Evaluation of apple cultivars for their resistance to premature leaf fall (*Marssonina coronaria*)

T. Wöhner[1], S. Radatz[1] and M.V. Hanke[1]

Abstract

Apple blotch, caused by Marssonina coronaria *(Ellis & Davis) Davis, is a fungal pathogen and since its recent description in Italy in 2003, the disease spread to northern Europe during the last decade. Until now, the pathogen plays an increasing role in integrated and organic apple production. The disease causes a premature defoliation before fruit harvest resulting in reduced tree vigor and decreasing yield. In this study, we developed a method for artificial inoculation of apple leaves to evaluate* Malus *genetic resources against premature leaf fall. No cultivar was found without typical symptoms but significant differences between apple cultivars in symptom development were observed.*

Keywords: *Marssonina coronaria,* apple*,* artificial inoculation, genetic resources

Introduction

Marssonina coronaria (teleomorph *Diplocarpon mali*) is a fungal disease on apple and occurs in Germany since 2010 (Hinrichs-Berger, 2013). The disease appears in early summer after rain periods with high humidity and temperatures between 20 °C and 22 °C (Sharma *et al*., 2004). Typical symptoms are tiny round spots coloured greyish brown with a purple periphery. Small black acervuli develop in these spots and the infected leaf tissue turns to chlorotic yellow before harvest. Subsequently, the tree throws off the infected leaves (Sutton *et al*., 2014). As a consequence, the physiological balance of the tree is disturbed. A reduced deposition of assimilates and a deficit in flower primordia formation results in reduced yield and fruit quality. During the last five years, the importance of the disease rose in organic managed or meadow orchards in the apple growing area near Lake Constance, in Switzerland and South Tirol. Especially scab resistant apple cultivars like ‘Topaz’ showed a high susceptibility to *M. coronaria*. In India the disease affected 90 % of the apple producers of Himachal Pradesh within three years (Sharma *et al*., 2004). In China annual losses caused by *M. coronaria* were estimated up to 28 % (Shou *et al*., 2009; Zhao *et al*. 2011). Schrader & Steinmöller (2013) assume an increasing importance of the disease in Germany for the next years. Until now, the majority of cultivars dominating the market for direct consumption or cider production are known to be susceptible to the disease (Vorley *et al*., 2014). Asian apple varieties like ‘Qinguan’ as well as the European variety ‘Pinova’ were described as resistant to *M. coronaria* (Yin *et al*., 2013). Such genotypes are suitable as resistance donors for further breeding programs. To evaluate the susceptibility of different apple genotypes, a method based on the scab assessment described by Würdig *et al.* (2015) was performed using the susceptible genotype ‘Topaz’ in a glasshouse trial. Subsequently, forty different European apple cultivars were evaluated for their susceptibility to premature leaf fall.

Material and Methods

Scions of *M.* ×*domestica* cv. ‘Topaz’ (up to 20 shoots) were grafted on M9 rootstocks and cultivated as potted trees as described by Peil *et al.* (2007). First inoculation experiments were performed in the glasshouse based on the method of scab assessments described

[1] Institute for Breeding Research on Fruit Crops, Julius Kühn-Institut, Germany, 01326 Dresden, thomas.woehner@jki.bund.de, stine.radatz@gmx.net, viola.hanke@jki.bund.de

by Würdig *et al.* (2015), using dried *M. coronaria* infested leaves for inoculum preparation. The leaves were collected in the orchards of the experimental field of the Julius Kühn-Institut in Dresden. A conidial suspension of 3,4 × 10^5 spores ml^{-1} was prepared. Both sides of the youngest developed leaves (Shou *et al*., 2009) were spray inoculated and immediately covered with wet paper tissue and transparent polythene bags to assure high humidity conditions during incubation. After 72 hours, leaves were released and plants were kept in the glass house at 25 °C and irrigated every 45 minutes. The infection was evaluated up to 56 days post inoculation (dpi) by visually estimating the percentage of infested leaf area. Up to five leaves per genotype were evaluated.

To examine the reproducibility and variation of the inoculation procedure the experimental block design for the first and second trial was performed as follows: up to eight plants were inoculated in a block and three blocks were used in each trial (see Table 1). Ten plants were inoculated with water as negative control.

To test the influence of artificial inoculums, ten plants of *M.* ×*domestica* cv. 'Topaz' were inoculated with a conidial suspension of a single spore culture of *M. coronaria,* isolated from infested orchard leaves of the Julius Kühn-Institut in Dresden. The isolate was cultured on potato carrot dextrose agar as described by Zhao *et al*. (2010). The molecular characterization of the isolate was described in Wöhner (2015). Inoculation procedure was performed as outlined above. The conidial concentration of the inoculum was 3,7 × 10^6 spores ml^{-1}.

In parallel, forty apple cultivars were inoculated and tested for their susceptibility to *M. coronaria.* Three sets of apple genotypes (two sets including 15 genotypes and one set including 10 genotypes) were tested in a randomised block trial (no. 4). Each block consisted of one plant per genotype. *M. coronaria* infection was assessed up to 30 dpi.

Results were summarized by calculating the average percentage of infested leaf area (PILA). The total infected leaf area was divided to the total amount of assessed leaves per genotype. Duncan multiple range test (SAS 2010) was used to compare the PILA between different trials or apple genotypes.

Results

First symptoms of *M. coronaria* appeared at seven dpi as tiny red spots on the upper surface of inoculated 'Topaz' leaves. After 14 dpi, small black acervuli developed and chlorotic lesions started to expand. The first leaf fall was observed 28 dpi on the cultivar 'Topaz'. After 56 dpi no changes in symptom development were observed between the first and second trial and assessment was finished. No symptoms were observed on the lower leaf surface. The PILA of the cultivar 'Topaz' ranged from 30.7 % in the first and 28.7 % in the second trial (Table 1). No significant differences were found after Duncan test between the results of the first and the second trial, but significant differences were found between the PILA of all three blocks of trial two. The negative control remained healthy.

To examine the influence of artificial inoculum, a single spore culture was used for preparation of the conidial suspension. In this experiment (trial no. 3) the first leaf fall was observed after 18 days on leaves of the cultivar 'Topaz'. The PILA developed from 12.4 % (11 dpi) to 51.5 % (30 dpi). In trial no. 4, forty apple cultivars were tested for their susceptibility to *M. coronaria.* First leaf fall was observed 11 dpi on the cultivar 'Ariwa' (not shown). All tested cultivars were susceptible but no significant differences between the PILA of genotypes with the lowest infestation were determined using Duncan multiple range test (Table 1). These genotypes were significant different to the cultivar 'Reka', which showed the first leaf fall 18 dpi and showed a total PILA of 93.3 % respectively.

Table 1: Summarized results of artificial inoculation trials to assess the susceptibility of different apple genotypes to *M. coronaria.*

Trial no.	Genotypes of *M. ×domestic*a	No. of individuals	PILA (%)	Standard deviation	First observed leaf fall (dpi)	Duncan multiple range test[c]
1[a]	Topaz	20	30.7	26.2	28	A
2[a]	Topaz	20	28.7	29.1	36	A
	Topaz[d]	10	0.0	0.0	-	B
3[b]	Topaz	10	51.5	28.9	18	C
4[b]	Reka	3	93.3	16.3	18	D
	Topaz	3	43.3	29.5	18	C,E
	Ahra	3	26.7	37.1	25	C,E
	Astillisch	3	23.3	12.1	-	C,E
	Astramel	3	19.2	13.6	-	E
	Pinova	3	17.1	12.6	-	E
	Akane	3	16.7	8.2	-	E

PILA - percentage of average infested leaf area; [a] estimation of PILA was performed 56 dpi; [b] estimation of PILA was performed 30 dpi; [c] Genotypes with the same letter were not significantly different after Duncan multiple range test; [d] negative control inoculated with water.

Discussion

The scab resistant apple cultivar ‘Topaz’ is known to be highly susceptible to *M. coronaria,* the causative agent of premature leaf fall (Vorley *et al*., 2014). In this study we used this genotype in trials to establish an artificial glasshouse inoculation method for the evaluation of apple genetic resources. Using a conidial suspension prepared from infected leaves, first symptoms appeared at seven dpi. Yin *et al*. (2013) observed first symptoms on susceptible genotypes after three dpi under in vitro conditions. In our study the development of yellow chlorotic lesions started after 14 dpi, however Lee *et al*. (2011) described initial yellowing 21 dpi and observed natural occurrence of *M. coronaria* symptoms after 45 days of inoculation. In our study 56 dpi the assessment was finished and symptoms were fully developed. No significant differences were determined after Duncan test between the first and second trial, but within the second trial, significant differences between two blocks were detected. Consequently, inoculation experiments for resistance screenings should be performed in randomized block trials with several replicates per genotype to compensate variations resulting from differences in the environmental conditions. Using a higher inoculum concentration a faster infection progress and leaf fall was observed 10 days earlier in comparison to the first and second trial. Vorley *et al.* (2014) also described a successful inoculation with a suspension prepared from a single spore isolate and leaf yellowing of ‘Topaz’ 21 dpi. Our results confirm the application of cultured inoculums. Forty apple cultivars were tested in a randomized block trial in parallel. No cultivar without symptoms was observed. The most robust cultivars developed an averaged infected leaf area between 16.7 % and 19.2 %. Yin *et al.* (2013) determined ‘Pinova’ as resistant although typical symptoms were described after inoculation. Our results confirm a higher robustness of the apple cultivars ‘Akane’, ‘Pinova’ and ‘Astramel’ but more apple genetic resources have to be screened to find resistant genotypes without symptoms which can be used as donors for further breeding purposes.

Acknowledgements

We gratefully acknowledge Beatriz Lauria-Pluschkell and Prof. Hans Scheinpflug for sharing experiences and the good advices for culturing *Marssonina coronaria.* We are also grateful to S. Bartsch for technical assistance.

References

Hinrichs-Berger, J. & Müller, G. (2013). Zum Auftreten von Marssonina coronaria an Apfel in Baden-Württemberg. *Journal für Kulturpflanzen* **65**: 347-350.

Lee, D.H., Back, C.G., Win, N.K., Choi, K.H., Kim, K.M., Kang, I.K., Choi, C., Yoon, T.M., Uhm, J.Y. & Jung, H.Y.(2011). Biological characterization of *Marssonina coronaria* associated with apple blotch disease. *Mycobiology* **39** (3): 200-205.

Peil, A., Garcia-Libreros, T., Richter, K., Trognitz, F., Trognitz, B., Hanke, M.-V. & Flachowsky, H. (2007). Strong evidence for a fire blight resistance gene of *Malus robusta* located on linkage group 3 detected by rapid genome scanning. *Plant Breed* **126:** 470-476.

SAS (2010). Administering SAS® Enterprise Guide®4.3.SAS Institue Inc. Cary, NC.

Schrader, G. & Steinmöller, S. (2013). Express-PRA zu *Diplocarpon mali* Y. Harada & Sawamura. Online:http://pflanzengesundheit.jki.bund.de/dokumente/upload/7687c_marssonina_coronaria_express-pra.pdf.

Sharma, J.N., Sharma, A. & Sharma, P. (2004). Outbreak of *Marssonina* blotch in warmer climates causing premature leaf fall problem of apple and its management. *Acta Horticulturae* **662**: 405-409.

Shou, Y.Y., Li, C.M., Zhao, Y.B., Chen, D.M. & Zhang, X.Z. (2009). *In vitro* evaluation of resistance to *Marssonina mali* in apple. *Journal Fruit Scietificia* **26**: 912-914.

Sutton, T.B., Aldwinckle, H.S., Agnello, A.M. & Walgenbach, J.F. (2014). Compendium of apple and pear diseases and pests, Second Edition. *APS Press* **1**: 48-49.

Vorley, T., Oberhänsli, T., Tamm, L. & Schärer, H.J. (2014). Testing susceptibility of apple cultivars against Marssonina coronaria. Ecofruit-short contributions 1: 191-194.

Wöhner, T. (2015). *Marssonina coronaria* (Ellis & Davis) Davis (teleomorph *Diplocarpon mali* Harada Y. Harada & Sawamura 1974) Erreger des frühzeitigen Blattfalls an Apfel. *JKI Datenblätter Pflanzenkrankheiten und Diagnose* **2**: 1-16.

Würdig, J., Flachowsky, H., Saß, A., Peil, A. & Hanke, M.-V. (2015). Improving resistance of different apple cultivars using the *Rvi6* scab resistance gene in a cisgenic approach based on the Flp/*FRT* recombinase system. *Mol Breeding* **35** (95): 1-18.

Yin, L., Li, M., Ke, X., Li, C., Zou, Y., Liang, D. & Ma, F. (2013). Evaluation of *Malus* germplasm resistance to *Marssonina* apple blotch. *European Journal of Plant Pathology* **136**: 597-602.

Zhao, L., Zhao, Y., Wan, Y. & Dang, Z. (2011). A RAPD marker linked to the resistance gene to Alternaria leaf blotch (*Alternaria mali* Roberts) in 'Qinguan' apple. *Acta Horticulturae* **903:** 101-106.