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FACTORS AFFECTING TAN SPOT ON WINTER WHEAT IN THE GRAND-DUCHY OF LUXEMBOURG

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SUMMARY

Tan spot of wheat caused by *Drechslera tritici-repentis* was identified for the first time in the Grand-Duchy of Luxembourg (GDL) in 1999 on the basis of morphological characters. In order to optimize disease control measures in this country, tillage methods, cultivar resistance, and fungicide effects were investigated during 1999-2009 in four sites. Over this period, only three years (i.e. 1999, 2000, and 2009) with epidemic outbreak were recorded. Field experiments showed a significant difference in disease severity between sites ($P < 0.001$), cultivars ($P < 0.0001$) and years ($P < 0.001$). In years with epidemic outbreaks, the interaction of cultivars with non-inversion tillage, intensive winter wheat production, and favorable weather conditions caused an early outbreak of the disease and a significant severity at growth stage 83 (early dough). Non-inversion tillage was found to be a major factor increasing tan spot severity compared with conventional tillage. Furthermore, the analysis revealed that disease severity was related to the cultivar's susceptibility. For cultivars with similar phenology, disease severity beetween the most and the least susceptible cultivars differed by a factor of two to four. The study also showed that no fungicide (mix of triazoles and strobilurins) effect was observed in the epidemic years, except in 2000.

Key words: *Drechslera tritici-repentis*, cultivar, tillage methods, disease control.

INTRODUCTION

Winter wheat (*Triticum aestivum* L.) is an economically important crop in the Grand-Duchy of Luxembourg (GDL): 12,969 ha with an annual production of *ca.* 86,040 tons in 2009 (El Jarroudi *et al.*, 2011). *Pyrenophora tritici-repentis* (*Ptr*) (Died.) Drechs (anamorph, *Drechslera tritici-repentis*) (Died.) Shoem., causal agent of wheat tan spot (WTS), is an economically important pathogen in many wheat-growing regions worldwide (Ali and Francl, 2002; Ali *et al.*, 1999; Fernandez *et al.*, 2002; Perelló and Dal Bello, 2011; Tadesse *et al.*, 2006; Wakulinski *et al.*, 2003). The fungus can cause considerable yield losses to winter wheat when epidemiological conditions are favorable (Fernandez *et al.*, 2002; Hosford, 1982; Hosford *et al.*, 1987; Rees and Platz, 1983; Tadesse *et al.*, 2006; Waltkins *et al.*, 1978). Numerous studies showed that the yield losses can reach up to 50% depending on cultivar susceptibility and climate (Tadesse *et al.*, 2006). In the GDL, *Ptr* was identified for the first time in 1999 and was characterized through lens-shaped necrotic lesions with a chlorotic halo on susceptible cultivars (El Jarroudi *et al.*, 2004).

The increase in the severity of WTS has been associated with changes in the spectrum of the varieties grown, the expansion of the area where low and no-tillage practices dominate, which allows the build-up of inoculum on wheat stubble over time (Perelló *et al.*, 2003). An integrated control approach including the use of resistant cultivars, fungicides and appropriate cultural practices is currently recommended for WTS management (Perelló and Dal Bello, 2011). However, the level of resistance in commercial wheat cultivars is relatively low.

Various studies have demonstrated the efficacy of fungicides in controlling foliar diseases of wheat and increasing grain yield. In Kansas, over a period of 6 years, applications of propiconazole significantly increased the yield of winter wheat (Kelley, 2001). Other authors reported that fungicides containing tebuconazole, tebuconazole plus prothioconazole, and pyraclostrobin were very effective in reducing leaf spots in winter wheat in North Dakota (Ransom and McMullen, 2008). Some fungicides were reported to become less effective in controlling WTS because of the development of resistance in *Ptr* populations (Jørgensen and Olsen, 2007). Furthermore, in the last 10-15 years, fungicide chemistry evolved considerably and new fungicides became available on the market.

The objectives of the present study were: (i) to evaluate the occurrence, severity and the damage potential of WTS in the GDL and (ii) to identify factors that affect the severity of WTS in this country.

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MATERIALS AND METHODS

Crop management. Four locations close to the Luxembourgish villages Burmerange [49°29'N, 6°19'E], Christnach [49°47'N, 6°16'E], Everlange [49°46'N, 5°57'E], and Reuler [50°03'N, 6°02'E] were selected to carry out field experiments between 1999 and 2009 (Fig. 1). Crop management data for these experiments are given in Table 1. Experimental fields were typically sown around mid-October. The sowing and harvest methods and crop practices used reflected the usual wheat production practices in the GDL. In each location and for each cropping season, winter wheat cultivars were sown in a randomized block design with four replicates. Susceptible (Bussard, Biscay, Drifter, Ritmo, Vivant), semi-susceptible (Aron, Akteur, Batis, Boomer, Privileg, Shamane, Dekan, Sponsor, Tommi), and weakly-susceptible cultivars (Achat, Cubus, Dream, Flair, Rosario, Parador, Urban) (Anonymous, 2002, 2008) were used in these trials. Individual plots measured 8.0x1.5 m. I growth stages (GS) were assessed according to a dec scale (Zadoks *et al.*, 1974). All plots received 40-7(N/ha (ammonium nitrate) at GS 25, followed by 6(kg N/ha at GS 32, and a final application of 65-95 N/ha at GS 59. Weeds were controlled by two herbi applications [pre-emergence: IP flow, 1.0 l/ha (Isopro on, Bayer Crop Science Germany); post-emerger Javelin, 2.0 l/ha (Didlufenican + Isoproturon, Bayer C Science, Germany]. Throughout the paper, reference be made to the specific leaf positions on the wheat st These leaves are numbered with reference to the upp most flag leaf, or L1 (for Leaf 1), with the leaf immedia ly below designated as L2, followed by L3, and so (Shaner and Buechley, 1995).

The fungicide treatment was always a mix of strol urin(s) and triazole(s). Over the 1999-2006 period, o two fungicide treatments were tested at each site. I tween 2006 and 2009, three fungicide treatments we

Table 1. Agronomic data of the trials at the four experimental sites over the 1999-2009 period in the Grand-Duchy of Luxembourg

Site	Year	Cultivar	Previous crop	Tillage system	Sowing date	Nitrogen (N/ha
Burmerange	2000	Bussard, Dream, Flair, Ritmo	Maize	No tillage	16 Oct. 1999	200
	2001	Bussard, Dream, Flair, Ritmo	Maize	Tillage	30 Oct 2000	n.a.[a]
	2003	Dekan	Oilseed rape	Tillage	4 Oct 2002	185
	2004	Cubus	Oilseed rape	No tillage	1 Oct 2003	185
	2005	Cubus	Oilseed rape	No tillage	13 Oct 2004	185
	2006	Cubus	Oilseed rape	No tillage	30 Sep. 2005	192
	2007	Cubus	Oilseed rape	No tillage	11 Oct 2006	192
	2008	Cubus	Oilseed rape	No tillage	06 Oct 2007	228
	2009	Cubus	Oilseed rape	No tillage	06 Oct 2008	228
Christnach	2000	Bussard, Dream, Flair, Ritmo	Maize	No tillage	18 Oct 1999	200
	2001	Bussard, Dream, Flair, Ritmo	Maize	Tillage	28 Oct 2000	n.a.
	2003	Flair	Oilseed rape	Tillage	2 Oct 2002	200
	2004	Flair	Oilseed rape	Tillage	13 Oct 2003	200
	2005	Rosario	Maize	Tillage	27 Oct 2004	200
	2006	Flair	Maize	Tillage	12 Oct 2005	200
	2007	Tommi	Maize	Tillage	12 Oct 2006	200
	2008	Flair	Maize	Tillage	23 Oct 2007	200
	2009	Boomer	Maize	Tillage	23 Oct 2008	200
Everlange	1999	Batis, Flair, Ritmo, Sponsor, Vivant	Oilseed rape	No tillage	09 Oct1998	190
	2000	Bussard, Dream, Flair, Ritmo	Oilseed rape	No tillage	15 Oct 1999	195
	2001	Bussard, Dream, Flair, Ritmo	Oilseed rape	No tillage	18 Oct 2000	230
	2002	Achat, Biscay, Drifter	Pea	Tillage	12 Oct 2001	175
	2003	Achat	Oilseed rape	Tillage	4 Oct 2003	165
	2004	Achat, Urban, Aron	Oilseed rape	Tillage	14 Oct 2003	195
	2005	Achat (2x), Parador, Akteur	wheat, oilseed rape	Tillage	22 Oct 2004	190
	2006	Akteur, Flair	Fallow	Tillage	10 Oct 2005	225
	2007	Achat, Akteur	Pea	Tillage	10 Oct 2006	195
	2008	Rosario	Fallow	Tillage	09 Oct 2007	195
	2009	Achat, Privileg	Oilseed rape	No tillage	13 Oct 2008	195
Reuler	2003	Bussard	Oilseed rape	Tillage	5 Nov.2002	213
	2004	Bussard	Oilseed rape	Tillage	16 Oct 2003	200
	2005	Flair	Oilseed rape	Tillage	5 Oct 2004	200
	2006	Dekan	Maize	Tillage	13 Oct 2005	200
	2007	Akteur	Maize	Tillage	07 Oct 2006	200
	2008	Schamane	Oilseed rape	Tillage	10 Oct 2007	200
	2009	Schamane	Oilseed rape	Tillage	10 Oct 2008	200

[a] n.a.: not available.

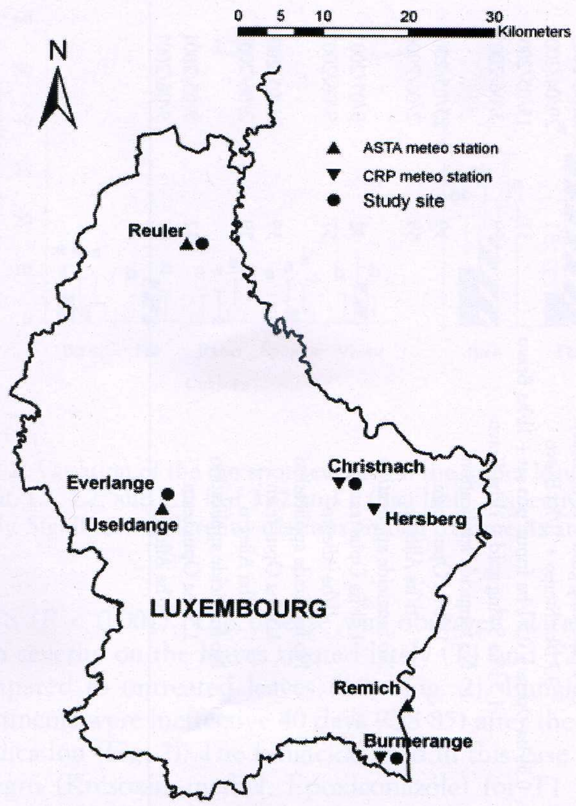

Fig. 1. Location of the experimental sites (filled circles) and meteorological stations (triangles). CRP: Centre de Recherche Publique-Gabriel Lippmann. ASTA: Administration des Services Techniques de l'Agriculture.

tested at each site (Table 2). Patterns of fungicide treatment were associated with wheat GS, and the products used were commercially available (Table 2). The products Allegro, Opus team, Opera, Input pro set, Bravo, Opus team, Stereo, and Swing gold contained the active ingredients Epoxiconazole (125 g/l) and Kresoxim-methyl (125g/l), Epoxiconazole (84 g/l) and Fenpropimorphe (250 g/l), Epoxiconazole (50 g/l)+Pyraclostrobin (133 g/l), Prothioconazole (250 g/l) and Spiroxamine (500 g/l); Chlorothalonil (500 g/l); Epoxiconazole (84 g/l) and Fenpropimorph (250 g/l); Cyprodinil (250 g/l) and Propiconazole (62.5 g/l), and Epoxiconazole (50 g/l) and Dimoxystrobine (133 g/l), respectively. Fungicide treatments were aimed at controlling leaf and ear diseases (Table 2).

Crop growth monitoring. At the beginning of GS 30 up to GS 87, plant samples from the field were inspected for tan spot occurrence. The percentage of infected leaf area was recorded using the standard area diagrams for tan spot and other cereal diseases, which were available to evaluators, who assessed the percentage of infection in the field prior to making observations (James, 1971; Tomerlin and Howell, 1988).

Laboratory analyses and characterization of the fungus. To recover *Ptr* from field samples, infected leaves were collected from both fungicide-treated and untreated plots at Everlange in 2000. Leaves were cut into 2-cm long fragments from symptomatic tissues and 10 leaf pieces per plot (40 leaves in total per treatment) were plated in plastic 9 cm Petri dishes containing three layers of dampened Whatman no. 1 filter paper. To dampen filter papers, 3 ml of sterilized distilled water were added to each plate. Leaves were incubated in an alternating cycle of 24 h light at 22°C and 24 h in dark at 16°C for 96 h to induce conidiophore and conidia formation. Incubated leaf pieces were examined under a stereoscope, and conidia were picked individually with a flamed steel needle that had been cooled in a plate containing V8PDA (150 ml of V8 juice, 10 g of Difco potato dextrose agar, 3 g of $CaCO_3$, 10 g of Bacto agar, and 850 ml of distilled water) (Lamari and Bernier, 1989). This procedure produces a sticky needle tip for picking of a single conidium which was then transferred onto an individual V8PDA plate for 7 days.

The mycelium produced by fungal isolates (coming from fungicide treated and untreated leaves) recovered from the sampled leaves was flattened with the bottom of a test tube to induce the formation of conidiophores (continuous light for 24 h) and conidia (18-24 h dark at 16°C). Conidia were photographed with a Hamamatsu digital charge-coupled-device camera (Hamamatsu, Japan) and a Zeiss Axioskop II microscope.

Statistical analyses. Statistical analyses were carried out using the SAS System (version 9.01, SAS Institute, USA). Comparison of tan spot between different areas, years, cultivars and tillage system was carried out in linear mixed model using the percentage of infected leaf area of L3, L2 and L1 as dependant variables, and the field site, cultivars and treatment as independent variables. Post-hoc tests (Tukey's test) were carried out following the Fisher-F test. P-values below 0.05 (2-sided) were considered as significant.

RESULTS

The mean comparison by linear mixed models showed that the cultivars, sites, year, treatment and the interaction between cultivars x sites x year x treatment were highly significant ($P < 0.001$) (Table 3)

1998-1999 cropping season. Severe damage caused by WTS occurred in the 1998-1999 cropping season, but no symptoms were seen before late April/early May. There were significant differences ($P < 0.01$) in the level of disease severity among cultivars assessed at GS 85 (Fig. 2). Commonly grown cultivars, e.g. Ritmo, Batis and Vivant, revealed a high degree of susceptibility to

Table 2. Dates, growth stages of the different fungicide sprays and disease assessments at each studied site in the Grand-Duchy of Luxembourg.

Sites	Year	First assessment	GS[a]	Last assessment	GS	Experimental Code	Stages of fungicide application	Fungicide treatment	Growth stage	Date
Everlange	1999	16/06/1999	65	16/07/1999	85	T0	Control	No fungicide application		
						T1	GS39	1l/ha Allegro	39	24/05/1999
						T2	GS31	1l/ha Allegro	33	12/05/1999
							GS59	1.5l/ha Opus team	55	5/06/1999
	2000	16/04/2000	30	12/07/2000	87	T0	Control	No fungicide application		
						T1	GS59	1l/ha Allegro	55	2/06/2000
						T2	GS31	1.5l/ha Opus team	31	4/05/2000
							GS59	1l/ha Allegro	59	2/06/2000
	2001	9/04/2001	24	19/07/2001	90	T0	Control	No fungicide application		
						T1	GS37	1l/ha Allegro	37	19/05/2001
						T1	GS45	1l/ha Allegro	49	31/05/2001
						T1	GS59	1l/ha Allegro	61	12/06/2001
						T2	GS31	1.5l/ha Opus team	32	9/05/2001
							GS59	1l/ha Allegro	65	14/06/2001
	2009	16/04/2009	30	22/07/2009	90	T0	Control	No fungicide application		
						T1	GS31	1.6l/ha Input®pro set + 1l/ha Bravo	31	29/04/2009
						T1	GS37	1.6l/ha Input®pro set + 1l/ha Bravo	37	13/05/2009
						T1	GS59	1.6l/ha Input®pro set + 1l/ha Bravo	59	3/06/2009
						T2	GS31	0.5l/ha Opera + 0.5 l/ha Input®pro set + 1l/ha Bravo	31	29/04/2009
							GS59	1.5 l/ha Swing gold + 1l/ha Bravo	59	3/06/2009
						T3	GS31	0.7 l/ha Stereo + 1l/ha Bravo	31	29/04/2009
							GS37	0.5l/ha Opera + 0.5 l/ha Input®pro set + 1l/ha Bravo	37	13/05/2009
							GS59	1.5 l/ha Swing gold + 1l/ha Bravo	59	3/06/2009
Christnach	2000	20/04/2000	30	12/07/2000.	85	T0	Control	No fungicide application		
						T2	GS31	1.5l/ha Opus team	39	23/05/2000
							GS59	1l/ha Allegro	59	2/06/2000
	2001	9/04/2001	24	19/07/2001	87	T0	Control	No fungicide application		
						T2	GS31	1.5l/ha Opus team	32	9/05/2001
							GS59	1l/ha Allegro	57	9/06/2001
Burmerange	2000	20/04/2000	30	12/07/2000	89	T0	Control	No fungicide application		
						T2	GS31	1.5l/ha Opus team	39	23/05/2000
							GS59	1l/ha Allegro	59	2/06/2000
	2001	9/04/2001	30	9/07/2001	89	T0	Control	No fungicide application		
						T2	GS31	1.5l/ha Opus team	32	9/05/2001
							GS59	1l/ha Allegro	67	9/06/2001

[a]GS: growth stage (Zadoks *et al.*, 1974); T1, T2, and T3: One, two and triple fungicide treatment, respectively.

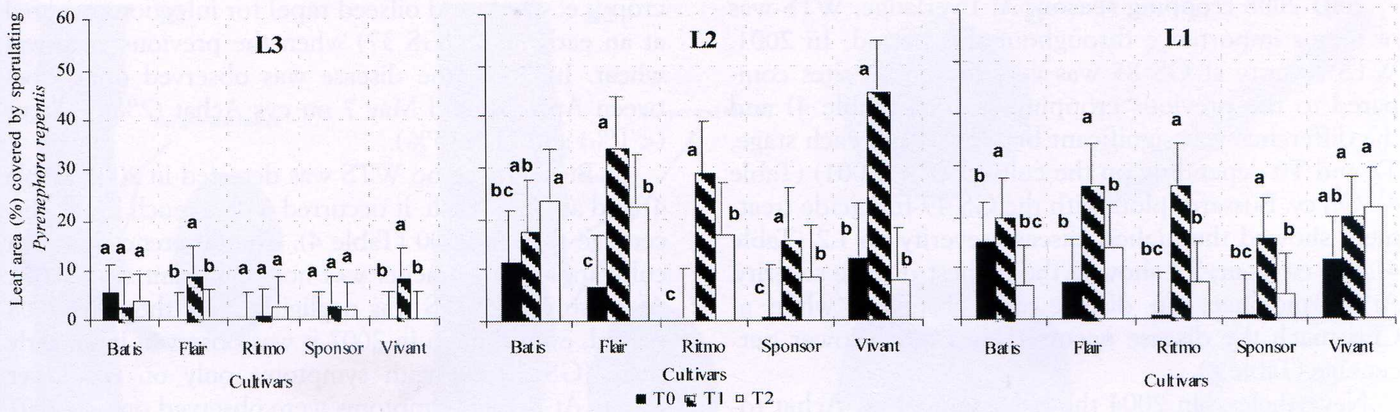

Fig. 2. Variation of the tan spot severity on the upper leaves at GS 85 (July 14, 1999) at Everlange according to the fungicide treatment. L3, L2, and L1: leaf 3, 2 and 1 (flag leaf), respectively; T0: Control; T1 and T2: One and two fungicide treatments respectively. Significant different variations among treatments are indicated by different letters ($P < 0.05$).

WTS ($P < 0.001$). The disease was observed at rather high severity on the leaves treated lately (T1 and T2) as compared to untreated leaves (T0) (Fig. 2). Fungicide treatments were ineffective 40 days (GS 85) after the last application (Fig. 2). The fungicide used in this case was Allegro (Kresoxim-methyl, Epoxiconazole) for T1 and Opus team (Fenpropimorph, Epoxiconazole) and Allegro (Kresoxim-methyl, Epoxiconazole) for T2 (Table 2). The DMI (demethylation inhibitors) fungicides Epoxiconazole and the strobilurin Kresoxim-methyl less efficient and did not stop the disease (Fig. 2).

1999-2000 cropping season. In 2000, WTS was detected in all experimental fields, its severity being significantly different between locations ($P < 0.05$). The highest severity was found at Everlange (West), followed by Burmerange (South) and Christnach (Center). Differences among cultivars were highly significant ($P < 0.001$) within each field as well as between fields (Fig. 3).

The treatment effect was highly significant ($P < 0.01$) at GS 75 on L1 (flag leaf), but this treatment did not show any effect at GS 85. Cultivars Bussard and Ritmo appeared as the most sensitive to WTS ($P < 0.0001$) at GS 75. While no significant difference (P=0.73) in disease severity was observed between cvs Flair and Dream at the same GS. The range of fungicide products used in our tests provided some protection against the disease at GS 75. However, these products did not stop a very important outbreak of the disease at GS 85. Indeed, disease severity reached 50% (cvs Bussard and Ritmo) at the beginning of July (July 10) for T1 and T2 treatments 38 days after the last fungicide spray. Laboratory characterization of the fungus from treated and untreated leaves identified two strains (Fig. 4) denoted: (i) strain "LB2038-1" for treated leaves, and (ii) strain "LB2038-3" for untreated leaves.

At Burmerange, WTS severity was important at GS 85 (Fig. 3). T0 showed the highest disease severity on L1 with 15% for cv. Bussard and 25% for cv. Ritmo, whereas the lowest severity (< 1%) was observed for T2.

At Christnach, cv. Ritmo differed significantly from the three other cultivars ($P < 0.004$) only for L2, whereas for L1 no significant difference between cultivars was observed. However, for this leave, the disease severity was much more important in T2 than T0 (Fig. 3).

Table 3. Analyses of variances (ANOVAs) for the effects of year, site, cultivars, and treatment and the interaction between year, site, cultivars, and treatment.

Source	Type III Sum of Squares	Mean Square	F	P^a
Year	18186.09	18186.09	351.28	***
Site	5596.08	2798.04	54.05	***
Cultivars	10616.02	3538.67	68.35	***
Treatment	2567.63	2567.63	49.60	***
Year*Site*Cultivars*Treatment	72322.63	1808.07	34.92	***

[a] Significance levels: *= $P<0.05$; **= $P < 0.01$; *** = $P < 0.001$; and NS= $P>0.05$.

2001-2008 cropping season. At Everlange, WTS was of minor importance throughout this period. In 2001, WTS severity at GS 85 was very low in all sites compared to the previous cropping seasons (Table 4) and the difference was significant between T1 at each stage, T2 and T0 depending on the cultivar ($P < 0.001$) (Table 4). On cv. Bussard, plots with the GS 49-fungicide treatment showed the highest disease severity on L2 (Table 4). On cv. Flair, T2 showed the highest disease severity. At Burmerange, no disease was observed; while at Christnach the disease occurred in a much lower percentage (Table 4).

Nevertheless, in 2004 the behaviour of cv. Achat towards WTS severity differed depending on the previous crop (i.e. wheat and oilseed rape) for infections o at an early stage (GS 37) when the previous cro wheat. In 2007, the disease was observed on I tween April 30 and May 7 on cvs Achat (2%), A (< 1%) and Flair (5%).

At Burmerange no WTS was detected in 2001 4) and at Christnach, it occurred with a much lowe centage than in 2000 (Table 4). The difference be cultivars and treatments was not significant (P > At these sites, WTS was negligible over the 200 period, even though in 2007 it was observed in ar stage (GS 30-31) with symptoms only on the leaves. At Reuler, symptoms were observed only i on L5 at the beginning of the season (GS 30, mid-

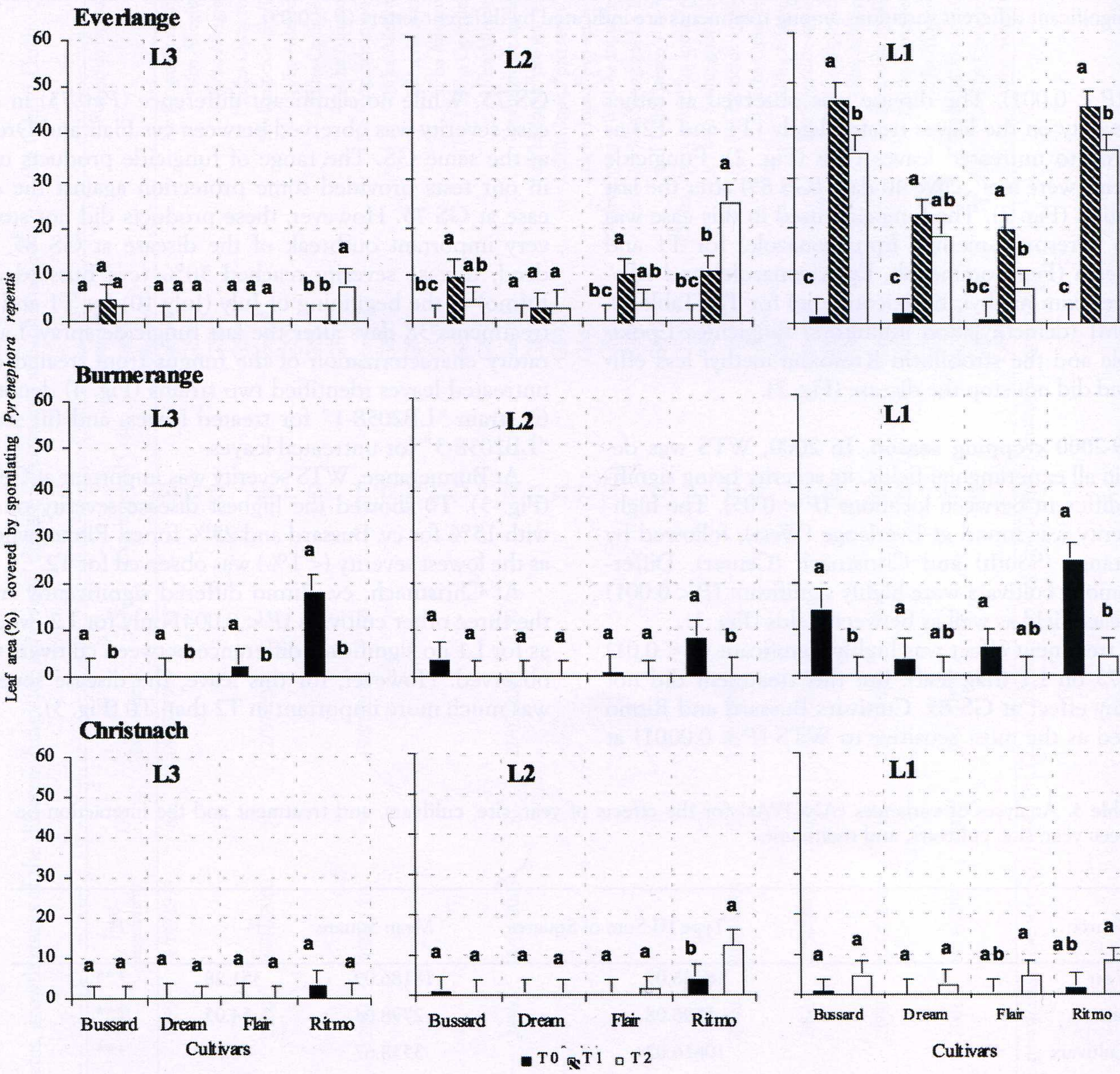

Fig. 3. Variation of the tan spot severity on the upper leaves at GS 85 (July 10, 2000) at three studied sites as affected by th cide treatment. L3, L2, and L1: leaf 3, 2 and 1 (flag leaf), respectively; T0: Control; T1 and T2: One and two fungicide tre respectively. Significant different variations among treatments are indicated by different letters ($P < 0.05$).

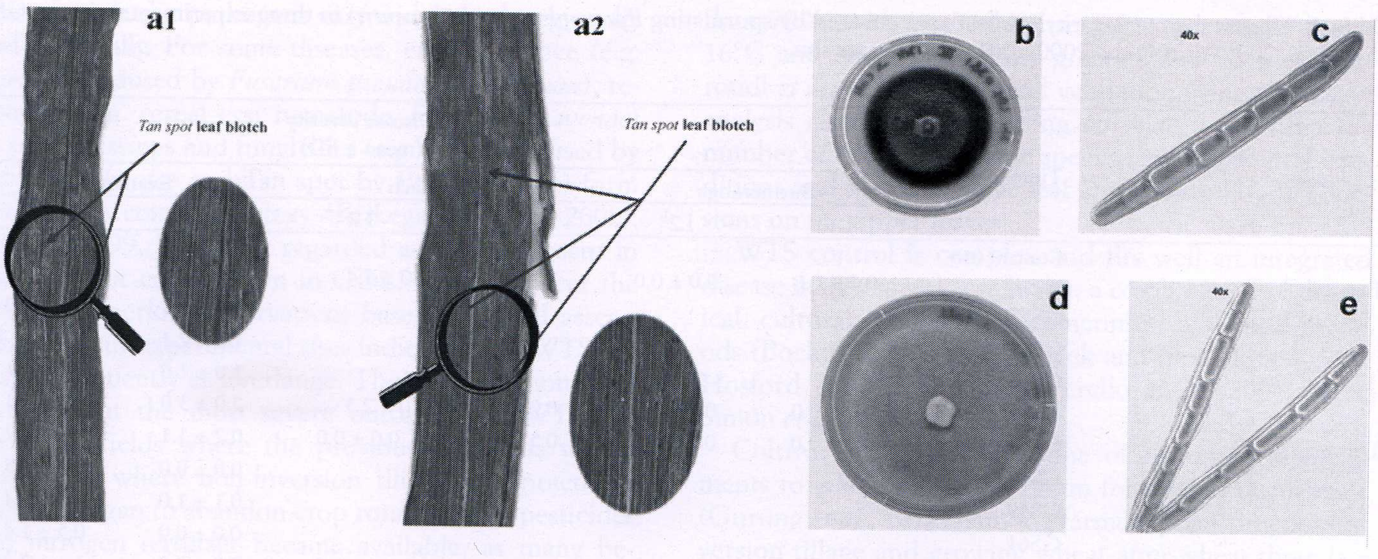

Fig. 4. Tan spot blights of winter wheat in the Grand-Duchy of Luxembourg. a1, L3 from cv. Bussard at Everlange (sample harvested on July 10, 2000). a2, L3 from cv. Bussard treated at Everlange (sample harvested on July 10, 2000). b, strain LB2038-1 of treated L3 pushed on PDA. c, conidia of LB2038-1 of treated L3. d, strain LB2037-3 of control L3 pushed on PDA. e, conidia of LB2037-3 of control L3.

2008-2009 cropping season. WTS was observed only at Everlange. The difference between the cultivars and the treatments was highly significant ($P < 0.01$) (Table 5). Disease severity was very low on cv. Achat for the two upper leaves (L1 and L2) while it was very high on the cv. Privileg. For this last cultivar, the comparison between the treatments (T1 and T2) and T0 revealed that fungicide-treated plots showed the highest disease severity. The highest severity (26%) on L1 occurred in plots with T1 performed at GS 39 (Table 5). T2 and T3 plots showed a severity of 11% and 10%, respectively.

The comparison of the number of days between the first appearance of WTS and the latest fungicide application for each treatment (Fig. 5) showed that the disease appeared before the end of the product protective activity. WTS was observed in T2 and T3 plots while the number of days since the last application was only 19 days (Fig. 5).

DISCUSSION

Wheat crops grown repeatedly in sequence (monocropping) can suffer from various soil and stubble-borne disease, although the range and aggressive-

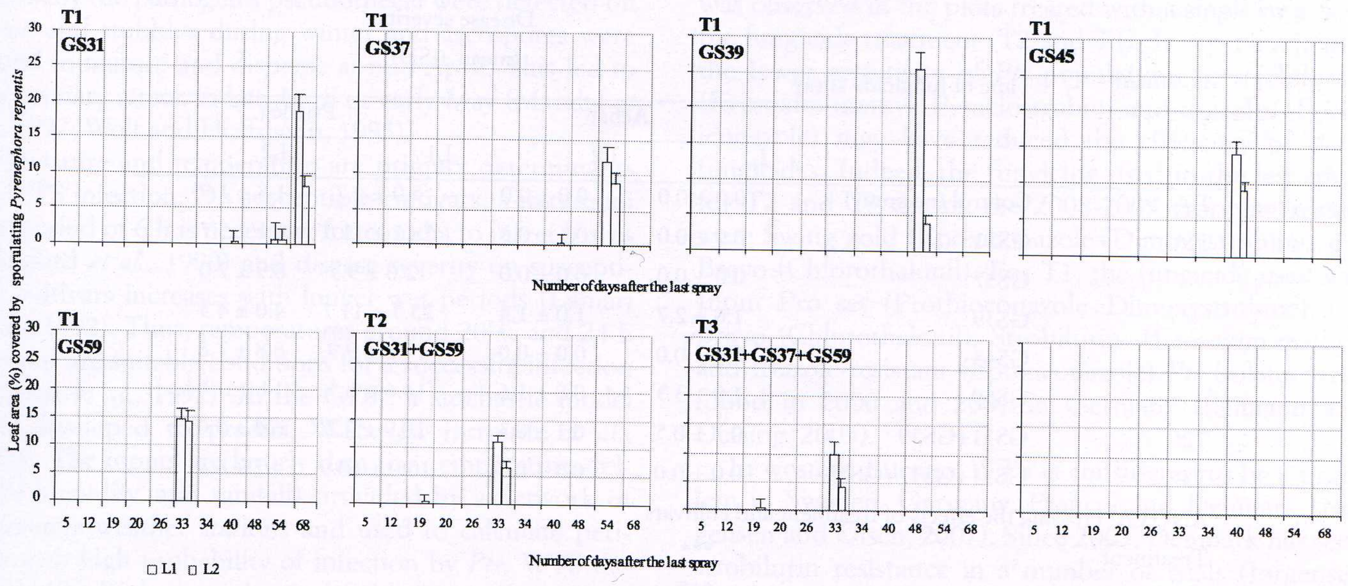

Fig. 5. Variation of tan spot severity on the two upper leaves of cvs Privileg at Everlange in 2009 after the last fungicide spray. T0, control; T1, T2, and T3, one, two, and triple fungicide treatment, respectively; L1 and L2, leaf 1 and leaf 2.

Table 4. Wheat tan spot severity (% leaf area covered by sporulating *Pyrenophora tritici-repentis*) in three experimental site 85 (July 6, 2001) during the 2000-2001 cropping season.

Cultivars	Experimental code	Time of spray	Disease severity (mean ± SD)					
			Burmerange		Christnach		Everlange	
			L1[b]	L2[b]	L1	L2	L1	
Bussard	T0[a]	Control (no spray)	0.0 ± 0.0	0.0 ± 0.0	1.5 ± 1.8	1.0 ± 1.4	0.2 ± 1.1	0.2
	T1[a]	GS37					2.2 ± 3.1	5.0
		GS49					1.2 ± 2.1	11.2
		GS61					1.0 ± 1.6	5.5
	T2[a]	GS31+GS59	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	2.0 ± 2.3	2.0 ± 3.0	3.4
Dream	T0	Control	0.0 ± 0.0	0.0 ± 0.0	0.5 ± 0.8	0.0 ± 0.0	0.2 ± 1.1	0.8
	T1	GS37					0.0 ± 0.0	0.6
		GS49					0.1 ± 1.0	0.5
		GS61					0.0 ± 0.0	0.5
	T2	GS31+GS59	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.5 ± 0.8	0.0 ± 0.0	1.1
Flair	T0	Control	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.1 ± 0.4	0.0 ± 0.0	0.6
	T1	GS37					0.3 ± 1.2	1.0
		GS49					1.1 ± 2.0	1.0
		GS61					0.0 ± 0.0	1.1
	T2	GS31+GS59	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	1.1 ± 2.0	7.3
Ritmo	T0	Control	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	1.7 ± 2.6	2.6
	T1	GS37					1.0 ± 1.9	1.8
		GS49					0.5 ± 1.4	1.2
		GS61					0.0 ± 0.0	1.2
	T2	GS31+GS59	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	1.0 ± 1.3	0.2 ± 1.1	2.0

[a] T0: Control; T1 and T2: One and two fungicide treatments, respectively.
[b] L1 and L2: Leaf 1 and leaf 2.

Table 5. Wheat tan spot severity (% leaf area covered by sporulating *Pyrenophora tritici-repentis*) at Everlange during the 2008-2009 cropping season in cvs Achat and Privileg.

Treatment	Time of fungicide spray	Disease severity (mean ± SD)			
		Achat		Privileg	
		L1	L2	L1	L2
T0[a]	Control (no spray)	0.0 ± 0.0	0.0 ± 0.0	5.0 ± 4.7	1.8 ± 1.9
T1[a]	GS31	0.0 ± 0.0	0.1 ± 0.3	18.1 ± 11.5	8.1 ± 5.7
	GS37	0.0 ± 0.0	0.0 ± 0.0	12.0 ± 9.5	8.9 ± 7.0
	GS39	1.6 ± 2.7	1.0 ± 1.4	25.5 ± 11.7	4.0 ± 4.3
	GS45	0.0 ± 0.0	0.0 ± 0.0	14.2 ± 9.9	8.8 ± 7.8
	GS59	1.9 ± 3.3	0.8 ± 1.6	14.6 ± 8.5	14.0 ± 9.4
T2[a]	GS31+GS59	0.3 ± 0.5	0.1 ± 0.3	10.9 ± 7.2	6.6 ± 5.0
T3[a]	GS31+GS37+GS59	0.0 ± 0.0	0.3 ± 0.7	10.0 ± 10.9	4.2 ± 6.5
ANOVA[b] crossing the effects of treatment and cultivars					
Treatment		***			
Cultivars		***			

[a] T0: Control; T1, T2 and T3: One, two, and triple fungicide treatment, respectively.
[b] A multifactorial ANOVA evidences significant differences. Significance levels: *= P<0.05; **= P < 0.01; *** = P < 0.001; and NS= P > 0.05.

ness of particular pathogens vary widely both regionally and seasonally. For some diseases, crop tolerance (e.g. crown rot caused by *Fusarium pseudograminearum*), resistance (e.g. cereal cyst nematode, *Heterodera avenae*) or seed dressings and fungicides (e.g. eyespot caused by *Tapesia yallundae* and Tan spot by *Ptr* in Europe) form part of the control strategy (Kirkegaard *et al.*, 2008). Prior to 1999, WTS was regarded as rare or absent in winter wheat crops grown in GDL. However, over the 1999-2009 period, observations based on visual assessments in four experimental sites indicated that WTS occurred frequently at Everlange. These observations also showed that the most severe outbreaks of WTS occurred in fields where the previous crop was winter wheat and where non-inversion tillage was practised. Farmers began to abandon crop rotation after pesticides and nitrogen fertilizer became available, as many believed that they could replace the benefits of crop rotations (Karlen *et al.*, 1994). Some authors indicate that crops grown in short rotations or monoculture often suffer from yield decline compared to those grown in longer rotations or for the first time (Bennett *et al.*, 2011). It should be noted that between late 1999 and early 2000, the Luxembourgish farmers began to practice non-inversion tillage and intensified the wheat-after-wheat cropping practice. In 2000, WTS was observed in all experimental sites. In these sites and for this year, the non-inversion tillage was practised. In the same year, the disease was also found in several GDL neighbouring countries, mainly France and Germany (Jørgensen and Olsen, 2007; Moreno and Perelló, 2010; Reimann and Deising, 2005). WTS introduction coincided with the spread of non-inversion tillage and wheat-after-wheat cropping systems (Sutton and Vyn, 1990). Under the climatic conditions of Belgium and Germany the pathogen's pseudothecia were detected on straw and stubbles during winter and ascospores were found to mature and disperse at mid-April. This led to the primary attack in late April or early May (Maraite *et al.*, 1992; Wolf and Hoffmann, 1995).

Moisture and temperature are primary determinants of WTS infection. On susceptible cultivars, a minimum wet period of 6 h is necessary for conidia to infect leaves (Hosford *et al.*, 1990) and disease severity on susceptible cultivars increases with longer wet periods (Lamari *et al.*, 1992). Thus, temperatures around 20°C and 24 h wetness are suitable conditions for a successful infection (Lamari *et al.*, 1992). In the GDL, a stochastic model was developed to predict WTS (El Jarroudi *et al.*, 2004). The inputs are hourly data (i.e. temperature, relative humidity, and rainfall) provided by a network of automatic weather stations and used to calculate periods with high probability of infection by *Ptr*. WTS development in the experimental field sites requires a period of at least 24 consecutive hours with temperatures between 4 and 25°C and a relative humidity greater than 70%, with optimal values varying between 8 and 16°C and relative humidity greater than 90% (El Jarroudi *et al.*, 2004). Statistical validation using regression analysis also showed a strong correlation between the number of hours with these specific meteorological conditions and the percentage leaf area covered by WTS lesions on the upper leaves.

WTS control is complex and fits well an integrated disease management system, i.e. a combination of chemical, cultural, genetic and, sometimes, biological methods (Bockus *et al.*, 1992; Duczek and Jones-Flory, 1994; Hosford and Busch, 1974; Perelló *et al.*, 2003, 2006; Simón *et al.*, 2011).

Cultivar susceptibility is one of the important elements to take into consideration for disease occurrence (Gurung *et al.*, 2012). Among farmers practising non-inversion tillage and growing wheat-after-wheat there is a need to grow cultivars with good resistance to WTS since this cropping system facilitates disease establishment. In our study, the difference between the cultivars in terms of susceptibility was highly significant ($P < 0.001$). Cultivars Bussard, Ritmo and Privileg were the most susceptible to WTS, whereas cv. Achat was among the most tolerant thus representing a good option to reduce disease risk. Some variation in the ranking of the cultivars was seen over the years, but the overall trend between years was similar for the most resistant and the most susceptible cultivars.

Regarding treatments, our study showed that for years with major WTS oubreaks, and during the early crop development (GS75), the disease severity was well controlled by single (T1) and double (T2) fungicide treatments. This was consistently observed in 1999, 2000 and 2009. However, at later growth stages (GS85), the contrary occurred for the highest disease severity was observed in the plots treated with a single or a double fungicide treatment (T2 and T1). It is probable that the lower sensitivity of *Ptr* populations to strobilurins (Kresoxim-methyl, Pyraclostrobin) and triazoles (Epoxiconazole) may have reduced the efficiency of these fungicides. Indeed, the fungicide used in the last spray for T2 and T3 during the 2008-2009 cropping season were Swing gold (Epoxiconazole+Dimoxystrobine) and Bravo (Chlorothalonil). For T1, the fungicide used was Input Pro set (Prothioconazole+Dimoxystrobine) and Bravo (Chlorothalonil). Strobilurin- (Kresoxim-methyl) and triazole-resistant (Epoxiconazole) *Ptr* isolates were found in 2000 and 2001 in Germany (Reimann and Deising, 2005).

In western Europe, WTS is considered to be a problem in Sweden, Germany, France, and Denmark (Jørgensen and Olsen, 2007). Since 2003, Denmark has seen strobilurin resistance in a number of trials (Jørgensen and Olsen, 2007) and strobilurin resistance has also appeared in Germany and Sweden (Jørgensen and Olsen, 2007). The development of strobilurin (Kresoxim-methyl, Pyraclostrobin) and Epoxiconazole resistance means that the recommendations for WTS control must changed in favour of other active ingredients. It is probable that this resistance will be more frequent in the future and this should be monitored closely. Another hypothesis is that the fungicide treatments strongly affect the microbial consortia present on the wheat leaf surface. Among these microbes, yeast-like fungi, hyphal fungi, and bacteria that are known to play an important antagonistic role towards leaf surface pathogens (Bashi and Fokkema, 1977; Rodgers-Gray and Shaw, 2001; Wachowska, 2005), are very susceptible to some fungicides. The effect of a fungicide may differ according to the microbial consortia involved. For example, the reducing effect of chlorothalonil on fungal numbers (i.e. *Ascomycetes*) was often compensated for by an increase of bacterial numbers (Rodgers-Gray and Shaw, 2001). It is highly possible that under fungicide pressure, useful antagonistic organisms on wheat leaf surface are inhibited, leaving an open space for colonisation by pathogenic fungi. Very little is known about naturally occurring organisms at the surface of wheat leaves and their role in protecting the plant against pathogenic fungi. This research area deserves particular attention when developing disease control strategies based on the use of fungicides.

The years 1999, 2000 and 2009 showed that WTS could be an acute problem for wheat production in the GDL. The increase in conservation tillage and the intensification of wheat production through shorter rotations and monoculture are favouring WTS and are unlikely to change. Successful management of WTS will depend on research efforts such as disease forecasting technology and monitoring of fungicide resistance, and research on the interaction between the microclimatic conditions and the structure of the population as well as its architecture.

Currently, susceptible cultivars are closely monitored in non-inversion tillage and wheat-after-wheat cropping systems for recommending the appropriate fungicide treatments. In all other situations, the severity of WTS was too low to justify special control in 3 out of 10 years during the monitoring period.

The trend in farming today is towards using more reduced and non-inversion tillage systems to optimizing the cost of production. In order to reduce the WTS risk and the use of fungicides, it is, however, important to couple non-inversion tillage with resistant cultivars and adequate crop rotation.

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REFERENCES

Ali S., Francl L.J., 2002. A New Race of *Pyrenophora* *pentis* from Brazil. *Plant Disease* **86**: 1050-1050.

Ali S., Francl L.J., De Wolf E.D., 1999. First r *Pyrenophora tritici-repentis*, race 5 from North *Plant Disease* **83**: 591.

Anonymous, 2002. BSA, Beschreibende Sortenliste, Mais, Ölfüchte, Leguminosen, Hackfrüchte. *Landwirtschaftsverlag GmbH*: 10-17.

Anonymous, 2008. BSA, Beschreibende Sortenliste, Mais, Ölfrüchte, Leguminosen, Hackfrüchte. *Landwirtschaftsverlag GmbH*: 78-127.

Bashi E., Fokkema N.J., 1977. Environmental factors growth of *Sporobolomyces roseus*, an antag *Cochliobolus sativus*, on wheat leaves. *Transactio British Mycological Society* **68**: 17-25.

Bennett A.J., Bending G.D., Chandler D., Hilton S., 2011. Meeting the demand for crop production: lenge of yield decline in crops grown in short rota *ological Reviews of the Cambridge Philosophical So* 52-71

Bockus W.W., Davis M.A., Shroyer J.P., 1992. Effect fungicide application on seed size of winter wheat *of Applied Seed Production* **10**: 1-6.

Duczek L.J., Jones-Flory L.L., 1994. Effect of timing conazole application on foliar disease and yield of spring wheat in Saskatchewan from 1991 to 1992. *an Journal of Plant Science* **74**: 205-207.

El Jarroudi M., Tychon B., Maraite H., Hoffmann L. Epidemiological importance of the Tan Spot on wheat crop in the Grand-Duchy of Luxembourg cobsen S.E., Jensen C.R., Porter J.R. (eds.). *Procee the 8th European Society for Agronomy Congress: E Agriculture in a Global Context, Copenhagen. De* 87-88.

El Jarroudi M., Giraud F., Tychon B., Hoffmann L., H., Delfosse P., 2011. Prévoir la septoriose. Un belge au Luxembourg. Modélisation et simulatio sionnelle de la septoriose des feuilles par PROCU pour une gestion durable du blé d'hiver. *Phy Défense des Végétaux* **642**: 43-46.

Fernandez M.R., Clarke J.M., De Pauw R.M., 2002. fect of plant height on tan spot on durum wheat i ern Saskatchewan. *Crop Science* **42**: 159-164.

Gurung S., Hansen J.M., Bonman J.M., Gironella A.I. hikari T.B., 2012. Multiple disease resistance to f spot diseases in winter wheat accessions from the USDA national small grains collection. *Crop Science* **52**: 1640-1650.

Hosford J.R.M., 1982. Tan spot-developing knowledge 1902-1981. In: Hosford Jr R.M. (ed.). *Proceedings of the Tan Spot of Wheat and Related Diseases Workshop, Fargo, ND. USA.*: 1-24.

Hosford R.M.J., Busch R.H., 1974. Losses in wheat caused by *Pyrenophora trichostoma* and *Leptosphaeria avenaria* f. sp. *triticea*. *Phytopathology* **64**: 184-187.

Hosford J.R.M., Larez C.R., Hammond J.J., 1987. Interaction of wet period and temperature on *Pyrenophora tritici-repentis* infection and development in wheats of differing resistance. *Phytopathology* **77**: 1021-1027.

Hosford J.R.M., Jordahl J.G., Hammond J.J., 1990. Effect of wheat genotype, leaf position, growth stage, fungal isolate, and wet period on tan spot lesions. *Plant Disease* **74**: 385-390.

James C.A., 1971. Manual of Assessment Keys for Plant Diseases. Canada Department of Agriculture Publication No.1458. APS Press, St. Paul, MN, USA.

Jørgensen L.N., Olsen L.V., 2007. Control of Tan spot (*Drechslera tritici-repentis*) using cultivar resistance, tillage methods and fungicides. *Crop Protection* **26**: 1606-1616.

Karlen D.L., Varvel G.E., Bullock D.G., Cruse R.M., 1994. Crop rotations for the 21st Century. *Advances in Agronomy* **53**: 1-45.

Kelley K.W., 2001. Planting date and foliar fungicide effects on yield components and grain traits of winter wheat. *Agronomy Journal* **93**: 380-389.

Kirkegaard J., Christen O., Krupinsky J., Layzell D., 2008. Break crop benefits in temperate wheat production. *Field Crops Research* **107**: 185-195.

Lamari L., Bernier C.C., 1989. Virulence of isolates of *Pyrenophora tritici-repentis* on 11 wheat cultivars and cytology of the differential host reactions. *Canadian Journal of Plant Pathology* **11**: 284-290.

Lamari L., Bernier C.C., Ballance G.M., 1992. The necrosis-chlorosis model in tan spot on wheat. In: Francl L.J, Krupinsky J.M., McMullen M.P. (eds). Advances in Tan Spot Research. *Proceedings 2nd International Tan Spot Workshop, Fargo, ND, USA, North Dakota Agricultural Experiment Station* 1992: 10-15.

Maraite H., Berny J.F., Goffin A., 1992. Epidemiology of tan spot in Belgium. In: Francl L.J., Krupinsky J.M., McMullen M.P. (eds). Advances in Tan spot Research. *Proceedings 2nd International Tan Spot Workshop, Fargo, ND, USA. North Dakota Agricultural Experiment Station* 1992: 73-79.

Moreno M.V., Perelló A.E., 2010. Occurrence of *Pyrenophora tritici-repentis* causing tan spot in Argentina. In: Arya A.P., Perelló A.E. (eds). Management of Fungal Plant Pathogens, pp. 275-290. CABI North America, Cambridge. MA, USA.

Perelló A.E., Moreno M.V., Simón M.R., Sisterna M., 2003. Tan spot of wheat (*Triticum aestivum L.*) infection at different stages of crop development and inoculum type. *Crop Protection* **22**: 157-169.

Perelló A., Mónaco C., Moreno M.V., Simón M.R., Cordo C., 2006. *Trichoderma spp.* isolates as potential biological agents for foliar wheat diseases under field conditions in Argentina. *Biocontrol Science and Technology* **16**: 803-813.

Perelló A.E., Dal Bello G.M., 2011. Suppression of tan spot and plant growth promotion of wheat by synthetic and biological inducers under field conditions. *Annals of Applied Biology* **158**: 267-274.

Ransom J.K., McMullen M.V., 2008. Yield and disease control on hard winter wheat cultivars with foliar fungicides. *Agronomy Journal* **100**: 1130-1137.

Rees R.G., Platz G.J., 1983. Effects of yellow spot on wheat: comparison of epidemics at different stages of crop development. *Australian Journal of Agricultural Research* **34**: 39-46.

Reimann S., Deising H.B., 2005. Inhibition of efflux transporter-mediated fungicide resistance in *Pyrenophora tritici-repentis* by a derivative of 42-hydroxyflavone and enhancement of hungicide activity. *Applied and Environmental Microbiology* **71**: 3269-3275.

Rodgers-Gray B.S., Shaw M.W., 2001. The effect of incorporating straw or manure into the soil on the natural microflora of winter wheat. *Plant Pathology* **50**: 537-545.

Shaner G., Buechley G., 1995. Epidemiology of leaf blotch of soft red winter wheat caused by *Septoria tritici* and *Stagonospora nodorum*. *Plant Disease* **79**: 928-938.

Simón M.R., Ayala F., Golik S., Terrile I., Cordo C., Perelló A., Moreno V., Chidichimo H., 2011. Influence integrated foliar disease management to prevent yield loss in Argentinian wheat Production. *Agronomy Journal* **103**: 1441-1451.

Sutton J.C., Vyn T.J., 1990. Crop sequences and tillage practices in relation to diseases of winter wheat in Ontario. *Canadian Journal of Plant Pathology* **12**: 358-368.

Tadesse W., Hsam S.L.K., Wenzel G., Zeller F.J., 2006. Identification and monosomic analysis of Tan spot resistance genes in synthetic wheat lines (*Triticum turgidum L. x Aegilops tauschii Coss.*). *Crop Science* **46**: 1212-1217.

Tomerlin J.R., Howell A., 1988. Distrain: a computer program for training people to estimate disease severity on cereal leaves. *Plant Disease* **72**: 455-459.

Wachowska U., 2005. The Role of Pesticides in the Development of Populations of Microorganisms Colonizing the Leaves of Winter Wheat. *Polish Journal of Environmental Studies* **14**: 95-102.

Wakulinski W., Kachlicki P., Sobiczewski P., Schollenberger M., Zamorski C., _otocka B., Sarova J., 2003. Catenarin production by isolates of *Pyrenophora tritici-repentis* (Died.) Drechsler and its antimicrobial activity. *Journal of Phytopathology* **151**: 74-79.

Waltkins J.E., Odvody G.N., Boosalis M.G., Patridge J.E., 1978. An epidemic of tan spot of wheat in Nebraska. *Plant Disease Report* **62**: 132-134.

Wolf P.F.J., Hoffmann G.M., 1995. Quantitative resistance of wheat cultivars against *Drechslera tritici-repentis* and pathogenicity of different isolates of the fungus. *Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz* **102**: 478-495.

Zadoks J.C., Chang T.T., Konzak C.F., 1974. A decimal code for the growth stages of cereals *Weeds Research* **14**: 415-421.