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Evaluation of the resistance of apple cultivars to *Diplocarpon coronariae* for the cultivation in meadow orchards

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Abstract

Meadow orchards are an important cultural heritage in Germany and provide biotopes for numerous animal and plant species. They are also valuable resources for maintaining the diversity of local and regional fruit varieties. A major issue for this form of production and utilization is the supply of adequate plant material that is not only adapted to local environmental conditions, but also robust against diseases and pests. In Germany, Apple blotch caused by the pathogen Diplocarpon coronariae ((Ellis and Davis) Wöhner and Rossmann) is increasingly spreading in meadow orchards. It is unknown which apple cultivars are robust against the pathogen. Therefore, it is necessary to evaluate the susceptibility of varieties to the disease in order to prevent the spread of the pathogen and supply robust cultivars for future cultivation. About a thousand old and historical cultivars are maintained in the German Fruit Genebank and it is unknown whether they are susceptible to apple blotch disease. For this purpose, artificial tests were performed to characterize symptoms of the disease on a first set of cultivars. Results show that differences in the susceptibility of apple cultivars exist and that there are cultivars exhibiting lower symptom expression of the disease.

Introduction

Meadow orchards are an important natural and cultural heritage in Germany. The number of sites is declining significantly (Kilian *et al.* 2020) due to insufficient maintenance and overaging of trees, especially in combination with insect calamities or weather extremes, such as summer droughts. Affected trees possess a predisposition for the spread of pathogens like *Diplocarpon coronariae*. The fungus causes apple blotch disease and has widely spread, especially in southern Germany (Hinrichs-Berger & Müller, 2013). Humid and warm conditions (20°C and 25°C) often promote the multiplication of apple blotch on the leaves of its host (Sharma *et al.,* 2004). Symptoms include the formation of black leaf spots, chlorotic areas, necrosis and result in premature leaf fall before fruit harvest (Sutton et al., 2014). Long-term infestation in combination with other impacts reduce the plant vigor (Yin *et al*. 2013) with effects on yields as described by Gravalon 2020 for cider apple crops. The cultivation of robust varieties is an alternative for sustainable control of the fungus. However, varieties used in conventional cultivation are often susceptible to the pathogen, such as the scab-resistant variety 'Topaz' (Vorley *et al*., 2014). Only a few Asian cultivars are described as resistant (Sharma *et al.* 2011, Li *et al.* 2012) and apple wild species also show high resistance to *D. coronariae* (Wöhner *et al*. 2020). However, there are no comprehensive studies on the resistance of old and historical apple cultivars adapted to local conditions and suitable for cultivation in meadow orchards. Varieties with a socio-cultural, local or historical relation to Germany as well as varieties with important orchard characteristics are preserved in the German Fruit Genebank (DGO, https://www.deutsche-genbank-obst.de/)

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(Flachowsky & Höfer 2010). In this study, we inoculated 144 cultivars of the apple cultivar collection held at the Julius Kühn-Institut (JKI), Institute for Breeding Research on Fruit Crops in Dresden-Pillnitz to proof their resistance towards the pathogen. The results indicate that robust cultivars may be useful for further utilization as genetic resources, important for cultivation in low fungicide treatment, such as meadow orchards or small gardens.

Material and Methods

Plant material. For the experiments, scions of 144 apple cultivars were grafted onto the rootstock 'M.111' and cultivated in the greenhouse in pots with a diameter of 12 cm at a diurnal temperature range between 25°C by day and 20°C at night under natural light conditions. Four leaves from three plants of each cultivar were collected from the middle of the plant shoot and washed with tap water to remove external contaminants. For inoculation, leaves were immediately transferred into plastic petri dishes which contained a layer of filter paper, a layer of paper tissue, and a metal grid on top. The chamber was moistened with 10 ml of tap water.

Inoculum and inoculation. The inoculum was washed from the surface of infested frozen stored leaves (-20°C) with typical symptoms of apple blotch disease harvested in the experimental field at JKI Dresden-Pillnitz. Frozen leaves were transferred to a 5 l beaker and moistened with 0.5 l tap water. After mixing the leaves and dissolving the spores into the suspension, the solution was filtered and finally concentrated to $1*10^5$ conidia ml^{-1} with a hemocytometer (Thoma). Tween20 (0.005%) was added to ensure improved wetting of the leaves. Inoculation was performed according to a method of Wöhner *et al*. (2019). Leaves in petri dishes were sprayed on the abaxial and adaxial site using a laboratory spray bottle. After 24 h, the remaining spore solution was again applied to the adaxial site. Petri dishes were packed in plastic bags and incubated at 24 °C, with diffuse light each for 13 days. Leaves kept humid during inoculation and where sprayed with tap water when the top of the leaf dried out.

Phenotypic characterization of disease expression. To evaluate the susceptibility of apple cultivars, phenotyping of inoculated leaves was performed by counting the number of acervuli grown after 13 days. In addition, quantification of infested leaf area was measured using the phenotyping software "Leaf Doctor" (Pethybridge & Nelson 2015).

Statistical analysis. Statistical analysis of the results was performed using the Mann-Whitney U test. An online calculator was used for this purpose (https://www.socscistatistics.com/tests/mannwhitney/).

Results

First symptoms appeared 7 days post-inoculation (dpi) as small spots on the leaf surface of several cultivars, for example the cultivar that served as a positive control, 'Golden Delicious'. The total number of acervuli formed on the leaf surface and infested leaf area were evaluated 13 dpi, since earlier experiments showed no further disease progress after that time point. A mean number of 32.4 (±2.71) acervuli was counted on all cultivars. The mean percentage of infested leaf area obtained was 9.7% (±0.75). Ten varieties with the least symptom expression and ten varieties with the highest symptom expression per trait are shown in Table 1. The smallest mean number was obtained for the apple cultivar 'Knäckerla 0.5 (±0.29) whereas 'Pidi' was assessed as the most susceptible cultivar with a mean number of 190.75 (±88.41) acervuli per leaf. The lowest mean percentage of infested leaf area was exhibited by the cultivar 'Elise Rathke' with 0.18 % (±0.11). The cultivar 'Aneta' showed the largest mean percentage infested leaf area of 63.9% (±17.79). It is visible that the varieties with the lowest number of acervuli and the varieties with the smallest infested area differed significantly ($p<0.05$) in this traits. However, some cultivars showed low values for both traits, such as the cultivars 'Martha', 'Schmalzprinz' and 'Elise Rathke'.

Table 1: Results of the inoculation of selected cultivars with *D. coronariae.* The traits 'Number of acervuli per leaf' and 'Leaf necrosis in %' were studied. The ten out of 144 cultivars with the lowest symptom expression and the ten cultivars with the highest symptom expression are shown below with the average number of acervuli per leaf (n=4) and the standard error.

Number of acervuli	**average**	**Standard error**	**Leaf necrosis / %**	**average**	**Standard error**
all cultivars	***32,4***	***2,71***	***all cultivars***	***9,7***	***0,75***
Cultivar			**Cultivar**		
Knäckerla	0,5	0,29	Elise Rathke	0,18	0,11
Weißer Astrachan	1,25	0,29	Reinette Evagil	1,01	0,64
Schöner aus Berwangen	1,25	0,48	Schmalzprinz	1,15	0,42
Martha	1,5	0,48	Akerö	1,23	0,32
Gustavs Dauerapfel	1,5	0,48	Alkmene	1,37	0,10
Schmalzprinz	1,75	0,63	Finkenwerder Herbstprinz	1,42	0,65
Wohlschmecker aus Vierlanden	1,75	0,63	Leupoldsdorfer Süßapfel	1,74	0,35
Zuccalmaglios Renette	2,25	0,65	Martha	1,76	0,47
Elise Rathke	2,5	0,75	Uphuser Tietjenapfel	1,78	0,31
Salome	2,5	0,87	Adams Parmäne	1,80	0,65
Pfirsichroter Sommerapfel	90,5	17,66	Iduna	24,82	18,13
Northern Spy	100,25	21,63	Golden Delicious	25,15	9,34
Vaterapfel ohne Kern	101,5	22,28	Antonovka	27,14	14,82
Reka	101,75	28,05	Sulinger Grünling	27,91	6,64
Golden Resistant	107	34,38	Vaterapfel ohne Kern	28,47	6,79
Iduna	107	34,70	Kaschaker	30,03	12,20
Golden Delicious	118,75	35,52	Sossenheimer Roter	35,40	3,93
Kaschaker	148,5	39,77	Pidi	37,32	14,03
Aneta	172,25	50,92	Rosenapfel von Schönbuch	40,29	8,07
Pidi	190,75	88,41	Aneta	63,87	17,79

Discussion

In this study, 144 apple varieties were tested for resistance towards apple blotch with artificial tests. Some cultivars were detected which can be classified as low susceptible, e.g. 'Martha', 'Schmalzprinz' and 'Elise Rathke', due to a low number of observed acervuli and percentage of affected leaf area. None of the evaluated varieties formed no acervuli, which was previously observed in wild species (Wöhner *et al.* 2020). Some varieties of the DGO collection also developed a high mean number of acervuli and mean percentage of affected leaf area (e.g. 'Pidi' and 'Aneta') as well as the cultivar 'Golden Delicious', described in the literature as highly susceptible (Li *et al.* 2012). However, these results of this study first have to be confirmed by a second test, which is planned in the current project. Moreover, traits like the time point of symptom occurrence or time point of leaf fall may also be favourable to distinguish between levels of low to high susceptibility. In general the results contribute to the utilization of apple genetic resources as valuable planting material in meadow orchards and resistance donors in breeding.

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