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BUDAPEST

Fitness and pathogenicity of *Venturia inaequalis* isolates

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Summary

Apple scab, caused by the ascomycete fungus *V. inaequalis*, is an economically devastating disease of apples especially in production regions where moist conditions and cool temperatures prevail during the spring months. The most visible symptoms occur on leaves and fruits, when heavily infected they may drop prematurely. Currently, fungicide application is the main management method in order to control the spread of the apple scab pathogen and produce commercially acceptable fruits, with up to twenty sprayings every growing season. The intensive chemical control has become questionable due to the current regulations and the adverse effects reported, including negative impacts on the environment, workers and consumers health, and the emergence of populations resistant to systemic fungicides e.g., dodine and QoIs. Therefore, a long-term strategy should be applied to provide high-quality fresh products to future generations taking into consideration economic viability and ecological sustainability. The first step toward this strategy consists in using existing scab-resistant cultivars and breeding new ones, for which it is important to understand the interaction between *V. inaequalis* and its host. However, the complex system *V. inaequalis – Malus* is dynamic, is conditionate to evolutionary pressures, and has evolved through time. Thus, keeping on collecting new data about *V. inaequalis – Malus* makes it possible to produce more eco-friendly, robust, and resilient apple fruits on the long run.

The current research aimed to quantify different fitness parameters of four *V. inaequalis* races of an apple cultivar collection (Budapest, Hungary) and to better understand the relationship between these parameters by using traditional methods. The race isolates are originated from four scab race indicator cultivars, 'Gala' (no *R*-gene), 'Golden Delicious' *(Rvi1),* 'Geneva' (*Rvi3*, complex), and OR45t132 *(Rvi5),* thus representing the corresponding pathogen races. We evaluated parameters that play a major role in the pathogen's survival ability through time (fitness), including vegetative growth and reproductive success, and also the ability to infect different apple cultivars *in vitro* (pathogenicity).

The steps carried out at the laboratory included the morphological characterization of isolates, mycelium growth of *V. inaequalis* isolates under different lab conditions, cellophane method for conidia production, and pathogenicity testing by detached leaf assay - DLA. The cellophane experiment aimed to produce asexual conidia spores *in vitro* and evaluate its dynamics in 27 days in order to evaluate the reproduction success of the race isolates. On the other hand, DLA was carried out to evaluate *V. inaequalis* pathogenicity by observing microscope (structures from the interaction between fungus– apple) and macroscope (symptoms range) responses on apple leaves infected *in vitro.*

We observed that the most significant differences in terms of fitness attributes relied on 'Gala' and OR45t132. We found significative differences among groups of fungal monosporic isolates ('Gala', 'Golden Delicious', 'Geneva' cultivars, and OR45t132) in terms of color ($p<0.005$), density ($p<0.005$), and texture ($p<0.05$) of the main air mycelium. The outcome of mycelium growth evaluation under different conditions (T1 – 19ºC, T2 – 14ºC, and T3 – H_2O_2) showed that 'Gala' was larger than OR45t132 isolates in every case ($p < 0.001$), even when the stressor (T3)

was added. This outcome showed a higher vegetative growth capacity of 'Gala' isolates over the other groups under different temperature and stress conditions. This was in line with the outcome of cellophane method which showed that 'Gala' isolates reached the highest spore density yield followed by 'Geneva', and then OR45t132 ($p < 0.001$). Therefore, 'Gala' isolates showed high fitness attributes regarding reproduction success. Following the course of experiments, DLA conidial suspensions were used to evaluate the germination rate of the spores *in vitro.* Once again, 'Gala' had the highest rate of germination which is according with the curve of conidial production from isolates. On the other hand, OR45t132 isolates had the smallest mycelium growth and lowest spore density yield thus presenting weak fitness attributes regarding vegetative growth and reproduction success, respectively. Conidial suspension of OR45t132 had the lowest germination rate among groups, which supports its poor reproduction capacity compared to 'Gala'.

Furthermore, based on the signs and symptoms of scab on the apple leaves during the DLA, the four conidia suspensions represent three different pathogenicity profiles: the conidia from 'Gala' and 'Golden Delicious' belong to race (1); the conidia from 'Geneva' belongs to race (1, 3); and the conidia from OR45t132 to race (1, 5). All conidia suspensions caused infections on 'Gala' and 'Golden Delicious' leaves during the DLA, but their conidial suspensions were unable to infect the resistant cultivars ('Geneva' and OR45t132). On the contrary, the OR45t132 isolate (race 1, 5) and the 'Geneva' isolate (race 1, 3) could infect the leaves of susceptible 'Gala' and 'Golden Delicious' cultivars although not the other resistant cultivar.

The fitness attributes and pathogenicity of *V. inaequalis* isolates evaluated in this study are subject to constant change through time due to the dynamism of the *V. inaequalis – Malus* system given by environmental and genetic factors. Therefore, characterization of different races of *V. inaequalis*, differential host, and further studies of *V. inaequalis – Malus* system are required to be reviewed constantly to obtain updated information. In this way, the scientific community would be able to identify the races of *V. inaequalis* that could defeat an individual source of resistance, and then develop apple breeding programs to produce high quality fruits in a more ecofriendly way.