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Population Biology

e-Xtra*

Population Genetics of *Fusarium graminearum* at the Interface of Wheat and Wild Grass Communities in New York

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ABSTRACT

Fusarium graminearum is primarily understood as an agricultural pathogen affecting cereal crops, but its host range also includes diverse, noncultivated grasses ubiquitous across agricultural and natural environments. Wild grasses may select for the production of diverse toxin variants (chemotypes) and serve as reservoirs of genetic diversity or sources of disease-inciting inoculum. Populations at the intersection of wheat and wild grass communities were described using 909 isolates collected from wheat spikes, wild grass spikes, and overwintered wild grass stems found at natural and agricultural sites in regions of high and low crop production. Trichothecene (TRI) genotypes correlated to pathogen chemotype were predicted from two loci, and multilocus genotypes (MLGs) were determined using eight microsatellite loci. The genetic diversity of wild grass and wheat-derived populations was comparable, and their differentiation was low. Duplicate MLGs were rare even in samples collected from a single square meter, although they could be found in multiple hosts, environments, regions, and years. TRI genotype frequencies differed between region and land use. Admixture between TRI genotype-defined populations, which correspond to three previously described sympatric North American populations, was detected and was highest in a region with remote host communities and little agricultural production. Nonagricultural environments may maintain different pathogen TRI genotypes than wheat fields and provide an opportunity for recombination between isolates from different *F. graminearum* populations. A lack of structural barriers suggests that pathogen gene flow is uninhibited between wheat and wild grass communities, and the recovery of putative clones from multiple hosts and environments provides initial evidence that noncultivated grasses are a source of local and regional inoculum.

Keywords: ecology and epidemiology, *Fusarium graminearum*, grasses, mycology, population biology, wild hosts

Fusarium graminearum Schwabe is best known for causing Fusarium head blight of small grains and Gibberella ear rot of maize but has also been recovered from dozens of noncultivated plants. Able to colonize plants from at least 26 families, *F. graminearum* is most often associated with species in the true grass family, Poaceae (Farr and Rossman 2019). Noncultivated grasses are ubiquitous features of both agricultural and unmanaged landscapes, often found in the margins of crop fields, occupying fallow land, or dominating natural spaces in close proximity to agricultural production (USDA National Agricultural Statistics Service 2019). Despite their widespread distribution and the high frequency of asymptomatic *F. graminearum* colonization reported (Inch and Gilbert 2003; Lofgren et al. 2017; Turkington et al. 2011), the role these hosts play in pathogen evolution and crop disease epidemics is unknown. Understanding pathogen populations at the intersection of crop and noncrop host communities may help determine the importance of these hosts.

F. graminearum sensu lato is a globally distributed species complex (O'Donnell et al. 2000, 2004; Starkey et al. 2007; van der Lee et al. 2015) and *F. graminearum* sensu stricto is the dominant species in North America (McMullen et al. 2012; O'Donnell et al. 2004). Three sympatric North American populations exist with overlapping distributions and can be differentiated largely by their production of trichothecene toxin variants, which are commonly referred to as chemotypes and correlated with trichothecene (TRI) genotypes (Kelly and Ward 2018). The chemotypes corresponding to North American populations are the 15- and 3-acetylated forms of deoxynivalenol (15ADON and 3ADON, respectively) and the more recently described NX2 (Varga et al. 2015). The NX2 genotype has been found with unusually high frequency in northeastern New York (Lofgren et al. 2018). The distribution of *F. graminearum* TRI genotypes and populations has been the subject of many studies (Astolfi et al. 2012; Guo et al. 2008; Malihipour et al. 2012; Pasquali et al. 2016; Scoz et al. 2009; van der Lee et al. 2015; Wang et al. 2011; Yang et al. 2018; Zhang et al. 2012), though the role of diverse, noncultivated hosts in the maintenance of TRI genotype diversity and structuring of populations has not been investigated.

Pathogen overwintering in crop debris, primarily corn stubble, is implicated as the most significant source of disease-causing inoculum (Leplat et al. 2013). Pathogen survival on infested crop residues results in both local and regional disease pressure (Keller et al. 2014), but the importance of local inoculum production on the tissue of segetal grasses and of regional inoculum resulting from long-term pathogen build-up in unmanaged grasslands where host tissue remains in situ has not been explored. Direct tracking of propagule movement is possible, though challenging (Keller et al. 2011; Prussin et al. 2014); however, drawing inferences about pathogen movement from population genetics is another approach to studying the connection between wild grass debris and disease incidence in crops.

This study was conducted to determine (i) whether wild grasses serve as reservoirs of *F. graminearum* genetic diversity and (ii) how New York populations are structured at the intersection of host communities. If wild grasses were reservoirs of *F. graminearum* diversity, we expected to find higher genotypic or allelic diversity in populations derived from wild grass spikes than from wheat spikes. By extension, we might find higher genetic diversity in

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nonagricultural field sites or in natural environments than in a wheat field or a region containing intensive agricultural production. Although many factors can influence population structure, this study was primarily concerned with the influence of host, physical distance, and predicted membership to previously defined North American populations. Because *F. graminearum* is capable of kilometer-scale dispersal, it was hypothesized that physical distance would only be associated with differentiation between the most separated isolate sources. This dispersal, coupled with the well-mixed nature of aerial populations (Schmale et al. 2006), led to the expectation that wild grass and crop-derived isolates would show signs of gene flow but that slight population subdivision could occur due to the persistence of local populations surviving between years in accumulated wild grass debris or crop residues. Although sympatric North American populations have been identified, admixture between them has been recorded (Liang et al. 2015; Ward et al. 2008). Because the northern United States and eastern Canada are putative centers of origin for 3ADON- and NX2-producing isolates (Kelly et al. 2015, 2016), we expected admixture between populations would be greater in the northeastern region of New York, where all three populations may have coexisted for a longer period of time.

MATERIALS AND METHODS

Sampling and Isolates. The isolates used in this study were collected during a 3-year survey of wild grass spikes, naturally senesced and overwintered wild grass stems, and winter wheat spikes (M. R. Fulcher, unpublished data). During the summers of 2015 to 2017, asymptomatic wild grasses (Supplementary Table S1) and symptomatic wheat (unknown winter wheat cultivars) were collected from 23 field sites in two regions of New York that differ in host density (USDA National Agricultural Statistics Service 2019) and *F. graminearum* TRI genotype frequencies (Kuhnem et al. 2015) (Fig. 1). Two land uses, agricultural and nonagricultural (natural), were included in the survey. In all cases, samples were collected from within 1-m^2 quadrats placed 10 m apart. At agricultural sites, wheat spikes were collected along transects placed randomly into the field whereas wild grass spikes and stems were collected from field margins. At natural sites, wild grass spikes and stems were collected along transects laid haphazardly in accessible portions of natural preserves. Wild grass stem debris was collected early in the spring to capture only individuals overwintering in place. The collection of spikes from wheat and 12 common wild grass species that have flowering phenology similar to winter wheat was timed to capture the pathogen population after the primary infection cycle occurred but before secondary spike-to-spike infection was likely to take place. The isolates chosen for this study ($n = 909$) were morphologically identified as *F. graminearum* sensu lato (Leslie and Summerell 2006) and selected to represent hosts, regions, land uses, and sampling sites as equally as possible (Table 1). Of these isolates, 150 were included specifically because they were collected within single 1-m^2 quadrats and could be used to observe fine-scale population structure.

Genotyping. Cultures were grown for 2 weeks on potato dextrose agar (PDA) under 12 h of fluorescent light, mycelium was scraped from the surface of PDA, and samples were frozen at –20°C before tissue disruption. DNA extraction was performed using the QIAGEN DNeasy Plant Mini Kit according to the manufacturer's instructions (Holden, Germany). The TRI genotype of all 909 isolates was determined using two loci. First, a portion of the *TRI12* gene was amplified and fragment size was visualized with gel electrophoresis to predict whether isolates would produce nivalenol (NIV), 15ADON, or 3ADON (Starkey et al. 2007). Following this, isolates with a 3ADON genotype were used in a *TRI1* PCR-digestion assay to identify NX2 genotypes (Liang et al. 2014).

A subset of 800 isolates was genotyped at eight previously described microsatellite loci (Supplementary Table S2) (Naef and Défago 2006; Suga et al. 2004; Vogelgsang et al. 2009). Fluorescently labeled microsatellite primers (Applied Biosystems G5 dye set) were split evenly between two multiplex reactions. The QIAGEN Multiplex PCR Plus Kit was used, and reaction mixtures were 25 µl with 0.2 µM primer concentrations. The same cycling conditions were used for both reactions: 95°C for 5 min; followed by 30 cycles of 95°C for 30 s, 58°C for 1 min 30 s, and 72°C for 30 s; with a final extension at 72°C for 10 min. Amplified products were diluted 1:10 in water, and 1 µl of diluted product was mixed with 10 µl of HiDi Formamide and 0.2 µl of GeneScan 500 LIZ size standard (Life Technologies, Woolston, United Kingdom). Fragments were separated on an ABI 3730xl DNA Analyzer at the Cornell Biotechnology Resource Center, and alleles were sized in Geneious Prime (version 2019.0.4; Biomatters, Auckland, New Zealand) using the Microsatellite Analysis plugin, version 1.4.6. A genotype accumulation curve was generated to check the completeness of genotype discovery. Data analyses were performed in RStudio, version 1.1.453 (RStudio Development Team 2016). Microsatellite data were formatted in GenAlex, version 6.503 (Peakall and Smouse 2006, 2012), and individuals with missing allele data were removed prior to analysis in R with the 'poppr', 'adegenet', and 'mmod' packages (Jombart 2008; Kamvar et al. 2014; Winter 2012).

Sources of genetic diversity. Wild grasses were hypothesized to serve as reservoirs of pathogen genetic diversity. In order to test this hypothesis using TRI genotypes and microsatellite multilocus genotypes (MLGs), populations were defined by region, land use, and host source. TRI genotype frequency was analyzed with a multinomial logistic regression implemented in the 'nnet' package (Venables and Ripley 2002) and analysis of variance using TRI genotypes as the response variables and a three-way interaction between region, land use, and host source as the predictor. TRI genotype probabilities were contrasted with 95% confidence intervals around least-squares means using the 'emmeans' package (Lenth 2019). MLG diversity was measured using rarified MLG counts, Shannon's H (Shannon 1948), Stoddart and Taylor's G (Stoddart and Taylor 1988), Simpson's λ (Simpson 1949), and evenness (Pielou 1975). Allele diversity was measured in mean alleles per locus, Simpson's λ, Nei's gene diversity H_{exp} (Nei 1978), evenness, and unique alleles.

Sources of genetic structure. Several hypotheses were tested to understand *F. graminearum* genetic structure in New York. First, populations were defined by year, region, land use, and host source. The structure of these populations was evaluated with analysis of molecular variance (AMOVA) using a hierarchical model. A

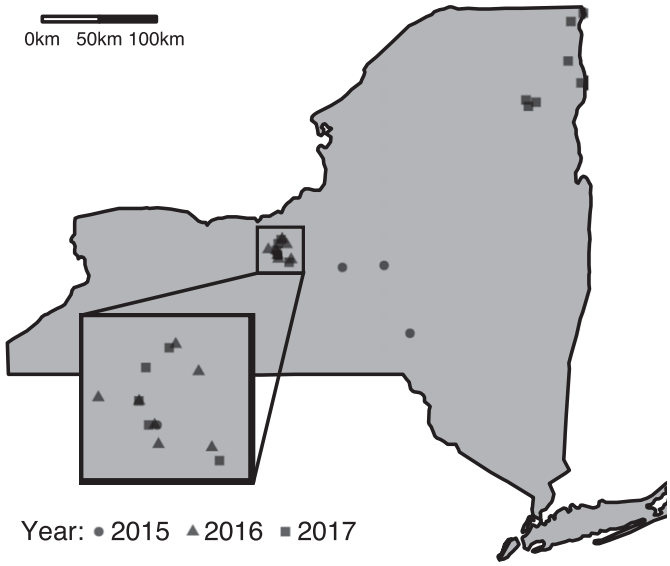

Fig. 1. *Fusarium graminearum* isolates used in this study were collected over 3 years from 23 field sites situated in two regions of New York.

random permutation test provided the significance of variance apportioned to each level of the model.

Next, populations were defined solely by host source. Fixation and differentiation indices similar to F_{st} (Meirmans and Hedrick 2011), including Hedrick's G'_{st}, Jost's D, and Meirmans φ_{st} (Hedrick 2005; Jost 2008; Meirmans 2006), were calculated to estimate structure between these populations. Bruvo's genetic distance (Bruvo et al. 2004) was also calculated between all pairs of isolates and used to build a minimum spanning network (Prim 1957).

Last, spatial structuring was assessed on two scales. The Bruvo's genetic distance matrix was compared with a physical distance matrix to check for spatial correlation using a Mantel test and a null distribution generated with 10,000 random permutations (Oksanen et al. 2018). In order to measure structure on a fine scale, a subset of the data was used to determine the proportion of duplicate MLGs collected from within 1-m^2 sampling quadrats. These probabilities were determined based on 11 wild grass debris quadrats from which 5 or 6 isolates were recovered and 12 wild grass spike quadrats from which 5 to 10 isolates were recovered.

Admixture rates. Populations were redefined using TRI genotype, and the hypothesis that admixture between these populations would vary between regions in New York was tested. Five isolates with a NIV genotype were removed from this analysis because of the small sample size. Pairwise differentiation statistics were calculated as described above. A discriminant analysis of principal components (DAPC) was used to visualize the genetic similarity of isolates and to determine the posterior probability of isolate membership to these predefined TRI genotype populations (Jombart et al. 2010). An arbitrary threshold of 80% posterior probability was used to define isolates showing admixture.

RESULTS

Sources of genetic diversity. TRI genotype frequencies were contrasted between region, land use, and host source. The production of 15ADON was predicted for 679 isolates, 3ADON for 201 isolates, NX2 for 24 isolates, and NIV for 5 isolates (Table 1). No variation in TRI genotype frequency was detected between wheat spikes, wild grass spikes, and wild grass debris (Table 2). The probability of a given TRI genotype occurring was significantly affected by both region and land use ($P \leq 0.001$). The occurrence of 3ADON and NX2 genotypes was greatest in northeastern New York, where agricultural and natural sites contained similar TRI genotype frequencies (Fig. 2). In central New York, the 15ADON genotype was most common but a significant increase in 3ADON genotypes was detected at natural sites compared with agricultural sites (Supplementary Fig. S1).

TABLE 2. Analysis of variance output from a multinomial logistic regression of trichothecene genotype frequencies

Predictor	χ^2 Statistic	Degrees of freedom	*P* value[a]
Region	62.345	3	**0.001**
Land use	45.416	3	**0.001**
Host	7.514	6	0.276
Region: Land use	6.987	3	0.072
Region: Source	2.490	6	0.870
Land use: Source	5.689	6	0.459
Region: Land use: Source	4.567	6	0.600

[a] Data in bold indicate predictors that explained a significant amount of variation in genotype frequency.

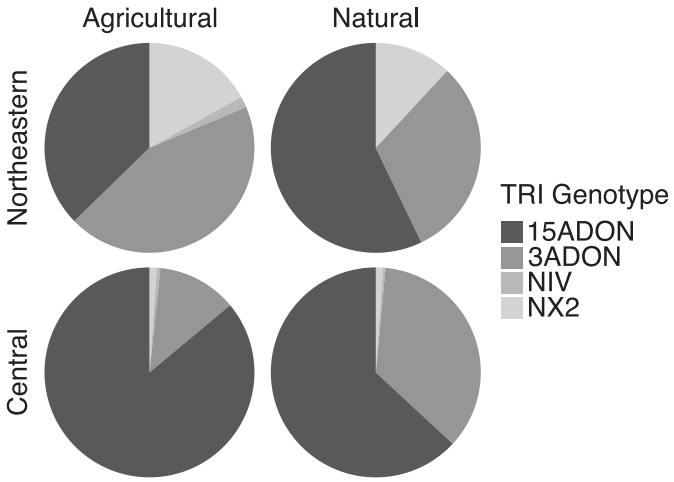

Fig. 2. Trichothecene (TRI) genotype frequencies varied by land use and region. Abbreviations: 15ADON and 3ADON = 15- and 3-acetylated forms of deoxynivalenol, respectively, and NIV = nivalenol.

TABLE 1. Population trichothecene (TRI) genotype frequencies and multilocus genotype (MLG) diversity metrics by year, region, land use, and host source[a]

Year, region	Land use	Host[b]	Isolates TRI genotype	15ADON	3ADON	NIV	NX2	Isolate MLG	MLG	Simpson's λ	Evenness
2015											
Central	Agricultural	Spikes	53	41	10	1	1	52	51	0.98	0.99
		Wheat	50	36	11	1	2	49	49	0.98	1.00
	Natural	Spikes	12	9	3	0	0	12	11	0.90	0.96
2016											
Central	Agricultural	Debris	236	201	33	0	2	89	88	0.99	0.99
		Spikes	3	3	0	0	0	2	2	0.50	1.00
		Wheat	16	12	3	1	0	10	10	0.90	1.00
	Natural	Debris	103	64	36	1	2	102	95	0.99	0.96
		Spikes	7	7	0	0	0	7	7	0.86	1.00
2017											
Central	Agricultural	Debris	53	50	2	0	1	51	49	0.98	0.98
		Spikes	59	54	5	0	0	59	59	0.98	1.00
		Wheat	68	66	2	0	0	68	67	0.98	0.99
	Natural	Debris	35	13	21	0	1	35	32	0.97	0.96
		Spikes	113	77	36	0	0	111	106	0.99	0.98
Northeastern	Agricultural	Debris	18	6	10	0	2	18	16	0.93	0.95
		Spikes	12	6	4	0	2	20	20	0.95	1.00
		Wheat	29	10	12	1	6	23	23	0.96	1.00
	Natural	Debris	4	3	1	0	0	4	4	0.75	1.00
		Spikes	38	21	12	0	5	22	22	0.95	1.00
			909	679	201	5	24	734	680	1.00	0.96

[a] Abbreviations: 15ADON and 3ADON = 15- and 3-acetylated forms of deoxynivalenol, respectively, and NIV = nivalenol.
[b] Spikes = wild grass spikes and Debris = wild grass debris.

Microsatellite genotypes were successfully determined for 734 isolates. Samples were removed from the analysis if any microsatellite failed to amplify or if any allele calls were ambiguous. A genotype accumulation curve indicated that almost all genotypes could be detected with seven of the loci chosen (Supplementary Fig. S2). Genotypic diversity was high across all isolate populations, and 680 MLGs were recorded (Tables 1 and 3). Duplicate MLGs were found in isolates collected from different host sources (n = 42, MLGs = 19), land uses (n = 38, MLGs = 18), regions (n = 2, MLG = 1), and years (n = 3, MLG = 1). Allele diversity was even across host sources (Table 4).

Sources of genetic structure. According to AMOVA, the majority of genotypic diversity was found within populations (98%). Year was not a significant source of structure, though region, land use, and host source accounted for slight but significant structure (Table 5). Little genetic differentiation was seen between isolates collected from wild grass spikes, wild grass debris, and wheat spikes (G'_{st} = 0.029, D = 0.017, φ_{st} = 0.015). Plotting the genetic distance between isolates as a minimum spanning network showed no clustering based on host source (Fig. 3). A slight, significant positive correlation was found between genetic and physical distance (r = 0.03, P < 0.038). The chance of recovering duplicate MLGs from within 1-m^2 quadrats ranged from 0 to 10% for wild grass debris and 0 to 20% for wild grass spikes, with each averaging 4%.

Admixture rates. Differentiation between TRI genotype-defined populations was greater than for host sources (Table 6), and TRI genotype-defined populations could be separated by a DAPC (Fig. 4). The NX2 population was less differentiated than the 3ADON and 15ADON populations. The posterior probability of isolate assignment to TRI genotype populations is displayed in Figure 5. The NX2 genotype was highly admixed and, for all TRI genotypes, admixture was greater in northeastern New York than in central New York (Table 7).

DISCUSSION

This is the first comprehensive study of the genetic relationship between wild-grass- and wheat-derived *F. graminearum* isolates. The primary goal of this work was to determine whether wild grasses serve as reservoirs of *F. graminearum* diversity. Pathogen genetic diversity was universally high. Most individuals contained a unique MLG, and wheat- and wild-grass-derived populations contained similar levels of allelic diversity at neutral microsatellite loci. However, in central New York, the wild-grass-derived population from nonagricultural sites did contain a different ratio of TRI genotypes than was found in either the wild grasses or wheat at agricultural sites. The wild grasses found in remote, isolated sites in northeastern New York also harbored a different mixture of TRI genotypes, and these isolates showed signs of significant admixture. This is evidence that wild grasses found in nonagricultural environments may support different pathogen populations than are found at agricultural sites, and that hosts found in remote areas could harbor recombined genotypes and serve as a source of novel genotypic diversity.

The genetic diversity recorded in this study was high and comparable with that found in previous studies focused on crop-infecting *F. graminearum* populations (Liang et al. 2014, 2015; Miedaner et al. 2008) and in one study containing isolates collected from various noncultivated hosts (Sneideris et al. 2018). This high

TABLE 5. Partitioned genotypic variance and significance from hierarchical analysis of molecular variance

Source of variance	σ	Variance (%)	P value
Between years	–0.05	–0.86	0.849
Between regions	0.05	0.76	<0.001
Between land uses	0.08	1.28	<0.001
Between host sources	0.04	0.64	0.013
Within populations	6.23	98.18	<0.001
Total	6.35	100	…

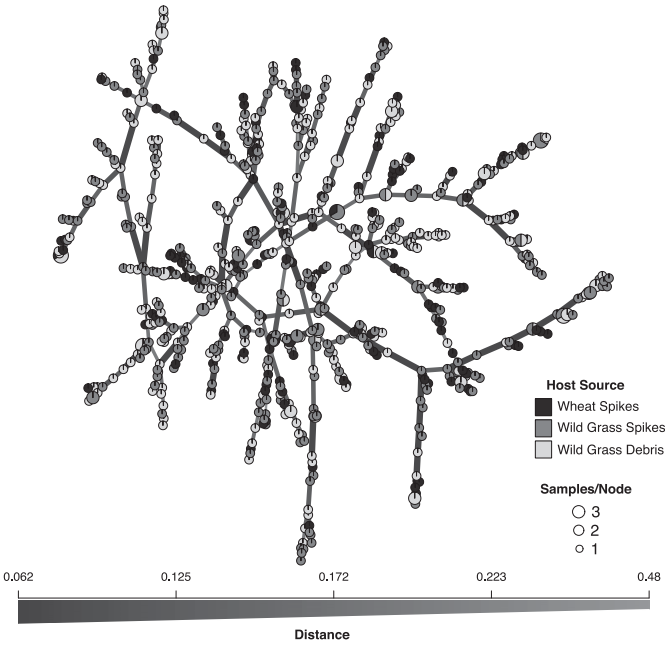

Fig. 3. Minimum spanning tree depicting Bruvo's distance between *Fusarium graminearum* isolates showed no clustering based on host of origin.

TABLE 3. Genotypic diversity of host defined populations[a]

Host	N	MLG	eMLG	Shannon's H	Stoddart and Taylor's G	Simpson's λ	Evenness
Wheat spikes	150	148	148	4.99	146.10	0.99	0.99
Wild grass spikes	285	274	147	5.59	264.57	0.99	0.97
Wild grass debris	299	277	144	5.59	259.13	0.99	0.96
Total	734	680	148	6.49	632.34	0.99	0.95

[a] MLG = multilocus genotype and eMLG = rarified MLG counts.

TABLE 4. Allelic diversity

Host	Alleles per locus	λ	H_{exp}	Evenness	Unique alleles
Wheat spikes	14	0.80	0.80	0.74	13
Wild grass spikes	16	0.78	0.78	0.71	13
Wild grass debris	17	0.79	0.79	0.72	20
Total	16	0.78	0.78	0.72	…

genotypic diversity is a result of outcrossing by *F. graminearum*, and the mutation rate associated with some markers, such as the microsatellites used at present. Duplicate MLGs were rare even within 1-m^2 patches of wild grass debris and wild grass spikes. On this fine scale, little spatial structure was found and multiple MLGs could be recovered from the spikes or stems of a single host plant. Given this, even small patches of noncultivated grasses may harbor multiple pathogen genotypes or allow unique isolates to recombine.

TABLE 6. Differentiation between populations defined by predicted trichothecene genotypes was measured with Hedrick's G'_{st} (Jost's D) and Meirmans φ_{st}[a]

Genotypes	15ADON	3ADON	NX2
15ADON	…	…	…
3ADON	0.31 (0.26)	…	…
NX2	0.17 (0.14)	0.19 (0.15)	…
Universal Meirmans φ_{st}	0.28	…	…

[a] Abbreviations: 15ADON and 3ADON = 15- and 3-acetylated forms of deoxynivalenol, respectively.

The balance of *F. graminearum* TRI genotype frequencies has been the subject of many investigations (Astolfi et al. 2012; Guo et al. 2008; Malihipour et al. 2012; Pasquali et al. 2016; Scoz et al. 2009; van der Lee et al. 2015; Wang et al. 2011; Yang et al. 2018; Zhang et al. 2012). It has been suggested that hosts, environmental factors such as temperature, or the virulence of isolates from different populations are determinants of TRI genotype distribution. In this study, it was clear that pathogen populations collected from winter wheat, wild grass spikes, and wild grass stems occurring in the same field did not differ in TRI genotype composition. This mirrors the results of an earlier study in New York that compared TRI genotype frequencies in isolates collected from wheat, corn, and aerial populations (Kuhnem et al. 2015). As shown in the past, northeastern New York has a uniquely high frequency of the NX2 genotype compared with the other regions where it has been observed (Kelly et al. 2015; Lofgren et al. 2018). The low recovery of NIV genotype isolates is in agreement with previous findings that show a north-south cline and a large population of NIV producers in Louisiana (Gale et al. 2011; Kelly et al. 2015; Schmale et al. 2011). Unlike previous studies that

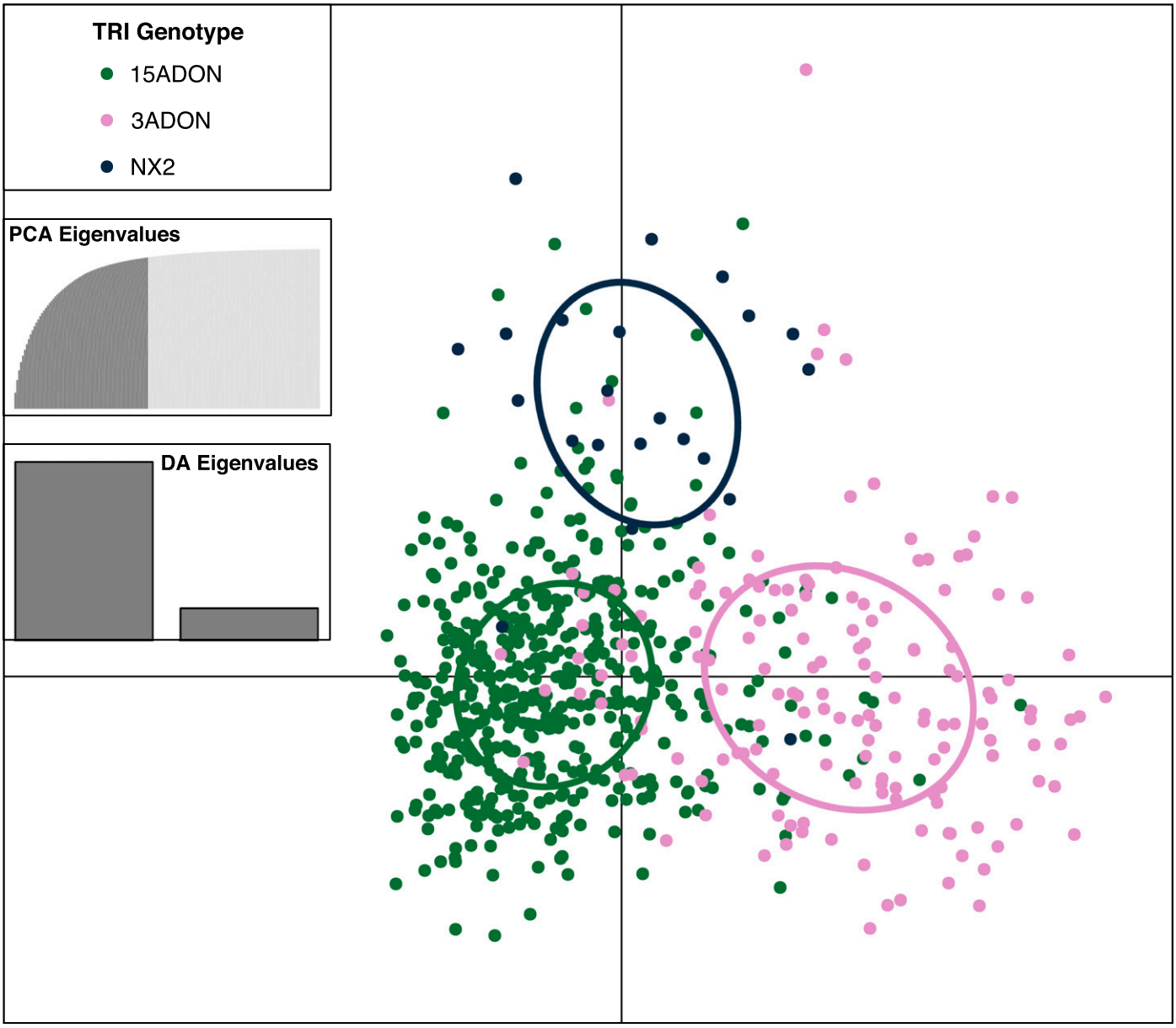

Fig. 4. Scatterplot from a discriminant analysis (DA) of principal components could distinguish three overlapping groups of isolates defined by their trichothecene (TRI) genotype. Abbreviations: PCA = principal component analysis and 15ADON and 3ADON = 15- and 3-acetylated forms of deoxynivalenol, respectively.

revealed changes in the relative abundance of 15- and 3ADON genotypes over time in other parts of North America (Burlakoti et al. 2011; Liang et al. 2014; Ward et al. 2008), the present survey of TRI genotypes from 2015 to 2018 populations presented findings nearly identical to collections made from 2012 to 2013 (Kuhnem et al. 2015) and in 2006 (Schmale et al. 2011). The conditions leading to those well-documented changes in population composition are unlikely to be present in New York.

The reason for the difference in TRI genotype frequency between wild grasses in nonagricultural environments and wild grasses in agricultural fields separated by only 3 to 5 km is not clear. The increased 3ADON frequency in these nonagricultural sites in central New York reflects a similar increase in 3ADON frequency in northeastern New York, observed at both agricultural and nonagricultural sites and in all host sources. The similar TRI genotype distributions in these two areas implicates commonalities between their environments such as lower host density or greater host diversity as potentially important factors in shaping TRI genotype distributions. This interpretation is bolstered by previous work associating the relative abundance of different hosts with *F. graminearum* sensu lato species and TRI genotype distributions (Boutigny et al. 2011; Kuhnem et al. 2016; Sampietro et al. 2011). A shortcoming of this study was an explicit focus on predominantly cool-season wild grasses. Another explanation for differences in TRI genotype frequency in natural spaces could be that warm-season wild grasses flowering later in summer are infected with higher levels of 3ADON-producing isolates and contribute these to local cool-weather wild grasses in subsequent years.

The second goal of this study was to assess pathogen population structure. *F. graminearum* populations in New York were not strongly structured by host, land use, or year. A weak spatial correlation was found that may relate to the regional difference in TRI genotype distributions. The New York population of *F. graminearum* is strongly structured by TRI genotypes, which correspond to three previously defined North American populations (NA1, NA2, and NA3) (Kelly and Ward 2018). 15ADON (NA1) is predominant in central New York while 3ADON (NA2) and NX2 (NA3) genotypes are found with greater frequency in northeastern New York.

The rates of admixture and differentiation between North American populations of *F. graminearum* has been recorded by several other researchers since Gale et al. (2007) first defined the subdivision of populations associated with 3- and 15ADON TRI genotypes. Although direct comparison is made difficult by variation in the methods and approaches used, prior estimates of admixture and differentiation between these groups are comparable with those reported here. Kelly et al. (2015) described spatial variation in the incidence of recombinant genotypes, and we similarly found elevated signs of admixture in one region of New York. The increased levels of admixture in northeastern New York could be a result of relatively even TRI genotype frequencies and the prolonged co-occurrence of these populations allowing for more recombination events to occur.

Recent work has identified genomic regions under selection or prone to recombination events (Kelly and Ward 2018, Talas and McDonald 2015). Isolates from noncrop hosts may be of particular interest in future genome analysis projects attempting to identify genes with adaptive function. *F. graminearum*-infected wild grasses are typically asymptomatic under natural conditions and do not accumulate mycotoxins to the extent observed in crop infection (Lofgren et al. 2017), indicating that successful colonization is facilitated by yet-to-be-discovered traits. For this reason, the host–fungus relationship between *F. graminearum* and wild grasses should be further characterized, particularly with respect to the selective pressures that exist in nonagricultural environments and how they contribute to the maintenance of agriculturally relevant pathogen phenotypes (for instance, fungicide resistance or toxin production).

Wild grasses may be important for their contribution of inoculum to crop disease epidemics. The movement of pathogen propagules between hosts and land uses does not appear inhibited by population structure, and several putative clones were found across multiple hosts and land uses. Because the number of putative clones identified based on isolates containing identical MLGs can be inflated due to size homoplasy of microsatellite alleles, this finding should be considered with caution (Estoup et al. 2002). Despite this, the combination of direct and indirect evidence suggests that noncultivated grass hosts are a substrate for the production of both local and regional inoculum. The relative importance of this compared with inoculum arising from agricultural crop residues such as corn stubble remains to be determined.

TABLE 7. Admixture rates of trichothecene (TRI) genotype-defined populations

TRI genotype[a]	Region	Admixed proportion[b]
3ADON	Central	0.35
	Northeastern	0.53
15ADON	Central	0.12
	Northeastern	0.19
NX2	Central	0.67
	Northeastern	0.72

[a] Abbreviations: 15ADON and 3ADON = 15- and 3-acetylated forms of deoxynivalenol, respectively.
[b] Having ≤80% assignment probability to the TRI genotype-defined population.

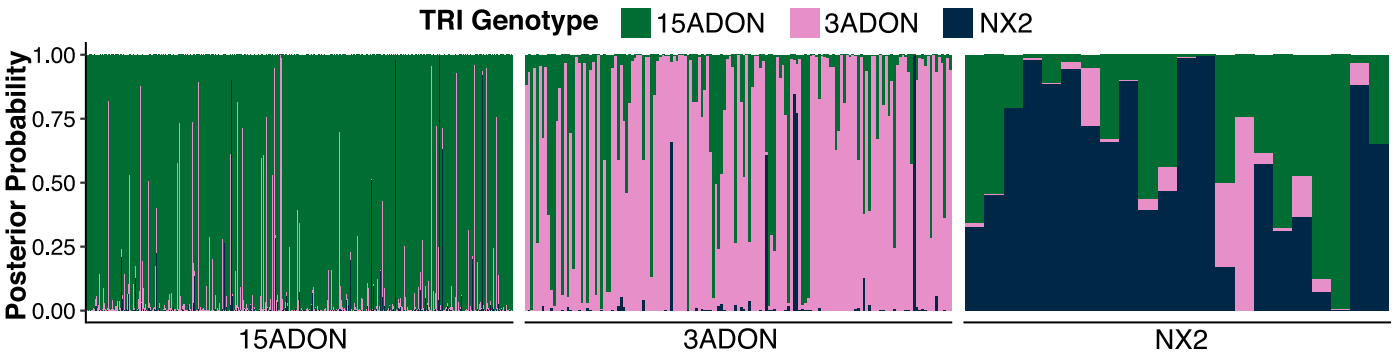

Fig. 5. Posterior probabilities of isolate assignment to trichothecene (TRI) genotype defined populations after a discriminant analysis of principal components indicated significant admixture. Abbreviations: 15ADON and 3ADON = 15- and 3-acetylated forms of deoxynivalenol, respectively.

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