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Yield gain due to fungicide application in varieties of winter wheat (*Triticum aestivum*) resistant and susceptible to leaf rust

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Abstract. In three independent experiments in Turkey and Kazakhstan, winter wheat germplasm with variable degrees of resistance to leaf rust was subjected to fungicide protection. The yield loss of genotypes susceptible to leaf rust varied from 30% to 60% depending on the environment and severity of infection. Genotypes completely or moderately resistant to leaf rust also responded positively to fungicide protection, with average yield increases in the range 10–30%. This increase was observed even in one season without leaf rust infection. The main character affected by fungicide was 1000-kernel weight. There was stable expression of the magnitude of yield gain in resistant genotypes in different seasons, confirming genetic variation for this trait. Possible mechanisms of yield gain from fungicide protection in resistant genotypes are related to a positive physiological effect of the chemical used as well as a possible 'cost of resistance' to wheat plants. The magnitude of yield gain by resistant germplasm justifies its capture in breeding programs to develop varieties resistant to diseases and with greater benefits from the fungicide protection.

Additional keywords: breeding, grain yield, leaf rust, wheat.

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Introduction

Winter wheat (*Triticum aestivum* L.) is an important crop in the region of Central and West Asia (CWA), stretching from Kazakhstan and Afghanistan to Iran and Turkey. The crop is grown under both irrigated and rainfed conditions on ~13 Mha, and average grain yield is ~2.5 t ha^{-1}, which is far below the potential of 4–5 t ha^{-1} (Sharma *et al*. 2013). There are several reasons for poor wheat grain yield in the region, including improper variety selection, lack of optimal input application, and poor agronomy due to lack of appropriate machinery. However, diseases and pests also play an important role in yield reduction. Yellow rust epidemics occurred in the region in 2009 and 2011, reducing the yield of susceptible varieties by up to 20–30% (Ziyaev *et al*. 2011). Leaf rust also occurs regularly and reduces yield. The damage from wheat rusts and other diseases is a constant threat because susceptible varieties are cultivated on large areas, providing pathogen population survival. Increasingly, crops of winter wheat are protected with foliar fungicides to reduce losses: 880 000 ha in Argentina; 2.3 Mha in Brazil; 5.27 Mha in Australia, and 6 Mha in China (Gianessi and Williams 2011). Wheat protection from diseases is driven by farming communities and policy makers. In some countries of CWA, fungicide applications are provided by crop protection agencies free of charge to farmers, paid by government agencies. Sometimes disease protection is applied as a preventive measure in the absence of a pathogen development and independent of the resistance of a wheat variety. Application of fungicides is becoming an integral element of wheat production technologies, especially under irrigated or high-rainfall conditions.

The International Winter Wheat Improvement Program (IWWIP, www.iwwip.org), based in Turkey, develops germplasm for CWA. IWWIP is a cooperative program between the Ministry of Food, Agriculture and Livestock of Turkey, CIMMYT and ICARDA, and has been operating since 1986. Its breeding activities are implemented through a

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multi-locational network in Turkey with close cooperation with the key wheat-breeding programs in the region. The main emphasis of IWWIP breeding is broad adaptation and disease resistance, especially to rust pathogens. Annually, IWWIP germplasm is sent to co-operators throughout the CWA region and globally for evaluation and selection. More than 60 varieties originating from IWWIP germplasm have been released in the CWA region to date. Most of these varieties, especially those recently developed, demonstrate excellent grain yield combined with resistance to diseases (Sharma *et al*. 2013). The breeding program in Turkey utilises disease hotspots and artificial inoculation for evaluation and selection of the germplasm, with response to yellow rust evaluated near Ankara, Turkey, in a Central Anatolian environment; leaf rust in Adapazari and Edirne, in higher rainfall, western regions of Turkey; and stem rust in the Kastamonu region of Turkey, close to the Black Sea. Selection for rusts is based primarily on an adult-plant resistance concept (Singh *et al*. 2000). Although the majority of IWWIP germplasm possesses a high degree of resistance to yellow rust and leaf rust, there is also group of genotypes that are high yielding but with a variable degree of susceptibility to the rusts. In order to evaluate the degree of yield losses associated with susceptibility to leaf rust, our first experiment was conducted at Adapazari, Turkey, a hot spot for leaf rust, in 2012 and 2013. Winter-wheat breeding lines and varieties were evaluated under high disease pressure with and without fungicide protection. Two more experiments were conducted in 2012 and 2013 near Almaty, Kazakhstan, using a diverse set of germplasm to evaluate response of winter wheat to fungicide protection.

Research on the application of fungicides to winter wheat previously focused primarily on Western Europe, where long growing seasons and relatively cool and moist environments create very favourable conditions for disease development. Fungicide protection of winter wheat is part of production technology, and research mainly focuses on how to reduce the number of applications depending on variety resistance, pathogen population development and weather (Jørgensen *et al.* 2008). Economic and cost–benefit analyses also play important roles in applying of fungicides based on studies in the USA (Wegulo *et al*. 2011). In Argentina, integrated foliar disease management combines nitrogen application, fungicide protection and resistant varieties (Simón *et al.* 2011). The wheat-breeding community at large has often focused its wheat disease-protection strategies on genetic resistance as an alternative for chemical protection, which is considered environmentally unfriendly, expensive and unnecessary if resistant varieties are available. However, fungicide application is a fast and simple means to increase yield and is gaining in popularity in developing countries. Fungicide application may play an even larger role in the future, given the need to increase grain production because of population growth and wheat-yield stagnation in many wheat-producing countries (Ray *et al.* 2012). The interactions of fungicides with wheat varieties has been well documented, showing that in the absence of disease, green leaves stay functional longer and provide assimilates for the grain (Dimmock and Gooding 2002) as well as improving photosynthesis (Serrago *et al*. 2009). Wheat varieties susceptible to diseases have different responses to protection, and yields reduced by diseases vary. Wheat varieties differ in their tolerance to diseases, and this phenomenon is used in production, allowing susceptible but tolerant varieties to be maintained by farmers. Tolerance to diseases has been recognised by breeders but is largely ignored in the breeding programs because of the strategies utilising genetic resistance. The reaction of resistant varieties to fungicides is not well studied. Some reports suggest modest positive yield responses (Zefelippo 1992; Jørgensen *et al.* 2008) that are more pronounced under higher disease pressure (Varga *et al*. 2005). The objective of the present study was evaluation of yield responses to fungicides in winter wheat genotypes with variable degrees of resistance to leaf rust.

Materials and methods

Experiment 1

Twenty-two breeding lines and two varieties were used in the study (Table 1). The germplasm is representative of IWWIP breeding materials destined for irrigated or high-rainfall environments, with short stature and high grain-yield potential. The cross ID ‘TCI’ indicates that the lines originate from crosses made by IWWIP. The cross ID ‘OCW’ belongs to lines originating from crosses made at Oklahoma State University and subsequently selected in Turkey from F_3 onwards. Entry 12 originated from a cross made in Iran. The entries were divided into four groups according to their reaction to leaf rust: resistant (R); moderately resistant (MR); moderately susceptible (MS) and susceptible (S). The trial was planted at the Maize Research Station in Adapazari, Turkey, in 2012 and 2013. A randomised complete block experimental design was used. Plot size was 1.2 by 5 m with six replications, three of which (nos 2, 4 and 6) were protected by the fungicide Opera® (with 12.5% active ingredients (a.i) pyraclostrobin and epoxiconazole; Nufarm Ltd, Laverton North, Vic.). The concentrations and rates of application in all three experiments followed the manufacturer’s recommended practices for wheat. The fungicide was applied twice in May in 2012 and three times in April–May in 2013, resulting in complete disease protection. In both years, naturally occurring leaf rust infections were observed. Leaf rust readings were taken three times in 2012, starting from 25 May (anthesis), with 7–10-day intervals. In 2013, leaf rust was observed earlier and the infection was higher, with the first reading taken on 26 April (flag leaf) and two more readings conducted at 10-day intervals.

Area under the disease progress curve (AUDPC) was calculated using a computer program developed at CIMMYT. Days to heading, plant height, 1000-kernel weight, test weight and grain yield of entire plots were recorded in both years. Yield components were evaluated based on 10 random plants. Protein content and Zeleny sedimentation were evaluated by standard methodologies in 2012 only. Weather conditions were close to long-term average, although higher temperatures and rainfall in 2013 resulted in higher disease pressure. Molecular marker analysis for *Lr* genes was provided by the CIMMYT Biotechnology Group in Mexico. Three-way ANOVA (24 varieties × 2 treatments × 2 years) for design of unorganised replications was used to estimate the effects of main factors and their interactions, using AGROS2.13 software (Vavilov Institute, Russia). ANOVA for each individual trial, treatment and year was performed using JMP software (SAS Institute,

Table 1. Expt 1: winter wheat genotypes used in the study and their leaf rust (LR) reaction in Adapazari, Turkey, 2012–13

AUDPC, Area under disease progress curve; R, resistant; MR, moderately resistant; MS, moderately susceptible; S, susceptible

Entry	Variety name or line pedigree	Cross ID	*Lr* genes	Unprotected LR final disease reading (%)		Control LR AUDPC		Resistance group
				2012	2013	2012	2013	
2	ATTILA/2*PASTOR//YUMAI 29	OCW02S567S		2	20	41	136	R
3	PFAU/WEAVER/3/MASON/JGR//PECOS	OCW02S369S	*Lr26*, *Lr34*	0	5	0	28	R
6	TAM200/KAUZ//BECUNA-6	TCI021152	Lr24	3	0	18	0	R
13	KAMBARA1/KALYOZ-17	TCI021034	*Lr34*	0	0	0	5	R
18	BACANORA/3/MASON/JGR//PECOS	OCW02S471S	*Lr34*	7	8	36	72	R
20	ADMIS/5/SMB/HN4//SPN/3/WTS//YMH/HYS/4/SAB	TCI02-87		3	5	18	33	R
	R Group average			**3**	**6**	**19**	**45**	
4	BACANORA /3/MASON/JGR//PECOS	OCW02S471S	*Lr34*	20	25	55	138	MR
5	KAMBARA1/KALYOZ-17	TCI021034	*Lr34*	43	17	238	123	MR
10	KAUZ/RAYON//JGR	OCW02S500S	*Lr37*	28	23	241	154	MR
14	SERI.1B*2/3/KAUZ*2/BOW//KAUZ/4/BAGCI2002	TCI021198		37	23	201	124	MR
15	VORONA/KAUZ/3/ALTAR 84/AE.SQ//2*OPATA	TCI021441	*Lr26*, *Lr34*	13	40	73	427	MR
17	ATTILA/2*PASTOR//YUMAI 29	OCW02S567S		47	20	91	209	MR
	MR Group average			**31**	**25**	**150**	**196**	
1	OK98697/5/SITE/MO/4/NAC/TH.AC//3*PVN/3/MIRLO/BUC/6/JGR/CUSTER//JGR* (OK0062278)	OCW02S155T	*Lr37*	27	50	302	285	MS
19	PASTOR//HXL7573/2*BAU/3/F12.71/COC//ATTILA	OCW02S596S	*Lr34*	47	43	284	445	MS
21	SOM//1D13.1/MLT/3/VORONA/3/TOB*2/7C//BUC	TCI021013		53	67	369	590	MS
22	DORADE-5/3/ES14/SITTA//AGRI/NAC	TCI021068	*Lr34*	43	67	266	496	MS
24	DORADE-5/3/ES14/SITTA//AGRI/NAC	TCI021068	*Lr34*	57	53	462	355	MS
25	AGRI/NAC//KAUZ/4/55.1744/MEX67.1//NO57/3/ATTILA	TCI011031		47	57	312	622	MS
	MS Group average			**46**	**56**	**333**	**466**	
7	JI5418/MARAS//SHARK/F4105W2.1/3/SHARK/F4105W2.1	TCI022086		93	93	749	1747	S
8	BEZOSTAYA		*Lr34*	80	60	733	605	S
9	JI5418/MARAS//SHARK/F4105W2.1/3/SHARK/F4105W2.1	TCI022086		87	100	702	1767	S
11	JI5418/MARAS//SHARK/F4105W2.1/3/SHARK/F4105W2.1	TCI022086		87	100	932	2378	S
12	LR64/IZ1813//093-44/3/NO57/4/SUT66/5/SABALAN/6/BEZ//BEZ/TVR/3/KREMENA/LOV29/4/KATYA1	IRW2000-01-246		93	80	1313	1007	S
16	KATIA			80	60	820	793	S
	S Group average			**87**	**82**	**875**	**1383**	

Cary, NC, USA). Linear regression and correlations were calculated using MS Excel (Microsoft Corp., Redmond, WA, USA).

Experiment 2

One winter wheat variety, Zhetisu, and two breeding lines, L286 (Almaly/Obryi) and L372 (Almatinskaya polukarlikovaya/Progress), were tested in randomised complete block yield trials in 2012 and 2013 near Almaty, Kazakhstan. The plot size was 1 by 5 m with three replications. Three irrigations were applied starting from pre-heading at 10-day intervals. Two fungicide treatments applied at heading stage in late May, ALTO Super® (3.4% a.i. cyproconazole; Syngenta, Basel Switzerland) and TILT® (25% a.i. propiconazole; Syngenta), were compared with unprotected controls. Common agronomic traits, protein content, Zeleny sedimentation and grain yield were recorded on entire plots, and yield components were determined based on 10 random plants. No diseases were observed in 2012, whereas leaf rust and tan spot infected wheat naturally in 2013 and severity of infection was recorded. Statistical analysis was limited to three-way ANOVA (3 varieties × 3 treatments × 2 years) for design of unorganised replications using AGROS2.13.

Experiment 3

A set of 60 winter wheat varieties and breeding lines varying in leaf rust reaction was studied in a two-replication small-plot (1 by 3 m) experiment in 2013. The set comprised diverse germplasm representing Kazakh breeding lines and germplasm from IWWIP and Eastern Europe. The germplasm was divided into three groups of 20 genotypes: completely resistant (R) to leaf rust; intermediate (MR), with leaf rust severity from 5% to 20%; and susceptible (S), with leaf rust severity $\geq$30%. One set of germplasm was sprayed with the fungicide TILT® at heading at the very beginning of rust infection; the other set was left unprotected. Yield was not determined but yield components were analysed based on 10 random plants from unprotected and protected plots. Days to heading and plant height measurements were taken, as well as normalised difference vegetation index (NDVI) spectral reflectance by using a hand-held GreenSeeker (Trimble, Sunnyvale, CA, USA) was measured, starting from heading, at 10-day intervals. As in Expt 2, three irrigations were applied during the vegetative growing period. Significance of differences between the groups for different traits was determined using *t*-criteria in Excel.

Results

Experiment 1

In both years, epidemic levels of leaf rust were observed in the trial. Final disease readings on susceptible varieties exceeded 80%. The AUDPC was almost twice as high in 2013 because of early disease infection. The four resistance groups were clearly distinct, with similar leaf rust development within the groups yet difference between groups: leaf rust severity <10% for R, 25–30% for MR, 45–55% for MS, and >80% for S. Gene *Lr34* was found in 10 genotypes, either singly or in combination. However, the reaction of genotypes carrying this gene varied from R to S. Based on the performance of Thatcher near-isogenic lines, the following genes provided complete resistance to leaf rust at Adapazari: *Lr19*, *Lr22a*, *Lr23*, *Lr25*, *Lr28* and *Lr35*. Genes *Lr24*, *Lr26* and *Lr37* provided severity of 30% with MSS reaction. These results are in agreement with previous detailed study of leaf rust virulence in Turkey by Kolmer *et al.* (2013).

Analysis of variance showed significance for all three factors of genotype, treatment and year, as well as all three bilateral interactions (Supplementary Material 1, available on the journal's website). However, the triple interaction was not significant ($P > 0.05$). Yield gain due to fungicide protection in 2012 was comparable for R, MR and MS groups, varying from 18.3% to 19.9% (Table 2). The yield gain was slightly higher (24.4%) for the S group. In 2013, the gain was 31.3%, 28.5%, 36.0% and 69.5% for R, MR, MS and S groups, respectively. Early infection in 2013 and favourable conditions for leaf rust resulted in substantially higher yield losses. The yield gain on protected plots compared with unprotected for MS and S groups are easily understood and interpreted. However, substantial yield gain for MR and especially R was not anticipated. Number of days to

Table 2. Expt 1: changes in yield and its components and grain quality due to fungicide application in winter wheat genotypes varying in leaf rust resistance, Adapazari, Turkey, 2012–13

R, Resistant; MR, moderately resistant; MS, moderately susceptible; S, susceptible; UPT, unprotected treatment; PT, protected treatment. *$P < 0.05$ for significance of difference between unprotected and protected treatments

Trait	Year	R group UPT	R group PT	R group Gain (%)	MR group UPT	MR group PT	MR group Gain (%)	MS group UPT	MS group PT	MS group Gain (%)	S group UPT	S group PT	S group Gain (%)
Yield (kg ha^{-1})	2012	6396	7564	18.3*	6044	7172	18.7*	4982	5972	19.9*	5495	6833	24.4*
	2013	5839	7669	31.3*	5624	7226	28.5*	5050	6866	36.0*	3993	6770	69.5*
Test weight, (kg hL^{-1})	2012	77.3	79.4	2.6*	76.2	78.9	3.6*	76.2	78.8	3.4*	76.3	79.2	3.8*
	2013	78.0	80.9	3.7*	77.7	80.8	4.0*	77.1	80.6	4.6*	75.0	80.1	6.8*
1000-kernel weight (g)	2012	40.1	41.7	3.9*	38.7	40.7	5.2*	38.5	41.8	8.5*	41.3	44.3	7.5*
	2013	38.2	43.5	13.9*	37.7	42.5	12.8*	39.0	44.6	14.6*	35.2	45.1	28.2*
Spike length (cm)	2013	9.7	9.5	–2.2	10.3	10.2	–1.4	9.6	9.5	–0.5	10.0	10.2	1.8
No. of spikelets per spike	2013	19.1	19.1	0.0	20.9	20.9	0.0	19.5	19.4	–0.7	19.0	19.3	1.3
No of grains per spike	2013	51.7	51.4	–0.7	55.6	53.7	–3.3	53.7	50.2	–6.5*	50.2	50.6	0.9
Spike grain weight (g)	2013	2.20	2.23	5.6*	2.24	2.41	8.0	2.15	2.39	10.7*	1.86	2.39	29.0*
Protein content (%)	2012	15.2	15.0	–1.2	15.2	15.4	1.0	14.7	15.2	3.6*	15.0	15.6	3.6*
Zeleny sedimentation (mL)	2012	32.6	35.4	8.8*	32.1	34.1	6.1*	28.9	31.6	9.3*	35.4	39.1	10.5*

heading and plant height are not presented, but they were not affected by fungicide application. There were slight but significant increases in test weight in both years. Grain size was the trait most affected, as reflected by changes in 1000-kernel weight. In 2012, there was a progressive increase in this trait from 3.9% for R, to 5.2% for MR, to 8.5% for MS and 7.5% for S. In 2013, the gain in grain size was much larger, at 13.9%, 12.8%, 14.6% and 28.2%, respectively. Spike length and number of spikelets per spike did not change with fungicide application. Number of grains per spike showed some non-significant tendency to reduction, except in the MS group where the protected treatment had significantly fewer grains. Protein content was increased significantly by 3.6% for MS and S groups in 2012. Zeleny sedimentation, which reflects bread-making properties of grain, increased significantly by up to 10.5%. Overall, there was benefit from application of fungicides for all four groups of genotypes independent of their reaction to leaf rust, although naturally, the greatest benefit was for the S group.

Table 3 and Fig. 1 show response to leaf rust protection and gains of individual genotypes. There were significant differences in yield gain within each of the four groups. The yield increase due to disease protection was not significant for some breeding lines in some years: genotypes 3, 13 and 20 from the R group and genotypes 4 and 17 from the MR group in 2012, and genotypes 18 in the R group and 4 in the MR group in 2013. On the other hand, responsive genotypes differed significantly in grain yield ($P < 0.05$). Genotypes 2 and 6 from the R group demonstrated the greatest response in both years: entry 2, 33.3% for 2012 and 32.6% for 2013; entry 6, 30.9% and 50.2%. The highest and stable responders in the MR group were entries 5, 14 and 15; in the MS group, entry 25; and in the S group, entry 9. This substantial and significant gain due to fungicide application was uniformly independent of the level of leaf rust resistance and was demonstrated even for genotypes completely free from leaf rust in unprotected conditions. From the production and farmers' perspectives, the yield gain as such is meaningless because a variety with low yield can have great gain and still be lower yielding than other varieties. The objective is to have a variety that provides high and stable yield under both conditions. For 2012, entry 18 from the R group, which ranked second under unprotected and protected treatment, fits

Table 3. Expt 1: yield performance of 24 winter wheat genotypes under leaf rust infection and protected by fungicide in Adapazari, Turkey, 2012–13
Within columns and resistance groups, means followed by the same letter are not significantly different at $P = 0.05$, based on l.s.d. calculated separately for each trial, treatment and year (RCBD ANOVA). *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$, for significance of increase due to fungicide protection for individual variety in each year, calculated based on l.s.d. ($P = 0.05$) = 1041 kg ha^{-1} for three-way ANOVA (24 varieties × 2 treatments × 2 years) for design of unorganised replications

Entry	2012					2013				
	Unprotected		Protected		Gain	Unprotected		Protected		Gain
	(kg ha^{-1})	Rank	(kg ha^{-1})	Rank	(%)	(kg ha^{-1})	Rank	(kg ha^{-1})	Rank	(%)
Resistant group										
2	5813bc	14	7750ab	5	33.3**	5917ab	8	7847b	4	32.6***
3	6366abc	7	7131b	11	12.0	6683a	2	8795a	1	31.6***
6	5634c	16	7373b	9	30.9**	5208bc	12	7822b	6	50.2***
13	6820ab	3	7430b	7	8.9	6092ab	6	7957ab	3	30.6**
18	7072a	2	8613a	2	21.8**	6105a	5	6892c	17	12.9
20	6672abc	4	7089b	12	6.3	5032c	13	6704c	19	33.2**
Moderately resistant group										
4	7235a	1	8192a	4	13.2	6927a	1	7718ab	7	11.4
5	6584ab	5	8553a	3	29.9***	6298a	4	8455a	2	34.2***
10	6315ab	8	7407ab	8	17.3*	6078a	7	7185bc	11	18.2*
14	4470c	23	5596c	22	25.2*	4759b	17	6425c	24	35.0**
15	5403bc	18	6765bc	15	25.2*	4733b	18	7058bc	13	49.1***
17	6256ab	9	6517bc	17	4.2	4948b	16	6514c	22	31.7**
Moderately susceptible group										
1	6461a	6	7467a	6	15.6	5901a	9	7342a	10	24.4**
19	5826ab	13	6332ab	19	8.7	5380ab	11	6694a	20	24.4*
21	4106c	24	4922c	25	19.9	4432c	21	6775a	18	52.9***
22	4802bc	21	5790bc	20	20.6	5018ab	14	6913a	14	37.8***
24	4861bc	20	5746bc	21	18.2	4996b	15	6570a	21	31.5**
25	3833c	25	5572bc	23	45.4**	4570b	20	6902a	16	51.0***
Susceptible group										
7	5262ab	19	6376b	18	21.2*	3852b	23	6911a	15	79.4***
8	4565b	22	5123c	24	12.2	3436c	24	4923c	25	43.3**
9	5943a	11	7337b	10	23.5**	3885b	22	7690a	8	97.9***
11	5931a	12	6648b	16	12.1	2462d	25	6432b	23	61.3***
12	5681ab	15	8657a	1	52.4***	4717b	19	7504a	9	59.1***
16	5585ab	17	6858b	14	22.8*	5605a	10	7158ab	12	27.7**
l.s.d. ($P = 0.05$)	1213		1178			887		864		

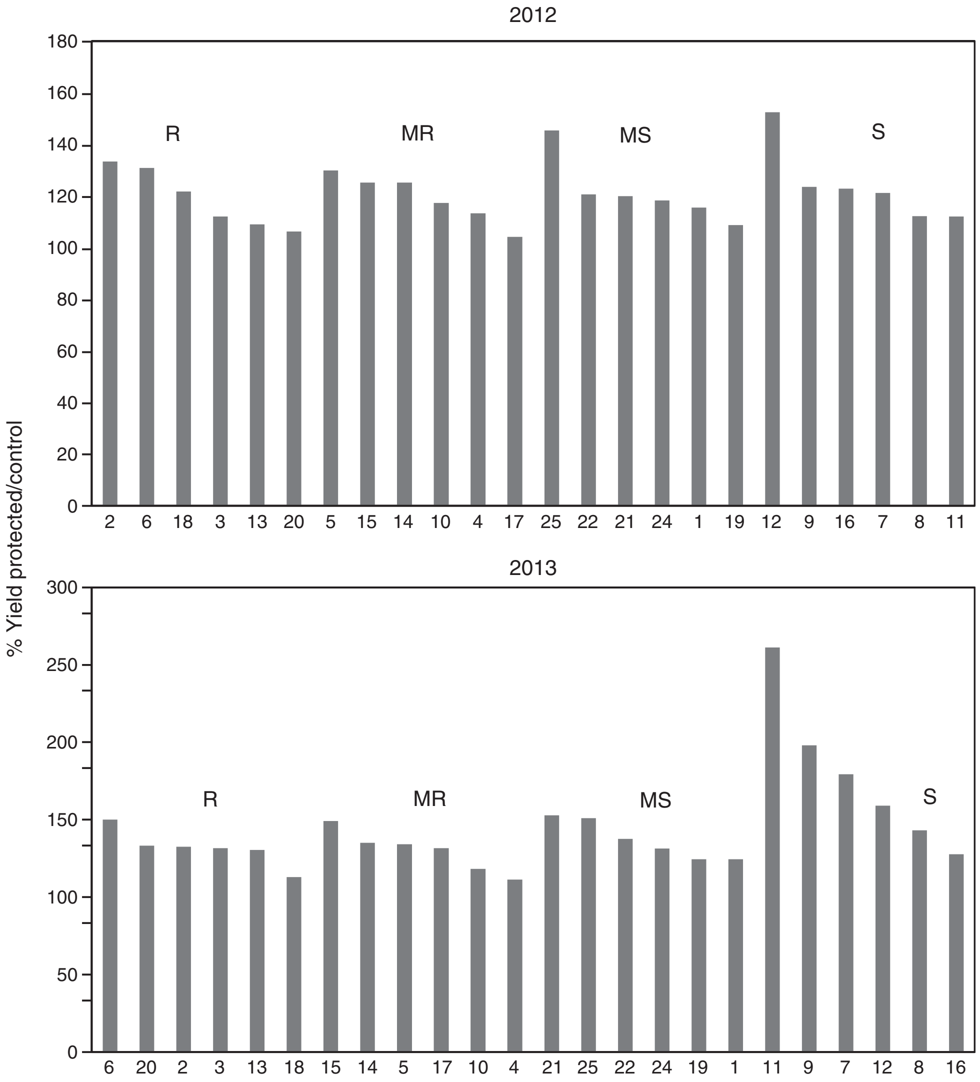

Fig. 1. Expt 1: yield increase (%) for 24 winter wheat genotypes due to fungicide application in 2012 and 2013, Adapazari, Turkey, 2012–13. Significance of yield increase for each genotype and year is shown in Table 3.

these criteria (Table 3). However, it still gained 21.8% from chemical application. For 2013, it would be entry 3, ranked second non-protected and first after gaining 31.6% of yield with protection. From the MR group, entry 4 was ranked first under unprotected conditions in both years. However, due to its poor response to fungicide application of 11–13%, it was outyielded by other, more responsive genotypes. When selecting a variety from the MS or S groups (if such selection is justified at all), we should look for high yield and minimal yield loss due to leaf rust rather yield gain from protection. This would be entry 1 from the MS group in 2012 and 2013, entry 9 from the S group in 2012, and entry 16 from the S group in 2013. These are the genotypes demonstrating tolerance to leaf rust through less yield reduction than other genotypes. Note, however, that none of the MS and S genotypes is competitive with the R and MR groups under leaf rust pressure or without it. This again confirms the need for genetic resistance. However, the genetic variation for response to fungicides shall be utilised for variety selection to increase benefits to farmers if and when wheat protection takes place.

Higher yielding genotypes would be expected to have less response to protection than lower yielding genotypes. Such a tendency is demonstrated in Fig. 2, but even within the highest yielding group, there were genotypes with above-average responses. The contribution of different traits to higher yield response for R and MR groups, or to yield loss in the case of MS and S genotypes, is not clear. No consistently significant correlations were observed between the expression of the traits (days to heading, plant height, 1000-kernel weight, test weight, spike productivity traits) and yield gain from fungicide application. Grain size seems to be the trait most affected by leaf rust, and its stability is important. In 2013, the gain in 1000-kernel weight correlated highly significantly with yield gain (0.87***; $P < 0.001$). However, in 2012, this coefficient of correlation was close to zero. In addition, the gain from fungicide did not depend on the grain size; varieties

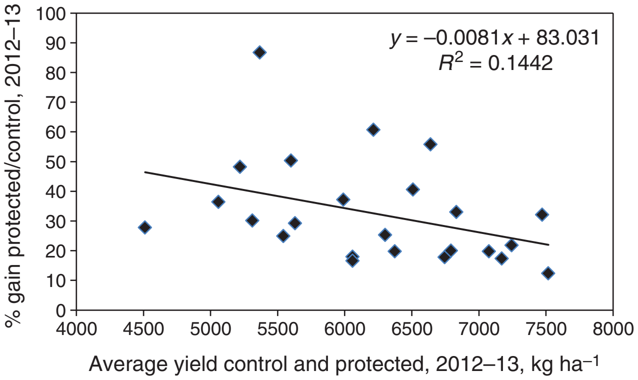

Fig. 2. Expt 1: relationship between grain yield and gain due to fungicide application, Adapazari, Turkey, 2012–13.

with low and high 1000-krnel weight demonstrated variable responses.

Experiment 2

In 2012, with relatively dry weather and a lack of natural leaf rust infection, the crop was almost free from diseases (Table 4). In 2013, leaf rust developed after heading and reached a severity of 40–50% on susceptible varieties Zhetisu and L372 under natural, unprotected conditions. Breeding line L286 demonstrated complete resistance to the pathogen. Tan spot was present in both years at a low severity and affected all genotypes equally. Both ALTO Super® and TILT® controlled leaf rust effectively on susceptible genotypes in 2013. The 2012 season without diseases was less favourable for winter wheat, with grain yield varying from 5.1 to 6.4 t ha^{-1}. In 2013, the more favourable conditions resulted in leaf rust infection but also raised the yield to 6.5–7.9 t ha^{-1}. This once again demonstrates the deceptive nature of rusts that occur in higher yielding years with yield losses less pronounced. The important result of 2012 was a substantial and significant yield increase due to fungicide application in the absence of disease. Application of ALTO Super® increased grain yield by 11–12.8% depending on variety and application of TILT® by 5.8–10.2%. In the presence of disease in 2013, the gain due to fungicide was smaller: 3.8–10.7% for ALTO Super® and 2.8–7.1 for TILT®. Interestingly, the highest yield gain was observed for resistant line L286 for both fungicides. It is paradoxical that yield increase due to fungicides in the season without diseases (2012) was higher than in the year with diseases (2013).

There were substantial differences in the responses of different yield components to fungicide application in different years. In 2012 in the absence of disease, modest but significant gains from ALTO Super® were observed for the number of grains per spike, 1000-kernel weight and, as a result, grain weight per spike. Application of TILT® did not affect 1000-kernel weight, although number of grains per spike and spike grain weight were higher. In 2013, in the presence of leaf rust, a significant double-digit increase was observed for the number of productive tillers per plant and number of grains per spike, whereas almost no increase was detected for 1000-kernel weight. Three varieties had slightly different responses to disease protection. Variety Zhetisu responded to ALTO Super® application by a significant increase in 1000-kernel weight in both years, whereas in L372, no significant gain was observed. A high degree of interaction between variety, reaction to disease, fungicide, disease pressure and environment was detected. Protein content and Zeleny sedimentation were not affected by fungicide application in 2012. In 2013, both parameters were generally lower and there were only three cases of modest, significant increase in either protein content or sedimentation.

Experiment 3

Application of fungicide (TILT®) at heading on a set of 60 genotypes partially controlled leaf rust (Table 5). Mean severity for the intermediate group without protection was 11.2% and with protection 2.2%. For the susceptible group, average leaf rust severity was 39.5% for unprotected and 22.0% for protected plots. As in the other experiments, fungicide protection did not affect days to heading or plant height. However, the rust-susceptible group was on average 10 cm taller than the intermediate group and almost 30 cm taller than the resistant group. This is explained by the fact that the susceptible group comprised either old or semiarid-type tall varieties. Significant differences in the last NDVI reading (24 June 2013) between unprotected and protected treatments for resistant and intermediate groups indicate that fungicide application extended the green canopy functioning period (Table 5). This is probably the main reason for the significantly higher 1000-kernel weight observed in protected plots across all three groups. Once again, as in the two previous experiments, we observed positive responses of resistant genotypes to fungicide application, which in this experiment were mostly expressed in larger grains. Number of grains per spike was not affected by crop protection. Again, there was very wide variation in gains in 1000-kernel weight with fungicide application in three groups of genotypes (Fig. 3). The three most responsive genotypes from the resistant group increased 1000-kernel weight by 10–14%—almost the same increase as in the intermediate group and the susceptible group highly affected by pathogens. However, the gains in 1000-kernel weight observed in this experiment were highest for the genotypes with smaller grain. Nevertheless, there was obvious genetic variation for response of 1000-kernel weight to fungicide treatment independent of leaf rust resistance, and this opens an avenue for breeding improvement of this trait.

Discussion

The present study again proved the devastating effect that leaf rust can have on susceptible wheat varieties. Yield losses up to 60% were observed in some varieties in 2013 in Turkey. The main outcome of the study was a clearly documented, positive response of leaf rust resistant and moderately resistant genotypes to fungicide protection. This response was observed in all three experiments even though they were conducted in different locations with different sets of germplasm, different fungicides and different disease pressure. Yield gain of leaf rust resistant germplasm from fungicide protection in the presence, as well as in the absence, of disease in this study was unexpected. The gain was substantial, exceeding 10%, and it cannot be ignored. Although this has been reported

Table 4. Expt 2: changes in disease severity, agronomic traits and grain quality due to fungicide application in three winter wheat genotypes, Almaty, Kazakhstan, 2012–13

UPT, Unprotected treatment. Protected treatments: ALTO, ALTO Super® (a.i. cyproconazole); TILT, TILT® (a.i. propiconazole). $*P < 0.05$ for significance of difference between unprotected and protected treatments based on three-way ANOVA (3 varieties × 3 treatments × 2 years) for design of unorganised replications

Trait	Year	Zhetisu					Line 286					Line 372				
		UPT	ALTO	Gain (%)	TILT	Gain (%)	UPT	ALTO	Gain (%)	TILT	Gain (%)	UPT	ALTO	Gain (%)	TILT	Gain (%)
Leaf rust (%)	2012	0	0	–	0	–	0	0	–	0	–	0	0	–	0	–
	2013	40	5	–	5	–	0	0	–	0	–	50	5	–	5	–
Yield (kg ha^{-1})	2012	5104	5760	12.8*	5626	10.2*	5527	6233	12.8*	5880	6.4*	5788	6423	11.0*	6127	5.8*
	2013	6463	6808	5.3*	6645	2.8*	6672	7386	10.7*	7149	7.1*	7636	7930	3.8*	7879	3.2*
Test weight (kg hL^{-1})	2012	797	800	0.4	801	0.5	802	805	0.4	806	0.5	796	798	0.3	799	0.4
	2013	766	783	2.2*	775	1.2*	777	781	0.5	777	0	776	783	0.9	782	0.8
1000-kernel weight (g)	2012	40.4	42.3	4.7*	40.4	0	41.2	43.2	4.9*	41.5	0.7	43.1	43.8	1.6	43.4	0.8
	2013	45.3	46.4	2.4*	46.2	1.2	46.3	46.8	1.1	46.5	0.4	46.7	47.5	1.7	47.1	0.9
No. of productive tillers per plant	2012	2.97	3.22	8.4	3.22	8.4	3.39	3.57	5.3	3.57	5.3	3.45	3.59	4.0	3.81	10.4*
	2013	3.97	4.71	18.6*	4.57	15.1*	3.79	4.23	11.6*	4.22	11.3*	4.59	5.24	14.2*	4.76	3.7
No. of grains per spike	2012	39.7	41.4	3.5*	41.1	3.5*	37.1	38.2	3.0*	37.5	1.1*	37.0	38.0	2.7*	39.0	5.4*
	2013	48.0	49.0	2.1*	50.4	5.0*	44.1	47.6	7.9*	48.9	10.9*	50.4	54.7	8.5*	54.4	7.9*
Spike grain weight (g)	2012	1.48	1.65	11.5*	1.61	8.8*	1.60	1.84	15.0*	1.61	0.6	1.72	1.94	12.8*	1.83	6.4*
	2013	2.06	2.17	5.3*	2.07	0.5	2.29	2.34	2.2	2.37	3.5*	2.39	2.40	0.4	2.42	1.3
Protein content (%)	2012	16.5	16.5	0	16.5	0	16.6	16.6	0	16.8	1.2	14.5	14.6	0.7	14.9	2.8
	2013	14.0	14.5	3.6*	14.0	0	16.1	16.1	0	16.8	4.4*	15.9	16.3	2.5	16.1	1.3
Zeleny sediment. (mL)	2012	64.0	64.0	0	64.3	0.5	65.1	65.1	0	65.9	1.2	47.0	47.0	0	48.5	3.2
	2013	45.3	46.3	2.2	46.2	2.0	63.7	64.3	0.9	64.0	0.5	61.2	63.4	3.6*	62.2	1.6

Table 5. Expt 3: effect of fungicide treatment for leaf rust on agronomic traits in resistant, intermediate and susceptible winter wheat genotypes, Almaty, Kazakhstan, 2013

Mean values for 20 genotypes in each group. Within trait and resistance group, means followed by different letters are different significantly between treatments at $P = 0.05$ based on *t*-criteria

Trait	Resistant group		Intermediate group		Susceptible group	
	Unprotected	Protected	Unprotected	Protected	Unprotected	Protected
Leaf rust (%)	0	0	11.2a	2.2b	39.5a	22.0b
Days to heading from 1 Jan.	142.8	142.6	143.3	143.2	144.0	143.8
Plant height (cm)	91.5	91.7	109.4	109.7	119.5	112.5
Normalised difference vegetation index:						
27 May	0.79	0.79	0.79	0.79	0.76	0.76
05 June	0.75	0.74	0.75	0.74	0.72	0.72
14 June	0.69	0.70	0.69	0.70	0.68	0.68
24 June	0.52b	0.57a	0.52b	0.57a	0.52	0.53
1000-kernel weight (g)	42.9b	45.2a	44.3b	46.8a	45.5b	48.3a
No. of grains per spike	58.2	57.4	55.9	58.2	55.9	55.3
Grain weight per spike (g)	2.39	2.51	2.38b	2.64a	2.47	2.60

previously (Zefelippo 1992; Varga *et al*. 2005; Jørgensen *et al*. 2008), its magnitude and genetic nature are poorly understood. There is a need to understand the nature of the yield gain in resistant varieties due to fungicide protection. Because no precise pathological or physiological study was made in this experiment, we can only hypothesise about different mechanisms.

The first and most obvious mechanism is protection from fungal diseases other than leaf rust. Powdery mildew was observed in Expt 1 in both years and was partly controlled by the fungicide treatment. However, its severity was too low (10–20%) to cause the observed yield reduction of 20–30% and the germplasm tested was relatively uniformly affected by disease. Tan spot was observed in Expt 2 but it was hardly affected by fungicides, and again genotypic responses were similar. The second possible cause is the physiological effect of fungicides on wheat. An effect of pyraclostrobin (Opera®) on crops was reviewed by Venancio *et al*. (2003). It is proven to have universal positive effect on crops even in the absence of diseases through a 'greening' effect as well as enhancement of stress tolerance through hormonal regulation and assimilation of carbon and nitrogen by plants. In a greenhouse experiment, durum wheat plants exposed to pyraclostrobin had higher nitrate accumulation and 20% higher biomass at 2 weeks after application without disease (Kohle *et al*. 2002). Propiconazole (TILT®) and cyproconazole (ALTO Super®) belong to the triazole chemical family, which affects sterol synthesis in fungi. Propiconazole was proven to have both fungicidal action and plant-growth regulating function as a growth retardant (Fletcher *et al*. 1986). In many species, treatment with triazole leads to inhibition of ethylene biosynthesis, delaying senescence and leaf abscission (Fletcher *et al*. 2000). Delayed senescence was also observed in our Expt 3, as reflected by NDVI measurements. In Expt 2, two different fungicides were applied and there were clear differences in their effect on grain yield. Although ALTO Super® and TILT® are from the same triazole family, wheat response to the former was higher. Further studies on this subject are needed to account for the role of the interaction between different chemicals and wheat germplasm in varying yield potential and genetic resistance to diseases. The third possible explanation of the yield increase in resistant varieties is that genetic resistance incurs cost to the plants and requires energy (Bertelsen *et al*. 1999). Fungicides relieve this pressure and the energy saved is utilised for biomass and grain yield. All three mechanisms, and possibly others, may contribute to yield gains. Study on this topic is warranted. In addition, yield gain from fungicide is likely to be more general than disease-specific and, once identified with one pathogen, may be expressed with other diseases.

The yield gains of resistant and moderately resistant varieties associated with fungicide application are substantial, and capturing this trait in wheat-breeding programs deserves consideration. Identification of responsive parents would be relatively easy, and some of the lines identified in this study can be used for this purpose. Such a trait is unlikely to be under simple genetic control. Nevertheless, it is worthwhile to test the interaction between known *Lr* genes and fungicide response by using near isogenic lines or bi-parental populations. If certain resistance genes do provide higher response to protection, they could be utilised in the breeding program. Handling of segregating populations to combine resistance to pathogen and response to fungicide represents a significant challenge. On one hand, selection of individual plants for response in early generations under protection would be logical and most likely efficient. Grain size can be used as a proven selection criterion (Bokus *et al*. 1992). On the other hand, protection in early generations would not allow selection of resistant plants. Alternating cycles of selection under disease pressure and under chemical protection is a possible solution. Once fixed lines are developed, the breeding methodology should be based on parallel testing under disease pressure and protection to select resistant and high-yielding lines under both conditions. Physiological tools may play an important role in identification of the stay-green trait under protection (Serrago *et al*. 2009). Multi-locational testing with exposure to different diseases and their combinations as well as protection by different chemicals would be needed for variety candidates in order to study genotype × disease × fungicide × environment interaction and select the high-yielding, disease-resistant and fungicide-responsive varieties.

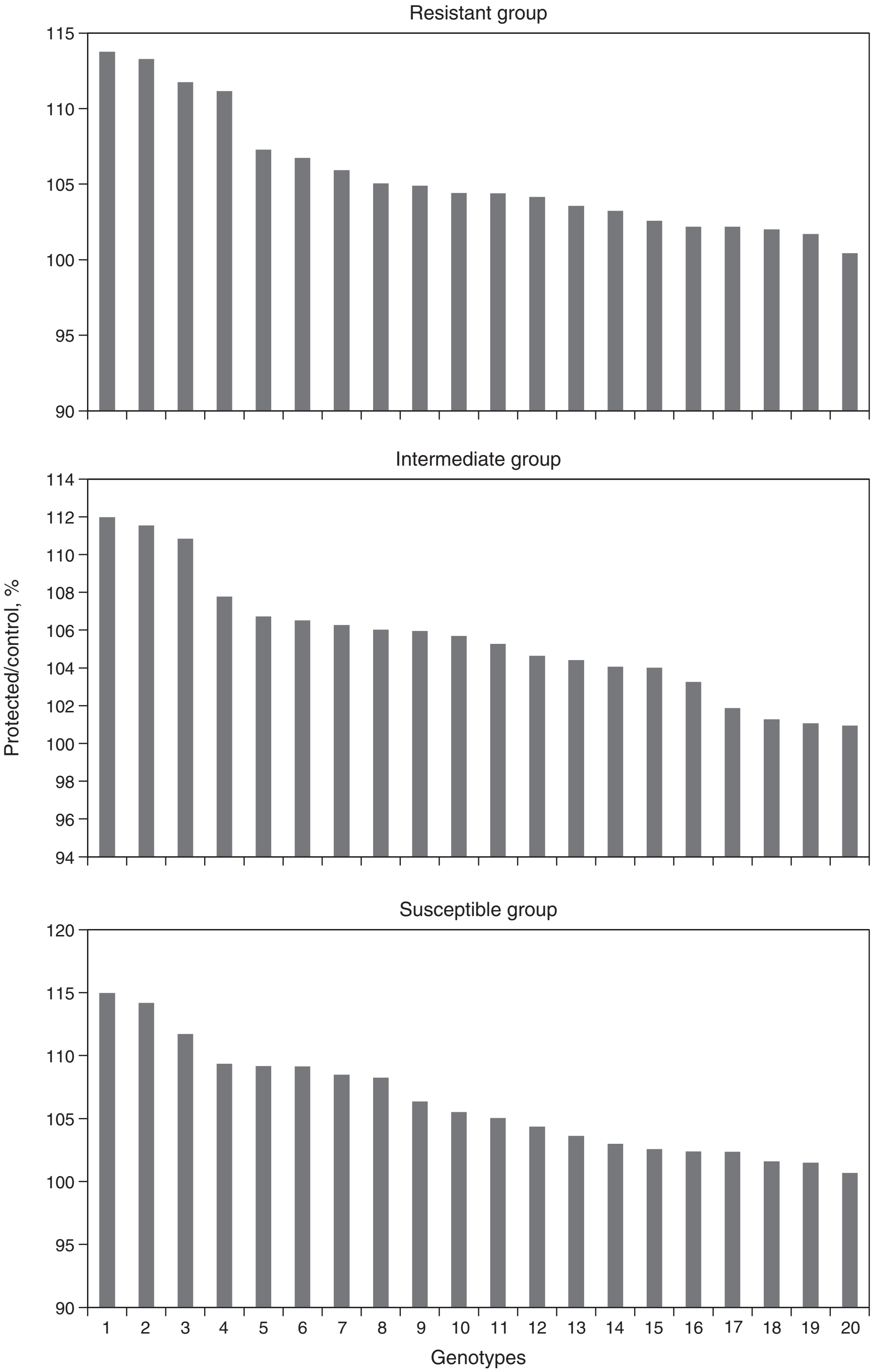

Fig. 3. Expt 3: increase (%) in 1000-kernel weight in leaf rust protected treatment over untreated for resistant, intermediate and susceptible groups of winter wheat genotypes, Almaty, Kazakhstan, 2013.

The outlook for maintaining the pace of production increase to meet growing population and wheat-grain demand does imply utilisation of crop protection measures on increasing scale. Despite the goal of wheat breeders for genetically determined, durable resistance, huge areas are still grown to varieties with different degrees of disease susceptibility. Fungicide protection is an easy and efficient way of increasing yield, and frequently it is applied preventively, even on resistant varieties. When this happens, optimising the response to fungicides demonstrated in this study appears highly desirable.

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References

Bertelsen JR, Neegard E, Smedegaard-Petersen V (1999) Reasons for improved yield when using azoxystrobin in winter wheat. *DJF Rapport, Markburg* **10**, 175–186.

Bokus WW, Davis MA, Shroyer JP (1992) Effect of foliar fungicide application on seed size of winter wheat. *Journal of Applied Seed Protection* **10**, 1–16.

Dimmock JPRE, Gooding MJ (2002) The effect of fungicides on rate and duration of grain filling in winter wheat in relation to maintenance of flag green area. *The Journal of Agricultural Science* **138**, 1–16. doi:10.1017/S0021859601001666

Fletcher RA, Hofstra G, Gao JG (1986) Comparative fungitoxic and plant growth regulating properties of triazole derivatives. *Plant & Cell Physiology* **27**, 367–371.

Fletcher RA, Gilley A, Sankla N, Davis TD (2000) Triazoles as plant growth regulators. *Horticulture Review* **24**, 55–138.

Gianessi L, Williams A (2011) Fungicides protect the world's wheat crop from rust. International Pesticide Benefits Case Study No. 31. CropLife Foundation. Available at: https://croplifefoundation.files.wordpress.com/2012/07/31-wheat-rust.pdf

Jørgensen L, Nielsen G, Orum J, Jensen J, Pinnschmidt H (2008) Integrating disease control in winter wheat – optimizing fungicide input. *Outlooks of Pest Management* **19**, 206–213. doi:10.1564/19oct04

Kohle H, Grossmann K, Jabs T, Stierl R, Gerhard M, Kaiser W, Glaab J, Conrath U, Seehaus K, Herms S (2002) Physiological effects of the strobilurin fungicide F 500 on plants. In 'Fungicides and antifungal compounds. III'. pp. 61–74. (AgroConcept: Bonn, Germany)

Kolmer JA, Mert Z, Akan K, Demir L, Unsal R, Sermet C, Keser M, Akin B, Morgounov AI (2013) Virulence of *Puccinia triticina* in Turkey and leaf rust resistance in Turkish wheat cultivars. *European Journal of Plant Pathology* **135**, 703–716. doi:10.1007/s10658-012-0107-1

Ray DK, Ramankutty N, Mueller N, West P, Foley J (2012) Recent patterns of crop yield growth and stagnation. *Nature Communications* **3**, 1293.

Serrago RA, Carretero R, Bancal M, Miralles DJ (2009) Foliar diseases affect the eco-physiological attributes linked with yield and biomass in wheat (*Triticum aestivum* L.). *European Journal of Agronomy* **31**, 195–203. doi:10.1016/j.eja.2009.06.002

Sharma RC, Rajaram S, Alikulov S, Ziyaev Z, Hazratkulova S, Khodarahami M, Nazeri SM, Belen S, Khalikulov Z, Mosaad M (2013) Improved winter wheat genotypes for Central and West Asia. *Euphytica* **190**, 19–31. doi:10.1007/s10681-012-0732-y

Simón M, Ayala F, Golik S, Terrile I, Cordo C, Perello A, Moreno V, Chidichmo H (2011) Integrated foliar disease management to prevent yield loss in Argentinian wheat production. *Agronomy Journal* **103**, 1441–1451. doi:10.2134/agronj2010.0513

Singh RP, Huerta-Espino J, Rajaram S (2000) Achieving near-immunity to leaf and stripe rusts in wheat by combining slow rusting resistance genes. *Acta Phytopat et Entomol Hungarica* **35**, 133–139.

Varga B, Svecnjak Z, Mac'esic' D, Uher D (2005) Winter wheat cultivar responses to fungicide application are affected by nitrogen fertilization rate. *Journal of Agronomy & Crop Science* **191**, 130–137. doi:10.1111/j.1439-037X.2004.00133.x

Venancio WS, Rodrigues M, Begliomini E, de Souza NL (2003) Physiological effects of strobilurin fungicides on plants. *Siências Exatas e da Terra, Ciências Agrárias e Engenharias* **9**, 59–68.

Wegulo S, Zwingman M, Breathnach J, Baenziger P (2011) Economic returns from fungicide application to control foliar fungal diseases in winter wheat. *Crop Protection* **30**, 685–692. doi:10.1016/j.cropro.2011.02.002

Zefelippo M (1992) Response of cultivars to crop protection treatments. *Informatore Agrario* **48**, 47–49.

Ziyaev ZM, Sharma RC, Nazari K, Morgounov AI, Amanov AA, Ziyadullaev ZF, Khalikulov ZI, Alikulov SM (2011) Improving wheat stripe rust resistance in Central Asia and the Caucasus. *Euphytica* **179**, 197–207. doi:10.1007/s10681-010-0305-x