4.3. Influence of Transgenosis on the Plant-Insect-Relationships, in particular on Chemically Mediated Interactions

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Cross-Validations

The biological assessment of impacts of transgenic plants on non-target organisms is, in general, based on a simple comparison, e.g. between transgenic *Bt*-plants and non-transgenic plants lacking *Bt*. For the present investigation, we chose a novel biological system to assess the impact of a transgene on non-target organisms. A comparison was made between the original cultivar and two transgenic equivalents, with and without the target gene. For additional comparison, a cultivar was included in the analysis in which the target gene was introduced through classical breeding, but which has otherwise a different genetic background.

Healthy food, fresh food and choice

Society demands the supply of fresh and healthy foodstuff. To fulfill these demands, pest management has moved into the limelight. Currently, apple trees require more than a dozen treatments per year, even when cultivated in ideal conditions. These treatments are particularly important to control scab diseases, which are distributed worldwide. The wild apple (*Malus floribunda* 821) contains the *Vf* resistance, providing increased resistance against this fungal disease. This *Vf* resistance was transferred through transgenesis to the susceptible apple cultivar (*Malus* x *domestica*) 'Gala' ('Gala-trans*Vf*'). A second line 'Gala-trans0' used in this study – a vector control – contains promoter and selective marker (*nptII*) but not *Vf*. The two cultivars used in this study for comparison, were the scab susceptible cultivar 'Gala' and the scab resistant cultivar 'Florina', which obtained the *Vf* resistance gene through classical breeding.
To summarize, the following cultivars were included in the green house experiments, in order to be able to formulate well-founded conclusions.

Identifier	Gala	Gala-trans0	Gala-trans*Vf*	Florina
Properties	**Cultivar**	**transgene**	**transgene**	**Cultivar**
Genotype	Without gene for resistance	With CaMV 35S promoter, selection marker *nptII,* <u>without</u> gene for resistance "Vector control"	With CaMV 35S promoter, selection marker *nptII,* **with gene for resistance *HcrVf2***	**with Resistance *Vf***
Scab	Susceptible	Susceptible	Resistant	Resistant

Current Research Questions

To understand the impact of transgenic apple cultivars on the plant-insect-relationships, in particular on chemically mediated interactions, the following research questions were addressed:

1. How is the apple cultivar influencing the **development time** of the spotted tentiform leafminer (*Phyllonorycter blancardella*)? The leafminer develops as a caterpillar inside the apple leaf, which is visible on the leaf surface. It is a non-target organism for transgenic scab resistance. The obvious hypothesis would be that there is no difference in the development of the leafminer between scab resistant transgenic cultivar 'Gala-trans*Vf*' and the corresponding scab susceptible cultivar 'Gala'.
2. How is the apple cultivar influencing the content of the long-chained terpene **squalene on the leaf surface of plants** infested with the leafminer? This secondary plant metabolism product plays a central role for the natural leafminer predator, the ichneumon fly *Pholetesor bicolor*. This parasitoid locates the harmful leafminer hidden in the apple leaf by specific and critical concentrations of squalene on the leaf surface, as was demonstrated earlier with the apple cultivar 'Golden Delicious'. Since the parasitoid of scab resistant transgenic apple cultivars is a non-target organism, the obvious hypothesis is that no difference to the parasitoid will be observed between scab resistant transgenic cultivar 'Gala-trans*Vf*' and the corresponding scab susceptible cultivar 'Gala'.
3. How is the **apple cultivar affecting the emission of scents**? The scents released from healthy or leafminer-infected apple cultivars can influence the behavior of insects in the environment, and henceforth have a considerable impact on the state of health of the plant. Changes in the composition of scents can e.g. lead to the situation that detrimental insects are suddenly attracted to or that useful insects are diverted from apple cultivars, because these non-target organisms cannot decipher the chemical message in the scent mixture. The straightforward null-hypothesis would again suggest that there should be "no difference between scab resistant transgenic cultivar 'Gala-trans*Vf*' and the corresponding scab susceptible cultivar 'Gala'".

Comparative reports with different apple cultivars.

1. Development time of the spotted tentiform leafminer and number of hatched butterflies

The results of the biological experiments can be summarized as follows:
The genotype affects the differences in development time, which is subject to natural variability due to the phenotypic state of the plant.
1.1. In this project the leafminer developed faster in the scab resistant transgenic apple cultivar 'Gala-trans*Vf*' than in the corresponding cultivar 'Gala'. Faster development of the miners is a disadvantage for plant health because it provides an opportunity for more generations of detrimental insects to complete their life-cycle within one season increasing damage accordingly. Increased leaf miner activity can lead to premature leaf fall and yield losses, that can persist even in the following year. Compounding these problems, the window of opportunity to attack leafminers is reduced. Natural enemies, like the caterpillar parasitoid *Pholetesor bicolour,* have a shorter period during which to parasitize the leafminer resulting in greater damage. If one would restrict the analysis to this comparison (cultivars 'Gala' and 'Gala-trans*Vf*'), a slightly negative effect of the transgenic plants could be deduced.
1.2. The classically bred apple cultivar 'Florina', containing the same gene for resistance as the transgenic cultivar 'Gala-trans*Vf*' was included in the experimental analysis. The cultivar 'Florina' plays an important role in organic agriculture. The development time of the miner in the transgenic plant 'Gala-trans*Vf*' was comparable with the 'Florina' apple cultivar. This result suggests that the fungal resistance of the apple cultivar, mediated by the *HcrVf2* resistance gene and not the fact that the cultivar 'Gala-trans*Vf*' was transgenic, caused the differences measured. The non-desired effect on the leafminer could therefore not be attributed to the transgenic nature of the apple cultivar 'Gala-trans*Vf*'.
1.3. Often, it is suggested that the transgenic construct itself may change the quality of a cultivar. To answer this question, the transgenic cultivar 'Gala-trans0' containing only the marker and promoter (i.e. without gene for resistance), was included in the experiment for comparison. It is noteworthy that the development time of the miner in this cultivar did not differ from the development time of miners in the corresponding non-transgenic cultivar 'Gala', nor from the scab resistant transgenic cultivar 'Gala-trans*Vf*'. There was no undesired effect of the transgenic plant 'Gala-trans0'.
1.4. The number of hatched leafminer butterflies was not different across the cultivars of the 'Gala'-strains. However, the number of hatched butterflies with the cultivar 'Florina' was lower than in the cultivar 'Gala', indicating again the sensitivity of the chosen system.

2. Content of the terpene squalene on the leaf surface

The results of the chemical analyses can be summarized as follows.
2.1. Based on the published experiments with seedlings of the cultivar 'Golden Delicious', it was expected that extracts of leaves invaded by the leafminer would have a higher content of squalene than the ones of healthy leaves of the same cultivar. This was, however, not the case for 'Gala-trans*Vf*'; the content of squalene was in the same range. To be able to interpret this results correctly, additional experiments must be included.

2.2. The resistant cultivar ‘Florina’ showed a lower content of squalene in the healthy plants, similar to ‘Gala-trans*Vf*’. The content in the leafminer-infected plants was, however, considerably higher. This means that ‘Florina’ behaves like ‘Golden Delicious’.
2.3. The cultivar ‘Gala’ showed a lower content of squalene than was found in healthy plants. Interestingly, there was no visible difference in the content of squalene in healthy and leafminer infected plants. There was also no difference between the cultivars ‘Gala-trans0’ and ‘Gala-trans*Vf*’. This suggests that the plants in the ‘Gala’-line behave in the same way.

3. Emissions of Scents

First results of our chemical analyses indicate the following conclusions:
3.1. Different emissions among genotypes were measured for the terpenes (*E,E*)-α-farnesene, caryophyllene and ocimene. For the sesquiterpene (*E,E*)-α-farnesene an effect on approaching detrimental insects (apple coddling moth; *Laspeyresia pomonella*) has been documented. Depending on the concentration, it ranges from attraction to repulsion, indicating its function in influencing the insect’s behavior.
3.2. The differences of the scent emissions are significant among the cultivars ‘Gala’ and ‘Florina’.
3.3. Selective differences among the ‘Gala’-lines require further analyses. The results of ‘Gala-trans*Vf*’ and ‘Gala-trans0’ fall consistently between the values for both cultivars.

4. Conclusions

The selected system (including the methodology) allows the measurement of subtle changes among two apple cultivars. It is thus sufficiently sensitive and qualifies for the monitoring of undesired side effects. All values measured for the assayed transgenic plants fall in the range between the two cultivars.