Accurate Assessment of Wheat and Triticale Cultivar Resistance to *Septoria tritici* and *Stagonospora nodorum* Infection by Biotin/Avidin ELISA

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ABSTRACT

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Specific and quantitative biotin/avidin–enzyme-linked immunosorbent assays (BA-ELISA) were evaluated for their ability to assess resistance of wheat and triticale cultivars to *Septoria tritici* (leaf blotch) and *Stagonospora nodorum* (leaf and glume blotch) in field trials. Using BA-ELISAs, the antigen amounts of *S. tritici* and of *Stagonospora nodorum* were measured in the flag leaf (F) and the first leaf below it (F-1) of five cultivars of triticale at Zadok's growth stage (GS) 75-80 and in 11 cultivars of wheat at GS 73-75 in 2001 and 2002. The presence of the pathogens was found to be specific to parts of the plants, cultivar, and plant species. *Stagonospora nodorum* was the dominant leaf blotch pathogen in triticale, while both *Septoria tritici* and *Stagonospora nodorum* occurred commonly in wheat. Close correlations were obtained between the pathogen amount measured by BA-ELISA and the percentage of necrotic leaf area in the tested cultivars. The BA-ELISA values for the tested triticale and wheat cultivars were ranked, and they correlated well with the susceptibility ratings given in the cultivar list recommended by Bundessortenamt (German Federal Office of Plant Variety), which is based on visual assessment of the leaf blotch complex caused by *S. tritici* and *Stagonospora nodorum*. The relative susceptibilities of individual wheat cultivars to both pathogens were similar. In conclusion, BA-ELISA provided for an accurate diagnosis and quantification of *S. tritici* and *Stagonospora nodorum* in infected plant tissue, and therefore can be used to assess resistance to these fungi in a disease complex in both early-stage breeding lines and field trials.

Additional keywords: Septoria leaf blotch

Wheat leaf blotch, caused by *Septoria tritici* (teleomorph: *Mycosphaerella graminicola*) and *Stagonospora* (= *Septoria*) *nodorum* (teleomorph: *Phaeosphaeria nodorum*), is a major disease complex of wheat worldwide (12). In Germany, these diseases have been reported to cause yield losses up to 25% in selected field trials (4). *Septoria* and *Stagonospora* diseases are also common on triticale, barley, and rye (6,12,31). The use of resistant cultivars is one of the most effective methods for managing this disease complex (7,9,15).

Assessment of the resistance of wheat and triticale plants to *S. tritici* and *Stagonospora nodorum* is generally based on visual, quantitative measurement of the percentage of necrotic leaf area or pycnidial density as determined by visual or microscopic examination (7). In practice, both these methods have limitations. Vis-

ual assessment is simple and easy to perform, but lacks accuracy and specificity (13,28). For example, similar symptoms can be caused by *S. tritici*, *Stagonospora nodorum*, and other leaf pathogens, such as *Drechslera tritici-repentis*. Thus, it is difficult to identify diseases caused by these pathogens based solely on visual symptoms (13,19,20). As a consequence, under natural infection conditions, visual assessment is suitable for assessing combined resistance to all the pathogens involved, but is not suitable for assessing resistance to individual pathogens in a disease complex (19,20). Lesion color, size, and shape, and appearance of pathogen sporulation structures can be used to distinguish one disease from another in the disease complex (12,20). However, sporulation is heavily influenced by physiological stress, nutritional status, and other environmental factors (2,15). Furthermore, disease symptoms, as indicated above, are often nonspecific and may be confused with stress-related symptoms or even normal plant development. For example, the coalescing lesions and pale pycnidia associated with infections by *Stagonospora nodorum* are often difficult to distinguish from natural senescence (13,31). Addition-

ally, in damp locations and during wet seasons, *Didymella exitialis* can be found on damaged and senescing tissue, where it causes brown, oval, or irregularly shaped lesions with brown or black pseudothecia and pycnidia, which can be easily confused with the pycnidia of *S. tritici* (12,13).

Enzyme-linked immunosorbent assay (ELISA) is regarded as a specific, accurate, and routine test for detection of pathogens in plants, and has been applied to studying host–pathogen interactions by estimating certain protein antigens of the pathogens (3,10,17,26,31,33). Different ELISA tests have been developed for *S. tritici* and *Stagonospora nodorum* (14,29), but they are mostly used as a guide for when to make fungicide sprays (13,16,24).

Recently, the polymerase chain reaction (PCR) has been used to evaluate wheat cultivar resistance to *S. tritici* and *Stagonospora nodorum*, as well as to *Puccinia* spp. in field trials (9). PCR may be the most specific and accurate method of detection, but this method is expensive, prone to contamination, and requires expensive equipment and highly trained individuals to perform (23,25).

The objectives of this study were (i) to determine whether the biotin/avidin (BA)-ELISA method can be used to more accurately assess the resistance of wheat and triticale to infections by *S. tritici* and *Stagonospora nodorum* than the classical method based on visual assessment of disease symptoms, (ii) to determine if BA-ELISA is effective in detecting and quantifying disease severity related to infection by the two pathogens in plants, and (iii) to compare the disease severity in different parts of the plants.

MATERIALS AND METHODS

Inoculum preparation. The fungal isolates used in studies included *S. tritici* strain BW57, obtained from the Fungi Collection, Department of Plant Pathology, University Goettingen, Germany, and strain Pn403 of *Stagonospora nodorum*, obtained from H. Wolf (38). To produce pycnidia, *S. tritici* was cultured on malt-yeast-sucrose medium (22) for 5 to 7 days, while *Stagonospora nodorum* was cultured on V8-cellophane medium (38) for 2 weeks, both at 20 ± 2°C under near-UV light (12-h light/dark cycle). After *Stagonospora nodorum* pycnidia were

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formed, fungal cultures were flooded with sterile distilled water (approximately 10 ml per petri dish) and scraped with a sterile microscope slide to release pycnidiospores. Spore suspensions were filtered through two layers of cheesecloth, and the spore concentration of both fungi was adjusted to 1×10^6 spores per ml using sterile distilled water.

Inoculation procedure. Pots containing 15 seedlings of the wheat cv. Bussard were grown in climate chambers with a relative humidity of 100% and light intensity of 8,000 to 10,000 lx. Plants were inoculated at the three-leaf stage by spraying spore suspensions mixed with 0.2% (vol/vol) Tween 20 at a rate of 4 ml per pot using a hand atomizer. To achieve successful infection, plants inoculated with *S. tritici* were held at 20°C for 48 h, whereas plants inoculated with *Stagonospora nodorum* were held at 25°C for 24 h. Control plants were sprayed with 0.2% Tween 20 and kept under the same conditions. After inoculation, plants were placed on benches in a greenhouse at $20 \pm 5^\circ\text{C}$ with a light intensity of 6,000 to 8,000 lx until further use.

Sample preparation for comparison of three drying methods. Wheat leaves from the greenhouse experiments (inoculated and noninoculated) were collected at 2 or 4 weeks after the inoculation with *Stagonospora nodorum* and *Septoria tritici*, respectively, and visually assessed for leaf blotch disease (see below). The leaves were cut into pieces no longer than 0.5 cm, mixed, and divided into three replicate portions (10 g per portion). The different portions were then dried using the following procedures: (i) in a freezer dryer (-50°C for 24 to 48 h), (ii) in an oven (30°C for 48 to 72 h), or (iii) at room temperature for 72 to 120 h. The dried leaf materials were ground to powder with a biomiller (IKA-Werke, Staufen, Germany) and stored at -20°C until use.

Field experiments. The field sites were located at Reinshof–Agricultural Research Station of the Goettingen University, Goettingen, Germany (165 m above sea level; annual rainfall 635 mm; annual mean temperature 8.5°C). The soil type is a loam with pH 6.8 to 6.9. Eight cultivars of winter wheat and three cultivars of winter triticale were planted on 26 September 2000 in 2×10 m plots, row width of 15 cm. The second-year experiment involved 11 cultivars of winter wheat and four cultivars of triticale planted on 6 October 2001. Seedling rates in both experiments were 280 seeds per m^2 for triticale and 350 seeds per m^2 for wheat (exception: 200 seeds per m^2 for wheat cv. Hybnos, a hybrid cultivar). A synthetic pyrethroid insecticide, Esfenvalerat (BASF, Ludwigshafen, Germany), was applied at 10 g a.i./ha at growth stage (GS) 51 on the Zadok's scale (37). The herbicides Carfentrazone + Flupyrsulfuron (10 + 20 g a.i./ha; DuPont,

Bad Homburg, Germany) and Fluoxypyr (90 g a.i./ha; DOW Agro Sciences, München, Germany) were sprayed at GS 13 and GS 31, respectively, in both experiments. A urea ammonium nitrate solution was applied at GS 27, GS 30, and GS 51 (180 kg N/ha in 2001 and 195 kg N/ha in 2002, respectively). Two growth regulators were used in experiments. Chlormequat (720 g a.i./ha; BAYER, Leverkusen, Germany) was sprayed on wheat and triticale at GS 27, whereas Trinexapac-Ethyl (Syngenta, Basel, Switzerland) was applied to wheat and triticale at GS 32 at rates of 75 g a.i./ha and 150 g a.i./ha, respectively.

Samples consisting of 25 leaves per plot were arbitrarily collected at GS 73–75 for wheat and at GS 75–80 for triticale, and were visually assessed for Septoria leaf blotch as described below. Additionally, in order to get information on the distribution of the two fungi over different parts of plants, four types of samples were collected from the wheat cv. Ritmo: flag leaf and F-1 (both at GS 75), ear (GS 80), and harvested grain. All the field samples were dried in an oven and then cut, ground, and stored as described above.

Visual disease assessment. Disease severity was visually assessed as percentages of necrotic leaf area with respect to total leaf area. Each leaf was placed into one of the following eight categories: 0, 1, 5, 10, 25, 50, 75, and 100% of leaf area affected (8). The data per leaf were averaged to give percent disease severity for each plot.

Antigen extraction. Leaf or ear powder was added at a rate of 0.5 g to 10 ml of extraction buffer (0.01 M phosphate-buffered saline + 0.05% [vol/vol] Tween 20 [PBST] containing 1% [wt/vol] polyvinylpyrrolidone [PVP], pH 7.2), and the samples were extracted at 4°C overnight with shaking. For grain flour, 5 g was added to 20 ml of the extraction buffer. The extract suspension was centrifuged at $12,000 \times g$ at 4°C for 10 min. The supernatant was tested in immunoassays.

BA-ELISA. Two BA-ELISAs (biotin/avidin format ELISA, a modified double antibody sandwich [DAS]-ELISA), recently developed in our laboratory (34,35), were used for measuring *S. tritici* and *Stagonospora nodorum* antigens present in samples. Polyclonal antisera were raised by the immunization of rabbits with total protein fraction extracted from shaken cultures of *S. tritici* (four strains) or *Stagonospora nodorum* (two strains). The immunization was carried out at the Federal Biological Research Centre for Agriculture and Forestry, Institute of Plant Virology and Microbiology, Braunschweig, Germany. The fungal antigens, row antisera, purified antibodies, and biotinylated antibodies were kept at the Institute of Plant Pathology and Plant Protection, University Goettingen, Germany. Both BA-ELISAs have been applied to

evaluating fungicide efficacies to special pathogen in wheat under field conditions (18,36).

The BA-ELISA tests were performed in 96-well convex-bottomed plastic plates (Immuno Plate Maxisorb, Nunc International, Denmark). After each step, the plate wells were emptied and washed thoroughly three times for 3 min each with washing buffer (50% PBST), and dried by pounding the plate on a stack of paper towels after the last washing step. All the reaction volumes, except in the second step, were 100 μl per well. The plate was coated with the required antibody (IgG) diluted with a coating buffer (0.05 M carbonate buffer [$\text{Na}_2\text{CO}_3/\text{NaHCO}_3$], pH 9.6) to a tested concentration. The plate was incubated at 4°C overnight. On the next day after washing, the plate was blocked with 200 μl per well of 1% (wt/vol) skim milk powder (Roche Diagnostics, Mannheim, Germany) dissolved in the coating buffer and incubated at 37°C for 1 h or at room temperature for 2 h. The sample extract or fungal protein was added and incubated at 4°C overnight. The biotinylated antibody as the second antibody diluted in conjugate buffer (0.2% [wt/vol] bovine serum albumin + PBST, pH 7.2) was placed in each well and the plate incubated at 4°C overnight. Next, streptavidin-alkaline phosphatase conjugate (Roche Diagnostics) diluted to 1:10,000 (vol/vol) with the conjugate buffer was added and the plate was incubated at room temperature for 1 h. Finally, substrate (1 mg/ml of *p*-nitrophenylphosphate in diethanolamine-buffer, pH 9.8) was added and incubated at room temperature in darkness for 2 to 3 h. Absorbance (optical density [OD]) was read at 405 nm (reference: 592 nm) with a photometer (Spectra II, SLT Laborinstruments, Austria).

The BA-ELISA values were then converted to fungal protein equivalent (FPE) using a standard protein curve, and the final value was used as an estimate of the amount of fungal protein (antigen, μg FPE/g leaves) in the corresponding sample. Antigen amounts greater than 0.03 μg FPE/g for *S. tritici*, and 0.04 μg FPE/g for *Stagonospora nodorum* were regarded as positive for infection. In the immunoassay, plates always included two control reactions: (i) positive (inoculated wheat leaves) and (ii) negative (noninoculated). Each BA-ELISA result was based on the average value of three replicates.

Assessing cultivar resistance. Results of the BA-ELISA measurements (i.e., fungal antigens) were compared with visual susceptibility ratings for cultivars to Septoria leaf blotch complex obtained from the Bundessortenamt (BSA, German Federal Office of Plant Variety) recommended cultivar list for winter wheat and winter triticale (3). Moreover, information about the susceptibility of two EU wheat cultivars tested (cvs. Contur and Bandit) was

recently recorded in the results of cultivar trials undertaken by the Hesse Serve Centrum for Agriculture, Horticulture and Nature conservation (Hessisches Dienstleistungszentrum für Landwirtschaft, Gartenbau und Naturschutz [HDLGN]), Kassel (11), and the Chamber of Agriculture Schleswig-Holstein (Landwirtschaftskammer Schleswig-Holstein [LWKSH]), Kiel (21), Germany, respectively.

Statistical analysis. The standard deviation between replicates and the correlation (r) was determined using SigmaPlot 2000 (SPSS Inc., Chicago, IL). Multiple comparisons of means were performed using one-way ANOVA (Tukey-Kramer, Systat8, SPSS Inc.).

RESULTS

Standardization of BA-ELISA. Standard calibration curves of the immunoassays used to calculate the amounts of fungal antigen in samples for *S. tritici* and *Stagonospora nodorum* are presented in Figure 1.

Comparison of the drying methods. Wheat leaves that were dried in an oven or at room temperature contained less antigen for both pathogens after extraction than did freeze-dried materials (Fig. 2). Nevertheless, high antigen amounts (84% for *S. tritici* or 74% for *Stagonospora nodorum*) were detected in oven-dried leaves.

Distribution of pathogens in different parts of wheat plants. Based on BA-ELISA results (Fig. 3), there was a greater quantity of *S. tritici* antigen in F-1 than in flag leaves, while minimal amounts were detected in ears, and none in grains. In comparison with these results, antigen of *Stagonospora nodorum* was found in equal relation, but smaller amounts, in F-1 and flag leaves, while high quantities of antigen were present in ears.

Testing of resistance of triticale and wheat cultivars to infections by *S. tritici* and *Stagonospora nodorum*. Results of visual disease assessment and BA-ELISA analyses for flag leaf samples collected from wheat cultivars (GS 73-75) and triticale cultivars (GS 75-80) in 2001 and 2002 are presented in Table 1. *Stagonospora nodorum* was detected by BA-ELISA in all triticale cultivars in both years; antigen levels varied significantly among the cultivars. In contrast, no *S. tritici* antigen was found in the flag leaves of triticale cultivars tested in either year. To further check for the presence of *S. tritici* in triticale, 2002 F-1 samples with higher levels of leaf necrosis (i.e., 2.5 to 11% leaf necrosis) were analyzed by BA-ELISA. Only two out of four cultivars tested had minimal *S. tritici* antigen amounts: 0.06 µg FPE/g leaves for cv. Modus and 0.13 µg FPE/g leaves for cv. Trimaran.

S. tritici was detected in flag leaves of all wheat cultivars analyzed by BA-ELISA (Table 1). Antigen amounts were much higher in 2002 (7 to 288 µg FPE/g leaves)

than in 2001 (0.6 to 21 µg FPE/g leaves). This difference corresponded to differences in visual disease data between the two years. Antigen results obtained with BA-ELISA correlated well with percent necrotic leaf area ($r = 0.96$, $P < 0.001$) for all wheat cultivars tested, except for cvs. Flair and Contur in 2001 (Table 1). A close correlation ($r = 0.91$, $P < 0.001$) was also obtained between visual assessment and antigen amounts for *Stagonospora nodorum*. As was the case for *S. tritici*, antigen levels of *Stagonospora nodorum* were higher in 2002 (0.7 to 16 µg FPE/g) than in 2001 (0.4 to 2.5 µg FPE/g). Based on the visual assessments and BA-ELISA results from the flag leaves, the relative susceptibilities of individual wheat cultivars tested were similar for both pathogens (Table 1).

For most wheat and triticale cultivars tested, the quantitative data obtained from BA-ELISAs and our visual assessments were consistent with the susceptibility ratings published by the BSA (3) (Table 1). Two EU wheat cultivars, Contur and Bandit, preliminarily classified as moderately susceptible were found to be highly susceptible to both pathogens.

DISCUSSION

ELISA tests have been applied to studying cultivar disease resistance because results are highly specific and reliable (10,17,26,33). This is especially true for field trials where different pathogens frequently occur in a disease complex. In the present study, quantitative BA-ELISA tests were used to assess cultivar resistance of

Fig. 1. Standard protein curves for quantification of *Septoria tritici* (A, ▲) and *Stagonospora nodorum* (B, ●) in biotin/avidin enzyme-linked immunosorbent assay.

Fig. 2. Effects of different drying methods for leaf samples on amounts of *Septoria tritici* antigen (black) and *Stagonospora nodorum* antigen (white) in biotin/avidin enzyme-linked immunosorbent assay. RT: room temperature. Same letters were not significantly different according to the Tukey test ($P = 0.05$).

triticale and wheat to infections by *S. tritici* and *Stagonospora nodorum* under field conditions in two consecutive years.

In ELISA, a direct comparison of spectrophotometric measurements of plant samples expressed in optical density units may be influenced by uncontrollable factors such as temperature fluctuations, operator precision, and minor differences in reagent activities (2,14). In this study (Fig. 1), concentrations of *S. tritici* and *Stagonospora nodorum* antigen reagents were used to generate a standard curve for each immunoassay performed. The standardization of the immunodiagnosics allowed a good

comparison of assay results obtained from independent experiments.

In general, freeze drying is used to prepare plant materials for ELISA (2,26). In our investigation (Fig. 2), an oven drying method was selected because in comparison to the freeze-dried samples prepared, a relatively high amount of fungal antigens remained. In addition, oven drying is very practical as it is cheaper and more easily performed than freeze drying. Also, it is particularly useful for processing a large number of samples at one time.

When comparing the relative susceptibility of different parts of the wheat plant

(Fig. 3), it was found that *Stagonospora nodorum* was abundant in leaves, ears, and grain. In contrast, *S. tritici* was detected in high levels in leaves, but at very low levels in ears and not at all in grain. These findings confirm the results of Fraaije et al. (9), who used a PCR-based assay to test for the presence of these pathogens in wheat tissues.

In the triticale cultivars, little *S. tritici* was detected in F-1 leaves with BA-ELISA, while none was detected in the flag leaves, even in 2002 when there were abundant infections of wheat by *S. tritici*. This result supports findings of Mielke (27) and of Schröder and Gabriele (31) that *Stagonospora nodorum* is the predominant pathogen causing leaf blotch of triticale in Germany, while *S. tritici* causes only minimal levels of infection. In contrast, *S. tritici* was reported to be the primary causal agent of triticale leaf blotch in Alabama, USA (5). This difference suggests that we need to compare and analyze *Septoria/Stagonospora* infections in more triticale cultivars from a variety of geographic regions.

A wet spring and early summer was likely responsible for the extremely high infection levels of both *S. tritici* and *Stagonospora nodorum* observed in wheat during 2002 studies (Table 1). In response to elevated infection levels, BA-ELISA values correlated more closely with the susceptibility ratings published by BSA for both pathogens in 2002 than in 2001, when disease pressure was limited. Wheat cv. Ritmo varied in susceptibility levels in two trial years (relatively higher in 2001, lower in 2002), as compared with the more consistent reactions for cvs. Contur and Bus-

Fig. 3. Quantification of *Septoria tritici* (black) and *Stagonospora nodorum* (white) in F-1 leaves, flag leaves, ears, and grains of wheat (cv. Ritmo, 2001) by biotin/avidin enzyme-linked immunosorbent assay. *: no *S. tritici* was detected, antigen amount for grain described as µg FPE/g grain. Vertical bars represent standard error of means.

Table 1. Visual assessment of diseases and antigen amounts of *Septoria tritici* and *Stagonospora nodorum* from flag leaves of wheat and triticale cultivars at Goettingen, 2001 and 2002

Cultivars	Susceptibility rating ^a	2001				2002			
		Diseases ^b (GS 75/73)	Necrotic leaf area (%) ^c	(µg FPE/g leaves)		Diseases ^b (GS 80/75)	Necrotic leaf area (%) ^c	(µg FPE/g leaves)	
				<i>S. tritici</i>	<i>S. nodorum</i>			<i>S. tritici</i>	<i>S. nodorum</i>
Triticale									
Lupus	4	— ^d	—	—	—	SN	0.5	0	2.1
Modus	4	SN,PS	0.2	0	1.0	SN	0.3	0	1.6
Trinidad	4	—	—	—	—	SN	0.5	0	1.2
Alamo	5	SN,PS	0.1	0	0.8	—	—	—	—
Trimaran	6	SN,PS	21.3 ^e	0	2.3	SN,BG	2.1	0	7.0
Wheat									
Flair	4	ST,SN,PS	29.3 ^e	0.9	0.4	ST,SN	3.2	7.8	0.8
Cardos	4	ST,SN	2.3	1.6	0.6	ST,SN	5.1	13.6	1.5
Pegassos	4	ST,SN	2.1	1.0	0.4	ST,SN	5.4	6.8	0.8
Xanthos	4	ST,SN	3.5	0.6	0.8	ST,SN	5.1	6.7	1.1
Hanseat	4	ST,SN	2.5	2.6	0.2	ST,SN	6.5	10.2	1.0
Hybnos1	5	—	—	—	—	ST,SN	25.3	116.0	2.7
Ritmo	6	ST,SN	6.9	18.0	1.5	ST,SN	35.4	154.1	3.1
Contur	M	ST,SN,PS	47.5 ^e	12.9	2.4	ST,SN	40.2	201.0	7.9
Bandit	M	—	—	—	—	ST,SN	90.4	298.1	15.3
Bussard	6	ST,SN	6.5	20.7	1.5	ST,SN	80.1	285.2	13.3
Kanzler	6	—	—	—	—	ST,SN	92.0	301.0	13.5

^a Susceptibility ratings (1–9) of the BSA cultivar list (3). M: moderate susceptibility for Contur (HDLGN, 11) and Bandit (LWKSH, 21).

^b SN: *S. tritici*, ST: *Stagonospora nodorum*, PS: *Puccinia striiformis*, BG: *Blumeria graminis* f. sp. *tritici*.

^c Samples of 25 flag leaves per cultivar.

^d Not sown.

^e Caused by a complex of *Septoria* diseases and a severe *P. striiformis* infection.

sard. This variation in apparent resistance levels may be due to the effect of environment (15,32). This also emphasizes the fact that a reliable resistance classification in field trials requires high disease pressure and multiyear assessments (7).

The EU cultivars Contur and Bandit were considered moderately susceptible to Septoria/Stagonospora leaf diseases in previous reports (11,21). In our study, the results of these cultivars indicate a high to very high susceptibility to both pathogens.

Similar studies were conducted by Schnieder and Fehrmann (30) using ELISA kits supplied by DuPont Agrochemicals (Stevenage, UK), and by Fraaije et al. (9) using PCR assays. Schnieder and Fehrmann (30) found a close correlation between percentage of necrotic leaf area and ELISA values of *S. tritici* or *Stagonospora nodorum* in wheat cultivars in greenhouse experiments, while a weak correlation was observed in field trials. The authors attributed this discrepancy to interferences resulting from necrosis caused by other pathogens or to natural senescence under field conditions (30). Results from the present study and those of Fraaije et al. (9) revealed close correlations between visual assessment data and ELISA measurements of *S. tritici* in cultivars tested under field conditions. In our study, interference from other pathogens and natural senescence was not a factor, except for high infection by *Puccinia striiformis* (stripe rust) in three cultivars in 2001. In addition, natural senescence was observed only occasionally in both years as small areas of necrotic tissue of flag leaves sampled during GS 73-80. Good correlations for our BA-ELISA data or Fraaije et al.'s PCR (9) data with observed necrosis were confirmed by the susceptibility ratings from the BSA (3) or from the NIAB (9) recommended cultivar lists.

Our BA-ELISA tests are valuable for detecting fungal antigens in plants before disease symptoms are visible, and when disease level is low. In the latter case, different disease levels between cultivars were shown more clearly by BA-ELISA than that by visual assessments (Table 1). The BA-ELISAs can also distinguish a particular pathogen from other pathogens involved in a disease complex. For example, in 2001, a severe infection by *P. striiformis* in several susceptible cultivars prevented a reliable visual assessment of Septoria diseases on these cultivars, whereas BA-ELISA was able to quantify the differences between the cultivars under these conditions. The obtained BA-ELISA results corresponded with the susceptibility ratings for these cultivars obtained from the BSA cultivar list (3,11), and were also confirmed by the data from 2002 when no stripe rust occurred. Our results highlight the advantages of quantitative BA-ELISA for detection of pathogen antigen quantities in plant tissue. Assessment of cultivar

resistance using ELISA has also been made for other pathogens, such as *Microdochium nivale* and *Fusarium* spp. (26), *Polymyxa betae* (17), and *Bean common mosaic necrosis virus* (33). BA-ELISA is, therefore, a sensitive, specific, and accurate method for assessing levels of resistance of plant cultivars against pathogens. Its implementation would be most useful in breeding programs that involve identification of plant resistance to pathogens within a disease complex, and in other situations where visual disease assessment may be hindered.

In conclusion, we demonstrated that the BA-ELISAs were suitable for accurate assessment of resistance of winter wheat and triticale cultivars to *S. tritici* and *Stagonospora nodorum*. BA-ELISA tests for additional leaf pathogens such as *D. tritici-repentis*, an increasingly important pathogen on wheat (1), will be developed to study plant resistance against multiple diseases.

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