# Influence of Plant Height and Heading Date on the Expression of the Resistance to Septoria Tritici Blotch in Near Isogenic Lines of Wheat

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## ABSTRACT

**The effects of plant height and heading date on the expression of the resistance to Septoria tritici blotch, which is caused by the fungus *Mycosphaerella graminicola* (Fuckel) Schroeter, in Cohn (*Septoria tritici* Rob. ex Desm.), were investigated in wheat (*Triticum aestivum* L.) near isogenic lines in the Mercia and Cappelle-Desprez background and differing in dwarfing genes (*Rht*) or in genes for insensitivity to photoperiod (*Ppd*). The experimental design was a randomized block design with four replications conducted over 2 yr. Necrosis percentage at boot (growth stage GS 49), milk (GS 73), and dough stage (GS 85); area under disease progress curve (AUDPC); plant height; and heading date were recorded. The shortest lines, Mercia *Rht*12, Mercia *Rht*3, and Cappelle-Desprez *Rht*3, showed higher necrosis percentage and AUDPC values than their respective controls. Mercia lines with *Ppd*1 and Cappelle-Desprez *Ppd*1 and *Ppd*2, the earliest heading lines, showed lower necrosis values than their respective controls. Multiple regression models including plant height and heading date accounted for 44.3 to 99.1% of the variation in necrosis percentage and were statistically significant in most cases. Reduced plant height was usually associated with more necrosis because of the highest necrosis percentage of the shortest lines. Late heading date was not associated or positively associated with more necrosis because of favorable weather conditions for late heading cultivars. When weather variables were included in the models, several of them replaced days to heading, suggesting that the positive relationship between necrosis and days to heading was caused by environmental conditions. Corrections of disease severity values for heading date and plant height should be done in breeding programs when selecting for resistance.**

Septoria tritici blotch is an important disease in many areas where wheat is grown (Shipton et al., 1971; King et al., 1983; Eyal et al., 1987). Progress in breeding has been slow because of different factors, including a great variability in the pathogen, a certain degree of specificity, and the fact that breeding has concentrated on monogenic resistance, which implies specificity to some isolates is readily broken down (Lee and Cough, 1984; Perelló et al., 1991).

Furthermore, one of the most confounding factors in selecting for resistance to Septoria tritici blotch could be the reported interaction between resistance and plant height or heading date. There were several reports of genetic associations between increased disease severity with earliness (Rosielle and Brown, 1979; Eyal, 1981; Camacho Casas et al., 1995) or short stature (Rosielle and Brown, 1979; Baltazar et al., 1990; Camacho Casas et al., 1995); however, Arama et al. (1999) and Simón et al. (2001) did not detect genetic associations between resistance and plant height or heading date. Van Beuningen and Kohli (1990) indicated that the associations between plant height and heading date are due to epidemiological factors.

Assuming no genetic associations, it is important to (i) determine whether plant height or heading date affects expression of resistance to Septoria tritici blotch and (ii) detect the influence of epidemiological or environmental factors. Genotypes that are genetically similar except for plant height or heading date and evaluated at the same phenological crop stage are necessary to separate genetic differences for resistance from environmental or epidemiological factors.

Possible association between Septoria tritici blotch severity and plant height or heading date is important when setting targets for incorporating genetic resistance to the disease in short or early heading lines. Knowledge about such associations contributes to optimizing management decisions in areas where Septoria tritici blotch is a major disease. The objective of this research was to determine the effect of plant height and heading date on the development of Septoria tritici blotch by means of near isogenic lines of wheat differing in dwarfing genes or insensitivity to photoperiod genes.

## MATERIALS AND METHODS

### Genotypes Included

Eight isogenic lines from the British winter wheat cultivar Mercia and nine isogenic lines from the French winter wheat cultivar Cappelle-Desprez developed at the John Innes Center were sown in a randomized block design with four replications each year. Lines with their unique genes were Mercia control, Mercia *Rht*1 (from 'Norin 10'), Mercia *Rht*1 (from 'Saitama 27'), Mercia *Rht*2 (from Norin 10), Mercia *Rht*3 (from 'Tom Thumb'), Mercia *Rht*12 (from 'Karkagi 522'), Mercia *Ppd*1 (from 'Mara'), Mercia *Ppd*1 (from 'Ciano 67'), Cappelle-Desprez control, Cappelle-Desprez *Rht*1 (from Norin 10), Cappelle-Desprez *Rht*1 (from Saitama 27), Cappelle-Desprez *Rht*1 (from 'Bezostaya'), Cappelle-Desprez *Rht*2 (from 'Ai-bian'), Cappelle-Desprez *Rht*2 (from Norin 10) Cappelle-Desprez *Rht*3 (from Tom Thumb), Cappelle-Desprez *Ppd*1 (from Mara), and Cappelle-Desprez *Ppd*2 (from 'Chinese Spring'). McIntosh et al. (1998) describes the individual genes.

### Experimental Details

Two field experiments were conducted at the Estación Experimental J. Hirschhorn, Facultad de Ciencias Agrarias y Forestales, Universidad Nacional de La Plata, Argentina, during 2000 and 2001. Plots were 1 m long by 0.60 m (three rows) wide. Seeds were vernalized for 4 wk at 4 to 8°C in a growth

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chamber before sowing in the field. Between plots, three rows of oat were sown to avoid interplot interference. The same was done between blocks. Plots were sown on 5 July 2000 and 26 June 2001. The 2001 experiment was planted close to a no-till wheat experiment.

### Inoculations

Inoculations were done with a mixture of isolates from the collection of the Facultad de Ciencias Agrarias de La Plata, Argentina (FALP 3096; FALP 4396; FALP 3398) in both years. The isolates were grown on malt extract agar at 19°C with 12 h of alternating light and dark cycles. Inoculum was prepared by aseptically scraping sporulating colonies with a scalpel and suspending conidia in deionized water. The conidial suspension was adjusted to $3 \times 10^6$ spores $mL^{-1}$ and sprayed at the 4-leaf stage (GS 21, Zadoks et al., 1974) in both years until runoff with a manually operated sprayer. Inoculum was applied at an early stage of development to simulate a natural infection. After inoculation, plants were kept moist by sprinkling water several times a day over a 3-d period.

### Data Collection

Necrosis percentage was visually estimated on the upper three leaves of 10 to 15 plants from the central row of each plot for each replication when each line reached the following growth stages (Zadoks et al., 1974): GS 49 (booting, first awn visible), GS 73 (early milk stage), and GS 85 (soft dough stage). Average necrosis of the three upper leaves was calculated for each plant. Height from the soil surface to the flag leaf, height from the soil surface to the tip of the tallest spike (total plant height) at dough stage, and days to 50% heading also were assessed on 10 to 15 plants for each plot and each replication. Although height to flag leaf was used in the data analysis, total height also was measured to determine the real range in plant height of the lines. Area under the disease progress curve (AUDPC) for each plot was calculated to summarize the progress of the disease, according to the formula of Shaner and Finney (1977).

### Data Analysis

Each isogenic data set (derived from Mercia or Cappelle-Desprez) was analyzed separately by ANOVA by a randomized block design in a combined analysis for both years at each growth stage and for the AUDPC values.

Multiple linear and nonlinear regression analyses, considering necrosis percentage as the dependent variable and days to heading and height to flag leaf as independent variables were computed to determine the percentage of the variation in necrosis accounted for by those morphophysiological traits. Height to flag leaf was used instead of total plant height considering that Septoria tritici blotch generally progresses up to the flag leaf in the Argentinean wheat growing area.

Also, a stepwise multiple linear regression analysis, including weather variables together with height to flag leaf and days to heading, was performed to assess the influence of weather conditions on the expression of the disease in lines with different morphophysiological characteristics. Necrosis percentage was the dependent variable and the independent variables were mean temperature (°C), sum of precipitation (mm); number of days with precipitation of at least 1 mm; mean relative humidity (%) and mean radiation (W $m^{-2}$) for periods of 3, 7, 15, or 30 d before the date of evaluation of each line for each growth stage [indicated as $T_3$ (temperature 3 d before evaluation); $T_7$ (temperature 7 d before evaluation); $T_{15}$ (temperature 15 d before evaluation); $T_{30}$ (temperature 30 d before evaluation); $P_3$ (precipitation 3 d before evaluation); $P_7$ (precipitation 7 d before evaluation); $P_{15}$ (precipitation 15 d before evaluation); $P_{30}$ (precipitation 30 d before evaluation); $Dp_3$ (days with precipitation 3 d before evaluation); $Dp_7$ (days with precipitation 7 d before evaluation); $Dp_{15}$ (days with precipitation 15 d before evaluation); $Dp_{30}$ (days with precipitation 30 d before evaluation); $H_3$ (humidity 3 d before evaluation); $H_7$ (humidity 7 d before evaluation); $H_{15}$ (humidity 15 d before evaluation); $H_{30}$ (humidity 30 d before evaluation) and $R_3$ (radiation 3 d before evaluation); $R_7$ (radiation 7 d before evaluation); $R_{15}$ (radiation 15 d before evaluation); $R_{30}$ (radiation 30 d before evaluation), respectively]. Averages for the period from inoculation to evaluation also were considered for all weather variables except the sum of precipitation and number of days with precipitation, because these variables are affected by the duration of that period.

If regression models are significant, the data should be corrected for plant height and heading date by covariance adjustment. The formula to adjust them is

$$Y \text{ adjusted} = Y(\text{data}) - b1\ (x1i - X1) - b2\ (x2\text{i} - X2)$$

where $Y$ (data) is the necrosis percentage value to be corrected, b1 is the regression coefficient for plant height; $x1i$ is the value of plant height corresponding to the data to be corrected, $X1$ is the average of plant height for all genotypes, $b2$ is the regression coefficient of heading date, $x2$i is the value of heading date corresponding to the data to be corrected, and $X2$ is the average of plant height for all genotypes.

## RESULTS

### Mercia Data Set

The ANOVA for the near isogenic lines from Mercia showed statistically significant differences for necrosis percentage at GS 49, GS 73, and GS 85, for the AUDPC, for plant height (to flag leaf and total) and for days to heading between lines and years (Table 1). The interaction of line × year was significant for necrosis percentage at GS 49 and GS 85 and for plant height (to flag leaf and total) and days to heading. Mean necrosis percentage for the three growth stages and for the AUDPC was higher in 2001 than in 2000 at the three growth stages. Plant height (to flag leaf and total) was lower and days to heading longer in 2001 than in 2000 because of the earlier planting in 2001. For the average of the two years, genes *Rht*1 (Norin 10), *Rht*2, *Rht*3, and *Rht*12 significantly reduced plant height (to flag leaf and total). Gene *Rht*1 coming from Saitama 27, which is known to have a less potent allele than *Rht*1 (Norin10) (C. Law, personal communication, 2002) did not reduce the height of Mercia relative to the control. *Ppd*1 (Mara) and *Ppd*1 (Ciano 67) significantly decreased the days to heading compared to the Mercia control. The shortest lines Mercia *Rht*12 and Mercia *Rht*3 showed higher necrosis percentage and higher AUDPC values than the Mercia control at the three growth stages. Mercia *Ppd*1 and *Ppd*2 showed lower necrosis values at the three growth stages and lower AUDPC values than the Mercia control (Table 2).

The significant line × year interaction for days to heading was due to lines with *Ppd*1 and *Ppd*2 being insensitive to photoperiod, thus having similar days to heading in both years, whereas the other lines were later in 2001 than in 2000 because of earlier planting. The

**Table 1. Mean squares for necrotic percentage caused by *Mycosphaerella graminicola* at three growth stages, plant height to flag leaf and heading date in eight near isogenic lines of the Mercia wheat cultivar, differing in plant height or days to heading evaluated over 2 yr.**

| Source of variation | df | Necrosis percentage GS49 | GS73 | GS85 | AUDPC | Height to flag leaf | Height (total) | Days to heading |
|---|---|---|---|---|---|---|---|---|
| Line | 7 | 1 099.7 ($P < 0.001$)† | 1 195.1 ($P < 0.001$) | 397.4 ($P < 0.001$) | 1 432 750 ($P < 0.001$) | 2 393.3 ($P < 0.001$) | 3 748.1 ($P < 0.001$) | 835.6 ($P < 0.001$) |
| Year | 1 | 7 543.5 ($P < 0.001$) | 20 592.3 ($P < 0.001$) | 6 136.6 ($P < 0.001$) | 20 683 200 ($P < 0.001$) | 2 074.8 ($P < 0.001$) | 3 084.4 ($P < 0.001$) | 1 198.9 ($P < 0.001$) |
| Line × year | 7 | 233.4 ($P < 0.002$) | 90.7 ($P < 0.001$) | 215.0 ($P < 0.001$) | 32 881 ($P < 0.001$) | 59.4 | 106.3 ($P < 0.004$) | 4.35 ($P < 0.0747$) |
| Error | 45 | 48.6 | 102.3 | 28.9 | 68 427 | 15.2 | 20.2 | 9.19 |

† $P > F$.

line × year interaction for plant height was due to the shortest lines (Mercia *Rht*3 and Mercia *Rht*12) having similar values in both years, whereas the other lines were shorter in 2001 (data not shown). Significant line × year interaction for necrosis percentage at GS 49 was observed because in 2000 necrosis was low and similar in all cultivars except for Mercia *Rht*12, which had a higher necrosis percentage value. In contrast, in 2001, the earliest heading lines *Ppd*1 (Mara) and *Ppd*1 (Ciano 67) showed lower values than the control, whereas Mercia *Rht*12 and Mercia *Rht*3 showed higher values than the control (Fig. 1A). At GS 85 the interaction line × year can be explained by the 2001 differences between lines being small because all lines reached high and similar disease severity. Whereas, in 2000, there was discrimination between the lines, with Mercia *Ppd*1 (Mara) and Mercia *Ppd*1 (Ciano 67) showing lower necrosis percentages than the control and Mercia *Rht*3 and Mercia *Rht*12 showing higher values than the control (Fig. 1C).

## Cappelle-Deprez Data Set

The ANOVA for Cappelle-Desprez set showed significant differences between lines and years for necrosis percentage at the three growth stages, for the AUDPC values, and for plant height and days to heading (Table 3). The line × year interaction also was significant for necrosis percentage at the three growth stages. All *Rht* genes significantly reduced plant height compared with the control and *Ppd* genes shortened the period to heading. On average, Cappelle-Desprez *Ppd*1 showed lower disease severity than the control at the three growth stages and Cappelle-Desprez *Ppd*2 at GS 73 and GS 85. Cappelle-Desprez *Rht*3, the shortest line, showed higher necrosis percentages than the control at GS 49 and GS 73 and Cappelle-Desprez *Rth*2 (Norin) was higher at GS 49. For the AUDPC, line *Rht*3 showed higher values than the control and *Ppd*1 and *Ppd*2 showed lower values (Table 4). There was a higher disease severity in 2001 than in 2000 at each of the three growth stages.

The line × year interaction at GS 49 can be explained by low and similar severity values for all the lines in 2000 but not in 2001. In 2001, Cappelle-Desprez *Ppd*1 showed lower necrosis percentage than the control and Cappelle-Desprez *Rht*1 (Bezostaya), Cappelle-Desprez *Rht*2 (Ai-bian), Cappelle-Desprez *Rht*2 (Norin 10), and Cappelle-Desprez *Rht3* higher values than the control. At GS 73, Cappelle-Desprez *Ppd*1 and Cappelle-Desprez *Ppd*2 showed significantly lower values than the control in 2000, whereas in 2001, only Cappelle-Desprez *Ppd*1 was lower than the control. At GS 85, only Cappelle-Desprez *Ppd*1 and Cappelle-Desprez *Ppd*2 in 2000 showed lower values than the control (Fig. 2A,B,C). The line × year interaction for the AUDPC values was due to the fact that in 2000 there was less discrimination between lines and none of the lines had significantly higher values than the Cappelle-Desprez control, whereas in 2001 Cappelle-Desprez *Rht*1 (Bezostaya), Cappelle-Desprez *Rht*2 (Norin 10) and Cappelle-Desprez *Rht*3 (Tom Thumb) had higher necrosis percentage than the control (Fig. 3B).

## Multiple Regression Model

Multiple linear regression models including plant height and days to heading as independent variables and necrosis percentage as dependent variable showed for the Mercia set plant height to flag leaf was negatively associated with necrosis at GS 73 in both years, at GS

**Table 2. Means for necrosis percentage caused by *Mycosphaerella graminicola* at three growth stages in eight isogenic lines of the Mercia wheat cultivar differing in plant height or days to heading evaluated over 2 yr.**

| Line | Necrosis percentage GS 49 | GS 73 | GS 85 | AUDPC | Height to flag leaf | Height (total) | Days to heading |
|---|---|---|---|---|---|---|---|
| Mercia control | 15.2 c† | 61.7 b | 88.2 b | 1977 b | 64.5 d | 81.2 d | 129.4 bcd |
| Mercia *Rht*1 (Norin 10)‡ | 12.7 bc | 55.5 b | 82.1 ab | 1885 b | 53.7 c | 69.4 c | 126.6 b |
| Mercia *Rht*1 (Saitama 27) | 15.0 c | 57.4 b | 83.7 ab | 1963 b | 66.7 d | 85.2 d | 126.9 b |
| Mercia *Rht2* (Norin 10) | 17.4 cd | 60.3 b | 89.0 b | 2100 b | 47.7 b | 62.7 b | 128.1 bc |
| Mercia *Rht*3 (Tom Thumb) | 22.6 d | 72.8 c | 95.6 c | 2456 c | 24.8 a | 33.8 a | 132.1 d |
| Mercia *Rht12* (Karkagi 522) | 42.9 e | 73.9 c | 100.0 c | 2763 d | 25.8 a | 32.9 a | 130.9 cd |
| Mercia *Ppd*1 (Mara) | 5.4 a | 38.7 a | 80.9 a | 1448 a | 64.2 d | 82.0 d | 106.5 a |
| Mercia *Ppd*1 (Ciano 67) | 6.9 ab | 44.3 a | 81.4 a | 1584 a | 63.4 d | 82.3 d | 108.1 a |
| Years | | | | | | | |
| 2000 | 6.4 a | 40.9 a | 77.8 b | 1453 a | 57.0 a | 73.1 a | 119.2 a |
| 2001 | 28.1 b | 76.0 b | 97.4 b | 2590 b | 45.7 b | 59.3 b | 127.9 b |

† Means followed by the same letter in the same column are not statistically significant ($P < 0.05$).
‡ Source of *Rht* gene in parentheses.

**Table 3. Mean squares for necrotic percentage caused by *Mycosphaerella graminicola* at three growth stages, AUDPC, plant height, and heading date in nine near isogenic lines of the wheat cultivar Cappelle-Desprez differing in plant height or days to heading evaluated over 2 yr.**

| Source of variation | df | Necrosis percentage GS49 | GS73 | GS85 | AUDPC | Height to flag leaf | Height (total) | Days to heading |
|---|---|---|---|---|---|---|---|---|
| Line | 8 | 553.6 ($P < 0.001$)† | 1 403.6 ($P < 0.001$) | 570.2 ($P < 0.001$) | 1 432 750 ($P < 0.001$) | 1 528.6 ($P < 0.001$) | 2 070.5 ($P < 0.001$) | 764.4 ($P < 0.001$) |
| Year | 1 | 19 367.7 ($P < 0.001$) | 16 379.3 ($P < 0.001$) | 1 723.8 ($P < 0.001$) | 21 668 700 ($P < 0.001$) | 2 389.7 ($P < 0.001$) | 5 038.4 ($P < 0.001$) | 2 167.0 ($P < 0.001$) |
| Line × year | 8 | 268.7 ($P < 0.002$) | 392.6 ($P < 0.001$) | 472.0 ($P < 0.001$) | 2 174 690 ($P < 0.001$) | 27.3 | 74.9 ($P < 0.004$) | 41.26 ($P < 0.075$) |
| Error | 51 | 75.6 | 41.0 | 18.3 | 51 169.8 | 23.9 | 33.6 | 21.33 |

† $P > F$.

49 in 2001 and at GS 85 in 2000 ($P \leq 0.05$) (Table 5). Heading date was positively associated with necrosis (at $P \leq 0.10$) at GS 49 in 2001, and at GS 73 and GS 85 in 2000. Models including both independent variables accounted for 35.3 to 98.3% of the variation in necrosis percentage, being significant for all cases except at GS 49 in 2000 and at GS 85 in 2001 (Table 5).

A large set of nonlinear regression models was tested too. However, only one model with better probability of the $R^2$ values than for multiple linear regression was identified. This model corresponded to the Mercia set at GS 49 in 2000.

For the Cappelle-Desprez set, plant height had a significant negative association with necrosis percentage (Table 6) at GS 73 in both years ($P \leq 0.10$) and at GS 49 in 2001 ($P \leq 0.05$). Days to heading had a significant positive association with necrosis percentage at GS 73 in 2001 and GS 85 in both years and at GS 49 in 2001 ($P \leq 0.05$). Models accounted for 66.1 to 99.1% of the variation in necrosis percentage, being significant for both years and at all three growth stages. No nonlinear-regression models with higher probabilities of the $R^2$ values than for multiple linear models could be identified.

Mean temperatures for the period from inoculation to evaluation varied from 14.1 to 15.8 (GS 49), 15.8 to 17.4 (GS 73) and 16.8 to 18.4°C (GS 85) for early and late Mercia lines in 2000 and from 15.2 to 20.5 (GS 49), and 16.8 to 17.9 (GS 73) and 18.2 to 19.9°C (GS 85) in 2001. Precipitation 15 d before evaluation (25.5–48.6 mm for early and late cultivars, respectively), accounted for part of the variation in necrosis percentage at GS 73.

For the Cappelle-Desprez lines, mean temperature ranged from 14.1 to 16.1 (evaluation at GS 49), 14.1 to 16.1 (GS 73) and 16.8 to 18.9°C (GS 85) for early and late lines in 2000 and from 14.4 to 17.6 (evaluation at GS 49), 17.1 to 18°C (GS 73) and 18.0 to 19.4°C (GS 85) in 2001. Precipitation 30 d before evaluation (70–153 mm for late and early cultivars respectively), accounted for part of the variation in necrosis percentage at GS73 in 2001.

The stepwise multiple linear regression analysis including environmental variables together with heading date to account for variation in necrosis percentage, determined that for the Mercia set plant height to flag leaf remained negatively associated with necrosis for most of the models. However, days to heading was primarily replaced by the environmental variables, mean temperature from inoculation to evaluation, or temperature or rainfall during the 15 d before evaluations (Table 7).

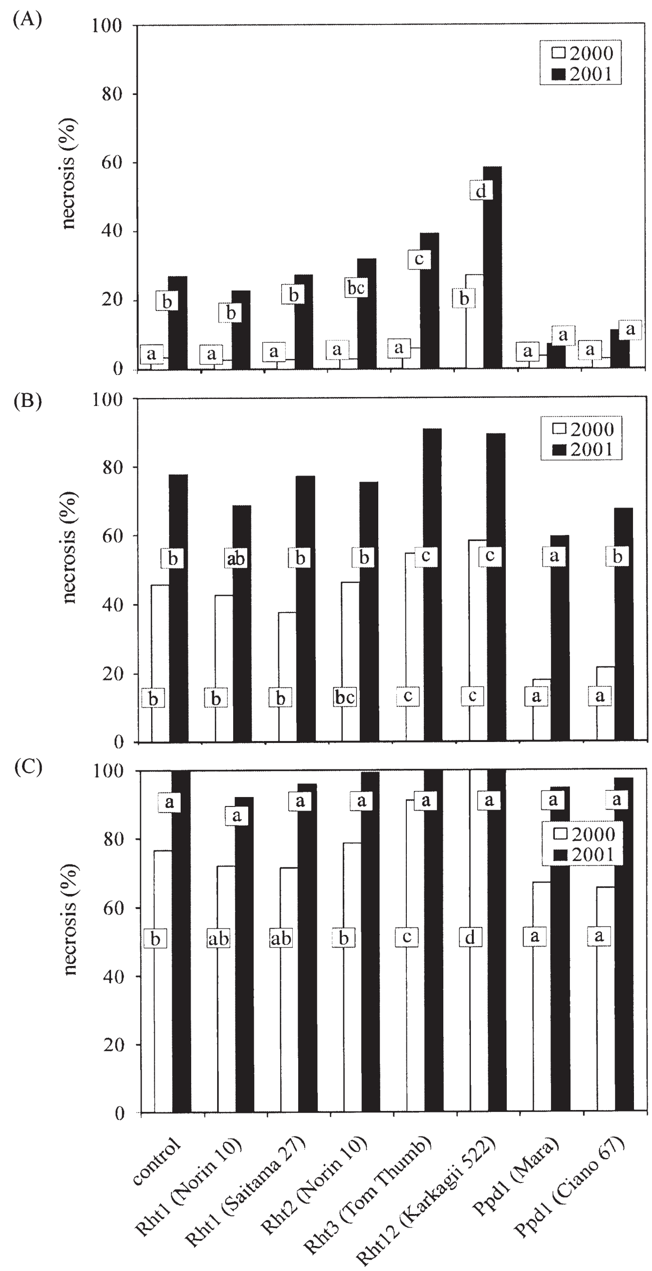


**Fig. 1. Line × year interaction for necrosis percentage caused by *Mycosphaerella graminicola* in eight near isogenic lines of the wheat cultivar Mercia, differing in plant height and heading date at (A) boot stage (GS 49), (B) milk stage (GS 73), (C) dough stage (GS 85). Means with the same letter within the same year are not significantly different, LSD ($P < 0.05$).**

**Table 4. Means for necrotic percentage caused by *Mycosphaerella graminicola* at three growth stages in nine isogenic lines of the wheat cultivar Cappelle-Desprez differing in plant height or days to heading.**

| Line | Necrosis percentage | | | AUDPC | Height to flag leaf | Total height | Days to heading |
|---|---|---|---|---|---|---|---|
| | GS49 | GS73 | GS85 | | cm | | |
| Cappelle-Desprez control | 22.6 bcd† | 71.6 c | 99.7 cd | 2526 d | 70.4 e | 89.5 e | 135.1 cd |
| Cappelle-Desprez *Rht*1 (Norin 10) | 20.1 abc | 70.5 c | 98.3 cd | 2265 cd | 60.4 d | 77.6 d | 130.7 c |
| Cappelle-Desprez *Rht*1 (Saitama 27) | 25.8 bcd | 72.8 c | 95.5 c | 2495 d | 59.8 d | 75.8 d | 132.6 cd |
| Cappelle-Desprez *Rht*1 (Bezostaya) | 31.3 def | 74.7 c | 98.6 cd | 2624 de | 46.7 b | 60.8 b | 135.0 cd |
| Cappelle-Desprez *Rht*2 (Ai-bian) | 28.2 cde | 71.7 c | 98.9 cd | 2527 d | 52.3 c | 68.1 c | 134.2 cd |
| Cappelle-Desprez *Rht*2 (Norin 10) | 35.1 ef | 75.5 cd | 98.6 cd | 2688 de | 56.5 cd | 73.0 cd | 136.1 d |
| Cappelle-Desprez *Rht*3 (Tom Thumb) | 37.5 f | 81.2 d | 99.8 d | 2843 e | 29.1 a | 39.0 a | 136.4 d |
| Cappelle-Desprez *Ppd*1 (Mara) | 11.8 a | 39.9 a | 77.9 a | 1856 b | 70.4 e | 87.3 e | 108.7 a |
| Cappelle-Desprez *Ppd*2 (Ciano 67) | 18.3 ab | 50.8 b | 81.4 b | 1531 a | 72.7 e | 88.3 e | 117.4 b |
| Years | | | | | | | |
| 2000 | 9.2 a | 52.4 a | 89.4 a | 1824 a | 63.4 a | 81.6 a | 124.1 a |
| 2001 | 42.0 b | 82.6 b | 99.2 b | 2921 b | 51.8 b | 64.9 b | 135.1 b |

† Means followed by the same letter in the same column are not statistically significant, LSD ($P = 0.05$).

**Table 5. Multiple linear regression of days to heading and plant height to flag leaf (independent variables) on necrosis percentage (dependent variable) caused by *Mycosphaerella graminicola* in eight near isogenic lines of the wheat cultivar Mercia at three growth stages in 2 yr.**

| Growth stage-year | Estimates | | | $R^2$ model (%) |
|---|---|---|---|---|
| | Constant | Plant height | Days to heading | |
| GS 49 | | | | |
| Year 2000 | 8.45 (0.883)† | −0.26 (0.182) | 0.11 (0.799) | 44.27 (0.232) |
| Year 2001 | −16.22 (0.706) | −0.62 (0.034) | 0.57 (0.084) | 86.22 (0.007) |
| GS 73 | | | | |
| Year 2000 | −84.35 (0.003) | −0.34 (0.001) | 1.20 (<0.001) | 98.28 (<0.001) |
| Year 2001 | 49.91 (0.141) | −0.42 (0.039) | 0.35 (0.122) | 83.59 (0.011) |
| GS 85 | | | | |
| Year 2000 | 34.37 (0.348) | −0.43 (0.009) | 0.57 (0.071) | 89.72 (0.003) |
| Year 2001 | 101.23 (0.001) | −0.11 (0.248) | 0.0082 (0.939) | 35.26 (0.337) |

† Probability, *t* test.

For the Cappelle-Desprez set, plant height to flag leaf was consistently one of the most important variables in predicting variation in necrosis percentage in several of the models (Table 8). Days to heading was replaced by environmental variables temperature from inoculation to evaluation, precipitation and temperature 30 d before evaluation, or relative humidity. For GS 73 in 2001, also a negative association with precipitation 30 d before evaluation was shown when precipitation 30 d before evaluation varied from 70 to 153 mm for late and early cultivars, respectively. In some cases, single linear regression on weather data yielded highly significant models. Humidity 15 d before evaluation accounted for 75% of the variation in necrosis percentage among lines of Cappelle-Desprez at GS 49 in 2000, whereas the temperature from inoculation to evaluation accounted for 98.5 and 76.6% of the variation in necrosis of Cappelle-Desprez lines at the dough stage in 2001 and 2000, respectively.

As an example, the true and the adjusted values according to plant height and heading date for Mercia at GS 73 in 2000 are shown (Table 9). This set of data was selected because the multiple regression model of plant height and heading date on necrosis percentage was highly significant ($P > 0.001$), indicating that values should be corrected to assess the true resistance of the lines. A similar procedure can be used to adjust data in a set of lines from a breeding nursery. In our case, all adjusted values were similar because the genotypes tested are near isogenic lines. In a breeding program, this procedure will allow lines to be selected in segregating populations on the basis of true resistance values.

## DISCUSSION

The increase in the severity of disease in 2001 most likely is attributed to the inoculation in the previous year and proximity to a no-till wheat crop. Although the necrosis percentage of near isogenic lines followed a similar pattern in both years, the line × year interaction for both sets of isogenic lines was mainly due to a higher inoculum pressure in 2001 than in 2000. This resulted in less discrimination between lines at the boot stage (GS 49) in 2000, because of low necrosis percentages for most of the lines and no discrimination at the dough stage (GS 85) in 2001, because of very high values for all the lines. For the Mercia set, there were two lines with extremely short plant height to flag leaf (Mercia *Rht3* and Mercia *Rht12*). For Cappelle-Desprez, there was only one line with extremely short plant height (Cappelle *Rht3*) because *Rht12* was not incorporated in this set. Lines with higher necrosis percentages were those carrying these genes, indicating that necrosis percentage is negatively associated with plant height only in very short wheats. For the AUDPC values, line × year interaction was only evident for the Cappelle-Desprez set. This also can be explained by the fact that under low inoculum pressure (in 2000), the discrimination between lines was lower than in 2001.

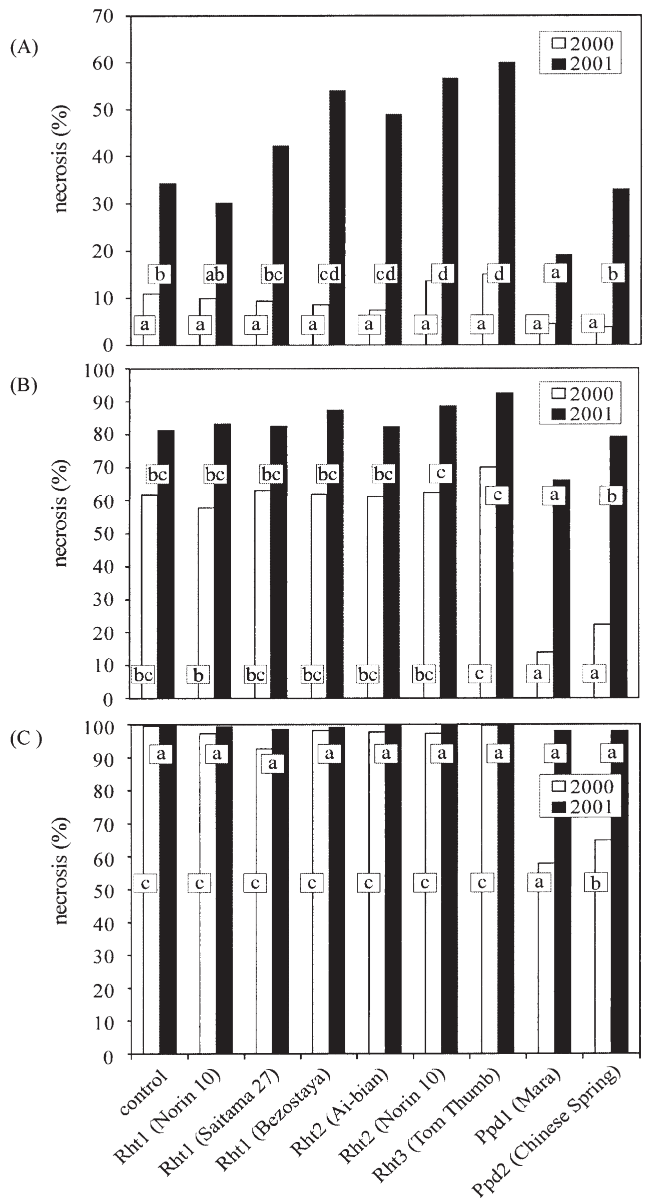


**Fig. 2. Line × year interaction for necrosis percentage caused by *Mycosphaerella graminicola* in nine near isogenic lines of the wheat cultivar Cappelle-Desprez, differing in plant height and heading date at (A) boot stage (GS 49), (B) milk stage (GS 73), (C) dough stage (GS 85). Means with the same letter within the same year are not significantly different, LSD ($P < 0.05$).**

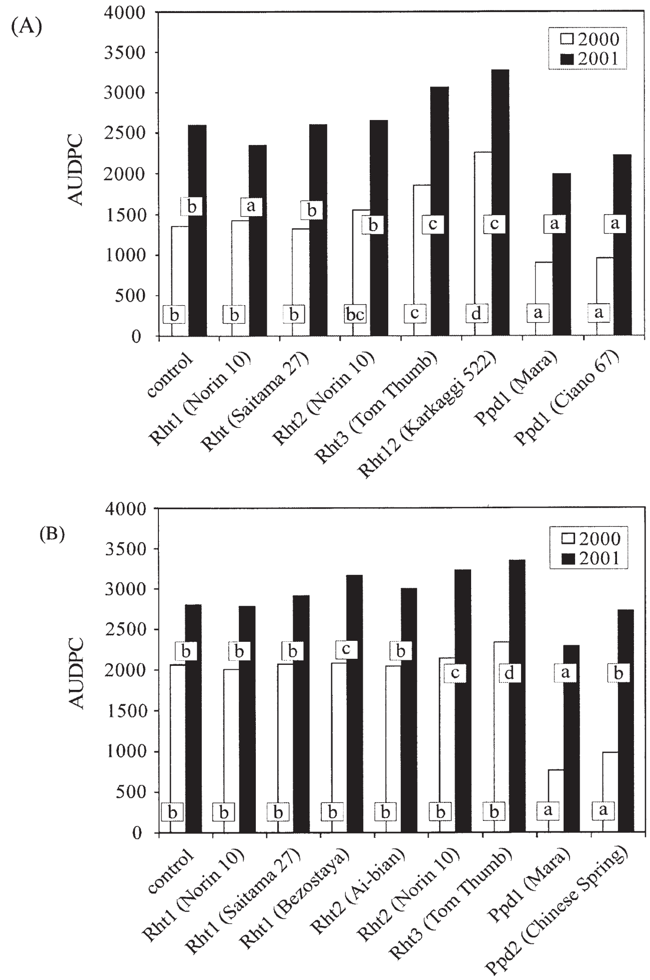


**Fig. 3. Line × year interaction for the area under disease progress curve (AUDPC) caused by *Mycospharella graminicola* in (A) eight isogenic lines of the wheat cultivar Mercia, (B) nine near isogenic lines of the wheat cultivar Cappelle-Desprez. Means with the same letter within the same year are not significantly different, LSD ($P < 0.05$).**

Overall, multiple regression models showed plant height was negatively associated with disease levels and heading date was positively associated. Thus, the highest necrosis values were observed on the shortest lines and the lowest values on the two lines which headed earlier. Lack of associations between necrosis and the morphophysiological traits was more evident in both sets at GS 49 in 2000 because of the very low and similar necrosis percentages for all lines and at GS 85 in 2001 because of the extremely high and similar necrosis percentages reached by all lines. However, when weather variables were included in stepwise regression models together with the morphophysiological traits, plant height to flag leaf remained negatively associated, whereas heading date was replaced by relevant weather variables such as temperature, rainfall, and relative humidity in different periods before evaluation. This suggested that the positive association between heading date and resistance was due to weather conditions in these years being conducive to the development of Septoria tritici blotch, especially in the lines with late heading. Several studies showed negative associations between necrosis with plant height and heading date, which were attributed in some cases to genetic linkages and in others to epidemiological or environmental factors (Rosielle and Brown, 1979; Eyal et al., 1987; Camacho Casas et al., 1995; Baltazar et al., 1990; Van Beuningen and Kohli, 1990). Previous studies by Arama et al. (1999); Simón et al. (2001, 2002) indicated no genetic associations within a wide set of cultivars between resistance, plant height, and heading date. Instead these, associations are due to epidemiological or environmental factors. Reduced plant height is generally associated with increased ne-

**Table 6. Multiple linear regression of days to heading and plant height to flag leaf (independent variables) on necrosis percentage (dependent variable) caused by *Mycosphaerella graminicola* in nine near isogenic lines of the wheat cultivar Cappelle-Desprez at three growth stages for 2 yr.**

| | Estimates | | | |
|---|---|---|---|---|
| Growth stage-year | Constant | Plant height | Days to heading | $R^2$ model |
| GS 49 | | | | |
| Year 2000 | −9.31 (0.591)† | −0.09 (0.254) | 0.20 (0.109) | 67.49 (0.034) |
| Year 2001 | −41.56 (0.326) | −0.51 (0.030) | 0.81 (0.02) | 88.65 (0.001) |
| GS 73 | | | | |
| Year 2000 | −140.37 (<0.001) | −0.24 (0.029) | 1.68 (<0.001) | 98.53 (<0.001) |
| Year 2001 | 14.82 (0.441) | −0.18 (0.073) | 0.57 (0.002) | 91.68 (0.001) |
| GS 85 | | | | |
| Year 2000 | −92.58 (<0.001) | −0.06 (0.317) | 1.50 (<0.001) | 99.13 (<0.001) |
| Year 2001 | 88.73 (<0.001) | 0.003 (0.894) | 0.077 (0.029) | 66.07 (0.039) |

† Probability, *t* test.

**Table 7. Best models explaining variation in necrosis caused by *Mycosphaerella graminicola* (dependent variables) using plant height, days to heading and some weather variables (mean temperature, rainfall, days with precipitation, radiation) as independent variables (by stepwise multiple regression) in eight near isogenic lines of the Mercia wheat cultivar.**

| Growth stage-year | Model† | $R^2$ (%) |
|---|---|---|
| GS 49 | | |
| 2000 | 22.87 (0.030)‡ − 0.288 (0.070) Hfl | 44.27 (0.232) |
| 2001 | −44.56 (0.270) − 0.54 (0.030) Hfl + 5.96 (0.020) Ti | 91.50 (0.002) |
| GS 73 | | |
| 2000 | −111.87 (0.002) + 0.18 (0.090) P15 + 9.3 (<0.001) T15 − 0.39 (<0.001) Hfl | 99.50 (<0.001) |
| 2001 | 101.66 (0.001) − 0.56 (0.011) Hfl | 68.74 (0.011) |
| GS 85 | | |
| 2000 | −29.83 (0.580) + 7.32 (0.040) Ti − 0.43 (0.006) Hfl | 91.71 (0.002) |
| 2001 | 101.23 (0.001) + 0.008 (0.939) Hd − 0.11 (0.248) Hfl | 92.94 (<0.001) |

† Abbreviations: Hfl is plant height to flag leaf; Ti is mean temperature from inoculation to evaluation; P15 is rainfall during the 15 d before evaluation; T15 is mean temperature during the 15 d before evaluation; Hd is heading date.
‡ Probability, *t* test.

crosis percentages because there are shorter distances between leaf layers making inoculum transfer easier.

Weather variables, depending on the growing region, year, or sowing date also could be neutral or favorable for development of Septoria tritici blotch in early heading cultivars, as has been found in other studies (Simón et al., 2001, 2002, 2003). The data indicated that some weather conditions were more conducive to Septoria tritici blotch in the latest heading near isogenic lines, which had higher necrosis percentage than the earliest heading near isogenic lines. Under greenhouse conditions, temperatures from 17 to 25°C are optimum for disease development (Hess and Shaner, 1987; Shaw, 1990; Wainshilbaum and Lipps, 1991; Magboul et al., 1992; Chungu et al., 2000). In this investigation, temperature was the main environmental factor positively associated with necrosis, especially at GS 49 and GS 73, mean temperatures were lower for early heading cultivars than for late heading cultivars. Precipitation, relative humidity and radiation were similar for all cultivars. At GS 73 for the Cappelle-Desprez set, negative associations were found with precipitation 30 d before evaluation probably because of rainfall in this phase being too heavy for normal transport of spores.

Evaluating the disease at the same crop phenological stage is crucial. When the disease is assessed at the same chronological time, leaves of late cultivars are younger and less exposed to the pathogen. Moreover, for *Septoria* spp. resistance decreases with leaf age, and the older lower leaves are more susceptible than the younger upper leaves (Jonsson, 1991). Because of this effect, late maturing germplasm is often considered more resistant than early maturing lines.

In this research, strong associations with plant height were only found in very short wheats (in general with differences in height to flag leaf higher than 38 cm compared with the control), indicating that moderately short wheats are not necessarily more susceptible to Septoria tritici blotch than the taller ones. Although a wide spectrum of wheat cultivars fell far from plant height values of the shortest lines used in this study, cultivars with intermediate to high plant height should be selected in areas where Septoria tritici blotch is a major disease. Knowing the climatic characteristics of the wheat growing region, it is possible to adjust planting dates and cultivar choice (on the basis of heading date) to avoid periods with weather conditions predisposed to the disease during critical phases of wheat growth.

Taking into account the influence of plant height and heading date due to epidemiological and environmental factors on the expression of the Septoria tritici blotch, breeders should correct their data according to a covariance analysis using the coefficients of the multiple regression line, with the disease score as independent variable and heading date and plant height as independent. This regression line needs to be calculated with the data for each specific year within their breeding materials. In our example, only necrosis data corresponding to very short or very early heading lines were significantly modified.

**Table 8. Best models explaining variation in necrosis caused by *Septoria tritici* (dependent variables) using plant height, days to heading and some weather variables (mean temperature, rainfall, days with precipitation, radiation) as independent variables (by stepwise multiple regression) in nine isogenic lines of the Cappelle-Desprez wheat cultivar.**

| Growth stage/year | Model† | $R^2$ (%) |
|---|---|---|
| GS 49 | | |
| 2000 | −69.83 (0.050)‡ + 0.92 (0.002) H15 | 75.19 (0.002) |
| 2001 | −53.46 (0.220) − 0.53 (0.016) Hfl + 7.43 (0.010) Ti | 91.50 (0.002) |
| GS 73 | | |
| 2000 | −325.61 (<0.001) − 0.25 (0.011) Hfl + 22.80 (<0.001) Ti | 98.97 (<0.001) |
| 2001 | −73.66 (0.210) − 0.16 (0.040) Hfl − 0.15 (0.002) P30 + 10.08 (0.016) T30 | 96.95 (<0.001) |
| GS 85 | | |
| 2000 | −262.91 (<0.001) + 19.18 (<0.001) Ti | 98.46 (<0.001) |
| 2001 | 62.18 (<0.001) + 1.97 (0.002) Ti | 76.58 (0.002) |

† Abbreviations: H15 is mean relative humidity during the 15 d before evaluation; Hfl is plant height to flag leaf; Ti is mean temperature from inoculation to evaluation; P30 is precipitation during 30 d before evaluation; T30 is mean temperature during 30 d before evaluation.
‡ Probability *t* test.

**Table 9. Example of unadjusted and adjusted values (by plant height and heading date) of the Mercia set at GS 73 in 2000.**

| Line | Unadjusted necrosis percentage GS 73 (Year 2000) | Plant height to flag leaf | Heading date | Adjusted necrosis percentage GS 73 (Year 2000) |
|---|---|---|---|---|
| Mercia control | 45.7 | 74.8 | 124.5 | 45.5 |
| Mercia *Rht*1 (Norin10) | 42.5 | 57.9 | 121.0 | 40.8 |
| Mercia *Rht*1 (Saitama 27) | 37.6 | 74.8 | 120.7 | 42.0 |
| Mercia *Rht*2 (Norin 10) | 46.2 | 53.2 | 122.2 | 41.4 |
| Mercia *Rht*3 (Tom Thumb) | 54.7 | 27.5 | 127.2 | 35.2 |
| Mercia *Rht*12 (Karkagi 522) | 58.3 | 29.0 | 124.5 | 42.6 |
| Mercia *Ppd*1 (Mara) | 17.9 | 68.0 | 106.5 | 37.0 |
| Mercia *Ppd*1 (Ciano 67) | 21.3 | 71.0 | 108.1 | 39.5 |

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