defense in minimizing bacterial cross-contamination during food preparation is adequate hand washing. Both the Centers for Disease Control and Prevention (CDC) and the Food and Drug Administration (FDA) in the United States recommend vigorously rubbing hands with soap followed by rinsing with water to effectively remove pathogens from food handler's hands (Garner et al., 1986; FDA, 2009). The recommended amount of time spent washing hands with soap is variable with different hand washing signs reporting various timeframes between 10 and 20 s (Jensen et al., 2015). Educating food handlers about the effects of poor hygiene and incorporating hand washing with soap and water significantly reduces the bacterial levels present on hands (Prado et al., 2015; Shojei et al., 206).

Even with regulations and training programs in place, food service workers rarely adhere to recommended hand washing methods (Stronbehn et al., 2008). With the pressure of completing multiple tasks within short timeframes, appropriately washing hands after completing various tasks are more likely to be neglected (Green et al., 2007). Due to the inability to enforce hand washing compliance, mandatory glove use has been adopted in several states, yet the efficacy of glove use in food protection is often debated. One study found no statistically significant differences in coliform bacteria recovered from flour tortillas that were handled by bare or gloved hands in fast food restaurants (Lynch et al., 2005). In another study that investigated bacterial levels on employees' hands at a poultry slaughter plant, it was elucidated that disposable glove surfaces contained less aerobic mesophilic bacteria and *S. aureus* than bare hands (Chen et al., 2001). However, wearing disposable gloves did not significantly reduce the amount of bacteria transferred to boneless chicken breasts compared to bare hands (Chen et al., 2001).

Food handlers who wear gloves are more likely to neglect washing their hands as frequently during food preparation (Lynch et al., 2005), yet gloved hands are touching the exact same potentially contaminated sources as bare hands, and therefore, gloves need to be changed frequently (Snyder 1994). If hands have been inadequately washed, pathogens can transfer to the outside surfaces of gloves while being donned (Snyder 1997). In studying bacterial transfer rates from food to hands, it was stated that gloves were permeable to

bacteria, and the rates of bacterial transfer were measured accordingly (Montville et al., 2001). However, it was unclear if the bacteria actually permeated the glove or were present on the outside glove surfaces while being donned by participants. Transient bacteria can be efficiently transferred from bare hands to other surfaces, but only a few studies have been conducted to understand exact mechanisms (Montville et al., 2001; Rusin et al., 2002).

Therefore, the primary goal of this study was to better understand bacterial transfer from hands to gloves under heavy inoculum levels, and compare bacterial transfer rates to food as affected by hand washing time and glove use. By artificially contaminating hands with heavy bacterial loads, the efficacy of different hand washing scenarios and the variability of the recovered bacteria were measured and compared. Finally, the effects of hand washing method and glove use were observed by quantifying the bacterial transfer rate to tomato samples and outside glove surfaces.

Methods and Materials

Bacterial strain and growth conditions

Enterobacter aerogenes B199A generously provided by Dr. Donald Schaffner (Rutgers University, NJ, USA), is a nonpathogenic microorganism with similar growth and attachment characteristics to *Salmonella* (Liu & Schaffner, 2007) used as a surrogate organism for this study. This *E. aerogenes* strain is nalidixic acid resistant which allows selective enumeration in the presence of residential microorganisms on the hands of participants. Nalidixic acid sodium salt and phosphate-buffered saline (PBS) tablets were purchased from Sigma-Aldrich (St. Louis, MO, USA). *E. aerogenes* cells were grown overnight at 37°C with shaking (150 rpm) in tryptic soy broth (Difco, San Jose, CA, USA) containing 50 μg/ml nalidixic acid. To determine the cell density of the inoculum 10-fold dilutions were made in PBS and the 2 lowest dilutions were plated on tryptic soy agar. Aliquots of the stationary phase growth were used to inoculate participants' hands.

Study participants

Nine participants (four males and five females) were recruited in this study to provide 27 data points for every sample in each hand washing scenario. Each participant was informed of the experimental procedures they would be required to perform and signed a consent form before taking part in the experiments. All participants' hands were inspected before testing to verify that there were no visible cuts or scratches. Both hands of each

participant were samples so that handedness would not be a factor. Each participant was informed as to the general nature of the experimental procedures and signed a consent form prior to taking part in the study. Participants were trained in hand washing procedures by watching a standardized video clip describing and demonstrating the hand washing and tomato dicing techniques.

Hand inoculation and hand washing

Before inoculation each participant washed their hands with nonantimicrobial soap for 20 s followed by rinsing with municipal tap water for 10 s. Flow rate of the water from the sink was set to a standardized level (12 L/min) by the researchers and maintained throughout the hand washing scenarios. Hands were dried with paper towels and left to air dry for 1 min before being inoculated. A 1 ml aliquot of inoculum (~9 log_{10} CFU) was placed on the participant's hands, and the participants spread the inoculum over the entire surface by rubbing their hands for 1 min.

After hands were inoculated, participants performed one of three different hand washing scenarios. The hand washing scenarios were: (1, 4) 10 s of rinsing only with water (rinsing), (2, 5) 3 s of rubbing with soap followed by 10 s of rinsing with water (non-compliant hand washing), or (3, 6) 20 s of rubbing with soap followed by 10 s of rinsing with water (compliant hand washing). Compliance is in reference to the US FDA Food Code which states that hands must we vigorously rubbed with soap for at least 10 to 15 s. One pump (2 ml) of nonantimicrobial soap (Softsoap, Colgate-Palmolive, New York, NY, USA) was used for all scenarios where hands were rubbed with soap. Participants then dried their hands using two paper towels and left their hands to air dry for 1 minute before dicing a tomato. Participants then either proceeded to dice a tomato with bare hands (1, 2, 3) or wear gloves before dicing (4, 5, 6).

Preparation of tomato

Each tomato (~220 g) was rinsed with deionized water and dried with a paper towel after purchasing. All contact surfaces of the knife, cutting board, and weighing tray were sterilized with 70% isopropyl alcohol before being used by the participants. Tomatoes were diced by the participants within 24 h of purchasing in the same manner as a supplied instructional video. After dicing the tomato, the participants picked up pieces and weighed 20 g to be placed in a sterile stomacher bag.

Sample analysis

Once dicing was completed, the tomato and participant's hands were sampled to determine how much *E. aerogenes* remained. For the tomato samples, 180 ml of tryptic soy broth was added to each stomacher bag before homogenizing on high (120 rpm) for 2 min using a stomacher (Seward Ltd., Worthing, UK). The homogenized tomato sample was then poured into a sterile stomacher bag with a filter so that the liquid portion could be removed. 500 µl of the crude extract, or 10-fold dilutions in PBS were plated on MacConkey agar containing 50 µg/ml nalidixic acid with an additional 1% agar to facilitate spreading. Plates were incubated at 37°C overnight before counting colonies.

For scenarios where the tomato was diced with bare hands sterile loose fitting "gloves" (polyethylene bags, 18 × 30 cm) containing 20 ml PBS were donned to each hand and secured above the wrist. The glove juice method was performed, wherein each hand was massaged for 1 min before the bags were removed and samples were collected. This same method was used to sample the outside of gloved hands, after gloved fingertips were stamped onto MacConkey Agar. Stamping involved pressing all gloved fingertips onto the agar surface simultaneously. After each gloved hand was sampled using the above method, 20 ml of PBS was placed inside each glove worn by the participant and secured above the wrist. Each hand was massaged for 1 min before samples were collected. Glove juice samples were diluted 10-fold in PBS to determine levels of *E. aerogenes* remaining on the hands.

Data analysis

Total bacterial counts for tomato, hands, and glove surfaces were determined, and the resulting values were $\log_{10}$-transformed. Frequency histograms of the distributions of $\log_{10}$ CFU recovered from each sample were created in Excel (Microsoft Corp., Redmond, Washington, USA).

Statistical analysis

The distributions of $\log_{10}$ CFU recovered from each sample were analyzed using SPSS (IBM Corp., New York, USA). Differences between distributions as affected by hand washing scenario were determined using a one-sided Wilcoxon's matched pairs signed rank test. Differences between proportions of positive outside glove samples were determined using a two-proportion z-test. A statistically significant difference was defined as when $p<0.05$.

Results

The concentration of *E. aerogenes* B199A inoculum used was 9.23 ± 0.15 $\log_{10}$ CFU/ml. With a heavy bacterial load on the participant's hands, differences in hand washing scenarios showed no significant differences in the amount of *E. aerogenes* B199A recovered from hands after dicing a tomato (Table 3.1). The distribution of $\log_{10}$ cell counts recovered from participants' hands is normally distributed (Figure 3.1).

The amount of *E. aerogenes* B199A recovered from tomato samples diced with bare hands after different hand washing scenarios showed a decrease in cell counts as hand washing time increased (Table 3.2). The differences in the distribution of cell counts recovered from diced tomatoes can be clearly seen using cumulative distribution functions (Figure 3.2). Cell counts recovered from tomatoes diced with bare hands were washed to compliance were significantly less (p=0.009) than counts recovered from hands that were not washed to compliance, but the difference was less than 1 $\log_{10}$.

Wearing gloves significantly reduced the incidence of tomato samples containing detectable amounts of *E. aerogenes* B199A. When hands were either rinsed or non-compliantly washed, only 1 out of 27 (3.7%) replicates contained cell counts at 3.65 and 3.95 $\log_{10}$ CFU, respectively. Interestingly, the same participant was responsible for the single positive result for both scenarios. When hands were washed to compliance before donning gloves, no tomato sample contained detectable cell counts.

Increasing hand washing duration decreased the incidence of *E. aerogenes* B199A recovered from outside surfaces of gloves. For hands that were just rinsed, 12/54 (22%) outside glove samples from both hands displayed *E. aerogenes* B199A cell counts ranging from 1.60 to 4.38 $\log_{10}$ CFU/ml. For hands that were inadequately washed, 8/54 (15%) outside glove samples displayed *E. aerogenes* B199A cell counts ranging from 1.90 to 3.95 $\log_{10}$ CFU/ml. The difference in incidence between rinsing and non-compliant hand washing was not statistically significant (p=0.349). When hands that were washed to compliance before wearing gloves, 3/54 (5.6%) outside glove samples displayed *E. aerogenes* B199A cell counts ranging from 1.90 to 3.48 $\log_{10}$ CFU/ml. The difference in incidence between rinsing and hand washing to compliance was statistically significant (p=0.013).

Increasing hand washing duration also decreased the incidence of *E. aerogenes* recovered from prints taken with gloved hands after dicing a tomato. Of 54 prints collected

for each hand washing scenario 12 (22%), 8 (15%), and 3 (6%) showed colonies after hands were rinsed only, inadequately washed, and adequately washed, respectively.

Discussion

Inherent variability in transferring bacteria between surfaces makes determining differences between treatments difficult to measure. The $\log_{10}$ CFU reductions of a previous study using a ~6 $\log_{10}$ CFU *E. aerogenes* B199A inoculum varied by four orders of magnitude during the testing of four hand washing scenarios (Jensen et al., 2015). In our study, the range of $\log_{10}$ CFU recovered from samples varied by approximately two orders of magnitude. In two separate studies that investigated *E. aerogenes* B199A transfer rates from cutting boards to lettuce, distributions using individual rates by over 30 participants were much broader than distributions that were generated from averaging replications from fewer participants (Brizio & Prentice, 2014; Zhao et al., 1998). In comparing *E. aerogenes* B199A transfer rates between common surfaces involved in food preparation the least variability was seen for bacterial transfer between cutting boards and lettuce while the greatest variability was seen on hands after hand washing (Brizio & Prentice, 2014). The transfer rates involving hands were consistently more variable than transfer rates involving inanimate objects.

When participants' hands were inoculated with ~9 $\log_{10}$ CFU/ml *E. aerogenes* B199A suspension, rinsing for 10 s displayed the same reductions in cell counts as washing hands for 3 s or 20 s with soap followed by rinsing. Previous studies using a ~8 $\log_{10}$ CFU/ml *E. aerogenes* B199A suspension found significant differences in log reduction, when hands were rinsed for 5 seconds without soap compared to hands washed with soap for 20 s (Jensen et al., 2015). However, when comparing hand washing for 20 s with or without soap, there were no significant differences in the log reduction. They also demonstrated that using paper towels significantly reduced the amount of bacteria remaining on hands as opposed to air drying when hands were washed for 20 s without soap. Paper towels were used in this study when participants dried their hands, and paper towel use increases the variability of the resulting distributions (Jensen et al., 2015). Since paper towels cause a physical removal of bacteria from the skin's surface, their effect may overtake any difference in hand washing with or without soap (Blackmore 1989).

Similar log reductions were observed after hands were washed with soap for 3 or 20 s. Previously, researchers used nonantimicrobial soap to wash participants' hands artificially

contaminated with ~6 log_{10} CFU *Serratia marcescens* and showed that washing hands for 15 or 30 s displayed no differences in log reduction (Fuls et al., 2008). In the same study, increasing the volume of nonantimicrobial soap from 0.75 g to 1.5 g caused significant reductions in bacteria from hands, while increasing the volume from 1.5 g to 3 g didn't cause significant differences in reduction. It was suggested that there is a maximum level of reduction achievable during hand washing with soap, and increasing the volume or time may not produce additional benefits. Since nonantimicrobial soaps reduce bacterial levels through surfactants, wash time doesn't improve bacterial reductions past maximum removal. This effect was also observed at normal baseline microbial levels when participants washed their hands with either 1 or 3 ml nonantimicrobial soap, as the log reductions were similar after one wash. Only after 15 iterations of hand washing did the increased volume of soap used produce significant reductions in microbial counts (Larson et al., 1987).

Interestingly, even with such a high bacterial burden on our participants' hands the effect of wearing gloves significantly reduced the incidence of detectable cross-contamination. The transfer of *E. aerogenes* B199A from hands to lettuce through a glove barrier was significantly reduced when hands possessed a ~6.5 log_{10} CFU inoculum as opposed to a ~4 log_{10} CFU inoculum (Montville et al., 2001). It has been shown that as the inoculum level on the source increases, the transfer rate decreases (Montville et al., 2003). A high inoculum was used in this study to better determine the full capacity of soap to reduce bacterial loads on hands, as a previous meta-analysis of literature data highlighted the importance of testing using high inoculum levels (Patrick et al., 1997).

Glove use doesn't completely contain heavy levels of bacterial contamination, as evidenced by the periodic enumeration of *E. aerogenes* B199A collected from outside glove surfaces. Positive samples were contaminated with ~2-4 log_{10} CFU regardless of hand washing scenario. However, increasing the hand washing time with soap decreased the incidence of detectable colonies recovered from outside glove surfaces. The proportion of positive samples was significantly reduced when hands were adequately washed compared to hands that were just rinsed. The absence of colony growth from outside glove samples, even after being donned by hands containing ~6 log_{10} CFU bacteria, may be attributed to decreased transfer rates associated with higher inoculum levels (Fravalo et al., 2009; Montville et al., 2003). In addition, participants' hands were dried with paper towels and left

to air dry for 1 min before donning gloves, and less residual moisture on the skin is correlated with decreased rates of transfer to surfaces (Patrick et al., 1997).

Hand washing alone is not sufficient in reducing the food safety risk of high bacterial loads on the hands of food service workers. Examples of situations where hands could have high bacterial loads include processing raw meats and fish, or illness such as diarrhea. Glove use is able to drastically reduce cross contamination to food products, but improper hand washing leads to bacteria deposited on outside glove surfaces. While glove use alone reduces the risk of bacterial contamination, washing hands in conjunction with glove use reduces the risk further. In order to maximize food safety, both glove use and adequate hand washing are necessary to reduce bacterial cross-contamination.

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Table 3.1 Amount of *Enterobacter aerogenes* B199A recovered from both hands after each hand washing scenario

	Scenario[a]	**Soap rubbing time (s)**[b]	**Log_{10} CFU/mL**	**Log_{10} Reduction Factor**
Bare hands	1	0	6.55 ± 0.64	2.68 ± 0.69
	2	3	6.58 ± 0.69	2.65 ± 0.75
	3	20	6.53 ± 0.66	2.73 ± 0.70
Gloved hands	4	0	6.48 ± 0.80	2.82 ± 0.82
	5	3	6.53 ± 0.62	2.76 ± 0.63
	6	20	6.47 ± 0.69	2.82 ± 0.67

[a] Scenarios 1,4 = rinsing only; 2,5 = non-compliant hand washing; 3,6 = compliant hand washing

[b] All durations of soap rubbing were followed by 10 s rinsing with water, hands were dried using two paper towels

Table 3.2 *Enterobacter aerogenes* B199A cell counts and statistical analysis of tomato samples diced with bare hands.

Hand Washing Method	**Log_{10} CFU**	**Log_{10} Transfer Rate (%)**	**Significance of Difference**[a]
Rinsing only	5.78 ± 0.79	-3.45 ± 0.83	
Non-compliant hand washing	5.58 ± 0.76	-3.65 ± 0.80	$P = 0.056$
Compliant hand washing	5.36 ± 0.52	-3.88 ± 0.49	$P = 0.009$

[a]Determined using Wilcoxon's matched pairs signed rank test (one sided)

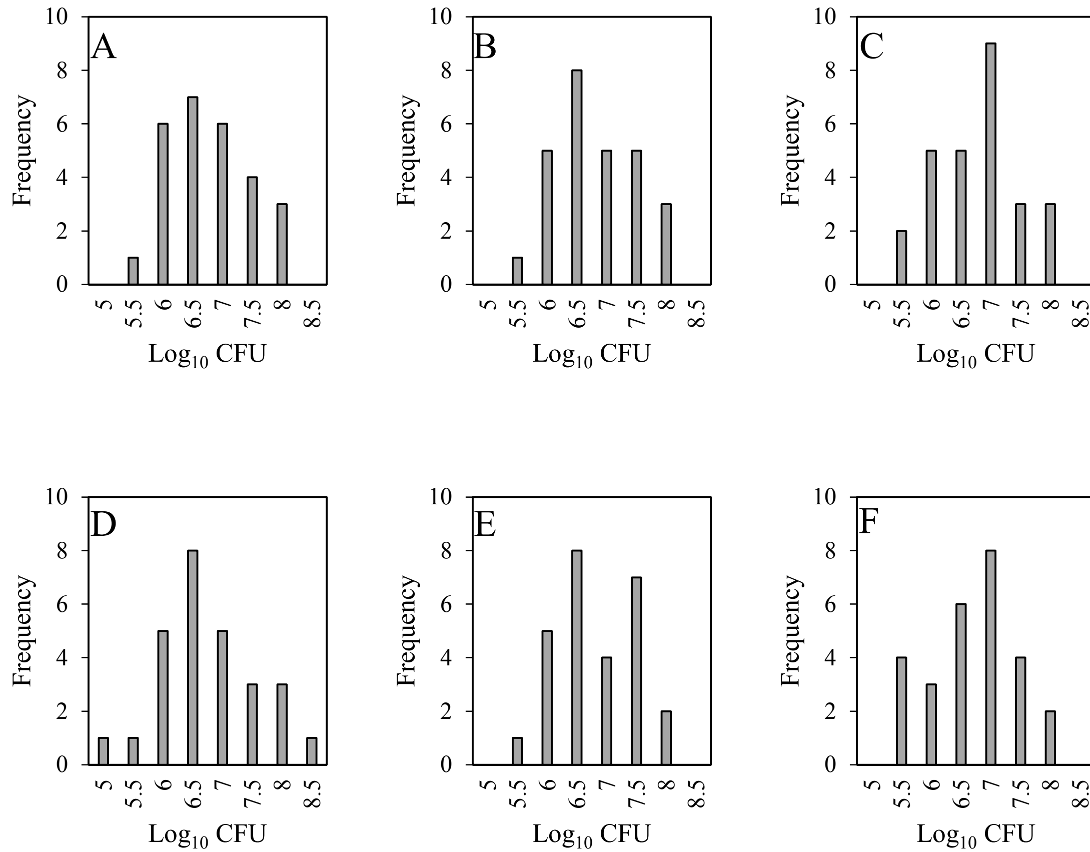

Figure 3.1 Distributions of *Enterobacter aerogenes* B199A recovered from both hands during (A) scenario 1, (B) scenario 2, (C) scenario 3, (D) scenario 4, (E) scenario 5, and (F) scenario 6.

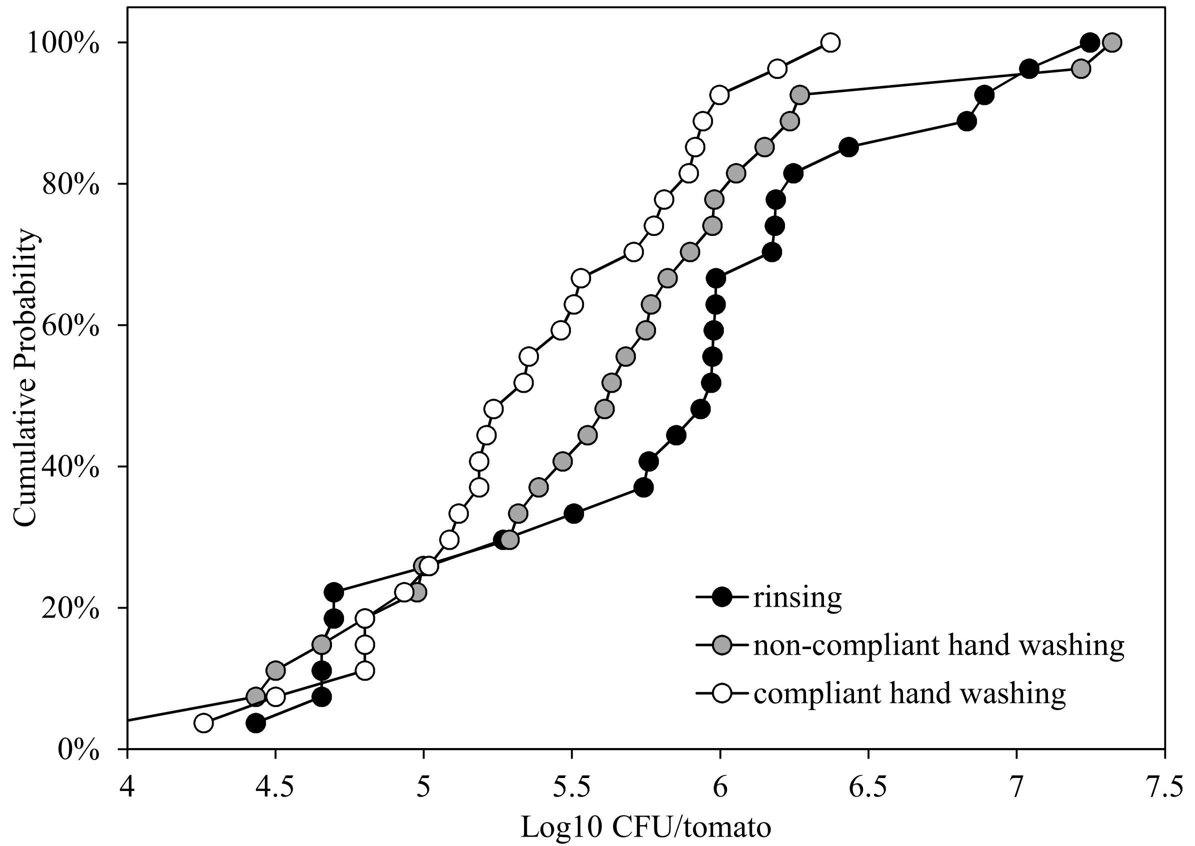

Figure 3.2 Cumulative distribution functions of *Enterobacter aerogenes* B199A collected from tomato samples diced with bare hands during different hand washing scenarios.

Chapter 4: Utility of a multilevel-modelling approach to investigate differences in isolation frequency of *Fusarium culmorum* in agricultural soil across the Inland Pacific Northwest

Robinson, A., Ryu, D., & Lee, H. J. (2019). Utility of a multilevel modeling approach to investigate differences in isolation frequency of *Fusarium culmorum* in agricultural soil across the Inland Pacific Northwest. *Phytopathology*, *109*(6), 1062-1073.

Abstract

The plant pathogen *Fusarium culmorum* represents an inoculum source capable of contaminating grains with deoxynivalenol (DON) in the Inland Northwest (INW) region of the United States. A multilevel modelling approach utilizing varying intercepts for different sampling quadrats, fields, and iterations in the dataset was performed to characterize differences in isolation frequency of *F. culmorum* collected during a two-year soil survey. Differences in the isolation frequency of *F. culmorum* varied the most by sampled field followed by quadrat and iteration, respectively. Higher relative elevation within the sampling region of a field limited the amount of *F. culmorum* recovered. The effect of annual climate variables was investigated using combinations of single and multivariable model equations with linear and polynomial terms. The same data analysis approach was applied to an external dataset of *F. culmorum* isolation frequencies in grains from fields across eastern Australia. These results represent a case study for investigating variability within datasets containing overdispersed fungal counts and incorporating climate summaries as predictor variables.

Introduction

Fusarium culmorum (W.G. Sm.) Sacc. is a causal agent of *Fusarium* Head Blight (FHB) and *Fusarium* Root Rot (FRR) of cereals and limits the production of winter wheat (*Triticum aestivum L.*) across the Inland Northwest (INW) region of the United States (Poole et al., 2013). Mycotoxin production in the form of trichothecenes, e.g. deoxynivalenol (DON) and nivalenol, is a common feature among *F. culmorum* isolates collected worldwide (Bakan et al., 2002; Gang et al., 1998; Jennings et al., 2004; Laraba et al., 2017; A. Quarta et al., 2005; Yekkour et al., 2015). Infection of grains by *F. culmorum* is of concern for both agriculture and food safety as yield reduction is highly correlated with DON content in grains(C. Snijders & Perkowski, 1990).

The INW currently produces winter wheat with some of the lowest levels of average DON in the continental United States (Bianchini et al., 2015). The primary causal agent of FHB, *F. graminearum*, is rarely present in this region as the primary inoculum source for *F. graminearum* is corn residues rather than wheat or its stubbles(Dill-Macky & Jones, 2000). It was reported that only one isolate was recovered out of 831 *Fusarium spp.* isolated from 2,250 wheat crowns collected across eastern Washington and Oregon(R W Smiley & Patterson, 1996). The Mediterranean climate regime of the INW is not favorable for the development of corn or FHB as most annual rainfall only occurs from October through March. While wheat is most susceptible to FHB during mid-anthesis(Lacey et al., 1999), the dry conditions in the INW during anthesis limit fungal infection and DON production in grains which is dependent upon high relative humidity during flowering(Beyer et al., 2005; De Wolf et al., 2003).

However, the climate of the INW is forecasted to change in the upcoming decades (Abatzoglou et al., 2014), with warmer winters, springs, and summers in addition to wetter springs and drier summers predicted by 2070 (Pan et al., 2017). Warmer winters may allow *F. culmorum* populations in soil to increase and cause increased infection pressure during the following growing season(Lukas et al., 2014). In addition, hot and dry conditions during summer can initiate plant water stress which predisposes crops to *F. culmorum* infections (Papendick & Cook, 1974). Warmer and wetter springs are a concern since both the incidence of FHB and concentration of DON increases with increasing periods of wetness and temperature (Cowger et al., 2009; Xu et al., 2007). Flowering typically occurs in mid to late May for winter wheat grown in the INW, but rising temperatures may shift flowering dates earlier in the year (Hu et al., 2005). To address the food safety concerns of DON contamination in grains from the INW under climate change the current inoculum source needs to be quantified and related to climatic factors.

Quantifying the prevalence of *F. culmorum* out of total *Fusarium* spp. in agricultural soil is difficult due to the high degree of variability accompanied by the methods of enumeration. Population densities of soil *Fusaria* can vary widely within sampling quadrats as small as 10 × 10 cm (Rodríguez-Molina et al., 2000). In addition, population densities of *F. culmorum* are known to fluctuate seasonally (Bateman & Murray, 2001). Fortunately, the regional

distribution of *F. culmorum* prevalence is more relevant to the issue of climate change in the INW and has been related to local climate at that scale (Poole et al., 2013).

Isolation frequency of *F. culmorum* from infected wheat and barley roots across 13 southern Idaho counties was higher than in counties with greater annual precipitation (Carl A. Strausbaugh et al., 2004). Isolation frequency of *F. culmorum* in crops is related to their soil populations since the population density of the fungus in soil is proportional to FRR disease severity (R.J. Cook, 1968). The population density of *F. culmorum* recovered from field soil was greater in Pullman, WA compared to Ritzville, WA where the climate is hotter and drier (R.J. Cook & Bruehl, 1968b). The longevity of chlamydospores of *F. culmorum* in artificially inoculated soil samples which were buried in Pullman, WA was greater than compared to Lind, WA (Inglis & Cook, 1986b; Sitton & Cook, 1981a). Lind, WA receives less annual rainfall than Pullman, WA while also having warmer summers. An extensive survey across the INW combined with factor analysis and logistic regression determined that greater annual precipitation increased the isolation frequency of *F. culmorum* out of total *Fusarium* spp. from winter wheat stubble (Poole et al., 2013). In general, *F. culmorum* was found more frequently in areas of the INW with higher elevations and moisture and with cooler temperatures.

Research into the regional distribution of *Fusarium* spp. pathogenic to grains has also been extensively surveyed across Australia. The range and mean of 16 different climate parameters was calculated for the distribution of *F. culmorum* in cereals across Australia using accession records from multiple lyophilized culture collections and the BIOCLIM version 2.0 program (Backhouse & Burgess, 1995). A survey of stem bases collected from crops across the eastern Australian grain belt confirmed that *F. culmorum* was common and widespread in cooler and wetter areas of Victoria and South Australia (Backhouse et al., 2004). Isolation frequency of *F. culmorum* correlated best with November rainfall measured from the nearest meteorological station.

The objective of this work was to evaluate the relationship between climatic variables and the prevalence of *F. culmorum* using isolation records from a study to collect potentially toxigenic isolates of soil *Fusaria*. As isolation frequency varies by time and location, multiple fields were sampled seasonally for two years. While the culture collection will be utilized for food safety research, the present manuscript attempts to characterize differences

in isolation frequency of a fungal plant pathogen in soil based on various experimental clusters within the dataset.

Inferences gained can provide the scaffolding to guide the construction of more complex models in the future for use with climate change ensembles. The data analysis approach herein provides a case study for plant pathologists who also work with count data of fungal plant pathogens to better investigate how experimental units within their datasets vary. A further applied approach is presented using an external dataset from Backhouse et al. (2004) to compare estimates for parameters using similar climatic variables.

Methods and Materials

Soil sampling and weather monitoring

Field locations were selected based on grower cooperation with the help of extension specialists from both the University of Idaho and Washington State University. All fields were within 50 miles of Moscow, ID. The survey consisted of 3 separate fields each within the annual crop, transitional, and grain-fallow agroecological classes delineated by Douglas et al (1992) giving a total of 9 fields.

The sampling region within each field consisted of 9 quadrats 1 acre (4047 m^2) in size arranged in a 3 × 3 grid pattern. The position of all 16 vertices of the sampling grid were calculated using an offset of 63.62 m and converting the distance to decimal degrees for each field. Elevation, in meters, for each vertex was estimated using FreeMapTools (https://www.freemaptools.com/elevation-finder.htm). The elevation of each sampling quadrat was the average of 4 vertices. Maps of sampling regions were created using the Google Maps API interface and uploaded onto a smartphone (Samsung Galaxy S7) as a geo-reference while sampling.

During each sampling iteration, 6 subsamples were taken from each quadrat of a field using a systematic unaligned grid methodology (Franzen & Cihacek, 1998). A subsample was defined as a soil core 10 cm in depth taken using a 7/8” soil probe (AMS, American Falls, ID). Subsamples were collected from random locations within each quadrat and the GPS accuracy radius was always completely within a quadrat during sample collection. Subsamples for each quadrat were pooled into a labeled plastic bag for transport. Once in the laboratory soil samples were dried in a biosafety cabinet in weighing dishes for 48 hr prior to processing. Soil samples were ground with a pestle and mortar to pass a 2 mm sieve and

mixed thoroughly prior to dilution. Soil moisture was measured using a moisture meter (HB43-S, Mettler Toledo, Columbus, OH). Soil sampling occurred every 3 months from 11 June of 2016 through 17 March of 2018.

The primary inoculum capable of causing FHB within the INW is soilborne (T. C. Paulitz et al., 2002). Spatio-temporal spread of *F. culmorum* was not considered in the present study as the long term survival of *F. culmorum* chlamydospores in soil is considered the reason inoculum levels within a field remain constant despite crop rotations (R.J. Cook & Bruehl, 1968b; Windels et al., 1993). It was assumed that *F. culmorum* had a cosmopolitan distribution in soils across the INW and that environmental factors could be used to determine the relative amount present in a field (Poole et al., 2013; R W Smiley & Patterson, 1996).

Site-specific weather data was collected using Climate Engine (https://clim-engine.appspot.com/). The GPS coordinates from the center of each field's sampling region were used as inputs for Climate Engine to collect daily weather data from the corresponding cell within a 4 km raster dataset (Abatzoglou, 2013). Daily values for precipitation, maximum temperature, minimum temperature, and grass reference evapotranspiration were collected from January 1 1980 through December 31 2018. Grass reference evapotranspiration was calculated using the Penman-Montlieth equation (Walter et al., 2001). Daily maximum and minimum temperatures were used to calculate growing degree-days (GDD) with a base temperature of 0 °C. GDD are used to describe the heat energy received by a crop over a given interval of time. Maximum and minimum temperature was referenced against the base temperature prior to averaging (McMaster & Wilhelm, 1997). For each year in the dataset (N=38) the total sum of each variable was calculated for each field to produce an annual average.

Soil dilution plating

Ten grams of soil from each quadrat sampled was suspended in 100 ml of sterile 0.05% water agar (WA) and mixed for 30 min using a wrist action shaker (Burrell Model 75, Pittsburgh, PA). Further dilutions were performed using additional 0.05% WA first to 1 g/100 ml followed by serial two-fold dilutions up to 1 g/800 ml. A 1 ml aliquot of each 2-fold dilution was plated onto Nash-Snyder media (Nash & Snyder, 1962). The source of pentachloronitrobenzene (PCNB) was Blocker ® 4F generously provided by Amvac

Chemical Corporation. Soil dilution plating was performed in duplicate for each quadrat. Plates were incubated in a greenhouse placed adjacent to a north-facing window for sunlight for 5 d. Colonies which exhibited unrestricted growth were assumed to be *Fusarium* species (Nash & Snyder, 1962).

After incubation, the soil dilution for each replicate which produced approximately 10 to 25 colonies per plate was selected for subculturing. This range is based on 10 colonies being the minimum necessary to ensure sampling adequate species diversity and plates with above 25 colonies were typically overcrowded (Sangalang et al., 1995). Colonies of interest were labeled and transferred to homemade potato dextrose agar (PDA) (Nelson et al., 1983). To conserve space and resources when handling thousands of isolates, 1 ml of homemade PDA was dispensed into reservoirs of 24-well plates (Primaria, Corning, Durham, NC) with each fungal isolate occupying a single well. After 3 days only red-pigmented colonies were transferred to carnation leaf agar and identified further as they represented all species known to be pathogenic to cereals and also potential tricothecene producers (Backhouse et al., 2004). After incubation for 2 weeks, isolates were identified as *F. culmorum* using morphological criteria (Leslie & Summerell, 2007; Nelson et al., 1983; Samson et al., 2004). The isolation frequency of *F. culmorum* out of total soil *Fusaria* was first calculated by summing both replicates for a given quadrat. Isolation frequency was also calculated by summing all replicates from a field after a single sampling iteration.

Data analysis approach

The dataset contained counts of *F. culmorum* colonies (c) from field (i) at quadrat (j) during sampling iteration (k) out of total soil *Fusaria* (n). "Sampling iteration" was defined as every unique sampling point for 9 fields across 8 seasons (N=72) and letting each vary independently as there was no consistent temporal trend across consecutive sampling periods. The reported outcome is a binary result of the number of identified *F. culmorum* isolates out of all *Fusarium* colonies observed during soil dilution plating. Determining the proportion of a plant pathogen of interest within a specific fungal genus is a familiar research outcome in plant pathology. The proportion of *F. culmorum* in the soil out of total soil *Fusaria* (p_{ijk}) was modelled using a binomial likelihood distribution where $c_{ijk} \sim$ Binomial (n_{ijk}, p_{ijk}). The term c_{ijk} represents the number of *F. culmorum* colonies for the ith field, j^{th} quadrat, and k^{th} iteration, respectively. The term p_{ijk} represents the unobserved true proportion of *F.*

culmorum which will be estimated using the dataset. The logit link, where logit(p) = ln(p/(1-p)), was used to build a regression model for p_{ijk} using varying intercepts for i, j, and k. The equation used for determining the unique log-odds given the dataset was (Eq, [1]):

$$\text{logit}(p_{ijk}) = a + a_i + a_j + a_k$$

where a_i, a_j, and a_k represent the additive effects of the ith field, j^{th} quadrat, and k^{th} iteration, respectively, which modify the overall population parameter a. The prior distribution for a was Normal (0, 100) which is weakly regularizing. A regularizing prior is used to both minimize overfitting and allow the Markov chains to sample properly. Prior distribution for offsets was Normal (0, σ^2).The prior distribution for the variance parameters $\sigma^2{}_i$, $\sigma^2{}_j$, *and* $\sigma^2{}_k$ was Half Cauchy (0, 1) for offsets i, j, and k. A Half Cauchy prior was used for the variance estimates as found in McElreath (2016). The Half Cauchy distribution is approximately uniform in the tail and is considered “weakly informative” (Gelman, 2006). Increasing the prior for the variance parameters by a factor of 10 did not change the posterior distribution of the additive effect parameters (Fig 4.1).

This approach accounted for overdispersion in the dataset by assigning a unique intercept for all unique fields (N=9), quadrats (N=81), and sampling iterations and allowing them to vary independently. Definitions of “sampling iteration” based temporal sequence (N=8) or season (N=4) were also analyzed. The posterior probabilities for p_{ijk} were estimated using Bayesian analysis through the rethinking package in R (McElreath, 2016a). The rethinking package in R converts model specifications into a format that can be utilized in Stan, which is a probabilistic programming language (Carpenter et al., 2017). Stan uses the Hamiltonian Monte Carlo algorithm to sample from a target posterior distribution, and an introduction can be found in Betancourt (2017)

Parameters for the Markov chain included a warmup of 1,000 iterations followed by 5,000 sampling iterations using 4 chains distributed across 4 cores. Parameters for the Markov chains remained constant for all models presented. The output was checked for convergence using the Gelman-Rubin statistic $\hat{R}$ (Gelman & Rubin, 1992).Markov chains were assumed to have converged when the value of $\hat{R}$ was 1.00. The model output consists of a distribution of 20,000 parameter estimates, the frequency of which correspond to their relative plausibility given the dataset and model formula. Posterior distributions for parameters are summarized by either mean ± standard deviation or the 95% highest posterior

density interval (HDPI). A HDPI is the narrowest range of values containing a specified probability mass, which in this study was 95%. While there are many possible intervals with the same mass, the HDPI answers which interval is the densest. The resulting range utilizes parameter values with the highest posterior probability while also being the narrowest (McElreath, 2016a). A 95% HDPI which excluded zero was used as justification for a parameter value being predominantly positive or negative.

Adding annual climate summaries as predictor variables

The effect of a climate variable x was first modelled as a slope b where x was either average annual precipitation, evapotranspiration, or GDD calculated using the historical daily dataset from 1980 through 2018. Annual averages were used as a proxy for field differences based on climatic summaries. Shorter timescales were not analyzed due to the stochastic changes within fields across sampling iterations. The updated link equation becomes (Eq. [2]):

$$\text{logit}(p_{ijk}) = a + a_i + a_j + a_k + bx$$

Climate variables were standardized to mean 0 with standard deviation 1 prior to analysis. The prior distribution for b was Normal(0, 10), and all the following equations utilize the same prior for all parameters associated with climate variables. This approach gives unique fields, quadrats, and sampling iterations their own intercept and assumes a fixed linear relationship between the climate parameter being analyzed and the log odds of a *Fusarium* colony isolated from soil being *F. culmorum*. Polynomial regression using a quadratic parameter was also investigated. For a single climate variable x the updated link equation becomes (Eq. [3]):

$$\text{logit}(p_{ijk}) = a + a_i + a_j + a_k + b_1x + b_2x^2$$

This approach gives unique fields, quadrats, and sampling iterations their own intercept and assumes a nonlinear relationship between the climate parameter being analyzed and the log odds of a *Fusarium* colony isolated from soil being *F. culmorum*.

Multivariable models using two climate variables were also investigated. For climate variables x and y assuming a multivariable linear relationship the updated link equation becomes (Eq. [4]):

$$\text{logit}(p_{ijk}) = a + a_i + a_j + a_k + b_1x + b_2y + b_3xy$$

All combinations of climate variables x and y were tested with and without the interaction term. For climate variables x and y assuming a multivariable quadratic relationship the updated link equation becomes (Eq. [5]):

$$\text{logit}(p_{ijk}) = a + a_i + a_j + a_k + b_1x + b_2y + b_3xy + b_4x^2 + b_5y^2$$

Generalization of methodology to previous literature

The original dataset used in Backhouse et al. (2004) was generously provided by Dr. Backhouse. Annual precipitation and GDD were calculated using the nearest meterological station to a field which was available from the Australian Bureau of Meteorology at the time of publication (Backhouse, personal communication). The dataset consists of *F. culmorum* counts (c) out of total *Fusaria* in grains (n) collected from 163 fields. The same modelling equations as the current study were used with the Australian dataset without a_j or a_k as only the parameters for each ith field were available. Climate variables were standardized to mean 0 with standard deviation 1 prior to analysis and posterior distributions of parameters were compared to the current study.

Evaluation of model predictions

Model output using Eq. [2] through Eq. [5] contained 20,000 values for each parameter with a relative frequency proportional to relative plausibility given the dataset used. The distribution of each parameter value is based on the uncertainty in the dataset for which the model equation was conditioned upon. Uncertainty is propagated forward into model predictions by using the same equation used during model fitting and calculating the value of the linear model using samples from the posterior distribution. Model predictions were generated by first simulating a new varying intercept using the posterior distributions of σ for field, quadrat, and iteration from the model being evaluated. The simulated intercept was then used with the posterior distribution of the effect parameters for annual climate variables to simulate predictions. The effect of the climate variables based on the model equation is visualized by the trend of the simulated intercept. By simulating and plotting multiple intercepts the variation in proportions of *F. culmorum* due to differences in fields, quadrats, and sampling iterations can be visualized. When evaluating multivariable models using Eq. [4] and Eq. [5] the climate variable y was set to the average when simulating predictions for a climate variable x. All data and R scripts to reproduce the analyses shown here are deposited publicly on GitHub (https://github.com/nosnibor27/PHYTO).

Results

Frequency of isolation records

A total of 20,675 soil *Fusaria* were collected over 2 years of sampling and yielded 2,293 isolates of *F. culmorum*. The number of *F. culmorum* isolates out of total soil *Fusaria* collected from 1 acre quadrats across 9 fields and 8 seasons is presented in Table 4.1. The proportion of *F. culmorum* ranged from 0% to 85% of all soil *Fusaria* within a 1 acre quadrat. The total number of *F. culmorum* isolates collected from a field during each sampling iteration is presented in Table 4.2. The resulting proportion is the average of all 9 quadrats, but reporting proportions in this manner neglects the variability across field quadrats. In general, isolation frequency of *F. culmorum* within a field was highest in the winter and spring and lowest in the summer and fall. Surveys of *F. culmorum* conducted during harvest between July and August coincide with the period of the year where soil populations are the lowest, which may lead to biased inferences.

Total proportion data from both the current study and Backhouse et al. (2004) is shown in Figure 4.2. The prevalence of *F. culmorum* in soil across the INW is much greater than in wheat across eastern Australia. *F. culmorum* was not recovered from soil samples in 244 out of 648 cases in the current study, and not recovered from 101 wheat samples out of 163 cases in the dataset provided by Backhouse et al. (2004).

Posterior distribution of cluster offsets

Bayesian analysis was used to assess the variability in the isolation frequency of *F. culmorum* in soil based on sampling field, quadrat, and sampling iteration by assigning a unique intercept for each index across each cluster. The mean ± standard deviation of the posterior distribution for a, the population average, was -6.37 ± 0.58 using Eq. [1]. Units are in log-odds, so the average percentage of a soil *Fusaria* being *F. culmorum* given the data is 0.17% for the INW.

The mean ± standard deviation of the posterior distributions using Eq. [1] for σ were 1.64 ± 0.49, 0.92 ± 0.1, and 0.63 ± 0.08 for field, quadrat, and sampling iteration, respectively. These values are conditional on the dataset used and show that isolation frequency of *F. culmorum* in soil varies the most by field followed by quadrat and sampling iteration. Tabulated summaries of marginal posterior distributions for sampling iteration and quadrat are presented in Table 4.3 and Table 4.4, respectively. The mean ± standard

deviation of the posterior distributions for σ_k for different interpretations of sampling iteration were 0.43 ± 0.14 and 0.48 ± 0.32 for temporal sequence (N=8), and season (N=4), respectively. Posterior distributions for σ_k using different interpretations of sampling iteration are visualized in Figure 4.3.

The quality of model fit using Eq. [1] is visualized in Figure 4.4 by simulating 100 counts of *F. culmorum* from the posterior distribution for each case in the dataset and comparing the result to the enumerated value. The predicted range of simulated counts does not always contain the enumerated value. This can be seen where the range of simulated counts is less than the empirical data for high counts (>30) of *F. culmorum* within a specific quadrat. The posterior predictive check visualized in Figure 4.4 does not change based on different interpretations of sampling iteration (data not shown).

Effect of local climate on isolation frequency of F. culmorum *in soil*

Posterior means and 95% HDPI of field offset parameters using Eq. [1] are shown in Figure 4.5A. These distributions are marginal, meaning that the field offsets are averaged over the other parameters. No climate data was used when calculating the field offsets shown in Figure 4.5. The average historic precipitation, evapotranspiration, and GDD for a sampled field is plotted against the corresponding posterior field offset in Figure 4.5B, 4.5C, and 4.5D, respectively. Fields with historically greater annual precipitation had an increased isolation frequency of *F. culmorum* in soil. Conversely, fields with historically greater annual evapotranspiration had a decreased isolation frequency of *F. culmorum* in soil. Fields 1, 2, 3, 4, and 5 all had annual evapotranspiration below 1150 mm and 96%, 56%, 98%, 99%, and 96% of their marginal posterior distribution was positive, respectively. Fields 6, 7, 8, and 9 all had annual evapotranspiration above 1150 mm and 69%, 96%, 99%, and 99% of their marginal posterior distribution was negative, respectively. Annual GDD exhibited a similar trend to evapotranspiration since both incorporate daily maximum and minimum temperature during their calculation. Simple linear relationships may be insufficient for this dataset, as can be seen with the non-linear relationship between annual precipitation and isolation frequency in Figure 4.5B.

Relative elevation within a field and marginal posterior distributions of sampling quadrats

Most sampling quadrats provided no substantial increase or decrease in odds to the final observed isolation frequency of *F. culmorum* in soil (Supplemental Table 3). For 81

total quadrats sampled, only 26 quadrats had a marginal posterior probability distribution that was predominately positive or negative (i.e. their 95% HDPI excluded zero). For clarity, only those quadrat offsets are shown in Figure 4.6 where they are plotted against the relative elevation difference for that quadrat compared to the mean elevation for a given field. Centering of field elevations was performed as only relative differences within a field are of interest. All quadrat offset parameters which were predominately negative occurred at quadrats which were above the average elevation for the field's sampling region. However, quadrat offsets which were predominately positive were not always from quadrats which were below average elevation for a field's sampling region. Relative elevation alone is not sufficient in explaining an increased isolation frequency of *F. culmorum* within a sampling quadrat. In addition, estimates of quadrat offsets can be positively skewed by a few high observed counts of *F. culmorum*. This can be seen in Figure 3 where high counts of *F. culmorum* above the predicted range increase the range of predicted values for the same quadrat in the same field across seasons.

Comparison of slope estimates

The posterior distribution of slopes after incorporating annual precipitation, evapotranspiration, and GDD using Eq. [2] through Eq. [5] for each field is summarized in Table 4.5. The same data analysis approach was also applied to previously published literature on *F. culmorum* isolation frequency in grains from eastern Australia (Backhouse et al., 2004). *F. culmorum* isolation frequency in soil was positively related to annual precipitation and negatively related to annual evapotranspiration as the 95% HDPI of estimated slope parameters excluded zero when used individually in Eq. [2]. The distributions of slope parameters for precipitation and GDD using data from Backhouse et al. (2004) were the same sign as the current study. However, the variability of slopes estimated using isolation frequencies of *F. culmorum* was different across datasets. The effect of annual precipitation and GDD on *F. culmorum* isolation frequency was lower in grains from Eastern Australia than in soil from the INW.

The correlations between annual climate variables used in both datasets is shown in Figure 4.7. Annual precipitation is negatively correlated with annual growing degree days across both datasets. Annual growing degree days is positively correlated with annual evapotranspiration. The posterior distributions of slope parameters for specific climate

variables change when combinations are used in the same equation (Table 4.5). In the current study, the posterior distribution of the slope parameter for annual precipitation is 0.42 ± 1.00 when used in combination with annual evapotranspiration using Eq. [4] without an interaction term, meaning that there is no information gained incorporating annual precipitation after conditioning upon annual evapotranspiration. A similar pattern is seen with incorporating annual GDD after conditioning upon annual precipitation. The posterior distribution of slope parameters for GDD changed signs when used in combination with annual evapotranspiration using Eq. [4] without an interaction term. Using the dataset from Backhouse et al. (2004), the posterior distribution of slope parameters for annual precipitation changed signs when used in combination with annual GDD. Correlations between predictor variables needs to be addressed prior to incorporation into a model equation as the resulting coefficients may change erratically and be potentially misleading.

Posterior distributions of parameters for quadratic terms are also presented in Table 2, but there is little interpretability in their values when viewed alone. Therefore, predictions based on different model equations had to be generated for comparison.

Evaluation of model predictions

Predicted proportions of *F. culmorum* based on annual precipitation, evapotranspiration, or GDD using both datasets is shown in Figure 4.8. The effect of an annual climate variable is visualized in the trend of a line representing a simulated proportion of *F. culmorum*. The variability in proportions of *F. culmorum* due to differences in fields, quadrats, and sampling iterations is visualized by plotting 100 simulations from the posterior distribution of each model. Predictions are highly variable independent of if a single or multivariable linear or quadratic model equation is used as the random effects of field, quadrat, and iteration remain relatively constant across all model types. Most simulated proportions of *F. culmorum* remain close to 0 across the range of annual climate variables being investigated which is seen by the dark overlapped black lines in the bottom region of the subplots. Simulated proportions of *F. culmorum* in wheat using the dataset from Backhouse et al. (2004) remain near zero regardless of the effect of annual precipitation or annual growing degree days. Most of the variability in the proportion of *F. culmorum* is due to differences in sampled fields, quadrats, and iterations and not differences in an annual climate variable from the corresponding field of isolation.

The marginal posterior distribution of field offsets after using Eq. [2] with annual precipitation were centered at zero (data not shown). This represents a model equation which fits the data well, as there was no residual pattern in the field offsets which occurred when using other model equations. Improved model fit to the dataset does not guarantee that model predictions will also be improved (Fig. 4.8).

Discussion

This study provides an example of utilizing a multilevel modelling approach to study variability of counts for a fungal plant pathogen in soil based on sampling quadrat, field, and iteration. This approach provided information suggesting that most of the observed variability is due to the field being sampled from. Investigation into variability within sampling quadrats showed lower frequencies of isolation from higher elevations within a given field. To further test if differences in isolation frequency of *F. culmorum* in a field could be attributed to local climate, different combinations of effect parameters were incorporated into the model equation.

Probability distributions for highly variable counts of fungal plant pathogens in soil have classically been assigned to the negative binomial (Dillard & Grogan, 1985; Hau et al., 1982; Stanghellini et al., 1982; Taylor et al., 1981; Punja et al., 1985). This approach accounts for overdispersion in the data by utilizing a conceptual construct which extends the Poisson count distribution such that the Poisson rate parameter follows a gamma distribution (McElreath, 2016). While this formulation accounts for unobserved variability in fungal counts it does not give much more information apart from confirming that the population of a fungal plant pathogen is spatially aggregated in soil. The data analysis approach in this study accounted for overdispersion by allowing unique clusters within the dataset to vary independently. Differences in unique cluster parameters derived from the dataset were used to make inferences on possible sources of variability.

Out of 81 unique acre quadrats sampled the marginal posterior distribution of only 8 were predominately negative with a 95% HDPI that excluded zero (Table 4.4). All of those quadrats were from higher relative elevations within the sampling region for a given field (Fig 4.6). The landscape of the INW is known for its hilly topography, and it has been known for a long time that agricultural practices such as tilling have made the high points within fields of the INW drier and less productive for plant growth which negatively impacts wheat

yields (Rockie, 1951; Yang et al., 1998). Precipitation occurs primarily during the winter months when cropland is seeded to winter wheat and provides only minimal cover until spring (Frazier et al., 1983). It follows that isolation frequency of *F. culmorum* in soil is decreased in the eroded high points of fields across the INW. There have been observed cases of FRR caused by *F. culmorum* in the INW where the pathogen density was highest halfway along a slope but undetectable at the hilltop (Cook, 1980). Lower soil moisture within high points of a field may have accounted for a marginal decrease in isolation frequency when accounting for relative elevation. However, differences in fields accounted for more of the variability in isolation frequency of *F. culmorum* compared to differences in sampling quadrats as the posterior distribution of σ was greater for the field parameter compared to the quadrat parameter using Eq. [1].

Isolation frequencies of *F. culmorum* within a field fluctuated seasonally as well but the differences were not consistent across fields (Table 4.3). In general the isolation frequency of *F. culmorum* in the soil of an agricultural field was highest in the winter and spring and the lowest in the fall. Previous monthly observations of *F. culmorum* population densities in wheat field soils of the United Kingdom also fluctuated differently according to the field sampled yet multiple increases in *F. culmorum* population densities were observed during the spring following rainfall events (Bateman & Murray, 2001) To account for differences in how isolation frequency changed across fields over the same season all sampling points were treated as random representing a conservative choice. Models which combine the results from fields together using the same temporal sequence or season can also be evaluated (Fig 4.2). A marginal decrease in isolation frequency in the fall can still be observed when using temporal sequence or season.

After averaging over the uncertainty in both sampling quadrat and iteration, the marginal posterior distributions for field offsets still showed differences (Suppl. Table 2). Plotting the field offset against historical averages for annual precipitation and evapotranspiration showed a positive and negative relationship, respectively, with isolation frequency of *F. culmorum* (Fig. 4.5). This relationship was further investigated by adding a slope term into the varying intercepts model using Eq. [2], and the posterior distributions were predominately positive and negative for annual precipitation and evapotranspiration, respectively (Table 4.5). The slope term investigates the ability of a single climate variable to

predict the isolation frequency of *F. culmorum* assuming there is a proportional relationship between the two. The same modelling approach was applied to a dataset containing 163 fields with isolation frequency of *F. culmorum* in grains, annual precipitation, and annual GDD. The slopes were predominately the same sign as the current study, though the magnitudes of the slope parameters were less when using the dataset from Backhouse et al. (2004).

Isolation frequency of *F. culmorum* in soil was typically higher in fields within the INW with greater annual precipitation and lower evapotranspiration. This is in agreement with an earlier survey of wheat stubble from the region where the researchers demonstrated that *F. culmorum* was isolated more frequently from cooler and wetter locations and negatively correlated with temperature parameters (Poole et al., 2013). Prevalence of *F. culmorum* out of total *Fusarium* spp. in wheat tissues was higher in the INW following a wet winter and spring compared to following a dry summer and autumn (Smiley & Patterson, 1996).

F. culmorum has a cosmopolitan distribution in soil (Nelson et al., 1983; Samson et al., 2004), but is most commonly found in temperate regions (Leslie & Summerell, 2007). However, surveys of grains have shown that *F. culmorum* is not found in areas with high summer temperatures and low rainfall in Australia and the INW (D. Backhouse & Burgess, 2002a; Poole et al., 2013). Comparison of the 2 datasets suggests that the effect of rainfall on the distribution of *F. culmorum* is more variable in plant tissues as opposed to soil samples. While *F. culmorum* can be considered cosmopolitan in soil and was frequently isolated from the INW, isolation of *F. culmorum* from plant tissues is a rarer event which is dependent on conditions for successful colonization.

Whilst all slope parameters for a given variable were predominantly the same sign, their variability is too large to provide useful predictions for growers even after increasing complexity with multivariable models and polynomial terms (Fig. 4.8). The annual summary of a climate variable combined with sampling only once a year may be too coarse of resolution to adequately address how distributions of plant pathogens may shift under climate change. Correlations between predictor variables need to be considered when constructing additive model equations as annual climate variables were highly correlated in both the INW and eastern Australia (Fig.4).The posterior distributions of slope parameters can potentially

change signs when used in combination which arises from multicollinearity (Table 2). However, the results from using combinations of climate variables suggest that temperature related variables such as annual evapotranspiration may have greater predictive power than annual precipitation. Polynomial regression using annual precipitation reduced the posterior distribution of field offsets compared to linear regression, and the predicted range of *F. culmorum* proportions was also lower compared to linear regression. These results suggest that there is only a slight relationship between annual precipitation and isolation frequency of *F. culmorum* in soil. Annual evapotranspiration showed greater predictive power than annual precipitation when the two variables were used in combination (Table 4.5). The predicted range of *F. culmorum* in wheat in eastern Australia also did not increase under higher annual precipitation or under lower annual GDD (Fig. 4.4, C & D). There is substantial variability in the proportion of *F. culmorum* in soil or in wheat due to differences in fields, quadrats, and sampling iterations which is not explained by an annual summary of a climate variable such as precipitation or growing degree-days. The original dataset is quite variable, and no clear trend was initially seen when plotting the raw data versus an annual climate variable (Fig. 4.2). This uncertainty is carried forward into the model predictions and visualized by simulating new varying intercepts which incorporate the random effects due to differences in sampled fields, quadrats, and iterations. Models which fit the dataset well, such as Eq. [2] with annual precipitation, still produce predictions with considerable uncertainty.

Fungal proportion data provides information about the relative incidence of a plant pathogen of interest referenced against the total amount of fungi within a particular fungal genus which allows researchers to address ecological questions while accounting for species diversity. It is difficult to report these proportions to growers in a clear and interpretable way. In general, higher proportions of *F. culmorum* could lead to increased disease incidence and yield losses (Cook, 1968; Hollaway et al., 2013). It is likely that growers would require additional information to provide context for fungal proportion data. Reporting proportions out of the number of wheat plants sampled can be adapted to provide field estimates of the risk associated with a particular plant pathogen. Incorporating the soil dilution factor could provide an estimate of population density which could be interpreted in a similar manner with seeding density for a field. However, the population density estimates are based on

highly variable fungal counts and new methods of summarizing the variability within a field are needed.

The tools and methodologies provided in this study should facilitate a more comprehensive analysis of the effect of local climate on the distribution of *F. culmorum* across the INW now that differences in experimental clusters have been investigated. Predictive values of climate variables from climate change ensemble datasets can be used as inputs to generate predicted ranges of plant pathogen proportions. Model output provides a framework for inferences that can lead to managerial decisions for future cropping practices under climate change tailored to the relevant data being conditioned upon to minimize yield losses and limit mycotoxin contamination of grains.

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Table 4.1 Site and seasonal differences in isolation frequency of *F. culmorum* out of total Fusaria in agricultural soil across the Inland Pacific Northwest

F[a]	Q[b]	Jun 2016			Sep 2016			Dec 2016			Mar 2017			Jun 2017			Sep 2017			Dec 2017			Mar 2018		
		C[c]	T[d]	%	C[c]	T[d]	%	C[c]	T[d]	%	C[c]	T[d]	%	C[c]	T[d]	%	C[c]	T[d]	%	C[c]	T[d]	%	C[c]	T[d]	%
1	1	11	63	17	0	29	0	9	22	41	9	37	24	3	24	13	1	35	3	2	20	10	15	27	56
1	2	4	39	10	2	31	6	2	20	10	3	26	12	4	30	13	0	23	0	5	26	19	2	27	7
1	3	2	46	4	2	33	6	0	23	0	1	30	3	2	27	7	1	49	2	11	32	34	4	28	14
1	4	2	28	7	9	35	26	2	29	7	4	40	10	9	37	24	0	31	0	1	21	5	0	20	0
1	5	0	36	0	4	33	12	0	28	0	1	32	3	0	36	0	0	35	0	7	25	28	1	28	4
1	6	2	31	6	1	43	2	0	28	0	9	43	21	1	30	3	1	26	4	0	26	0	4	44	9
1	7	2	42	5	17	30	57	30	40	75	2	30	7	8	31	26	6	33	18	3	20	15	5	31	16
1	8	1	33	3	6	31	19	12	37	32	4	35	11	6	30	20	4	24	17	11	31	35	30	50	60
1	9	2	21	10	4	31	13	11	31	35	4	30	13	2	29	7	4	33	12	33	48	69	32	60	53
2	1	4	27	15	2	26	8	1	30	3	9	41	22	2	38	5	6	33	18	4	32	13	7	37	19
2	2	5	34	15	2	24	8	4	37	11	8	41	20	1	36	3	2	35	6	8	28	29	1	40	3
2	3	1	45	2	5	39	13	0	32	0	2	47	4	2	39	5	1	41	2	0	29	0	6	49	12
2	4	0	23	0	0	32	0	3	28	11	1	44	2	0	33	0	0	24	0	0	20	0	1	23	4
2	5	1	21	5	1	28	4	2	35	6	0	44	0	0	35	0	0	34	0	0	30	0	0	39	0
2	6	0	21	0	1	29	3	0	31	0	2	49	4	0	30	0	0	38	0	1	59	2	1	38	3
2	7	0	23	0	1	34	3	1	27	4	8	33	24	0	23	0	2	24	8	0	24	0	0	25	0
2	8	0	24	0	1	30	3	1	32	3	5	34	15	1	26	4	1	28	4	2	29	7	0	23	0
2	9	2	25	8	1	21	5	3	38	8	1	35	3	1	29	3	1	22	5	3	45	7	0	39	0
3	1	1	44	2	2	31	6	1	32	3	2	31	6	2	29	7	3	36	8	33	59	56	8	40	20
3	2	1	40	3	1	31	3	2	32	6	3	27	11	7	42	17	1	27	4	8	50	16	0	30	0
3	3	1	44	2	0	42	0	2	25	8	7	33	21	3	27	11	7	33	21	5	44	11	8	32	25
3	4	5	41	12	1	26	4	2	28	7	13	27	48	0	28	0	5	35	14	8	31	26	11	30	37
3	5	5	40	13	2	26	8	0	28	0	9	34	26	9	34	26	0	23	0	30	52	58	10	42	24
3	6	0	32	0	1	29	3	5	33	15	17	41	41	10	28	36	2	28	7	2	36	6	8	45	18
3	7	1	41	2	2	38	5	0	54	0	8	27	30	10	40	25	0	31	0	2	23	9	4	32	13
3	8	10	44	23	1	20	5	23	46	50	15	36	42	15	28	54	6	29	21	12	31	39	24	41	59
3	9	8	34	24	15	31	48	2	21	10	7	32	22	4	28	14	14	34	41	4	33	12	8	30	27
4	1	0	51	0	4	31	13	0	36	0	4	26	15	2	24	8	1	28	4	1	24	4	0	20	0
4	2	6	35	17	2	29	7	1	40	3	2	37	5	14	30	47	0	25	0	8	25	32	9	23	39
4	3	4	27	15	15	34	44	16	44	36	15	32	47	12	32	38	9	32	28	14	46	30	17	35	49
4	4	1	27	4	2	32	6	0	36	0	4	26	15	1	30	3	0	24	0	3	20	15	9	21	43
4	5	4	24	17	4	26	15	6	35	17	12	31	39	15	43	35	2	31	6	17	27	63	29	37	78
4	6	3	31	10	1	28	4	13	43	30	8	32	25	5	34	15	3	33	9	38	50	76	15	36	42
4	7	1	38	3	7	22	32	30	36	83	19	38	50	14	31	45	9	35	26	15	21	71	22	29	76
4	8	2	26	8	16	31	52	19	55	35	16	37	43	17	33	52	9	39	23	6	27	22	19	28	68
4	9	1	22	5	2	28	7	1	33	3	7	31	23	3	41	7	5	52	10	18	40	45	4	25	16
5	1	1	21	5	0	21	0	2	28	7	1	34	3	1	37	3	6	35	17	1	28	4	2	31	6
5	2	9	25	36	5	31	16	14	35	40	16	34	47	21	39	54	10	30	33	30	36	83	22	33	67
5	3	14	39	36	26	35	74	2	33	6	11	28	39	13	33	39	10	31	32	6	28	21	28	33	85
5	4	0	18	0	0	35	0	2	37	5	0	20	0	1	43	2	0	20	0	0	24	0	2	24	8
5	5	3	31	10	2	24	8	12	35	34	1	33	3	8	25	32	3	21	14	6	40	15	6	21	29
5	6	6	37	16	31	38	82	2	36	6	9	22	41	10	26	38	11	33	33	11	37	30	13	37	35
5	7	7	29	24	5	22	23	9	31	29	14	33	42	14	32	44	3	23	13	12	31	39	5	28	18
5	8	0	27	0	4	35	11	1	31	3	0	33	0	1	27	4	5	23	22	1	34	3	9	38	24
5	9	0	16	0	2	34	6	0	32	0	2	32	6	1	28	4	0	23	0	0	25	0	0	43	0
6	1	0	39	0	0	46	0	0	29	0	0	32	0	5	25	20	0	21	0	1	31	3	0	26	0
6	2	0	38	0	11	35	31	4	32	13	0	34	0	3	26	12	0	38	0	2	32	6	0	32	0
6	3	0	19	0	0	25	0	0	35	0	0	30	0	0	25	0	0	25	0	0	41	0	0	26	0
6	4	0	34	0	0	28	0	1	32	3	0	28	0	0	24	0	0	22	0	0	32	0	0	40	0
6	5	2	43	5	3	41	7	12	43	28	10	35	29	1	34	3	1	21	5	0	27	0	0	35	0
6	6	0	38	0	0	20	0	0	52	0	0	40	0	0	41	0	0	30	0	0	37	0	4	36	11
6	7	0	35	0	0	36	0	7	43	16	0	32	0	0	36	0	0	29	0	1	32	3	0	33	0
6	8	1	31	3	0	36	0	15	54	28	5	37	14	6	31	19	0	22	0	4	54	7	1	47	2
6	9	4	40	10	1	30	3	10	54	19	4	35	11	4	30	13	0	29	0	0	41	0	1	54	2
7	1	0	22	0	0	29	0	0	25	0	0	34	0	0	31	0	0	22	0	0	20	0	0	31	0
7	2	19	34	56	0	37	0	0	35	0	0	30	0	0	30	0	0	22	0	0	34	0	0	25	0
7	3	0	26	0	0	31	0	0	25	0	0	28	0	0	34	0	1	23	4	0	23	0	0	33	0
7	4	0	35	0	0	36	0	1	33	3	0	31	0	0	40	0	0	21	0	0	23	0	0	21	0
7	5	1	34	3	0	27	0	2	35	6	0	34	0	0	29	0	0	32	0	0	20	0	1	32	3
7	6	0	27	0	0	24	0	0	47	0	0	31	0	0	47	0	0	28	0	6	42	14	0	22	0
7	7	0	28	0	1	26	4	0	50	0	0	26	0	0	36	0	0	20	0	0	16	0	0	20	0
7	8	1	27	4	0	30	0	1	34	3	1	33	3	3	37	8	0	20	0	0	32	0	0	24	0
7	9	0	30	0	0	24	0	0	43	0	0	28	0	22	46	48	0	31	0	0	27	0	0	22	0
8	1	0	31	0	0	38	0	0	34	0	1	29	3	1	35	3	0	21	0	0	32	0	1	24	4
8	2	0	32	0	1	27	4	0	34	0	0	32	0	0	34	0	0	24	0	2	36	6	0	30	0
8	3	0	25	0	0	35	0	0	25	0	0	38	0	0	45	0	0	38	0	0	32	0	0	35	0
8	4	1	29	3	0	24	0	0	37	0	2	32	6	0	23	0	0	20	0	0	24	0	0	23	0
8	5	0	28	0	0	35	0	0	30	0	0	34	0	1	37	3	0	22	0	0	30	0	0	40	0
8	6	1	19	5	0	26	0	0	37	0	0	34	0	0	33	0	0	21	0	0	36	0	0	25	0
8	7	1	35	3	0	20	0	2	36	6	0	38	0	2	39	5	0	24	0	0	32	0	0	27	0
8	8	1	25	4	0	24	0	0	50	0	1	28	4	0	26	0	0	27	0	0	20	0	0	20	0
8	9	1	26	4	0	30	0	0	39	0	0	25	0	0	37	0	1	22	5	0	20	0	0	29	0
9	1	0	44	0	0	38	0	0	29	0	0	30	0	0	33	0	0	28	0	0	30	0	0	31	0
9	2	0	30	0	0	38	0	0	26	0	0	25	0	0	46	0	0	29	0	0	25	0	0	30	0
9	3	0	36	0	0	29	0	0	34	0	0	30	0	0	34	0	1	34	3	0	36	0	0	24	0
9	4	0	32	0	0	27	0	0	24	0	0	32	0	0	34	0	0	21	0	0	25	0	0	29	0
9	5	0	28	0	0	30	0	0	29	0	0	34	0	0	34	0	0	24	0	0	25	0	0	21	0
9	6	0	33	0	0	33	0	0	32	0	0	34	0	0	42	0	0	29	0	0	27	0	0	28	0
9	7	0	36	0	0	43	0	0	32	0	0	26	0	0	32	0	0	31	0	0	24	0	1	29	3
9	8	1	34	3	0	30	0	0	35	0	0	38	0	1	37	3	0	25	0	0	53	0	2	24	8
9	9	0	29	0	1	37	3	0	32	0	1	35	3	1	45	2	0	29	0	0	37	0	0	29	0

[a]Field number
[b]Sampling quadrat (1 acre = 4047 m^2)
[c]Number of *F. culmorum* isolates
[d]Number of total soil *Fusaria* processed

Table 4.2 Seasonal differences in total field isolation frequency of *F. culmorum* out of total Fusaria in agricultural soil across the Inland Pacific Northwest

Date	Field	C[a]	T[b]	%	Date	Field	C[a]	T[b]	%
2016-06-11	1	26	339	**8**	2017-06-03	1	35	274	**13**
2016-06-11	2	13	243	**5**	2017-06-03	2	7	289	**2**
2016-06-11	3	32	360	**9**	2017-06-03	3	60	284	**21**
2016-06-18	4	22	281	**8**	2017-06-10	4	83	298	**28**
2016-06-18	5	40	243	**16**	2017-06-10	5	70	290	**24**
2016-06-18	6	7	317	**2**	2017-06-10	6	19	272	**7**
2016-06-25	7	21	263	**8**	2017-06-17	7	25	330	**8**
2016-06-25	8	5	250	**2**	2017-06-17	8	4	309	**1**
2016-06-25	9	1	302	**0**	2017-06-17	9	2	337	**1**
2016-09-03	1	45	296	**15**	2017-09-02	1	17	289	**6**
2016-09-03	2	14	263	**5**	2017-09-02	2	13	279	**5**
2016-09-03	3	25	274	**9**	2017-09-02	3	38	276	**14**
2016-09-10	4	53	261	**20**	2017-09-09	4	38	299	**13**
2016-09-10	5	75	275	**27**	2017-09-09	5	48	239	**20**
2016-09-10	6	15	297	**5**	2017-09-09	6	1	237	**0**
2016-09-17	7	1	264	**0**	2017-09-16	7	1	219	**0**
2016-09-17	8	1	259	**0**	2017-09-16	8	1	219	**0**
2016-09-17	9	1	305	**0**	2017-09-16	9	1	250	**0**
2016-12-03	1	66	258	**26**	2017-12-02	1	73	249	**29**
2016-12-03	2	15	290	**5**	2017-12-02	2	18	296	**6**
2016-12-03	3	37	299	**12**	2017-12-02	3	104	359	**29**
2016-12-10	4	86	358	**24**	2017-12-09	4	120	280	**43**
2016-12-10	5	44	298	**15**	2017-12-09	5	67	283	**24**
2016-12-10	6	49	374	**13**	2017-12-09	6	8	327	**2**
2016-12-15	7	4	327	**1**	2017-12-16	7	6	237	**3**
2016-12-15	8	2	322	**1**	2017-12-16	8	2	262	**1**
2016-12-15	9	0	273	**0**	2017-12-16	9	0	282	**0**
2017-03-04	1	37	303	**12**	2018-03-03	1	93	315	**30**
2017-03-04	2	36	368	**10**	2018-03-03	2	16	313	**5**
2017-03-04	3	81	288	**28**	2018-03-03	3	81	322	**25**
2017-03-11	4	87	290	**30**	2018-03-10	4	124	254	**49**
2017-03-11	5	54	269	**20**	2018-03-10	5	87	288	**30**
2017-03-11	6	19	303	**6**	2018-03-10	6	6	329	**2**
2017-03-18	7	1	275	**0**	2018-03-17	7	1	230	**0**
2017-03-18	8	4	290	**1**	2018-03-17	8	1	253	**0**
2017-03-18	9	1	284	**0**	2018-03-17	9	3	245	**1**

[a]Total number of *F. culmorum* isolates
[b]Total number of total soil *Fusaria* processed

Table 4.3 Posterior distributions of marginal parameter offsets for field and sampling iterations using counts of *F. culmorum* in soil across a 2 year soil survey. Values are presented as mean ± standard deviation

Field	Field offset[a]	Sampling iteration offset[a]							
		June 2016	September 2016	December 2016	March 2017	June 2017	September 2017	December 2017	March 2018
1	1.17 ± 0.67	-0.45 ± 0.28	0.06 ± 0.26	0.43 ± 0.25	-0.13 ± 0.27	-0.18 ± 0.27	-0.82 ± 0.30	0.53 ± 0.25	0.78 ± 0.25
2	0.14 ± 0.67	-0.14 ± 0.32	-0.08 ± 0.32	-0.02 ± 0.32	0.81 ± 0.28	-0.60 ± 0.36	-0.14 ± 0.32	0.14 ± 0.31	0.04 ± 0.31
3	1.44 ± 0.68	-0.42 ± 0.27	-0.64 ± 0.28	-0.29 ± 0.26	0.48 ± 0.25	0.18 ± 0.25	-0.26 ± 0.26	0.73 ± 0.24	0.48 ± 0.25
4	1.59 ± 0.68	-1.00 ± 0.28	-0.21 ± 0.26	0.27 ± 0.25	0.28 ± 0.25	0.23 ± 0.25	-0.52 ± 0.27	0.61 ± 0.24	0.64 ± 0.24
5	1.20 ± 0.67	-0.35 ± 0.27	0.27 ± 0.25	-0.25 ± 0.26	-0.06 ± 0.26	0.20 ± 0.25	-0.17 ± 0.26	0.15 ± 0.25	0.42 ± 0.25
6	-0.30 ± 0.67	-0.41 ± 0.37	0.20 ± 0.32	1.37 ± 0.28	0.41 ± 0.31	0.41 ± 0.31	-1.16 ± 0.43	-0.32 ± 0.36	-0.51 ± 0.37
7	-1.17 ± 0.69	1.25 ± 0.34	-0.70 ± 0.47	-0.19 ± 0.42	-0.69 ± 0.46	1.43 ± 0.33	-0.69 ± 0.47	0.09 ± 0.40	-0.70 ± 0.47
8	-1.61 ± 0.70	0.43 ± 0.45	-0.34 ± 0.51	-0.12 ± 0.49	0.27 ± 0.46	0.27 ± 0.46	-0.34 ± 0.51	-0.13 ± 0.49	-0.34 ± 0.51
9	-2.47 ± 0.75	-0.08 ± 0.55	-0.08 ± 0.54	-0.37 ± 0.56	-0.08 ± 0.54	0.18 ± 0.52	-0.08 ± 0.54	-0.37 ± 0.56	0.42 ± 0.51

[a]Offset from population average in log-odds derived from a hierarchical model using varying itercepts by field (N=9), quadrat (N=81), and sampling iteration (N=72)

Table 4.4 Posterior distributions of marginal parameter offsets for 1 acre sampling quadrats from the population mean for isolation frequency of *F. culmorum* out of total Fusaria in agricultural soils across the Inland Pacific Northwest*. Values are presented as mean ± standard deviation.

	Field								
Q[a]	1	2	3	4	5	6	7	8	9
1	0.39 ± 0.34	1.08 ± 0.35	0.18 ± 0.33	-1.36 ± 0.39	-0.77 ± 0.38	-0.37 ± 0.47	-1.24 ± 0.67	0.21 ± 0.58	-0.55 ± 0.78
2	-0.41 ± 0.37	0.96 ± 0.36	-0.62 ± 0.36	-0.20 ± 0.35	1.41 ± 0.32	0.75 ± 0.39	1.41 ± 0.41	0.22 ± 0.58	-0.55 ± 0.79
3	-0.37 ± 0.36	0.35 ± 0.38	-0.27 ± 0.34	0.69 ± 0.33	1.26 ± 0.32	-1.66 ± 0.63	-0.87 ± 0.62	-0.87 ± 0.72	-0.02 ± 0.72
4	-0.22 ± 0.36	-0.74 ± 0.47	0.03 ± 0.34	-0.91 ± 0.37	-1.58 ± 0.45	-1.35 ± 0.59	-0.87 ± 0.61	0.20 ± 0.59	-0.54 ± 0.78
5	-0.90 ± 0.39	-0.89 ± 0.48	0.40 ± 0.33	0.55 ± 0.33	0.25 ± 0.34	1.13 ± 0.37	-0.10 ± 0.52	-0.44 ± 0.66	-0.55 ± 0.77
6	-0.60 ± 0.37	-0.74 ± 0.47	0.03 ± 0.34	0.51 ± 0.33	1.08 ± 0.32	-0.68 ± 0.49	0.25 ± 0.49	-0.44 ± 0.67	-0.54 ± 0.78
7	0.78 ± 0.33	0.02 ± 0.40	-0.47 ± 0.35	0.83 ± 0.33	0.78 ± 0.33	-0.13 ± 0.44	-0.87 ± 0.62	0.67 ± 0.53	-0.02 ± 0.72
8	0.79 ± 0.33	-0.06 ± 0.41	0.90 ± 0.32	0.71 ± 0.33	-0.40 ± 0.36	1.24 ± 0.37	0.25 ± 0.49	-0.08 ± 0.62	1.06 ± 0.62
9	1.01 ± 0.33	0.02 ± 0.40	0.35 ± 0.33	-0.23 ± 0.35	-1.58 ± 0.45	0.94 ± 0.38	1.56 ± 0.40	-0.08 ± 0.62	0.77 ± 0.64

*Offset from population average in log-odds derived from a hierarchical model using varying itercepts by field (N=9), quadrat (N=81), and sampling iteration (N=72)

[a]Sampling quadrat (1 acre = 4047 m^2). The sampling region for a field consisted of a 3 × 3 grid pattern

Table 4.5 Posterior distributions of parameters used in different multilevel model equations using different combinations of annual climate summaries. Values are presented s mean ± standard deviation

Data source	Quadratic parameter			Linear parameter			Interaction parameter	Intercept parameter[c]
	P[b]	E[b]	G[b]	P[b]	E[b]	G[b]		
Current study				1.05 ± 0.45				-6.37 ± 0.45
					-1.23 ± 0.39			-6.36 ± 0.38
						-0.82 ± 0.53		-6.35 ± 0.56
				-0.42 ± 1.00	-1.64 ± 1.00			-6.35 ± 0.41
				1.15 ± 0.76	-0.17 ± 0.73		-1.47 ± 0.48	-5.03 ± 0.49
				1.10 ± 0.82		-0.05 ± 0.80		-6.35 ± 0.51
				2.38 ± 0.83		1.10 ± 0.76	1.39 ± 0.61	-5.28 ± 0.60
					-2.68 ± 0.68	1.60 ± 0.68		-6.38 ± 0.28
					-3.19 ± 0.51	2.31 ± 0.53	-0.84 ± 0.30	-5.61 ± 0.32
	-1.22 ± 0.20			-1.56 ± 0.20				-5.17 ± 0.26
		-1.15 ± 0.37			-0.75 ± 0.67			-5.62 ± 0.75
			-0.25 ± 0.72			-0.70 ± 0.65		-6.10 ± 0.91
	-3.39 ± 2.94	-1.93 ± 2.60		1.41 ± 0.77	-0.33 ± 0.72		-4.27 ± 5.58	-4.97 ± 0.61
	-1.62 ± 0.45		-0.92 ± 0.68	1.28 ± 0.53		-0.02 ± 0.62	-1.35 ± 0.90	-4.92 ± 0.39
		-1.49 ± 1.12	-1.32 ± 1.05		-2.33 ± 0.78	1.55 ± 0.75	-1.81 ± 1.83	-5.21 ± 0.48
Backhouse et al. (2004)				0.59 ± 0.21				-6.25 ± 0.29
						-0.89 ± 023		-6.28 ± 0.29
				-0.47 ± 0.38		-1.33 ± 0.43		-6.31 ± 0.29
				0.29 ± 0.52		-1.09 ± 0.42	0.60 ± 0.29	-5.86 ± 0.34
	-0.60 ± 0.24			1.26 ± 0.36				-5.68 ± 0.33
			-0.45 ± 0.21			-1.07 ± 0.25		-5.88 ± 0.32
	-2.18 ± 0.98		-1.41 ± 1.00	0.01 ± 0.62		-1.26 ± 0.61	-3.09 ± 1.82	-5.31 ± 0.48

[a]The equation used was different combinations $\text{logit}(p) = a + b_1x^2 + b_2y^2 + b_3xy + b_4x + b_5y$ for climate variables x and y with and without the quadratic parameters or interaction parameters where p is the proportion of *F. culmorum*. Climate variables were also tested individually. Parameters for each unique observation within a given cluster of the dataset (fields, iterations, and quadrats) were included in the equation as offsets from a but are not shown.

[b]P = annual precipitation; E = annual evapotranspiration; GDD = annual growing degree-days. Values were standardized to mean 0 and standard deviation 1 prior to model fitting

[c]The intercept a is the population parameter and the hierarchical term in the multilevel model

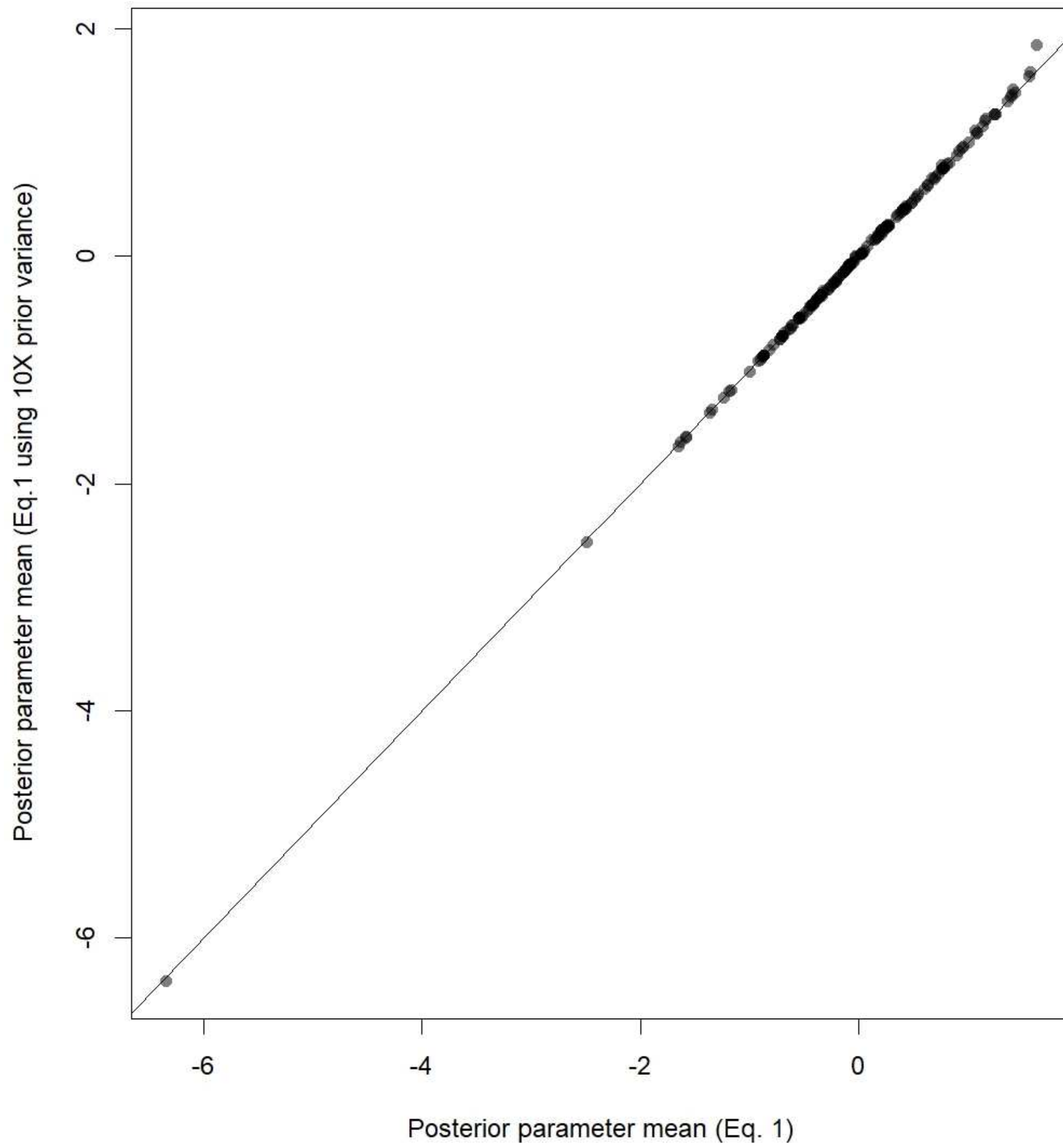

Figure 4.1 Comparison of posterior distributions parameter estimates using Eq. [1] where the priors for the variance components were all increased by an order of magnitude prior to model fitting.

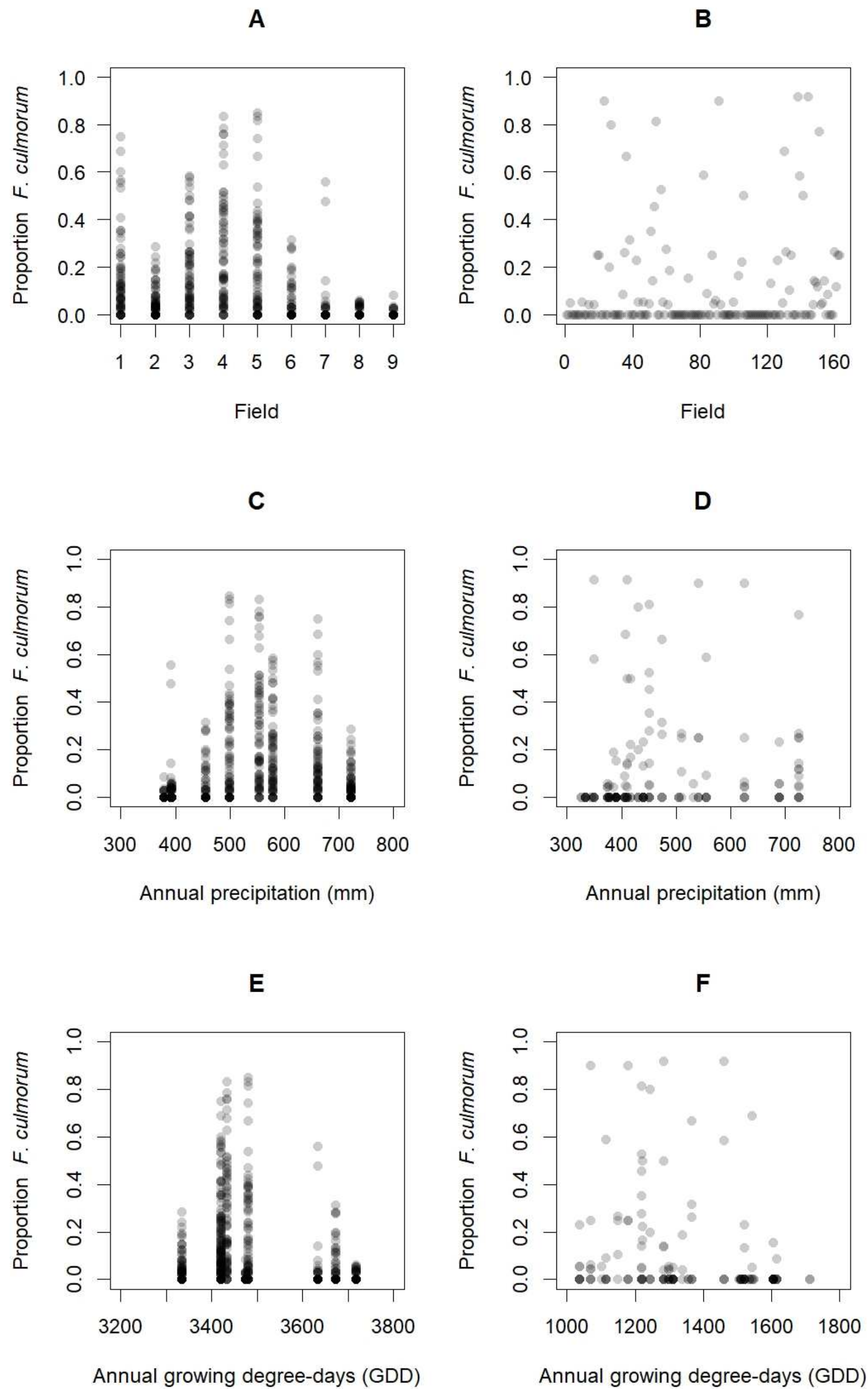

Figure 4.2 Comparison of raw data collected from the current study (A, C, E) and Backhouse et al., (2004) (B, D, F). Proportion of *F. culmorum* is out of total *Fusarium* spp in soil from the current study, and out of total *Fusarium* spp in wheat from Backhouse et al. (2004). Proportions are plotted against field designation (A, B), average annual precipitation (mm) (C, D), and average annual growing degree days (GDD) (E, F).

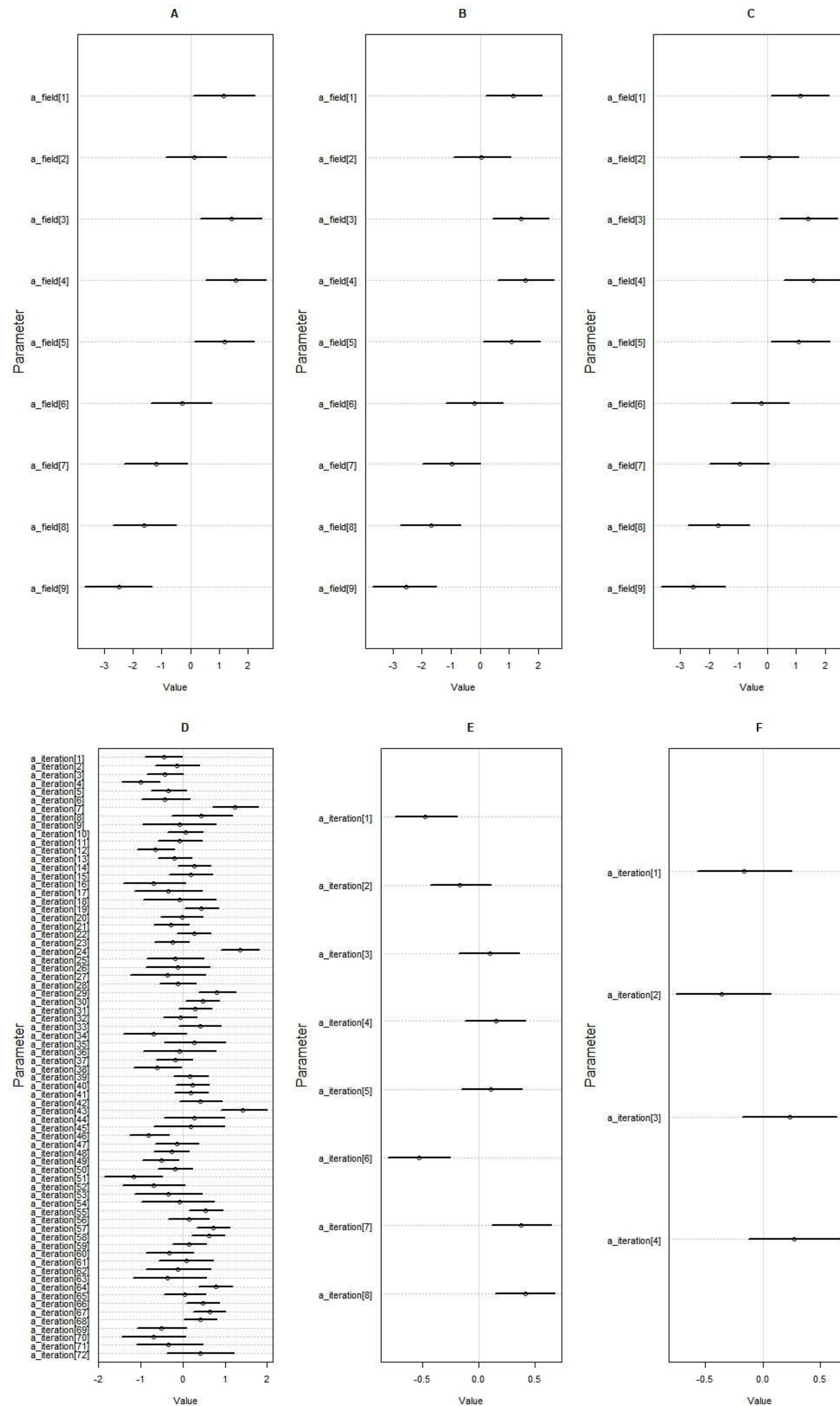

Figure 4.3 Comparison of posterior distributions of parameter offsets for field (A, B, C) and sampling iteration (D, E, F) based on number of sampling iterations used. All models used Eq. [1] with either all sampling iterations independent (N=72) (A, D), sampling iterations by time point (N=8) (B, E), or sampling iterations by season (N=4) (C, F). Error bars denote standard deviation.

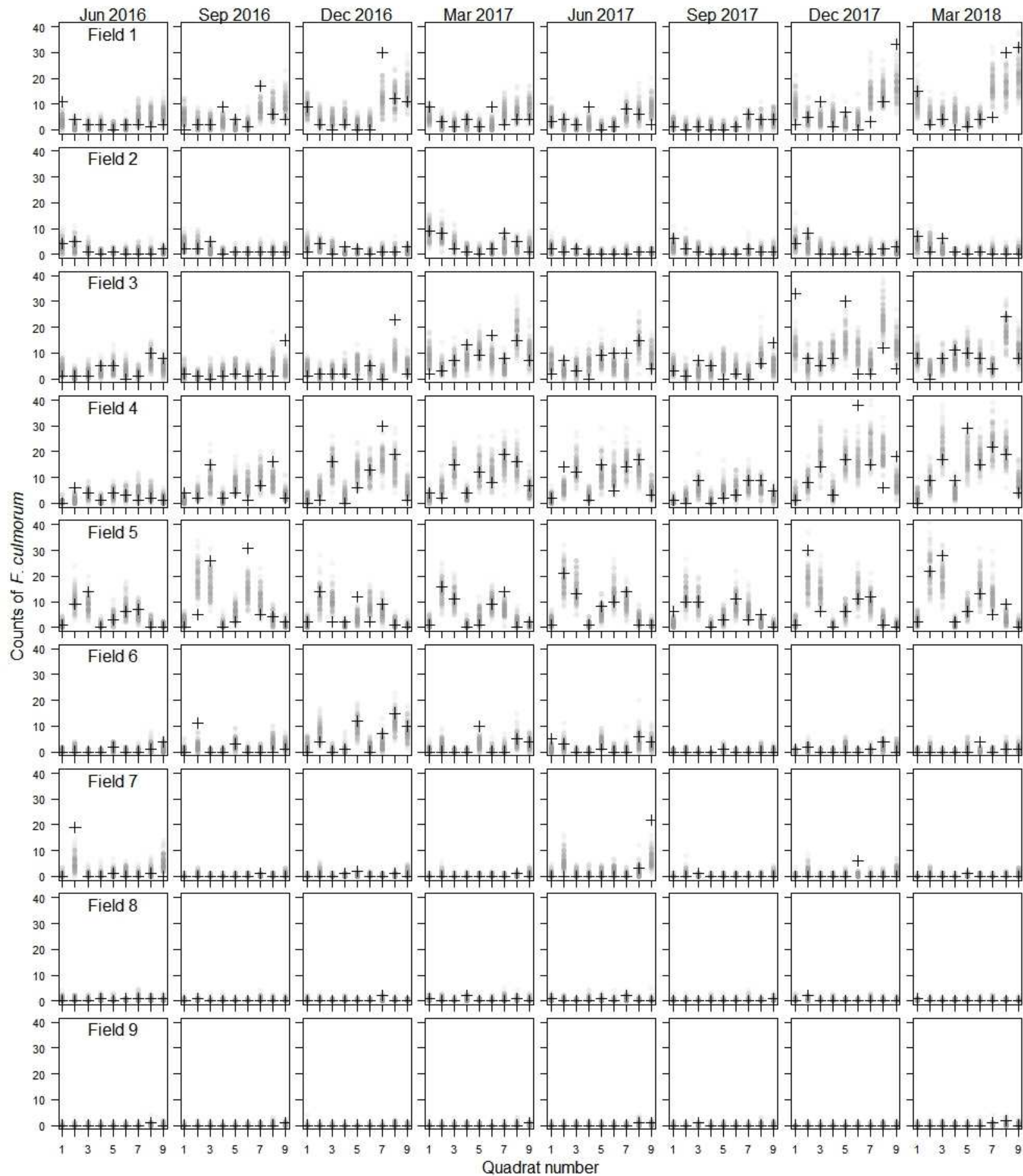

Figure 4.4 Comparison of model predictions (gray) with empirical data (crosses) for counts of *F. culmorum* in agricultural soil using a posterior distribution of offsets for unique quadrats, field, and sampling iterations derived from the dataset. Uncertainty in the posterior distribution is visualized by simulating 100 random samples from the posterior distribution.

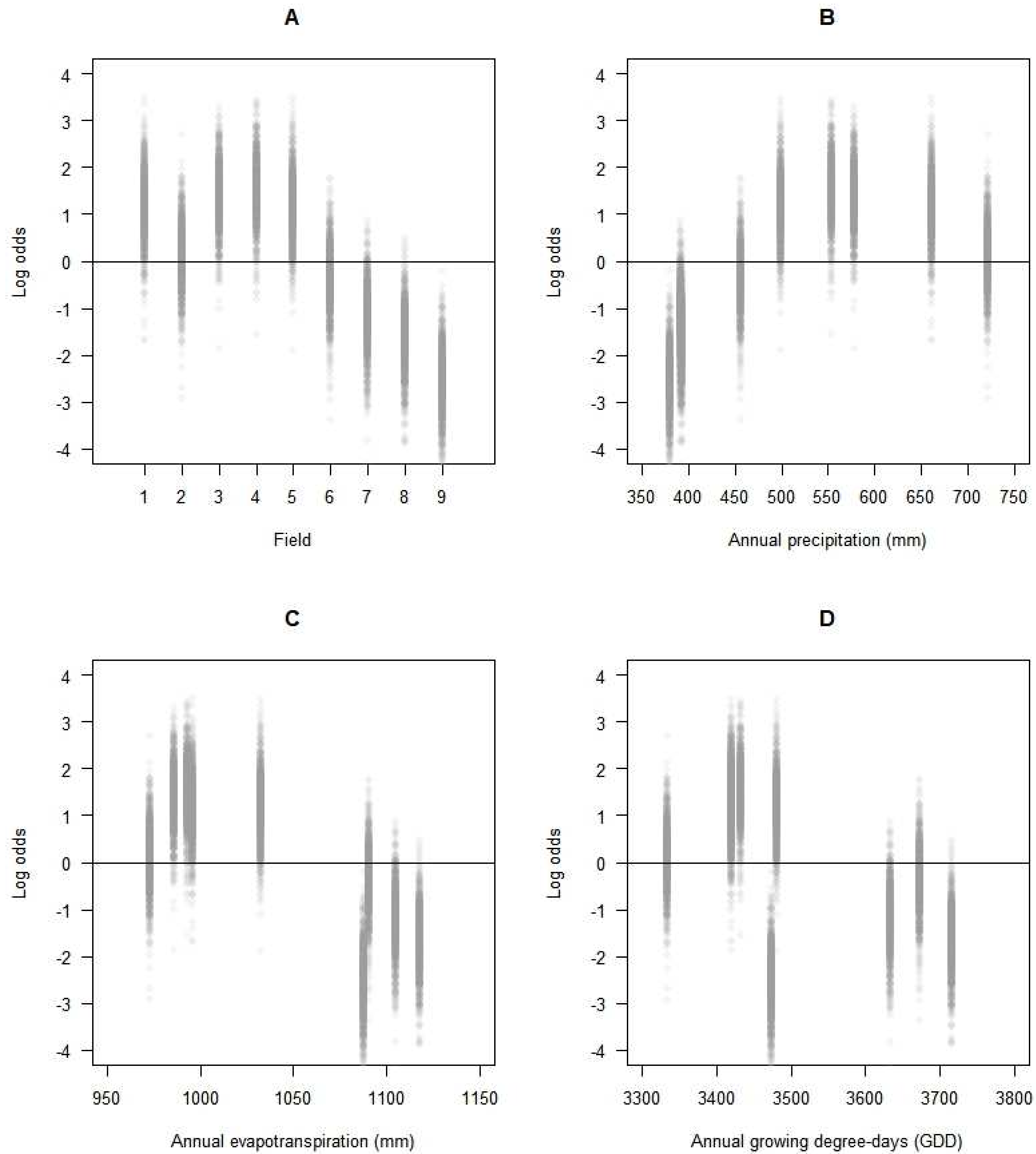

Figure 4.5 Marginal posterior distributions of field offsets using only isolation frequency records of *F. culmorum* plotted against (A) field sampled, (B) annual precipitation (mm), (C) annual grass reference evapotranspiration, and (D) annual growing degree-days (°C/day). Climate variables are long-term historic means of 38 years from 1980 to 2018. Uncertainty in the posterior distribution is visualized by plotting 1000 samples from the Markov chain used during approximation.

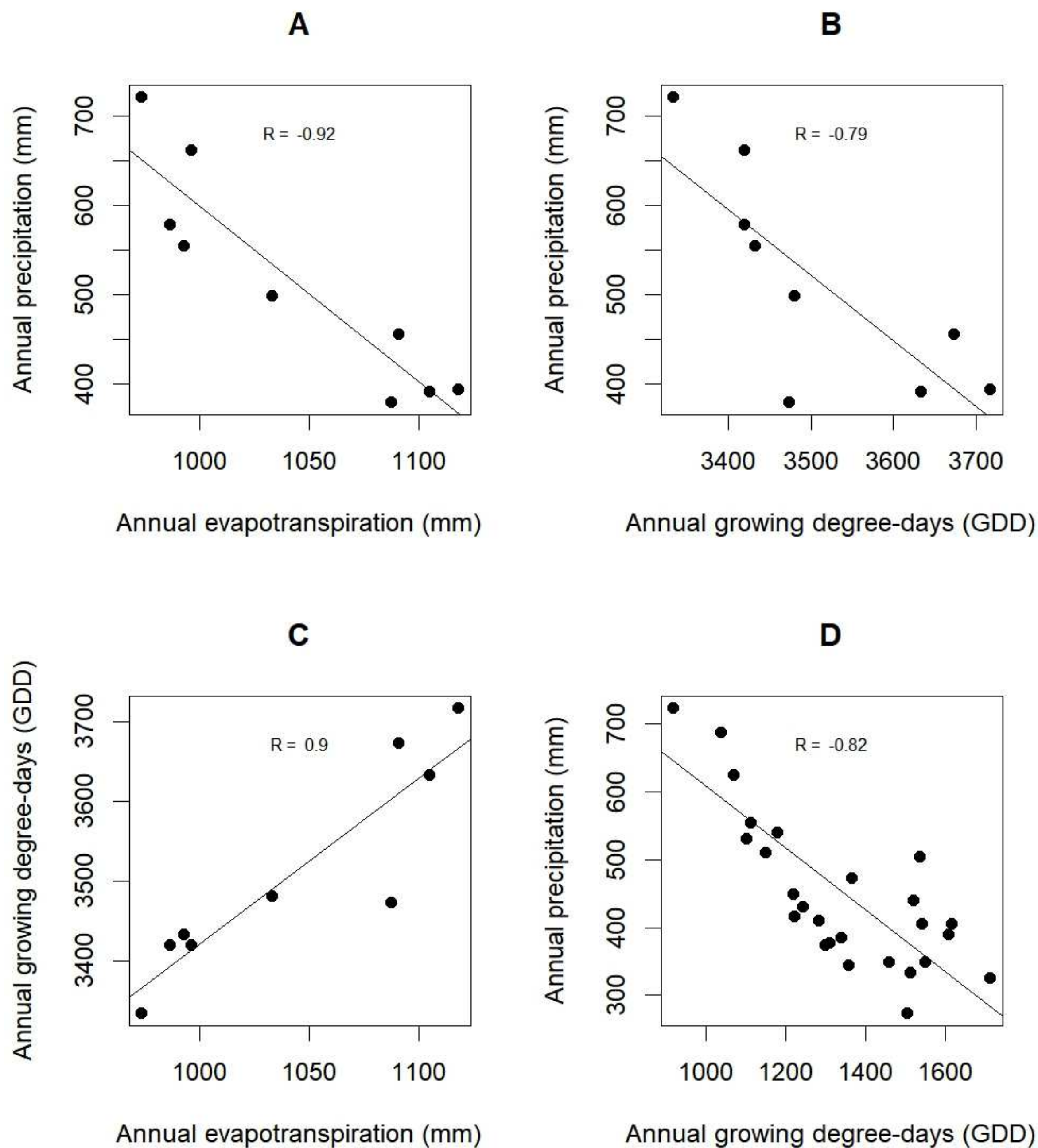

Figure 4.6 Correlations between predictor variables in the dataset from the current study (A, B, C) or Backhouse et al. (2004) (D). The correlations are between (A) annual evapotranspiration and annual precipitation, (B) annual growing degree-days and annual precipitation, (C) annual evapotranspiration and annual growing degree days, and (D) annual growing degree-days and annual precipitation. The Pearson correlation coefficient between two predictor variables is denoted using R.

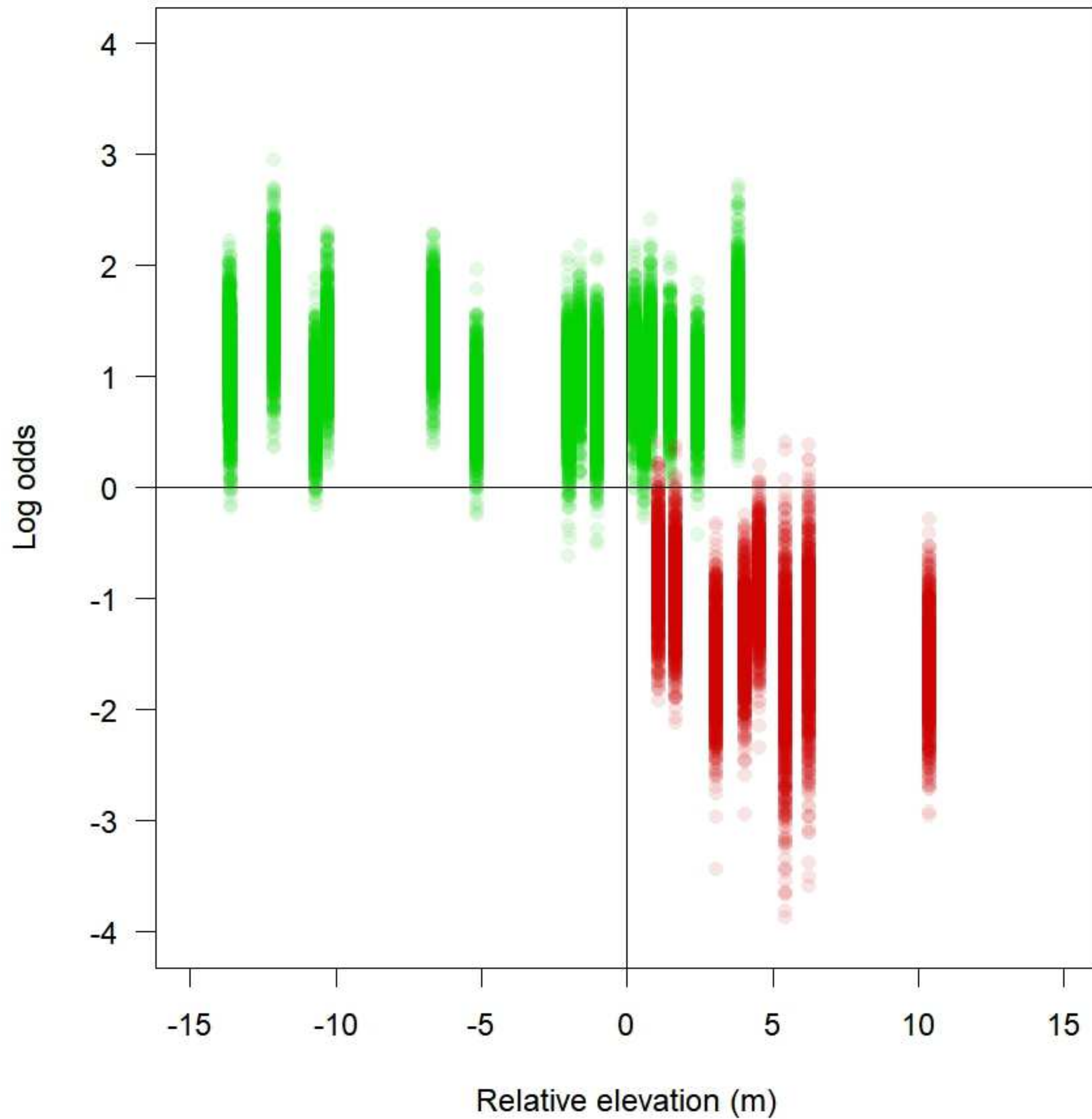

Figure 4.7 Marginal posterior distributions of quadrat offsets plotted against elevation. Only distributions where the 95% highest posterior density interval excluded 0 are shown (N=26) out of all quadrats sampled (N=81). The mean elevation for a field was subtracted from the elevation for a given quadrat, centering all values at 0. Quadrats which were predominately positive or negative are colored green and red, respectively. Uncertainty in the posterior distribution is visualized by plotting 1000 samples from the Markov chain used during approximation.

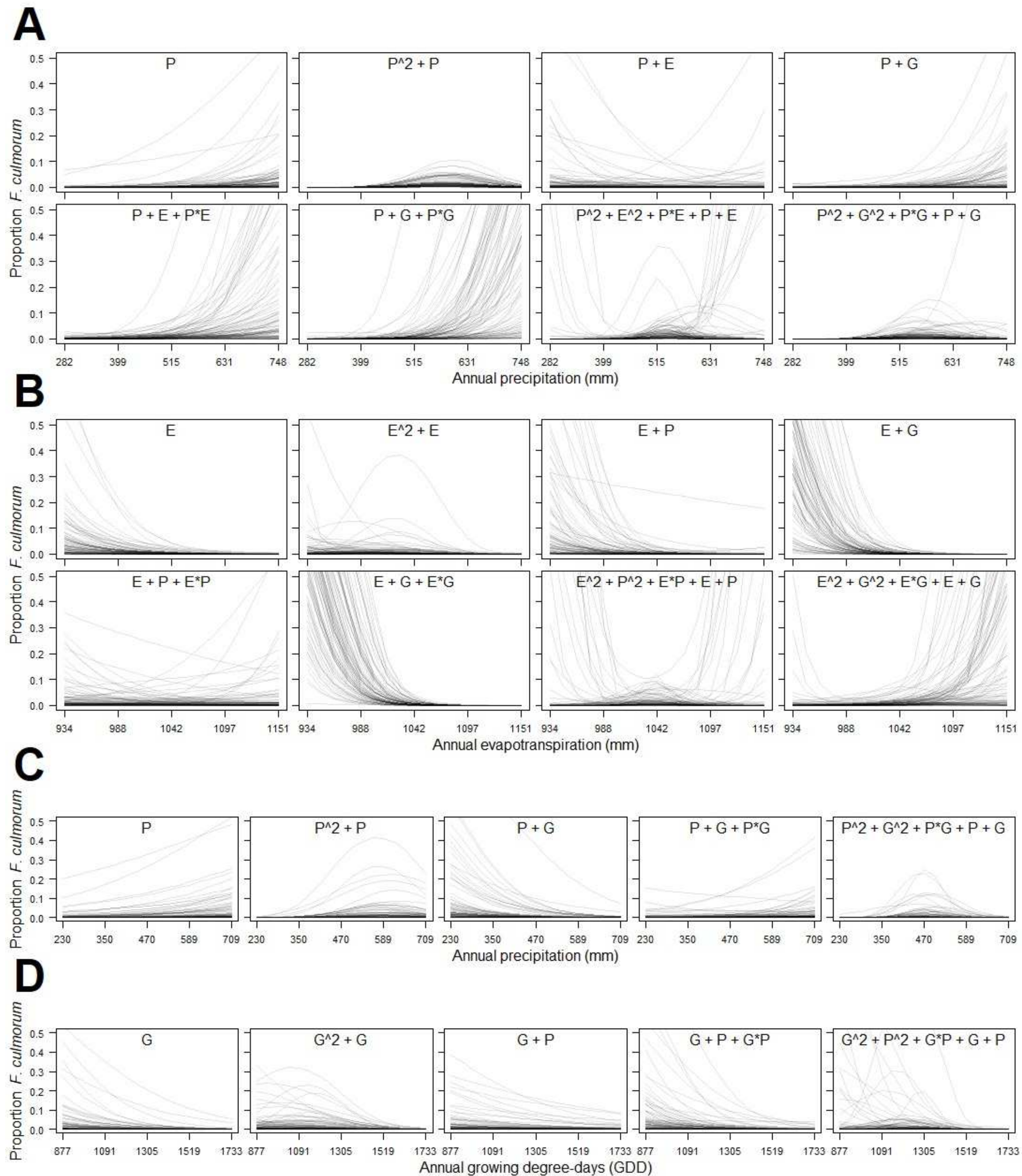

Figure 4.8 Predicted proportions of *F. culmorum* out of total *Fusarium* spp. in soil based on (A) annual precipitation or (B) annual evapotranspiration and predicted proportions of F. culmorum out of total Fusarium spp. in wheat based on (C) annual precipitation or (D) annual growing degree-days using the dataset from Backhouse et al. (2004). Variables used were annual precipitation (P), annual evapotranspiration (E), and annual growing degree days (G). Letters in each sub-plot indicate which climate variables were used in the model equation and "^2" and "*" denote a quadratic and interaction parameter, respectively. Each line represents the impact of the climate variable by simulating a new proportion of *F. culmorum* using the posterior distribution of each multilevel model. Variation among sampled fields, quadrats, and iterations is visualized by plotting 100 simulated proportions of *F. culmorum*.

Chapter 5: Probabilistic forecasts of *Fusarium culmorum* populations in agricultural soil across the Inland Pacific Northwest under climate change

Abstract

Fusarium culmorum is a plant pathogen capable of causing economic damage through yield losses and mycotoxin contamination of wheat. It remains unknown how climate change may influence future shifts in the population density of *F. culmorum* in agricultural soil. Isolation records from a two year soil survey across a precipitation gradient within the Inland Pacific Northwest (INW) region of the United States were used to construct a multilevel climate model. Varying intercepts and slopes were assigned to each unique agricultural field and a weather-based proxy for soil moisture, termed atmospheric water balance (AWB) was used as a predictor variable. Values of AWB derived from downscaled global climate models were used to forecast future shifts in the proportion of *F. culmorum* across all sampled fields. Population densities of *F. culmorum* are forecasted to remain constant during the winter and spring but decrease over the summer and fall under climate change, with the magnitude differing across fields. This study presents a means of quantifying a highly variable fungal plant pathogen in soil and presenting the results in terms of a frequency of exceedance. Results supplied within represent a scaffold for the development of more complex climate models which are needed to provide growers with the means to adapt to a changing climate.

Introduction

The Inland Pacific Northwest (INW) region of the United States (US), spanning across southeastern Washington and north-central Idaho, is one of the most highly productive wheat producing regions in the world (*Triticum aestivum*). Wheat yields in the INW typically average 5.4 to 6.7 Mg/ha, nearly double that in the Midwestern US (Scheinost et al., 2001; Richard W. Smiley et al., 2005). Wheat grown in the Midwestern US is also susceptible to a fungal disease called *Fusarium* Head Blight (FHB) (McMullen et al., 1997, 2012) which results in the mycotoxin deoxynivalenol (DON) in infected kernels (Placinta et al., 1999; Tanaka et al., 1988). By contrast, wheat grown in the INW has among the lowest levels of DON found in the US (Bianchini et al., 2015).

There are two primary hypotheses that explain the relatively low incidence of FHB in the INW: (i) the phenological mismatch between wheat flowering and annual rainfall, and (ii) the relatively low extent of corn cropping systems in the INW. Observed cases of FHB in the INW have been primarily collocated with central-pivot-irrigated fields in Washington where the localized high moisture content favors the release of *F. graminearum* ascospores (C. Strausbaugh & Maloy, 1986). Wheat is most susceptible to FHB around flowering in combination with humid conditions (Beyer et al., 2005; De Wolf et al., 2003; Lacey et al., 1999). In the Midwestern US the flowering of wheat coincides with periods of heavy rainfall and likely contributes to the higher incidence of FHB (Hatfield, 2012). Most annual rainfall in the INW occurs between October and March, and conditions during flowering in June are typically too dry for the development of FHB (Zobeck et al., 2010).

The primary causal agent of FHB, *Fusarium graminearum*, is rarely present in the INW (R W Smiley & Patterson, 1996). The primary inoculum source for *F. graminearum* is corn residue (Dill-Macky & Jones, 2000), and the fungus does not survive for prolonged periods in wheat stubble after burial in soil (Pereyra et al., 2004). Irrigated fields are the only source of corn growth in the INW, which are also where observed cases of FHB occur (C. Strausbaugh & Maloy, 1986; Wang et al., 2007). Recent cases of FHB in southern Idaho caused by *F. graminearum* have resulted from an increased acreage of corn planted to support the dairy industry (Marshall, 2007). However, *Fusarium culmorum*, another causal agent of FHB is considered to be ubiquitous in soil across the INW (R W Smiley & Patterson, 1996). *F. culmorum* can infect wheat heads through splash dispersal if sufficient rainfall occurs during flowering (Jenkinson & Parry, 1994b). In the INW, *F. culmorum* typically causes economic damage by yield losses through a disease known as *Fusarium* Root Rot (FRR) (R. Smiley & Machado, 2013; Richard W. Smiley et al., 2004, 2005).

While climatic conditions and the limited extent of corn planted in the INW are believed to have reduced the occurrence of FHB and contributed to the high wheat productivity of the region, changes in climate and agricultural markets may alter this balance. The INW is projected to see substantial increases in temperature in addition to wetter winter and springs and drier summers over the 21st century (Abatzoglou et al., 2014; Marlier et al., 2017; Rupp et al., 2013, 2017). It remains unclear how the changing climate of the INW will

influence populations of *F. culmorum* present in agricultural soil, which represents an inoculum source capable of causing FHB. Warmer winters with less freeze-thaw cycles may allow for increases in *F. culmorum* populations and cause increased infection pressure during the following growing season (Lukas et al., 2014). Conversely, the warmer and drier summers may limit populations of *F. culmorum* in soil as they are outcompeted by *F. pseudograminearum* in regions of the INW with lower annual soil moisture (Poole et al., 2013).

Soil moisture in the INW fluctuates seasonally with soil recharge occurring during the months of winter and spring and evaporation dominating in the summer and fall. Soil moisture can be modelled using moisture indices derived from climate data, such as the difference between prior precipitation minus prior potential evapotranspiration (Dymond et al., 2014; Hogg, 1997).However, soil moisture within a field is also influenced by cropping practices as fields under a no-till regime keep soils moister during the winter months compared to conventional tillage (Fuentes et al., 2003). Different rates of nitrogen application can also influence the resulting soil moisture(Papendick & Cochran, 1971).

The ratio of fungi to bacteria in soils exhibits a seasonality with a fungal maximum in the spring and minimum in the fall (Lipson et al., 2002; Schutter et al., 2001). These seasonal changes are also related to soil moisture (Bardgett et al., 1999; Bell et al., 2009). We hypothesized that seasonal fluctuations in *F. culmorum* population densities were related to soil moisture during sampling. Growth of *F. culmorum* in Palouse silt loam decreased linearly as the water potential decreased from -1.5 to -73 bar, with growth reduced by half at -40 bar (R.J. Cook et al., 1972). Bacterial activity in soil decreases as the soil dries and ceases at -10 to -15 bar (R.J. Cook & Papendick, 1971) which also coincides with the highest water potential at which germlings of *F. culmorum* can grow in natural soil without lysing (R. J. Cook & Papendick, 1970).Production of macroconidia and germination of chlamydospores by *F. culmorum* also decreases with lower water potentials (Sung & Cook, 1981). Survival of *F. culmorum* chlamydospores is reduced as soil temperature increases and soil water content decreases (Sitton & Cook, 1981b). The change in soil water potential also places *F. culmorum* at a greater or lesser relative competitive advantage compared to other unmeasured microorganisms in the soil (R J Cook & Papendick, 1972). Soil water potential

is related to soil moisture through the water retention curve, and influenced by various soil properties such as particle size (Assouline et al., 1998).

Modelling populations of soil fungi is difficult due to the high degree of spatial variability. Highly variable counts from a grid of contiguous quadrats can be arranged into a frequency table and used with a maximum likelihood procedure to estimate the parameters of a given probability distribution (Campbell & Noe, 2003). A negative binomial distribution has typically been used to indicate nonrandom spatial patterns in inoculum densities of plant pathogens (Jeger, 1983; Martin et al., 1983; Stanghellini et al., 1982; Taylor et al., 1981; Smith et al., 1985). These approaches only quantify the degree of spatial variability and the results were not used as a response variable in modelling equations. A multilevel model for *F. culmorum* isolation frequency was developed to account for over-dispersed counts and annual climate variables were used as predictors, using data collected from both the INW and eastern Australia (Robinson et al., 2019). However, the residual variability in the counts resulted in highly variable predictions which could not be of use to growers. New modelling efforts are needed, and additional information is required to supplement fungal count data.

In this study we sought to quantify the relationship between the levels of *F. culmorum* inoculum present in agricultural fields across the INW with the climate conditions during sampling. We supplemented count data with the soil dilution factor used during isolation and utilized a weather-based proxy for soil moisture which could also be derived from the output of global climate models. We utilized a multilevel modelling approach with varying effects to account for unknown confounds across sampled fields. This manuscript represents a case study for quantifying a highly variable fungal soil population, utilizing climate information to model seasonal fluctuations, and attempting to quantify projected exposure of a FHB inoculum source in the INW under future climatic conditions.

Materials and Methods

Data sources and description

Population density estimates of *F. culmorum* were derived using frequency of isolation records from a two year soil survey across 9 fields within the INW (Robinson et al., 2019). Previous work demonstrated the utility of a multilevel modelling approach using the recorded counts of *F. culmorum* out of total soil *Fusaria*. However, even after modelling the

highly variable isolation frequency with a binomial likelihood and varying intercepts for all experimental clusters in the dataset there was still too much residual variation to provide adequate predictions for growers. As count data alone was found to be insufficient, we sought to supplement the count data with the soil dilution factor used during isolation. Estimates of isolation frequency become estimates of population density for a given field.

The soil survey was performed across 9 fields within a 80 km radius of Moscow, ID which reside along a precipitation gradient within the INW (Douglas et al., 1992). Exact locations have been withheld to preserve grower anonymity as requested, but we provide the location of nearby towns in the text as reference for clarity. Soil samples were collected every 3 months from June 11th 2016 to March 17th 2018. The sampling region within each field consisted of 9 quadrats 1 acre (4047 m^2) in size arranged in a 3 × 3 grid pattern that remained constant during the entire soil survey. The position of all 16 vertices of the sampling grid were calculated using an offset of 63.62 m and converting the distance to decimal degrees for each field. During each sampling iteration, 6 subsamples were taken from each quadrat of a field using a systematic unaligned grid methodology (Cihacek et al., 1990). A subsample was defined as a soil core 10 cm in depth taken using a 22 mm soil probe and pooled into a labelled plastic bag for transport.

Once in the laboratory soil samples were dried in a biosafety cabinet in weighing dishes for 48 hr prior to processing. Soil samples were ground with a pestle and mortar to pass a 2 mm sieve and mixed thoroughly prior to dilution. After mixing 10 g of soil in 100 mL of sterile 0.05% water agar for 30 min, further two-fold dilutions were performed yielding 1 g soil per 100, 200, 400, and 800 mL water agar, respectively. Soil dilution plating was performed in duplicate for all sampled quadrats by dispensing a 1 mL aliquot onto Nash-Snyder media (Nash & Snyder, 1962). After incubation only the plate with 10 to 25 colonies was selected for subculturing as 10 colonies was determined the minimum necessary to ensure adequate species diversity and plates with above 25 colonies were typically too overcrowded (Sangalang et al., 1995). The identification procedure of *F. culmorum* was carried out as previously described (Robinson et al., 2019).

Counts of *F. culmorum* colonies were multiplied by the dilution factor of the plate used for sub-culturing, to obtain an estimate of *F. culmorum* propagules per gram soil (PPG).

The reported values for a given quadrat represent the mean of two replicates. Soil moisture was measured prior to soil dilution plating, and the mean PPG values for a given quadrat were adjusted by multiplying by the percent solids. PPG values are considered as continuous responses in this study, and do not reflect standardized counts across fields and seasons based on the soil moisture of the sample being diluted during plating.

Historical daily weather data for each field was estimated from corresponding pixels within the 4 km gridMET spatial dataset of the contiguous United States (Abatzoglou, 2013). Pixels corresponding to specific samples were determined using the GPS coordinates of the central quadrat within each field's sampling region. The weather variables of interest were daily precipitation and grass-reference evapotranspiration during the time frame of January 1st 1979 to March 31st 2018. Information on daily soil moisture was obtained in a similar manner from corresponding pixels of the NOAA NWS National Centers for Environmental Prediction 19.2 km spatial reanalysis dataset (Saha et al., 2010). Daily values for percent soil moisture in the upper 5 cm were recorded for the period of January 1st 2010 to March 31st 2018. Field-specific values for both datasets were downloaded as a time series using Climate Engine (https://clim-engine.appspot.com/).

For climate assessment, daily climate data was obtained from 20 global climate models (GCMs) which participated in the fifth phase of the Climate Model Intercomparison Project (CMIP5). This data encompassed both historical (1950 – 2005) information, as well as future model based predictions (2006 – 2099). The outputs from the GCMs were rescaled to a 6-km spatial resolution using the Multivariate Adaptive Constructed Analogs (MACA) approach (Abatzoglou & Brown, 2012) and a training dataset provided by Livneh et al. (Livneh et al., 2015). This was necessary to ensure compatibility between the historical GCM experiments (1950 – 2005) and the observed record data (1950 – 2011). Additionally, this allows for data at an appropriate scale that is relevant for growers, while also preserving the time scales of meteorological patterns simulated by the GCMs. The weather variables supplied in the GCM output that were used for our modeling included: maximum and minimum near-surface air temperature, mean near-surface specific humidity, total precipitation, surface downwelling shortwave radiation, and mean near-surface wind speed.

All variables except precipitation were used to calculate daily grass-reference potential evapotranspiration according to the Penman-Montlieth equation (Walter et al., 2001).

We calculated an atmospheric water balance (AWB) as a standardized 90-day moving sum of precipitation minus reference evapotranspiration following the general concepts of the standardized precipitation evaporation index (SPEI) (Vicente-Serrano et al., 2010). However, while SPEI standardizes data for the calendar date, we standardize water balance data over the entire period of record. The AWB was devised as a simple weather-based proxy for relative soil moisture throughout the year that could also be derived from available GCM outputs. We used the prior 90 days as it was the approximate length of time between sample collections during the soil survey. Shorter durations can also be used to capture the flashier nature of surface soil moisture after recent rainfall. We present only the prior 90-day AWB as the posterior distribution of effects remained similar across prior durations ranging from 10 to 90 days (data not shown).

Reference model

We assigned an exponential likelihood distribution to *F. culmorum* PPG values with a rate parameter λ. A reference multilevel model was constructed by assigning a varying intercept for each unique field (N=9) and sampling iteration within the dataset. We define “sampling iteration” as every unique sampling trip for 9 fields across 8 seasons (N=72) and letting each vary independently. A log link function was used to map the varying intercepts equation onto positive real numbers. For every unique field *i* and sampling iteration *j* in the dataset the link equation becomes (Eq. [1]):

$$\log(\lambda_i) = \alpha + \alpha_i + \alpha_j$$

The prior for the vector of intercept parameters α_i and α_j was a normal distribution with mean 0 and standard deviation σ_i and σ_j, respectively. The prior for α was a normal distribution with mean 0 and standard deviation 1, while the prior for σ_i and σ_j was an exponential distribution with a rate parameter of 1.

Climate model

The multilevel model with varying effects is design such that the intercepts and slopes are expressed as means and offsets. For each case *i* , the overall intercept and slope (α

and β) are estimated with their respective offsets α_field and β_field, due to individual fields. The updated link equation becomes (Eq. [2]):

$$\log(\lambda_i) = A_i + B_i(AWB_i) \quad \text{where}$$

$$A_i = \alpha + \alpha_field_i \text{, and}$$

$$B_i = \beta + \beta_field_i$$

This expands to: $\alpha + \alpha_field_i + \beta * AWB_i + \beta_field_i * AWB_i$ The informative prior for α was a normal distribution with mean -2.87 and standard deviation 0.99 which is based on the posterior distribution of α from the reference model in Eq. [1]. The prior distribution for β was a normal distribution with mean 0 and standard deviation 1. The population of varying effects α_field and β_field was assigned a joint Gaussian prior with mean 0, a correlation matrix Rho, and standard deviation σ_field. We used the Lewandowski Kurowicka and Joe (LKJ) distribution as a prior for the correlation matrix (Lewandowski et al., 2009). The prior for the correlation matrix Rho was a LKJ distribution with η of 7 which is strongly regularizing to minimize divergent iterations during sampling. The LKJ prior was chosen so that priors could be set for both the correlations and the standard deviations separately unlike the inverse-Wishart which cannot separate the prior correlation from standard deviation parameters (Ignacio et al., 2014). The prior for σ_field was an exponential distribution with a rate parameter of 1.

While the same approach utilized in the varying effects multilevel models could be extended to the slope, more information can be pooled together by exploiting the correlation structure between the population of intercepts and slopes (McElreath, 2016b).

For each field's location we acquired downscaled daily weather data from 20 GCMs to calculate daily precipitation and potential grass reference evapotranspiration. For each model AWB was calculated as described above and standardized using the same grand mean and standard deviation. We then calculated the average AWB for a given month of interest for each model across all the years in the historical simulation (1950 – 2005) or in the forcing experiments (2006 – 2099) for all 20 GCMs.

Parameter estimation

We used a Bayesian approach to estimate parameters within multilevel models by combining prior information with an exponential likelihood to generate a posterior

distribution for unknown quantities. We simulated samples from the posterior using a derivative of Markov chain Monte Carlo (MCMC) called Hamiltonian Monte Carlo (HMC), a conceptual introduction for which can be found in Betancourt (Betancourt, 2017). Model equations were specified using the rethinking package in R (McElreath, 2016b) which convert specifications into a format which can be utilized by the probabilistic programming language Stan (Carpenter et al., 2017). We ran four chains using the No-U-Turn Sampler for 3500 iterations each after a warm-up of 1000 iterations (Hoffman & Gelman, 2014). Convergence was assessed both visually by plotting the resulting 4 chains for each parameter and by verifying that the Gelman-Rubin convergence statistic $\hat{R}$ was less than 1.1 (Gelman & Rubin, 1992). All data and code required to reproduce the analysis are publicly available on GitHub (https://github.com/nosnibor27/soil_fusarium_model).

Results and Discussion

Mapping

We began our analyses by plotting the enumerated *F. culmorum* population densities for all sampled quadrats within the soil survey dataset as a heat map (Fig 5.1). There was a high degree of variability in the population density of *F. culmorum* within a given field, and often an acre quadrat would be in excess of 1000 PPG while an adjacent quadrat would contain undectable quantities *F. culmorum*. This pattern has been documented in the region previously, with *F. culmorum* being detected in a wheat field near Pullman, WA at 2000 PPG halfway up the slope but undetectable at the hilltop (Cook, 1980). The maximum population density of *F. culmorum* observed was approximately 6700 PPG, from Field 1 in Troy, ID during March 2018.

The first reports of *F. culmorum* population densities in the INW ranged from undetected to a maximal of 3208 PPG during a soil survey of Oregon, Washington, and Idaho from 1965 to 1967 (Cook, 1968). The enumerated values were averages of a single composite sample from 10 locations across an agricultural field. Other recorded population densities for *F. culmorum* in soil include around 3000 PPG near Ritzville, WA in 1965 and 1,600 PPG near Colfax, WA in 1966 (Cook & Bruehl, 1968). Population densities of up to 10,000 PPG have been observed (Cook, 1980). It is unknown what the theoretical maximum population density is for *F. culmorum*, but previous soil burial experiments in the INW used

8,000,000 PPG as a starting inoculum (Inglis & Cook, 1986a). Population densities of *F. culmorum* at 100, 1,000, and 10,000 PPG in non-sterile soil were utilized to demonstrate proportionality with the incidence of FRR in wheat (Cook, 1968). A population density of 3000 PPG translates to approximately 3 to 5 propagules per mm^3 of soil and it was suggested that a wheat plant growing in such soil could not avoid actual contact with several propagules during growth (Cook, 1968).

Model performance

To evaluate the compatibility of our model assumptions with the observed population densities of *F. culmorum* we sampled 100 rate parameters from the posterior distribution of both the reference model and the climate model. The quantile function for the exponential distribution was applied to the set of rate parameters and compared to the ordered population densities for the corresponding 9 quadrats by plotting exceedance curves (Fig 5.2). The posterior distribution of predicted frequencies of exceedance show good agreement with the observed measurements. Ordered data from sampling iterations which do not show as defined of an exponential pattern also have more uncertain distributions of rate parameters and a wider spread of possible exceedance curves. Model performance was also visualized by plotting the measured PPG values against the range of predicted values at the same frequency of exceedance (Fig 5.3).

We recognize that using an exponential distribution may produce biased estimates for higher population densities of *F. culmorum* which can be seen in the plotted curves at less than 25% frequency of exceedance. The observed population densities for a given quadrat represent the average of 6 pooled samples giving the possibility for higher population densities at smaller length scales. We recognize these limitations and retain the uncertainty in estimated rate parameters forward when plotting exceedance curves to provide a more probabilistic framework for interpreting our results. The predicted ranges of *F. culmorum* population densities remain within the bounds expected from the literature (Cook, 1980).

The climate model shows good agreement with the observed record, but the multilevel approach does not match the data exactly as information has been pooled through the population parameters α and β in the multilevel model. This effect is called shrinkage and can be seen in cases such as Field 6 in December 2016. The multilevel pools information

across all sampled fields when computing parameter estimates and is more skeptical of large departures from the average population density in Field 6 given other observations from the same field across time. The multidimensional shrinkage through information pooling adaptively regularizes the parameter estimates to minimize overfitting (McElreath, 2016b). This criterion is important as an out-of-sample prediction is required when forecasting *F. culmorum* population densities under climate change.

The highly variable population density of *F. culmorum* can now be summarized and communicated to growers as a frequency of exceedance. This methodology is similar to reporting 100-year floods using long term stream-flow records (USGS, 2014). The estimated rate parameters are scale invariant and frequencies of exceedance can be applied to any sized field, given the assumption that the rate parameter for given field is constant across space. This assumption assumes that variability in PPG values will remain constant regardless of quadrat size, and highly variable counts of *F. culmorum* have been observed in sampling quadrats as small as 10 cm^2 (Rodríguez-Molina et al., 2000). The proportion of a field which exceeds a given threshold PPG value can also be reported and used to provide more informed managerial decisions. There is little information on what a threshold for the population density of *F. culmorum* could be, and high population densities of up to 2000 PPG may not ensure disease as plant water stress also plays an important role in the development of root rot symptoms (Cook, 1980; Papendick & Cook, 1974). We present the exceedance curve in its entirety and recognize that this approach can be adapted in the future as more information becomes available.

Quantification of differences in population density by field

We estimated field-level differences in the population density of *F. culmorum* by incorporating a unique intercept parameter into the multilevel model equation. The distribution of median values of *F. culmorum* PPG for all 9 sampled fields is visualized by plotting 100 samples from the posterior distribution of the multilevel model (Fig. 5.4A). Fields with the highest predicted median value of *F. culmorum* PPG were 1, 3, 4, and 5. Overall differences in posterior medians were related to total annual precipitation (Fig. 5.4B) and total annual potential evapotranspiration (Fig. 5.4C). In general, fields with greater annual precipitation and lower annual potential evapotranspiration had higher distributions of

median values for *F. culmorum* population densities. The exception was Field 2, which typically received both the highest annual rainfall and lowest annual potential evapotranspiration of all sampled fields.

F. culmorum is considered to be cosmopolitan in soil (P. E. Nelson et al., 1983; Samson et al., 2010) but most commonly found in temperate regions (Leslie & Summerell, 2007). *F. culmorum* predominates in the cooler regions of north, central, and western Europe (Wagacha & Muthomi, 2007). Most of the prevalence data for *F. culmorum* comes from surveys of wheat segments, and the relative composition of *F. culmorum* out of total *Fusarium* spp can differ between soil, wheat, and residues within the same field which can confound comparisons based on differing inoculum sources (Landschoot et al., 2013). Incidence of *F. culmorum* in wheat roots and crowns differed across agroecological zones of Turkey but the distribution appeared random (Tunali et al., 2008).

Differences in the incidence of *F. culmorum* is more pronounced across geographic regions with a large gradient in annual precipitation and temperature. Eastern Australia represents such a region and extensive research has been done to better understand differences in the geographic distribution of *Fusarium* spp. An initial survey of soil samples across eastern Australia recovered *F. culmorum* from 33% of the sites in the temperate area south of 32°S, 20% of the sites in the subtropical area between latitudes 32°S and 28°S, and none were detected in the tropical one north of latitude 28°S (Burgess et al., 1988). Prevalence of *F. culmorum* was greater from regions in eastern Australia which have winter dominant rainfall and lower summer temperatures compared to tropical areas with summer dominant rainfall and hotter summer temperatures. A similar trend was observed when collecting wheat samples across the eastern Australian grain belt as *F. culmorum* isolation was greater in the high rainfall regions of Victoria compared to low rainfall regions, and the proportion of *F. culmorum* correlated best with the November rainfall for a given field (Backhouse et al., 2004). The range and mean of 16 different climate parameters was calculated for the distribution of *F. culmorum* in cereals across Australia using accession records from multiple lyophilized culture collections and the BIOCLIM version 2.0 program (Backhouse & Burgess, 2002). *F. culmorum* was found to be distributed over a wide range of annual rainfall but restricted to sites with mean summer temperatures less than 22°C.

The Columbia Basin within the INW encompasses a diverse range of local climates within a relatively small geographic area. The western edge of the Rocky Mountains forms a precipitation gradient which influences agronomic practices (Hall et al., 1999). The landscape across the INW varies from fields which receive enough annual precipitation to crop annually to fields which utilize a grain-fallow rotation so that wheat can be cultivated using two years of accumulated moisture (Douglas et al., 1992). *F.culmorum* is considered to be ubiquitous in soils across Washington and Oregon, with yearly variation in detection frequency according to differences in the prior year's annual precipitation (Smiley & Patterson, 1996). A comprehensive survey of wheat crowns across the INW combined factor analysis determined that the distribution of *F. culmorum* dominated under locations with cooler annual temperatures and higher elevations and the probability of isolation increased with increasing precipitation and snowfall (Poole et al., 2013). *F. culmorum* was also isolated more frequently from counties with higher annual precipitation in southern Idaho (Carl A. Strausbaugh et al., 2004), Montana (Moya-Elizondo et al., 2011), and Minnesota (Wilcoxson et al., 1988).

The prior survey of the INW also found a decreasing probability of *F. culmorum* isolation frequency as cropping intensity increased under environments with high annual precipitation (Poole et al., 2013). Field 2, near Moscow, ID, had the highest annual precipitation of all sites sampled, yet the posterior median of *F. culmorum* PPG was lower than Field 1 near Troy, ID and Fields 3 and 4 near Pullman, WA (Fig. 4). Field 2 was the most intensely cropped field sampled and has been under a continuous winter wheat and spring pea rotation. Other nearby fields had more periods of fallow during the winter and spring during the sampling period. The increased cropping intensity at Field 2 may play a role in the decreased population density of *F. culmorum* compared to other nearby fields under similar levels of annual precipitation.

Climate model predictions

We utilized the total precipitation minus total potential evapotranspiration over 90 days prior to sampling as a seasonal predictor variable termed atmospheric water balance (AWB). Seasonal variations in AWB were closely related to seasonal variations in modelled daily soil moisture (Fig. 5.5). We utilized AWB as a predictor variable as it can be calculated

from the output of GCMs and also serves as an interpretable dimensionality reduction which uses all available climate variables supplied by GCMs. Multicollinearity between weather variables needs to be considered when deriving equations, as the resulting parameter estimates can lead to differing predictions when different variables are used in combination (Robinson et al., 2019).

To evaluate differences in seasonal predictions across fields we performed a posterior predictive check using the distributions of α_i and β_i for every field along with the population parameters α and β. The distribution of expected values of *F. culmorum* PPG for all 9 sampled fields conditional on seasonal variation in AWB is visualized by plotting 100 samples from the posterior distribution of the multilevel model (Fig. 5.6). Fields 3,4, and 6 displayed the greatest range of predicted seasonal changes in the median value of *F. culmorum* PPG. Fields 1, 2, 5, 8, and 9 were not predicted to fluctuate substantially across seasons. Posterior predictions for Field 7 displayed an opposite trend as the other fields, which is due to a sampling quadrat yielding 3900 and 2279 PPG during June of 2016 and 2017, respectively.

Values of AWB derived from downscaled forcing experiments for each field was used to forecast changes in seasonal fluctuations of *F. culmorum* PPG values under climate change (Fig. 5.7). The median PPG value is presented for each field to center the results across fields. Typically, a greater proportion of a field is above the posterior median value for the winter and spring and below for the fall and summer. The exception is Field 7 outside of Hay, WA which had maximal values in the summer and fall. Under climate change the proportion of *F. culmorum* within a field greater than its posterior median is forecasted to be lower in the summer and fall, with little difference across forcing experiments. Fields 3, 4, and 6 which had the greatest amplitude of seasonal fluctuation would be most affected. Fields with a relatively stable population density of *F. culmorum* are forecasted to remain as similar levels under climate change.

F. culmorum has been commonly isolated from scabby wheat heads in the Netherlands from the 1980s up to the early 1990s (Daamen et al., 1991; De Nijs et al., 1996, 1997), but has been isolated less frequently since the early 2000s (van der Fels-Klerx et al., 2012; Waalwijk, Kastelein, De Vries, et al., 2003; X. M. Xu et al., 2005). This decreasing

trend in isolation from wheat heads has also been observed in Poland (Stępień et al., 2008; Wakulinski & Chelkowski, 1993), and Norway (Bernhoft et al., 2012; Kosiak et al., 2003; Langseth et al., 1998). However, isolation of *F. culmorum* has remained relatively constant in Denmark (Bottalico & Perrone, 2002; Nielsen et al., 2011; Thrane, 2000), and Belgium (K. Audenaert et al., 2009; Landschoot et al., 2011). It is important to consider changes in this plant pathogen over time, as regions of Europe my become more favorable to FHB in the future under climate change (Madgwick et al., 2011; Parikka et al., 2012; West et al., 2012).

In the INW, *F. culmorum* population densities in agricultural soil are forecasted to remain relatively constant during the winter and spring but decrease during the summer and fall. No increase in *F. culmorum* population densities was predicted for the winter and summer months based on forecasted shifts in AWB under climate change. Interannual variability does influence the prevalence of *F. culmorum* in the INW (Poole et al., 2013; Smiley & Patterson, 1996), yet chlamydospores of *F. culmorum* can survive in agricultural soil for a decade or more, suggesting the ability to potentially persist in the region under climate change (Inglis & Cook, 1986a; Sitton & Cook, 1981b; Windels et al., 1993). *F. culmorum* is likely under balancing selection between a saprophytic phase and a parasitic phase (Miedaner et al., 2001). It remains unclear what role this balance my play in the future if *F. culmorum* becomes displaced from plant tissues by competing microorganisms, similar to what has been observed in Europe. In order to persist, *F. culmorum* populations will need to either migrate to more favorable environments or evolve to changing climate conditions (Chakraborty, 2013). *F. culmorum* was isolated most frequently from regions in the INW classified as annual cropping or annual crop-fallow-transition (Poole et al., 2013; Robinson et al., 2019). However, a 46% decrease in area for those agro-ecological classes is forecasted for the INW (Kaur et al., 2017). The extent of *F. culmorum* may become more restricted in the INW under climate change. More research is needed into forecasting weather conditions during anthesis under climate change.

Additional agronomic factors are likely to influence the magnitude of seasonal fluctuations of *F. culmorum* in the INW. A better understanding of how crop management can dampen inoculum sources of plant pathogens is needed. Agronomic practices can potentially reduce the population density of *F. culmorum* below what would be expected

given the annual precipitation, such as Field 2, or even exhibit a reversal in trend, such as Field 7. Growers can potentially adapt to climate change by adjusting their farming practices to limit the amount of inoculum present capable of initiating plant diseases.

Conclusions

In this study we modelled the highly variable population density of the fungal plant pathogen *F. culmorum* in agricultural soils by assigning an exponential likelihood distribution to observed population density measurements collected over a two year soil survey across the INW. Rate parameters for were first estimated using a multilevel model with varying intercepts for each unique field and sampling iteration in the dataset. The posterior distributions of rate parameters were reflective of the uncertainty in the observed measurements.

Taking inspiration from frequency analysis in hydrology, the estimated rate parameters can be utilized in calculating exceedance curves for the population density of *F. culmorum* within an agricultural field. Reporting population densities as a frequency of exceedance provides a more interpretable means of communicating uncertainty to growers. The proportion of a field which is in excess of a given threshold population density can be utilized when making management decisions. The proportion estimates can be applied to any sized field due to the memorylessness of the exponential distribution. We provide a case study that can be adapted for use with other plant pathogens which are highly aggregated in agricultural soil.

Differences in population densities of *F. culmorum* were greater across fields than across sampling iterations. Fields with higher population densities also had greater uncertainty in their posterior median. Fields with higher population densities tended to be from locations with greater annual rainfall and less annual potential evapotranspiration.

We constructed a weather-based proxy for daily soil moisture using a moving sum of total precipitation minus total potential evapotranspiration 90 days prior to sampling (AWB). This moving sum is reflective of seasonal variations in soil moisture content and was used as a predictor variable in a multilevel model. Varying intercept and slope parameters for each field was used to investigate differences in seasonal fluctuations of *F. culmorum* population densities. Some fields exhibited strong seasonal fluctuations while others remained relatively

constant at the average level estimated by the unique intercept. Differences in the effect of AWB across fields is indicative of additional agronomic factors influencing seasonal fluctuations of *F. culmorum*..

We demonstrated the ability to use downscaled daily climate data from global climate models as model inputs to forecast changes in population density under climate change. Forecasts are specific to each field based on their derived parameters in the multilevel model with varying intercepts and effects. The forecasts suggest that population densities of *F. culmorum* would both remain constant during the winter and spring and decrease during the summer and fall relative to the historical baseline. The varying effects model only accounts for seasonal variation using a weather proxy for soil moisture and soil survey data collected over 2 years.

More studies are needed to better elucidate how populations of fungal plant pathogens may shift under a changing climate in addition to elucidation of how agronomic practices can influence the direction and magnitude of their seasonal fluctuations. The climate model provided herein may provide scaffolding for future modelling efforts to incorporate additional agronomic information using additional parameters.

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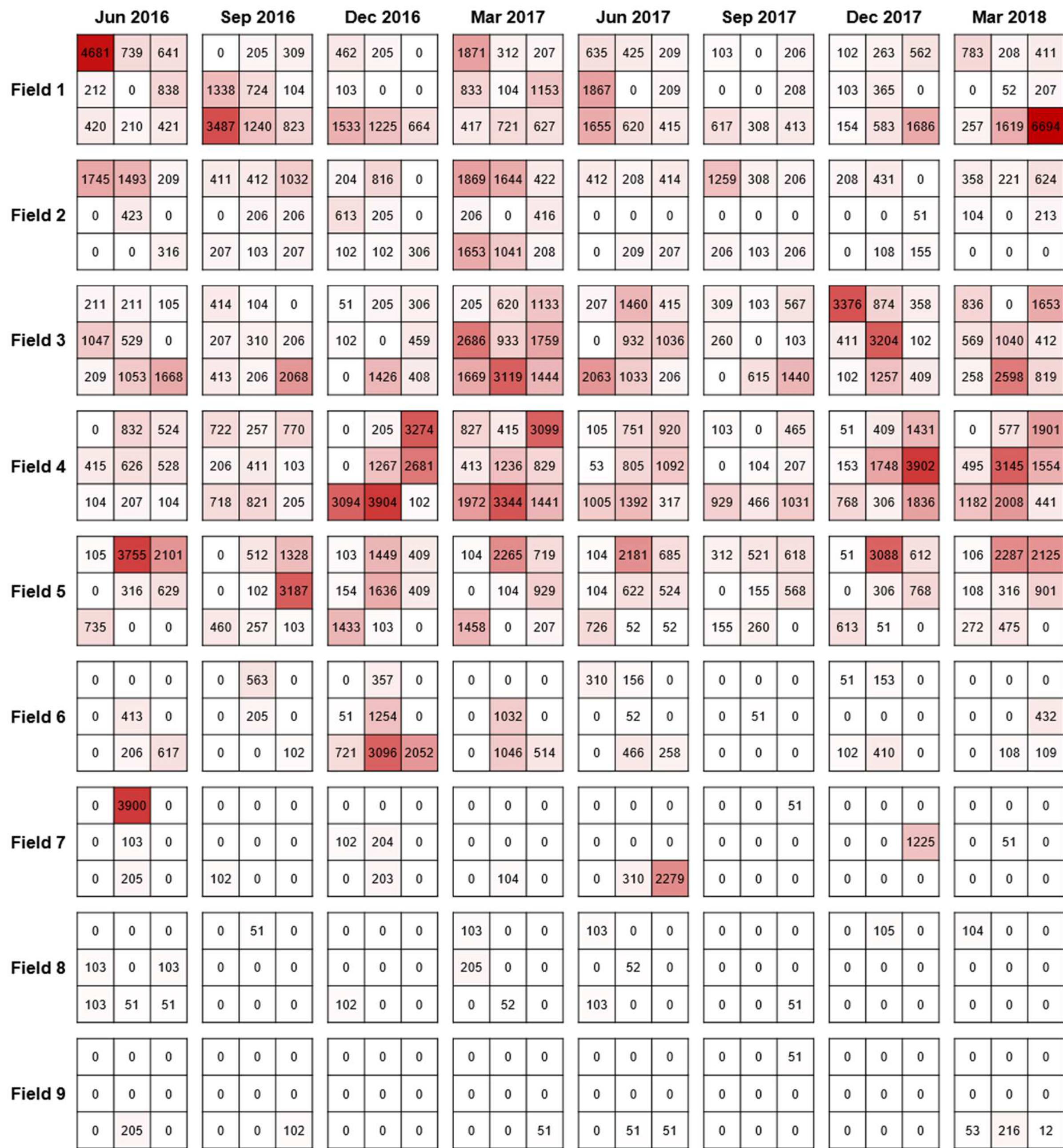

Figure 5.1 Heat maps of a 2 year agricultural soil survey across the Inland Pacific Northwest. Each subplot represents a 3 × 3 sampling region consisting of 9 quadrats 1 acre (4047 m2) in size. Rows of subplots represent different fields (N=9), columns represent different sampling iterations (N=8). Values within each quadrat are *F. culmorum* propagules per gram soil (PPG). The specific values do not represent the precision of the soil dilution procedure, but are the result of standardizing counts by the moisture content of the soil samples. The sampling region within a field, along with quadrat positions, remained constant across all sampling iterations.

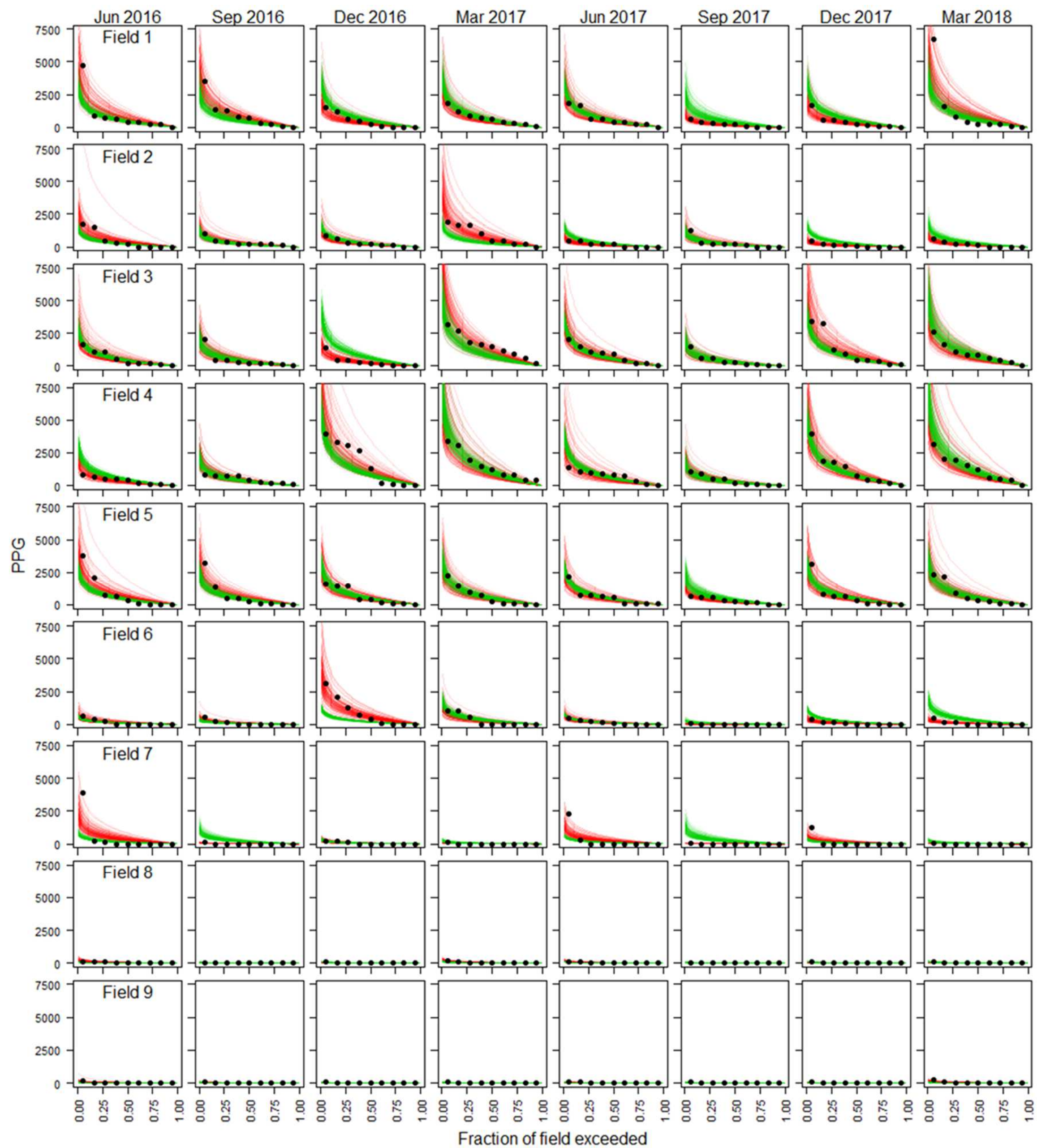

Figure 5.2 Exceedance curves of *F. culmorum* propagules per gram soil (PPG) in agricultural soil across the Inland Pacific Northwest over a 2 year soil survey. Rows of subplots represent different fields (N=9), columns represent different sampling iterations (N=8). Black points indicate observed values. Uncertainty in modelled rate parameters is visualized by plotting 100 samples from the posterior distribution as frequency of exceedance curves for both the reference model (red) and climate model (green).

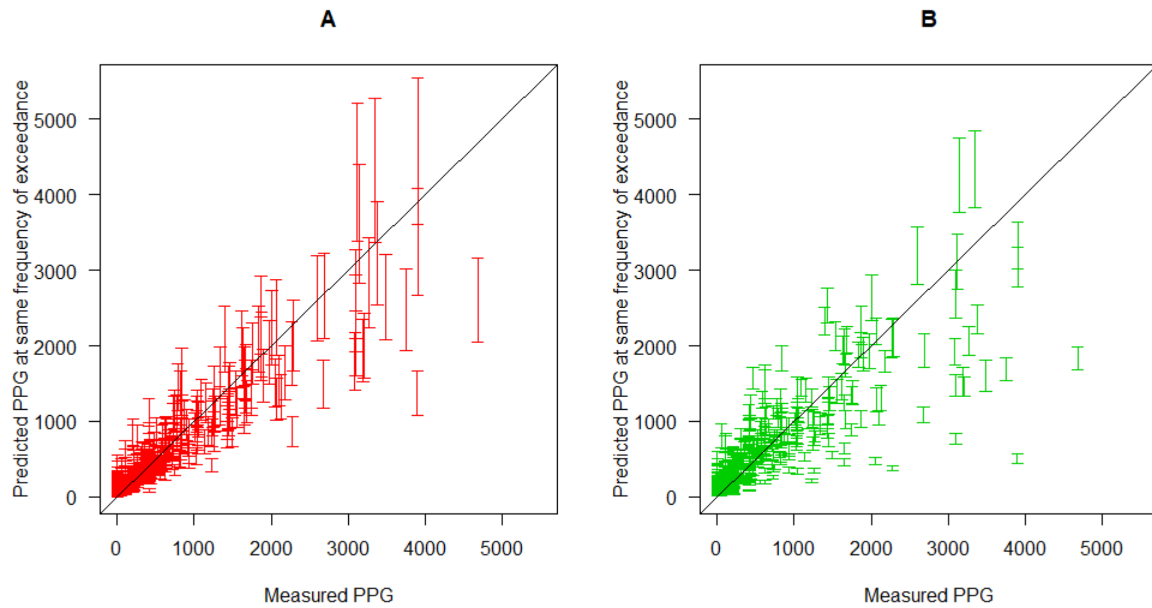

Figure 5.3 Measured versus predicted *F. culmorum* propagules per gram soil (PPG) in agricultural soil. Measured values of F. culmorum PPG plotted against the predicted range of PPG at the same frequency of exceedance as observed for both (A) the reference model and (B) the climate model. Error bars denote the inter-quantile range of the posterior distribution.

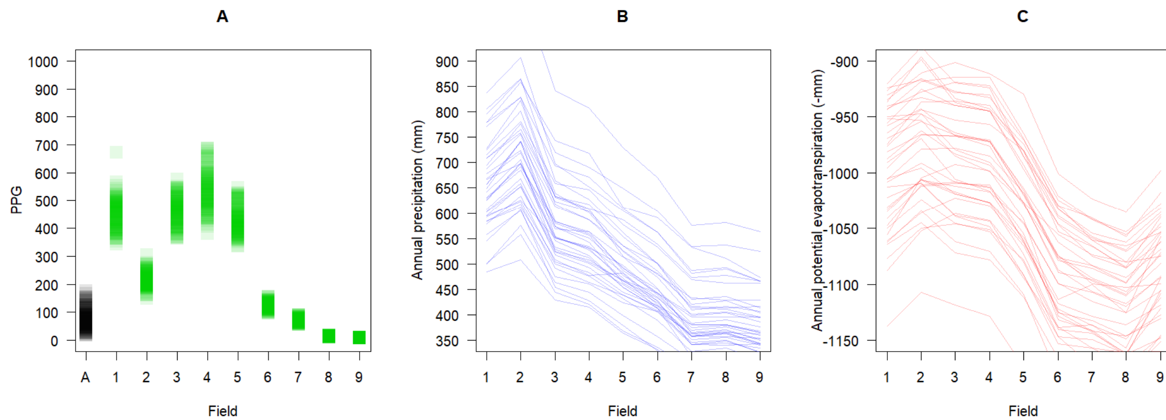

Figure 5.4 Posterior distributions of the median value of *F. culmorum* propagules per gram soil (PPG) from 9 fields across the Inland Pacific Northwest and their relationship with annual climate variables. (A) The median value of the population average (A) is in black. Uncertainty is visualized by plotting 100 random samples from the posterior distribution. The resulting distribution is corresponding to the uncertainty in estimating rate parameters and is not representative of the total variability of PPG values that would be observed within a field. (B) Annual precipitation (mm) for all sampled fields, from 1979 to 2017. Each line represents total annual precipitation across fields for a given year (N=39). (B) Annual potential evapotranspiration (-mm) for all sampled fields, from 1979 to 2017. Each line represents total annual precipitation across fields for a given year (N=39).

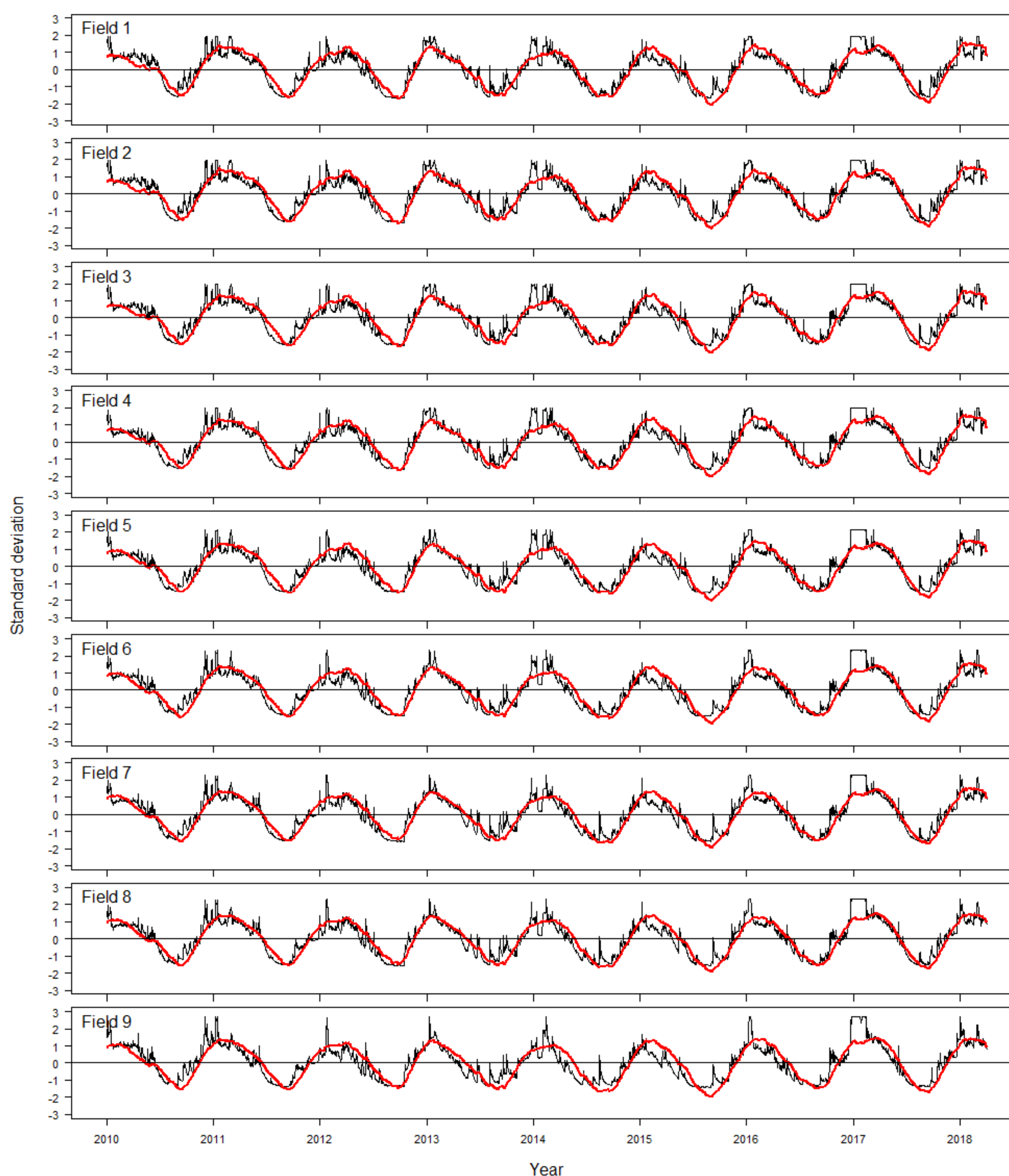

Figure 5.5 Daily modelled surface (5 cm) soil moisture (black) and daily atmospheric water balance (red) from January 21st 2010 to March 31st 2018 for all sampled fields (N=9). The atmospheric water balance (AWB) represents the prior 90 day total precipitation minus prior 90 day grass-reference potential evapotranspiration. Both soil moisture and AWB were standardized to mean 0 and standard deviation 1 prior to plotting to have complimentary scales. The modelled daily soil moisture came from the NCEP Climate Forecast System Reanalysis dataset.

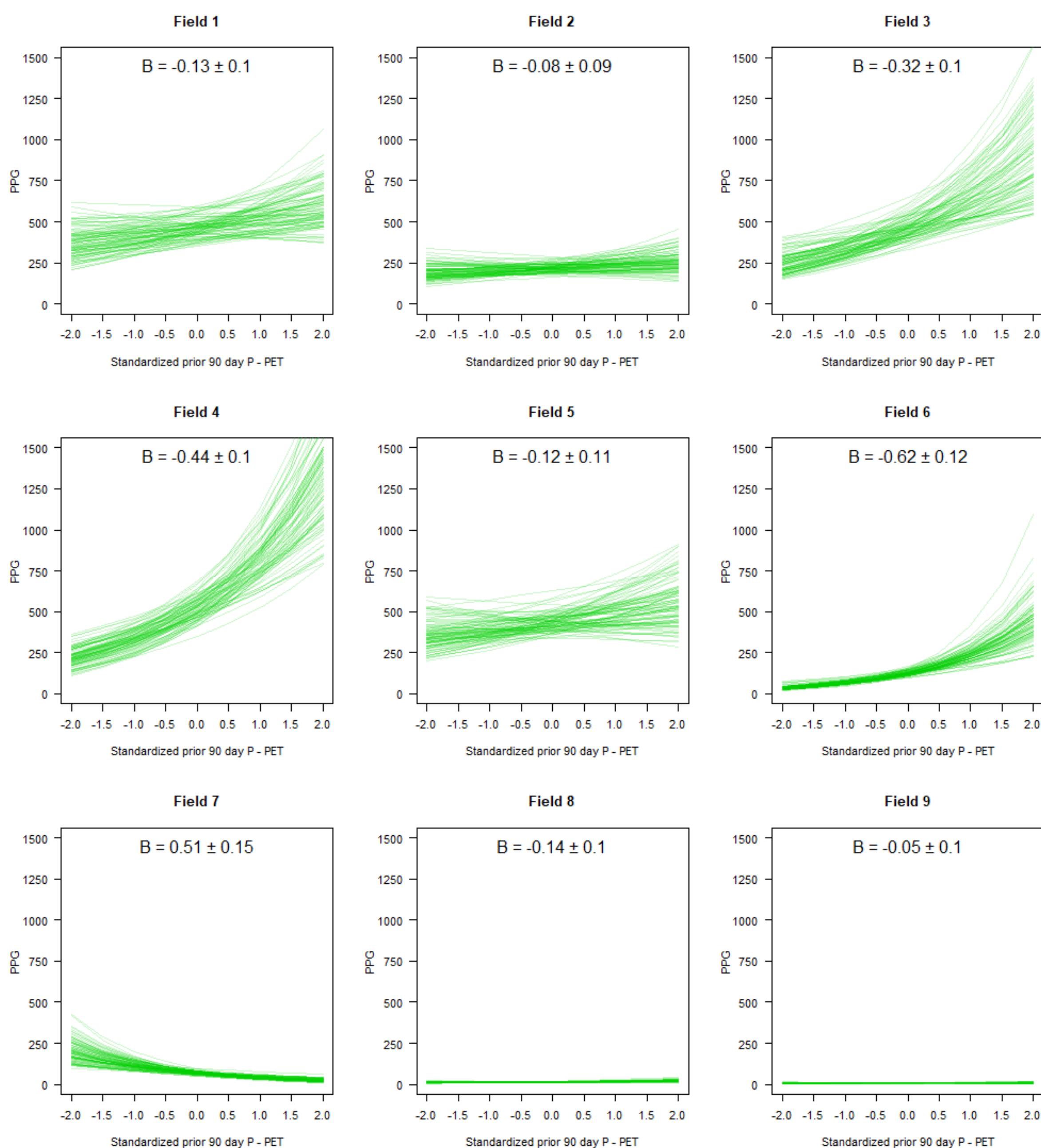

Figure 5.6 Predicted seasonal changes in the expected value of *F. culmorum* propagules per gram soil (PPG) based on different fields across the Inland Pacific Northwest (N=9). The model input parameter was the 90 day total precipitation (P) minus prior 90 day grass-reference potential evapotranspiration (PET) prior to sampling date. Values were standardized to mean 0 and standard deviation 1 prior to initializing the multilevel model and standardized values are shown on the top axis of each subplot. Green lines represent predictions for the expected value of *F. culmorum* and uncertainty is visualized by plotting 100 random samples from the posterior distribution of a multilevel model with varying intercepts and slopes for all fields. The mean and standard deviation of the posterior distribution for the effect of AWB (B) is shown for each field. The resulting distribution is corresponding to the uncertainty in estimating rate parameters and is not representative of the total variability of PPG values that would be observed within a field.

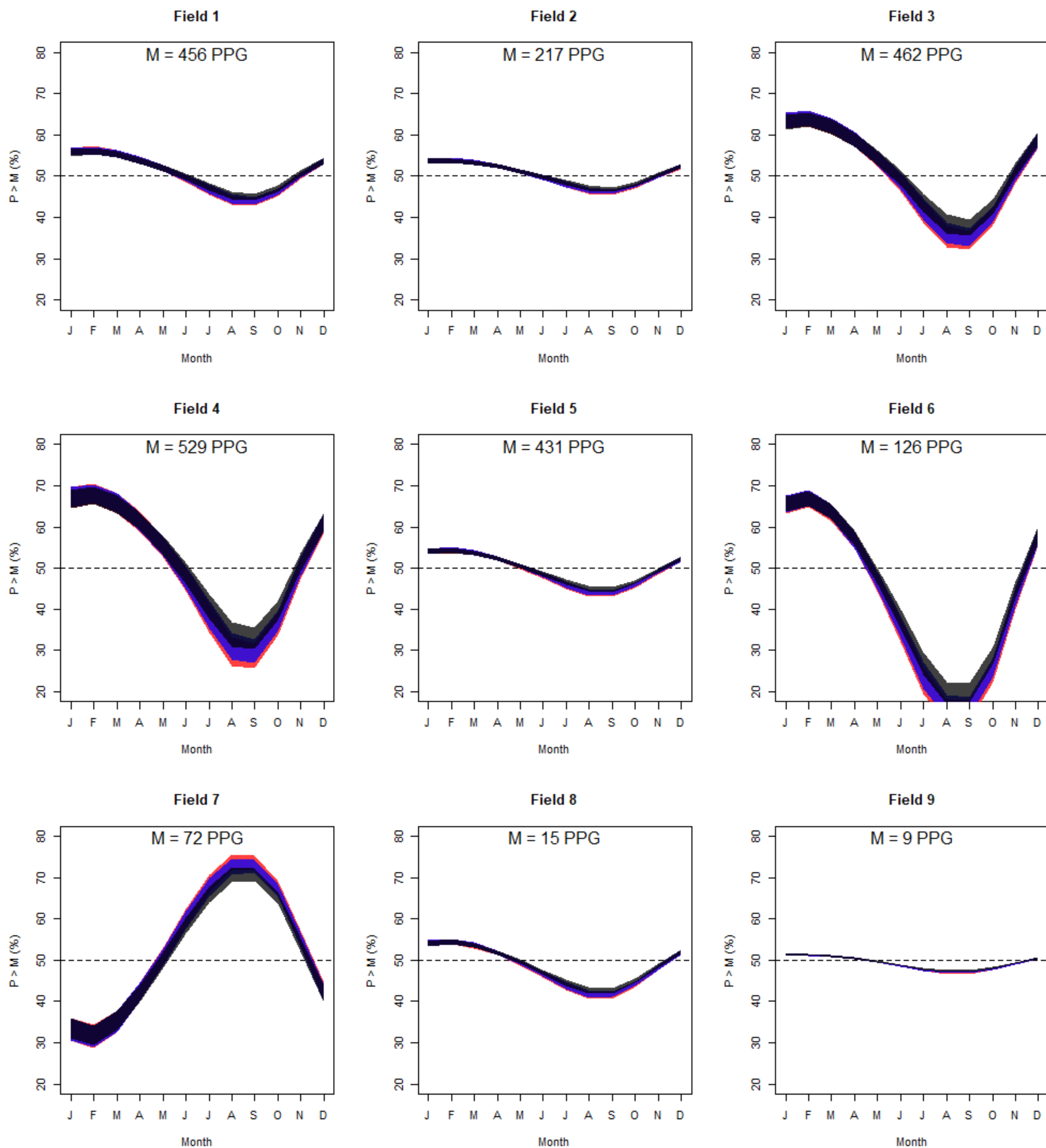

Figure 5.7 Forecasted changes in median *F. culmorum* soil populations across the Inland Pacific Northwest under climate change. The posterior median (M) value for *F. culmorum* propagules per gram soil (PPG) is shown for each sampled field (N=9). The proportion of a given field which is greater than M for all months is shown using either the historical baseline from 1950 – 2005 (black), RCP 4.5 from 2006-2099 (blue), and RCP 8.5 from 2006 – 2099 (red). The colored range is the inter-decile range of all years within the historical (N=54) and forcing (N=93) experiments. The resulting distribution is corresponding inter-annual variability and is not representative of the total variability of PPG values that would be observed within a field.

Chapter 6: Conclusions

The development of new methods to expand mycotoxin detection, compare bacterial transfer rates, and forecast fungal inoculum under climate change was described in further detail in this dissertation. In the first study the novel use of an insoluble phenol absorbent, polyvinylpolypyrrolidone (PVPP), was incorporated into the ochratoxin A (OTA) extraction procedure for a commercially available ELISA kit. Pistachio skins were used as a test material as they both contain high quantities of phenolic compounds and had been previously demonstrated to cross react with the antibodies in commercial ELISA kits for OTA detection in previous experiments. Increasing the amount of PVPP reduced the amount of gallic acid and catechin remaining in the pistachio skin extracts, and there was a concomitant decrease in the degree of interaction observed in the commercial ELISA kit. The utility of using PVPP was demonstrated as a proof-of-concept using pistachio skins and represents a case study for other researchers who may be attempting to selectively remove phenolic compounds because they might potentially interfere with their downstream analysis. In addition, this work demonstrated that PVPP can still bind phenolic compounds in 50% methanol. Previous literature stated that PVPP binding could only occur in aqueous environments. The PVPP binding might not be optimal, but the incorporation into the OTA extraction protocol still reduced cross-reactivity and did not interfere with the amount of OTA present.

The incorporation of PVPP to the existing protocol is simple and practical as it is the only modification to the OTA extraction protocol. The extraction solvents and shaking times remain the same, there is only the additional step of collecting the supernatant afterwards. PVPP was purchased as 110 micron sized particles which are insoluble in water. In our study the settling of PVPP particles into the bottom of the sample tube was accelerated by centrifugation at approximately 12,000 *g* for 10 minutes. Outside of a university setting, laboratory workers can simply leave the sample tubes on the bench and allow PVPP to settle by gravity in a similar timeframe. This simple addition to a commercial protocol increases the chances of these results being adopted in the future as no new specialized equipment is needed. Workers may need to follow a similar experimental design for their food matrix to determine how much PVPP is sufficient to reduce the resulting cross-reactivity.

The second study assessed bacterial transfer from hands to gloves and compared bacterial transfer rates to a tomato with different soap washing times and glove use, by inoculating participants hands with ~10^9 CFU *Enterobacter aerogenes* B199A, a test bacterium which had previously shown similar attachment characteristics as *Salmonella*. The main conclusion is that both hand washing and glove use is necessary to reduce bacterial cross contamination from hands to food. Glove use alone was not sufficient as bacteria were still able to transfer from a participant's hand to the glove surface and then to the tomato during dicing. Soap washing alone was also not optimal as adding gloves greatly reduced the amount of bacteria recovered from the tomato for participants who used similar soap washing times. While bacterial transfer is still quite variable, visualizing the results as cumulative distribution functions showed differences across soap rubbing times in a clear and interpretable manner. The differences were subtle since a high bacterial inoculum was used and the rate of transfer is limited. However, a high bacterial inoculum was necessary to get results which were consistent enough across a small sample size of volunteer participants and achieve statistical significance.

Future research would need to evaluate lower bacterial concentrations, incorporate a larger sample size of participants, and utilize enumeration methods which scale up and allow for higher throughput of more samples. Plating samples is quite time consuming and takes a considerable amount of training for the operator to produce plates with evenly dispersed bacterial colonies. One potential solution is to simple plate 100 µL drops of a bacterial suspension onto the agar plate. Up to a dozen separate drops can fit onto a standard 90 cm plate, and if plates are incubated at room temperature the growth can be more closely monitored and controlled. The procedure already disperses the colonies onto the plate without the need for spreading, and if growth is monitored early enough one can still resolve anywhere from 10 to 60 colonies. When attempting to study something which is inherently variable, it is vital to collect as much information through as many replicates as possible.

The third study demonstrated the utility of specifying a multilevel model to highly variable *F. culmorum* isolation frequency data in order to better understand potential sources of variability across experimental clusters. This approach is essentially a Bayesian equivalent to a classical ANOVA, and was used to show that isolation frequency of *F. culmorum* was

greater across fields as opposed to within fields or over time. This study also investigated the marginal offsets of different experimental clusters, essentially probing the posterior distribution for trends in different residuals. One notable finding was the isolation frequency of *F. culmorum* was marginally lower at the higher regions of a grower's field. The offsets for each field was related to annual climate and a pattern emerged where isolation frequency was greater from fields with higher annual precipitation and lower annual temperature.

Another important result is the importance of understanding the potential effect of multicollinearity. Originally model equations used only a single annual climate variable, such as total annual precipitation or average annual temperature, to both investigate the strength of a given relationship and construct a potentially predictive model. During review the researchers questioned why the climate variables were not combined into a singular model equation, not understanding the potential effects of multicollinearity. As scripting additional models allowed for an automated response and figure generation, the manuscript now represents a case study for various pathologies associated with incorporating correlated variables into the same multiple regression equation. Essentially, when preforming a multiple regression, the computer is attempting to answer what the affect of a particular variable is on the outcome assuming all other variables are held constant. In a different sense, multiple regression is answering how much more information is gained conditioning on one variable after already conditioning on all the others. In the case of fields across the Inland Pacific Northwest, locations with greater annual rainfall also have lower annual temperatures. The result is that there is little information gain conditioning on annual rainfall after already knowing annual temperature, and vice versa. More worryingly, the resulting predictions become different depending on which terms are included and if a researcher is unaware of how correlation between variables can influence the result they might unknowingly report incorrect conclusions. In addition, the issue of multicollinearity is also one for which polynomial regression doesn't solve. Ultimately, count data alone and annual climate summaries do not provide enough information to produce models which can provide useful predictions to growers.

The final study aimed to resolve the issues discovered and expressed in the previous chapter. Additional information in the form of the soil dilution factor was used to append the

isolation frequency records and produce an estimate of population density in units of propagules per gram soil (PPG). Values of PPG are still quite variable across the same field at the same point in time, with a standard deviation approximately equivalent to the mean. This hint, along with using cumulative distribution functions after their utility was demonstrated in the handwashing study, allowed for elucidation that collections of PPG values could be modelled using exponential distributions. The empirical data is used to estimate a rate parameter, and the rate parameter can then be used to simulate a new field. More importantly for growers, the results can be expressed in a similar manner as flood data: in terms of frequency of exceedance.

After an output variable was determined, the next step was to develop a new predictive model. The result, termed atmospheric water balance (AWB), incorporates all 6 variables available in the output of global climate models. The difference between precipitation and potential evapotranspiration share the same units and both provide a more interpretable predictor variable while also performing dimensionality reduction and avoiding potential pitfalls with multicollinear terms. Using AWB is flexible, and a researcher can adjust the prior time window to their domain of expertise. In this study the prior 90 day AWB was used as a proxy for seasonal changes in soil moisture, and was the approximate time between sampling. Researchers interested in shorter time scales could simply calculate AWB for a shorter interval.

The multilevel modelling approach was utilized again, with varying slopes in addition to varying intercepts. Different fields had different annual fluctuations in PPG values and the resulting seasonal change is expressed in the distribution of slope parameters. The posterior distribution of the multilevel model was then used with daily climate data provided by the downscaled global climate models. Populations of *F. culmorum*, a potential deoxynivalenol producer in the region, are forecasted to remain relatively constant in the region under climate change. Looking seasonally, lower population densities are forecasted during the summer and fall months as temperatures increase and rainfall becomes more limited.

This approach represents a case study with both a flexible output, in the form of an exponential distribution, and a flexible input, in the form of AWB for a given timeframe. Other soil microbes, or possibly even concentrations of metals such as arsenic which are

distributed unevenly in soil, can also potentially be modelled using an exponential distribution. The exponential distribution can also be used classically to model time between events, such as time between detection of *Listeria* in drains. The multilevel modelling approach could then be adapted to provide predictions for individual drains and also determine the extent of variability across different drains and different facilities.

Food safety is a diverse discipline and learning Bayesian data analysis yields a probabilistic framework for generating models and providing predictions which properly incorporate the inherent variability in the system of interest. This uncertainty is then communicated to managers and consumers through model predictions. Lessons learned in these case studies can be readily adapted to new disciplines, hopefully providing the greatest good to the greatest amount of people.

Chapter 7: Appendix

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