Functional Variation of Plant–Pathogen Interactions: New Concept and Methods for Virulence Data Analyses

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ABSTRACT

Classical virulence analysis is based on discovering virulence phenotypes of isolates with regard to a composition of resistance genes in a differential set of host genotypes. With such a vision, virulence phenotypes are usually treated in a genetic manner as one of two possible alleles, either virulence or avirulence in a binary locus. Therefore, population genetics metrics and methods have become prevailing tools for analyzing virulence data at multiple loci. However, a basis for resolving binary virulence phenotypes is infection type (IT) data of host–pathogen interaction that express functional traits of each specific isolate in a given situation (particular host, environmental conditions, cultivation practice, and so on). IT is determined by symptoms and signs observed (e.g., lesion type, lesion size, coverage of leaf or leaf segments by mycelium, spore production and so on), and assessed by IT scores at a generally accepted scale for each plant–pathogen system. Thus, multiple IT profiles of isolates are obtained and can be subjected to analysis of functional variation within and among

operational units of a pathogen. Such an approach may allow better utilization of the information available in the raw data, and reveal a functional (e.g., environmental) component of pathogen variation in addition to the genetic one. New methods for measuring functional variation of plant–pathogen interaction with IT data were developed. The methods need an appropriate assessment scale and expert estimations of dissimilarity between IT scores for each plant–pathogen system (an example is presented). Analyses of a few data sets at different hierarchical levels demonstrated discrepancies in results obtained with IT phenotypes versus binary virulence phenotypes. The ability to measure functional IT-based variation offers promise as an effective tool in the study of epidemics caused by plant pathogens.

Keywords: analytical and theoretical plant pathology, ecology and epidemiology, genetics and resistance, population biology

Virulence of a pathogen is defined as its ability to infect or damage a host, although the degree of damage may vary. Assuming the gene-for-gene relationship between a resistance gene in the host and the corresponding avirulence gene in the pathogen, virulence in plant pathology mainly refers to a pathogen's ability to infect a susceptible host (Brown 1998). Virulence analysis has served plant pathologists from the middle of 20th century as one of the major approaches to study populations of plant pathogens aimed to predict an epidemic expansion in time and space caused by those pathogens, to provide practical recommendations to growers, and to develop cultivars with effective resistance genes. Virulence analysis is based on discovering virulence phenotypes of isolates with regard to a composition of resistance genes in a differential set of host genotypes. Virulence phenotypes have usually been treated in a genetically binary manner, with two alleles of virulence and avirulence at a binary locus, so that population genetics metrics and methods have become the prevailing tools of virulence data analyses at multiple loci (Brown 2006; Kosman and Leonard 2007). However,

an empirical basis for resolving binary virulence phenotypes is determining the infection type (IT) of a plant–pathogen interaction, which is a phenotypic expression of a functional trait of each specific isolate in a given situation (particular host, environmental conditions, cultivation practice, and so on). ITs can be described in different ways, including by lesion type, lesion size, area covered by symptoms on the leaf or leaf segments, spore production, and so on, and combinations thereof. They are mainly quantitative and usually determined by scores using a generally accepted assessment scale, often an ordinal scale developed for each plant–pathogen system (e.g., the 0-to-4 scale for *Blumeria graminis* (DC.) Speer f. sp. *hordei* Marchal). Thus, multiple quantitative profiles of isolates are obtained and can be subjected to analysis of functional variation (Gregorius and Kosman 2017, 2018; Scheiner et al. 2017a,b) with no need to transform original scores into the binary “alleles”. Such an approach may allow better utilization of information available in the raw data (ordinal scale versus binary one) and, consequently, result in more accurate and comprehensive analyses of virulence data. In addition, ecological aspects of plant–pathogen interactions could also be addressed, because changes in IT may result from inconstant fitness of pathogen isolates or host plants under different environmental conditions or cultivation practices.

IT ASSESSMENT SCALES AND DISSIMILARITY BETWEEN IT SCORES

IT assessment scales may have different numbers of categories, depending on the plant–pathogen systems for which they were developed, but commonly they range either from 0 to 4 (McCallum et al. 2010; McIntosh et al. 1995; Torp et al. 1978) or from 0 to 9

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(Line and Qayoum 1992; Wan et al. 2016). The 0-to-4 scale does not provide a sufficient resolution for measuring extent of infection or damage observed, so that intermediate scores (e.g., 2+, 4–, and so on) with possible additional designations based on some other symptoms (see below for an example of wheat leaf rust in Canada) are commonly used during assessment. Therefore, ITs assessed using any scale with low resolution (e.g., the 0-to-4 scale) could be easily transformed to the corresponding scores in a scale of higher resolution (e.g., the 0-to-9 scale). We would recommend using the assessment scale from 0 to 9 because that range allows satisfactory determination of the degree of damage. However, any other appropriate, empirically determined scale of relatively high resolution can be used, subject to the needs of a specific plant–pathogen system. However, an optimal scale resolution should not be too high to allow for clear-cut visual determination and differentiation of infection by IT scores. For simplicity, we further considered only the 0-to-9 assessment scale, with avirulence and virulence scores from 0 to 5 and 7 to 9, respectively, while 6 is considered the borderline score that can be interpreted as either avirulence or virulence.

A key issue of analysis of variation of plant–pathogen interactions is a proper evaluation of dissimilarity between ITs (scores in the ordinal scale from 0 to 9). Such dissimilarities may differ from one plant–pathogen system to another, so that the corresponding expert assessments of dissimilarities need to be developed for each specific system. However, common features of dissimilarities between IT scores seem to be predetermined by the shape of the dependence relationship between pathogen effects on the host plant and related IT scores. We suppose that such a dependence relationship can be expressed by a sigmoidal (S-shaped) curve produced when changing effects are plotted over IT scores. The curve is a slightly increasing convex function in the domain of avirulence scores from 0 to 5, a steep increase between the avirulence and virulence domains from 5 to 7 with the inflection point at the borderline score 6, and continues gradually increasing in the domain of virulence scores from 7 to 9. Such a sigmoidal shape provides a strong differentiation between scores of avirulence and virulence—two absolutely different functional or genetic states—whereas differences between the virulence scores are rather small, as are those between the avirulence scores. Then, dissimilarity between any two IT scores can be naturally determined in terms of the absolute difference between the corresponding effects. For convenience, one can assume that the effect of a pathogen isolate on a given host genotype is measured in the $[0, 1]$ interval, so that dissimilarities between IT scores also range between 0 and 1. Importantly, the pathogen effects surge from the avirulence to virulence domain, which is in accordance with experimental quantitative assessments of the reproductive capacity of the pustules of different ITs in the oat stem rust pathosystem (Leonard 1969 [Table 2]). Consequently, dissimilarities between IT scores within each of the avirulence and virulence domains are much smaller than those between the domains. An example of dissimilarity matrix for IT scores based on expert assessments is presented in Table 1. This dissimilarity matrix is further used for analyses of experimental data to demonstrate the performance of a newly suggested approach; we denote it as $P = (p_{ij})$, where $p_{ij} = \rho(i, j)$ is dissimilarity between IT scores i and j , $0 \leq p_{ij} \leq 1$, and $i, j = 0, 1, 2, \dots, 9$.

FUNCTIONAL VARIATION OF PLANT–PATHOGEN INTERACTIONS

Hierarchical structure of functional variation. Standard approaches to analyze pathogen phenotypic variation are based on binary virulence data (virulence or avirulence). Virulence phenotypes (races) of a pathogen are shaped by a set of differential lines or cultivars, and relationships between them predetermine pathogen variation at different hierarchical levels. A local population is the bottom level in a hierarchical structure of the pathogen in studying

its variation whereas, at higher levels, either spatial (e.g., regional) or temporal (e.g., annual) variation is usually considered.

IT data allow for analysis of pathogen variation among isolates within a given race because the corresponding binary profile of this race can be generated by numerous IT profiles due to multiple scores interpreted as either avirulence or virulence. Therefore, variation within a race makes sense, and the race level becomes the lowest for analyzing functional variation, when a hierarchical structure of the pathogen is considered. Consequently, IT profiles of isolates and relationships between them turn out to be basic elements for performing analyses of functional variation at different hierarchical levels.

Distances between different operational units based on IT data. The elementary operational unit (OU) we deal with is an isolate of a plant pathogen, which is represented by its IT profile. Assuming that isolates are tested on a set of n differentials, the IT profile of each isolate is a vector of lengths n with coordinates $s_1, s_2, \dots, s_n$, where s_k is the IT score of that isolate on k th differential (remember, s_k is one of the scores from the 0-to-9 assessment scale). Then, the distance between two isolates $a = (s_{1a}, s_{2a}, \dots, s_{na})$ and $b = (s_{1b}, s_{2b}, \dots, s_{nb})$ can be determined as follows:

$$d_{ab} = d(a, b) = \frac{1}{n} \cdot \sum_{k=1}^n \rho(s_{ka}, s_{kb}) \quad (1)$$

where ρ is the corresponding entry from the matrix of dissimilarities between IT scores. Such distances range between 0 for isolates with identical IT profiles and 1 for isolates with the most contrasting IT scores on every differential (i.e., if $s_{ka} = 0$, then $s_{kb} = 9$, and vice versa). These distances are the basis for measuring functional variation of plant–pathogen interaction within a race.

Analyses of functional variation at the next hierarchical level needs a proper definition of distances between races (virulence phenotypes). This can be done using an average-based or assignment-based approach (Kosman 1996, 2014; Kosman and Leonard 2007). Formally, IT profiles of a given race can be considered a race-specific population, so that the distance between any two races A and B could be either $DAD_d(A, B)$ or $KB_d(A, B)$ with regard to distance d (equation 1) between IT profiles of isolates from race-specific populations of A and B (for definitions and details regarding the DAD and KB distances, see Kosman and Leonard [2007 (equations 2 and 5)] or Kosman [2014 (equations 16 and 17)]). Both of these distances range from 0 to 1. If there is only one IT profile for each race ($A = \{a\}$ and $B = \{b\}$), then these distances reduce to distance d between IT profiles: $DAD_d(A, B) = KB_d(A, B) = d(a, b)$. To simplify further explanations, we denote $D(A, B)$ distance between races A and B ($0 \leq D(A, B) \leq 1$). Such distances between races are the basis for measuring functional variation of plant–pathogen interactions within a population (group of races).

TABLE 1. Infection type (IT) score dissimilarities^a

IT	0	1	2	3	4	5	6	7	8	9
0	0.00	0.05	0.10	0.15	0.20	0.30	0.50	0.85	0.95	1.00
1	0.05	0.00	0.05	0.10	0.15	0.25	0.45	0.80	0.90	0.95
2	0.10	0.05	0.00	0.05	0.10	0.20	0.40	0.75	0.85	0.90
3	0.15	0.10	0.05	0.00	0.05	0.15	0.35	0.70	0.80	0.85
4	0.20	0.15	0.10	0.05	0.00	0.10	0.30	0.60	0.70	0.75
5	0.30	0.25	0.20	0.15	0.10	0.00	0.20	0.50	0.60	0.65
6	0.50	0.45	0.40	0.35	0.30	0.20	0.00	0.30	0.40	0.50
7	0.85	0.80	0.75	0.70	0.60	0.50	0.30	0.00	0.10	0.15
8	0.95	0.90	0.85	0.80	0.70	0.60	0.40	0.10	0.00	0.05
9	1.00	0.95	0.90	0.85	0.75	0.65	0.50	0.15	0.05	0.00

^a IT scores in the avirulence domain and dissimilarities between them are in italics, a borderline IT score (6) and its dissimilarities from all other IT scores are in bold italics, and dissimilarities between IT scores from different domains (avirulence 0 to 5 and virulence 7 to 9) are in bold.

Similarly, the distance between populations H and L can be determined: $DAD_D(H,L)$ or $KB_D(H,L)$ with regard to distance $D = D(A,B)$ between races. They range in a $[0,1]$ interval and serve for calculating functional variation among populations (within a set of populations grouped on spatial, temporal, or any other basis). In the same way, distances between OUs at higher hierarchical levels can be defined and employed for measuring functional variation of plant–pathogen interactions at that level.

Metrics of functional variation. Variation of an OU can be described by variable relationships among its entities (e.g., IT profiles of isolates or IT phenotypes within a population), and “dispersion” indices are used to quantify this variation. On the other hand, “diversity” describes variation of an OU by numbers and frequencies (representations) of qualitatively distinct types (e.g., richness and frequency of IT phenotypes in a population, respectively) among the entities without any reference to their interrelations (all distinct types are assumed equally distant). Thus, dispersion (an expression of relationships among entities) and diversity (an expression of amount and proportion of distinct qualitative types among those entities) are different facets of variation, and estimation of an overall variation of an OU needs to incorporate both the dispersion and diversity concepts.

Many dozens of approaches to analyze functional variation (mainly for species communities in ecology) were developed during the last two decades. However, they usually take into account a single attribute of functional variation, and only a few of them have been commonly used (e.g., the simplest one: Rao’s entropy) (Rao 1982). Villéger et al. (2008) suggested three metrics for independent measurement of different traits of functional variation: functional richness, divergence, and evenness [the latter is mathematically incorrect and has been frequently criticized (Legrasa and Gaertner 2018; Ricotta et al. 2014)]. The most comprehensive approaches for determining functional variation were suggested by Chiu and Chao (2014) and Scheiner et al. (2017a) and, in more general form, by Gregorius and Kosman (2017, 2018). They combine both diversity and dispersion perspectives and reflect all of the relevant aspects of functional variation. We will further consider the metric of Functional-Trait Dispersion (FTDis) (Scheiner et al. 2017a) and apply it to analyses of actual IT data.

Let N be the total number of entities in a given OU with distances d_{ab} (equation 1) between them. Then, the amount of dispersion within this OU can be estimated by the mean distance

$$M = \sum_{a,b=1}^N d_{ab} / N^2 \quad (2)$$

which ranges between 0 and $(N-1)/N$. In its normalized form

$$M' = \frac{N}{N-1} \cdot M = \sum_{a,b=1}^N d_{ab} / [N(N-1)] \quad (2')$$

The values of this average-based dispersion belong to a $[0,1]$ interval. We further consider this as metric of dispersion, though the amount of dispersion can be defined in different ways (e.g., assignment based dispersion) (Kosman 2014; Kosman and Leonard 2007).

Functional-Trait Diversity (FTDiv) of a given OU, ${}^qD(T)$, is defined by Scheiner et al. (2017a [equations 3 and 4]) based on the proportional contribution of each pairwise distance between entities (e.g., IT profiles of isolates or IT phenotypes) to the total sum of the pairwise distances $f_{ab} = d_{ab} / \sum_{a,b=1}^N d_{ab}$:

$${}^qD(T) = \frac{1}{2} \cdot \left(1 + \sqrt{1 + 4{}^qH(T)}\right) \quad (3)$$

where ${}^qH(T) = (\sum_{a=1}^N \sum_{b=1}^N f_{ab}^q)^{1/(1-q)}$ and ${}^1H(T) = \exp(-\sum_{a=1}^N \sum_{b=1}^N f_{ab} \log f_{ab})$ for nonnegative $q \neq 1$ and $q = 1$, respectively. This metric indicates the effective number of equally distant entities

(e.g., isolates) and ranges from 1 (when all entities are identical) to N (when all entities are equally distant).

FTDiv is a typical diversity metric, which is the invariant of a scaling effect of pairwise distances d_{ab} (each $d_{ab}(s) = s \cdot d_{ab}$, $s > 0$); thus, it is not able to distinguish between OUs with different extents of dispersion (e.g., M versus sM) but the same values of $f_{ab} = f_{ab}(s) = (sd_{ab}) / \sum_{a,b=1}^N (sd_{ab}) = d_{ab} / \sum_{a,b=1}^N d_{ab}$. To avoid this ambiguity in analyzing functional variation within an OU, two concepts of the diversity and the dispersion are combined. The following is one possible definition of the resulting measure (FTDis) recently suggested by Scheiner et al. (2017a) with diversity, ${}^qD(T)$, and dispersion, M or M' , integrated in a multiplicative way:

$${}^qD(TM) = 1 + {}^qD(T) \times M = 1 + (N-1) \times {}^qE(T) \times M' \quad (4)$$

where ${}^qE(T) = {}^qD(T)/N$ is the evenness of relationships among entities [$0 \leq {}^qE(T) \leq 1$], and TM indicates that FTDis is measured based on traits and dispersion. The range of ${}^qD(TM)$ is from 1 to N , and it expresses the effective number of distinct entities (e.g., isolates or phenotypes) in a given trait space (n -dimensional space for n differential lines with IT score values). In general, for any metric of dispersion $\hat{M}$ that ranges from 0 to 1 (e.g., assignment-based dispersion KW_d from Kosman and Leonard [2007 (equation 5)] or Kosman [2014 (equation 14)]), FTDis is determined as follows:

$${}^qD(TM) = 1 + \frac{N-1}{N} \times {}^qD(T) \times \hat{M} = 1 + (N-1) \times {}^qE(T) \times \hat{M} \quad (4')$$

To compare FTDis estimates of OUs with different numbers of entities, one can use the following normalized metric:

$${}^qND(TM) = {}^qD(TM)/N \quad (5)$$

with a range from 0 to 1. Metrics (3 to 5) are further used for $q = 1$, assuming that all entities equally contribute to measuring functional variation.

COMPARATIVE ANALYSES OF VARIATION ESTIMATED WITH IT AND STANDARD VIRULENCE DATA

Functional variation within races of the wheat stripe rust pathogen in the United States. The IT data of isolates of the wheat stripe rust pathogen (*Puccinia striiformis* f. *tritici* Erikss.), collected in 2011 and 2012 from the United States (Wan et al. 2016), were analyzed using the new approach, and the results were compared with those obtained from the analysis of the binary categorized virulence (V) and avirulence (A) data. The three most abundant *P. striiformis* f. *tritici* races (PSTv-11, PSTv-14, and PSTv-37) were chosen. The numbers of obtained IT profiles and different IT phenotypes of isolates of the three races collected in 2011 and 2012 are presented in Table 2. The minimum numbers of the IT profiles of isolates and IT phenotypes were obtained for race PSTv-14 in 2011 (16 and 14, respectively), while the corresponding maximum numbers were for race PSTv-37 in 2012 (151 and 78, respectively). Different IT phenotypes were used as basic entities for measuring functional variation within each race. The effective numbers of IT phenotypes expressed by the FTDis metric (4) and its normalized version (5) demonstrate significant differences between the functional variation within distinct races in each separate year and between the same races in different years (the normalized FTDis metric is more relevant here because of considerably different numbers of IT phenotypes for each race). The most prominent result here is that the functional variation (normalized FTDis) of all races was much higher in 2011 than in 2012; approximately 10, 75, and 547% for races PSTv-11, PSTv-14, and

PSTv-37, respectively (Table 2). Thus, the IT data allowed for analyzing variation within a group of isolates with the same virulence phenotype.

Relationships between isolates of the barley powdery mildew pathogen in the Czech Republic. Random samples of powdery mildew caused by the airborne fungus *B. graminis* f. sp. *hordei* originating from cultivated spring and winter barley fields in the Czech Republic were collected from the air in 2016 and 2017 (Dreiseitl 2019). Isolates of the pathogen were tested on a set of 45 barley differential varieties comprising genotypes carrying different resistance genes against *B. graminis* f. sp. *hordei* (Dreiseitl 2019 [Table 1]). The IT based on the reaction of each differential to an isolate was scored 8 days after inoculation on a scale of 0 to 4 (Torp et al. 1978), where IT scores 4 or 3–4 (= strong mycelial growth and strong sporulation on leaf segments) were considered virulent to the corresponding resistance genes. The original scores from 0 to 4 were transformed to the corresponding scores in the 0-to-9 assessment scale.

The IT profiles of 100 and 115 isolates of *B. graminis* f. sp. *hordei* in the Czech Republic were obtained in 2016 and 2017, respectively. These data were transformed to binary avirulence or virulence (a/v) data, so that isolates with IT scores from 0 to 6 were interpreted as avirulent and those with IT scores from 7 to 9 as virulent. Structure of relationships between isolates was analyzed with both types of data using unweighted pair-group method with arithmetic mean (UPGMA) dendrograms generated by the SAHN program of the NTSYSpc package (version 2.2; Exeter Software, Setauket, NY, U.S.A.). Distances between IT profiles were calculated according to equation 1 and dissimilarities between IT scores as in Table 1, while distances between virulence profiles were calculated with the simple mismatch coefficient. The UPGMA dendrograms with regard to the corresponding distances were constructed, and their schematic forms are presented in Figure 1. The annual *B. graminis* f. sp. *hordei* populations are nearly perfectly separated with IT data (Fig. 1A), with only two isolates assigned to a wrong cluster (there were seven outliers). On the other hand, two distinct clusters obtained with binary a/v data included isolates from both the annual populations (Fig. 1B), with isolates from 2016 and 2017 separated within each of the clusters and 31 isolates allocated to the wrong group (there were 9 outliers). Thus, the IT data allowed for superior separation between the Czech *B. graminis* f. sp. *hordei* populations in 2016 and 2017 when compared with the corresponding binary a/v data.

Relationships between regional populations of the wheat leaf rust pathogen in Canada. The leaf rust fungus (*P. tritici* Erikss.) was sampled throughout Canada to conduct national virulence surveys in 2007 (McCallum et al. 2010) and 2008 (McCallum et al. 2011). Urediniospores were scraped from infected leaves and used to inoculate a universal susceptible wheat line; then, single-pustule isolates were made from the subsequent infections. All isolates were tested for virulence at the seedling stage on the North American standard set of 16 Thatcher near-isogenic wheat lines each with a different resistance gene (*Lr1*, *Lr2a*, *Lr2c*, *Lr3*, *Lr9*, *Lr16*, *Lr24*, *Lr26*, *Lr3ka*, *Lr11*, *Lr17*, *Lr30*, *LrB*, *Lr10*, *Lr14a*,

and *Lr18*) and also on 12 supplementary Thatcher differentials with different genes (*Lr2b*, *Lr3bg*, *Lr14b*, *Lr15*, *Lr19*, *Lr20*, *Lr21*, *Lr23*, *Lr25*, *Lr28*, *Lr29*, and *Lr32*). The isolates used in the current study constitute a subset of all the isolates obtained in 2007 and 2008, chosen to represent all the unique virulence phenotypes found on

Fig. 1. Schematic forms of the unweighted pair-group method with arithmetic mean dendrograms of 215 isolates of *Blumeria graminis* f. sp. *hordei* in 2016 and 2017 (100 and 115 isolates, respectively) for **A**, infection type and **B**, virulence data. **A**, Two isolates from 2017 were allocated in the 2016 cluster, whereas the 2017 cluster did not include any isolate of 2016. Number of outliers = 7 (2 from 2016 and 5 from 2017). **B**, In all, 25 isolates from 2017 were allocated in the 2016 clusters, whereas the 2017 clusters included 6 isolates from 2016. Number of outliers = 9 (2 from 2016 and 7 from 2017).

TABLE 2. Functional variation of infection type (IT) phenotypes within three *Puccinia striiformis* f. sp. *tritici* races collected in the United States in 2011 and 2012

Race	Number of IT profiles or phenotypes							
	2011				2012			
	Isolates ^a	Actual ^b	Effective ^c	Normalized ^d	Isolates ^a	Actual ^b	Effective ^c	Normalized ^d
PSTv-11	100	39	2.07	0.053	33	24	1.14	0.048
PSTv-14	16	14	1.35	0.096	22	19	1.1	0.058
PSTv-37	19	16	1.67	0.104	151	78	1.51	0.019

^a Number of isolates collected and tested with available IT profiles.
^b Number of different IT phenotypes.
^c Functional-Trait Dispersion ¹*D*(*TM*) (equation 4).
^d Normalized version of Functional-Trait Dispersion ¹*ND*(*TM*) (equation 5).

the North American standard differential set, in each growing region. ITs were rated using a 0-to-4 scale where ‘0’ (no symptoms), ‘;’ (hypersensitive flecks), ‘1’ (small uredinia with necrosis), ‘2’ (small to medium sized uredinia with chlorosis), and ‘X’ (mesothetic or a mixture of pustule sizes) were considered avirulent, and those that produced ITs ‘3’ (medium-sized uredinia without chlorosis or necrosis) and ‘4’ (large uredinia without chlorosis or necrosis) were considered virulent. ITs with uredinia slightly smaller than normal are indicated by ‘-’ or ‘=’ and those slightly larger than normal by ‘+’. These ITs were converted to a 0-to-9 scale using the following conversion (‘ITs’ = scale value); ‘0’ = 0, ‘0;’ = 1, ‘;1’ = 2, ‘1-’ = 3, ‘1/1+’ = 4, ‘2-’ = 5, ‘2/2+’ = 6, ‘3-’ = 7, ‘3/3+’ = 8, and ‘4’ = 9.

The numbers of IT phenotypes detected in separate regional populations of *P. tritricina* varied from 3 in Prince Edward Island in 2007 to 41 in Manitoba and Saskatchewan in 2008 (Table 3). The corresponding a/v profiles were generated by interpreting isolates with IT scores from 0 to 6 as avirulent and those with IT scores from 7 to 9 as virulent. Because the same virulence phenotype could be obtained from several different IT profiles, the number of virulence phenotypes in a population was usually smaller than that of the corresponding IT phenotypes (e.g., 9 and 15, respectively, in Ontario in 2007) (Table 3). Despite the fact that the sample sizes are not big enough to justify valid comparison between the populations, they do allow weighting of the results obtained with IT data against the corresponding virulence data.

First, distances between IT profiles were calculated according to equation 1 and dissimilarities between IT scores as in Table 1, while distances between virulence profiles were calculated with the simple mismatch coefficient. Then, $KB_d(P_1, P_2)$ and $KB_m(P_1, P_2)$ distances between each pair of populations P_1 and P_2 were computed with regard to distance d (equation 1) and the simple mismatch coefficient (m) between IT and virulence phenotypes, respectively (Kosman 2014 [equation 17]; Kosman and Leonard 2007 [equation 5]). Second, the UPGMA dendrograms, with regard to the corresponding KB distances, were constructed (Fig. 2) to show structural relationships between the populations. The results clearly demonstrate that the IT data provide much better separation between the annual *P. tritricina* populations of 2007 and 2008 in Canada than the corresponding binary a/v data.

Populations of *P. tritricina* on wheat in Israel and Russia, and populations of the cucurbit powdery mildew pathogen in the Czech Republic. Annual and regional populations of *P. tritricina* in Israel and Russia, respectively, on wheat and isolates of the cucurbit powdery mildew pathogen *Podosphaera xanthii* (Castagne) U. Braun & Shishkoff collected in the Czech Republic in 2010 were studied for both the IT and virulence phenotypes. In Israel, isolates collected in 2010 to 2014 were tested on a set of 15 differentials (Thatcher near-isogenic wheat lines), whereas the number of IT phenotypes varied from 42 in 2014 to 102 in 2012 (unpublished data; Kosman et al. 2014). In Russia, isolates collected from six regions in 2016 (Dagestan, Central Europe, Northwest, Ural, West Siberia, and Volga) were tested on a set of 20 differential lines, whereas the number of isolates varied from 20 in Dagestan to 182 in Ural (unpublished data; Gulyaeva et al. (2017)). In both

TABLE 3. Number of infection type (IT) and avirulence/virulence (a/v) phenotypes in regional populations of *Puccinia tritricina* in Canada in 2007 and 2008^a

Year	Phenotype	AB	BC	MB_SK	ON	PEI	QC	Total
2007	IT	...	6	34	15	3	10	68
	a/v	...	4	23	9	3	7	46
2008	IT	6	...	41	27	11	5	90
	a/v	6	...	31	25	10	5	77

^a AB = Alberta, BC = British Columbia, MB_SK = Manitoba and Saskatchewan, ON = Ontario, PEI = Prince Edward Island, and QC = Quebec.

cases, KB distances between populations (annual and regional, respectively) were calculated with regard to the relevant distances between phenotypes for IT and virulence data (as in the previous section), and the corresponding UPGMA dendrograms were constructed. The relationships between the *Puccinia tritricina* populations obtained with the IT and virulence phenotypes were similar (results not shown).

The *Podosphaera xanthii* isolates were tested on a set of 21 differentials (Lebeda et al. 2018), and 26 IT phenotypes were detected. Distances between IT phenotypes were calculated according to equation 1, while distances between the corresponding virulence phenotypes were determined with the simple mismatch coefficient (in this case, the borderline IT score 6 was interpreted as a virulence reaction). The UPGMA dendrograms did not reveal significant differences in relationships among the *P. xanthii* isolates disclosed with IT and virulence data (results are not shown).

Fig. 2. Unweighted pair-group method with arithmetic mean dendrograms of regional populations of *Puccinia tritricina* in Canada in 2007 and 2008 for **A**, infection type (IT) and **B**, virulence data. Assignment-based KB distances between populations with regard to the distance between IT profiles of isolates (equation 1) and the simple mismatch coefficient were used for generating the dendrograms in **A** and **B**, respectively. Abbreviations: AB = Alberta, BC = British Columbia, MB_SK = Manitoba and Saskatchewan, ON = Ontario, PEI = Prince Edward Island, and QC = Quebec; for example, ON_07 designates population from Ontario in 2007.

DISCUSSION

IT data are based on originally recorded symptoms and signs of plant–pathogen interactions. They usually have been transformed to binary a/v data, to contrast the effects of the interaction between pathogens and their host plants. Nevertheless, a wide range of symptoms and signs observed can be interpreted as virulence or avirulence reactions. Therefore, transformation of ITs into a/v categories reduces the resolution of the actual extent of these plant–pathogen interactions.

The suggested new approach to analyzing a plant–pathogen interaction using IT data allows for retaining and processing all available information regarding the IT profiles of isolates. The key issue here is the selection of an appropriate assessment scale and determining dissimilarities between IT scores. Both the scale and dissimilarities are actually results of expert decision and estimates, respectively, that must become an agreeable universal standard for a given plant–pathogen system but should not necessarily be the same for different systems. However, we believe that the following property is generally required: dissimilarities between IT scores from the same avirulence or virulence domain (race) do not exceed those from different domains. One possible example of such dissimilarities is presented in Table 1. It should be noted that assessment scales and dissimilarities between scores for IT or virulence analyses differ from those for assessing plant disease severity in field experiments and epidemiological studies (Chiang et al. 2014). For virulence analysis, samples from infected host plants are collected in field experiments only once in a season; the obtained spores of separate isolates are used to inoculate seedlings of differential hosts in lab or greenhouse conditions, and IT scores are recorded in a few days (usually approximately 2 weeks). Diverse symptoms of infection may have a stronger effect on determining IT scores than the proportion of a plant unit diseased.

It was shown how dissimilarities between IT scores could be incorporated to measure distances between OUs at different hierarchical levels (isolates, races, populations, and so on). In parallel, metrics of functional variation based on the corresponding distances between OUs (equations 4, 4', and 5) were introduced for measuring variation of plant pathogens. The newly developed tools were applied to analyses of a few plant–pathogen systems with the idea to compare results obtained with IT data against the standard a/v data.

Intuitively, if variability of IT scores within each avirulence or virulence domain is low for the most differential lines/cultivars, then outcomes attained with IT and a/v data are expected to be similar with increasing resemblance at higher hierarchical levels. This was the case with the data from the wheat–*Puccinia triticina* pathosystem in Israel and Russia and the cucurbit–*Podosphaera xanthii* pathosystem in the Czech Republic (e.g., correlations of distances based on IT and a/v data between the phenotypes and annual populations of *Puccinia triticina* in Israel were 0.987 and 0.998, respectively; the correlation was 0.968 for the *Podosphaera xanthii* phenotypes). On the other hand, if variability of IT scores within each avirulence or virulence domain is high, then discrepancies between results achieved with IT and a/v data are expected to be significant and even become stronger at higher hierarchical levels. This was established with the isolates of *B. graminis* f. sp. *hordei* in the Czech Republic (the correlation between distances based on IT and a/v data were 0.896) and the isolates of *Puccinia triticina* in Canada (the correlations for distances based on IT and a/v data between the phenotypes and regional populations were 0.899 and 0.716, respectively). It is worthwhile to note that a relatively high correlation between two distance matrices (e.g., approximately 0.9) does not necessarily result in a congruency of structural relationships among OUs predetermined by those matrices. This is because small pairwise distances affect the structure much more strongly than larger distances but such an integral parameter as correlation is not

generally able to grasp this effect. Therefore, for example, somewhat different relationships between the isolates of *B. graminis* f. sp. *hordei* were uncovered for the IT and virulence phenotypes (Fig. 1) despite of the relatively high correlation (0.896) between the corresponding distance matrices.

Analyses from the three data sets at different hierarchical levels (the single virulence phenotypes of *P. striiformis* f. *tritici*, the isolates of *B. graminis* f. sp. *hordei*, and the regional populations of *P. triticina* in Canada) clearly demonstrated discrepancies of results obtained with IT and a/v data. The stronger annual differences were established with IT data in the three systems studied. Virulence phenotypes (races) are mainly predetermined by resistance genes deployed in host plants (Brown 2002). On the other hand, variation of IT phenotypes within the same race may result from variable fitness of both pathogen and host plant, which depends on agricultural practice and environmental and climate conditions. Assuming that agricultural practices do not change significantly in two consecutive years, discrepancies between structural variation within pathogen OUs determined by IT versus standard a/v data can be attributed to the changing environment. In addition to the virulent IT, there is often more than one distinct avirulent IT for a group of pathogen isolates inoculated onto a given host resistance line, containing a given resistance gene. This can be due to some isolates being homozygous and some being heterozygous for the corresponding avirulence gene. This difference is reflected in the IT data but not in the binary a/v data. The IT data has a lower score for isolates that elicit stronger host resistance compared with a slightly higher score for those that elicit a weaker host resistance, whereas the a/v data does not distinguish between more and less resistant host reactions and scores isolates with a wide range of host resistance as simply avirulent (Kolmer and Dyck 1994). Therefore, IT phenotypes allow consideration of the environment-dependent functional component of plant–pathogen interactions, along with the genetic component. This seems to be a decisive argument in favor of studying plant pathogen epidemics based on IT phenotypes rather than the corresponding virulence ones.

We do believe that analyses of plant–pathogen interactions with IT data are preferable as compared with the standard a/v data. However, it is also obvious that IT data collection is more laborious because it requires (i) higher sampling efforts; (ii) accuracy, disambiguation, and standardization of IT assessments and records by raters; and (iii) postulating a proper universal assessment scale and dissimilarities between IT scores for a given plant–pathogen system. Therefore, despite the fact that the introduced paradigm of functional IT variation has apparent theoretical advantages, the potential applied effectiveness of IT in epidemiological studies and disease management needs practical testing and validation.

Finally, metrics of functional variation (e.g., FTDis and its normalized version; equations 4, 4', and 5) are suggested for analyzing different OUs of plant pathogens. We used the metrics to measure IT variation within *P. striiformis* f. *tritici* races (Table 2). Comparing the newly proposed approaches with the standard ones commonly used in plant pathology, we think that determining variation within and among OUs in terms of effective numbers provides more transparent numerical estimates that incorporate both the diversity and dispersion perspectives of variation.

CONCLUSIONS

Analyzing IT variation may allow better utilization of information available in raw data and brings new knowledge, providing a different vision of a pathogen population as compared with the standard virulence data.

Metrics of functional variation (e.g., FTDis and its normalized version) could be useful and more effective for measuring variation of plant pathogens at different hierarchical levels as compared with standard approaches to diversity analysis.

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LITERATURE CITED

- Brown, J. K. M. 1998. Surveys of variation in pathogen populations and their application to disease control. Pages 73-102 in: *The Epidemiology of Plant Diseases*. G. D. Jones, ed. Kluwer Academic Publishers, Dordrecht, The Netherlands.
- Brown, J. K. M. 2002. Comparative genetics of avirulence and fungicide resistance in the powdery mildew fungi. Pages 56-65 in: *The Powdery Mildews, A Comprehensive Treatise*. R. R. Bélanger, W. R. Bushnell, A. J. Dik, and T. L. W. Carver, eds. The American Phytopathological Society, St. Paul, MN, U.S.A.
- Brown, J. K. M. 2006. Surveys of variation in virulence and fungicide resistance and their application to disease control. Pages 81-115 in: *The Epidemiology of Plant Diseases*. B. M. Cooke, D. G. Jones, and B. Kaye, eds. Springer, Dordrecht, The Netherlands.
- Chiang, K.-S., Liu, S.-C., Bock, C. H., and Gottwald, T. R. 2014. What interval characteristics make a good categorical disease assessment scale? *Phytopathology* 104:575-585.
- Chiu, C.-H., and Chao, A. 2014. Distance-based functional diversity measures and their decomposition: A framework based on Hill numbers. *PLoS One* 9:e100014.
- Dreiseitl, A. 2019. Great pathotype diversity and reduced virulence complexity in a Central European population of *Blumeria graminis* f. sp. *hordei* in 2015–2017. *Eur. J. Plant Pathol.* 153:801-811.
- Gregorius, H.-R., and Kosman, E. 2017. On the notion of dispersion—From dispersion to diversity. *Methods Ecol. Evol.* 8:278-287.
- Gregorius, H.-R., and Kosman, E. 2018. Structural type diversity: Measuring structuredness of communities by type diversity. *Theor. Ecol.* 11:383-394.
- Gulyaeva, E. I., Aristova, M. K., Shaidayuk, E. L., Mironenko, N. V., Kazartsev, I. A., Akhmetova, A., and Kosman, E. 2017. Genetic differentiation of *Puccinia triticina* Erikss. in Russia. *Russ. J. Genet.* 53:998-1005.
- Kolmer, J. A., and Dyck, P. L. 1994. Gene expression in the *Triticum aestivum*–*Puccinia recondita* f. sp. *tritici* gene-for-gene system. *Phytopathology* 84:437-440.
- Kosman, E. 1996. Difference and diversity of plant pathogen populations. A new approach for measuring. *Phytopathology* 86:1152-1155.
- Kosman, E. 2014. Measuring diversity: From individuals to populations. *Eur. J. Plant Pathol.* 138:467-486.
- Kosman, E., Ben-Yehuda, P., and Manisterski, J. 2014. Diversity of virulence phenotypes among annual populations of wheat leaf rust in Israel from 1993 to 2008. *Plant Pathol.* 63:563-571.
- Kosman, E., and Leonard, K. J. 2007. Conceptual analysis of methods applied to assessment of diversity within and distance between populations with asexual or mixed mode of reproduction. *New Phytol.* 174:683-696.
- Lebeda, A., Křístková, E., Sedláková, B., McCreight, J. D., and Kosman, E. 2018. Virulence variation of cucurbit powdery mildews in the Czech Republic – population approach. *Eur. J. Plant Pathol.* 152:309-326.
- Legrasa, G., and Gaertner, J.-C. 2018. Assessing functional evenness with the FEve index: A word of warning. *Ecol. Indic.* 90:257-260.
- Leonard, K. J. 1969. Factors affecting rates of stem rust increase in mixed plantings of susceptible and resistant oat varieties. *Phytopathology* 59:1845-1850.
- Line, R. F., and Qayoum, A. 1992. Virulence, aggressiveness, evolution, and distribution of races of *Puccinia striiformis* (the cause of stripe rust of wheat) in North America, 1968-87. U.S. Dep. Agric. Tech. Bull. No. 1788.
- McCallum, B. D., Seto-Goh, P., and Xue, A. 2010. Physiologic specialization of *Puccinia triticina*, in Canada in 2007. *Can. J. Plant Pathol.* 32:229-236.
- McCallum, B. D., Seto-Goh, P., and Xue, A. 2011. Physiologic specialization of *Puccinia triticina*, the causal agent of wheat leaf rust, in Canada in 2008. *Can. J. Plant Pathol.* 33:541-549.
- McIntosh, R. A., Wellington, C. R., and Park, R. F. 1995. *Wheat Rusts: An Atlas of Resistance Genes*. CSIRO, Sydney, NSW, Australia, and Kluwer Academic Publishers, Dordrecht, The Netherlands.
- Rao, C. R. 1982. Diversity and dissimilarity coefficients: A unified approach. *Theor. Popul. Biol.* 21:24-43.
- Ricotta, C., Bacaro, G., and Moretti, M. 2014. A new measure of functional evenness and some of its properties. *PLoS One* 9:e104060.
- Scheiner, S. M., Kosman, E., Presley, S. J., and Willig, M. R. 2017a. Decomposing functional diversity. *Methods Ecol. Evol.* 8:809-820.
- Scheiner, S. M., Kosman, E., Presley, S. J., and Willig, M. R. 2017b. The components of biodiversity, with a particular focus on phylogenetic information. *Ecol. Evol.* 7:6444-6454.
- Torp, J. H., Jensen, P., and Jørgensen, J. H. 1978. Powdery mildew resistance genes in 106 northwest European spring barley varieties. Pages 75-102 in: *Royal Veterinary and Agricultural University Yearbook 1978*. Copenhagen, Denmark.
- Villéger, S., Mason, N. W. H., and Mouillot, D. 2008. New multidimensional functional diversity indices for a multifaceted framework in functional ecology. *Ecology* 89:2290-2301.
- Wan, A. M., Chen, X. M., and Yuen, J. 2016. Races of *Puccinia striiformis* f. sp. *tritici* in the United States in 2011 and 2012 and comparison with races in 2010. *Plant Dis.* 100:966-975.