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Barley yield losses due to defoliation of upper three leaves either healthy or infected at boot stage by *Pyrenophora teres* f. *teres*

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Abstract This paper evaluates, in the greenhouse and under natural conditions, barley yield losses due to defoliation treatments of the upper three leaves either healthy or infected at the boot stage by *Pyrenophora teres* f. *teres*. Defoliation was assumed as a loss of a similar leaf area caused by net blotch disease severity of 100%. Contribution to grain yield was defined herein as a difference between defoliation treatments and a treatment where plants lost all their upper three leaves. In contrast, yield losses referred to differences in yield between defoliations and the control. In the greenhouse, removal of the antepenultimate leaf did not affect any yield component. For main stems, defoliating upper three leaves reduced grain yield by 30% and this was mainly due to flag leaf removal. These losses were similar to those induced by net blotch disease under natural conditions, but were of 42% for all tillers. Grain yield losses due to disease severity were not equivalent to the defoliation effect of a similar healthy leaf area. On the other hand and for a significant contribution to grain yield, flag leaf was dependent on the presence of the other two leaves. Inoculation and defoliation of 21 cultivars induced similar grain yield losses of 32%. However, biotic stress reduced by 40% the contribution of their upper three leaves. Under field conditions, yield losses were not significant until barley plants lost more than one upper leaf and flag leaf contribution was equivalent to that of the remaining leaves. Characteristic roots, defined as leaf coefficients for plant performance, were 0.13, 0.06 and 0.01 for the flag, penultimate and antepenultimate leaves, respectively. Because antepenultimate leaves become trivial at the boot stage, we propose that coefficients of the remaining leaves should be used when modelling yield losses due to barley foliar diseases.

Keywords Barley (*Hordeum vulgare*) · Characteristic roots · Defoliation · Leaf contribution · Net blotch · Yield loss

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Introduction

In cereals, the period from initiation of terminal spikelets to anthesis is important for the determination of the potential number of kernels per spike (Fischer and Stockman 1986), while the period from anthesis to physiological maturity is critical for the determination of final grain yield (Borrell et al. 1989). Assimilates contributing to grain yield are from two major sources: current photosynthesis and the mobilisation of carbohydrates and proteins stored temporarily in the vegetative tissues (Niu et al. 1998; Gaunt

and Wright 1992). Blum (1988) specified that grain growth is supported by transient photosynthesis, primarily by the flag leaf and inflorescence, and translocation of stored reserves. Since photosynthates produced by all green parts of small-grain cereal plants are translocated to seeds, any biotic or abiotic stress e. g., drought, insects and diseases, may induce losses in grain yield.

Net blotch caused by *Pyrenophora teres.* (anamorph: *Drechslera teres*) is a predominant foliar disease of Moroccan barley crops (Douiyssi et al. 1998; El Yousfi 2002) and prevails elsewhere in any barley-growing region of the world (Steffenson and Webster 1992; Serenius et al. 2007). The causal agent occurs in two forms, one producing net-type symptoms and the other producing spot-type symptoms (Tekauz 1990). However, the net form caused by *P. teres* f. *teres* was reported to be more prevalent than *P. teres* f. *maculata* producing the spot form (Tekauz 1990; Serenius et al. 2007). In Morocco, yield losses range from 14 to 29% and reach 39% on susceptible cultivars (El Yousfi and Ezzahiri 2001).

Most studies dealing with the contribution of upper leaves to grain yield were carried out on wheat using different approaches such as defoliation, shading or inoculation of functional leaf area (Seck et al. 1991; Buntin et al. 2004). Unfortunately, few reports have investigated leaf removal effects on the grain yield of barley. Jenkyn and Anilkumar (1990) studied defoliation effects on growth and yield of spring barley at different growth stages and in different grain-filling environments (outside and inside the greenhouse). In their work, they pointed out the importance of the contribution of the upper two leaves to grain yield. The only significant yield losses were recorded at the boot stage and at the start of grain filling, especially under greenhouse conditions. Therefore, assimilates before anthesis have little importance to grain yield as estimated by Bidinger et al. (1977) using carbon-14 methodology. Contribution of these assimilates in irrigated culture were evaluated to be 12% and 13% of grain yield for barley and wheat, respectively.

Our earlier work pointed out that the defoliation of the upper three leaves of barley during the growth cycle from the three-leaf stage to heading, reduced grain yield over three successive seasons by a mean of 51% (Jebbouj and El Yousfi 2006). On the other hand, the flag leaf contributed to grain yield by 39% and to 1000-kernel weight by 24%. Moreover, simple or combined removal of these upper three leaves elongated the period of the growth cycle. However, the defoliation effect of the upper three leaves, during the growth cycle, on grain yield was significantly lower than the net blotch disease effect induced by successive inoculations. Furthermore, net blotch disease reduced by 47% the plant performance defined as the combined effect of the upper three leaves on biomass, grain yield and the growth cycle (Jebbouj and El Yousfi 2006).

The current study, conducted in the greenhouse and in the field, aims to reveal the importance of the upper three leaves of barley (the flag leaf, the penultimate leaf and the antepenultimate leaf), and to estimate their effective contributions to grain yield under healthy and diseased conditions. Clipping these upper three leaves at the boot stage simulated a loss of their total leaf areas due to a high net blotch disease severity of 100%.

Materials and methods

Inoculum production

Isolates of *P. teres* f. *teres* (hereafter *P. ter*es) used for inoculations were isolated from samples of infected barley leaves showing typical symptoms of net blotch. Leaf samples were collected from Jemaat Shaim (32° 24′ 09″ N—008° 46′ 55″ W) and Sidi El Aidi (33° 07′ 16″ N—007° 37′ 48″ W) experimental stations of INRA (National Institute of Agronomical Research), Morocco. Leaves with net blotch lesions were cut into sections of 1 to 2 cm, rinsed three times in sterile distilled water and dried between two layers of a filter paper, then placed on moistened filter paper in Petri dishes. Plates were incubated at 20±1ºC with a 12-h photoperiod to promote sporulation. After one to two days and with the help of a binocular microscope, a fine sterilised glass needle was used to transfer single conidia of *P. teres* to Petri dishes containing a homemade V8 juice agar (El Yousfi 2002). Plates were then incubated for ten days under previously described conditions.

Five isolates of *P. teres* used in this experimentation were previously identified in our earlier study dealing with virulence quantification and pathotyping. These five isolates presented different pathotypes, high virulence levels and induced high infection

responses on susceptible barley cultivars. Inoculum was prepared by scraping conidia from 10 day-old cultures using a small volume of distilled water. Tween 20 was added as one drop per 100 ml of distilled water. The suspension of conidia was filtered through three layers of cheesecloth and the concentration adjusted to 30,000 conidia ml^{-1}. At the boot stage (GS 45) of the Zadoks scale (Zadoks et al. 1974), 50 cm^3 per pot was sprayed on plants using a manual sprayer. Inoculated plants were covered with transparent polyethylene bags in order to provide a high relative humidity to promote infection. Bags were removed whenever first symptoms were visible.

Plant material

In the first trial, one cv. Arig8 was used, a Moroccan six-row barley cultivar known to be moderately sensitive to *P. teres* (Douiyssi et al. 1998). The experiment was carried out in the greenhouse where plants were grown in 20 cm-diam plastic pots filled with approximately 4.5 kg of natural soil brought from Sidi El Aidi experimental station. Five pockets per pot were sown with five seeds each. After germination, five plants were kept per pot. Sowing was made the first week of November for three consecutive growing seasons (2002-2003, 2003-2004 and 2004-2005). The greenhouse was maintained at 20°C to 25°C with a natural photoperiod of daylight.

Treatments

Two similar trials were placed in the greenhouse. In the first trial, plants were kept healthy until they reached the boot stage, when they were subjected to different treatments of leaf removal. For the second trial, plants were inoculated with *P. teres* and ten days later, were subjected to eight defoliation treatments of the upper three leaves. For each trial, the first three treatments consisted of a single removal of the flag leaf (F1), the penultimate leaf (F2) and the antepenultimate leaf (F3). These treatments were called simple defoliations. The following four treatments involved the removal of combined leaves of F1 and F2, F1 and F3, F2 and F3 and F1, F2, F3. These four treatments were called combined defoliations. Finally, the last treatment for each trial was the control with no leaf removal. The experimental design was a randomised complete block with four replications and eight treatments each, for both trials (healthy and inoculated).

Before any defoliation, disease severity was recorded for barley plants in each treatment of the inoculated trial. It was scored by estimating percentages of infected leaf area (necrosis and chlorosis) 10 days after inoculation (Weierang et al. 2002). Dates of maturity for plants in each treatment were evaluated before harvest. Data for dry biomass, tiller number, ear number, weight and number of kernels were determined. Grain yield data for five main tillers from each treatment were treated separately from other tillers.

To investigate the contribution to grain yield of the upper three leaves for some barley cultivars, two other separate trials were carried out in the greenhouse for the 2004–2005 growing season. For each trial at the boot stage, a total of 21 barley cultivars, sown as in the same previous tests, were subjected to simultaneous clipping of their upper three leaves (F1, F2, F3). In the first trial, plants were kept healthy while in the second, they were first inoculated with a mixture of *P. teres* isolates, as previously described, then defoliated. Each barley cultivar was represented by a pot containing five plants. Two plants per pot were kept as a control and left intact and the remaining plants received defoliation of their upper three leaves (F1F2F3). Moroccan cultivars used were: Igrane (1777), Amira (1776), Tissa, Orge628, Aglou, Amalou, Azilal, Acsad176, Acsad68, Asni, Rabat071, Massine, Arig8, Aanacer, Acsad60, Taffa, Tamellalt, Rabat077, Tiddas, Oussama and one line NDB112 from North Dakota (USA). At harvest time, grain weight and number of kernels per spike were evaluated.

Field trials

In order to study the contribution of the upper three leaves of barley to grain yield under natural conditions, a trial was carried out at Sidi El Aidi experimental station. Crop husbandry was the same as described elsewhere (El Yousfi 2002). The same cv. Arig8 was used in this experimentation and the design was a randomised complete block with four replicates. Each block involved ten treatments: the same seven treatments of simple and combined defoliations mentioned previously and a control not defoliated and replicated three times within a block. Defoliation treatments were applied at spike emergence. The net-type of barley net blotch was the

prevailing disease within the station and the experiment was carried out using no disease control. Sowing was done the first week of November and a plot of 5×2 m² containing six rows, 30 cm apart represented each treatment. Clipping of leaves was applied on the two central rows and at harvest time, grain weight was evaluated. Because of severe drought, only results from one season are presented although three replicates were carried out during three consecutive agricultural seasons (2003–2004, 2004–2005 and 2005–2006).

Statistical analysis

Yield data from the greenhouse were analysed by SAS 9.1 (SAS Institute 1990) based on a hierarchical linear model using Proc Mixed (Littell et al. 2006). The model consisted of two fixed effects: one health status (inoculated and healthy) and the other one was relative to the eight defoliation treatments. The random effects were years (3 years), blocks (four blocks) and error. The model can be written as: $y_{ijkl} = \mu + \alpha_i + \beta_j + (\alpha\beta)_{ij} + bl_l + y_k + e_{ijkl}$
where μ: the general mean.

α_i	the mean effect of i-th (health status) (fixed)
β_j	the mean effect of j-th (defoliation treatments) (fixed)
$\alpha\beta_{ij}$	the interaction of the i-th (health status) and j-th (defoliation treatments) (fixed)
bl_l	the effect of l-th block (random)
y_k	the effect of k-th year (random)
e_{ijkl}	the mean plot error (random)

The degrees of freedom were estimated with the satterthwaite option of Proc Mixed using balanced data (Spilke et al. 2005). With the help of the procedure GLM in SAS, a MANOVA analysis was used based on dry biomass, growth cycle period and grain yield to estimate the importance of different leaves in relation to plant performance (SAS Institute 1990; Sanogo and Yang 2004). Contrast statement of the GLM procedure was used to compare treatment main effect (Johnson and Wichern 2001). In the first experiment involving cv. Arig8, five spikes of main stems per treatment were threshed and analysed separately to those of the secondary tillers. The remaining tillers were also treated and analysed in the same way.

Percentage yield loss due to any treatment was defined as a reduction of yield compared to the control, while the contribution of the flag leaf (F1) to grain yield, for example, was calculated according to the difference between yield corresponding the to F1, F2, F3 defoliation treatment and the F2, F3 treatment. In the same way, contribution of the other leaves was evaluated (Fig. 1).

Yield data of the twenty-one barley cultivars from the greenhouse experiment were analysed in the same way. The model consisted of two fixed effects; the first was the health status (inoculated and healthy) and the other was relative to the eight defoliation treatments within varieties. The error was the only random effect left in the model. Field data were treated with mixed model analysis where the model consisted of one fixed effect relative to the eight defoliation treatments. The random effects were represented by four blocks and the error.

Results

Our results did not reveal any significant effect of years, blocks, block × treatment interaction, year × treatment interaction and health status × treatment interaction for all measured variables (Table 1). Therefore, treatment main effects were used for comparison. In addition, inclusion of growth cycle data in the analysis model as a covariate improved the analysis and reduced the residual error for some components (Table 2). Neither diseased nor healthy antepenultimate leaves had any significant effect on any grain yield component. Under the inoculated treatment, mean disease severity on all plants was 48%.

Yield losses and contributions

Main stems Flag leaf removal significantly affected all grain yield components of the main stems. It reduced grain weight by 25%, 1000-kernel weight by 8% and number of kernels ear^{-1} by 14% (Table 3). In addition, grain weight losses induced by single leaf removal showed a decreasing trend from the flag leaf (F1) to the antepenultimate leaf (F3). Losses were 25%, 16% and 10% (P=0.073), respectively for clipping F1, F2 and F3. Moreover, grain weight was significantly reduced by combined defoliations

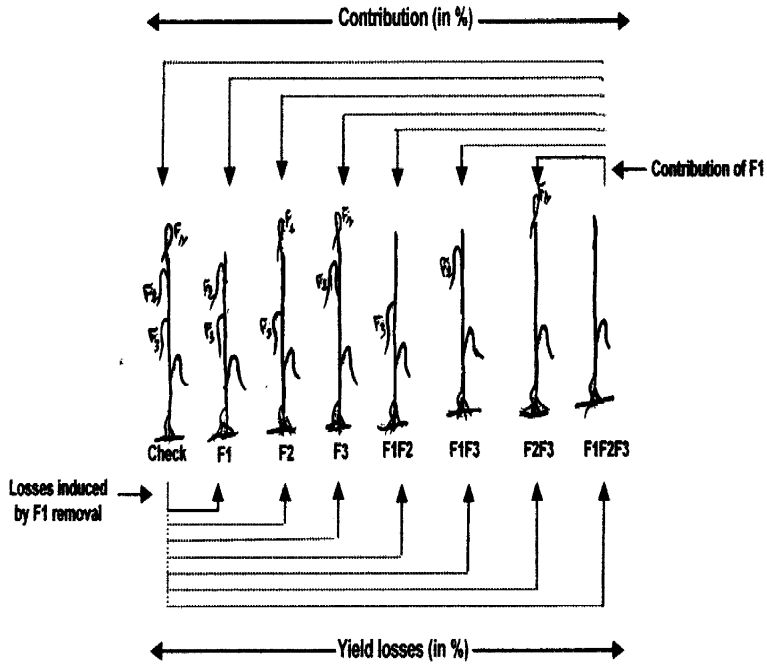

Fig. 1 A graphical representation of simple and combined defoliation treatments of the upper three leaves of barley plants used to illustrate estimation of yield losses and contributions. F1: Flag leaf defoliated, F2: Penultimate leaf (Flag leaf-1), F3: Antepenultimate leaf (Flag leaf -2)

(Table 3). This reduction was 17%, 19% and 14%, respectively for F1, F2, F1, F3 and F2, F3 while a loss of all leaves (F1, F2, F3) decreased grain weight by 30% and 1000-kernel weight by 14%. Losses in number of kernels ear^{-1}, estimated to be 14%, were only induced by combined defoliation of F1, F3 and F1, F2, F3 similar to those induced by simple defoliation of the flag leaf (F1) (Table 3).

Net blotch did not have a significant effect on 1000-kernel weight. However, it decreased grain weight and number of kernels ear^{-1} by 21% and 25%, respectively (Table 3).

Table 1 Mixed model analysis of treatments, health status and their interaction effects on grain yield including growth cycle as a covariate

Effect	Num DF	Den DF	*F* Value	*P*>F
Growth cycle	1	169	54.87	0.0001
Group[a]	1	169	116.34	0.0001
Treatment[b]	7	169	2.64	0.0131
Group × treatment[c]	7	169	0.39	0.9066

[a] Group: healthy and inoculated group.

[b] Treatment: all different leaf removal treatments of the upper three leaves of barley cv. Arig8.

[c] Group × treatment: group by treatment interaction.

Except for treatment F2, F3 and the probability obtained for the contribution of F2 (P=0.056) to the grain weight of the main stems, all simple and combined leaves contributed significantly to grain weight. Flag leaf contribution alone was approximately similar to the contribution of F1, F2 and F1, F3 which were 29 and 21%, respectively. This contribution was 23% and was greater than that of F2 and F3 which were 16 and 19%, respectively. Antepenultimate leaves and treatments F2, F1, F2 and F1, F3 were found to contribute significantly to grain yield (19%) and 1000-kernel weight respectively. However, the F2, F3 treatment contribution was not significant (Tables 3 and 4). On the other hand, the flag leaf did not contribute significantly to the 1000-kernel weight and number of kernels (Tables 3 and 4).

Secondary tillers Simple and combined defoliation treatments did not have any significant effect on 1000-kernel weight or on number of kernels ear^{-1}, while grain weight was significantly affected. Flag leaf removal (F1) and combined defoliation of F1, F2 and F1, F3 treatments decreased similarly the grain weight by 20%. In addition, a loss of all upper three leaves reduced grain weight by 26%.

Net blotch disease did not have any significant effect on the 1000-kernel weight. However, it de-

Table 2 Percent reduction of the residual errors by the inclusion of the growth cycle in mixed model analysis for cv. Arig8 yield component

Yield components	Residual Error		Reduction of residual error (%)	Significance of the growth cycle in the model
	Without growth cycle	With growth cycle		
Dry biomass	562.99	548.97	3	s[d]
Grain weight of ms[a]	3.46	3.38	2	s
Number of kernels ear^{-1} of ms	1690.01	1656.30	2	ns[e]
1000-kernel weight of ms	49.87	50.00	0	ns
Grain weight of st[b]	150.37	143.24	5	s
Number of kernels ear^{-1}of st	77663	71407	8	ns
1000-kernel weight of st	66.31	66.64	0	ns
Grain weight of tot[c]	175.50	166.61	5	s
Number of kernels ear^{-1}of tot	92676	85482	8	ns
1000-kernel weight of tot	49.87	50.00	0	ns

[a] ms: main stems.

[b] st: secondary tillers.

[c] tot: totality of tillers.

[d] s: significant.

[e] ns: not significant.

creased grain weight and number of kernels ear^{-1} by 47 and 49%, respectively (Table 3). While flag leaf removal significantly reduced grain weight, it did not show any significant contribution. Treatments F1, F2 and F1, F3 showed the greatest contributions to grain weight estimated to be 34% and 26%, respectively. Furthermore, neither simple nor combined defoliation treatments revealed any significant contribution to

Table 3 Mean losses in yield components (%) of main stems, secondary tillers and totality of tillers, induced by different leaf removal treatments of the upper three leaves of barley cv. Arig8

Treatments	Main stems			Secondary tillers	Totality of tillers		Widening of the growth cycle
	Grain weight	1000-kernel weight	Number of kernels ear^{-1}	Grain weight	Grain weight	1000-kernel weight	
F1	25*	8*	14*	20*	21*	6	3*
F2	16*	5	9	7	10	2	2*
F3	10	0	9	2	4	2	1
F1, F2	17*	6	9	20*	19*	8*	2*
F1, F3	19*	1	14*	21*	21*	3	2*
F2, F3	14*	7	5	12	13	8	2*
F1, F2, F3	30*	14*	14*	26*	27*	7	4*
Disease	21	Ns	25	47	42	Ns[a]	3

F1: flag leaf defoliated.

F2: penultimate leaf (flag leaf-1) defoliated.

F3: antepenultimate leaf (flag leaf -2) defoliated.

*: values are significant at $P \leq 0.05$.

[a] Ns: not significant.

Table 4 Contribution to grain yield and yield components (in %) of upper three leaves of main stems, secondary tillers and totality of tillers, of barley cv. Arig8

Treatments	Main stems			Secondary tillers	Totality of tillers		Growth cycle[b]
	Grain weight	1000-kernel weight	Number of kernels ear^{-1}	Grain weight	Grain weight	1000-kernel weight	
F1	23*	8	Ns [a]	20	20	1	−2*
F2	16	15*	Ns	8	9	4	−1*
F3	19*	9	Ns	9	11	1	−2*
F1F2	29*	17*	Ns	34*	33*	9*	−3*
F1F3	21*	10*	Ns	26*	24*	9*	−2*
F2F3	8	7	Ns	8	8	1	−1
F1F2F3	30*	16*	14*	26*	27*	7	−4*

F1: flag leaf defoliated.

F2: penultimate leaf (flag leaf-1) defoliated.

F3: antepenultimate leaf (flag leaf -2) defoliated.

*: values are significant at $P \leq 0.05$.

[a] Ns: not significant.

[b] growth cycle (−): contribution of leaf or leaves in reducing the widening of the growth cycle period when the remaining leaves are defoliated.

both 1000-kernel weight and number of kernels ear^{-1} (Tables 3 and 4).

Totality of tillers For the totality of tillers, including main stems and secondary tillers, losses in grain weight were induced by simple defoliation of the flag leaf (F1) and by the combined defoliation of F1, F2, and F1, F3 and F1, F2, F3. These were estimated to be 21%, 19%, 21% and 27%, respectively. 1000-kernel weight was only affected by the combined defoliation the treatments F1, F2 and F1, F2, F3. Losses were 8% and 7% (P=0.099), respectively. However, none of the defoliation treatments had any significant effect or contribution to number of kernels ear^{-1} (Tables 3 and 4). Contributions of leaves to grain weight and 1000-kernel weight of totality of tillers were similar to those of secondary tillers (Table 4).

Net botch disease decreased grain weight and number of kernels ear^{-1} by 42% and 44%, respectively without any effect on 1000-kernel weight.

Effect of defoliation on growth cycle

Except for the F3 treatment, flag leaf removal, as well as all upper three leaves F1, F2, F3, affected significantly the period of the growth cycle, and these effects were estimated to be 3% and 4%, respectively (Table 3). Removing the third leaf (F3) did not have any effect on the growth cycle but its contribution was significant and estimated to be 2% (Tables 3 and 4). Net blotch disease increased the period of the growth cycle by 3%.

Importance of leaves to plant performance

Coefficients in Table 5 are for characteristic roots obtained for all treatments under healthy and inoculated status using MANOVA analysis. For main stems, secondary tillers or totality of tillers, coefficients for grain yield losses revealed a gradual importance which decreased from the flag leaf (F1) to the antepenultimate leaf (F3), and became more important for the combined leaves F1, F2 and F1, F3 but decreased for F2, F3 and reached their maximum for all three leaves F1, F2, F3. These coefficients showed a similar tendency for secondary tillers and totality of tillers. However, coefficients seemed to be higher in main stems than in totality of tillers, especially for the flag leaf. For main stems, coefficients of grain yield losses were estimated to be 0.20, 0.08, 0.03, 0.10 and 0.33 for F1, F2, F3, and F1, F2 and F1, F2, F3 respectively and were 0.13, 0.06, 0.01, 0.09 and 0.29 for F1, F2, F3, and F1, F2 and F1,

Table 5 Characteristic roots for losses and contributions of different leaf removal treatments based on MANOVA of dry biomass, growth cycle and grain yield for main stems, secondary tillers and totality of tillers of barley cv. Arig8

	Main stems		Secondary tillers		Totality of tillers	
	Ch. root[a] (Losses)	Ch. root (Contribution)	Ch. root (Losses)	Ch. root (Contribution)	Ch. root (Losses)	Ch. root (Contribution)
F1	0.20*	0.09*	0.13*	0.08*	0.13*	0.08*
F2	0.08*	0.04	0.07*	0.04	0.06*	0.03
F3	0.03	0.08*	0.01	0.07*	0.01	0.07*
F1,F2	0.10*	0.20*	0.10*	0.21*	0.09*	0.20*
F1,F3	0.14*	0.09*	0.13*	0.08*	0.13*	0.08*
F2,F3	0.07*	0.03	0.06*	0.06*	0.06*	0.05*
F1,F2, F3	0.33*	0.33*	0.30*	0.30*	0.29*	0.29*

F1: flag leaf defoliated.

F2: penultimate leaf (flag leaf-1) defoliated.

F3: antepenultimate leaf (flag leaf -2) defoliated.

[a] Ch. root: characteristic root of the contrast statement in the MANOVA analysis.

*: values are significant at $P \leq 0.05$ for the contrast based on Roy's Greatest Root.

F2, F3, respectively for totality of tillers (Table 5). Coefficients for the antepenultimate leaf (F3) did not show any significant contribution to grain yield for main stems or totality of tillers.

For contribution to grain yield, coefficient of the flag leaf (F1) was less important than for grain yield losses and was similar to the F3 coefficient. The respective coefficients were 0.09 and 0.08 for main stems and totality of tillers, respectively. Furthermore, the coefficient for the contribution of the F2 treatment was not significant, while the flag leaf and penultimate leaf or all of the upper three leaves (F1, F2, F3) had superior coefficients for their contribution to grain yield. These coefficients for totality of tillers were 0.20 and 0.29 for F1, F2 and F1, F2, F3, respectively (Table 5).

Figure 2 represents losses in grain yield, number of kernels ear^{-1} and 1000-kernel weight of 21 barley cultivars, induced by removing all upper three leaves F1, F2, F3 under diseased and healthy status. Because of parallel curves, all varieties presented the same magnitude in their yield losses under either diseased or healthy status (Fig. 2).

Defoliation vs inoculation for barley cultivars

From Table 6, it is possible to deduce grain yield losses induced by defoliation and inoculation treatments. Defoliation of all upper three leaves under healthy status (healthy control — healthy defoliated plants)/healthy control × 100 decreased grain yield by a mean of 32% over all cultivars. No significant differences were obtained when comparing disease effect to defoliation under healthy status (Table 6). Net blotch disease generated a yield loss of 32%. Moreover, defoliation under disease status revealed a yield loss of 68%. However, net blotch disease under defoliation treatments reduced grain yield by 52%. Finally, defoliation under disease conditions reduced grain yield by 53% (Table 6).

Field results

Under field conditions, losses in grain weight induced by defoliation of the upper two leaves F1, F2 were found to be significant (P=0.066) and estimated to be 25%. While simple defoliations (F1, F2, and F3) and combined defoliations (F1, F3 and F2, F3) did not induce any significant losses, removing all upper three leaves F1, F2, F3 significantly decreased grain yield by 32%. Flag leaf removal did not induce any significant loss but its contribution was estimated to be 30%. When plants lost simultaneously their F2 and F3 leaves, they did not show any significant yield loss. However, contribution of these leaves (F2, F3) was estimated to be 30% (Table 7).

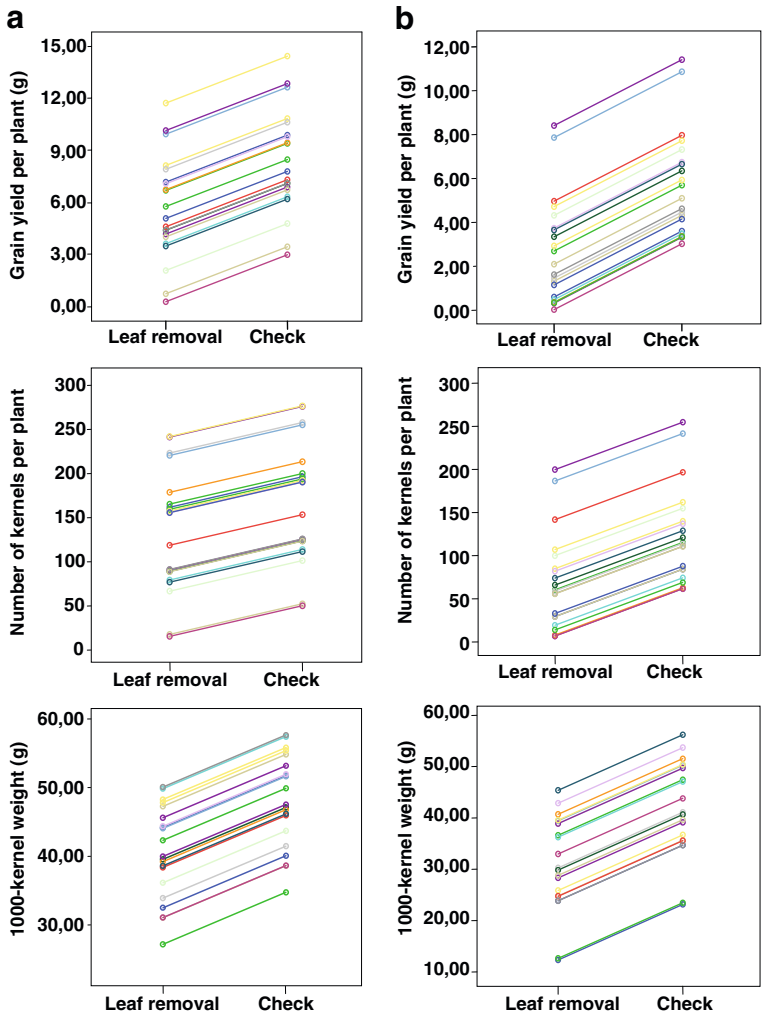

Fig. 2 Differential effect on grain yield of defoliation of the upper three leaves at the boot stage of twenty-one barley cultivars in the greenhouse under healthy **(A)** and disease (*Pyrenophora teres*) status **(B)**

Discussion

Removal of the upper three leaves of barley at the boot stage, was assumed to be equivalent to a loss of total leaf area resulting from a disease severity of 100%. In this regard, comparison between defoliation and inoculation treatments aimed to verify this hypothesis. Grain yield losses due to flag leaf removal were 25% and 21% for main stems and totality of tillers, respectively. This result supports the importance of the flag leaf as an important organ contributing to cereal grain yield and that any damage to its green leaf area would generate

Table 6 Differential effect of defoliation of the upper three leaves at the boot stage under healthy and diseased conditions on grain yield of twenty-one barley cultivars grown in the greenhouse

Treatments	Mean grain weight (g)	Standard Error
Inoculated and defoliated	2.69	0.5645
Inoculated and non-defoliated	5.69	0.6145
Defoliated	5.64	0.6853
Control non-inoculated and non-defoliated	8.34	0.7271

Table 7 Contributions (%) of single or combined leaves to grain yield and grain yield losses (%) induced by different leaf removal treatments of the upper three leaves of barley cv. Arig8 grown at Sidi El Aidi experimental station (2003–2004)

	Losses	Probabilities*	Contributions	Probabilities*
F1	2	0.895	30	0.075
F2	5	0.683	17	0.306
F3	15	0.271	7	0.682
F1, F2	25	0.066	17	0.298
F1, F3	15	0.262	27	0.112
F2, F3	2	0.879	30	0.072
F1, F2, F3	32	0.022	32	0.022

F1: flag leaf defoliated.

F2: penultimate leaf (flag leaf-1) defoliated.

F3: antepenultimate leaf (flag leaf -2) defoliated.

*: values are significant at $P \leq 0.05$.

significant yield losses (Sharma et al. 2003; Buntin et al. 2004; Jebbouj and El Yousfi 2006).

Removing upper leaves and consequently measuring losses in grain yield, scientists logically attribute the magnitude of losses to the contribution to grain yield by these defoliated leaves. However, based on our methodology in measuring contribution of upper leaves, the flag leaf contribution to grain yield in secondary tillers and totality of tillers was not significant in spite of the significant yield losses induced when the treatment was compared to the control. This discrepancy between our findings and the literature arises from the difference in the methodology used to assess the flag leaf contribution. Moreover, our results showed that a sustainable grain yield needs a threshold level of green leaf area from the upper three leaves, and the flag leaf area alone was not able to make a significant increase in grain yield. Effectively, when lower leaves are present, flag leaf contribution to the grain yield was higher than any contribution from other single leaves. Consequently, the importance of the flag leaf to sustain grain yield is dependent on the presence of the other lower leaves. On the other hand, the flag leaf seems to be a cornerstone in grain yield determination, because any loss in leaf area including the flag leaf generates similar losses. Furthermore, grain yield losses of main stems generated by flag leaf removal were similar to those induced by net blotch disease, whereas for secondary tillers and totality of tillers, flag leaf removal or removing the upper two leaves induced losses that were 50% less than those generated by the disease. This study suggested that for unbiased comparison between defoliation and disease effects on yield, estimation should take into account the whole plant.

Some defoliation treatments contributed significantly to yield components without causing any yield loss. The difference between contribution and yield loss, measured herein, may be due to an improvement of the photosynthetic activity of the remaining green parts on plants. This is in agreement with Koch (1996) who reported that in spite of the importance of the flag leaf during grain filling, its defoliation could improve the photosynthetic activity of the other leaves and, additionally, generate mobilisation of stored carbohydrates as also reported by Schnyder (1993). These mechanisms in plants avoid an interruption of grain filling as a way to avoid any source limitation (Richards 1996). Under our conditions, any removal of barley leaves except the antepenultimate leaf induced an elongation of the period of the growth cycle. Therefore, plants are using their growth cycle to alleviate the stress effect by having supplementary time to produce more carbohydrates to sustain grain yield. This mechanism is especially used when there is no water limitation as is the case under greenhouse conditions. The lack of an effect of the third leaf on any measured variable is attributed to its decreasing importance with the emergence of newer leaves throughout the growing cycle. In addition, the third leaf has a smaller role at anthesis because of its senescence compared to the flag leaf and the penultimate leaf. Furthermore, the third leaf was reported to be more susceptible to disease than the upper two leaves (El Yousfi 2002).

Tiller reaction within the same plant, at the boot stage, to defoliation treatments or to the combined effect of inoculation by *P. teres* and defoliation was not similar because of differences in their physiological age. Three to five tillers within the same plant constitute main stems, whereas the rest is made of immature tillers, as pointed out by Tekauz (1986). Our finding is supported by Metho and Hammes (1997) who evaluated the contribution to grain yield to be 68.6% for main stems, 24.8% for first tillers and 4.4% for secondary tillers of wheat and concluded that grain yield is largely dependent on main stems and first tillers. Also, Gan and Stobbe (1995) reported

that grain yield of main stems was relatively uniform while that of the remaining tillers was variable. Additionally, these tillers contribute less to yield because of the lower size and weight of their grains.

Coefficients of characteristic roots revealed the importance of upper leaves to plant performance because their evaluation included dry biomass, growth cycle and grain yield. These coefficients represent the explained variation of the MANOVA contrast (Johnson and Wichern 2001) and their comparison is similar to comparing the mean squares in ANOVA. These estimated coefficients support our previous conclusion that the main stems contributed more to yield than secondary tillers. Effectively, coefficients for grain yield losses of the flag leaf for the main stems were the same as the sum of those for the flag leaf and the penultimate leaf for secondary tillers. This difference between reactions of main stems and secondary tillers was also observed in the case of the effect of the disease on grain yield. However, yield losses for main stems were two-fold lower than those of secondary tillers or totality of tillers. Comparison of the coefficients of characteristic roots for the upper three leaves of main stems revealed that the flag leaf is 2.5 times more important for the plant than that of the penultimate leaf and almost seven times more than the antepenultimate leaf. Furthermore and for totality of tillers, the importance of the flag leaf is twice that of the penultimate leaf and ten times that of the antepenultimate leaf.

From these findings, we suggest that the estimation of disease severity for foliar diseases in barley should take into account these specific values, especially when epidemiologists are interested in relating disease severity to yield losses. In this regard, this relationship would be more straightforward and more meaningful. We propose a refinement of disease severity measured on individual leaves e. g., if we assess a disease severity of 25% on the flag leaf and 45% and 75% on penultimate and antepenultimate leaves respectively, the average disease severity would be of 48%. However, based on our data, the antepenultimate leaf is of no importance to the plant and only the flag leaf and the penultimate leaf matter. Consequently, the disease severity would be 32% (25%×2+45%)/3. We can conclude that the classical evaluation of foliar disease severity exaggerates the estimation when the relative importance of the leaves is not taken into account. The difference between these estimates may explain cases where pathologists encounter disease tolerance in some susceptible varieties or discrepancies in relating disease severity to grain yield.

Our results suggest that the net blotch disease effect on yield seems to be two-fold that of the effect of defoliation, especially when it is evaluated on secondary or totality of tillers. This means that disease severity is not equivalent to a loss of the same percentage of the healthy leaf area. Net blotch disease affects plant health by disturbing host physiology (Desjardins and Hohn 1997) rather than by decreasing photosynthetic leaf area alone. Therefore, the effect of the disease on plants is more than simple premature leaf drying, as it is described for barley net blotch. We can conclude that the contribution to grain yield of functional foliar area under biotic stress is physiologically restricted by the host/pathogen interaction. In our pathosystem, the plant is using its functional green area towards alleviating the harmful effect of the disease rather than only by sustaining grain filling. According to Gaunt and Wright (1992), stored carbohydrate reserves contribute less to grain yield in diseased than in healthy plants, especially when the yield potential is low.

The impact of defoliation treatments on the number of kernels ear^{-1} was only observed under net blotch disease pressure. Consequently, this reduction mainly arises from a decreasing number of fertile spikelets affected by the disease (Buntin et al. 2004). This study also demonstrated that removing all leaves, followed by flag leaf removal, had the greatest impact on wheat yield components during the flag leaf and spike emergence stages. Defoliation before spike emergence has a greater impact on all yield components than after anthesis. In the study of Saghir et al. (1968), removing the upper leaves of barley one week before anthesis induced losses in grain weight estimated to be 25.8% and this supports our results which showed that grain yield loss were of 26% to 30% (Table 3). Based on these findings, we can state that the infection of the upper leaves by net blotch, especially the flag leaf before anthesis, would eventually affect grain yield.

Removing the upper three leaves of Moroccan barley cultivars at the boot stage under healthy and biotic stress conditions revealed that the upper leaves had qualitatively a similar importance to grain yield for all cultivars. Differences between these cultivars

in grain yield losses is attributed to their grain yield potential. Following the comparison between various inoculation treatments by *P. teres* or defoliation (Table 6), the disease effect on grain yield, at the boot stage, was similar to the effect of removing the upper three leaves (F1, F2, F3) and was estimated to be 32%. This yield reduction was also obtained in the defoliation of the same leaves of cv. Arig8 under natural conditions (Table 7). However, a yield reduction of 53% due to the defoliation treatment under disease stress (comparison with inoculated control) is an outcome of plants under biotic stress that enhances the photosynthetic leaf area to alleviate both biotic and abiotic stresses. However when the combined effects of inoculation and defoliation were compared to the healthy control, grain yield losses were 68% and were similar to those due to the sum of each individual treatment effect.

In the field and because yield losses did not exceed 32%, our study emphasised that removing all upper three leaves of barley with no use of fungicide for disease control is likely to be a maximum threshold level that a plant could sustain regardless of any further leaf area loss. According to Buntin et al. (2004) grain yield was greatly affected by manual defoliation before spike emergence than at spike emergence or grain filling stages. This explains in our study the difference between losses obtained in the greenhouse and in the field.

Several studies relative to yield losses generated by foliar diseases, not affecting the ear, showed similar results which often attain approximately 30%, and this is in agreement with our findings in the field (32%). In Morocco and particularly in semi-arid regions, yield losses induced by net blotch disease were estimated to be 39% on susceptible cultivars (El Yousfi 2002). In the USA, yield losses induced by this disease were reported as 35% (Steffenson et al. 1991), and 21% in Western Australia with a maximum loss of 37% (Khan 1987).

We conclude that in barley, the contribution of plant parts to final yield depends on several factors. It depends on the methodology used and on the interaction of the host with the environment. Contributions of plant parts to grain yield seem to be a dynamic phenomenon, as described by Seck et al. (1991), who stated that leaf contributions to grain yield are related to the reference yield of healthy tillers.

The practical implication from this study is that whenever disease reaches the upper leaves, control measures should be cosidered to bring the disease under control. Furthermore, and for an unbiased estimation of the relationship between disease severity and yield losses, the flag leaf and penultimate leaf are of great importance. Their coefficients, developed herein for each upper leaf, should be used in conjunction with disease severity and leaf area when modelling yield losses and the epidemiology of foliar barley diseases.

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