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Application of pathogen surveys, disease nurseries and varietal resistance characteristics in an IPM approach for the control of wheat yellow rust

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Abstract The present paper presents the rationale for the use of pathogen surveys, inoculated and non-inoculated disease nurseries and varietal resistance characteristics in an integrated approach to control wheat yellow rust in Denmark. The non-inoculated disease observation plots, which gave valuable information about yellow rust at the year, site and variety level, served as the primary sample source for the pathogen survey revealing pathogen virulence dynamics. This survey was also the main source for isolates of new pathotypes, a prerequisite for the assessment of the resistance characteristics of varieties and breeding lines in inoculated nurseries, and the postulation of race-specific resistance genes. A simple grouping of varieties into four categories with respect to resistance to the current yellow rust population proved robust, and this grouping was used as a determinant in a web-based decision support system for pesticide applications in cereals, Crop Protection On-line (CPO). The interplay between the different research and survey activities in the integrated pest management (IPM) approach demonstrated the need for a coherent and long-term involvement at all stages from plant breeding to the official variety approval system, extension service and research in disease epidemiology and resistance genetics.

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Introduction

Yellow rust or stripe rust, caused by *Puccinia striiformis* f. sp. *tritici*, is a common disease of wheat in temperate regions of Europe and elsewhere (Zadoks 1961; Chen 2005). The pathogen is a biotrophic basidiomycete, which does not complete sexual reproduction, apparently due to the lack of an appropriate alternate host (Stubbs 1985). It has been recognized as harmful to wheat since Theophrastus (371–286 BC), one of the classical Greek authors, who made remarks on the susceptibility of wheat to cereal rusts and that disease development was affected by weather (Hermansen 1968). The first comprehensive review on the biology and host plant symptoms at different growth stages was published as early as the late nineteenth century (Eriksson and Henning 1896).

In recent years, the control of yellow rust by host resistance has been generally successful in NW Europe (Johnson 1992), although the emergence of new virulence phenotypes has sometimes caused disease epidemics on varieties with race-specific resistance genes (Hovmøller 2001; Enjalbert et al. 2005) resulting in subsequent fungicide sprays (Schmidt 2003). Resistance to *P. striiformis* may be based on genes which are expressed at all wheat growth stages or genes which are effective mainly at the post-seedling and adult plant stages; the latter is denoted as 'adult plant resistance' (APR) (Johnson 1984). Genes providing effective disease control at the seedling stage generally follow race-specificity (Manners 1988), although this may also be the case for some APR genes (Johnson 1992). Thus, the ratio of virulence/avirulence in the current pathogen population has a great influence on the expected disease severity on wheat varieties carrying different sources of *P. striiformis* resistance (Priestley et al. 1984; Hovmøller 2001). Virulence is defined as the qualitative ability to cause disease on plant genotypes possessing a particular resistance gene (Flor 1971; Brown 2003), i.e. virulence represents the lack of recognition between a specific pathogen avirulence gene and the corresponding resistance gene in the host (Flor 1971). Depending on the context, terms such as 'pathotype', 'race' and 'virulence phenotype' are often used synonymously, just as 'emergence of virulence' and 'loss of avirulence'.

Forecasts on expected yellow rust severity at the crop level is a challenging task due to variable disease loads in different years and regions, influenced by seasonal weather conditions as well as prior winter survival (Hovmøller 2001) and the patchiness by which the disease often appears (Buiel et al. 1989). Assessments of average or maximum disease severity for specific varieties, when challenged by specific pathogen isolates with well-defined virulence/avirulence characteristics under disease conducive field conditions, may therefore be valuable in estimating average and worst case scenarios for such varieties (Priestley et al. 1984; Pinnschmidt et al. 2006).

In the context of a wish to reduce the pesticide use in Danish crop production (Jørgensen and Kudsk 2006), the challenging features of the yellow rust—wheat system were tackled through an integrated pest management (IPM) strategy by a sequence of research and survey activities: disease observation plots and virulence surveys (Hovmøller 2001), assessment of host resistance genes (Hovmøller 2007), national and European wheat nurseries using yellow rust pathotypes typical for each area (Bayles et al. 2000), and the application of decision support systems for pesticide use (Hagelskjær and Jørgensen 2003) and choice of varieties (http://www.sortinfo.dk/). Although most pages on internet sites referred to in this paper were in Danish, they provided summaries in English. This paper presents the rationale for an IPM strategy for yellow rust on wheat in Denmark, illustrated by examples of data sources and how data were generated.

Materials and methods

Survey data were generated through ongoing activities in the official cereal variety approval system (Anonymous 2007a; http://www.pdir.dk/), the Danish Agricultural Advisory Service (http://www.lr.dk/) and pesticide efficacy evaluations (e.g., Jørgensen 2006).

Disease observation plots subject to natural disease occurrence

All varieties and advanced breeding lines considered for approval in Denmark, were grown in non-fungicide treated, non-inoculated disease observation plots distributed at 15–20 locations across Denmark (Pinnschmidt et al. 2006). The naturally occurring diseases on each variety were generally assessed visually once or twice during the growing season, when the disease severity on the most susceptible varieties had reached a substantial level. Disease scores were recorded as percent leaf area covered by symptoms of the individual disease, assessed visually using a scale comprising the following steps (in percent): 0–0.1–0.5–1–5–10–25–50–75–100. Mean values of two adjacent steps were used when a score was not assigned to a specific category with sufficient confidence.

Pathogen virulence surveys

Representative, single-lesion samples of yellow rust were collected from susceptible varieties as well as previously yellow rust-resistant varieties in disease observation plots (Hovmøller 2001). Samples were multiplied on seedlings of the standard susceptible variety 'Cartago' or the variety from which the sample was collected, and covered by spore-proof cellophane bags to minimize the risk of contamination. Seedlings with sporulating lesions were gently rubbed onto 12–16 day-old seedlings of a set of 20–30 differential wheat varieties and lines. Typically, these comprised 15 varieties from the 'world' and 'European' differential sets (Johnson et al. 1972), a range of near-isogenic wheat lines in the Avocet background (Wellings 2007), which duplicated some of the considered resistance genes, and supplementary commercial varieties of particular interest. Disease reactions were scored using a 0–9 scale (McNeal et al. 1971) in which infection types up to six indicated varying levels of incompatibility between host and pathogen, whereas types 7–9 were interpreted as compatible interactions (Hovmøller and Justesen 2007).

Assessment of sources of resistance with major effect at the seedling stage

Wheat varieties were assessed for race-specific resistance by analysing the patterns of disease reaction (qualitatively defined infection types) on seedlings when challenged by 10–16 yellow rust isolates of diverse origin and pathotype. A comparison with patterns of infection types on differential varieties with defined resistance genes (Johnson et al. 1972), pedigree information and occasional information on molecular markers in specific varieties (Christiansen et al. 2006), allowed an interpretation of data in terms of presence/absence of specific *Yr*-resistance genes in more than 150 European varieties (Hovmøller 2007).

Inoculated yellow rust nurseries at the adult plant stage

A range of yellow rust isolates obtained via the pathogen survey and selected according to pathotype, were used to investigate yellow rust resistance characteristics under field conditions. Most varieties were challenged by three to six individual isolates in multiple years, sown in six-rowed, 1 m^2 plots consisting of two varieties per plot and two rows of an isolate-specific, susceptible spreader (Hovmøller 2007). The spreaders were inoculated at GS 30–32 by gently rubbing seedlings with fresh spores, often twice at 2-day intervals, preferably prior to natural dew formation or light rain. Main plots were surrounded by winter barley for minimizing unintentional mix of isolates in the field. Disease scores were recorded as percent leaf area covered by lesions (scale shown above). First assessment in a trial was done at first symptom appearance, typically three weeks after inoculation, and then once for each subsequent yellow rust generation until senescence of leaves made reliable scoring impossible.

Comparative analysis of seedling data and adult plant data allowed conclusions about presence of components of resistance to *P. striiformis*, which were mainly expressed at the adult growth stages, and denoted as APR (Johnson 1984). Thus, the level of APR was not accessible for varieties being resistant to all isolates at the seedling stage.

Results and discussion

The general yellow rust disease level in Denmark, measured by yellow rust severity on a susceptible check, was highly variable across years (Fig. 1). Previous research has shown that these dynamics were much influenced by pathogen winter survival as well as the distribution of host varieties with specific resistance characteristics within Denmark (Hovmøller 2001). Random samples, as well as targeted sampling from varieties previously resistant to yellow rust, were obtained from these observations plots and from farmer's fields and assayed for pathotypes as described.

A total of 14 new pathotypes have emerged since 1997, i.e., an average rate of approximately one per

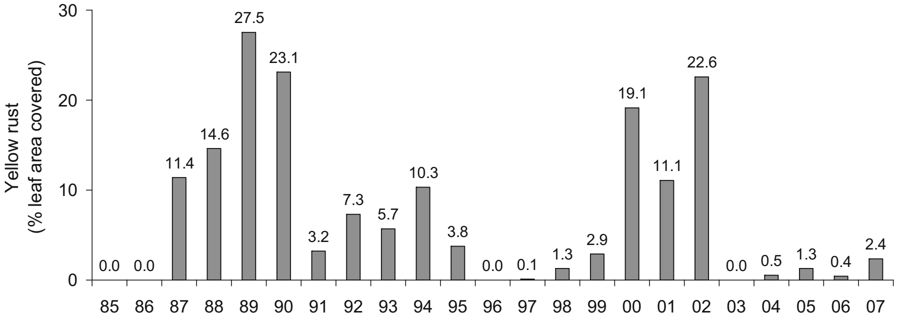

Fig. 1 Yellow rust attack on var. 'Anja' (susceptible check) in non-fungicide treated disease observation plots in Denmark, average of eight to ten locations at GS 65–71

year (Table 1). However, the time of first appearance, and virulence characteristics of new pathotypes were highly unpredictable. For instance, the most frequent pathotype in Denmark in 2007, which was virulent on host varieties possessing *Yr17* and *Yr32*, had not been observed in previous years. The same occurrence pattern of this particular pathotype applied for France (de Vallavieille-Pope, personal communication), although it had been present in moderate to high frequencies in the UK since 2000 (Bayles et al. 2004). This new pathotype may be of immediate agronomic relevance for Danish farmers because *Yr17* and *Yr32* were commonly present in NW-European breeding programmes (Hovmøller 2007). A similar chain of events has been observed several times in the past in northern Europe (e.g., Bayles et al. 2000).

Isolates of pathotypes XI and XII (Table 1), which represent the first yellow rust detected in a wheat crop in Denmark in 2007, were observed as randomly dispersed single lesions at the field scale, approximately ten lesions per square meter without focal development in the area, i.e., a clear indication of random spore dispersal by wind from distant source(s). This hypothesis was confirmed by highly atypical pathotypes as compared to the resistance characteristics of wheat varieties currently grown in Denmark. In fact, a range of 'atypical' pathotypes has been observed in recent years in Denmark (Hovmøller and Justesen 2007), suggesting relatively frequent, long-distance spread of airborne yellow rust spores from exotic sources into northern Europe.

The implementation of variety and pathogen characteristics in an IPM approach is illustrated in Table 2, where the resistance groupings in Crop Protection On-line (CPO) are presented for five varieties. CPO is a web-based decision support system (DSS) for pesticide use in cereals (Hagelskjær and Jørgensen 2003), which is widely used by farmers and extension service in Denmark (Jørgensen et al. 2007). The resistance grouping has immediate influence on the CPO-recommended disease management strategy. Most of the information in Table 2 is accessible online through a DSS for choice of variety in cereals, 'SortInfo' (http://www.sortinfo.dk). This system contains all public available information about growth and resistance characteristics of cereals, which have been marketed in Denmark since 1995.

The varieties Penta and Ambition were both in CPO group 0, i.e., sources of resistance expected to be effective for yellow rust control in the year considered. No yellow rust was detected in these varieties in the observation plots, or in inoculated field trials, although var. Penta was susceptible to several, frequent pathotypes at the seedling stage (Hovmøller 2007). Therefore, var. Penta was classified as a variety with a highly effective APR resistance. A similar conclusion could not be made for var. Ambition, in which the source(s) of seedling resistance has not yet been resolved due to the absence of virulent isolates.

Variety Robigus was initially grouped as 0 based on results in the disease observation plots, 2003. The grouping changed to 1 based on 2004 results in inoculated nurseries (>10% diseased leaf area on average during the epidemic caused by 71/93, a *Yr32*-virulent isolate). If *Yr32*-pathotypes had been present in the natural Danish *P. striiformis* population at that time, the variety would have been grouped as 2. Group 1 indicates 'partial susceptibility', i.e., there is a risk from yellow rust if a corresponding virulence emerges. *Yr32*-pathotypes reappeared in Denmark in 2007 (Table 1). The level of APR was classified as low based on disease severity levels when challenged by virulent isolates (>10% leaf area covered, seasonal average).

In recent years, vars Bill and Blixen were grouped as 2 and 3, respectively, as reflected by moderate to high levels of disease in inoculated nurseries. The APR in these varieties was classified as medium and very low, respectively. A moderate to low frequency of pathotypes virulent on var. Blixen may, in part, explain the annual fluctuations in disease severity on this variety in disease observation plots (0–7% leaf area covered).

The CPO-groupings for a larger set of varieties, which have covered a substantial part of the Danish wheat area in recent years, had changed for most varieties since 2002 (Table 3). The groupings were defined according to the procedures above, and generally based on results in a previous year. However, group changes may occur within the growing season, e.g., if a new pathotype suddenly emerges at a high frequency. In general, the groupings were robust in the sense that yellow rust was never observed to any significant extent on a non-inoculated variety with a 0-grouping. The relative distribution of the varieties, e.g. the low proportion of the highly resistant var. Penta, suggests that the choice of variety is a multifactor

Table 1 Frequency of *Puccinia striiformis* f. sp. *tritici* pathotypes in Denmark, 2000–2007

Number	Pathotype															Pathotype frequency (2000–2007)								First observation (DK)
																2000 *n*=61	2001 *n*=38	2002 *n*=72	2003 *n*=22	2004 *n*=12	2005 *n*=12	2006 *n*=6	2007 *n*=51	
I	1	2	3	4	6	–	–	9		–	17	–	Sd	Su	An	13	13	4	9	0	0	0	0	1994
II	1	2	3	–	–	–	–	–	–	–	17	–	Sd	–	An	44	34	47	9	0	0	0	0	1997
III	1	2	3	4	–	–	–	9	–	–	17	–	Sd	Su	An	41	42	6	5	0	0	0	4	1997
IV	1	2	3	4	–	7	–	9	–	–	17	–	Sd	Su	An	2	0	0	0	0	0	0	0	2000
V	–	(2)	–	–	6	7	8	9	–	–	–	–	(Sd)	–	An	0	11	8	14	8	0	0	0	2001
VI	1	2	3	–	6	–	–	9	–	–	17	–	Sd	–	An	0	0	29	18	50	42	0	0	2002
VII	1	2	3	–	–	–	–	9	–	15	17	–	Sd	–	An	0	0	6	0	0	0	0	0	2002
VIII	–	*	–	–	–	–	–	–	–	–	*	–	*	–	An	0	0	0	36	17	0	0	0	2003
IX	–	(2)	–	–	6	7	8	–	–	–	–	–	–	–	An	0	0	0	0	17	42	67	2	2004
X	–	–	–	–	6	7	8	–	–	–	–	–	*	–	–	0	0	0	0	0	0	33	0	2006
XI	–	–	–	–	6	–	–	–	–	–	–	–	–	Su	An	0	0	0	0	0	0	0	4	2007
XII	–	–	3	–	6	–	–	–	–	–	–	(32)	(Sd)	Su	An	0	0	0	0	0	0	0	4	2007
XIII	1	2	3	4	–	–	–	9	–	–	17	32	Sd	Su	An	0	0	0	0	0	0	0	78	2007
XIV	–	(2)	–	–	6	7	8	–	10	–	–	–	(Sd)	–	An	0	0	0	0	0	0	0	2	2007
XV	–	(2)	–	–	6	7	8	–	10	–	*	–	*	–	–	0	0	0	0	0	0	0	4	2007
																100	100	100	100	100	100	100	100	

Figures and symbols defining pathotype correspond to yellow rust resistance genes; parenthesis indicate interactions either influenced by unrecognised resistance in test-cultivars or a heterozygous state of pathogen isolates. Asterisk means not accessible based on present test varieties (Hovmøller and Justesen 2007).

n number of isolates

Table 2 Rationale for CPO yellow rust resistance grouping of five varieties based on variety and pathogen characteristics: percent diseased leaf area in non-inoculated observation plots, in inoculated nurseries using specific yellow rust isolates (for virulence profile cf. Table 1), varietal resistance characteristics and frequency of relevant *P. striiformis* pathotypes in Denmark

Variety	Year	CPO resistance grouping in following year	Observation plots (non-inoculated) mean across locations			Inoculated nurseries (mean of three assessment dates)					Resistance genes	Frequency of matching virulence in DK population 2001–2007 (average)	APR[b]
			Yellow rust	Septoria	Powdery mildew	111/02 (VII)	16/02 (VI)	70/99 (I)	08/97 (III)	71/93[a]			
Penta	2001	0	0.0	0.8	0.0			0.0	0.1	0.0	Sd, *Yr25*, +	84	Very high
	2003	0	0.0	1.5	0.0								
	2005	0	0.0	1.1	0.0								
	2006	0	0.0	1.6	0.0			0.0	0.0				
Ambition	2004	0	0.0	0.8	0.0						–	0	Unknown
	2005	0	0.0	0.9	0.1								
	2006	0	0.0	1.1	0.1			0.0	0.0				
	2007	0	0.0	2.5	2.9	0.0		0.0		0.0			
Robigus	2003	0	0.0	3.6	0.0						*Yr2*, *Yr32*	0	Low
	2004	1	0.0	5.0	0.1	0.0	0.0			10.4			
	2005	1	0.0	5.0	0.0								
	2006	1	0.0	16.0	0.1			0.0	0.0				
	2007	2	2.3	5.0	3.2	(1.4)		0.5		17.5		78	
Bill	2001	1	0.6	9.0	1.0			0.1	2.8	1.8	*Yr2*, *Yr3*, *Yr17*	84	Medium
	2002	1	1.7	8.0	1.6								
	2003	1	0.1	16.0	1.9								
	2004	2	0.0	9.0	3.8	1.4	5.6	2.7					
	2005	2	0.0	15.0	2.4								
Blixen	2001	2	0.8	6.0	2.6			18.9	0.1	1.5	*Yr6*, *Yr9*, +	12	Very low
	2002	2	7.0	8.0	3.8			20.6		(7.3)			
	2003	2	0.0	7.0	2.3								
	2004	3	1.6	5.0	2.5	(4.3)	30.7						
	2005	3	6.0	8.0	4.7								

Parentheses refer to incompatible host-pathogen combinations, thus indicating within-trial mixture of isolates.

[a] Pathotype Vyr1, 2, 3, 32, Sd

[b] Level of resistance of adult plant stage, measured by disease severity when exposed to virulent isolate

Table 3 Dynamics of CPO-groupings (0, 1, 2, 3) for 12 varieties 2001–2008 representing different resistance characteristics, and their relative distribution in Denmark based on amounts of certified seed (*t*), in the considered period

Variety	Race-specific resistance	APR[1]	2002	2003	2004	2005	2006	2007	2008	Av. distribution in DK (in %) based on amounts of certified seed
Ambition	–	–				0	0	0	0	1.0
Baltimor	*Yr17, +*	Very low	3	3	3	3	3			3.5
Bill	*Yr2, Yr3, Yr17*	Medium	1	1	1	2	2			8.1
Boston	*Yr15*	Low	0	1	1					1.0
Cardos	*Yr6*	Very low	2	2	2	3	3			1.2
Deben	*Yr2,Yr32+*	–	0	0	0	0	0	0		5.8
Penta	Sd, *Yr25, +*	Very high	0	0	0	0	0	0		0.1
Ritmo	*Yr1*	Low	2	2	2	2	2	2		12.8
Robigus	*Yr2,Yr32*	Low			0	1	1	1	2	6.7
Senat	*Yr3,Yr32+*	–	0	0	0	0				2.4
Smuggler	*Yr1, Yr17, +*	–			0	1	1	1	1	12.2
Terra	*Yr1*	High	0	0	0					0.9

[1] Level of resistance at adult plant stage, measured by disease severity when exposed to virulent isolate

decision, also depending on other characteristics than resistance to yellow rust, e.g., yield, market preferences, resistance to other diseases, etc.

A specific disease management recommendation by CPO depends on a range of information, e.g., disease records in the crop, growth stage, crop rotation, CPO resistance grouping, and to some extent weather forecasts. For simplicity, disease assessments were measured by disease incidence (% infected plants), where the basis for an assessment was dependent on growth stage: (1) GS 29–32, whole plant assessment; (2) GS 32–71, three top leaves of main tiller. Note that disease control by fungicides in wheat was only considered relevant between GS 29 and 71 (Anonymous 2007b). The end user (farmer) is asked to supply four types of data to the CPO-model: (1) variety, (2) crop growth stage, (3) previous records (and fungicide treatment) of disease in the crop, (4) disease incidence of yellow rust, leaf rust, powdery mildew, Septoria leaf blotch and tan spot, respectively. An observation above the threshold results in a fungicide recommendation, depending on product and pesticide prices, crop growth stage, presence of additional diseases (below their threshold value) and weather forecast (Table 4). If treatment was not recommended, the farmer will be recommended to make another assessment after 7 days. If the model recommends fungicide treatment against yellow rust, and the variety is susceptible/very susceptible, the model will recommend the treatment to be repeated after 3 weeks.

Past experiences from numerous field trials in >10 years have documented that CPO, in general, has resulted in the highest yield margin over product use and much reduced fungicide inputs as compared to treatments using the recommended standard doses, or treatments recommended by other European decision support systems (Hagelskjær and Jørgensen 2003). Part of these reductions may be ascribed to the CPO-resistance groupings (Jørgensen et al. 2003) who analysed fungicide inputs in 36 winter wheat field trials in three growing seasons and observed an almost 50% reduction in fungicide input in resistant varieties compared to varieties which were susceptible to Septoria leaf blotch or yellow rust.

Although approximately only 1,000 Danish farmers have bought access to CPO (Jørgensen et al. 2007), there are indications that CPO may have influenced the general recommendations by the

Table 4 Yellow rust thresholds in CPO according to resistance grouping, growth stage and disease incidence

CPO resistance grouping	Disease incidence (% infected plants)	
	GS 29–60	GS 61–71
0	>10	>75
1	>1	>50
2 and 3	>1	>10

Danish extension service for using reduced fungicide doses, compared to label standards, thereby contributing to achieving the goals laid down in the various Danish pesticide action plans since 1986 (Jørgensen and Kudsk 2006). Based on farmer interviews (Jørgensen et al. 2007), the main barriers for a wider use of the system may be lack of time for disease assessments, possibly due to increasing farm and field sizes, increasing focus on animal production as compared to crop production, problems in recognizing diseases, which may remain undetected until too late, and access to relatively cheap routine sprays.

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