ESSENTIAL OIL VAPOR TRIGGERS RESISTANCE PATHWAYS IN VITIS VINIFERA AND BLOCKS PLASMOPORA VITICOLA INFECTION

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Abstract:
The amount of synthetic pesticides applied in viticulture is relatively high compared to other agricultural crops, due to the high sensitivity of grapevine to diseases such as downy mildew (Plasmopora viticola). Alternatives to reduce fungicides are utterly needed to promote a sustainable vineyard-ecosystems and meet consumer acceptance.
Essential oils (EOs) are amongst the most promising natural plant protection agents and have shown their antifungal properties previously. However, the efficiency of EOs depends highly on timing and application technique. Additionally, the molecular interactions of host, pathogen and EO, which underlie the efficiency of EOs, are not understood. The presented study aimed to a) evaluate whether a continuous fumigation of EO can control downy mildew and b) decipher molecular mechanisms triggered in host and pathogen by EO. A custom made climatic chamber was constructed, which enabled a continuous fumigation of vines with different EOs during long term experiments.
Several experiments were carried out with vine cuttings infected with Plasmopora viticola and subsequently exposed to continuous fumigation of different EOs with different concentrations and application times (24 h to 10 d). Experiments were stopped when infection symptoms were clearly present on the control. Physiological parameters (photosynthesis, growth rate) were recorded and leaves were sampled at different time points for subsequent RNA extraction.
The post-infection oregano oil vapor treatment during 24h was sufficient to reduce downy mildew development to 95%. Leaf RNA sampled after 24 hours and 10 days of EO treatment was used for RNA-seq analysis. Sequenced reads were mapped onto the Vitis vinifera and Plasmopora viticola genomes. Less than 1% of reads could be mapped onto the Plasmopora genome from treated samples, whereas up to 30 % reads mapped from the controls, thereby confirming visual observation of P. viticola absence under treatment. An average of 80 % reads could be mapped onto the V. vinifera genome for differential expression analysis, which yielded 4800 modulated transcripts. Grapevine genes triggered by EO treatment were mainly linked to plant biotic stress response and plant-pathogen interactions. Key genes controlling ethylene synthesis, phenylpropanoids and flavonoid synthesis were also highly activated by EO. We report here for the first time the effects of EO treatments on the control of a grapevine pathogen, concomitantly with the molecular description of EO-host-pathogen interactions. These results strongly support the hypothesis that the antifungal efficiency of EO is indirect and mainly due to switching on resistance pathways of the host plants. These results are of major importance for the production and research on biopesticides, plant stimulation products as well as for resistance breeding strategies.
Keywords: Plant defense; essential oil; Plasmopara viticola; Grapevine

1. Introduction.

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Essential Oil vapor triggers resistance pathways in *Vitis vinifera* and blocks *Plasmopora viticola* infection

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Introduction

To promote a sustainable vineyard-ecosystems and meet consumer acceptance, alternatives to reduce synthetic fungicides are utterly needed. Amongst most potent natural plant protection products Essential oils (EOs), have shown their antifungal properties already on different agricultural crops. Their performance, is however highly depending on timing and application and molecular mechanisms, which underlie their efficiency are still to be elucidated. To circumvent drawbacks of direct EO application, our study aimed to evaluate whether a continuous fumigation of EO can control downy mildew of Grapevine (*Vitis vinifera*) and to decipher molecular mechanisms that are involved in EO efficiency against pathogens.

Material & Methods

A custom-made climatic chamber was constructed, which enabled a continuous fumigation of vines with different EOs (Fig. 1).

Experiments were carried with Chasselas cuttings infected with *Plasmopora viticola*, the causing agent of downy mildew, and subsequently exposed to continuous fumigation of different EOs during various application times (24 h to 10 d). Experiments were stopped, when first infection symptoms were clearly visible on control plants. Physiological parameters, such as photosynthesis and growth rate, were recorded. Leaves were sampled at different time points for subsequent RNA extraction, sequencing and differential gene expression analysis .

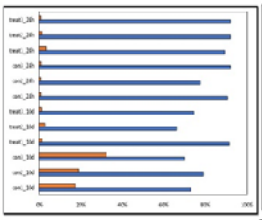

Figure 3: Mapped reads: Reads (%) mapped on *V. vinifera* genome (blue), reads (%) mapped on *P. viticola* genome (orange). **con**: Control; **treat**: EO vapor treatments post-infection for **24h** or **10days**

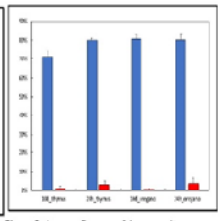

Figure 2: Average Downy mildew severity assessed after sporulation on 5 plants. Blue: non-treated control; Red: oregano treatments.

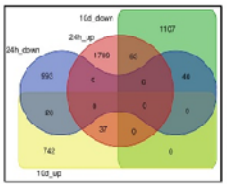

Figure 4 : Venn diagram of differentially expressed genes (DEGs, pval>0.01, fc<0.5) upon *O. vulgare* vapor treatment: 24h up and 24h_down: up - respectively downregulated genes after 24h treatment, 10d_up and 10d_down: up - respectively downregulated genes after 10 d treatment

Conclusion

We report here for the first time the effects of EO treatments on the control of a major grapevine pathogen, *P.viticola*, concomitantly with the molecular description of EO-host-pathogen interactions. Our results strongly support the hypothesis that the antifungal efficiency of EO is indirect and mainly due to switching on of resistance pathways of the host plants, that has never been shown previously. These results are of major importance for further research on plant innate immunity, the production and research on biopesticides, plant stimulation products as well as for resistance breeding strategies.

Acknowledgments

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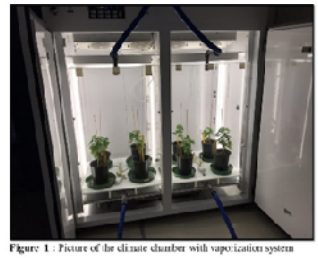

Figure 1 : Picture of the climate chamber with vaporization system

Results & Discussion

Remarkably, the post-infection EO vapor treatment during 24 h was sufficient to reduce fungi development to 95% (Fig 2). Reads from sequenced samples of 24 h and 10 d treatments were mapped onto both, *the V. vinifera* and *P.viticola* genomes (Fig 3). Fewer than 1% of reads could be mapped onto the *P.viticola* genome from treated samples, whereas up to 30 % reads mapped from the controls, which confirmed absence of *P.viticola* in treated plants. An average of 80 % reads could be mapped onto the V. vinifera genome for differential expression analysis, which yielded 4800 modulated transcripts.

Sequenced samples showed a good correlation for treatments and times (figure 5) and genes were allocated to different clusters according to their expression patterns (Fig 6)

Functional analysis of modulated genes indicates that EO vapor triggered innate plant immunity with major changes in genes linked to plant biotic stress response, plant-pathogen interactions as well as ethylene, jasmonic acid and salicylic acid signaling resulting in a upregulation of phenylpropanoid-, notably stilbene synthesis (Fig7), as well as other defense related pathways and resistance-genes.

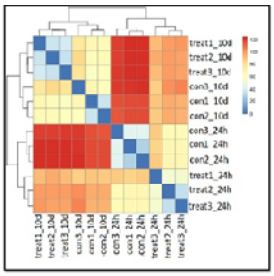

Figure 5: Correlation matrix calculated on normalized expressions; **con**: Control; **treat**: EO vapor treatments post-infection for **24h** or **10days**

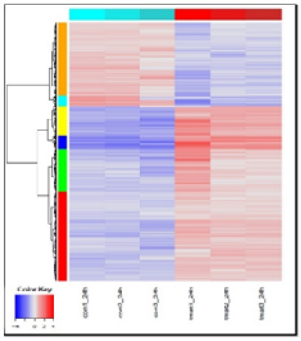

Figure 6: Hierarchical clustering of differentially expressed genes after 24 h treatment; **con**: Control; **treat**: EO vapor treatments post-infection for **24h** or **10days**

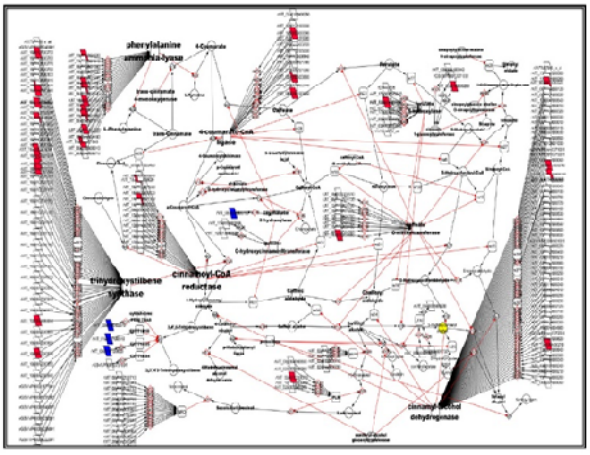

Fig. 7. Cytoscape graph of affected transcripts within the phenylpropanoid synthesis pathway. Blue: repressed, Red: induced transcripts by 24 h EO vapor treatment. Parallelograms: RNA, Round rectangles: Proteins; Ellipse: Simple molecules Diamonds: State transitions, transcription and translation