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An RNAi-based Control of *Fusarium graminearum* Infections Through Spraying of Long dsRNAs

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ABSTRACT

RNA interference (RNAi) is a conserved and integral aspect of gene regulation that utilizes small RNAs (sRNAs) to direct the silencing of gene expression at the transcriptional or posttranscriptional level. The consequence of RNAi is a loss-of function phenotype that, ideally, is identical to that of a genetic null mutant. RNAi is associated with diverse regulatory processes, including regulation of gene expression at the transcriptional and translational levels, protection against viral infection, control of epigenetic modifications, regulation of genome stability, curbing of transposon movement and regulation of heterochromatin formation (Castel & Martienssen 2013). Over the last decade RNAi has emerged as a powerful genetic tool for scientific research. It has been utilized not only in fundamental research for the assessment of gene function, but also in various fields of applied research, such as human and veterinary medicine. In plants, RNAi strategies have the potential to allow manipulation of various aspects of food quality and nutritional content (Koch & Kogel 2014). Exploiting the RNAi mechanism in plants also has a strong potential for agricultural disease control. Indeed, expression of inhibitory dsRNAs in the corresponding host plant conferred protection from predation or infection by targeted gene silencing (Koch et *al*. 2013; Koch & Kogel 2014; Abdellatef et *al*. 2015), a phenomenon that has been termed **host-induced gene silencing (HIGS)**. Here we present a direct spray application of long dsRNAs to control Fusarium head blight of barley.

INTRODUCTION

Recently, we demonstrated that in Arabidopsis (*Arabidopsis thaliana*) and barley (*Hordeum vulgare*), transgenic expression of *CYP3*-dsRNA, a 791 nt long dsRNA targeting the three

fungal *CYP51* genes involved in ergosterol biosynthesis, confers resistance to infection with *Fusarium graminearum* (Koch et *al.* 2013) (Figure 1).

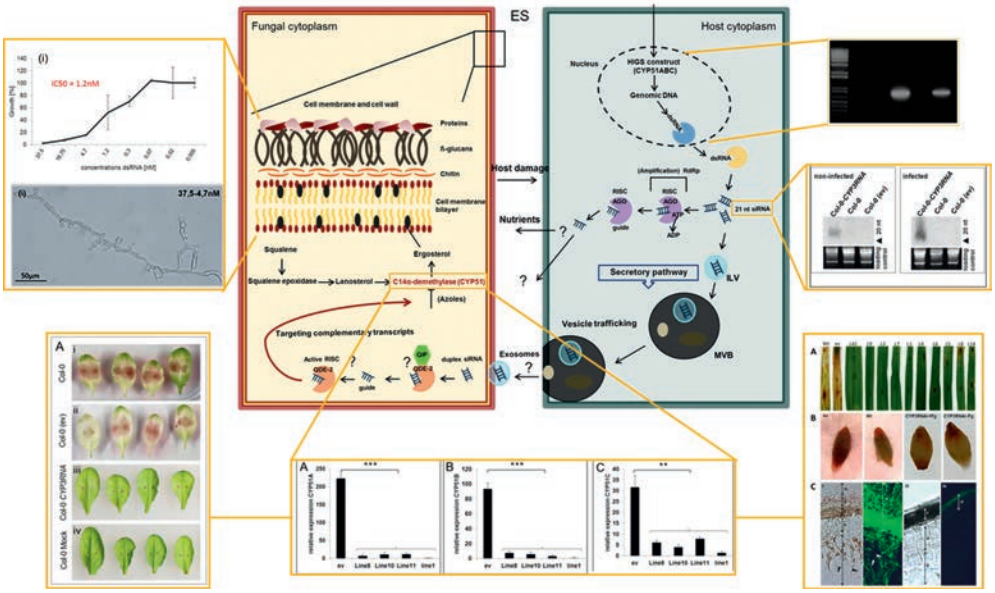

Figure 1 Summary of the data published in Koch et *al.* (2013). In axenic cultures of *Fusarium graminearum*, *in vitro* feeding of *CYP3RNA*, a 791-nt double-stranded (ds)RNA complementary to *CYP51A*, *CYP51B*, and *CYP51C*, resulted in growth inhibition as well as altered fungal morphology (upper left). Expression of the same dsRNA in Arabidopsis and barley (upper right) rendered susceptible plants highly resistant to fungal infection (bottom left /right). Microscopic analysis revealed that mycelium formation on *CYP3RNA*-expressing leaves was restricted to the inoculation sites, and that inoculated barley caryopses were virtually free of fungal hyphae (bottom right). This inhibition of fungal growth correlated with *in planta* production of siRNAs corresponding to the targeted *CYP51* sequences (middle right), as well as highly efficient silencing of the fungal *CYP51* genes (bottom).

While these results provided proof-of-concept that RNAi-based plant protection is an effective strategy for controlling diseases caused by devastating necrotrophic pathogens, the broad applicability of this transgenic tactic remains questionable due to the persisting weak acceptance of GMO strategies for food and feed production in many countries. Moreover, while delivery of inhibitory noncoding dsRNA by transgenic expression is a promising concept, it requires the generation of transgenic crop plants which may cause substantial delay for application strategies depending on the transformability and genetic stability of the crop plant species. Therefore, we established an RNAi-based crop protection strategy using direct spray applications of noncoding double-stranded RNA to target pathogens, termed **spray-induced gene silencing (SIGS)**.

MATERIALS, METHODS AND RESULTS

Using the agronomically important barley - *Fusarium graminearum* pathosystem, we alternatively demonstrate that spraying *CYP3*-dsRNA silences the expression of *CYP51* fungal genes and inhibits fungal growth (Koch et *al*. 2016) (Figure 2). The antifungal activity of *CYP3*-dsRNA and their siRNA derivatives was tested, by using a detached leaf assay that enabled us to assess fungal growth in local (directly sprayed) and distal (semi-systemic, non-sprayed) leaf segments. Using this approach, we could demonstrate that inhibitory dsRNA translocated via the plant vascular system and eventually was absorbed by the pathogen from leaf tissue (Figure 2). The profile of inhibitory dsRNA accumulation, as demonstrated by northern blot analysis and RNAseq, showed that both long *CYP3*-dsRNA and plant-processed *CYP3*-dsRNA-derived siRNA accumulate in the plant vascular system, though translocation of siRNA seems to be less efficient and thus siRNA concentration at the remote infection sites probably was not high enough to induced SIGS. Unexpectedly, efficient spray-induced control of fungal infections involved passage of *CYP3*-dsRNA via the plant vascular system and its processing into siRNAs by fungal DICER-LIKE 1 after uptake by the pathogen (Figure 2).

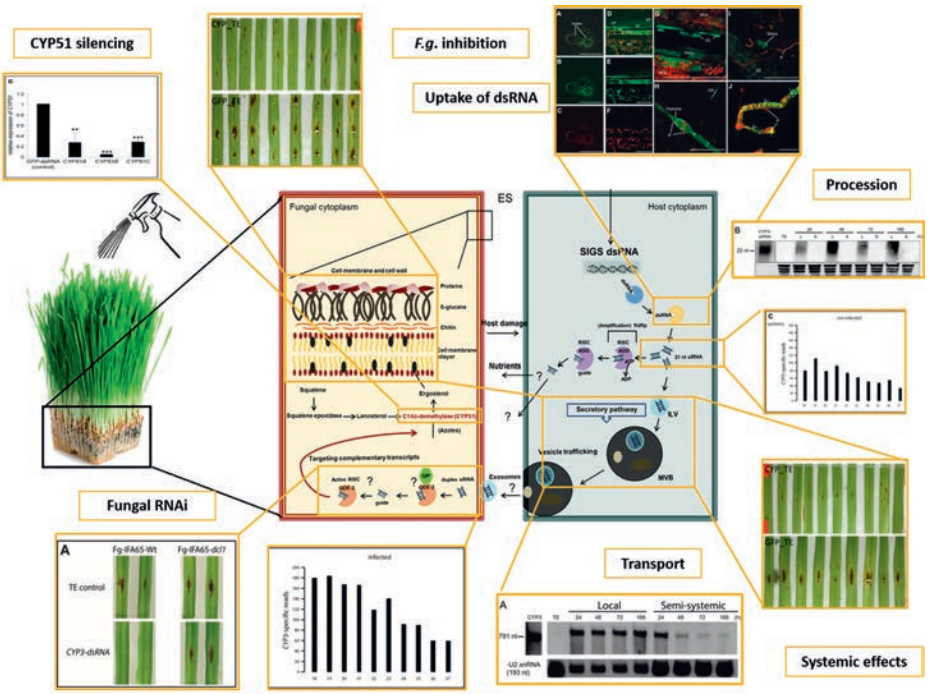

Figure 2 Summary of the SIGS studies (Koch et al. 2016). We showed that the 791 nt long dsRNA is taken up by the plant (upper right) and transferred via the vascular system to fungal infection sites (bottom right) where it is processed by the fungal RNAi machinery (bottom left) as a prerequisite for its antifungal activity (upper right). We showed a strong correlation between accumulation of *CYP3*-dsRNA at infection sites (bottom), silencing of CYP51 expression (upper left), and fungal inhibition (upper left/bottom right).

CONCLUSION

Given the ease of design, high specificity, and applicability to diverse pathogens, the use of target-specific dsRNA as an anti-fungal agent offers unprecedented potential as a new plant protection strategy.

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