Cite as: Richter, S., Höfer, M., Bohr, A., Buchleither, S., Flachowsky, H., Wöhner, T. (2024). Differences in apple cultivar susceptibility to apple blotch disease. *Proceedings of the 21st International Conference on Organic Fruit-Growing*, Filderstadt 2024.02.19-21. Ed. FOEKO e.V. 2024: 58-61

Differences in apple cultivar susceptibility to apple blotch disease

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Abstract

Apple blotch is a disease caused by the fungus Diplocarpon coronariae. Typical symptoms are brown spots on leaves and fruits resulting in impaired fruit quality and fruits that cannot be marketed. A promising strategy to reduce the impact of this disease is the cultivation of more robust cultivars. There has been limited research about the susceptibility of apple cultivars to the disease. In this study, 154 apple cultivars from the German Fruit Genebank (GFG) collection were evaluated for their susceptibility to apple blotch disease in the greenhouse. Initial resistance screenings were carried out using detached leaf assays in petri dishes in the laboratory and revealed differences in susceptibility between apple cultivars. In particular, the GFG collection comprised cultivars that exhibited reduced symptom development compared to cultivars with heavy symptom expression. In this study, the cultivars were further tested in greenhouse trials to validate these findings and to monitor the time point of leaf drop. Variations in the time point of leaf drop were observed. These results provide valuable insight about the identification of apple cultivars with reduced apple blotch symptom development.

Keywords: *Diplocarpon*, greenhouse trial, apple blotch, early leaf drop

Introduction

Apple blotch disease, caused by the fungus *Diplocarpon coronariae*, occurs during cultivation of apple trees in meadow orchards and private gardens (Hinrichs-Berger & Müller, 2013). First detected in Italy in 2001, this fungal pathogen has spread throughout Europe, including the more northern regions of Germany in the last decade (Hinrichs-Berger & Müller, 2013). The disease appears on leaves during warm and humid conditions, typically in mid-June, causing premature leaf drop and weakening overall tree health due to reduced photosynthesis (Yin et al., 2013; Sutton et al., 2014).

Robust cultivars such as 'Granny Smith' and 'Hunter Melba', as well as selected Asian rootstocks, have been recently identified (Li et al. 2012, Noh et al., 2020). Most cultivars planted in Germany are susceptible or the status of susceptibility is unknown. The German Fruit Genebank (GFG) contains around 1,000 apple cultivars with historical significance or potential for breeding new varieties and could be a valuable resource for resistant apple cultivars. In a project, so far 535 apple varieties of the GFG collection were evaluated for their susceptibility in detached leaf assays and eight showed a reduced symptom development compared to 'Golden Delicious' used as susceptible control. They could be considered as robust (Richter et al., unpublished). In this study, a greenhouse test was performed with 154 apple cultivars of the GFG collection that are already tested in laboratory, to verify these results.

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Material and Methods

Plant material.

For the greenhouse trials, scions of 154 apple cultivars were grafted on the rootstock 'M.9' and grown in the greenhouse in 12 cm diameter pots at a diurnal temperature range of 20 °C during the day and 17 °C at night under natural light conditions. During the inoculation trials, the temperature was increased to 25 °C during the day and 20 °C at night. In addition, the plants were sprayed with water for 10 seconds every 15 minutes to maintain leaf wetness and high humidity. Two leaves from three plants were tested for susceptibility. The cultivar 'Golden Delicious' was used as a susceptible control.

Inoculum and inoculation.

The inoculum was rinsed from infected leaves from a 'Topaz' orchard at the Lake Constance Research Station for Fruit Cultivation (47°46'04.2"N 9°33'18.6"E). The frozen (stored at - 20 °C) leaves were transferred to a beaker and mixed with water to obtain a spore solution. This solution was filtered and diluted to a final concentration of $1*10^5$ conidia ml^{-1}. Tween20 (0.005%) was used to reduce the surface tension of water, which allows the solution to wet more effectively and spread more evenly across the leaf surface. Finally, the leaves were sprayed with the inoculum from the adaxial and abaxial sides, wrapped in a wet paper towel and placed in a plastic bag to allow spore ingrowth (Wöhner et al., 2019). After three days, the bag and the paper towel were removed and the plants were cultivated as described above.

Phenotypic characterisation of the disease.

To assess symptom development, the time of leaf drop was observed for each individual leaf (TP_Leafdrop). In addition, the inoculated leafs of the cultivars were phenotyped after 14 days post inoculation (dpi) according to the method of Wöhner et al. 2019 with a five-level scoring: 1 - healthy leaves; 2 - tiny spots on the leaves; 3 - spots and necrotic areas larger than 3 mm; 4 - advanced symptoms and chlorotic areas; and 5 - leaf drop.

Statistical analysis.

Statistical analysis of the data was carried out using Microsoft Excel (Version 2016) (mean values, Standard deviation) and past 4.0 (histogram). Outliers in the data were identified using the 1.5quartile method and the Spearman correlation between 14 dpi scoring and leaf drop time was calculated using Rstudio (version 1.4.1717).

Results

The plants in the greenhouse were observed between 7 and 41 dpi for the time of leaf drop. In this period, scoring was carried out at 14 dpi to evaluate specific symptom development on the leaves of each cultivar.

Leaf drop of the tested apple varieties (n=154) occurred after eleven and up to 41 dpi (Figure 1 (a)). On average, leaves dropped from the plants 21.62 ± (7.11) dpi for all varieties. The susceptible control 'Golden Delicious (GD)' had a mean value of 21.14 ± (9.07) dpi. A total of 59% of the varieties had an earlier mean leaf fall and 41% had a later mean leaf fall than 'GD' (see Figure 1 (b)). Of this 41 %, 19 cultivars were observed that showed a significant delay in leaf drop compared to ‘GD’. These cultivars dropped their leaves between an average of 29.67 ± (7.61) dpi and 41 ± (0.00) dpi. However, none of the eleven cultivars classified as robust after detached leaf assay showed significant differences in the time of leaf drop compared to the susceptible control.

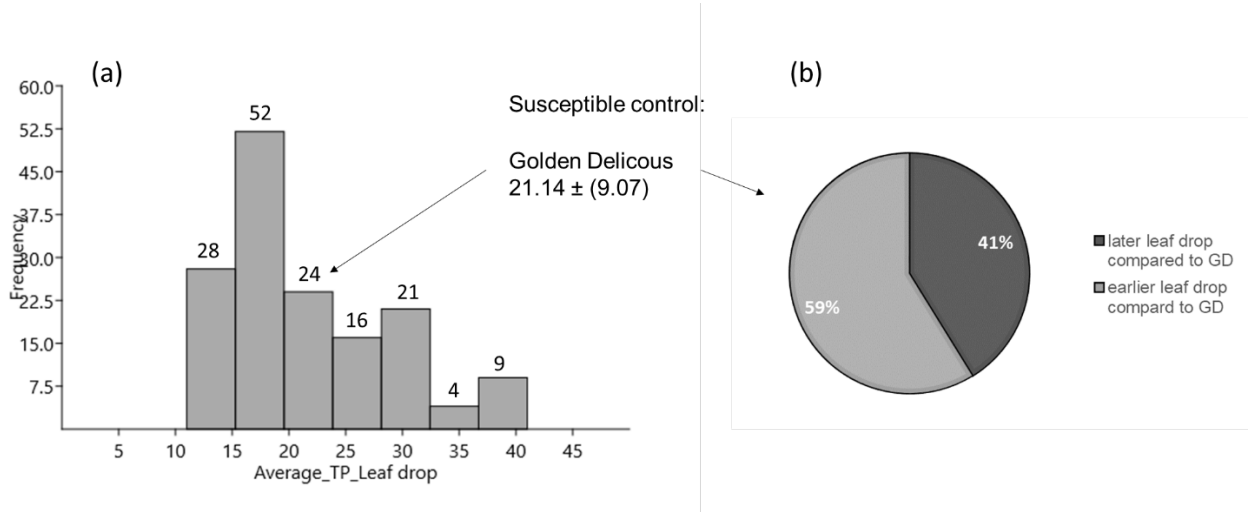

Figure 1: (a) Distribution of the time of leaf drop in Greenhouse of 154 cultivars. The count of the cultivars per class is shown over each bar. (b) Percentage of the cultivars with a later and earlier leaf drop compared to the susceptible control 'Golden Delicious (GD) ' (n=154).

To review if the leaf drop was due to the progressive infection with *Diplocarpon coronariae,* Figure 2 shows the negative correlation (-0.73) between the scoring 14 dpi in the green house and the time point of the leaf drop post inoculation. The leaves that fell from the tree early also showed strong symptom development at 14 dpi.

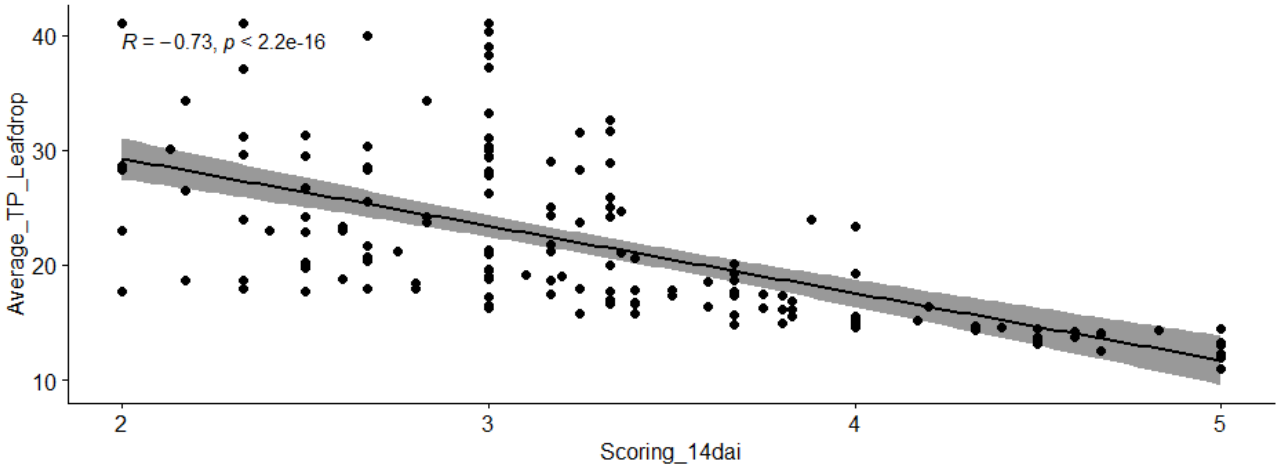

Figure 2: Correlation analysis between the scoring 14 days past inoculation and average time of leaf drop in greenhouse tests (spearman correlation with Rstudio) (n=154 cultivars).

Discussion

In this study, 154 apple cultivars were tested for the time of leaf drop in artificial greenhouse trials to verify the results of the detached leaf assay (Richter et al., (unpublished)). In addition, the symptom development of these cultivars was assessed after 14 dpi to assess susceptibility to *Diplocarpon coronariae*. Cultivars with a significantly later leaf drop than the susceptible control 'Golden Delicious' (Li *et al.*, 2012) were identified in these trials. Surprisingly, none of the cultivars classified as robust in the detached leaf assays (Richter et al., (unpublished)) showed significant differences in the greenhouse trails to the control genotype 'Golden Delicious'. This result confirms that there is no resistant cultivar to *D. coronariae*, as all cultivars tested developed symptoms of the disease.

We also showed, that the time of leaf drop is correlated with the development of the disease symptoms at 14 dpi. Cultivars with a later symptom development, also showed a later leaf drop due to the disease. However, the variation in the time of leaf drop is also large in some cultivars for example 'Golden Delicious', so the next trials will be carried out with more replicates.

Acknowledgements

We gratefully acknowledge the ’Deutsche Bundesstiftung Umwelt’ for funding the project (file number 20021/716). Furthermore, we acknowledge the Deutsche Genbank Obst (www.deutsche-genbank-obst.de) for providing plant material.

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