

# Experiment 4 - Data S5

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## Experiment 4 – BX-feedback on wheat insect resistance

A fourth experiment, using a fully randomized design for testing the factor conditioning (BX+ or BX-), was set up in a climate chamber to examine insect performance. Experiment 4 was conducted with a greenhouse-conditioned batch of Changins soil (Table S1). The conditioned soils (BX+ and BX- variants) were further sieved to 5 mm, filled in 300 mL pots and sown with four wheat plants. We used again the two wheat cultivars ‘Drifter’ and ‘Fiorina’ resulting in 40 experimental units (2 BX conditions \* 2 cultivars \* 10 replicates; Data S4). Plants were grown with a light period from 8:00 to 22:00 at 22 °C and 18°C at night, with 70% relative humidity. At five weeks, when we terminated the experiment, Drifter was at the vegetative stage while Fiorina started flowering. We approximated the chlorophyll content and used two fully expanded leaves per plant for a caterpillar performance assay.

Table 1: Number of replicates

	BX+	BX-
<b>Drifter</b>	10	10
<b>Fiorina</b>	10	9

Feedback experiment with wheat (Drifter and Fiorina) (*wheat\_line* variable) grown on Changins soil previously conditioned with maize B73 (BX+ soil) and B73(*bx1*) (BX- soil) (*BX\_condition* variable).

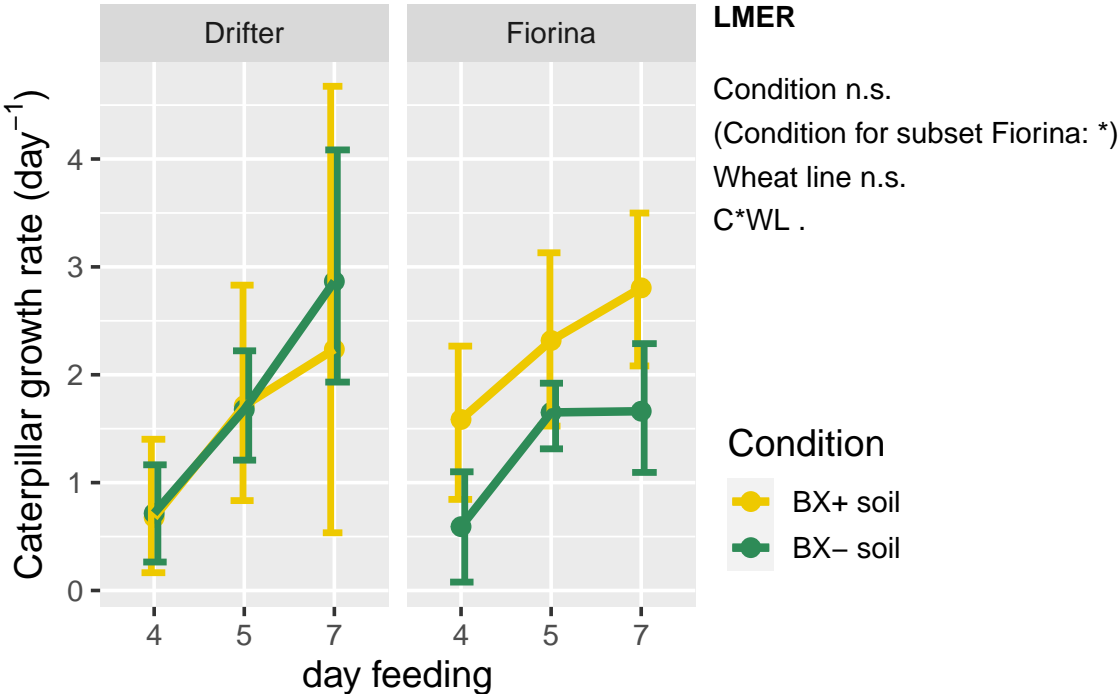
*S. littoralis* growth rate

Table 2: Linear mixed-effect model on Caterpillar growth rate, at 5 weeks. Model = caterpillar growth rate ~ day\_feeding \* BX\_condition \* wheat\_line, random=~1|individual

	numDF	denDF	F-value	p-value
(Intercept)	1	55	69.2	2.646e-11
day_feeding	2	55	26.72	7.79e-09
BX_condition	1	31	0.8084	0.3755
wheat_line	1	31	0.08093	0.7779
day_feeding:BX_condition	2	55	0.496	0.6116
day_feeding:wheat_line	2	55	1.546	0.2223
BX_condition:wheat_line	1	31	2.919	0.09756
day_feeding:BX_condition:wheat_line	2	55	1.424	0.2494

Table 3: Linear mixed-effect model on Caterpillar growth rate, only Fiorina. Model = caterpillar growth rate ~ day\_feeding \* BX\_condition, random=~1|individual

	numDF	denDF	F-value	p-value
(Intercept)	1	26	51.37	1.303e-07
day_feeding	2	26	17.33	1.651e-05
BX_condition	1	15	5.39	0.03474
day_feeding:BX_condition	2	26	0.8752	0.4287



**Figure 5 | BX-feedback on wheat insect resistance and chlorophyll content**

Wheat plants from cultivar Drifter and Fiorina were grown on 'BX+' and 'BX-' variants of Changins soil. Caterpillar performance of *Spodoptera littoralis* fed with leaves of 5 weeks old plants was measured after 4, 5 and 7 days of feeding. The LME (model:  $\sim$  day\_feeding \* condition (C) \* wheat\_line (WL), random factor = individual) results are reported next to the figure (significance code:  $P < 0.001$  \*\*\*;  $P < 0.01$  \*\*,  $P < 0.05$  \*;  $P < 0.1$  †; 'n.s.' = not significant).

# Wheat chlorophyll content

Table 4: ANOVA on chlorophyll content, at 5 weeks. Model = chlorophyll ~ BX\_condition \* wheat\_line

	Sum Sq	Pr(>F)
<b>BX_condition</b>	41.76	0.09075
<b>wheat_line</b>	6.24	0.5047
<b>BX_condition:wheat_line</b>	143.9	0.002844
<b>Residuals</b>	424.7	NA

Table 5: t.test on chlorophyll content, at 5 weeks

wheat_line	mean in BX+	mean in BX-	p.value
Drifter	30.47	29.53	0.3037
Fiorina	26.98	30.61	0.03066

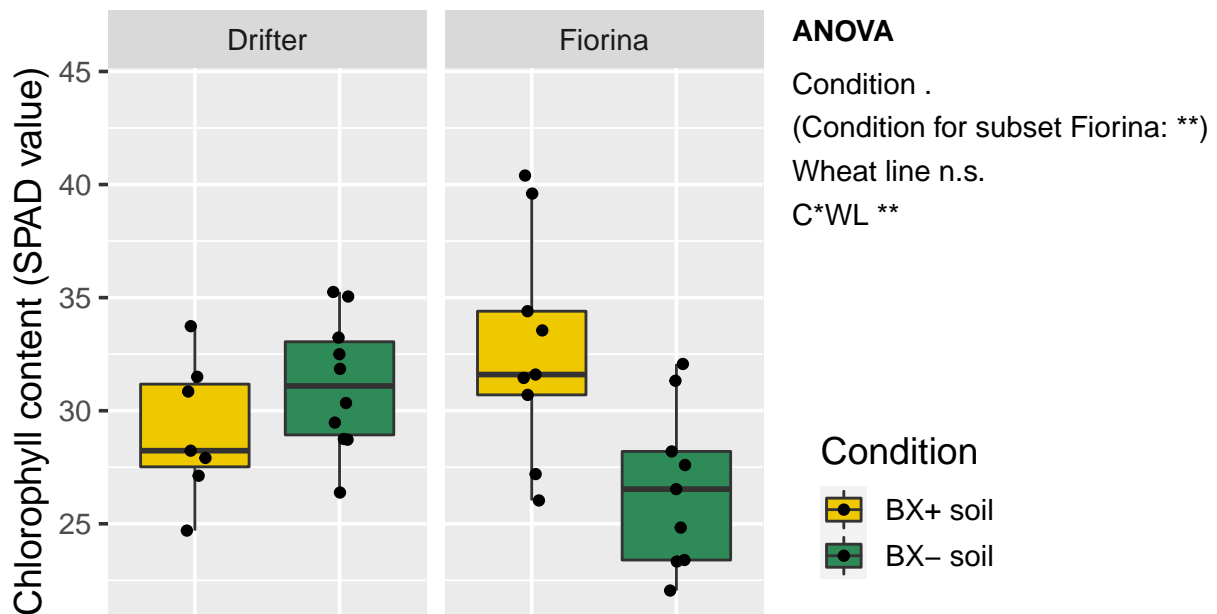


Figure 5 | BX-feedback on wheat insect resistance and chlorophyll content

Wheat plants from cultivar Drifter and Fiorina were grown on ‘BX+’ and ‘BX-’ variants of Changins soil. Leaf chlorophyll content (SPAD values) was measured on 6 weeks old plants. Data S4 documents the statistical analyses in detail. The ANOVA (model: ~ condition (C) \* wheat\_line (WL)) results are reported next to the figure (significance code: P < 0.001 \*\*\*, P < 0.01 \*\*, P < 0.05 \*, P < 0.1 ‘;’, ‘n.s.’ = not significant).

## Descriptive statistics

Table 6: Mean and standard deviation (sd) of measures. Dr = Drifter Fio = Fiorina (continued below)

	mean BX+ Dr	sd BX+ Dr	mean BX- Dr	sd BX- Dr
<b><code>insect_growth_rate</code></b>	1.468	1.919	1.753	1.509
<b><code>chlorophyll</code></b>	29.15	3.048	31.16	2.916

Table 7: Table continues below

	percent diff Dr	mean BX+ Fio	sd BX+ Fio
<b><code>insect_growth_rate</code></b>	17.67	2.236	1.265
<b><code>chlorophyll</code></b>	6.65	32.77	4.898

	mean BX- Fio	sd BX- Fio	percent diff Fio
<b><code>insect_growth_rate</code></b>	1.231	0.8766	-58.01
<b><code>chlorophyll</code></b>	26.59	3.545	-20.81