Testing susceptibility of apple cultivars against *Marssonina coronaria*

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Abstract

The apple pathogen Marssonina coronaria *(Ellis and Davis, 1914) (teleomorph* Diplocarpon mali *[Harada, Y. and Sawamura K. 1974]) has recently become a significant problem in Central European organic apple production, causing dark spots on both the leaves and fruit, and early leaf fall. In order to support selection of cultivars for fruit and cider production and to optimise plant protection strategies on these cultivars it is important to know their susceptibility to* M. coronaria*. In this study 16 commercially important or newly developed apple cultivars were screened for susceptibility to* M. coronaria*. 1 year old saplings were inoculated using conidia grown in liquid medium and kept at 23°C and under high humidity. The severity of any resulting infection was scored 3 times using a simple scale adapted to visual symptoms. Several cultivars showed good initial resistance to the disease, including Gala, Ariane and Elstar. However, at the last scoring, 21 days after inoculation, Gala had become one of the most severely infected cultivars, suggesting that resistance to the disease is complex, and involves a number of mechanisms.*

Keywords: *Marssonina coronaria*, Apple, Cultivars, Resistance

Introduction

The apple pathogen *Marssonina coronaria* (Ellis and Davis, 1914) (teleomorph *Diplocarpon mali* [Harada, Y. and Sawamura K. 1974]) has recently become a significant problem in Central European organic apple production (Lindner 2012). The pathogen causes dark spots on both the leaves and fruit, and early leaf fall, which may weaken the trees. Observations in the field indicate that most important cultivars for fruit or cider production are susceptible to the disease, but there is some variation in the expression of symptoms and there seem to be different levels of susceptibility or tolerance. In order to support selection of cultivars for fruit and cider production and to optimise plant protection strategies on these cultivars it is important to know their susceptibility to *M. coronaria*. In this study 16 commercially important or newly developed apple cultivars were screened for susceptibility to *M. coronaria* under controlled conditions. 1 year old saplings were inoculated using conidia grown in liquid medium and kept at 23°C and under high humidity. The severity of any resulting infection was scored 3 times using a simple scale adapted to visual symptoms.

Material and Methods

Apple plants used: One year old saplings of 16 cultivars (listed in table 2, 5 plants per cultivar) grafted onto M9-T337 rootstock.

Spore production and inoculation: *Diplocarpon mali* strain no. 30405 from NITE Biological Resource (NBRC) Center was propagated in potato carrot dextrose broth (PCDB) (Zhao *et al*. 2010), with 2g peptone added per litre of media. Cultures were kept on a mechanical shaker at 150 RPM under standard laboratory conditions for approx. 30 days or until the spore concentration reached 10^6 conidia per ml before being used as inoculum. The plants were inoculated twice, the first time on August 25th, and the second time on September

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20th. For the 1st inoculation 150ml of inoculum per wagon was sprayed on both surfaces of the leaves, for the 2nd inoculation 200ml of inoculum was used. For inoculation the 16 cultivars were placed together on one wagon, resulting in five wagons (replicates) with 16 plants each. Once inoculated, the wagons were wrapped in plastic wrap to maintain high levels of humidity and to encourage successful infection. After 72 hours of incubation the plastic wrap was removed and each replicate of 16 plants was placed on two wagons within the climate chamber with consistent conditions of 16 hours of light, 23°C daytime temperature, and between 40 % and 80 % relative humidity.

The plants were scored 11, 16 and 21 days after the second inoculation, using 4 rank scales based on visual symptoms such as necrosis, yellowing of the leaves, and leaf fall (Tab. 1). A similar scale was used by Yin *et al*. in 2013, though with less importance placed on just the presence of lesions, as we found in preliminary work that their presence does not always correspond to a successful infection by *M. coronaria*.

For data analysis a disease severity index (DSI) was calculated for each of the eight assessments according to the equation of Townsend and Heuberger (1943). A mean index per day of assessment was calculated and these three values were used for statistical analyses and for final classification of the cultivars (Tab. 2 and Fig. 1). Arc-sin transformed data were analysed with SPSS 21.0 using a general linear model and Duncan's Multiple Range test at P=0.05.

Table 1: Rank scales used for disease assessments of *M. coronaria* on apple cultivars

	Oct. 2nd		Oct. 6th		Oct. 11th			
Score	Necrosis	Leaf symptoms	Leaf symptoms	Leaf drop	Necrosis (density)	Necrosis (size)	Leaf drop and yellowing	Proportion green /yellow
0	No	No	No	No	No	No	<10 % of leaves are yellow / dropped	10 %
1	Few	Few	Few	Few	Low	Small spots	10 % - 50 % yellow / dropped	10 % - 50 %
2	Several	Many	Inter-mediate	Inter-mediate	Medium	Medium sized spots	50 % - 70 % yellow / dropped	50 %- 75 %
3	Many	Many and leaf drop	Many	Strong	High	Large sized spots	>70 % yellow / dropped	>75 %

Results

There are clear differences in susceptibility between the cultivars (Table 2, Fig. 1) but none of the tested cultivars showed a high level of resistance. Several cultivars showed good initial resistance to the disease, including Gala, Ariane and Elstar. Across the first two scorings, Gala was by far the most tolerant cultivar, with a mean DSI of 6.67. However, by the time of the third scoring, the severity of the disease had increased greatly, to a mean DSI of 68.33, on par with a susceptible cultivar, Galaxy, a variant of Gala. Galaxy had a more consistent DSI across the scorings, shown by a standard deviation of 36.21 for Gala, and 11.31 for Galaxy.

The range of DSIs was divided into susceptibility groups, based on Duncan's multiple range tests, to give a tolerance rating. Based on this data analysis only the cultivar Gala may be classified as moderately tolerant to the disease, though still showing symptoms. Elstar, Ariane, and Schneiderapfel as well as Pinova, Glockenapfel, Rewena, Gravensteiner and Otava were rated as intermediately susceptible. These cultivars presented strong symptoms across the scorings, with a disease index of between 30 and 50. Seven cultivars showed symptoms severe enough to be classed as susceptible, with a DSI of >50. These were Natyra, Galaxy, 11907, Topaz, Galiwa, Braeburn and Boskoop. These results confirm some observations in the field where it was noted that a tree of cultivar Schneiderapfel was more diseased than an adjacent tree of cv. Boskoop.

Table 2: Disease severity indices (DSI) for all individual scorings and all cultivars. DSI were calculated with the formula of Townsend and Heuberger (1943) to transform 0-4 scale values into percentage values. A mean per day of assessment was calculated and from these three values the "Mean index" was calculated. The susceptibility classes are MT = moderately tolerant, I = intermediate and S = susceptible.

	October 2nd 2013		October 6th 2013		October 11th 2013					
	Necroses	Leaf symptoms	Leaf symptoms	Leaf drop	Necroses (density)	Necroses (size)	Leaf fall and yellowing	Proportion of green leaves w/ necroses	Mean Index	Suceptibility class
Gala	0.0	0.0	20.0	6.7	80.0	86.7	33.3	73.3	27.2	MT
Elstar	20.0	13.3	40.0	26.7	66.7	53.3	40.0	46.7	33.9	I
Ariane	20.0	6.7	33.3	33.3	60.0	80.0	33.3	53.3	34.4	I
Schneiderapfel	40.0	13.3	26.7	26.7	53.3	33.3	66.7	60.0	35.6	I
Gravensteiner	20.0	26.7	60.0	26.7	46.7	73.3	33.3	33.3	37.8	I
Pinova	20.0	60.0	33.3	53.3	53.3	33.3	40.0	26.7	40.6	I
Glockenapfel	53.3	20.0	46.7	26.7	60.0	73.3	26.7	46.7	41.7	I
Rewena	46.7	33.3	60.0	46.7	60.0	40.0	46.7	53.3	47.8	I
Otava	20.0	33.3	73.3	46.7	40.0	80.0	73.3	60.0	50.0	I
Natyra	33.3	33.3	60.0	53.3	86.7	93.3	86.7	60.0	57.2	S
Galaxy	40.0	53.3	73.3	53.3	53.3	73.3	80.0	66.7	59.4	S
11907	60.0	66.7	73.3	53.3	40.0	80.0	60.0	53.3	61.7	S
Topaz	60.0	40.0	73.3	60.0	60.0	86.7	73.3	73.3	63.3	S
Galiwa	60.0	60.0	66.7	53.3	60.0	66.7	73.3	80.0	63.3	S
Braeburn	93.3	26.7	86.7	60.0	53.3	66.7	46.7	73.3	64.4	S
Boskoop	86.7	66.7	66.7	60.0	33.3	80.0	60.0	66.7	66.7	S

Discussion

Across the first two scorings, Gala was by far the most tolerant cultivar. However, the last scoring, 21 days after inoculation, Gala had become one of the most severely infected cultivars, suggesting that resistance to the disease is complex, and involves a number of mechanisms.

This could suggest that different cultivars differ in the modes of their resistance to the disease, with some showing a higher tolerance to a relatively low disease pressure, which is overcome when disease pressure becomes higher, for example when secondary infections start to occur after the primary infections have started to sporulate. Another possible explanation for the discrepancy is that the disease progression is slowed in some cultivars. It has been suggested that *M. coronaria* is hemibiotrophic (Zhao et al. 2013), initially living within the leaves as a biotroph without the death of any plant cells, before later becoming a necrotroph, penetrating and killing leaf cells at the site of infection. It may be that in Gala this progression from biotroph to necrotroph is delayed.

The results presented here are based on inoculation of cultivars with one (Japanese) strain of the pathogen. Although Lee e*t al*. (2011) have not found differences in pathogenicity of some selected isolates it may be that with different strains of marssonina there is a different result of resistance assessment as the strains may differ in aggressiveness or virulence. Yin *et al*. (2013) used a local strain isolated from a diseased apple leaf for testing resistance to marssonina apple blotch. In their overall evaluation cultivar Ariane was also classified as an intermediate type of resistance, as in our evaluation, but cultivar Pinova was classified as resistant while we found Pinova to have an intermediate type of resistance/tolerance. Another cause of discrepancy of results may be the method of inoculation and disease assessment. While Yin *et al*. (2013) worked with artificial inoculation on detached leaves *in vitro* and with inoculation of single leaves on potted plants *in vivo* we used a whole plant inoculation method. Different classification of a cultivar into a resistance/susceptibility group may be due to differences in physiological growth status of the experimental material in combination with the method of inoculation and the strain of the pathogen used.

In contrary to the results of Sharma *et al*. (2001) and Yin *et al*. (2013) no cultivar with good resistance to marssonina leaf blotch was found in the current work. As there are further discrepancies in resistance classification of cultivars more work is needed to clarify the resistance level of cultivars. The methodology of testing as well as the scales for scoring the disease need to be improved and standardised in order to allow better comparisons between different investigations. Additionally, more of the currently used European cultivars need to be screened for tolerance to this disease in order to find basic material for resistance breeding and to advise farmers on the choice of disease tolerant cultivars.

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