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Diversity, spatial variation, and temporal dynamics of virulences in the German leaf rust (*Puccinia recondita* f. sp. *secalis*) population in winter rye

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Abstract A large collection of German rye leaf rust isolates was analysed to characterize the diversity, spatial variation and temporal dynamics of virulences. Virulence-avirulence phenotypes (=pathotypes) were determined on 23 host differentials. We found 93 pathotypes among 177 single-uredinial isolates in 2000, 201 pathotypes among 437 isolates in 2001, and 125 pathotypes among 213 isolates in 2002. In total, the 827 analyzed isolates represented 317 pathotypes. Frequency of virulences on the individual differentials varied from 2% to 97%. Eight of the differentials showed a high resistance level with virulence frequencies <10%. Virulence complexity of the isolates ranged from 3 to 21 with a mean of nine. The percentages of highly virulent isolates (>14 virulences) increased from 4 to 15% during the sampling period. A high level of virulence diversity was observed within and between individual sampling sites with Simpson indices around 0.9. Evenness indices ranged from 0.88 to 0.92. Four of the five most frequent pathotypes were found in each year but their frequency never exceeded 10%. Isolates with unusual virulence combinations could be clearly separated by principal component analysis. Location-specific pathotype frequencies were revealed in each year, but the frequency patterns varied across years. On four fields a considerable increase of highly virulent pathotypes occurred within 6 weeks during the epidemic. The high diversity of pathotypes as well as the fast accumulation of highly virulent

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pathotypes favour the adaptation of the pathogen to race-specific host resistances. More durable resistance might be achievable by combining new effective race-specific resistances with adult-plant and/or race-non-specific quantitative resistances.

Keywords Complexity · Diversity · Leaf rust · Pathotype · Populations · Rye · Virulence

Introduction

Leaf rust (*Puccinia recondita* f. sp. *secalis* Roberge ex Desmaz) is one of the most common airborne pathogens of rye (*Secale cereale* L.) and appears regularly in all rye-growing areas. Sexual reproduction may occur on the alternate hosts *Anchusa* spp. and *Echium* spp. These wild plants are frequently found adjacent to agricultural fields in Central Europe. Rye leaf rust has recently been divided from wheat leaf rust, *Puccinia triticina*, due to differences in spore morphology and the failure to intercross both species (Anikster et al. 1997).

Winter rye is an important cereal crop in Middle Europe with an acreage of 750.000 ha in Germany and 1.4 million ha in Poland in 2009 (FAO 2010). Rye is the only cross-pollinating crop among the small-grain cereals with a strong self-incompatibility system. In Germany, about 50% of the total rye acreage is devoted to hybrid cultivars, the other half to population cultivars. Both types of cultivars, however, represent highly heterogeneous stands due to the outcrossing nature of rye and because the hybrids are composed of several lines (Geiger and Miedaner 2009).

Inoculation experiments resulted in losses of thousand-kernel weight ranging from 11 to 27% (Miedaner and Sperling 1995). In continental climates the epidemics of rye leaf rust start much earlier and cause yield losses up to 40% (Kobylanski and Solodukhina 1996). The application of fungicides may cause 29% higher yield in years with an early start of the epidemic compared with untreated plots (Hartleb et al. 1995). Apart from fungicidal treatment, breeding for resistance is the most economically and environmentally effective protection against leaf rust.

At the beginning of this study, all widely grown hybrid and some population cultivars in Germany were moderately susceptible (score 5–6 on the 1–9 scale with 1 = healthy), the population cultivars Amilo, Born, Danko, and Motto were moderately resistant (score 3–4, Anonymous 1998). The basis of resistance of all commercial rye cultivars, including their race-specific resistance genes, has never been analyzed. This is astonishing, because several leaf rust resistance genes from rye have been successfully used in wheat (Roelfs et al. 1992). A genetic study showed that most of the breeders' lines selected for leaf-rust resistance contained race-specific resistances (Miedaner et al. 2002). Genetic analysis of race-specific resistance genes in the pathosystem rye/leaf rust is still in its infancy, leading to five chromosomally located and characterized genes from breeders' material only (Wehling et al. 2003, Roux et al. 2004, Klocke 2004) that were, however, not known at the beginning of this study in 2000.

The long-term success of using monogenic, race-specific resistances depends on the structure and dynamics of the leaf rust populations. Mains (1926) and Gassner and Kirchhoff (1934) already recognized the presence of different pathotypes of rye leaf rust but systematic virulence analyses could not be conducted because of the absence of a suitable differential set in the cross-pollinating rye. With the development of hybrid breeding, a differential set on the basis of homozygous inbred lines and a few full-sib families was developed (Leßner and Sperling 1995). By hybrid breeding it is for the first time possible to introgress monogenic traits effectively in rye. Because hybrid cultivars consist of at least four inbred lines (Geiger and Miedaner 2009), it would be easy to combine several race-specific resistances in the same cultivar for potentially prolonging their durability. This concept has been suggested for self-pollinating crops by propagating cultivar mixtures (Wolfe and Finckh 1997). Whether it will work in rye largely depends on the adaptability of the local leaf-rust populations.

The objectives of this study were to: (1) analyse pathotype frequency and complexity of rye leaf rust across regions, and within individual fields and (2) establish spatial and temporal differences of virulences to test whether the use of race-specific resistances individually or in combinations might be a useful concept for hybrid rye breeding. In the following, we call a monosporic line 'isolate'. After determining its avirulence structure it is classified as 'pathotype'. It should be noted that, in contrast to other rust

pathosystems, a differential set for rye/leaf rust is a recent achievement and it is still not fully characterized concerning the race-specific genes it involves. Therefore, this is the first comprehensive study on structure and dynamics of leaf-rust populations in rye.

Materials and methods

Sampling design

Three experiments were performed within this study on diversity of leaf-rust populations, their spatial variation and temporal dynamics.

For the diversity analysis, isolates were collected randomly in the years 2000 and 2001 by a jet spore sampler, which was mounted on the roof of a car (Schwarzbach 1979; Limpert et al. 1984). The car was driven through different rye growing areas in June and July. Spores sampled from the air settled on detached leaf segments of a susceptible cultivar that were placed in petri dishes containing water agar (5 g l^{-1}agar) and benzimidazol (35 mg l^{-1}). Additionally, isolates on susceptible genotypes from 20 environments were arbitrarily collected from visibly infected winter rye leaves in the years 2000 to 2002 to analyse the spatial distribution of virulences. The environments were either breeders' locations or experimental sites where we expected to find highly complex and diverse isolates as a worst-case scenario.

For detecting spatial variation within individual fields, samples were collected at Hohenheim near Stuttgart (HOH), Bad Schönborn near Karlsruhe (BSB), and Petkus near Jüterbog (PET). From a breeding nursery of 0.5-1 ha, 50 leaf-rust infected leaves were arbitrarily collected. For analysing temporal dynamics of virulences, isolates were collected at three to four sampling dates from specially designed field trials (see below).

Establishment of single-uredinial isolates

Individual pustules that developed on the leaf segments were firstly subcultured by pipette transfer onto new leaf segments of the susceptible hybrid cultivar Ursus. To induce infection, the plates were incubated at 100% relative humidity and 20°C in the dark for 20 h. Afterwards, they were maintained at 20°C with continuous light. Secondly, single-pustule isolates were established by diluting spores collected from infected leaves on a susceptible genotype and subculturing of individual developing uredospore pustules as described above. In total, 827 single-pustule isolates were analysed.

Plant material and field trials

The differential set consisted of 17 inbred lines and six full-sib families homogeneous for their leaf-rust reaction provided by Hybro GmbH & Co.KG, Schenkenberg, KWS LOCHOW GmbH, Bergen, Julius Kühn-Institut (JKI), Institute of Breeding Research on Agricultural Crops, Groß Lüsewitz, and State Plant Breeding Institute, Universität Hohenheim. Seventeen of these genotypes derived from the study of Leßner and Sperling (1995); six new pretested genotypes were added to improve differentiation. Two of the new entries were full-sib families derived from a leaf rust-resistant population obtained from the Agricultural Research Institute of the Non-Chernozem Zone at Nemchinovka near Moscow, Russia. This population had been improved for leaf-rust resistance over several cycles of mass selection.

Rye seedlings used for pathotyping isolates of *Puccinia recondita* f. sp. *secalis* were potted in peat moss and cultivated in a growth room at 20°C under continuous light.

For analysing the temporal dynamics within a prolonged growing period, a diverse set of 33 heterogeneous winter rye synthetics and two full-sib families were used. These genotypes were developed for and described in detail in a companion study (Wilde et al. 2006) where we wanted to test the effect of heterogeneous genotypes (simulating cultivar mixtures) on the rye leaf rust popula-

Table 1 Key for the rating of leaf rust symptoms of rye according to Frauenstein and Reichel (1978)

Infection type	Infection symptoms
1	No visible reactions
2	Chlorotic flecks
3	Small pustules with chlorotic halo
4	Small to medium pustules with chlorotic halo
5	Large pustules with chlorotic halo
6	Large pustules without chlorosis

tions. By taking more samples in a time sequence, we wanted to analyse the temporal dynamics in the study presented here. Some of the differentials (H22/7, H23/8, S4083) were included in the plant materials grown. Winter rye was planted in April at two locations in Southwestern Germany (Stuttgart-Hohenheim, Bad Schönborn) in 2001 and 2002. These non-vernalized rye plants remained in the vegetative state and developed an extremely high disease severity by natural infection during the season. Three to four sampling dates were achieved. At each date a representative number of visually infected leaves was randomly collected.

Detached leaf-segment test and disease assessment

Detached leaf segments of 8-days-old primary leaves of each differential genotype were placed in square plastic dishes with 12 compartments containing water agar (5 mg l^{-1}agar) complemented by benzimidazol (35 mg l^{-1}). Single-pustule isolates were applied to the differential set using a settling tower, which was placed over the plastic dishes with the leaf segments. Spores were sucked into an eyedropper pipette using a rubber teat, which was then removed. The wider end of the pipette was placed through a hole in the top of the settling tower and spores were blown into the tower with a 20-ml syringe connected to the narrow end of the pipette. Infection was induced as previously described. Nine days after inoculation, leaf segment reactions were assessed according to the Frauenstein and Reichel (1978) scale for infection types (Table 1). Infection types 1 to 4 were interpreted as incompatible (resistant/avirulent) and infection types 5 and 6 as compatible (susceptible/virulent).

Frequency of virulences on differentials and virulence complexity

Virulence structure of the leaf-rust population was described by the frequency of virulent isolates on 23 differentials and virulence complexity in the seedling stage. Host genotypes displaying frequencies of virulent isolates of 0–10% were classified as highly resistant, 11–20% as resistant, 21–50% as moderately resistant and >50% as susceptible (Felsenstein and Jaser 2000). Virulence complexity specifies the number of virulences per isolate and designates the ability of an isolate to infect a host with multiple resistance genes (Welz 1986). For interpretation, isolates with complexities of 0–6 were classified as weakly, 7–14 as moderately and 14–23 as highly virulent.

Virulence diversity

Virulence diversity was described by the Simpson (S) index calculated as

$$S = 1 - \sum (n_i^2 - n_i)/(N^2 - N),$$

where N is the number of tested isolates, n_i is the number of observed isolates of pathotype i (Müller et al. 1996). A pathotype is a specific combination of virulences. The Simpson index accounts for the number of different pathotypes (richness) in the population while the Evenness index (EH) is based on the Shannon index (H) and was estimated as:

$$EH = H/\ln R, \quad \text{with } H = -\sum P_i x \ln P_i,$$

where P_i is the frequency of the *i*th pathotype and R is the total number of pathotypes in the sample. If the pathotypes occur in similar frequencies EH=1, if they occur in highly varying frequencies the value will be 0. Because of the varying sample sizes, only Simpson and the Evenness indices were used to measure the diversity of leaf-rust populations.

Principal component analysis (PCoA)

A principal component analysis (PCoA) as a multivariate approach was done by SAS (SAS Institute Inc. 1999) on the whole data set. PCoA is a common technique for finding patterns in data of higher dimension (Smith 2002). A number of possibly correlated variables is transformed into a number of uncorrelated variables called principal components, related to the original variables by an orthogonal transformation. The first principal component explains the largest amount of variation in the data set. The second principal component defines the next largest amount of variation and so on (Böker 2010). Two PCoA were realized to detect similarities between the 827 German isolates based on their reactions to the 23 differential genotypes and also to detect similarities between the 23 differential genotypes based on their reactions to the 827 German isolates.

Results

Frequency of virulences on differentials and virulence complexity

Frequency of virulences of a total of 827 isolates on 23 differentials varied on average from 2 to 97% (Table 2). Ten out of the 23 differential genotypes were infected by more than 50% of the tested isolates, suggesting a low resistance level to leaf rust. Eight genotypes were infected by <10% of the isolates and showed therefore a high resistance level, but for most of these genotypes the percentage of corresponding isolates increased during the 3 years, e.g., from 5 and 6% to 17 and 18% for genotypes 94107 and 94108, respectively. For some inbred lines, virulences were found only rarely, but no genotype remained without leaf-rust infection across all isolates.

Table 2 Frequencies of virulent isolates (%) of the German leaf rust population during the years 2000–2002 on 23 differentials tested at the seedling stage with a total of 827 isolates

Genotype	Breeders ID	Year			Mean
		2000	2001	2002	
1	1684004[a]	99	95	97	97
2	1684047[a]	99	93	95	95
3	H22/7	95	95	96	95
4	1684161[a]	96	89	93	92
5	S4087	79	83	86	83
6	1684266[a]	86	75	85	80
7	9126	86	77	79	80
8	N9a/86[a]	73	77	80	77
9	H23/8	77	73	68	72
10	9084	53	47	59	51
11	N75/81[a]	38	37	40	38
12	94104	11	13	17	14
13	94108	6	16	18	14
14	H26	6	14	17	13
15	94107	5	13	17	12
16	P54	3	11	11	9
17	P53	3	9	6	7
18	H54/2	5	3	9	5
19	P52	10	3	1	4
20	H54/9	2	3	9	4
21	S4083	1	3	9	4
22	S4084	1	3	9	4
23	P51b	1	3	3	2
No of isolates		177	437	213	827

[a] Full-sib families homogeneous for leaf-rust reaction

Virulence complexity for all tested German isolates ranged from 3 to 21 across the 3 years (Fig. 1); the most frequent isolates displayed nine virulences. The average complexity increased slightly from 9.4 virulences in 2000 to 10.1 in 2002, but the percentage of highly virulent isolates increased considerably from 4 to 15%.

Pathotype diversity and structure

In total, 317 pathotypes, i.e., different virulence combinations, were found among the 827 analysed isolates (Table 3). Within each year, a very high level of diversity was found with Simpson indices near the maximum value of 1. Evenness indices also had high values ranging from 0.88 to 0.92, indicating that no dominant pathotypes occurred.

A closer look into the structure of pathotypes showed that four of the five most frequent pathotypes could be found in every year, except that pathotype C was replaced by pathotype F in 2002 (Table 4). Their frequency of occurrence, however, was low ranging from 2.8 to 9.6% only. Pathotypes A–E had nine to 11 virulences, the newly occurring pathotype F, however, had 20 virulences. This highly virulent pathotype F was not detected in 2000 and occupied rank 18 in 2001.

Analyzing the within-field diversity as the lowest level of population differentiation revealed an extremely high pathotype diversity at three locations (Table 5). The frequency of unique pathotypes ranged from 61 to 78%, being considerably higher than the overall frequency of 38% (see Table 3). Simpson and Eveness indices reached maximal values ranging from 0.92 to 0.99.

The first two components of the principal component analysis explained 30% of the phenotypic variance in 2000 and 41% in 2001 (Fig. 2). In 2002, the first and the third components were plotted for a better differentiation of the isolates and together explained 37% of the total variance. In all 3 years, most of the isolates had similar coordinates. Only those with extreme virulence combinations can be distinguished. The isolate E008 showing in 2000 the largest distance from the main group had a complex-

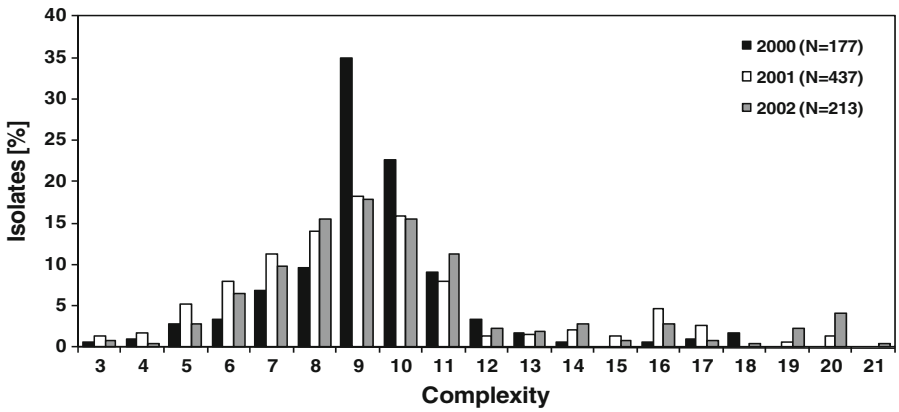

Fig. 1 Virulence complexity of 827 isolates of *Puccinia recondita* f. sp. *secalis* collected during the years 2000–2002 and tested on 23 differential cultivars at the seedling stage

ity of 18 and was the only isolate with virulence for the differentials S4083, S4084, H54/9 and H54/2. The identical isolates F008 and A016 possess also a high complexity of 18 and could infect the differentials P52, P53 and P54. Part of this group of highly complex isolates were C016, C014 and A015. In contrast, C013 and F006 were among the least virulent isolates infecting one and four differentials, respectively. In 2001, C162, C163, C166 and D109 showed identical reactions on the differential set and a similar distance from the main group because of their high virulence complexity of 20. To this group belong also the isolates H136 and H144 with complexities of 20 and 19, respectively. G101 and I110, on the other hand, had the largest distance to highly virulent isolates and were avirulent on all but one differential. In 2002, H272 was the most complex isolate infecting 21 of the 23 differentials. The isolates H249 and C214 were the only isolates with combined virulence to the highly resistant differentials P51b and P52.

Spatial differences of virulences

The virulence situation in individual environments was complex and differed highly among locations and years (Fig. 3) as seen when only the most contrasting differentials were analyzed. Interestingly, there was no location-specific pattern across years. In Eckartweier (EWE) in 2001, for example, the leaf-rust population was highly virulent infecting even three highly resistant differentials (H26, P53, P54), whereas in the following year no virulent isolate occurred for these lines. Similarly, in Hohenheim (HOH) in 2002 virulences for two differentials (P54, S4084) occurred in high frequencies that were not found in the years before; a third isolate (H26) increased considerably in frequency. For two differentials, P51b and P52, extremely low virulence frequencies were found in all environments ranging from 0 to 6% except for Bergen (BER) in 2001, where 19 and 14% of isolates were virulent for these lines, respectively.

Temporal dynamics of virulences

Leaf-rust populations from spring-sown winter rye showed an increase in virulence complexity across sampling dates in four environments when sampled from 33 genetically diverse host genotypes (synthetics, Fig. 4). This was most obvious in 2001 at both locations, but the same tendency occurred to a lesser extent in 2002. At the Hohenheim 2001 location, for example, the percentage of highly

Table 3 Pathotype diversity in Germany for the years 2000 to 2002 and Simpson and Evenness indices tested at the seedling stage

Year	No. of isolates	No. of pathotypes	Unique pathotypes (%)	Diversity index	
				Simpson	Evenness
2000	177	93	53	0.97	0.88
2001	437	201	46	0.98	0.89
2002	213	125	59	0.99	0.92
Total	827	317	38	0.98	0.85

Table 4 Ranking of the five most frequent pathotypes (PT) in the 3 years in Germany and their percentage (%) of occurrence and virulence complexity (C) tested at the seedling stage

Rank	Total			2000			2001			2002		
	PT	Percent (%)	C	PT	Percent (%)	C	PT	Percent (%)	C	PT	Percent (%)	C
1	A	6.0	10	E	9.6	9	B	5.9	11	B	6.6	11
2	B	5.9	11	A	9.0	10	A	5.0	10	A	5.6	10
3	C	5.0	9	C	9.0	9	C	5.0	9	D	5.2	10
4	D	4.8	10	D	5.1	10	D	4.6	10	F	4.2	20
5	E	4.8	9	B	4.5	11	E	3.7	9	E	2.8	9

virulent isolates increased from 3% at the first date to 55% at the fourth date. At the first date, isolates with a maximum of 14 virulences were found; whereas at the last date isolates with up to 20 virulences occurred. Similarly, in Bad Schönborn the percentage of highly virulent isolates increased. The frequency of a pathotype with 16 virulences, for example, increased in 2001 from 9% at the first date to 41% at the last date, illustrating that in contrast to the general German leaf-rust population, dominant pathotypes occurred in this experiment. Similar to Hohenheim, isolates with maximal complexities of 20 virulences occurred in Bad Schönborn preferentially at the later sampling dates. In 2002, such an isolate already occurred at the first date in Bad Schönborn and its frequency rose to 10% by the fourth date.

Optimization of the differential set

For the differentials, the first two components of the principal component analysis explained 69% of the total variance (Fig. 5). The differential set can be clustered into two groups. Group I with genotypes H26, 94104, 94107, 94198, P53, P54, S4084, S4083, H54/2, H54/9, P51b and P52 could be infected by less than 14% of the 827 isolates. Group II is composed of the susceptible genotypes 9126, 1684266, 1684004, 1684161, 1684047, H23/8, S4087, N9a/86 and H22/7 which could be infected by more than 70% of the 827 isolates. The differentials 9084 and N75/81 are intermediate with frequencies of virulence of 51% and 38% on the differentials. Group I could be further subdivided. Differentials S4084, S4083, H54/2 and H54/9 showed only a few differences with regard to their resistance reactions to the 827 tested isolates indicating that they most likely have the same resistance genes. Similarly, differentials 94104, 94107, 94108 and H26 revealed only a few differences.

Discussion

Differential set

Virulence analysis of 827 isolates revealed a high complexity of virulences in the German leaf-rust population as measured by 23 differential rye genotypes, most of them inbred lines. Based on reaction of the differentials, different clusters could be found by principal component analysis (see Fig. 5). Especially in group I comprising the most resistant differentials, two subgroups can be observed. Differentials within each of these subgroups most likely have the same resistance gene(s). This could be demonstrated for the differentials S4084 and H54/9 that carry most probably the same resistance locus on chromosome 1RS revealed by genetic mapping (Wehling et al. 2003; Roux et al. 2004; Klocke 2004). Similarly,

Table 5 Within-field variation of number of isolates and pathotypes, percentage of unique pathotypes and diversity indices Shannon and Evenness in Hohenheim (HOH), Bad Schönborn (BSB), and Petkus (PET) 2002 tested at the seedling stage

Location	No. of isolates	No. of pathotypes	Unique pathotypes (%)	Diversity index	
				Simpson	Evenness
HOH	38	23	61	0.92	0.92
BSB	45	34	76	0.99	0.98
PET	45	35	78	0.98	0.97

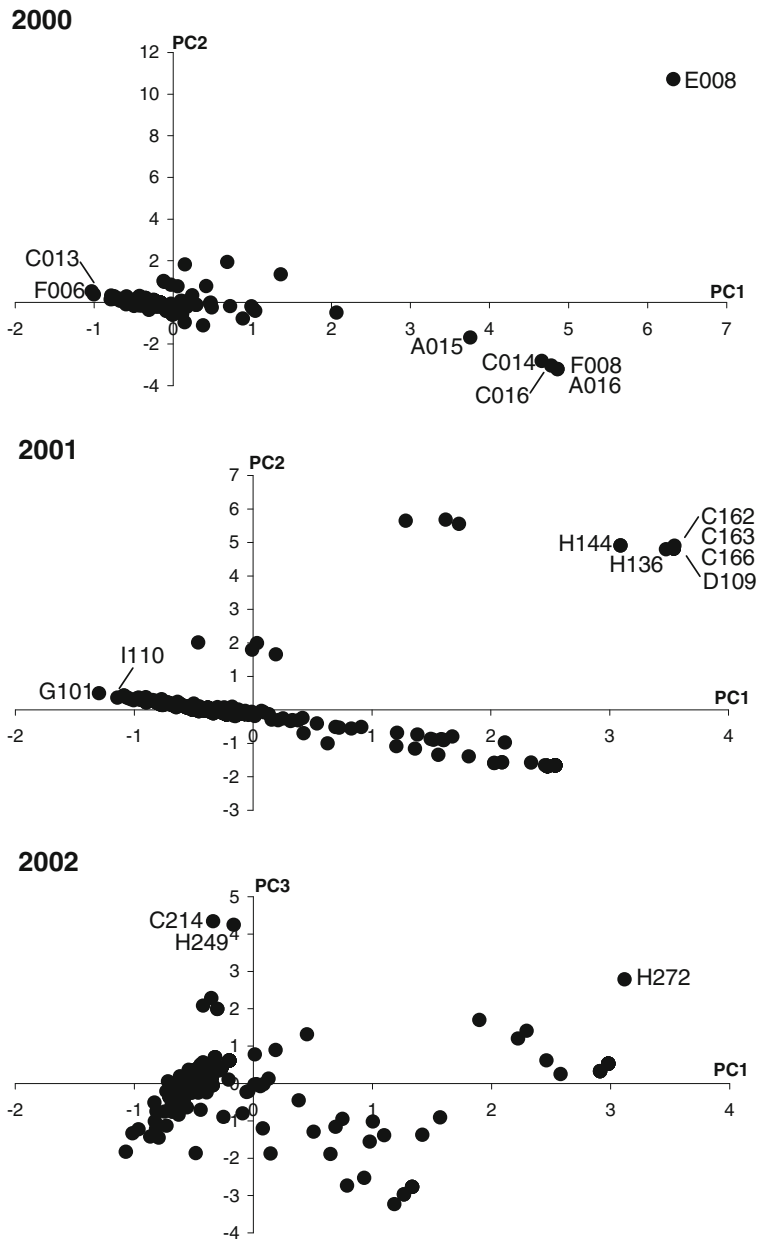

Fig. 2 Principal component analysis based on the virulences of 177, 437, and 213 isolates of *Puccinia recondita* f. sp. *secalis* in 2000, 2001, 2002, respectively

differentials H26 and 94107 of the second subgroup had an identical resistance locus on chromosome 4R (Klocke 2004). Assuming that the differentials clustering closely together also have the same resistance gene(s), the differential set could be reduced to 17. Because differentials of group II are highly susceptible showing differences only for a few isolates, the differential set might be further reduced for practical reasons to nine rye genotypes representing strongly deviating virulence combinations (see Fig. 3). This, however, does not affect the general outcome of this study because duplicates just increase absolute virulence complexity but do not considerably change the number of virulence combinations.

Diversity of virulences and pathotypes

In total, the 827 analyzed isolates represent 317 pathotypes with a mean virulence complexity of nine illustrating a high diversity in the German leaf rust

Differential genotype	BSB 2001	BSB 2002	EWE 2001	EWE 2002	HOH 2000	HOH 2001	HOH 2002	PET 2000	PET 2001	PET 2002	BEBU	BER 2001	BS 2001	DAH 2002	GL 2002	HAL 2002	RIE 2001	RL 2001	LZ 2001	120j. HAL
H23/8	52	60	97	56	100	50	89	90	76	64	59	95	50	88	65	52	67	73	93	67
9084	59	80	97	33	23	38	63	60	39	56	27	86	23	82	65	19	33	23	60	13
N75/81	33	40	35	22	31	25	53	50	44	38	36	52	19	35	57	26	50	27	27	27
H26	7	22	88	0	8	6	26	0	5	13	0	5	0	18	30	4	13	5	0	13
P54	7	4	74	0	0	13	26	0	10	7	0	0	0	0	35	4	4	9	0	7
P53	7	11	82	0	0	13	0	0	2	7	0	5	0	12	4	4	4	5	0	7
S4084	0	2	3	0	0	0	26	0	0	4	0	0	0	0	26	0	0	14	0	7
P52	0	0	0	6	0	0	3	0	2	2	0	14	4	0	0	0	4	0	7	0
P51b	4	4	0	6	0	0	3	0	0	4	0	19	0	0	4	0	4	5	0	0
N	*27*	*45*	*35*	*18*	*13*	*16*	*38*	*10*	*41*	*45*	*22*	*21*	*26*	*17*	*23*	*27*	*24*	*22*	*15*	*15*

51-100% 21-50% 0-20%

BSB = Bad Schönborn, EWE = Eckartsweier, HOH = Hohenheim, PET = Petkus, BEBU = Bernburg, BER = Bergen, BS = Braunschweig, DAH = Berlin-Dahlem, GL = Groß Lüsewitz, HAL = Halle, RIE = Rieste, RL = Rottmersleben, LZ = Lindau-Zerbst, 120j. Hal = ewiger Roggenanbau Halle

Fig. 3 Frequency of virulent isolates (%) on the nine most discriminating differentials from a total of 500 isolates (N) collected at 20 German rye-growing environments and tested at the seedling stage

population. Matching virulences were detected for all differentials and many of their combinations. The Evenness index showed high values indicating a similar pathotype composition among sampling areas. Accordingly, no dominating pathotype was found in the 3 years of investigation. Although four of the five

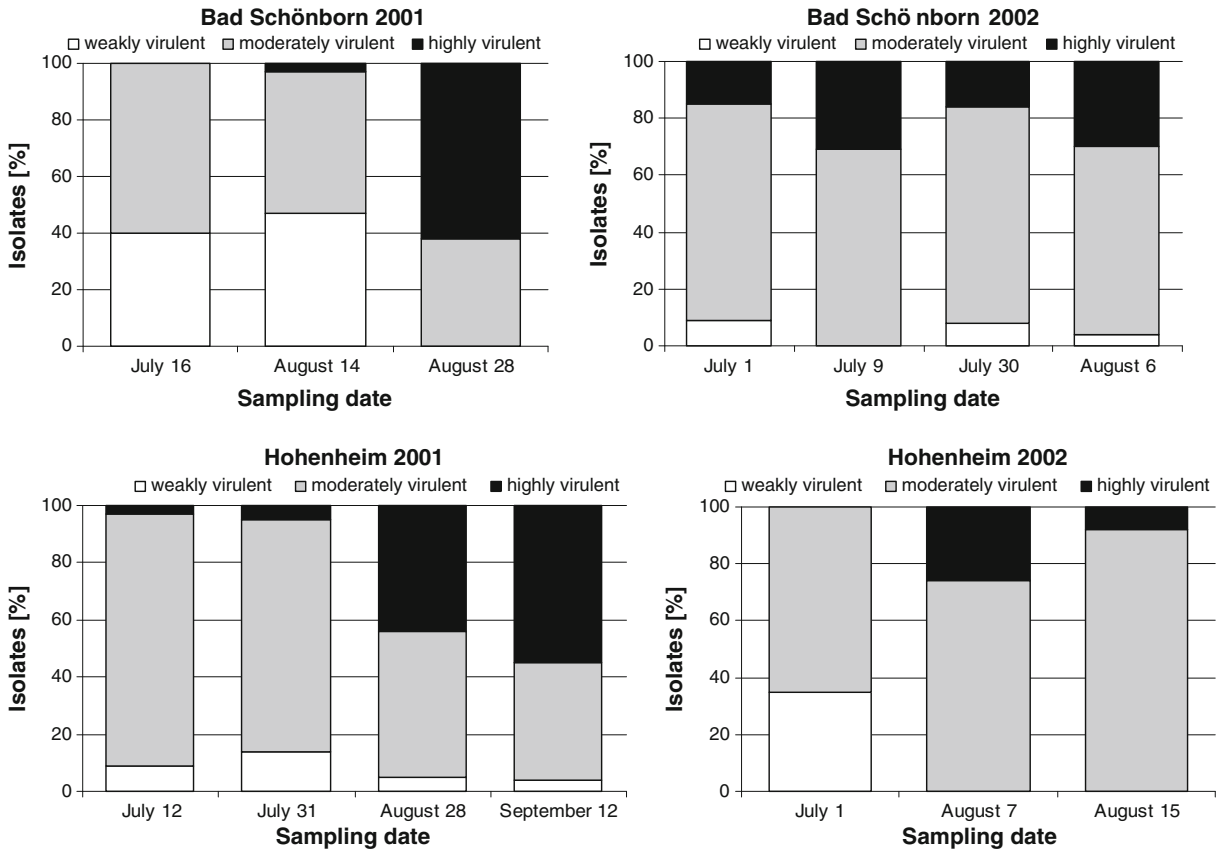

Fig. 4 Percentage of weakly, moderately and highly virulent isolates from samples (N=22–42) taken at three to four successive dates at two locations in 2 years and tested on 23 differentials at the seedling stage

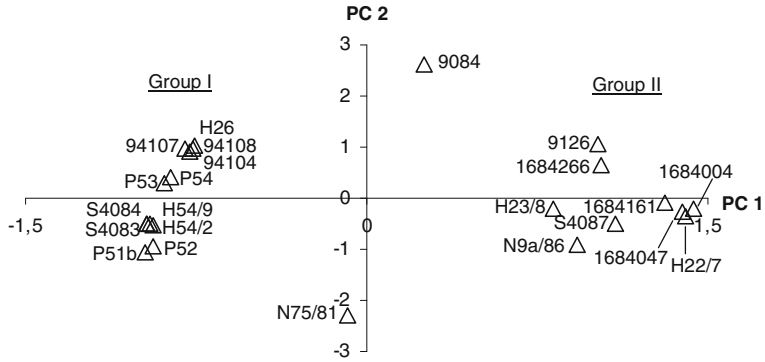

Fig. 5 Principal component analysis of 23 differentials based on their reaction to 827 German isolates at the seedling stage

most frequent pathotypes were detected in each of the 3 years, they accounted for only small percentages (2–10%). Principal component analysis (PCoA) confirmed that no dominating subpopulation existed in the leaf-rust population. While the Evenness and the Simpson indices ignore the number of virulence differences between the isolates (Kosman 1996), the multivariate procedure considers it. Results of the PCoA also demonstrate that most of the leaf-rust isolates differed only in one or a few virulences.

The presented results are in contrast to the composition of leaf-rust populations in wheat (*P. triticina*), where homogeneous cultivars with race-specific resistances are common in commercial growing. Accordingly, only a restricted number of pathotypes is found even across large geographic areas (Mesterházy et al. 2000, Long et al. 2002, McCallum et al. 2010). In Europe, the percentage of the four most frequently occurring pathotypes in wheat added up to 64% (Park and Felsenstein 1998; Mesterházy et al. 2000). The frequency of two dominating pathotypes in the South Atlantic States of the USA amounted to 73% of the total population (Kolmer 2002) and even one single pathotype reached 61% in the Canadian wheat leaf-rust population in 2007 (McCallum et al. 2010). Accordingly, a high rate of clonal reproduction was postulated by population analyses in France (Goyeau et al. 2007).

Temporal dynamics and spatial variation

The higher the virulence complexity and diversity of a population, the faster it can adapt to changes in the spectrum of race-specific host resistances (McDonald and Linde 2002). This was especially demonstrated in the spring-sown winter rye experiment. We planted 33 self-fertile rye synthetics containing race-specific resistances of varying complexity in four field environments (Wilde et al. 2006). This model experiment was designed as a worst-case scenario where the naturally occurring leaf-rust populations could parasitize rye plants that remained in the vegetative growth stage during the whole season. We expected that these conditions will accelerate leaf-rust epidemics and lead to greater changes in the pathotype composition than normal field conditions. The composition of leaf-rust populations indeed adapted quickly. The frequency of highly virulent isolates increased within weeks up to 60%, especially in 2001. The most complex isolate found in this experiment was H272 (Fig. 2) infecting all differentials except P51b and P53. But even for these highly resistant inbred lines other virulent isolates were detected.

Host-specific changes were observed among the individual differentials when analysing the leaf-rust populations at several observation dates. In HOH2001, frequencies of virulence on the differentials H26 and S4084 increased from about 1% at the first sampling date to >50% at the fourth date illustrating the crucial role of selection of pathotypes by resistant hosts. Also for the highly resistant differentials P53 and P54, the frequency of virulences increased by 12% although they were not grown in this experiment. Possibly, the matching virulences occurred together with some of the used virulences resulting in a "hitchhiking effect" as described by Hau and de Vallavieille-Pope (2006). Interestingly, the highly virulent pathogen populations at the end of the natural epidemic in 2001 were much less virulent at the beginning of the second year 2002 (Fig. 4). This suggests that a negative trade-off between virulence and other fitness parameters exists as shown by Thrall and Burdon (2003) for the wild pathosystem of *Linum marginale*/*Melampsora lini*. Here, the

number of virulences negatively correlated with spore production. This agrees well with our findings that isolates with >12 virulences occurred at a low frequency only (<5%, see Fig. 1).

A rapid temporal change also occurred over years in the natural epidemic situation at different locations (Fig. 3). At HOH, for example, virulences to P54 or S4084 did not occur in 2000 but were observed with 26% in 2002. Similarly, in PET, virulences to P53 and H26 increased from 0% to 7% and 13%, respectively. But this was not true for all virulences, again those for differentials P51b and P52 stayed near zero in all environments except BER2001.

Some German sites showed a complex pattern of virulences with varying frequencies on some differentials. For example, populations from EWE2001, HOH2002 and GL2002 had some frequent virulences against P53 or S4084, that occurred rarely at any other location and even at the same locations in other years. These results of seedling tests were substantiated by a virulence analysis at the adult-plant stage of 11 differentials at 11 field environments in the same seasons (Wilde et al. 2006). Here, as well, we observed a high diversity of virulences and the composition of rust populations varied considerably among environments. Possibly we overestimated the diversity and complexity of virulences in our study because we performed our sampling at breeding stations (BSB, EWE, HOH, PET, BER, GL, RIE) where a much broader spectrum of host genotypes is grown than in farmers' fields. This, however, may help to predict the effectiveness of race-specific resistances more properly. Considering the breakdown of stripe rust resistance gene *Yr17* in wheat within 3 years of commercially growing resistant cultivars (Bayles et al. 2000), the testing procedures for monogenic resistances should be as rigorous as possible. A companion study clearly illustrated that even highly heterogeneous synthetic populations of rye could not compete with the high pathogen diversity and spatial fluctuations of leaf-rust populations in rye fields (Wilde et al. 2006).

Factors of dynamics in leaf-rust populations

Possible causes of the complex and fluctuating composition of the German rye leaf-rust populations include: (1) highly heterogeneous host cultivars, (2) mixed reproduction mode (asexual/sexual) of the pathogen, (3) high gene flow of the wind-borne conidia.

For biotrophic fungi, it is well documented that a high diversity of host resistances inevitably leads to a corresponding diversity of virulences (Thrall and Burdon 2003; Goyeau et al. 2006). The spectrum of rye cultivars grown during the study comprised 15 open-pollinated cultivars and 18 multi-line hybrids occupying 55% and 45% of the total rye acreage, respectively (Anonymous 2001). None of the leading cultivars has been dominating for many years. Both open-pollinated and hybrid cultivars are genetically highly heterogeneous. It was shown that 7% to 54% of plants of the population cultivars Danko, Nikita, and Born possess race-specific seedling resistances (Klocke 2004). This should have contributed to the selection of various pathotypes. A mixed reproduction mode of rye leaf rust alternating between production of asexual spores on rye and sexual reproduction on alternate hosts, favours the fast propagation of successful pathotypes during the growing period as well as the recombination of accumulated virulences resulting in new pathotypes in the following year (McDonald and Linde 2002). In contrast, wheat leaf rust does not frequently reproduce sexually (Goyeau et al. 2007). Finally, leaf-rust spores may be dispersed by the wind over large distances in Europe. Thus, part of the spores sampled in a particular field may originate from other fields with other cultivars (migration). Interestingly, the results of our study agree well with the analysis of the natural pathosystem of heterogeneous wild barley stands (*Hordeum spontaneum*) and powdery mildew in the Near East (Löwer 2000, Heckelbacher et al. 1992). A similarly large diversity of virulences was detected there with a preponderance of simple virulences. The most frequent pathotype found in these studies reached a frequency of only 8.6% (Löwer 2000).

Taken all together, host-specific selection in rye leaf rust seems to be much weaker than in wheat leaf rust and allows the rye leaf-rust populations to maintain a high diversity of pathotypes including those with only a few virulences (see Fig. 1, Thrall and Burdon 2003).

Consequences for resistance breeding

The highly varying composition of pathogen populations in different environments has great implications

for resistance selection. Rye genotypes that would have been selected at some sites for their high resistance, may be confronted with virulent isolates in other sites and vice versa. Even the same location provided different virulence patterns in subsequent years (see Fig. 3). Site-specific pathogen populations result in high host genotype x environment interaction. Selection for stable resistance therefore requires tests across many environments.

For all tested differentials, even the most resistant ones, matching virulences already existed although the differentials had never been used as components of commercial cultivars. Even two full-sib families (N75/81, N9a/86), whose components were recently provided by Prof. Dr. A.A. Goncharenko from Russia, were already susceptible to 38 and 77% of the isolates, respectively, at the seedling stage. It is, therefore, necessary to find new, highly effective, and environmentally stable race-specific resistance genes. However, according to the large number of virulences already existing in the present-day leaf-rust populations, even gene deployment or pyramiding strategies will not provide sufficiently durable resistance (Wilde et al. 2006). More durable resistance might be achieved by an additional selection for race-specific adult-plant and/or quantitative resistances.

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