Cercospora spot on avocado – A preliminary report on the relook at the epidemiology of the pathogen

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ABSTRACT

From a quality point of view, Cercospora spot caused by the fungus *Pseudocercospora purpurea* is the most serious fungal disease on avocado. It can cause losses of up to 70% on unsprayed trees. A predictive model developed by Dr. J.M. Darvas in the early 1980s is currently used by the industry to determine when spores are released and when spraying should begin. The impact of climate change necessitated re-investigation of the existing model. The objective of this study was to determine if the equation developed by Dr. Darvas is still a valid model for forecasting spore release and the timing of the first spray for effective control. In the 2017/18 season, four spore traps per site were placed in an unsprayed 'Fuerte' orchard at both HL Hall and Sons and at the ARC-TSC (Nelspruit area). Slides were changed and examined weekly at both sites. At the ARC-TSC site, in addition to the weekly changed slides, slides were changed daily for a four-month period and changed hourly for a seven-day period. To determine the critical infection periods, a bagging trial was carried out at the Halls site. One thousand five hundred fruits were covered with paper bags when fruit were <40 mm in diameter. Every fortnight, 50 bags were removed to allow infection and then replaced at the end of the fortnight. The project is ongoing and fruit will be harvested in April/May 2018 and evaluated for Cercospora spot and the results expressed in terms of a disease index. The disease index data and spore trapping data will be correlated with weather data to develop a forecasting model.

INTRODUCTION

Cercospora spot of avocado, caused by the fungus *Pseudocercospora purpurea* (Cooke) Deighton (Darvas *et al*., 1987) is one of the most serious pre-harvest diseases of avocado in South Africa, causing losses of up to 70% in untreated orchards (Darvas and Kotze, 1987).

Symptoms occur on leaves, fruit, twigs and fruit stems at any time during the growing season. On leaves, symptoms first appear as small (1-5 mm), angular, purple to brown flecks or spots near the leaf margins. Lesions are first apparent on the abaxial surface of leaves, however, as the infection progresses, spots are evident on both leaf surfaces (Fig. 1). Older lesions are surrounded by yellow, chlorotic haloes. Sporophores with spores are produced on the abaxial surface under humid, wet conditions and are observed as grey, felty mycelial growths in lesion centres. Individual lesions often coalesce to form large, brown, irregular necrotic areas on the leaf.

On fruit, the first sign of infection is a darkening of the epidermis followed by swelling of the underlying tissues which raises a small dark spot. As the cells die and dry out, the spots become sunken and crack to form "scabby" spots (Fig. 2). Older sunken spots with cracks can create an entry point for secondary pathogens such as *Colletotrichum gloeosporioides,* the causal agent of anthracnose. Fully-developed spots are 3-6 mm in diameter, slightly irregular and often with white areas of sporulation in the center. In some cases, if the disease is stopped temporarily only minute, raised, shiny black spots are formed which are associated with the corking of lenticels. The disease is normally confined to the fruit rind, but the flesh may be invaded during advanced stages. Defoliation may occur and fruit can become chlorotic, shrivel and drop. Dark brown to black lesions (2-10 mm) may also develop on fruit stems and green twigs.

The fungus survives on old lesions and when the warm rainy period begins, spores are released which can infect new fruit and leaves. Spores are disseminated via wind, rain splash, insects, irrigation water and movement of contaminated orchard tools and equipment, to new infection sites. The movement of spore-infected fruit and plant material can cause the rapid spread of the disease over long distances to new areas (Darvas, 1982).

Figure 1: Small angular spots on the abaxial side of a 'Fuerte' avocado leaf.

Figure 2: A 'Fuerte' avocado fruit with Cercospora spot.

The South African avocado growers are currently making considerable use of the prediction models developed by Darvas (1982) for decision making in terms of when to commence spraying for Cercospora spot. The current models for the forecasting of the number of conidia in the atmosphere in a given area are:

- Z (number of conidia) = 24.8 (constant) – 0.93X (X is temperature in °C) + 0.25Y (Y is rainfall in mm) (Darvas, 1982; Darvas and Kotze, 1987)
- Z (number of conidia) = -58.99 (constant) + (3.22X (X is mean weekly temperature in °C) + 0.18Y (Y is the weekly rainfall in mm), spore release occurs when Z>0, and the potential for Cercospora infection is high when Z>20 (Darvas, 1982).

The latter equation is mostly used by the industry.

According to the Subtrop orchard management schedule for the 'Fuerte' cultivar, first copper sprays should commence when the fruit are >2.0 cm and or the Z value >15 and repeated every 4 weeks (Campbell, 2016).

The equation by Darvas (1982) was derived from only 10 data days from 27 January to 6 February in 1979, according to observations made and published in the 2009 SAAGA Yearbook by Dr. B.Q. Manicom. Two of these days account for most of the prediction efficiency. According to Manicom and Schoeman (2009), the equation is also only really valid for temperatures between 20 °C and 26 °C and on a weekly average data set over a 6-month period, no useful regressions could be derived. Uncertainty also exists regarding the growth stage at which the avocado becomes susceptible. It is commonly believed that fruit smaller than 40 mm in diameter are resistant to infection. No actual data is however available and this is probably based on work done by Darvas (1982). The only other report on this resistance is a report from 1922, but according to Manicom & Schoeman (2009) this report refers to resistance against anthracnose and not Cercospora spot. The critical periods for spray application also need to be re-investigated. Darvas (1982) did some bagging trials to determine infection periods and the trend identified was that fruits exposed in November had more Cercospora spot than those exposed later in the season.

Considering all the above, and with the impact of climate change, it became necessary to re-investigate the existing models. New models need to be developed which possibly include more weather parameters such as humidity and leaf wetness.

According to Darvas (1982), relative humidity plays an important role in spore release and most conidia in his study were caught in the early mornings when high humidity occurred. Further it was reported that the timing of the first application is very important and that the timing of follow-up sprays is of little importance, because the time period favourable for spore production is too long. The latent phase between infection and symptom development appears to be three months.

The objective of our study was to determine if the equation developed by Darvas (1982) is still a valid model for forecasting spore release and the timing of the first spray for effective control of *P. purpurea.* The second objective was to investigate the inclusion of humidity and/or leaf wetness in the formula as a determining factor.

MATERIALS AND METHODS

Spore trapping

The trial was carried out in a 'Fuerte' orchard at both HL Hall and Sons and at the ARC-TSC (Nelspruit area). 'Fuerte' avocados were used in this trial, as it is the most problematic cultivar for pre- and post-harvest disease problems (Partridge, 1990). In the 2016/17 season, four spore traps were placed in an unsprayed 'Fuerte' orchard at the Halls site. In the 2017/18 season, four spore traps were placed in the same orchard at Halls (Fig. 3) and four spore traps were placed in an unsprayed 'Fuerte' orchard with very high disease incidence at the ARC-TSC. Two of the four spore traps had four vaseline-coated slides held horizontally and two had four slides held vertically, facing the four different wind directions. Slides were held in position with clothes pegs attached to the end of two metal strips fixed at right angles to one another, on top of a 1.5 m pole. Trees with high disease incidence in the 2016/17 season were identified in the Halls orchard and at the ARC-TSC at harvest and spore traps for the 2017/18 season were placed in close proximity to these trees to ensure better spore trapping data.

Figure 3: A spore trap with horizontal vaseline slides.

Figure 4: 'Fuerte' avocado fruit covered with brown paper bags at Halls.

During the 2016/17 season, spore release was monitored from October 2016 to March 2017 by the University of Mpumalanga. Slides were changed weekly and examined. Readings were carried out using methyl blue dissolved in water at 0.25% [m/v] as a colourant for *P. purpurea* spores (Darvas, 1982).

During the 2017/18 season, spore release in the ARC-TSC orchard was monitored from 6 September 2017. Slides were changed weekly on the four traps and changed daily on the two horizontal traps from December to end of February. For the daily spore trapping, four additional clothes pegs were added onto the horizontal traps to hold these slides. In November, for a seven day period, spores were changed on an hourly basis from 23:00 in the evening until 03:00 in the morning. In the 2016/17 season, weather data were obtained from the ARC-TSC weather station and in the 2017/18 season Hobo sensors were placed in the orchards to record temperature and humidity. An EM50 data logger to record leaf wetness was placed at the ARC-TSC site. Other weather data were obtained from the nearest weather station.

Infection periods

A bagging trial was carried out at HL Hall & Sons to determine the critical infection periods of avocado fruit by Cercospora spot. In the 2016/17 and 2017/18 seasons, 1400 and 1500 fruits were covered with brown paper bags, respectively (Fig. 4). Every fortnight, fifty bags were removed to allow infection and then replaced at the end of the fortnight. In the 2017/18 season, this was carried out from 11 October 2017. In the 2017/18 season, fruit were covered earlier when fruit were between 20 and 30 mm in diameter. In the previous season the fruit were almost 40 mm in diameter and therefore some infection might have occurred before bagging of fruit. In the second season, brown paper bags were covered with duct tape at the top end to further prevent water from running onto fruit in addition to the use of non-absorbent cotton wool.

Fruit were harvested on 22 May 2017. Immediately after harvest, fruit were evaluated for the presence of Cercospora spot on a scale of 0-5 (Table 1).

0 = clean fruit
1 = 1-5 spots with diameter of combined lesion area 1-5 mm
2 = 1-5 spots with diameter of combined lesion area 6-10 mm
3 = 1-5 spots with diameter of combined lesion area >10 mm
4 = 6-10 spots
5 = >11 spots.

For Classes 1-3, all fruit had 1-5 spots but were differentiated on the basis of combined lesion diameter, as most of the fruit that was not clean fell into these 3 categories and it was necessary to differentiate between fruit with 1-5 large lesions and fruit with 1-5 very small lesions. Results are expressed in terms of a disease index according to Wheeler (1969), where infection index = (Sum of all numerical ratings/total number of fruit) x (100/Maximum disease category (5)).

RESULTS

Spore trapping

In January 2017, the ARC took responsibility for the project by appointing a MSc student to continue the study. Some of the slides of the 2016/17 season were read by University of Mpumalanga (UMP) at that stage and they found that the occurrence of Cercospora spores was very limited and two possible reasons for this observation were provided:

a. the preceding dry season had a negative effect on spore survival, and/or
b. the spray programme for managing the occurrence of Cercospora spores was very well maintained.

Slides from the 2016/17 season were further investigated by the ARC-TSC in 2017. As was found by

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Darvas (1982), reading of many slides is necessary to correctly identify spores and determining the correct staining method. Darvas (1982) stated that spore trapping was attempted in 1977 for the first time in the history of Cercospora spot investigations and the first year of research focused primarily on finding appropriate techniques and gaining sufficient experience in the identification of trapped conidia. Another difficulty experienced was that disease incidence was very low in the Halls orchard in the 2016/17 season, making trapping of spores very difficult. Because of very low conidia numbers trapped in the 2016/17 season, spore trapping data for the 2016/17 season are not shown.

In the 2017/18 season more than 1000 slides were placed in the orchards at the two trial sites from September 2017. At the Halls trial site, only weekly spore trapping data were collected, while at the ARC-TSC site both weekly, daily and hourly data were collected. More than 500 slides have been read so far. At this stage, no spores have been trapped at the Halls site. Weekly conidia trapped at the ARC-TSC site from September 2017 – December 2017 are presented in Figure 5.

Conidia were first detected in the period 20 to 27 September 2017. Conidia were detected in the next two periods, 27 September to 4 October and 4 October to 11 October, as substantial rain was recorded in these periods, 32.2 and 28.7 mm, respectively. As average fruit size was still below 40 mm in diameter in these periods, infection probably did not occur in these periods despite conidia being present, but this needs to be confirmed with disease index data which will only be obtained at harvest. Conidia were also detected in the period 25 October to 1 November, as some rain was recorded in this period. This period was the period when a Z value greater than 15 was calculated for the first time during the season, using Darvas' equation. Conidia numbers reached a peak in the period 15 to 22 November when more than 68 mm rain was recorded. As some rain also occurred in the following three weeks, conidia were still trapped, but fewer conidia were trapped due to there being less rain. In the period 13 to 20 December, conidia numbers dropped drastically as less than 5 mm rain was recorded. At the end of December, conidia numbers increased again when 74.17 mm rain was recorded. A good correlation (r=0.893) was found between conidia numbers and rainfall for the period September to December 2017. This is however only preliminary data (until December 2017) and all weather factors will be taken into account when developing a model at the end of the season.

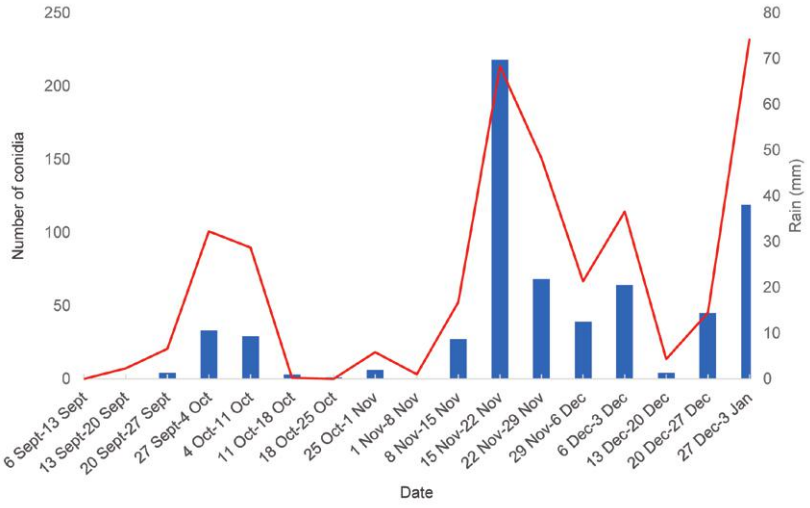

Figure 5: The mean weekly number of conidia trapped at the ARC-TSC site and weekly rainfall in the period September to December 2017.

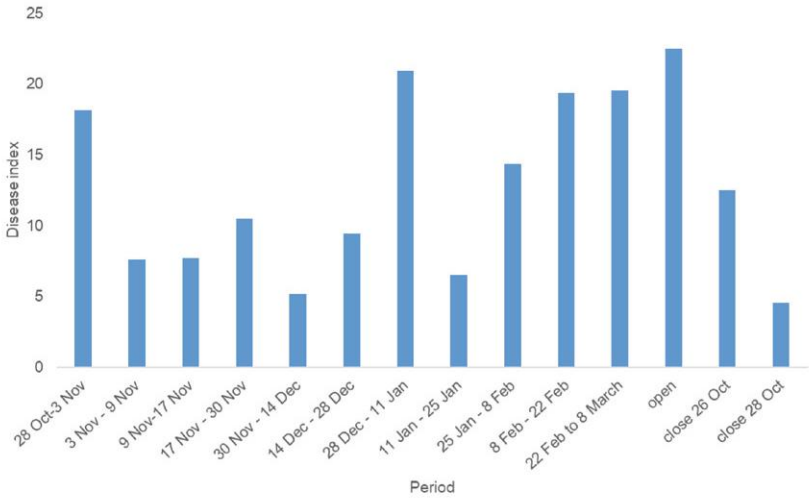

Figure 6: Cercospora disease severity in the unsprayed 'Fuerte' orchard at Halls.

Infection periods

The infection indices for each of the 11 periods as well as the index for fruit not exposed for the entire period and fruit exposed the entire period of the 2016/17 season's trial, are presented in Figure 6.

The highest disease indices for Cercospora spot were recorded in the periods 28 October to 3 November, 28 December to 11 January, 8 February to 22 February, 22 February to 8 March and for fruit exposed during the entire period. Fruit were covered with paper bags on 26 October and some infection also occurred on these fruit. The reason for this could have been that fruit were covered with bags too close to 40 mm in diameter and fruit might already

have been susceptible before covering and infection took place. Two days after most of the fruit were covered with bags, more fruit at an earlier developmental stage were covered (28 October). These fruit had a lower incidence of infection than the larger fruit. The reason for a degree of infection on this fruit might be that some leakage of the bags took place. In the 2017/18 season, these problems were addressed as described under Materials and Methods.

The disease index values for the 2016/17 season for each period were correlated with weather data and a preliminary model was developed:

Disease index = $5361.8 - 314.9 * Tx + 5.104 * Tx^2 - 66.46 * Tn + 1.897 * Tn^2 + 0.009635 * RH^2$
(Tx = max Temp, Tn = min Temp, RH – relative humidity)

Although isolation of *P. purpurea* is difficult, an attempt was made to isolate the fungus from infected fruit for verification on potato dextrose agar (PDA) at harvest in the 2016/17 season. In the 2017/18 season, isolations were made from fruit and leaves from January 2018. A few isolates which could be *P. purpurea* were obtained, but these isolates need to be verified. No culture of *P. purpurea* is available in any culture collection in SA. A *P. purpurea* culture, the only culture available in the world, was obtained from the Westerdijk Fungal Biodiversity Institute in Netherland that was isolated from avocado fruit in Mexico. This culture will be used in further studies to verify that we are trapping the correct spores and to verify the cultures isolated from avocado in our study.

The project is ongoing and fruit will be harvested in April/May 2018 and evaluated for Cercospora spot and the results expressed in terms of an index as described for the 2016/17 season. The disease index data and spore trapping data will be correlated with weather data to develop a forecasting model which can be used to forecast conidia production by *P. purpurea*. This model will then be used to determine high risk infection periods enabling accurate and more cost effective timing of fungicide sprays.

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