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EFFECT OF CYP51 GENE EXPRESSION LEVELS AND HAPLOTYPE DIVERSITY ON RESISTANCE PHENOTYPES OF FIELD ISOLATES OF *CERCOSPORA BETICOLA*

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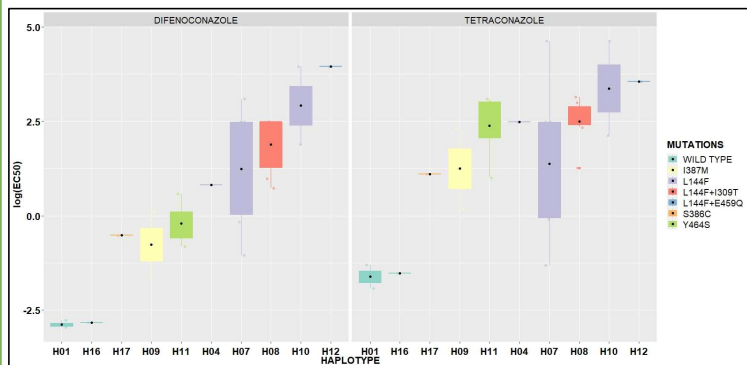
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Cercospora beticola, is one of the most widespread foliar diseases of sugar beet. A decrease in the susceptibility of *C. beticola* to azoles, a class of fungicides that inhibit the biosynthesis of sterol by acting on sterol 14-demethylase, has been reported in France over the past decade. The contribution of the overexpression of the *Cyp51* gene, coding for the sterol 14-demethylase, has been studied on a set of 27 isolates characterized for their sensitivity to azoles and sequenced for the *Cyp51* gene.

CONTEXT

Diversity of susceptibility to difenoconazole and tetraconazole of 27 *C. beticola* isolates from France (25) or Germany (2)



FINDING:

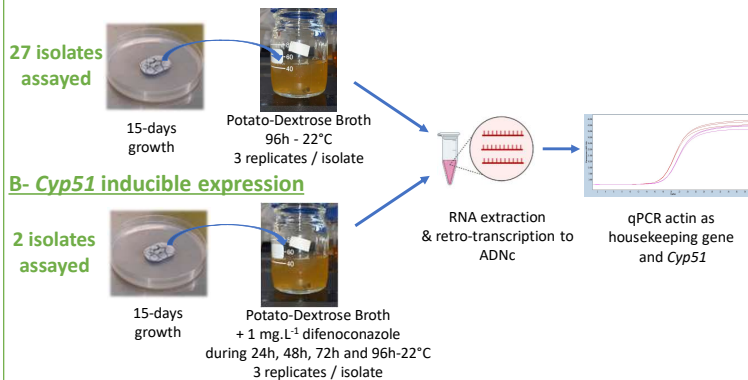
Sensitivity of *C. beticola* to azoles is highly variable between and within *Cyp51* haplotypes.

QUESTIONS:

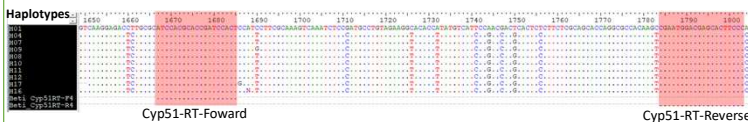
- What is the contribution of *Cyp51* overexpression to the resistance phenotype of *C. beticola* isolates?
- Is *Cyp51* overexpression constitutive or induced in *C. beticola* isolates?

APPROACH OF CYP51 GENE EXPRESSION

A- *Cyp51* constitutive expression



Cyp51 primers: based on different *Cyp51* haplotypes

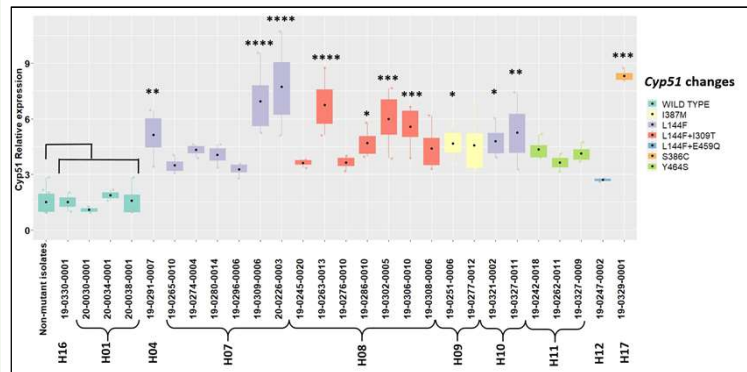


Actin primer: from Bolton *et al.* 2011

RESULTS

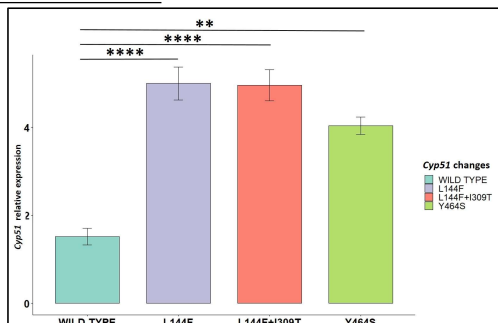
A-Constitutive expression

Level of constitutive expression of the *Cyp51* gene in a panel of 27 isolates of *C. beticola*



All resistant isolates carrying one or several mutations in *Cyp51* also overexpressed this gene compare to wild type isolates, but this overexpression is only statistically significant for some of these isolates (* = $p \leq 0.05$; ** = $p \leq 0.01$; *** = $p \leq 0.001$, **** = $p \leq 0.0001$).

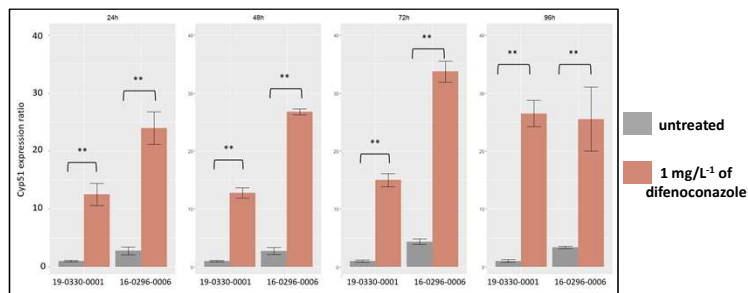
Level of constitutive expression of the *Cyp51* gene between groups of isolates carrying the same mutations



Groups of *C. beticola* carrying mutations (L144F, L144F + I309T, Y464S) on the *Cyp51* gene have a significantly higher level of *Cyp51* expression than wild type isolates. (** = $p \leq 0.01$; **** = $p \leq 0.0001$).

B-Inducible expression

Time-dependent evolution of inducible expression of the *Cyp51* gene in an azole-sensitive (19-0330-0001; H16) and azole-resistant (16-0296-0006; H04) of *C. beticola* isolate



Preliminary results show that both the resistant and susceptible isolates are able to induce the overexpression of *Cyp51* during at least 96 h after difenoconazole treatment.

Conclusion & Discussion

Only mutant isolates show constitutive overexpression of the *Cyp51* gene at a moderate level. The level of constitutive expression does not appear to explain the wide variability in azole sensitivity observed between and within *Cyp51* haplotypes.

→ Does constitutive overexpression of *Cyp51* really play a role in the variability of sensitivity to difenoconazole or could it be a compensatory mechanism present in mutant isolates?

Both azole-resistant and azole-sensitive isolates show an inducible expression in presence of difenoconazole. *Cyp51* induction could be a non-haplotype-specific response to difenoconazole treatment.

→ Is *Cyp51* inducible overexpression present in all sensitive and resistant haplotypes? Is inducible expression regulated by the same regulatory factors in susceptible and resistant isolates?

The roles of constitutive and inducible expression of *Cyp51* need to be clarified.

→ Do the levels of constitutive or inducible expression of *Cyp51* have an effect on ergosterol richness and thus contribute to the strengthening of cell membranes?