A RELOOK AT THE EPIDEMIOLOGY OF CERCOSPORA SPOT ON AVOCADO IN SOUTH AFRICA

By

Reshika Kallideen
(209536027)

Submitted in fulfilment
of the requirements for the degree of

MASTER OF SCIENCE

in the
Discipline of Plant Pathology

1. University of KwaZulu-Natal (UKZN), King Edward Avenue, Scottsville, Pietermaritzburg, Private Bag X01, Scottsville, 3209

and

2. Agricultural Research Council-Tropical and Subtropical Crops (ARC-TSC), Private Bag X11208, Mbombela, Mpumalanga, 1200
    Email: KallideenR@arc.agric.za

June 2020

<center>**Declaration**</center>

I, Reshika Kallideen, declare that:

i. The research reported in this dissertation except otherwise indicated is my original work;

ii. This dissertation has not been submitted for any degree or examination at any other university

iii. This dissertation does not contain other person's data, pictures or graphs or other information, unless specifically acknowledged as being sourced from other persons

iv. This dissertation does not contain other person's writing unless specifically acknowledged as being sourced from other researchers. Where other written sources have been quoted, their words have been re-written but the general information attributed to them has been referenced.

v. This dissertation does not contain text, graphics or tables copied and pasted from the Internet unless specifically acknowledged and the source being detailed in the dissertation and in the References section.

Signed …………………………………..   **Date** …8/05/2020…………………

R. Kallideen (Candidate)

Signed …………………………………..   **Date** … 8/05/2020………………….

Maritha Schoeman (Supervisor)

Signed Prof. M. Laing (Co-Supervisor)   Date. 6 June 2020

Dissertation summary

Avocado (*Persea americana* Mill.) belongs to the family Lauraceae and is one of the most economically important subtropical fruit crops in the world. The South African avocado industry contributed approximately "R1.2 billion to the total gross value of subtropical fruits (R3.4 billion) during the 2017/18 season", according to the latest available records. One of the most serious pre-harvest diseases affecting avocado in South Africa is Cercospora spot. Losses of up to 70% have been reported on unsprayed trees. This disease is commonly found in avocado producing regions where warm, humid and rainy conditions persist. It affects all commercial cultivars, with 'Fuerte' being recognized as the most susceptible cultivar. The plant pathogen responsible for this disease is *Pseudocercospora purpurea* (Cooke) Deighton. As with other *Cercospora* species, this fungus grows slowly and sporulate sparsely on artificial media. Typical disease symptoms are found on the leaves, stems and fruit. Lesions first appear on the underside surface of leaves. These lesions are minute and are brown in colour. As the disease progresses, lesions are observed on both sides of leaves and have distinctive chlorotic halos. On the fruit, small lesions form, later becoming sunken, irregular and brown to black in colour. The most commonly chemical control is copper oxychloride although there are other registered fungicides for use against Cercospora spot.

The South African Avocado industry currently uses a predictive model, developed by Dr J.M. Darvas, in the early 1980s, to predict the number of conidia and the timing for the first spray. The model is based on the temperature and rainfall that occurred in the week preceding the calculation of the prediction. As a result of climate change and adaptions in the fungal populations over the years, it was vital to re-evaluate this model. The primary aims of this study were to determine whether the current Darvas 2 model is still valid for forecasting the first spray for effective control; secondly, to evaluate whether the inclusion of humidity and/or leaf wetness values into a model would enhance its predictive accuracy; and thirdly, to evaluate the size of fruit that was susceptible to infection by *P. purpurea*.

In this study, spore trapping and critical infection trials (bagging trial) were conducted for two seasons. Spore traps were placed in two unsprayed 'Fuerte' orchards (HL Hall and Sons and the ARC-TSC) in the first season (2017/18). However, in the second

season (2018/19), only one orchard (ARC-TSC) was used for both trials because no conidia were trapped as a result of a low disease incidence at the Halls orchard that was used in Season One. At harvest, fruit were assessed for Cercospora spot using a disease rating scale. The disease index data for both seasons (2017/18 and 2018/19) were correlated with weather data using multiple stepwise linear regression analysis. In both seasons, the critical infection period was in the beginning of the season. It was also established that fruit exposed to natural infection early in the season from October to November developed significantly more Cercospora disease symptoms than fruit exposed later in the season.

The daily spore trapping results (2017/18 season) indicated that conidia were mostly trapped on days when rainfall occurred. The most significant correlation (r=0.893) was found between the weekly number of trapped conidia and weekly rainfall (September to December 2017). Based on the weekly spore trapping results of the 2018/19 season, for the period October to December, there was a strong correlation (r=-0.696) between conidia and mean maximum temperature. For the entire season (October to April 2018) the correlation between conidia and mean maximum temperature was slightly lower (r=-0.520). In the 2017/18 season, more rainfall fell and more conidia were trapped than in the 2018/19 season. Due to low rainfall during the 2018/19 season, a stronger correlation was found between conidia and temperature than conidia and rainfall. This negative correlation can be explained by the cooling effect of rain, as mentioned by Darvas (1982). For both seasons, the weekly weather parameters and the weekly spore trapping data were correlated with one another. Using multiple stepwise linear regression analysis of the weekly conidia trapped and weekly weather data, three models were developed for each season. It was found that all new models (for each season) followed a similar pattern to the Darvas 2 model, with some minor differences.

The spore trapping results confirmed that rainfall and temperature were the dominant environmental parameters. However, leaf wetness and relative humidity were not factors in the release of conidia but played a role in disease development, probably in the step of host infection. The study found that the Darvas 2 model was still an effective forecasting tool. However, the selected model/s (current Darvas 2 model or the new models) must be used in combination with fruit size monitoring to determine accurate and cost-effective timing of the first spray. This study determined that the first spray should be

applied around mid-October (depending on the geographic region, rainfall and Z values). In addition, it was also concluded that spraying should begin when the Z-value is 15, and fruit size is approximately 25mm in diameter, and not 40mm as previously recommended by Darvas (1982). This study showed that spraying when the fruit is 40mm in diameter would be too late to slow down disease development.

In support of the primary aims, experiments were conducted to determine the growth requirement/s of *P. purpurea*. The growth of *P. purpurea* was evaluated on several artificial media (potato dextrose agar (self-made), potato dextrose agar (commercial), malt extract agar, potato sucrose agar, oatmeal agar (self-made), oatmeal agar (commercial), and V8 juice agar. The fungus was grown on these media at temperatures ranging from 5°C to 35°C. The radial growth was recorded by measuring the colony diameter for a period of 28 days at seven-day intervals. The results of the growth study indicated that oatmeal agar was the best agar medium, and that 25°C was the optimal temperature for the growth of *P. purpurea* on artificial media.

In conclusion, this study showed that the Darvas 2 model is still an effective forecasting tool, irrespective of climate change and that when the model i.e., either the current Darvas 2 model or one of the newer models is used in combination with fruit size monitoring, we can achieve a more accurate and cost-effective time to apply the first fungicide spray.

Acknowledgements

Table of Contents

Introduction

Avocado (*Persea americana* Mill.) is a tropical fruit, belonging to the flowering plant family, Lauraceae. There are a number of commercial cultivars available. According to the South African Avocado Growers Association (SAAGA), the majority of the avocado trees (approx. 80%) produced in South Africa are the dark skinned 'Hass' and 'Hass'-type cultivars ('Carmen', 'Gem', 'Lamb-Hass' and 'Maluma'), whilst the rest (20%) is made up of 'Fuerte', 'Ryan', 'Pinkerton' and 'Reed.' (SAAGA, 2019).

South Africa is one of the world's leading avocado producers. Its annual production has been traditionally concentrated in the warm, humid subtropical regions of Limpopo, Mpumalanga and parts of KwaZulu-Natal. Due to the increase in global demand, plantings have been extended into other provinces namely the Western and Eastern Cape (SAAGA, 2019). South African avocado are exported to the Netherlands, United Kingdom, Spain, Namibia and Russia, in particular. Between 45 and 50% of South African avocados are exported and 10-15% is processed into oil and pulp, and the rest is traded locally (Louw, 2019).

Avocado is considered to be a highly nutritious fruit and have many health benefits. It contains higher levels of soluble and insoluble fibre and protein than other fleshy fruits. Additionally, it is a rich source of potassium (more than banana), vitamins E and C, and pro-Vitamin A (Cowan and Wolstenholme, 2003). Some of its purported benefits is that regular consumption of avocados can decrease the risk of heart disease, aid in weight loss, improve brain health, and reduce cholesterol levels (Robbins, 2019).

Nevertheless, avocado crops develop several diseases (Darvas, 1982; Lonsdale and Kotze, 1989). Darvas and Kotze (1987) reported that warm, humid conditions are favourable to a range of disease problems on avocados. The most serious pre-harvest fruit disease in South Africa is Cercospora spot. This disease is found in all avocado growing regions in the country (Darvas and Kotze, 1987). It is most severe in regions where ideal climatic conditions occur, i.e. high rainfall and favourable temperatures (Darvas, 1982). Crop losses of 70% have been reported on unsprayed trees. The most susceptible cultivars are 'Fuerte' and 'Ryan'. Inferior quality fruit is produced from

infected trees (Darvas and Kotze, 1987). *Pseudocercospora purpurea* (Cooke) Deighton, formerly known as Cercospora, is the causal organism responsible for the disease. Darvas (1982) found that rainfall was the most important environmental parameter influencing production of *P. purpurea* conidia, and that it had a significant effect on Cercospora spot incidence. Copper oxychloride is mostly used to control Cercospora but there are other registered products that can also be used e.g. copper hydroxide, basic copper sulphate, copper ammonium acetate, azoxystrobin and carbendazim.

A general spray recommendation is not suitable to control this disease in the different avocado production regions in South Africa because the climatic factors are not the same in different regions at a given time. To deal with this problem, Darvas (1982) developed two epidemiological models to predict the onset and development of Cercospora spot. The Darvas models for predicting the number of conidia in the atmosphere in a given area are:

Darvas 1 Z (number of conidia) = 24.8 (constant) – 0.93X (X is temperature in °C) + 0.25Y (Y is rainfall in mm).

Darvas 2 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)

Release of conidia occurs when Z>0, and the potential for Cercospora infection is high when Z>20 (Darvas, 1982). The Darvas 2 equation is still being used by South African avocado growers to determine when to commence spraying for Cercospora spot.

Significance of the research

Due to climate change and evolution in fungal populations (over 40 years since Darvas' research) it was necessary to re-investigate the existing forecasting model/s and determine if the Darvas 2 model is still valid as a tool to predict the release of conidia of *P. purpurea*. For example, very high temperatures may result in Z values ≥15, causing incorrect prediction of conidia release and therefore inaccurate timing of first sprays. The two models developed by Darvas (1982) only use temperature and rainfall figures, and do not use humidity and/or leaf wetness values, which are commonly used in predictive models in plant epidemiology (Rowlandson *et al.*, 2015; Park *et al.*, 2019). In addition, the formula in his study was derived from only 10 data days, of which two of those days contributed the most to the prediction model (Darvas 1982). Manicom

and Schoeman (2009) noted that the models were only effective for temperatures between 20 and 26°C. Therefore, it was decided to retest the Darvas models, and to develop new models that incorporated humidity and leaf wetness, in order to develop the best model based on current climatic conditions and the development of Cercospora spot on the avocado crop.

Another concern was to understand the growth stage at which avocado fruit become susceptible. Darvas (1982) believed that when fruit reaches 40 mm in diameter they become susceptible to infection. However, there is no objective data presented as a basis for this belief. As such, the fruit size at which avocado fruit becomes susceptible also needed to be determined.

Research aims and objectives

The overall aim of this research was to investigate the epidemiology of Cercospora spot on avocado in South Africa.

The main objectives of the current study were as follows:
1. To determine if the Darvas 2 model developed by Darvas in the 1980s is still a valid forecasting tool in predicting the timing of the first spray for effective control of *P. purpurea;*
2. To test the addition of leaf wetness and relative humidity to temperature and rainfall into new prediction models;
3. To determine the fruit size at which avocado fruit become susceptible to *P. purpurea.*
4. To determine the effect of various temperature ranges, and different solid media on the growth of *P. purpurea*.

Dissertation structure

This dissertation is divided into four chapters. Chapter 1 is a literature review that covers the avocado crop and its production, and details of the Cercospora spot disease of avocado. Chapter 2 focuses on the epidemiology of Cercospora spot disease and describes the results from the spore trapping and bagging trials, and subsequent predictive modeling activities. Chapter 3 evaluates the effects of various temperature ranges, and different solid media on the growth of the casual organism, *P. purpurea*. Chapter 4 is a general overview of the study, including the major findings and the way forward.

References

Cowan A.K., and Wolstenholme, B.N. 2003. Encyclopedia of Food Sciences and Nutrition (Second Edition). Academic Press, Oxford, UK.

Darvas, J.M. 1982. Etiology and control of some fruit diseases of avocado (*Persea americana* Mill.) at Westfalia Estate. D.Sc. (Agric.) thesis, University of Pretoria, Pretoria.

Darvas, J.M. and Kotzé, J.M. 1987. Avocado fruit diseases and their control in South Africa. South African Avocado Growers' Association Yearbook 10:117-119.

Lonsdale, J.H. and Kotzé, J.M. 1989. Chemical control of pre-and post-harvest diseases of avocados. South African Avocado Growers' Association Yearbook 12:68-70.

Louw, M. 2019. Avocado production: Avocados in South Africa. http://southafrica.co.za/avocado-production.html. Accessed on 11 June 2019.

Manicom, B.Q and Schoeman, M.H 2009. Evaluation of fungicides for *Pseudocercospora* control (II). South African Avocado Growers Association Yearbook 32:70-72.

Park, J., Shin, J., Kim, K. Rang, H., J. 2019. Leaf Wetness Duration Models Using Advanced Machine Learning Algorithms: Application to Farms in Gyeonggi Province, South Korea. 11:1-20.

Robbins, O. 2019. Everything You Need to Know About Avocados + 15 Science-Backed Reasons to Eat These Fabulous Fruits. https://foodrevolution.org/blog/avocado-health-benefits/. Accessed on 13 April 2019.

Rowlandson, T., Gleason, M., Sentelhas, P., Gillespie, T., Thomas, C., and Hornbuckle, B. 2015. Reconsidering Leaf Wetness Duration Determination for Plant Disease Management. Plant Disease. 99: 310-319.

South African Avocado Growers Association (SAAGA). 2019. Overview of South African avocado.https://www.avocado.co.za/overview-of-sa-avocado-industry/. Accessed on 10 April 2019.

CHAPTER 1

LITERATURE REVIEW

1.1 General introduction

Avocado black spot, also known as Cercospora spot, is the most severe pre-harvest fruit disease of avocado in South Africa (Willis and Mabunda, 2004; Pérez-Jiménez, 2008; Schoeman and Kallideen, 2018). From a quality perspective, while not affecting the flesh of the fruit, the disease can cause losses of up to 70% in unsprayed orchards e.g. unaccepted by consumers (Darvas and Kotze, 1987; Manicom, 2001). It affects all commercial cultivars including 'Edranol', 'Pinkerton' and 'Hass', but 'Fuerte' and 'Ryan' are considerably more susceptible (Ploetz *et al.*, 1994). The 2017 South African Avocado Growers' Association loss factor benchmark report stated that the total loss to the avocado industry for fruit delivered to the pack house due to Cercospora spot was 3%. The total loss on 'Fuerte' fruit alone, in 2017, was approximately 10% (SAAGA, 2017). Disease severity varies from season to season. The disease was first reported in Florida in 1920 but only became prominent in South Africa during the rainy seasons of the late 1960s (Ploetz *et al.*, 1994; Menge and Ploetz, 2003). Today, it can be found in all avocado-producing areas where warm, humid, and rainy conditions persist (Ploetz *et al.*, 1994; Manicom, 2001). A predictive model, originally developed by Darvas in the 1980s, is currently being used by the avocado industry to time the spraying of fungicides to control Cercospora spot. This model is used to predict the number of conidia in a given area (Z value) and the optimum timing of the first spray (Darvas, 1982). Copper oxychloride is the most commonly used chemical for the control of Cercospora spot, although copper hydroxide, basic copper sulphate, copper ammonium acetate, azoxystrobin and carbendazim are also registered (SAAGA, 2020a). The aim of this chapter is to provide a review of the current literature on Cercospora spot on avocado. This chapter briefly outlines the avocado crop, its production, economic importance, and uses, and an overview Cercospora spot and its control measures.

1.2 Avocado crop

Avocado (*Persea americana* Mill.) belongs to the flowering plant family Lauraceae and can be classified into three subspecies namely: *americana* (West Indian), *guatemalensis* (Guatemalan) and *drymifolia* (Mexican) (Sippel, 2001). Avocado is regarded as a tropical fruit as it is believed to have evolved mostly within geographically tropical latitudes (23.5°N to 23.5°S). Nowadays, it is among the most economically important subtropical/tropical fruit crops in the world (Schaffer *et al.*, 2013).

Numerous historical studies have suggested that avocados originated in Southern Mexico. Over time, and with the movement of people, avocado production expanded to the West Indies and to almost all parts of the tropical and subtropical areas with suitable environmental conditions. Initially avocado was grown only by small-scale farmers in and surrounding the area of origin. It was also consumed locally and principally as part of agricultural systems. However, during the last 150 years, production and consumption levels have increased drastically (Schaffer *et al.*, 2013). It is uncertain when avocados were first introduced into South Africa. Some studies propose that avocados were introduced into the country by settlers coming from the West Indies, whereas others report avocado being imported from Dutch colonies between 1652 and 1700 (Sihlobo, 2018).

1.3 Avocado production

1.3.1 World avocado production

Mexico is the leading avocado producing country in the world (Pariona, 2017). The total avocado production area is about 168,155 hectares, which produces 1.52 million metric tons p.a. The majority of the avocados in Mexico (86%) are grown in Puebla, Morelos, Michoacán, Nayarit, and Mexico City. Mexico produces more avocados than any other country in the world and produces more per hectare too. The avocados are harvested by hand by means of poles and baskets. This country has been growing in both production and exports over the last few years. The next largest avocado producing country in the world is the Dominican Republic. It produces 420,000 metric tons annually, and this has been gradually increasing over the last few years.

A large portion of this production is consumed nationally rather than exported. Other significant avocado producing countries include Peru, Colombia and Indonesia (Table 1.1) (Pariona, 2017).

Table 1.1: Top five avocado producing countries

Rank	Country	Production (millions of tonnes, 2014)
1	Mexico	1.52
2	Dominican Republic	0.42
3	Peru	0.35
4	Indonesia	0.31
5	Columbia	0.28

Source: (Pariona, 2017)

Global production has increased dramatically due to improvements in post-harvest technologies, reductions in trade barriers, strong health-related claims, increased incentives and cultivated regions in the producing countries (Duarte *et al.*, 2016).

1.3.2 South African avocado production and trade

In South Africa, avocado production is mainly focused in the warm subtropical regions, with Limpopo contributing 61% (9401 ha), Mpumalanga 30 % (4554 ha), and KwaZulu-Natal 8% (1319 ha) (DAFF, 2012) (Fig. 1.1). Due to the growing global demand, production has expanded into the Eastern and Western Cape provinces (up to 33 °S) (SAAGA, 2019). Annual rainfall in most of these parts is high (> 1000 mm per annum), but there are some orchards in semi-arid regions with rainfall of ± 400 mm per annum (SAAGA, 2019).

Eighty percent of avocado trees produced in South African nurseries are the dark-skinned 'Hass' and 'Hass'-type cultivars, for example, 'Carmen', 'Gem', Lamb-Hass' and 'Maluma'. Green-skinned cultivars including 'Fuerte, 'Pinkerton', 'Ryan' and 'Reed' contribute to the remaining 20% of nursery tree production (SAAGA, 2019).

The South African avocado season begins in February and ends in November, with the bulk of fruit being harvested between March and September. As a result of climatic variability between growing regions, the most important cultivars are available over an extended period during the season. 'Fuerte', for example, is harvested from March to May in the northern areas and from July to August in KwaZulu-Natal (SAAGA, 2019).

The avocado industry in South Africa grew progressively from the early 1970s to 2003, with plantings of ±2000 ha in 1970 increasing to ±12 000 ha in 2003. The number of new plantings was reduced from 2003 to 2008 as growth declined. But, later in 2009, the total number of plantings increased due to the rising consumer demand for avocados. The region under commercial avocado orchards is currently approximately 17,500 ha with new plantings amounting to almost 1,000 ha per annum (SAAGA, 2019).

Local production has responded to growing demand for avocado in domestic and global markets (Sihlobo, 2019). Although it is not among the top 10 worldwide avocado-producing countries, South Africa holds a prominent place in the avocado global export market. Fig. 1.2 shows the production trends for avocado for the past four decades (1978 to 2018). Production has increased from 1,500 MT in 1969/70, to a peak of 169,243 MT in 2017/18. This is mainly attributed to enhanced yields, better varieties, better water management and agricultural practices, as well as extensive investments in new orchard plantings especially from 2007/08 (Sihlobo, 2019).

In 2017, the total production was slightly lower (110 000 metric tonnes) compared with the 2018 season where 125 000 metric tonnes was produced. This is largely attributed to the drought, which many avocado-producing regions experienced previously, and also the cyclical on-year and off-year nature of avocado production (Fresh Fruit Portal, 2018; Sikuka, 2019).
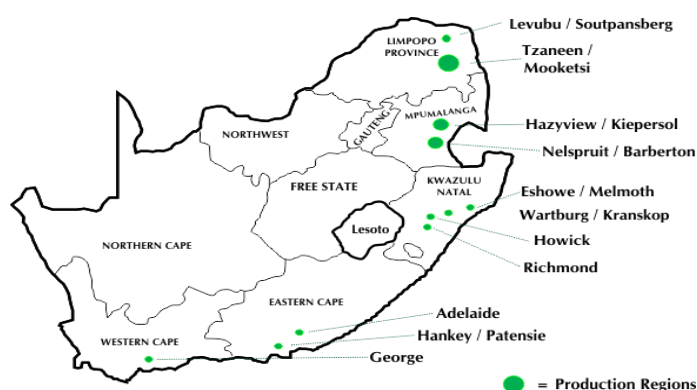

Figure 1.1: Avocado production regions in South Africa
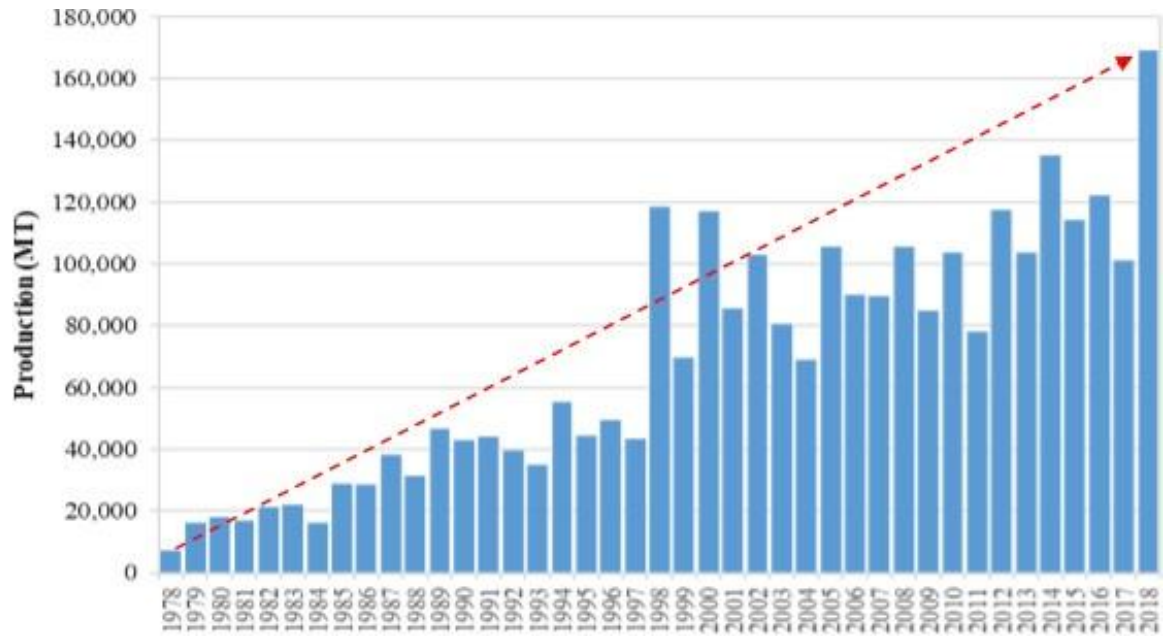Source: SAAGA, 2019

Figure 1.2: South African avocado production from 1978 – 2018

Source: Sikuka, 2019

1.4 Economic importance and uses of avocado

The South African avocado industry contributed approximately "R1.2 billion to the total gross value of subtropical fruits (R3.4 billion) during the 2017/18 season" according to the available records (DAFF, 2019).

Avocado is considered a major tropical fruit with a high nutritional value since it is rich in protein, low in carbohydrates and contains fat-soluble vitamins that are deficient in other fruits, for example, Vitamins A and B, and moderate levels of Vitamins D and E (Duarte *et al.*, 2016). It also has the highest energy value of any fruit (Frey, 2019). Avocado has the highest concentration of dietary fiber of any commonly eaten fruit. The pulp is relatively high in various oils, and therefore is extensively used in pharmaceutical and cosmetic industries, and for obtaining commercial oils similar to olive oil, because of its fatty acid composition (Duarte *et al.*, 2016). Additionally, this fruit has been renowned for its health benefits, especially due to the compounds present in the lipid fraction, for instance, omega fatty acids, phytosterols, tocopherols, and squalene (Duarte *et al.*, 2016). The ripe fruit can be eaten fresh, and can be used in preparing salads, to flavour ice-creams, as filling for sandwiches and in quick desserts (QD FRIENDS, 2012).

Other parts of the crop have medicinal benefits, for example; boiled leaves are sometimes used as a remedy for diarrhea (QD FRIENDS, 2012). These fruits also have a concentrated amount of cancer-preventing antioxidants, including potent carotenoids (QD FRIENDS, 2012).

South African consumers generally prefer the 'Fuerte' avocado variety despite the availability of other avocado varieties such as 'Hass', which are easily available in the market during the harvest season (February to August) (Sikuka, 2019). South Africa exported approximately two thirds of its avocado production between 1994/1995 and 2016/2017. South Africa exported 43 492 tons to the value of R853 million, which is approximately R19,612/ton, in 2017. However, this was a decrease of 24.8% compared with the export total in 2016, probably showing the effect of a drought during the 2016/17 season (Stone, 2019). South Africa was the world's eighth largest avocado exporter in value terms in 2018 (Motaung, 2019).

Some of the major importers for 2017 and 2018 have been the Netherlands, the United Kingdom, Spain, Russia, the United Arab Emirates (UAE), Portugal, Russia, Namibia, Turkey, Saudi Arabia and France, which collectively accounted for 97% of South Africa's avocado exports (Fig. 1.3) (Motaung, 2019). South African exports were expected to increase to 66,000 MT in 2019 compared with 43,700 MT in 2018 (Fresh fruit portal, 2018).
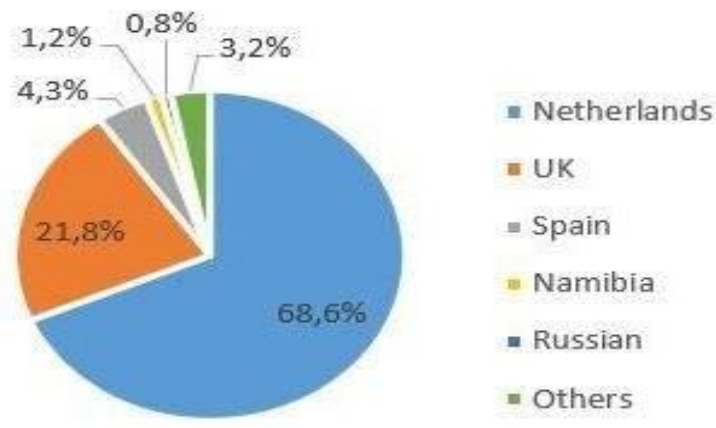
Source: Stone, 2019

Figure 1.3: Major importers of South African avocados (2017)

1.5 Export market, quality and standard of avocado

Avocado has been a growing market locally and internationally, for several years. Changes in production, planning and climate resulted in variation of supply and prices. Growers pursuing the best market and prices should comply with export standards thereof, so that the best quality fruit can be sold to the export market (Obi, 2017). In South Africa, the inspection of avocado quality is classified into three classes: Class 1, Class 2 and Lowest class (DAFF, 2014). Generally, avocado fruit should be intact, clean, free from pests, free from any visible signs of fungus growth, undamaged, free of abnormal external moisture, have a stalk no longer than 10 mm in length, and to be in a condition to withstand transport and handling. The quality standards allowed for trade of South African grown fruits are therefore, largely set by the UK standards and European Union (EU). Various factors affecting the quality of avocados are considered (see Table 1.2 below).

Table 1.2: Some factors considered for inspection of avocado quality in South Africa

Quality factors	Percentage allowed per class		
	CLASS 1	**CLASS 2**	**CLASS 3**
1. Blemishes, Skin damage, Cercospora, sunburn (all types, excluding dark sunburn), carapace skin, hail damage, sooty mould, insect damage, lenticel damage, netting, dark wind damage	15%	15%	*
2. Malformation			
(a) Epidermal notches	15%	15%	*
(b) Epidermal bumps	15%	15%	*
(c) Bent necks	15%	15%	*
3. Visible chemical residues	10%	10%	*
4. Cold damage a) Internal cold b) Frost damage c) Damage due to low storage temperatures	10% 10%	10% 10%	* *
5. Decay (e.g. stem end decay, vascular browning, internal spot, anthracnose, dothiorella rot)	5%	5%	5%

Source: DAFF, 2014

% represents the permissable deviation   * means unspecified

The most economically important pre-harvest disease of avocado fruits is Cercospora spot, which is caused by *Pseudocercospora purpurea* (*Cooke*) Deighton. Due to the unaesthetic look of black spots, consumers reject the infected fruit, resulting in an economic loss to markets, and ultimately, to the farmers.

It should also be noted, that in general, most countries have established Maximum Residue Limits (MRLs) for pesticides used in the control of pest and diseases, not only to protect consumer health but also to reduce the presence of these residues in the environment. Therefore, in order to gain access to the market, the products exported must follow these residue standards. For instance, the permitted residue limit for copper oxychloride used to treat Cercospora spot is 20 mg kg^{-1} (South Africa) (DAFF, 2014; SAAGA, 2020b).

1.6 The causal agent of Cercospora spot

P. purpurea (*Cooke*) Deighton is a fungal plant pathogen responsible for Cercospora spot, worldwide (Darvas 1982; Menge and Ploetz, 2003). Previously, the fungus was called Cercospora, but after a taxonomic revision of Cercospora and allied genera, the causal agent was renamed (Deighton, 1976). It is currently classified as follows: Kingdom: Fungi; Phylum: Ascomycota; Class: Dothideomycetes; Subclass: Dothideomycitidae; Order: Capnodiales; Family: Mycosphaerellaceae; Genus: *Pseudocercospora* and Species *P. purpurea* (EPPO, 2019).

Under high humidity conditions, *P. purpurea* produces dense fascicles of conidiophores on dark brown to black, spherical to irregular stomata, 15 – 125 µm in diameter and embedded in infected leaves and fruit (Darvas, 1982). These conidiophores can be tightly packed or divergent, 20 – 200 µm long, pale to olive brown, rarely branched, straight or with a zigzag growth, with scars formed on the tip or sides where conidia have detached (Darvas 1982; Menge and Ploetz, 2003). Conidia are rod-shaped to cylindrical, with a blunt end, pale olive, 9 – 11 septate, straight or curved and 20 – 200 x 2 – 5 µm (Fig. 1.4a). Recent literature has suggested that there is a *Mycosphaerella*-like state. However, it has been rarely noticed and thus it's function appears to be insignificant in the disease cycle (Menge and Ploetz, 2003). The fungus, being a slow growing organism, may be isolated from fresh symptomatic tissues on standard nutrient media e.g. potato dextrose agar (PDA). However, it can be challenging

to isolate it from older lesions because other fungi outcompete it on artificial media (Manicom, 2001). For example, *Colletotrichum gloeosporioides* grows faster than *P. purpurea* on agar media (Manicom, 2001).

Hence, precautionary measures such as surface disinfection of leaf tissues are crucial in eliminating other contaminating fungi. Macroscopically on oatmeal agar, it formed a tufted, leather-like mycelium that is first greyish-olive (Fig. 1.4b) i.e. depending on age and growth media (Darvas 1982; Menge and Ploetz, 2003).
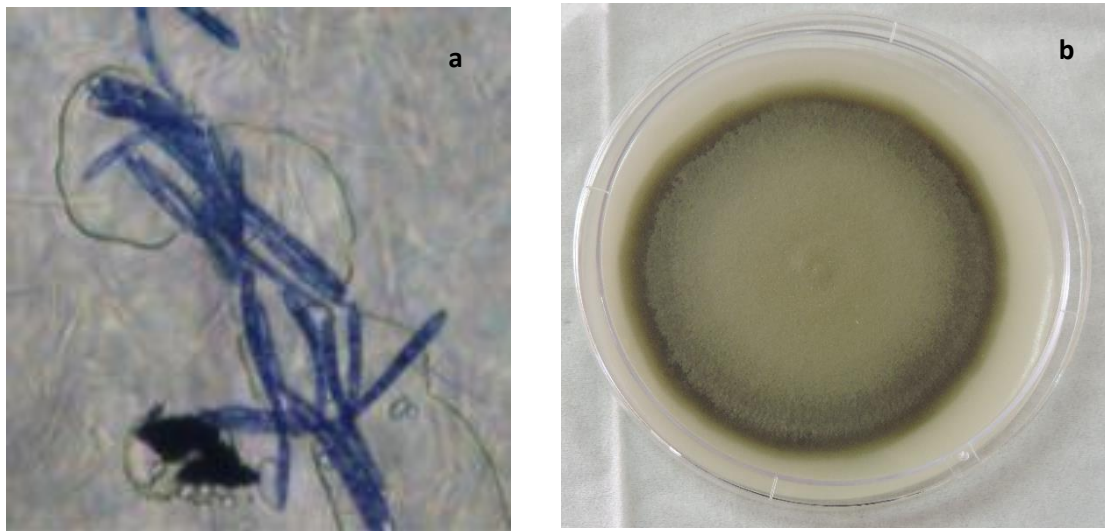
Figure 1.4: (a) *P. purpurea* conidia on a Vaseline slide stained with methylene blue solution and viewed under the light microscope (400x); (b) One-month old *P. purpurea* culture grown on oatmeal agar.

1.7 Geographic distribution

P. purpurea is found globally in all subtropical and tropical regions: *Asia*: India, Japan, Philippines; *Africa*: Cameroon, Democratic Republic of Congo, Côte d'Ivoire, Guinea, Kenya, South Africa; *North America*: Bermuda, Mexico, USA; *Central America and Caribbean*: Dominica, El Salvador, Honduras, Jamaica, Nicaragua, Panama, Puerto Rico, Trinidad and Tobago, United States Virgin Islands; *South America*: Argentina, Bolivia, Brazil, Chile, Guyana, Peru, Venezuela; *Oceania*: Australia, Palau (CABI, 2016).

1.8 Host/s

Avocado is the main host and belongs to the family Lauraceae. Other hosts include *P. borbonia* (redbay)*, and P. palustris* (swamp bay) (Schaffer *et a*l., 2013).

1.9 Symptoms

Signs of infection may occur on leaves, stems and fruit at any time during the growing season (Menge and Ploetz, 2003; Schaffer *et al.*, 2013). Various symptom descriptions on 'Fuerte' are available. However, Darvas (1982) noted some differences in the recognition of development stages of the disease. Younger leaves develop greenish-white spots whereas on mature leaves; small lesions, 3 – 6 mm in diameter, irregular and angular brown to purplish brown are visible (Darvas 1982: Ploetz and Menge 2003). These are typically first noticeable on the abaxial surfaces of leaves (Fig. 1.5) and are surrounded by distinctive chlorotic yellow halos (Ploetz *et al.*, 1994; Menge and Ploetz, 2003). Ultimately, lesions are seen on both leaf surfaces. Under high humidity or wet conditions, grey mycelium is observed in the centre of the lesions due to sporulation of the casual organism, *P. purpurea* (Ploetz *et al.*, 1994; Menge and Ploetz, 2003; Schaffer *et al.*, 2013). Single lesions often coalesce to form large, irregular areas of necrotic lesions (Pernezny and Marlatt, 2000). An infected young fruit shows apparent small, greenish-white spots which later develop into slightly sunken, irregular, and brown to brownish black lesions (Fig. 1.6) (Menge and Ploetz, 2003; Schaffer *et al.*, 2013). In South Africa, lesions appear on fruit as small, raised, shiny black spots often associated with cracking and corkiness of lenticels (Darvas, 1982; Menge and Ploetz, 2003). In older lesions, the development of cracks and fissures allows secondary pathogens to enter, such as *Colletotrichum gloeosporioides,* which causes anthracnose. The disease is typically confined to the rind of the fruit, however; the flesh may be invaded during advanced stages (Darvas 1982; Ploetz *et al.*, 1994; Menge and Ploetz, 2003; Schaffer *et al.*, 2013). Later, defoliation occurs, and fruit can become chlorotic, shrivel and drop. On fruit stems and green twigs, lesions (2 – 10 mm in diameter) may become dark brown to black (Menge and Ploetz, 2003).

Figure 1.5: Small brown-black angular Cercospora lesions on the underside of a 'Fuerte' avocado leaf.

Figure 1.6: An infected 'Fuerte' avocado fruit showing characteristic Cercospora spot symptoms.

1.10   Life cycle and epidemiology

Infected leaves serve as the primary inoculum for the fungus (Ploetz *et al.*, 1994). Avocado is an evergreen crop for most of the months during the year. It is deciduous for only a few weeks per year, and therefore sufficient inoculum is readily available to initiate *P. purpurea* infection on new tissues. Penetration by the fungus may be either

direct or by means of wounds (Ploetz *et al.*, 1994). In South Africa, the pathogen remains dormant for almost three months following penetration (Menge and Ploetz, 2003). Infection generally occurs through conidia (asexual spores), which grow into susceptible tissues. The pathogen is most likely to be present throughout the year, under favourable environmental conditions. The fungus form conidiophores (specialized hypha) that emerge from the plant surface in clusters through stomata. From the conidiophores conidia are generated continuously. The conidia are disseminated via wind, rain splash, insects, irrigation water and movement of contaminated orchard tools and equipment. The spread of spore infected fruit and plant material may be widespread over long distances to new areas (Ploetz *et al.*, 1994; Menge and Ploetz, 2003).

Developing fruit (<40 mm in diameter), and those at or near maturity, are immune, whilst intermediate sized fruits are susceptible (Ploetz *et al.*, 1994; Manicom, 2001). Fruits that are one quarter (25 %) to three quarters (75%) of their final size are most likely to be susceptible, particularly during periods of heavy rainfall (Manicom, 2001; Menge and Ploetz, 2003). High relative humidity is essential for conidial germination and plant infection. The fungus may overwinter as mycelium (stromata) in old infected leaves or shoots until favourable conditions permit the release of conidia, therefore initiating infection in new fruit and leaves.

1.11 Some factors that affect the severity of Cercospora

1. Environmental conditions – Cercospora spot has previously been correlated to humidity/rain and temperature (Darvas, 1982; Darvas and Kotze, 1987).
2. The high-risk infection period or availability of conidia, in addition to weather conditions favourable for infection (Darvas, 1982; Boshoff *et al.*, 1996)
3. The interval or latent period that must lapse between infection and symptom development (Darvas, 1982; Boshoff *et al.*, 1996)
4. Date of harvest; for example, with Cercospora spot the longer the fruit is left on the tree, the more severe the symptoms will be, if control measures are not effective (DAFF, 2014).

Cultivars differ in their susceptibility to the disease. For instance, 'Fuerte' is more susceptible than 'Ryan', 'Edranol' and 'Hass'. It is unknown whether 'Pinkerton' is

susceptible or not at this stage. However, the 'Hass' cultivar seems to be resistant to Cercospora spot (Ploetz *et al.,* 1994; Darvas and Kotze, 1987).

1.12 Management

1.12.1 Cultural control

a) Since, Cercospora spot is transmitted from plant debris that lies underneath the tree, it is advisable to remove fallen leaves, shed fruit and keep the area free of unwanted plants. Any remaining fruit from the previous season should also be removed.
(https://www.infonet-biovision.org/PlantHealth/MinorPests/Cercospora-fruit-spot/ last accessed 11 September 2019).

b) Thinning the inside branches of the tree will reduce humidity in the canopy and thus improve the quality of the fruit. Although the yield will be reduced, the quality will be significantly better.

c) In regions where the disease is problematic, pruning should be carried out during dry periods, or the plant residue should be ground in a shredder or removed from the orchard (Waterworth, 2018).

1.12.2 Chemical control

The control of Cercospora spot on avocados has been studied mainly in South Africa, and research conducted in other countries has been limited. The general recommendation is that copper-based chemicals should be used.
Up until 1982, benomyl (Benlate®) was the standard pre-harvest spray for the control of Cercospora spot on avocado (Darvas, 1982). However, it was substituted with copper

oxychloride, largely due to build-up of pathogen resistance to Benlate® (Darvas and Kotzé, 1987). In South Africa, the most effective control was achieved by using copper-based products for example copper oxychloride, copper sulphate and cuprous oxide. Other fungicides also registered against Cercospora spot on avocado include azoxystrobin and carbendazim (SAAGAa, 2020).

According to Darvas (1982), the timing of the spray is critical because the first spray should be applied at fruitlet stage during the rainy season. Copper oxychloride sprays

can effectively control several plant pathogens on many hosts due to their ability to adhere well to plant surfaces (Boshoff *et al.*, 1996). Several studies have been carried out to find alternative chemicals or to reduce copper sprays, including studies evaluating triazoles (e.g. cyproconazole, flusilazole, triadimenol), strobilurins (e.g. azoxystrobin, trifloxystrobin) and others (e.g. prochloraz) (Lonsdale, 1991, 1992; Duvenhage, 1994; Willis and Duvenhage, 2003; Manicom and Schoeman, 2009).

Manicom and Schoeman (2008) reviewed the literature on studies evaluating fungicides against Cercospora spot, and then later conducted a screening trial to further evaluate some of these fungicides (Manicom and Schoeman, 2009, 2010). They showed that copper oxychloride offered the best control for *P. purpurea*. It was also established that 15kg Copper/ha/year was the minimum dose required for disease control. However, they concluded that this may need to be increased to have good disease control. On the other hand, future EU regulations are likely to limit the quantity of copper that may be sprayed onto avocado crops.

Initially, later spray applications in November and December were believed to be important. However, this was found to be incorrect (Manicom and Schoeman, 2010). Sprays should actually commence late spring (October), depending on the rainfall and Z values (from the Darvas 2 model).

Currently, the avocado industry in South Africa uses a forecasting equation, (developed by Darvas in the 1980s), to predict the timing of the first spray. This equation can be used to predict the number of conidia likely to be produced and released into the atmosphere. Consequently, identification of 'high risk infection periods enable optimal timing of the first spray in his prediction and hence provides for better control of the disease (Darvas, 1982).

The Darvas equations use mean weekly air temperature (x) and total rainfall (y) as the key environmental parameters:

a) Z (number of conidia) = 24.8 (constant) – 0.93 X (X is temperature in °C) + 0.25 Y (Y is rainfall in mm) (Darvas 1982; Darvas and Kotze, 1987).

b) Z= - 58.99+3.22x (mean weekly temperature) + 0.18y (total weekly rainfall in mm) where Z represents the value indicating the likelihood of conidia in the atmosphere.

The latter equation is used by the industry. Conidia release occurs when Z>0. Cercospora infection takes place when the Z value is ≥15 and fruit is larger than pigeon egg size. The first copper spray should be applied when fruit is bigger than pigeon egg size, and the Z value is greater than five. If Z≥20, the possibility for infection is high. A second spray should be applied 4 weeks later and a 3rd spray three weeks after the second. A fourth and fifth spray should be applied at 4-week intervals after the third spray.

Darvas (1982) reported that the timing of follow up sprays is not as significant as the first spray, since the favourable period for conidia production is relatively long. The accurate timing of the first spray and spray intervals are sufficiently short to ensure good protection of fruit. Various factors, such as temperature, rainfall and fruit size, play a role in determining when the first spray should commence. Control is therefore most effective when the weather conditions that affect conidia release are monitored. A fixed spray programme is not recommended.

1.12.3 Biological control

Over the years, the desire for alternative control strategies has increased, due to unsightly copper residues, of the loss of benomyl due to pathogen resistance, the adverse effects of agrochemicals on human health and the environment, and the limited number of fungicides available (Denner and Kotzé, 1986; Darvas and Kotzé, 1987).

The definition of 'biological control' was defined by Baker and Cook (1974) as "the reduction of inoculum density or disease-producing activities of a pathogen in its active or dormant state, by one or more organisms, accomplished naturally or through manipulation of the environment, the host, or antagonist, or by mass introduction of one or more antagonists".

Biological control can be effective when antagonists are applied as pre-harvest treatments to control leaf and fruit diseases, e.g., Cercospora leaf spot on groundnuts caused by *Cercospora arachidicola*. In South Africa, the first commercial biocontrol agent developed against Cercospora spot was Avogreen (*Bacillus subtilis)*, which was

originally isolated from avocado leaf surfaces of a 'Fuerte' tree in Tzaneen, Limpopo (Korsten and Bornman, 2004).

Avogreen was evaluated in field trials for several seasons and at two major geographically distinct production areas for its ability to control pre-harvest (Cercospora spot) and postharvest (anthracnose and stem end rot) diseases (Korsten and Kotze, 1993, Korsten *et al.*, 1997) on commercially important cultivars, e.g., 'Fuerte' and 'Hass'. It was found that the most consistent and effective treatment for the disease was achieved with an integrated programme of fungicides and *B. subtilis* sprays. The timing of application of the biocontrol agent appeared to be critical to its efficacy (Korsten *et al.*, 1997). This finding makes integrated approach to managing the disease attractive to the avocado industry in South Africa. The single chemical application in the integrated approach may serve as a safeguard in years when the weather conditions do not favour the antagonistic activity of biocontrol agents. The efficacy of Avogreen was evident over several seasons in these trials, but once the product was used on a commercial scale, variation in effectiveness was reported from certain growers (Van Eeden and Korsten 2004). A further problem existed in the large-scale production and marketing of the product.

1.13 Conclusion

Avocado is a major tropical fruit with many beneficial uses. Cercospora spot is the most important pre-harvest fruit disease affecting avocados. Prevailing conditions in the regions i.e., warm temperatures favour the disease. Considering climate change, it is important that the existing model used in determining the application of the first spray is reinvestigated. Currently, copper oxychloride is the best available control measure. More research needs to be carried out to find alternative control strategies as a total dependence upon a few fungicides is a risky strategy for the avocado industry.

1.14 References

Boshoff M., Kotzé, J.M. and Korsten, L. 1996. Effect of staggered copper sprays on pre and post-harvest diseases of avocado in the KwaZulu-Natal Midlands. South African Avocado Growers' Association Yearbook 9: 49-51.

CABI. 2016. *Pseudocercospora purpurea* (spot blotch) (basic) datasheet. Crop Protection Compendium. https://www.cabi.org/cpc/datasheet/12266 Accessed 6 August 2019.

Darvas, J.M. 1978. Cercospora spot. SAAGA Research Report 2: 42 - 44.

Darvas, J.M. 1982. Etiology and control of some fruit diseases of avocado (*Persea americana* Mill.) at Westfalia Estate. D.Sc. (Agric.) thesis, University of Pretoria, Pretoria.

Darvas, J.M. and J.M. Kotzé. 1979. Cercospora spot of avocados. Research Report. South African Avocado Growers' Association 3:38-39.

Darvas, J.M., 1977. Cercospora spot. South African Avocado Growers' Association proceeding of the technical committee 1*:* 3-6.

Darvas, J.M., and Kotzé, J.M. 1987. Avocado fruit diseases and their control in South Africa. South African Avocado Growers' Association Yearbook 10:117-119.

Darvas, J.M., Kotze, J.M. and Wehner, F.C. 1987. Field occurrence and control of fungi causing postharvest decay of avocados. Phytophylactica 19(4): 453-455.

Denner, F.D.N. and Kotze, J.M. 1986. Chemical control of post-harvest diseases of avocados. South African Avocado Growers' Association Yearbook 9: 23 - 26.

Department of Agriculture Forestry and Fisheries (DAFF). 2014. Regulations relating to the grading, packing and marking of avocados intended for sale in the Republic of SouthAfrica.https://www.daff.gov.za/daffweb3/Branches/Agricultural-Production-Health-

Food-Safety/Food-Safety-Quality-Assurance/Export-Standards/Citrus-and-Subtropical-Fruit/Avocados. Accessed 10 August 2019.

Department of Agriculture Forestry and Fisheries (DAFF). 2015. A Profile of the South African Avocado Market Value Chain. Directorate of Marketing, DAFF, Pretoria, Republic of South Africa.

Department of Agriculture, Forestry and Fisheries (DAFF). 2012. Production Guideline Avocado. DAFF, Pretoria, RSA.

Department of Agriculture Forestry and Fisheries (DAFF). 2019. A Profile of the South African Avocado Market Value Chain. Directorate of Marketing, DAFF, Pretoria, Republic of South Africa.

Duarte, P.F., Chaves, M.A., Borges, C.D. and Mendonça, C.R.B., 2016. Avocado: characteristics, health benefits and uses. Ciência Rural 46(4):747-754.

Duvenhage, J.A. 1994. Control of Black Spot of Avocado by Organic and Inorganic Fungicides. South African Avocado Growers' Association Yearbook, 17: 49-52.

EPPO. 2019. *Pseudocercospora purpurea*. https://gd.eppo.int/taxon/CERCPU Accessed 11 August 2019.

Fresh fruit portal. 2018. South Africa Expects record avocado exports. https://www.freshfruitportal.com/news/2018/04/04/south-africa-expects-record-avocado-exports-in-2018. Accessed 29 August 2019.

Frey, M. 2019. Avocado Nutrition Facts. Calories, Carbs, and Health Benefits of Avocados. https://www.verywellfit.com/calories-in-an-avocado-3495640 Accessed 15 August 2019.

Infonet biovison. Cercospora fruit spot. https://www.infonet-biovision.org/PlantHealth/MinorPests/Cercospora-fruit-spot. Accessed 10 October 2019.

Korsten, L. and Bornman, C.H. 2004. Biological control in Africa: Can it provide a sustainable solution for control of fruit diseases. South African Journal of Botany *70*(1): 128-139.

Korsten, L. and Kotzé, J.M. 1993. Postharvest biological control of avocado postharvest diseases. Proceedings of the 2nd World Avocado Congress. University of California, Riverside, and California Avocado Society 2: 473-478.

Korsten, L., De Jager, E.S., Wehner, F.C. and Kotzé, J.M. 1997. Field sprays of *Bacillus subtilis* and fungicides for control of avocado pre-harvest fruit diseases of avocado in South Africa. Plant Disease 81: 455–459.

Lonsdale, J.H. 1991. Control of preharvest fruit diseases of avocado. Part 1: Efficacy of various triazole fungicides against Cercospora spot and sooty blotch. South African Avocado Growers' Association Yearbook*,* 14: 61-62.

Lonsdale, J.H., 1992. Evaluation of systemic fungicides as pre-harvest treatments of avocados. South African Avocado Growers' Association Yearbook 15: 35-38.

Manicom, B.Q and Schoeman, M.H. 2008. Fungicides for *Pseudocercospora* control on avocados: A review, a trial and observations. South African Avocado Growers' Association Yearbook 31: 35-41.

Manicom, B.Q and Schoeman, M.H. 2009. Evaluation of fungicides for *Pseudocercospora* control (II). South African Avocado Growers Association Yearbook 32: 70-72.

Manicom, B.Q. and Schoeman, M.H. 2010. Evaluation of fungicides for *Pseudocercospora* control – final report. South African Avocado Growers' Association Yearbook 33: 70-72.

Manicom, B.Q., 2001. Diseases. In: The Cultivation of Avocado. de Villiers, E.A. (Ed.). Institute for Tropical and Subtropical Crops, Nelspruit, South Africa, pp.170-171.

Menge, J. A. and R. C. Ploetz. 2003. Disease of Avocado in Diseases of Tropical Fruit Crops. CABI Publishing, Wallingford, UK pp. 527.

Motaung, N., 2019. Avocado extracts market overview. Farm Biz *5*(3): 44-44.

Obi, L. 2017. What the avocado export wants from you. https://www.nation.co.ke/business/seedsofgold/What-avocado-export-market-wants-from-you/2301238-4172194-h78exr/index.html. Accessed 11 October 2019.

Willis, A. and Duvenhage, J.A. 2003. Progress report on evaluation of alternative fungicides for control of Cercospora spot. South African Avocado Growers' Association Yearbook*,* 26: 45-49.

Pariona, A. 2017. The biggest avocado producing countries. In The World Atlas.https://www.worldatlas.com/articles/the-biggest-avocado-producing-countries-in-the-world.html/. Accessed 10 August 2019.

Pérez-Jiménez, R.M. 2008. Significant avocado diseases caused by fungi and *oomycetes.* European Journal of Plant Science Biotechnology *2*(1): 1-24.

Pernezny, K. and Marlatt, R.B. 2000. Diseases of Avocado in Florida. Plant Pathology Fact Sheet, University of Florida. Florida. USA. pp.21.

Ploetz, R.C. (Ed.) 1994. Compendium of Tropical Fruit Diseases (Vol. 88). APS Press, St Paul, Minn., USA.

QD Friends. 2012. Avocados. https://qdfriends.wordpress.com/2012/04/05/avocados-2/. Accessed on 5 September 2019.

SAAGA. 2017. Final report: South African Loss Factor Benchmark Report. pp. 1 - 64

SAAGA. 2019. Overview of the South African avocado industry by the South African Avocado Growers Association. https://www.avocado.co.za/overview-of-sa-avocado-industry/. Accessed on 30 September 2019.

SAAGA. 2020a. Products registered for disease control on avocados in South Africa.

https://www.avocado.co.za/wp-content/uploads/2020/01/Registered-FUNGICIDES-list-AVOCADO_January-2020.pdf. Accessed on 15 May 2020.

SAAGA. 2020b. Summary of Registered Chemicals, MRLs & PHIs on Avocados in South Africa.
file:///C:/Users/KallideenR/AppData/Local/Microsoft/Windows/INetCache/Content.Outlook/UDQ1C9JC/MRLs_Avocados_April%202020%20(004).pdf Accessed on 15 May 2020.

Schaffer, B.A., Wolstenholme, B.N. and Whiley, A.W. (Eds). 2013. The Avocado: Botany, Production and Uses. CAB International, Wallingford, UK.

Schoeman M.H and Kallideen, R. 2018. Cercospora spot on avocado – A preliminary report on the relook at the epidemiology of the pathogen. South African Avocado Growers Association Yearbook 41: 84-88.

Schoeman, M.H. and Kallideen, R. 2019. Validating spray timing for Cercospora spot. South African Avocado Growers Association Yearbook 42: 12-18.

Sihlobo, W. 2018. A Brief History of Avocado Production in South Africa.
https://wandilesihlobo.com/2018/08/13/brief-history-of-avocado-production-in-south-africa/#targetText=Some%20studies%20suggest%20that%20avocados,the%20regions%20around%20Mpumalanga%20province. Accessed 11 September 2019.

Sihlobo, W. 2019. Chinese Potential for SA Avocado Exports.
https://www.agriorbit.com/agrimarkets-chinese-potential-for-sa-avocado-exports/. Accessed 11 September 2019.

Sikuka, W. 2019. New Markets Critical for the Growing South African Avocado Industry.https://apps.fas.usda.gov/newgainapi/api/report/downloadreportbyfilename?filename=New%20Markets%20Critical%20for%20the%20Growing%20South%20African%20Avocado%20Industry_Pretoria_South%20Africa%20-%20Republic%20of_7-24-2019.pdf. Accessed 12 October 2019.

Sippel, A.D. 2001. Origin and history of the avocado. In: The Cultivation of Avocado. de Villiers, E.A. (Ed.). Agricultural Research Council-Institute for Tropical and Subtropical crops, Nelspruit, RSA. pp 9-10.

Stone, J. 2019. Guess Who's Snapping Up Most Of South Africa's Avos. https://www.2oceansvibe.com/2019/04/24/guess-whos-snapping-up-most-of-south-africas-avos/#ixzz62i99IBP3. Accessed 13 September 2019.

Van Eeden, M. and Korsten, L. 2004. Determining appropriate parameters for an integrated disease control system for biological predictive modelling. South African Avocado Growers' Association Yearbook 27: 83–95.

Waterworth, K. 2018. Avocado Black Spot: Learn About Cercospora Spot in Avocados. https://www.gardeningknowhow.com/edible/fruits/avocado/cercospora-   spot-in-avocados.htm/. Accessed 10 September 2019.

Willis, A. and Mabunda, R.S. 2004. Alternative control of Cercospora spot on Fuerte. South African Avocado Growers' Association Yearbook 29: 53-56.

CHAPTER 2
Epidemiology of Cercospora spot on avocados

Abstract

Cercospora spot, caused by *Pseudocercospora purpurea,* is the most severe pre-harvest disease of avocado in South Africa. Losses of up to 70% were reported on unsprayed trees. In the early 1980s, Darvas developed two predictive models to forecast the number of conidia, and the timing for the first spray, an approach still used today. He also proposed that avocado fruit was only susceptible when it was larger than 40mm in diameter. Due to climate change and possible adaptions in the pathogen populations, it was crucial to re-evaluate this model. The primary aim of this study was to determine if the current model is still valid, secondly, to consider including humidity and/or leaf wetness in the formula, and thirdly whether avocado fruit smaller than 40mm are susceptible. In the 2017/18 season, spore traps were placed in two unsprayed 'Fuerte' orchards (at HL Hall and Sons (Halls), and at the Agricultural Research Council -Tropical and Subtropical Crops (ARC- TSC)). During the 2018/19 season, the spore traps were only at the ARC -TSC site. Vaseline-coated slides on the spore traps were changed hourly, daily and weekly. Concomitantly, a bagging trial to determine the critical infection periods was carried out at the Halls site in the 2017/18 season, and at ARC -TSC in the 2018/19 season. About a thousand fruits (<25mm in diameter) per season were covered with paper bags. Every fortnight, between thirty and fifty bags were removed to allow for infection and were then replaced at the end of the fortnight. At harvest, fruit were evaluated for levels of Cercospora spot using a disease rating scale. The spore trapping data were correlated with weather data to develop forecasting models. In the 2017/2018 season, a strong correlation was found between weekly conidia trapped and weekly rainfall. In the 2018/19 season, the strongest correlation (negative) was found between weekly conidia trapped and mean maximum temperature. In both seasons, humidity and leaf wetness were not found to be significant factors in conidia release and were therefore not included in any model. Avocado fruit became susceptible at least at 25mm in diameter, which introduces a significant change to the timing of the first spray. The Darvas 2 model is still a valid forecasting tool but should be used in combination with monitoring of fruit size with a 25mm threshold to ensure accurate and effective timing of the first spray.

Key Words: *Pseudocercospora purpurea*, Cercospora, pre-harvest disease, avocado, 'Fuerte', epidemiology, temperature, humidity, spore count

2.1 Introduction

Avocado black spot, otherwise known as Cercospora spot, is the most severe pre-harvest fruit disease of avocado in South Africa (Darvas and Kotze, 1987; Willis and Mabunda, 2004; Schoeman and Kallideen, 2018). From a quality perspective, while not particularly affecting the flesh of the fruit, the disease can cause losses of up to 70% on unsprayed trees (Darvas and Kotze, 1987; Manicom, 2001). Infected leaves are the main source of inoculum for the continuation of the disease from one season to the next. Infections on fruits arise primarily from inoculum from infected leaves.

Currently, Cercospora spot control is predominantly based on timed spray applications of copper- containing fungicides, e.g., copper oxychloride, copper hydroxide and cuprous oxide. Other fungicides also registered against Cercospora spot on avocado include azoxystrobin and carbendazim (SAAGA, 2020a). The first spray is the most important spray of the season, as the whole control programme for the season is depedent on the first spray. Therefore, the timing of this spray and good coverage of the fruit will determine the level of control of Cercospora spot for that season. Control is also more effective when weather variables such as temperature, rainfall, etc. that affect conidia release, are monitored, and used as a basis for the timing of spray applications. Additionally, fruit size is a significant factor in deciding when to apply the first spray.

The South African avocado industry uses prediction models developed by Darvas in the 1980s to make an informed decision regarding copper spray applications for Cercospora spot disease. The current prediction models are based on temperature and rainfall:

Darvas 1 Z = 24.8 (constant) – 0.93X (X is temperature in C) + 0.25Y (Y is weekly rainfall in mm).

Darvas 2 Z = -58.99(constant) + 3.22X (X is mean weekly temperature in C) + 0.18Y (Y is the weekly rainfall in mm). "Z" is the value indicating the likehood of conidia in the atmosphere.

(Darvas, 1982; Darvas and Kotze, 1987).

The Darvas 1 model starts with a positive constant, from which is deducted a fraction of the temperature, but to which is added a fraction of the rainfall. In this model, the temperature and rainfall are opposing parameters: increasing temperatures will reduce the Z value, whereas increasing rainfall will increase the Z value.

The Darvas 2 model starts with a larger, negative constant to which is added a multiple of the mean weekly temperature and a fraction of the weekly rainfall. In this model, the temperature and rainfall are complementary parameters: increasing temperatures and increasing rainfall will both increase the Z value.

The Darvas 2 model is currently used by the industry to predict the number of conidia released in an agricultural area and the optimum timing of the first spray, based on critical Z values (Darvas, 1982). Conidia release ensues when Z is greater than 0, and the potential for Cercospora infection is high when Z exceeds 20. Hence, it is vital to apply the first spray before Z values exceed 20 (Darvas, 1982). Recommendations by SAAGA are to apply the first copper spray for the 'Fuerte' cultivar, when fruit size is >2.0cm and or the Z value >15 (Campbell, 2016).

Despite leaf wetness/humidity not being one of the weather variables included in these models, Darvas (1982) found that relative humidity played a key role in conidia release because most conidia were trapped when high humidity occurred, in the early mornings. It should be noted that Darvas' Z value models were established from only 10 days of data, of which two days' accounted for the accuracy of the model. Over those 10 days, the temperature ranged from 20 – 26°C in 1979. Considering all of the above, as well as the impact of climate change, it was therefore decided to re-assess the Darvas 2 model under current environmental conditions.

Raid *et al.*, (2008) evaluated the use of two forecasting models (the Tomcast and the Berger models) in scheduling fungicide sprays for the management of early blight of celery, caused by *Cercospora apii* (Fresen.)*.* Both models offered excellent control of early blight for two seasons, with three to four sprays compared with the weekly calendar spray of 13 sprays. Timely application of sprays using these two disease-forecasting models can assist growers in saving money. The Tomcast forecasting system is also

used in tomatoes for the prediction of infection by *Alternaria solani* (Sorauer) and has also accurately predicted the potential for *Alternaria dauci* (Groves & Skolko) blight in carrots (Chaput, 2000).

Kushalappa and Brodeur (1989) analysed the carrot growth stages affected by Cercospora blight, caused by *Cercospora carotae* (Kazn. & Siemaszko) in order to develop alternative methods for predicting the disease incidence threshold recommended to commence fungicide application. Regression equations were developed with days after the cotyledon stage (DAY), plant growth stage (GS), and degree-day with a base of 7°C (DD$_7$), respectively. The variation in the rate of blight development was 83, 86, and 85%, and the disease incidence threshold of 50% was reached at 48 DAY, GS 8.6, and 557 DD$_7$. These alternative action thresholds were used to time the first fungicide treatment for late carrots. Predictive equations were not developed for early carrots because the disease developed late, and few or no fungicide applications were needed earlier.

Olatinwo *et al.*, (2012) studied a prediction model used for monitoring early leaf spot in peanut, caused by *Cercospora arachidicola* (Hori). They examined the use of a high-resolution Weather Research and Forecasting (WRF) model for the management of early leaf spot disease in peanut caused by *C. arachidicola.* Since the development of early leaf spot on peanut and spread of the *C. arachidicola* spores depend on favourable weather conditions, accurate spatio-temporal weather data was essential for monitoring the development of favourable conditions and the potential threat of the disease. They combined the weather output of the WRF (which included the relative humidity and temperature variables) with the Oklahoma peanut leaf spot advisory model in predicting favourable conditions for early leaf spot infection. Spatial maps were generated of accumulated daily infection hours and an infection threat index was introduced. The infection hour was calculated, based on the combination of hourly weather conditions being met with the assumption that 36 infection hours is necessary for leaf spot infection initiation to take place. Growers could use this as an early warning tool to monitor imminent threat as the threshold level for favourable condition for infection approaches. Therefore, as soon as the threshold of 1.0 has been reached, a leaf spot threat warning would be issued, and the infection hour would revert to zero, given that suitable management measures had been taken (i.e., fungicide application).

They also found that the short-term prediction of weather parameters in the management of peanut diseases is a practical and promising technique that could help growers to make better decisions regarding the management of early leaf spot and thus reduce the effect of disease by optimal timing of spray applications.

Beetcast is a weather forecasting model used for Cercospora leaf spot on sugar beet (*Cercospora beticola* (Sacc.)*. This model uses temperature and leaf wetness data to generate a disease severity value (DSV) that is based on requirements for growth and development of *C. beticola*. Scouting should be used in combination with the Beetcast model (Poindexter, 2006). By using the model to time every fungicide application including the first, growers are able to make one or multiple fungicide applications before symptoms appear, allowing for improved Cercospora leaf spot disease management throughout the season (Poindexter, 2006).

The aims of this chapter were therefore: (1) to determine whether the Darvas 2 forecasting model developed by Darvas in 1982 is still a valid model for predicting conidia release and the timing of the first preventative fungicide spray for effective control of Cercospora spot; (2) to evaluate new models that would include humidity and/or leaf wetness in the formula as a third environmental parameter; and (3) to evaluate the size of the juvenile fruit at which stage the fruit becomes susceptible to the conidia of *P. purpurea*, and thereby to determine whether the size of 40mm, as recommended by Darvas (1982) is the ideal phenological age to start spraying.

2.2 Material and methods

2.2.1 Vaseline slide preparation

Slides with one frosted end were used. Petroleum jelly (Vaseline®) was placed into a 500ml beaker (two-thirds full) and slowly heated on a hot plate until it melted to a clear yellow colour. Two slides at a time were dipped into the beaker of melted Vaseline, holding the frosted ends. The slides were separated and then placed onto a paper towel so that the Vaseline could set. A scalpel was used to scrape off excess Vaseline on the edges of the slides to prevent the slides from smearing when placed into the microscope slide box.

2.2.2 Experimental site and Spore trapping for two seasons

The trial was conducted at two unsprayed 'Fuerte' orchards, namely at HL Hall and Sons (Halls) (25°23'31.96"S, 30°55'39.94"E) (Fig. 2.1) and at the Agricultural Research Council -Tropical and Subtropical Crops (ARC-TSC) (25°27'04.6"S, 30°58'09.1"E) (Fig. 2.2), both located in Nelspruit, Mpumalanga. 'Fuerte' avocados were used in this study because it is the most problematic cultivar for pre- and postharvest disease problems (Partridge, 1990). In the 2017/18 season, four spore traps were placed in each of the two orchards. A metal strip was also placed on the traps to prevent birds from alighting on the slides. The ARC-TSC orchard had a very high disease pressure, due to a build-up of inoculum in the trees over the past two decades because copper sprays were not applied for many years.

Two of the four spore traps had four petroleum jelly-coated slides (Vaseline®) held horizontally and two had four slides held vertically, facing the four different wind directions. Vaseline slides were held in place with clothes pegs that were attached to the end of two metal strips placed perpendicular to one another, mounted on top of a 1.5m metal pole (Fig. 2.3). Conidia release for the 2017/18 season was monitored at the ARC-TSC site from the 6th of September 2017 to the end of March 2018. The slides were changed weekly for the entire period, and daily for a period of three months beginning in late November. For the daily spore trapping four extra pegs were placed onto two horizontal traps used previously (weekly spore trapping). Hourly spore trapping was carried out for a period of 10 days' (November) and slides were changed hourly from 23.00 to 03.00 the next morning. Slides were stained with methylene blue dissolved in water at 0.05% [m/v] to visualize and count *P. purpurea* conidia

microscopically.

The weather data for the 2017/18 season was acquired from the ARC-TSC weather station for the ARC-TSC orchard. Additionally, a Decagon EM50 data logger (Decagon Devices, Washington, United States) was placed in the ARC-TSC orchard to measure leaf wetness. At the Halls orchard, Hobo U23 Pro v2 data loggers (Onset computer cooperation, Massachusetts, United States) were installed to measure temperature and humidity. Additional weather data were obtained from the nearest government weather stations. Weather data were correlated with spore trapping data.

An adjustment was made in the 2018/19 season whereby only two horizontal spore traps consisting of four Vaseline coated slides were placed at the ARC-TSC site, because this spore trap was most efficient in trapping conidia in the previous season (Fig. 2.4). The slides from each spore trap were changed weekly from October to the end of the season and changed daily from September to March 2019. The slides were viewed as was described previously. The weather data was obtained from the nearest weather station.

Figure 2.1: Spore trapping and bagging trial at the HL Hall & Sons site (2017/18 season)
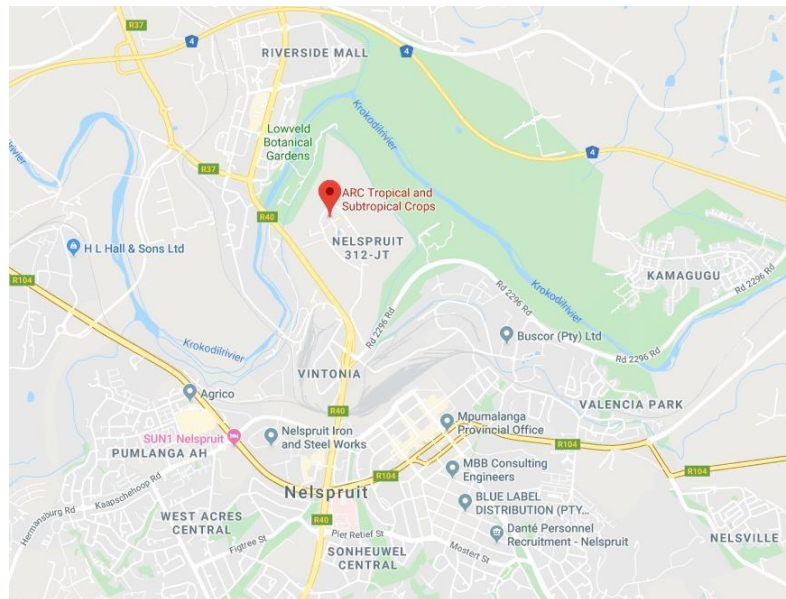
Figure 2.2: Experimental site at ARC-TSC used for spore trapping (2017/18 and 2018/19) and bagging (2018/19)

Figure 2.3: Spore trap placed in a 'Fuerte' orchard

Figure 2.4: Spore trap with two horizontal Vaseline slides

2.2.3 Critical infection periods

In 2017/18, a bagging trial was carried out at Halls to detect the critical infection period for fruit infection by *P. purpurea* under natural orchard conditions. Approximately one thousand five hundred fruits were covered with brown paper bags when they were 20-30mm in diameter before they reached the reported susceptible stage at 40mm diameter. The fruits were covered with brown bags and non-absorbent cotton wool, which were sealed with duct tape to prevent water runoff onto the fruit (Fig. 5).

Every two weeks, fifty bags were removed to allow natural infection, and were then covered again after the two week exposure period. The opening of bags for the two weekly exposure periods commenced from 11 October 2017 and continued until 28 March 2018, providing twelve exposure periods. Once the fruits were harvested in April 2018, they were evaluated for the presence of Cercospora spot using the following scale, from 0 to 5 (Table 2.1).

Table 2.1: Cercospora spot disease rating scale

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

Fruit categorised in Classes 1-3 were separated based on the combined lesion diameter. It was essential to differentiate between those fruit that had 1-5 large lesions and 1-5 small lesions. The disease index was calculated using a formula:

Infection index = (sum of all numerical ratings / total number of fruit) x (100 / maximum disease category (5)), (Wheeler, 1969).

The disease index data was correlated with weather data obtained during the season.

For the 2018/19 season, the bagging trial was carried out at ARC-TSC because the disease incidence was much higher than at Halls. At the beginning of October 2018, one thousand fruit that were between 20 – 25 mm in diameter were covered with brown paper bags. Initially, thirty bags were removed and replaced every two weeks but as the season progressed, the number of bags was reduced to twenty because many bags were lost due to severe weather conditions, and many fruits were lost due to a November fruit drop (i.e. the premature shedding of unripe fruit from a tree). Additionally, fruit were also exposed monthly from October 2018 to January 2019. In April 2019, the fruit was harvested and evaluated as described for the previous season.

Figure 2.5: Avocado fruit covered with brown paper bags

Statistical analysis

Using Multiple Stepwise Linear Regression Analysis (SAS software), the spore trapping data for both seasons (2017/18 and 2018/2019) were correlated with the weather data and the disease index data to develop the forecasting models.

Results

2.3.1 Weekly spore trapping (2017/2018 Season)

A weekly spore trapping and bagging trial was carried out at the Halls site, whilst at the ARC-TSC site both weekly, daily and hourly spore trapping data were collected. The following results refer to the 2017/18 spore trapping data season at the ARC-TSC site only (Fig.2.6), because no conidia were trapped at Halls due to a ow disease incidence at that site.
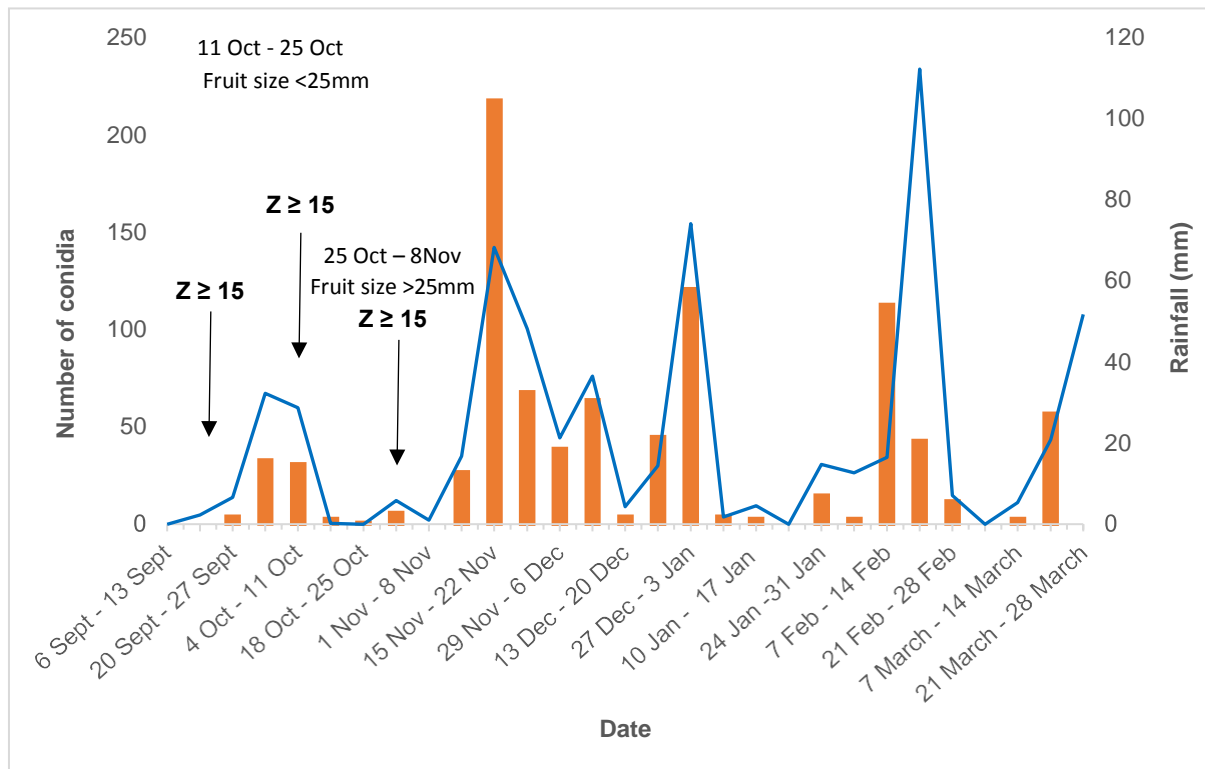
Figure 2.6: Number of conidia trapped weekly at the ARC-TSC site, and weekly rainfall in the period September 2017 to March 2018

The first time that conidia were found was in the period 20 to 27 September and thereafter in the following two periods upon commencement of the rainy season (Fig. 2.6). In the period 6 to 19 September conidia were not detected, despite conidia release being predicted by the Darvas 2 model (Z ≥ 15). High temperatures recorded in September were the most probable cause for the incorrect prediction. The next time that the Z value was Z ≥ 15 occurred on 5 October (a one-day period). At this stage, it would have been too early to spray because the fruit size was under 25mm in diameter. In the period 25 October to 1 November, some rain was recorded and conidia were trapped

Using the Darvas 2 model, it was established that spraying should have already begun before this period because Z ≥ 15, and the fruit was large enough to be susceptible. This spray was of utmost importance because spore numbers started to increase rapidly after this period. A large number of conidia were trapped in the period 15 to 22 November when more than 68 mm rain was measured. Throughout the season, several other peaks were detected following rainfall events, although this was not seen

39

near the end of the season. Conidia were detected even as late as the last week of March.

A good correlation (r=0.898) was obtained between the weekly number of conidia trapped and rainfall for the period September to December 2017 (Fig. 2.7). However, over the whole season (September to March 2018), the correlation (r=0.609) was slightly lower (Fig. 2.8) as a result of relatively few conidia being trapped, irrespective of high rainfall being experienced in mid-February. There were no significant correlations with any of the other weather variables measured (temperature, humidity, leaf wetness).

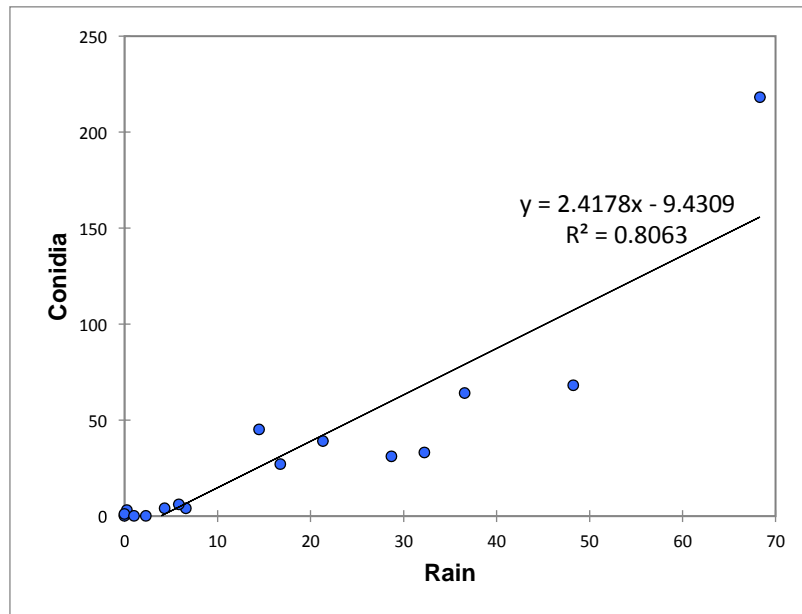The chart plots Conidia on the y-axis (0–250) against Rain on the x-axis (0–70), with a linear trend line labelled y = 2.4178x - 9.4309 and R² = 0.8063.

Figure 2.7: Correlation between counts of conidia and rainfall for September – December 2017/18
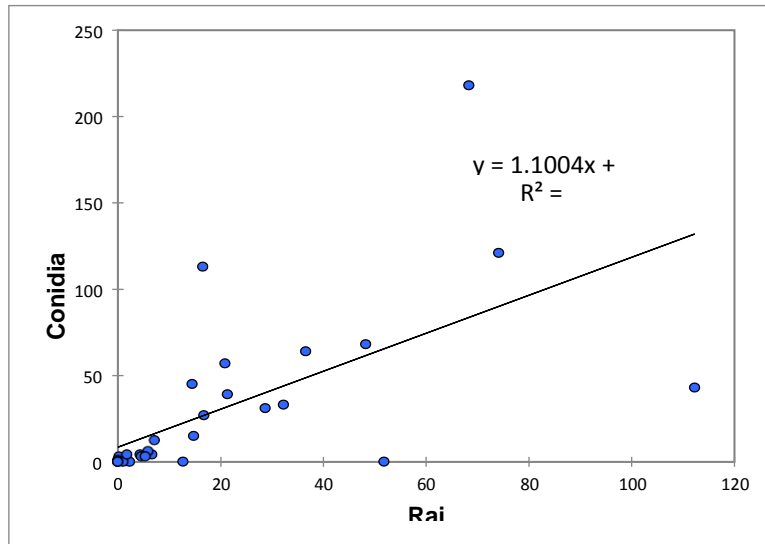
Figure 2.8: Correlation between counts of conidia and rainfall for September – March 2017/18

2.3.2 Daily spore trapping

The number of conidia trapped daily at the ARC -TSC site from 29 November 2017 to 27 February 2018 are presented in Fig. 2.9.

Figure 2.9: Number of conidia trapped at the ARC-TSC site, and weekly rainfall in the period November 2017 to February 2018. The daily data is shown as weekly counts. No data was collected from 23 December – 8 January.

A general finding was that conidia were trapped in periods where rainfall occurred, as was found with the weekly spore trapping. A poor correlation was obtained when the number of conidia was correlated with any of the other weather variables.
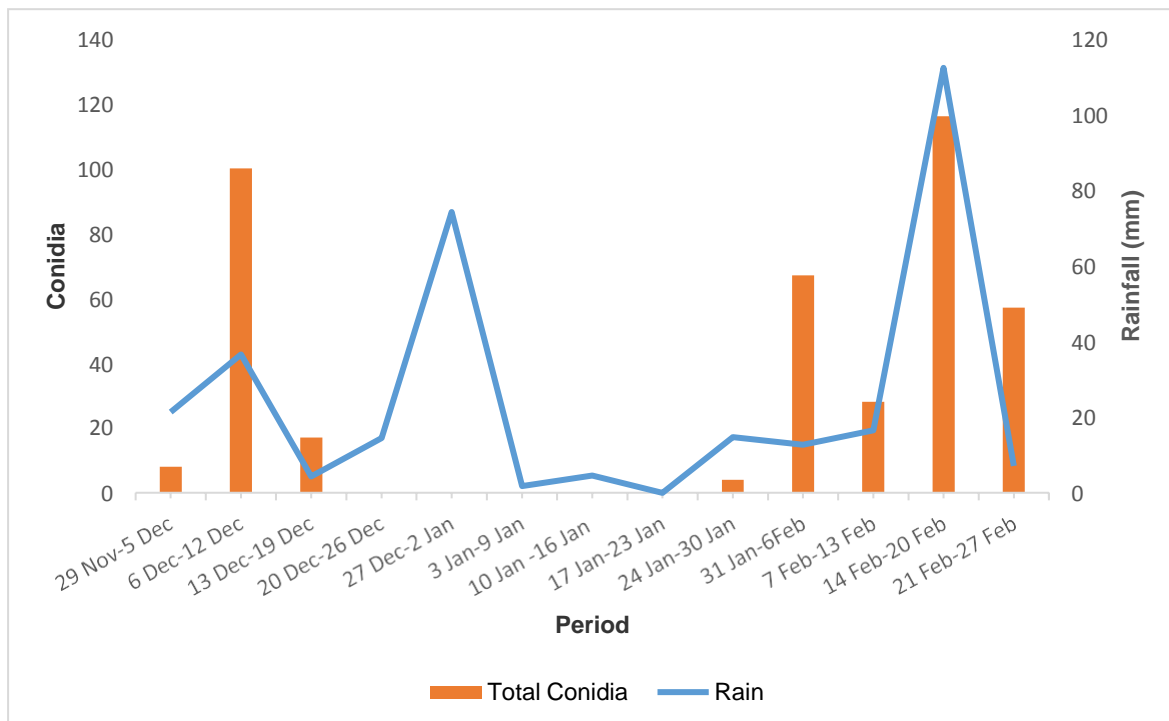
2.3.3 Hourly spore trapping

No conidia were found on the Vaseline slides that were changed hourly between 23.00 at night and 03.00 the next morning for a 10-day period (12-21 November 2017). The 4-hour period for the hourly replacement of slides was not sufficient to trap conidia. Although rainfall occurred during the 10- day trapping period in our study, we were unable to detect conidia in the hourly periods monitored as the effect of rainfall on spore release was only evident later and not in the period that the slides were being sampled.

2.3.4 Critical Infection periods of 2017/18 season

There were a total of twelve critical infection periods in addition to one index for 'fruit covered' for the whole period at the ARC-TSC site, and one index for 'fruit exposed' for the whole season (October 2017 – March 2018) at the Halls site (Fig. 2.10).
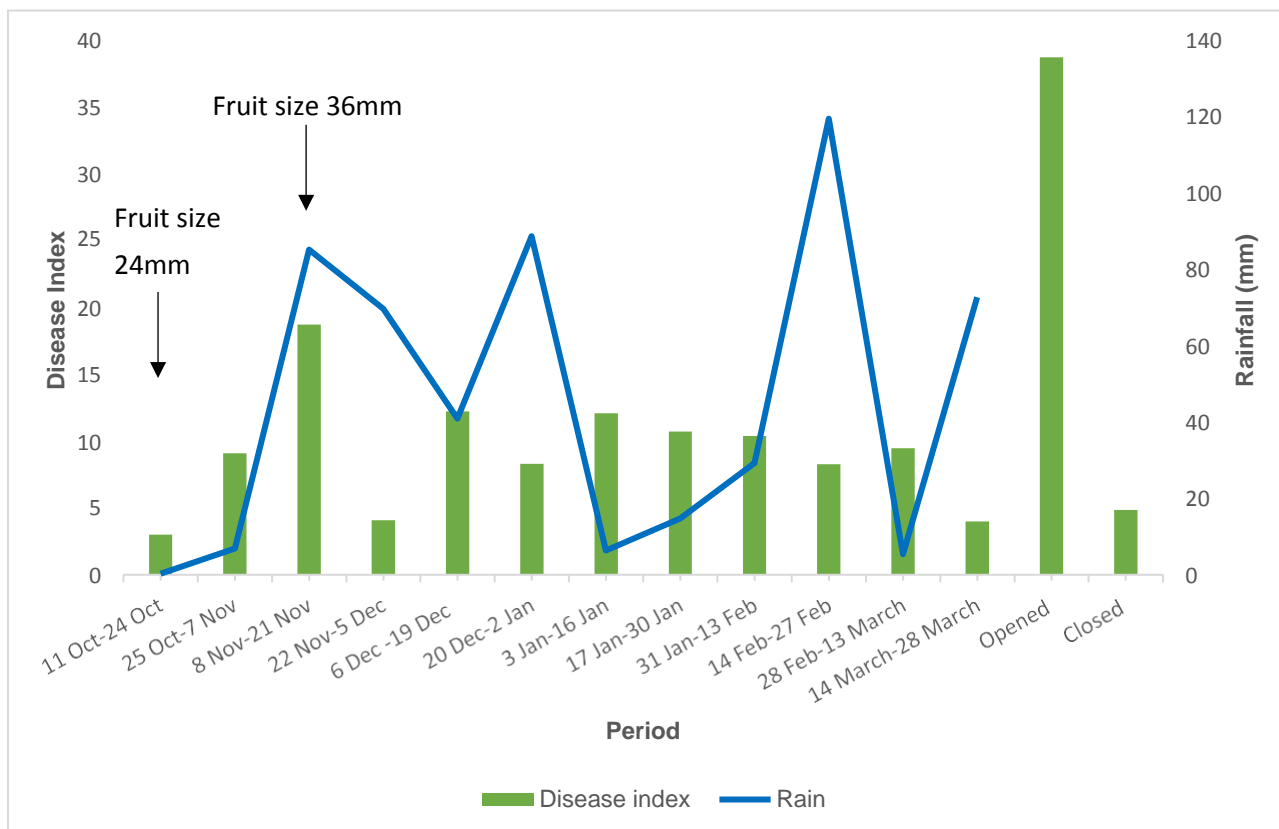
Figure 2.10: Disease severity of Cercospora spot, and rainfall at the Halls site for the 2017/18 season

Cercospora spot infection took place throughout the season from October 2017 to the end of March 2018. Fruit exposed to the natural inoculum of *P. purpurea* for the entire season had the highest infection indices. Infection had already taken place in the period 11 October to 24 October, and in that period fruit size was only 24mm in diameter. The highest two weekly infection indices were recorded in the period 8-21 November when 85mm of rainfall occurred. Fruit size at this stage was 36mm in diameter (<40mm). This two-weekly period corresponded to the two weekly periods (8 November to 14 November and 15 November to 21 November) where 27 and 218 conidia were found, respectively. It was also the period where the largest number of conidia (218) were trapped during the season. Fruit covered for the entire season were also infected, although the disease index was very low. Infection of the fruit may have occurred because some bags were damaged during the season, or that some leakage of bags occurred, or these infections occurred before bagging took place.

2.3.5 Weekly spore trapping (2018/19 Season)

The weekly spore trapping data and rainfall data for the 2018/19 season (ARC-TSC) are presented in Fig. 2.11.
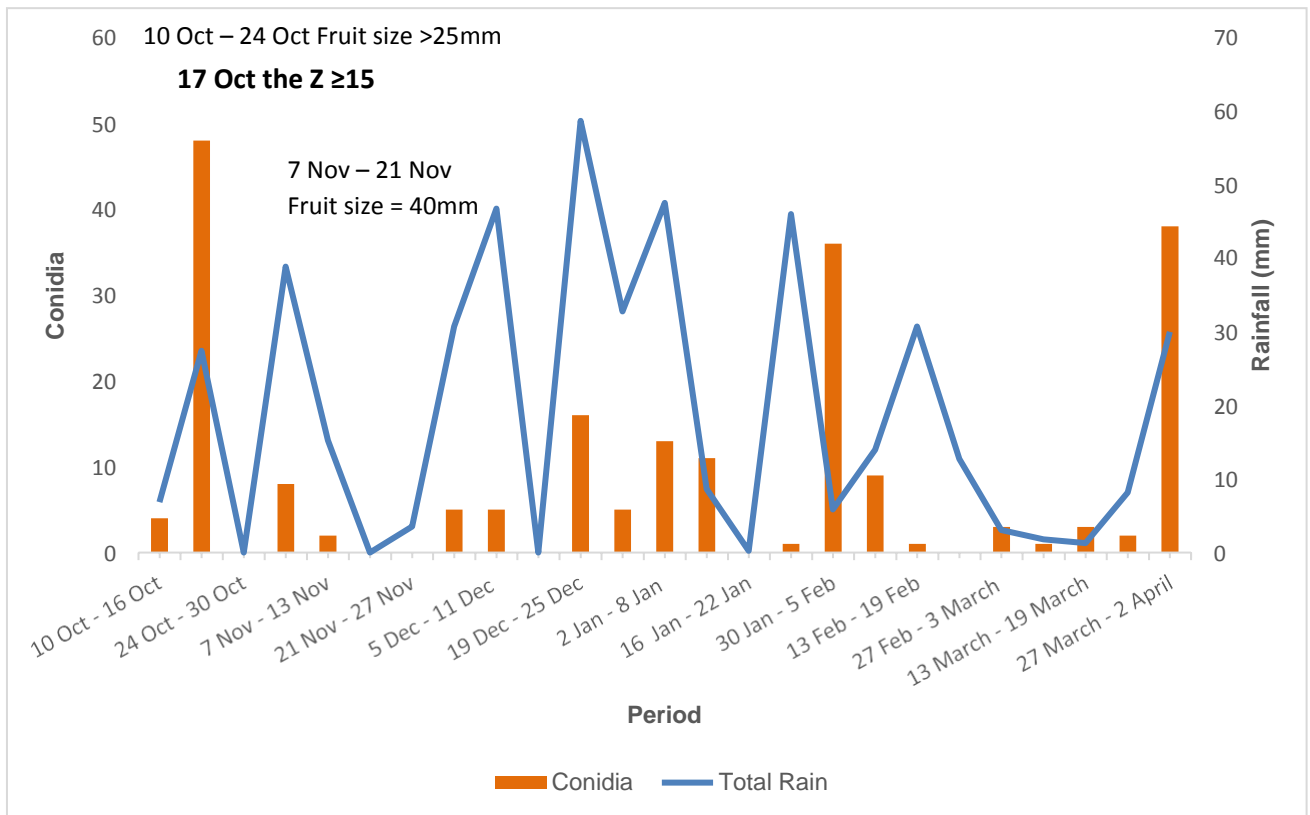

Figure 2.11: Number of *P. purpurea* conidia trapped weekly at the ARC-TSC site, and weekly rainfall from October 2018 to April 2019

The Z value was greater than 15 in the periods 18 to 23 September and 27 September – 2 October (not shown on graph). Very few conidia (4) were detected in the period 10 October to 16 October. However, rainfall increased in the next period (17- 23 October), and more *P. purpurea* conidia (48) were found. At this stage, the fruit was already larger than 25mm in diameter. The next time that Z ≥ 15 was on the 17 October but this was only for a one-day period. Few conidia (8) were detected in the period 31 October – 6 November when the Z value was calculated to be greater than 15. The Darvas 2 model predicted the presence of conidia in the period 14 – 20 November but none were detected. Several peaks occurred throughout the season following the pattern of rainfall. The overall trend observed was that no conidia were found in periods where rainfall was absent. In the last week of March (27 March to 2 April), 30mm of rainfall was recorded and the number of conidia increased (38). For the period October to December, a strong negative correlation was found (r= -0.696) between counts of

conidia and mean maximum temperature. For the entire season (October to April), the correlation between counts of conidia and mean maximum temperature was slightly lower (r= -0.520). This negative correlation between conidia and temperature may have been linked to the cooling effect of rain. Since the peak in conidia release occurred in the period 17-23 October, spraying was needed before this period. Disease index values in Fig. 2.13 indicates that infection had already taken place in the period 10-23 October, and that spraying before this period was crucial to prevent infection.

2.3.6 Daily spore trapping

The daily number of conidia trapped during the 2018/19 season at ARC-TSC are presented as weekly counts in Fig. 2.12.
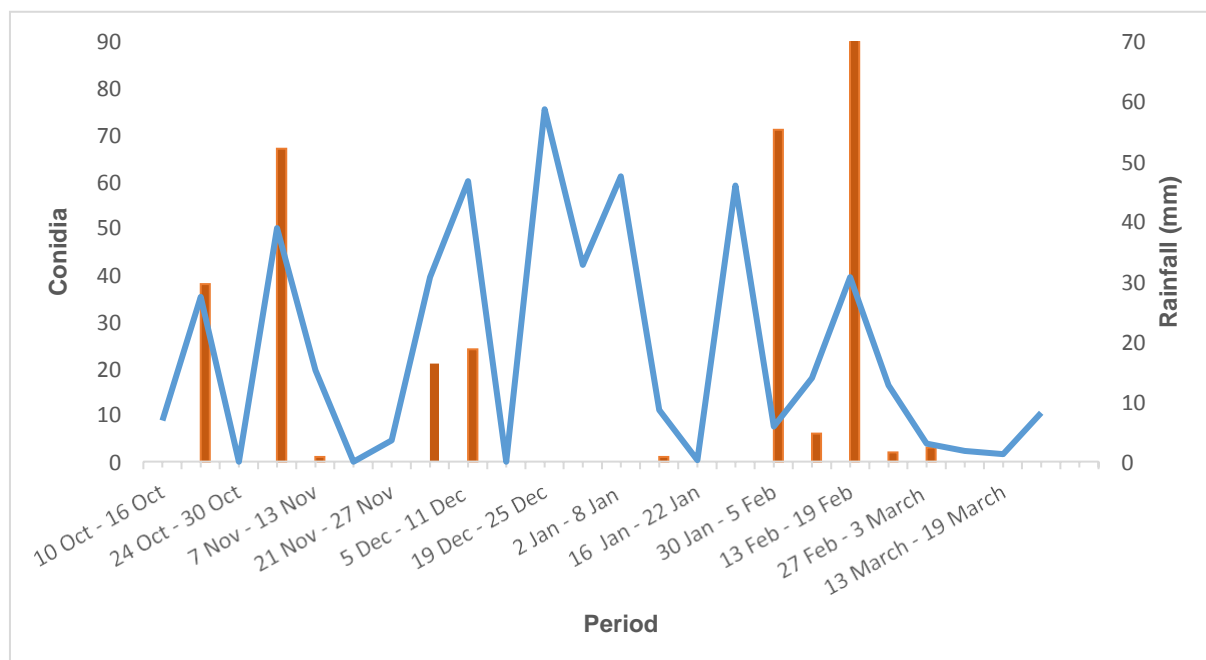
Figure 2.12: Numbers of conidia trapped at the ARC-TSC site, and weekly rainfall in the period October 2018 to March 2019. No data was collected from the 21 December – 6 January.

Conidia were found in the period 17-23 October as was found with the weekly spore trapping data (Fig. 2.12). Conidia were not found in periods were rainfall did not occur, as with the weekly spore trapping counts. When the daily spore trapping data was presented as weekly counts, a good correlation was found between weekly conidia and leaf wetness (r=0.526) for the entire season, whilst for the October to December period, rainfall was more significant (r=0.792). Negative correlations were found

between weekly conidia and temperature (maximum temperature, minimum temperature and mean temperature), as was found with the actual weekly data (Fig. 2.11).

2.3.7 Critical Infection periods

2.3.7.1 Two weekly infection periods

The two weekly infection periods were evaluated from October 2018 to February 2019. The rainfall values for each two weekly period and their disease index values are presented in Fig. 2.13.
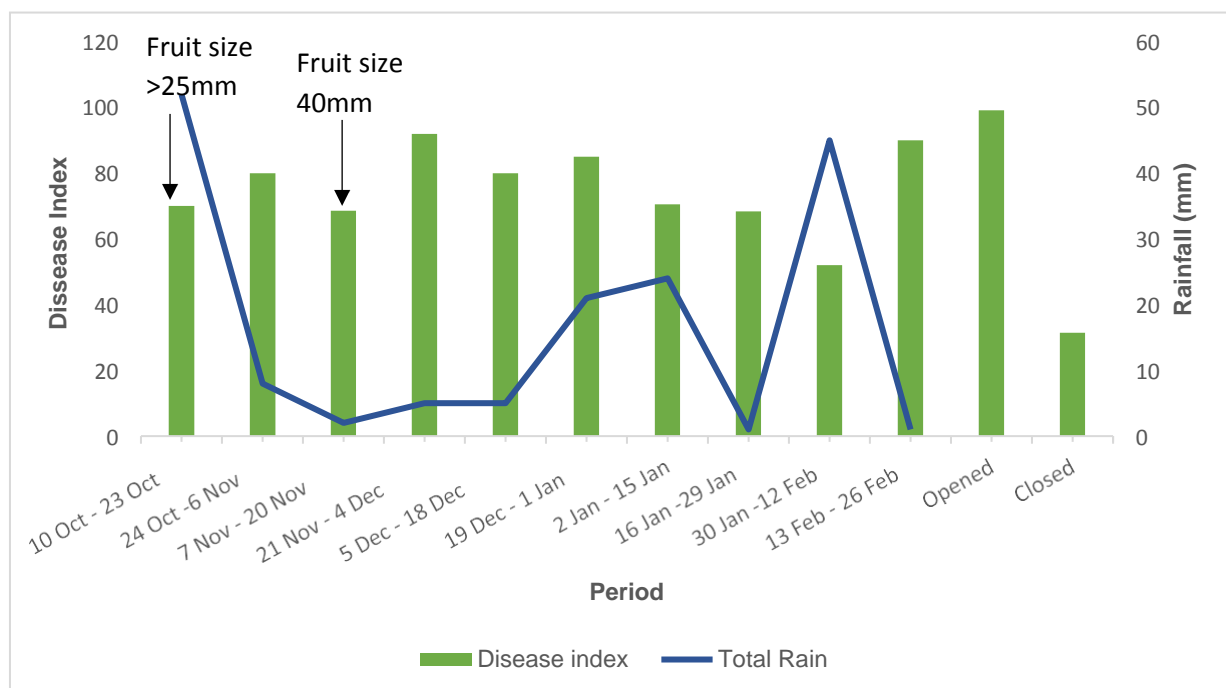
Figure 2.13: Cercospora disease severity vs rainfall during the 2018/19 season at ARC-TSC

There were several two weekly exposure periods where the infection indices for Cercospora spot were high (Fig. 2.13). Infection in the period 10 October – 23 October corresponded with the large number of conidia found in that period. The fruit size at that stage was 30mm in diameter, indicating that fruit are already susceptible when <40mm. The highest infection index was found in the period 21 November – 4 December at which point fruit size was greater than 40mm. Infection took place throughout the season, similarly to the previous season. Some infection was found in bags closed for the entire season, as was found the previous season, and this could

have been due to damage or leakage, or prior infections. For the period October to February a weak correlation (r= 0.442) was found between conidia and rainfall, whereas a strong correlation (r= 0.642) was found between conidia and mean temperature for the period October to December 2018.

2.3.7.2 Monthly infection periods

The monthly infection periods were evaluated from October 2018 to January 2019. The monthly disease index values are presented in Fig. 2.14.
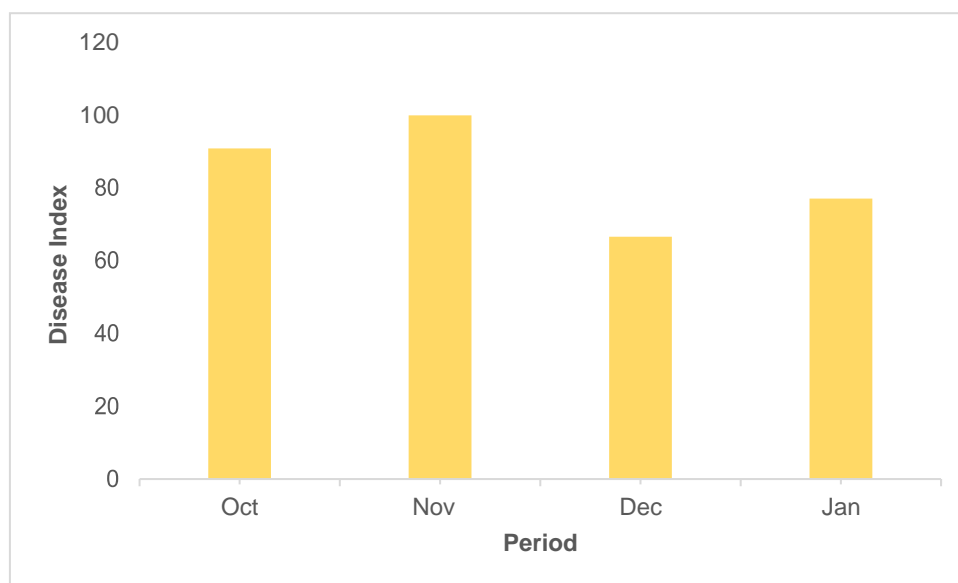
Figure 2.14: Monthly Cercospora disease severity at ARC-TSC.

Disease severity of Cercospora spot on fruit exposed for a monthly period was evaluated from October to January (Fig. 2.14). Infection occurred in all the periods but the first two periods (October and November) had a higher infection index than the last two periods (December and January). Negative correlations were established between the infection index and the weather variables. The highest negative correlation was found between infection index and maximum relative humidity (r=- 0.962) and the second highest correlation was found with infection index and mean minimum temperature (r=-0.744).

2.3.7.3 Comparison of monthly Darvas 2 critical infection equation and monthly Cercospora disease severity of 2018/19 at the ARC-TSC site

Darvas (1982), assessed the number of Cercospora spots per fruit on a monthly basis to detect the critical infection period of avocado fruit by *P. purpurea* between October 1977 to March 1978. The number of months was gradually extended, one month at a time until March. The resultant linear regression equation is Y=2.80+0.74X, with r=0.403 and where X is the exposure period. A non significant increase of Cercospora spots on the fruit was found with the increase in the length of exposure time. If exposure time is decreased on a monthly interval from the full seasons exposure to the end of March, a significant correlation (r= -0.859) was obtained.

In the 1978/79 season (Nov-March) a good correlation (r=-0.899) was found between the number of Cercospora spots on fruit exposed on a monthly basis and timing of these exposures. The resultant equation is Y=6.33-0.88X. Additionally, data from the 1978/79 fruit exposure experiments were used to analyse correlations between severity of Cercospora spot, rainfall and conidia counts. A good correlation was found between monthly Cercospora infection and rainfall where:

Y=1.65+0.009X with r=0.319.

However, in our study the best correlations between monthly disease index and weather variables were with mean minimum temperature (MinT) with r= -0.744, and with mean maximum relative humidity (RH) with r= -0.962. The disease model is given by

Y=804.273+3.436*Avg MinT-8.615*AvgMaxRH

where MinT is the minimum temperature and MaxRH the maximum relative humidity.

2.3.8 Comparison of new forecasting models and the Darvas 2 model

2.3.8.1 Models for the 2017/18 season
Various new models were developed using multiple stepwise linear regression analysis of the weekly weather parameters and the weekly spore trapping data (Fig. 2.15). The weather parameters included temperature, rainfall, relative humidity and

leaf wetness period. The best model obtained for the period September to December 2017 was given by the equation:

Y = -234.60 + 2.82 x weekly Rainfall + 7.44 x weekly MaximumT

with R^2 =0.92 and Adjusted R^2=0.91.

The best model obtained for the entire period, September to March 2018, was given by the equation:

September-to-March model Y = 8.4166 + 1.1 x weekly Rainfall

with R^2 = 0.371 and Adjusted R^2 = 0.348.

The best two weekly model for the September to December 2017 period was given by:

Y = -9.80233+2.24767 x two weekly Rainfall

with R^2=0.8532 and adjusted R^2=0.816.

In addition, the disease index values for the 2017/18 season for each period were also correlated with weather data and a disease index model was developed. The highest correlation obtained was between disease index and maximum humidity; the Disease Index Model therefore gave the resultant equation

Y = -110.149-4.92 x two weekly MinT + 2.08 x two weekly MaximumH

with R^2=0.816 and adjusted R^2=0.693 where T represents temperature and H the relative humidity (significant at 10% confidence level).
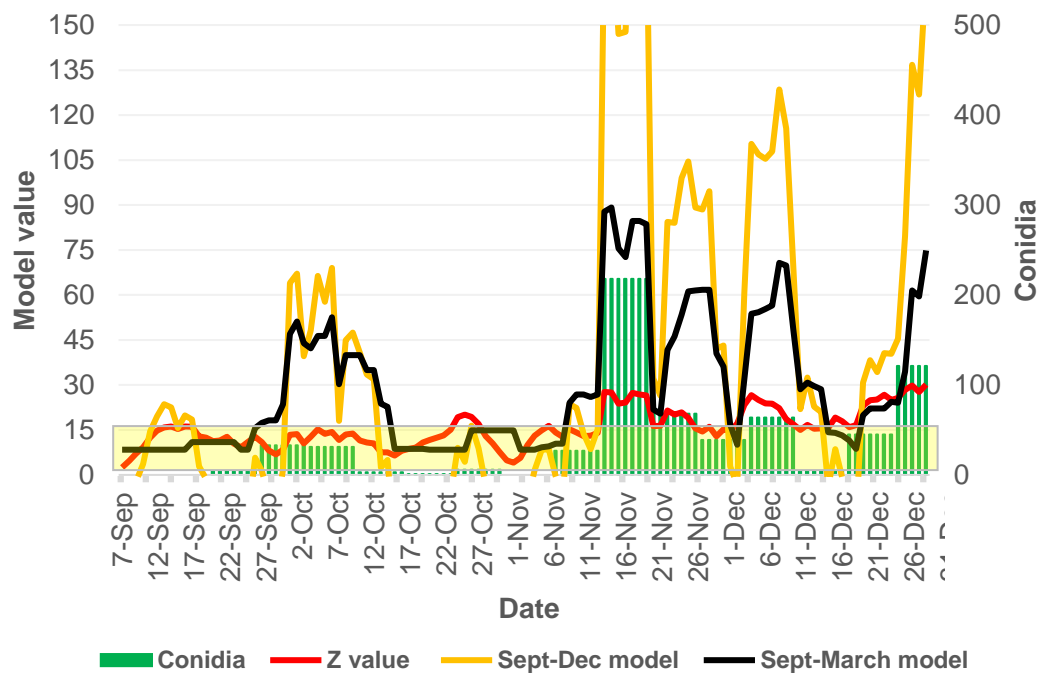
Figure 2.15: Prediction models for 2017/18 season

The Darvas 2 Z values and the values obtained for the Sept-Dec 2017 model and Sept-March 2018 models developed in 2017/18 using the weather data for the 2017/18 season are presented in Fig. 2.15, as well as the numbers of conidia trapped. The numbers of conidia trapped in a week period is the value that was used in the graph for each day in that period.

In the period 12 to 17 September 2017 the Darvas 2 Z value was for the first time calculated to be ≥15. Darvas 1 and 2 models use temperature and rainfall as their environmental parameters. The newly developed September-to-December 2017 model also uses temperature in the equation, and despite conidia release being predicted, no conidia were found in that period. This early prediction of conidia release by three models could have been a result of high temperatures experienced during that period. The September-to-March 2018 model, which uses only rainfall and not temperature as a factor in the equation, did not predict conidia release in that period. The next time that the Z value predicted the release of conidia was on the 5th of October (a one-day period). Two of the new models (September-to-December 2017

model and the September-to-March 2018 model) forecasted conidia release in this period. In the period 24 to 28 October the Z value was again greater than 15 and the fruit size at this stage was also above 25mm, but very few conidia (6) were detected. The September-to-December 2017 model and the September-to-March 2018 models also predicted conidia release but a few days later than the Darvas 2 model.

The Darvas 2 model indicated that spraying should start between 12 to 17 September because the Z value was ≥15. The September-to-December 2017 model also predicted conidia release but indicated spraying a day earlier than the Darvas 2 model. However, if fruit size were considered, then this still would have been too early for spraying, and would have been ignored because the fruit were not yet susceptible. The September-to-March 2018 model predicted conidia release much earlier and for a longer period, starting from the 26 September until the 15 October, compared with the Darvas 2 model and the weekly September-to-December 2017 model. Conidia release was predicted a few days earlier, from 1 October to 13 October, using the September-to-December 2017 model, compared with the Darvas 2 model that only predicted conidia release on the 5 October (a one-day period). At this stage, it would have been too early to spray because the susceptible fruit size was not yet reached. Fruit size was close to 25mm in diameter around the 11 October and infection was shown to have already taken place in the two weekly period 11 Oct – 24 October (Fig. 2.10), so spraying should have commenced before this period.

The two weekly September to December 2017 model first predicted conidia release from 27 Sept to 10 October and predicted conidia release a few days before the Darvas 2 model which only predicted conidia release on 5 October (one-day period). The Darvas Z value was ≥15 again from the 24 – 28 October. However, fruit were already infected therefore spraying would have been too late. Therefore, if the two weekly September to December 2017 model were to be considered, then the first spray would need to be applied as early as 10 October, when the fruit size was close to 25mm. The predictions made by the new models followed a similar pattern to the Darvas 2 model.

2.3.8.2 Models for the 2018/19 season

Using multiple stepwise linear regression analysis of the weekly conidia trapped and weekly weather data, various models were developed. The highest correlation (r= -

0.696) for the September-to-December model was between weekly conidia and mean maximum temperature. The best-fitted equation was the September-December 2018 model

$$Y = 91.368 - 2.768 \text{ x Avg.MaximumT}$$

with $R^2 = 0.485$ and adjusted $R^2 = 0.433$.

A lower correlation was found between weekly conidia trapped and mean maximum temperature (r= -0.520) for the entire season (September to March). The resultant equation for September-March 2019 model:

$$Y = 87.779 - 2.591 \text{ x Avg Tx}$$

with $R^2 = 0.270$ and adjusted $R^2 = 0.239$.

The two weekly September to December 2018 model is given by the equation:

$$Y = 242.806 - 16,667 \text{*Avg MaximumT} + 11.850 \text{*Avg T}$$

with $R^2 = 0.932$ and adjusted $R^2 = 0.886$.

The two weekly September to March 2019 model is given by the equation

$$Y = 179.588 - 5.383 \text{*Avg MaximumT}$$

with $R^2 = 0.301$ and adjusted $R^2 = 0.231$ (insignificant at 5% confidence level). T represents temperature in the equation.

The disease index values for each period were correlated with the weather data using multiple stepwise linear regression analysis. The highest correlation for the September to December period was between disease index and mean temperature (r= 0.642), insignificant at 5% confidence level, $R^2 = 0.413$ and adjusted $R^2 = 0.266$. The resulting disease index model for the September to December period is given by the equation:

$$Y = 22.111 + 2.452 \text{*AvgT, where T represents temperature.}$$

For the entire period the disease index model is given by the equation:

$$y = 64.852 + 0.253 \text{*Total Rainfall.}$$

The highest correlation was with rainfall (r=0.442), but this was not significant at a 5% confidence level, with $R^2 = 0.196$ and adjusted $R^2 = 0.095$.

The Z value and the values obtained for the September-December 2018 model, the September-March 2019 model and the two weekly September-December 2018 model using the weather data of the 2018/19 season are presented in Fig. 2.16. The numbers of conidia trapped in a one- week period was the value that was used in the graph for each day in that period.
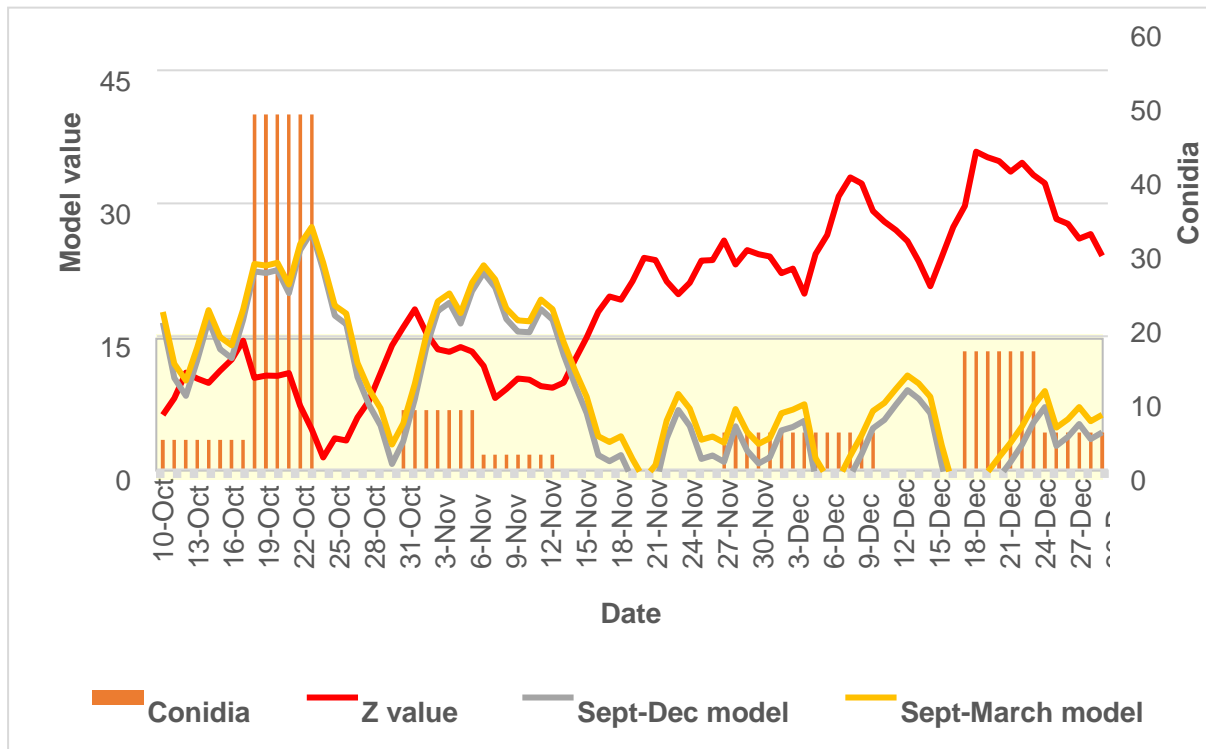
Figure 2.16: Prediction models for 2018/19 season

The Darvas 2 model uses mean temperature and rainfall in the equation, whereas both new models (the September-to-December 2018 model and September-to–March 2019 model) used mean maximum temperature as the parameter in the equation. The two-weekly model used mean maximum temperature and mean temperature in the equation. The first time in the 2018/19 season that the Darvas 2 Z value was found to be ≥ 15 was in the period 18 September to 23 September (not shown in Fig. 2.16). The September-to-December 2018 model, September-to-March 2019 model and two weekly September-to–December 2018 model did not predict conidia release in that period. The next time that the Darvas 2 model predicted conidia release (Z ≥ 15) was in the period 27 September to 2 October. The weekly September-to-December 2018, the weekly September-to-March 2019 and the two-weekly September-to-December 2018 models (not shown in graph) again did not predict conidia release in this period.

On 5 October and again on 8-10 October, only the September-to-December 2018 and the September-to-March 2019 models predicted conidia release for that period. The next time that the Z value was ≥ 15 was on the 17 October for a one-day period (Fig. 2.11). The fruit size at this stage was ≥ 25mm in diameter. The two weekly September-to- December 2018 model (not shown in graph) predicted the release of conidia a few days earlier, starting from 14 October and continuing for a longer period. Fruit size at this stage was greater than 25mm in diameter.

Considering all the above, the Z value was ≥15 in the periods 18 to 23 September and again in the period 27 September to 2 October (not shown in Fig. 2.16). The first period predicted conidia release earlier than the September-to-December 2018 model and the September-to-March 2019 model. In the period 27 September to 2 October, the Darvas 2 prediction date was only 3 days earlier than the prediction date the two new models had indicated. The September-to-December 2018 model and the September-to-March 2019 model predicted spore release only from 5 October and again from 8 to 10 October. Since infection had already taken place on fruit that was >25mm in our study, fruit size must therefore be considered and spraying should have commenced when fruit size was close to 25mm, because they were already susceptible at that stage. SAAGA has previously recommended that the Z value should be used in combination with fruit size, and this recommendation is in agreement with our findings. However, our data shows that fruit should be sprayed at a 25mm diameter or less, and not the 40mm fruit diameter as recommended by Darvas (1982). In our study, fruit size was approximately 25mm around 10 October. Since a peak in conidia release occurred in the period 18 to 23 October 2018 and infection had already taken place in the period 10-24 October, it was crucial that the first spray should have been applied before 17 October 2018. Using the Darvas 2 model the prediction of 27 September to 2 October is therefore the period that should have been used as the prediction period. Tying in the critical Z values with fruit size, as soon as fruit reached 25mm in diameter spraying should commenced, which would have been around 10 October for the 2018/19 season. For the September to December 2018 and September to March 2019 models, Z values ≥15 occurred from 5 October and indicated spraying anytime afterwards. As soon as the fruit became susceptible (>25mm fruit), which was around 10 October, then the first spray should have been applied, based on the predictions of either of these models. The two-weekly September to December 2018 model indicated that spraying was needed by 14 October. This prediction would have also been in time

for the peak in conidia release found in the period 18-23 October.

All models showed that the first spray should be applied before the peak of conidia release, which occurred in the period 17- 23 October. All three new models followed a similar trend to the Darvas 2 Z value, with a few differences, and that it was essential that fruit size also be taken into account.

These observations are summarized in Table 2.2

Table 2.2 A Summary of Positive Predictions of Conidia Release 2018/2019 Season

2018/19 Models	Dates when Z>15	Conidia numbers recorded	Fruit size
Darvas 2 Z value model	18-24 Sept	None	<25mm
	27 Sept-2 Oct	None	<25mm
	17 October(1 day period)	Peak in conidia (48)	29.7mm
Weekly Sept to Dec model	5 Oct, 8-10 Oct, 14 Oct	None	<25mm
Weekly Sept to March	5-6 Oct, 8-10 Oct, 14-15 Oct	None	<25mm
Two-Weekly Sept to Dec	14 October	4 conidia already from 10 Oct 4 days before peak in conidia of 48 on 17 Oct	>25mm
Weekly of daily Sept to Dec	4-9 October	None	<25mm

2.4   Discussion

During the 2017/18 season, for the period September - December 2017, a significant correlation was found between the weekly number of conidia and weekly rainfall. Pretorius (2005) found similar results in a study on leaf spot on citrus caused by *Pseudocercospora angolensis* (de Carvalho & Mendes) Crous & Braun) where conidia numbers peaked in periods where rainfall was high but decreased in periods where no or little rain was present. Additionally, they also found that conidia numbers increased when other factors such as temperature and relative humidity also increased. In the period September to December 2018, about 100mm less rain fell than in the previous season and a significant correlation was only found between the weekly number of conidia and the mean maximum temperature. Although the

correlation was negative, it could be explained by the cooling effect of rain, as mentioned by Darvas (1982). The Darvas 2 model uses mean weekly temperature and not maximum temperature. Darvas also found a significant correlation (r=0.543) between weekly conidia and weekly rainfall and mean temperature when using multiple regression analysis. However, when analysed separately no significant correlations were found between weekly conidia and rainfall or temperature. As was found in the summer of 1978/79 by (Darvas, 1982), a large number of conidia were trapped in our study in November (2017/18 season) when substantial rainfall occurred. Darvas (1982) concluded in his study that in a rainy season, the most consistent environmental parameter affecting the production of *P. purpurea* conidia is rainfall. However, in a study on *Cercospora beticola* (Sacc.) on sugar beet by Tedford *et al.*, (2018) air temperature was found to be the main weather variable influencing *C. beticola* conidia counts and not rainfall. However, the correlation between conidia and temperature was not strong (r= 0.329), and their final model did not include temperature but only included leaf wetness, rainfall and relative humidity (R^2 =0.1639). Although neither rainfall nor leaf wetness played a significant role in conidia production, it played an important role in *C. beticola* infection.

For both seasons (Fig. 2.6 and Fig. 2.11), a large number of conidia was detected in the last week of March, a similar finding to Darvas (1982). However, conidia released late in the season are of little importance for fruit harvested in April because the latent period is approximately 3 months. For late hanging fruit, this is important, and results in infected fruit being harvested. In a study on Cercospora spot on peanuts caused by *Cercospora arachidicola* (Hori) by Nuesry (1981), a peak in conidia numbers were also found towards the end of the season. They found that more inoculum was available due to secondary infections and thus more conidia were released into the atmosphere. This could also be the reason for more conidia being detected in our study towards the end of the season (March) because initial symptoms were already observed on the fruit in January. However, the role of conidia production from lesions observed on fruit was not determined in this study and further research is required to substantiate this. According to the literature, lesions on leaves produce more conidia than those on fruit and therefore constitute the main source of inoculum for primary and secondary infections in endemic areas (Seif and Hillocks, 1993).

When multiple regression analysis of the daily spore trapping data (presented as weekly counts, for a 6-month period) was carried out in the second season of our study, a good correlation was found between weekly conidia and leaf wetness (r=0.526) for the entire period. Rainfall (r=0.792) was found to be the most significant factor during the October to December period. This finding was similar to that of Darvas (1982) (when rainfall was evaluated independently). We also obtained negative correlations between weekly conidia and temperature, i.e., maximum temperature, minimum temperature and mean temperature, which was similar to our findings with the actual weekly data (Fig. 2.11). Daily spore trapping by Darvas (1982), which was done for a brief period of 11 days (27 January to 6 February 1979), found a highly significant correlation between conidia trapped daily in the orchard, and with rainfall and temperature data. However, when the variables in his study were evaluated independently, a negative correlation (r= -0.798) was found between the number of conidia and temperature, and the most significant correlation (r=0.905) was found between conidia and rainfall alone, as was found in our study for the October to Dec period. Darvas (1982) also found a significant correlation (r=0.798) between the number of conidia trapped daily and the mean relative humidity.

In a study, on *C. beticola* on sugar beet by Khan *et al*., (2009), daily mean temperatures below 10°C were found to have reduced *C. beticola* conidia numbers. They also found a significant relationship between the peaks of daily mean number of conidia (µg m^{-3}) and daily mean air temperature when relative humidity was greater than 87%. They found that as temperature increased and relative humidity was greater than 87%, there was a steady increase in conidia being released. Their study showed that temperature and relative humidity contributed to a high production of *C. beticola* conidia. However, we found negative correlations between weekly conidia counts and mean temperatures (with our daily and actual weekly data). Darvas (1982) found a similar result, and proposed that this was the result of rainfall, which caused a cooling effect, thus lowering temperatures in the atmosphere.

In the study of Darvas (1982), most of the conidia were trapped in the early hours of the morning from 01.00 to 06.00, when high humidity was present. In contrast, the hourly spore trapping in our study did not trap conidia during the 10-day period (12-21 November 2017) between 23.00 and 03.00. Two reasons are offered: firstly

conidia trapping was only carried out until 03.00. This may have missed a critical period during the early morning. Secondly, our passive spore trapping technique did not capture conidia as efficiently as the pumped air system used by Darvas, which sucks in a much larger volume of air, and therefore has the capacity to capture conidia far more efficiently. Although rainfall occurred in the trapping period in our study, no conidia was trapped in the hourly periods monitored as the effect of rain on spore release only become evident later and not in the period that the slides were being sampled. In a study by Tedford *et al.*, (2018) on *C. beticola* on sugar beet, it was found that during the hourly periods (6h intervals) of a 24-hour day period, airborne *C. beticola* conidia numbers increased most during the period 12.00 to 18.00, of which was determined as the warmest and most turbulent period. They probably were able to trap *C. beticola* conidia because the period of total hourly spore trapping was longer, and their spore trapping method could have been more effective than the spore trap we used.

The disease index data for both seasons (2017/18 and 2018/19) were also correlated with weather data. In the 2017/18 season (Fig. 2.10), the disease incidence was found to be very low at Halls. This may be attributed to good disease management in previous seasons, reducing field inoculum, and also the dry conditions experienced previously, that would have reduced the levels of pathogen inoculum present in the orchard. In the 2018/19 season at ARC-TSC, conidia release and host infections took place from October and continued throughout the season because favourable conditions were present (Fig. 2.13). It was evident that the fruit became susceptible early on in the season when less than 25mm in diameter, as opposed to the 40mm stage estimated by Darvas 1982, with infections being recorded as early as 10 October during both seasons (Fig. 2.10 and Fig. 2.13). Fruit only reached 40mm in diameter around 7 November. If sprays were only applied from this period onwards, infection would already have taken place, as evident in Fig 2.10 and 2.13.

In the 2018/19 season, fruit exposed to natural infection early in the season from October to November developed significantly more Cercospora spot than fruit exposed later (December to January) (Fig. 2.14). This finding is in agreement with Darvas (1982), who also found that early season fruit is more susceptible to Cercospora spot. Darvas (1982) concluded that levels of Cercospora spot were determined by two

factors: firstly, by the high-risk infection period or the availability of conidia along with weather conditions favourable for infection; and secondly, the latent period which must elapse between infection and symptom development. Therefore, the most critical infection periods appear to be the early months of the summer rainfall season.

Darvas (1982) suggested that preventative spray applications in November and January should be the most effective. However, Manicom and Schoeman (2008) subsequently showed that spray applications applied late in the season (after November) were too late to control the pathogen, and that the first spray should be much earlier for effective control. In this study, using the Darvas 2 model, the release of conidia and infection of small but susceptible fruit (25mm) was predicted to occur in early October. Therefore, spraying may need to commence in early October, depending on the size of the fruit, rainfall and Z values. This will be an important shift in the practices followed by avocado growers and will result in preventative sprays starting approximately one month earlier than Darvas initially recommended.In the 2017/18 season, for the period September to December 2018 when temperatures and rainfall events followed a normal pattern, the Darvas 2 model and the new models generated similar predictions for the release of conidia, and warnings for farmers. The 2018/19 season was much drier and hotter, with maximum temperatures as high as 40°C combined with no rain in September (Fig. 2.6). From 16 November to the end of the season, the Darvas 2 Z values were consistently greater than 15, whereas the new models did not predict conidia release.

In both seasons, the Darvas 2 model satisfactorily predicted conidia release throughout the season. In some cases, its predictions were slightly earlier or later than the new models (Table 2.2). However, the primary change needs to be to align its predictions with a recognition that avocado fruit is susceptible from when the fruit size is close to 25mm.

In this study, the most important parameters for conidia release were rainfall and temperature. Leaf wetness and humidity did not play as significant role in conidia release. In addition, the Darvas 2 model remains a valid forecasting tool, irrespective of climate change. It was determined that successful control of the disease requires monitoring of fruit size (to 25mm), and that this should be used in combination with the

preferred model (the Darvas 2 model and / or the new models). Predictive models forecast conidia release. However, infection cannot occur if the fruit crop is not at a susceptible stage. The corollary is that fruit size alone cannot be used to

determine the timing of the first spray because environmental conditions may not be favourable for conidia release, and consequently no infection can take place. Hence, spraying should commence when the Z value is ≥15 and fruit size is 25mm in diameter.

2.5 References

Campbell, T. 2016. Orchard management: January to March. Subtrop Quarterly Journal 16: 54-55.

Chaput, J. 2000. Management of carrot leaf diseases. http://www.omafra.gov.on.ca/english/crops/facts/00-045.html Accessed on 27 May 2020.

Danneberger, T. K., Vargas, J. M., Jr., and Jones, A. L. 1984. A model for weather-based forecasting of anthracnose on annual bluegrass. Phytopathology 74: 448-451.

Darvas, J.M. 1982. Etiology and control of some fruit diseases of avocado (*Persea americana* Mill.) at Westfalia Estate. D.Sc. thesis, University of Pretoria, Pretoria. South Africa.

Darvas, J.M. and Kotzé, J.M. 1987. Avocado fruit diseases and their control in South Africa. South African Avocado Growers' Yearbook 10: 117-119.

Fidanza, M. A., Dernoeden, P. H., and Grybauskas, A. P. 1996. Development and field validation of a brown patch warning model for perennial ryegrass turf. Phytopathology 86: 385-390.

Khan, J., Qi, A., and Khan, M. F. R. 2009. Fluctuations in number of *Cercospora beticola* conidia in relationship to environment and disease severity in sugar beet. Phytopathology 9(7): 796-801.

Kushalappa, A.C. 2001. BREMCAST: Development of a system to forecast risk levels of downy mildew on lettuce (*Bremia lactucae*), International Journal of Pest Management 47: 1-5.

Kushalappa, A.C., Boivin, G. and Brodeur, L., 1989. Forecasting incidence thresholds of Cercospora blight in carrots to initiate fungicide application. Plant Disease 73(12): 979-983.

Manicom, B.Q and Schoeman, M.H. 2008. Fungicides for *Pseudocercospora* control on avocados: A review, a trial and observations. South African Avocado Growers' Association Yearbook 31: 35-41.

Manicom, B.Q. 2001. Diseases. pp.17*0*-171. In: The Cultivation of Avocado. de Villiers, E.A. (Ed.). Institute for Tropical and Subtropical Crops, Nelspruit, South Africa.

Mousanejad, S., Alizadeh, A. and Safaie, N. 2009. Effect of weather factors on spore population dynamics of rice blast fungus in Guilan Province. Journal of Plant Protection Research 49(3): 319-329.

Nuesry, S.M. 1981. Survival of *Cercospora arachidicola*, *Cercosporidium personatum* and primary infection of peanuts in Oklahoma. MSc. Thesis. Oklahoma State University, USA.

Nutter, F. W., Cole, H., Jr., and Schein, R. D. 1983. Disease forecasting system for warm weather Pythium blight of turfgrass. Plant Disease 67: 1126-1128.

Olatinwo, R. O., Prabha, T. V., Paz, J. O. and Hoogenboom, G. 2012. Predicting favourable conditions for early leaf spot of peanut using output from the Weather Research and Forecasting (WRF) model. International Journal of Biometeorology 56: 259–268.

Pretorius, M.C., 2005. Epidemiology and control of *Pseudocercospora angolensis* fruit and leaf spot disease on citrus in Zimbabwe. MSc. Thesis. University of Stellenbosch. S.A.

Poindexter, S. 2006. Cercospora leafspot Newsletter: Identification and control. Michigan State University Extension, Michigan, USA. pp. 1-7.

Raid, R.N., Pernezny, K., Havranek, N., Sanchez, J. and Saddler, B., 2008. Weather-based forecasting systems reduce fungicide use for early blight of celery. Crop Protection 27(3-5): 396-402.

SAAGA. 2020. Products registered for disease control on avocados in South Africa.https://www.avocado.co.za/wp-content/uploads/2020/01/Registered-FUNGICIDES-list-AVOCADO_January-2020.pdf. Accessed on the 15 May 2020.

Samudra, A.A. 2018. Development of web based expert system for blast disease forecasting in rice plantation. MSc. Thesis. Bogor Agricultural University, Indonesia.

Samudra, A.A., Seminar, K.B., Widodo, D. 2019. Development of web-based system for blast disease forecasting in rice plantation. Journal of Informatics 6: 45-54.

Schoeman M.H and Kallideen, R. 2018. Cercospora spot on avocado – A preliminary report on the relook at the epidemiology of the pathogen. South African Avocado Growers Association Yearbook 41: 84-88.

Seif, A.A. and Hillocks, R.J. 1993. *Phaeoramularia* fruit and leaf spot of citrus with special reference to Kenya. International Journal of Pest Management 39: 44-50.

Tedford, S.L., Burlakoti, R.R., Schaafsma A.W. and Trueman, C.L. 2018. Relationships among airborne *Cercospora beticola* conidia concentration, weather variables and Cercospora leaf spot severity in sugar beet (*Beta vulgaris* L.), Canadian Journal of Plant Pathology 40: 1-10.

Uddin, W., Serlemitsos, K. and Viji, G. 2003. A temperature and leaf wetness duration-based model for prediction of gray leaf spot of perennial ryegrass turf. Phytopathology 93: 336-343.

Wheeler, B.E.J. 1969. An Introduction to Plant Diseases. John Wiley and Sons Limited, New York. pp. 301.

Willis, A. and Mabunda, R.S., 2004. Alternative control of Cercospora spot on Fuerte. South African Avocado Growers' Association Yearbook 27:19-23.

CHAPTER 3

Effect of temperature and nutritional sources on the artificial growth of *Pseudocercospora purpurea*

Abstract

Pseudocercospora purpurea (P. purpurea) is the fungal plant pathogen responsible for Cercospora spot on avocado. This fungus grows slowly similarly to other *Cercospora* species, which grow and sporulate sparsely on artificial media. The aim of this study was to investigate the effect of various nutritional solid media and temperature on the growth of *P. purpurea* in culture. Seven media (potato dextrose agar (self-made), potato dextrose agar (commercial) malt extract agar, potato sucrose agar, oatmeal agar (self-made), oatmeal agar (commercial) and V8 juice agar, and temperatures ranging from 5°C to 35°C were evaluated. The radial growth was recorded by measuring the colony diameter for a period of 28 days at 7-day intervals. There were significant differences (P≤0, 05) between radial growth for each of the solid media except for potato sucrose and malt extract agar. The pathogen grew best on oatmeal agar and potato dextrose agar. Significant differences (P≤0, 05) were found for each temperature. A low temperature (5°C) and a high temperature (35°C) inhibited growth. The optimal temperature for growth of *P. purpurea* was found to be 25°C.

Keywords: Cercospora spot, *P. purpurea*, avocado, media, temperature, growth, oatmeal agar

3.1 Introduction

Cercospora spot on avocado is caused by the fungus *Pseudocercospora purpurea* (Cooke) Deighton (Darvas 1982; Menge and Ploetz, 2003). This disease is a major limiting factor in the production of export quality avocado fruit, and is responsible for losses of up to 70% on unsprayed trees (Darvas 1982). Infection occurs on the leaves, stems and fruit at any point during the growing season (Menge and Ploetz, 2003; Schaffer *et al.*, 2013). Symptoms are first observed on the abaxial surface of leaves but later are observed on both sides of the leaves. Younger leaves develop greenish-white spots though on older leaves; minute irregular lesions (3 – 6 mm in diameter), and angular brown to purplish brown lesions are noticeable (Darvas 1982; Menge and Ploetz, 2003). An infected young fruit is recognized by apparent small, greenish-white lesions, which eventually becomes somewhat sunken, irregular, and of brown to black colour (Chapter 1, Fig. 1.6) (Menge and Ploetz, 2003; Schaffer *et al.*, 2013). The main source of inoculum is infected, mature leaves (Marias, 2007). According to Darvas (1982), rainy, warm conditions favour the development of the disease. The optimum conditions for infection of *P. purpurea* in the field are not well defined in the literature. However, the optimum conditions for infection of some *Cercospora* species, e.g., *Cercospora beticola*, has been reported to be 27 – 32°C during the day and above 16°C during the night. Infection does not occur if temperatures are below 15°C (http://www.sweetbeet.com/growernet/Resources/Pests/Diseases/cercospora.htm).

P. purpurea is slow growing, but is able to grow on standard nutrient media such as, potato dextrose agar (PDA) (Darvas, 1982). Generally, fungi obtain their food from the substrate on which they live in. However, in the laboratory fungi are grown by supplying the necessary elements and compounds in an artificial medium for their growth and other functions (i.e., reproduction). Not all media support growth of all fungus, nor is there a universal substrate that supports the growth of all fungi. Different organisms grow well in different environments and have a range of growth requirements, e.g., nutrients, temperature, osmotic conditions, etc. Basu *et al.*, (2015) stated that finding a suitable culture medium is one of the requirements to study microbial organisms. Temperature is another important factor, which plays a key role among the external factors that affect the development and reproduction of fungi. It affects practically every function of every organism. Each fungus has a minimum and maximum temperature,

below or above which they cannot grow since they are inactivated or killed (Basu *et al.*, 2015). Between these extremes, an optimal temperature exists.

This study was carried out to determine the growth requirements of *P. purpurea* under laboratory conditions. The purpose of this study was therefore to evaluate the effect of various solid media and a range of temperatures on the growth of *P. purpurea* in artificial culture.

3.2 Materials and Methods

3.2.1 Source and maintenance of culture

An isolate of *P. purpurea* (CBS strain 114163, Host: *Persea americana*; Country of origin: Mexico) was obtained from the Westerdjik Fungal Biodiversity Institute in the Netherlands. The culture was sub-cultured on 90mm petri dishes containing oatmeal agar (Sigma-Aldrich 03506) and incubated at $25^{\circ}C$ for further experiments. Additionally, the culture was maintained on oatmeal agar (OMA) and potato dextrose agar (PDA) slants that were stored at room temperature.

3.2.2 *In vitro* growth study experiments

3.2.2.1 Growth of culture on different solid media

A 4mm mycelial plug of a one-month-old *P. purpurea* culture was placed in the centre of seven different solid media (Table 3.1). The plates were placed randomly in a growth chamber and were incubated at $25^{\circ}C$ in constant darkness for a one-month period. Every seven days the colony diameter of the culture were measured. For each treatment (solid media), three petri plates per replicate were used. There were three replicates for each treatment (resulting in nine plates per treatment) and the experiment was repeated twice.

3.2.2.2 Growth of culture at different temperatures

Using a cork borer, mycelial plugs (4mm diameter) were cut from the margin of an actively growing culture of *P. purpurea* (one month old) and placed in the centre of petri dishes containing OMA (commercial powder, Sigma- Aldrich 03506). The plates were placed randomly in growth chambers set at a range of temperatures (5, 10, 15, 20, 25, 30 and $35^{\circ}C$) for a one-month period. Radial growth was determined by measuring the colony diameter of the culture every seven days. For each treatment

(temperature range), five petri plates per replicate were used. There were three replicates for each treatment, resulting in a total of 15 plates per treatment and the experiment was repeated twice.

3.2.3 Statistical analysis

Data were subjected to Analysis of variance (ANOVA) and the treatment means were compared using the Fishers protected LSD test ($p \leq 0.05$) using Genstat for Windows 18th Edition (VSN International, Hemel Hempstead, UK (Web page: www.vsni.co.uk).

Table 3.1: Composition of the culture media used

Medium/ abbrev.	Composition (per litre of distilled water)
V8 juice (V8)	200 ml of V8 juice, 3 g of $CaCO_3$ and 15 g of agar
Potato dextrose agar/PDA (Acumedia Lab Neogen Culture media LAB098)	4 g of potato extract, 20 g of dextrose, 15 g of agar
Potato dextrose agar/PDA*	200 g fresh potato, 20 g dextrose, 20 g agar
Oatmeal agar/OMA (Sigma-Aldrich 03506)	60 g oatmeal powder and 12.5 g of agar
Oatmeal agar/OMA*	30 g oats, 15 g agar
Potato sucrose agar/PSA	200 g potato, 20 g sucrose, 15 g agar
Malt extract agar/MEA (Neogen Culture Media NCM0093A)	30 g malt extract, 5 g peptone and 15 g agar

*Media was prepared using Basic Plant Pathology Methods by Dhingra and Sinclair, 2017

3.3 Results

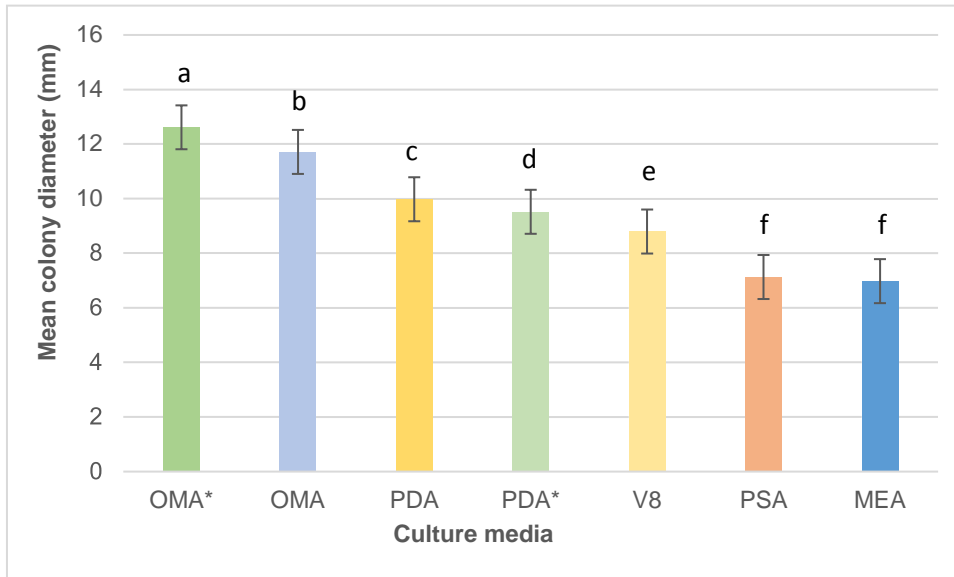Figure 3.1 shows the growth of *P. purpurea* on the seven solid media.

Figure 3.1: Growth of *P. purpurea* on seven different media at 25°C after 28 days

PDA*= potato dextrose agar (self-made), MEA= malt extract agar, PSA= potato sucrose agar, PDA= potato dextrose agar, OMA*= oatmeal agar (self-made), OMA= oatmeal agar, V8= V8 juice agar

Bars with different letters were significantly different (P≤0.05), F. pr <.001; CV %= 3.5 and LSD at 5% interval was 0.5

The fungus grew on all of the tested media but the best growth was on oatmeal agar, whilst poor growth (7mm) was recorded with potato sucrose and malt extract agar. There were significant differences in the growth of *P. purpurea* on each of the media, except for potato sucrose agar and malt extract agar, which resulted in poor growth (Fig. 3.1).

The effect of various culture temperatures on the growth of *P. purpurea* is shown in Fig. 3.2.
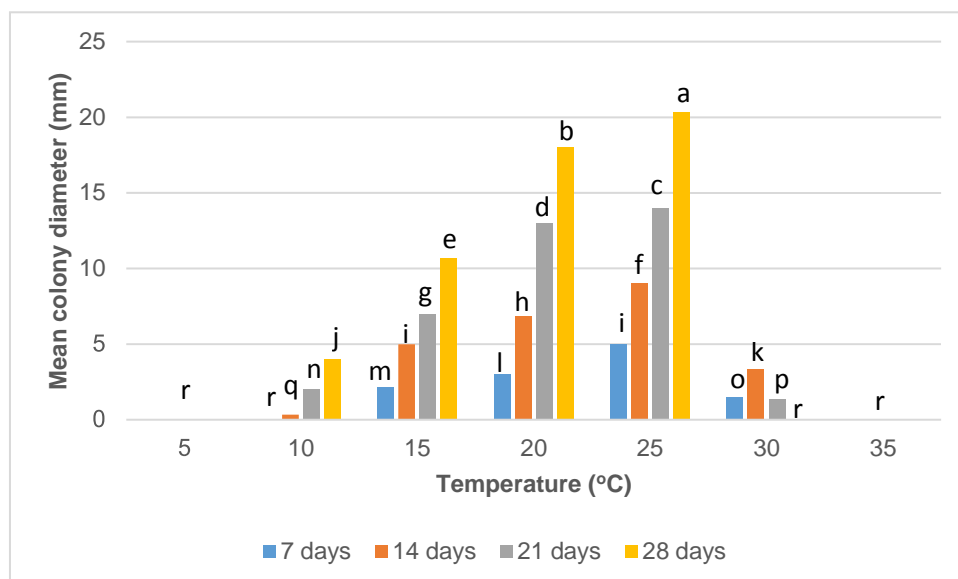

Figure 3.2: Growth of *P. purpurea* at different temperature ranges for a one-month period.

Bars with different letters were significantly different (P≤0.05), F. pr <.001, CV %= 6.4, LSD at 5% interval was 0.16

The fungus grew at a wide range of temperature, between 10 – 30ºC. However, maximum growth was recorded at 25ºC (day 28). No growth was recorded at 5ºC and 35ºC. There were significant differences between the radial growth of the fungus at each of the culture temperatures (Fig. 3.2).

3.4 Discussion

Several studies have indicated that *Cercospora* species grow slowly when cultivated on synthetic media (El-Gholl *et al*., 1982; Djebali *et al*., 2010). Among the seven solid media tested, maximum radial growth was found on oatmeal agar (day 28). (Fig. 3.1). This medium is favourable to many fungi because it is a balanced source of nitrogen, carbon, protein and nutrients essential for growth. The second-best medium was potato dextrose agar, which consists of dextrose, a carbohydrate source that functions as a growth stimulant, and potato extract, which serves as a nutrient base for most fungi. PDA is known to favour the growth of many *Cercospora* species, e.g., *C. zeae–*

maydis and *C. beticola* (Beckman and Payne, 1983; Djebali *et al.*, 2010, respectively). Hedge and Poornima (2014) also found that oatmeal agar and potato dextrose agar supported the best growth of *C. beticola*. Surendra *et al.*, (2015) found that potato sucrose agar (6%) and oatmeal agar were the best media for *Cercospora arachidicola*. However, in our study potato sucrose agar and malt extract agar resulted in poor growth of *P. purpurea*. We observed that on poor media, growth was less dense but on rich media such oatmeal and PDA the growth was more dense and slower. Although, Oatmeal agar is less rich in carbon than PDA several fungi grow more naturally (similar to natural habitat) on poor agar than a rich medium. If too much food is available, the fungus has no need to grow fast but if it had to adapt to a low energy environment than it can conserve energy by growing slower. For a fungus to survive in its niche it has to adapt to constantly changing parameters (Prell and Day, 2001). Since there is less sugar on the avocado skin, the fungus adapted to survive on less. Pathogen development remain influenced by factors for example, such as temperature, moisture, light, aeration, nutrient availability and pH (Gour, 2018). Most biotrophs for instance, can be cultivated in axenic media if necessary supplemented with certain vitamins or special nutrients, whereas obligate biotrophs can not be cultivated in artificial media since they seem to have an absolute requirement for nourishment by living and metabolizing host cell (Prell and Day, 2001). A typical saprophyte will be able to grow well on malt extract agar since it can recycle carbon, nitrogen and mineral nutrients. However, fungal growth requires an external food source, water, and appropriate environmental conditions. Growth ceases when any of these become limiting (Schaechter, 2009).

There was a steady increase in growth from 15 – 25°C (Fig. 3.2). The best temperature for the growth of *P. purpurea* was 25°C. This agreed with Verma (1972) who found that 26°C was the optimum temperature for *C. beticola.* The fungus grew slowly in culture at all temperatures ranging from 10 – 30°C but no growth was found at the lowest temperature (5°C) and highest temperature (35°C) as the fungus was inactive (Fig. 3.2).

This study concluded that oatmeal agar was the best solid medium for *P. purpurea* and that 25°C was the optimal temperature for growth. The colony colour on oatmeal agar was greyish olive however, on PDA it was more of a darker olive green colour but over time changed to a brown black colour (Darvas,1982).

3.5 References

Basu, S., Bose, C., Ojha, N., Das, N., Das, J., Pal, M. and Khurana, S. 2015. Evolution of bacterial and fungal growth media. Bioinformation 11(4): 182–184.

Beckman, P.M. and Payne, G.A. 1983. Cultural techniques and conditions influencing growth and sporulation of *Cercospora zeae-maydis* and lesion development in corn. Phytopathology *73*(2): 286-289.

Darvas, J.M. 1982. Etiology and control of some fruit diseases of avocado (*Persea americana* Mill.) at Westfalia Estate. D.Sc. (Agric.) thesis, University of Pretoria, Pretoria. South Africa.

Djebali, N., Gaamour, N., Badri, M. and Aouani, M.E. 2010. Optimizing growth and conidia production of *Cercospora medicaginis*. Phytopathologia Mediterranea 49(2): 267-272.

El-Gholl, N.E., Alfieri Jr, S.A., Ridings, W.H. and Schoulties, C.L. 1982. Growth and sporulation in vitro of *Cercospora apii*, *Cercospora arachidicola*, *Cercospora kikuchii*, and other species of *Cercospora*. Canadian Journal of Botany 60(6): 862-868.

Dhingra, O.D and Sinclair, J.B. 2017. Basic Plant Pathology Methods. 2nd edition. CRC press. UK.

Gour, H. N. 2018. Physiological and Molecular Plant Pathology. Scientific Publishers. India.

Hegde, Y.R. and Poornima, P. 2014. Cultural characteristics of *Cercospora beticola* Sacc. causing leaf spot of palak. International Journal of Plant Protection 7(2): 441-443.

Khare, K.B., Otanga, R.R.N., Achwanya, O.S. and Otaye, D.O. 2012. Effect of media, pH and temperature on growth and sporulation of *Cercospora zea-maydis*, the causal agent of grey leaf spot of maize. International Journal of Food, Agriculture and

Veterinary Sciences 2(2): 87-90.

Marais, L. 2007. Avocado diseases of major importance worldwide and their management. In: Diseases of Fruits and Vegetables: Volume II. Naqvi, S.A.M.H. (Ed.). Kluwer Academic Publishers, the Netherlands.

Menge, J. A. and Ploetz, R. C. 2003. Disease of Avocado. In: Diseases of Tropical Fruit Crops. CABI Publishing, Wallingford, UK.

Prell, H.H. and Day, P. R. 2001. Plant Fungal Pathogen Interaction: A classical and molecular view. Springer-Verlag Berlin Heidelberg, New York, USA.

Resources – Pests
http://www.sweetbeet.com/growernet/Resources/Pests/Diseases/cercospora.htm
Accessed 20 March 2020.

Schaffer, B.A., Wolstenholme, B.N. and Whiley, A.W. (Eds). 2013. The Avocado: Botany, Production and Uses. CAB International, Wallingford, UK.

Schaechter, M. 2009. Encyclopedia of Microbiology. 3rd Edition. Academic Press. UK.

Surendra, V., Zacharia, S., Reddy, K.R., Reddy, N.E. and Chowdappa, P. 2015. Effect of different media on growth and sporulation of *Cercospora arachidicola* causing early leaf spot of groundnut. 10:1825-1828.

Verma, P.R. and Agnihotri, J.P. 1972. Effect of nutrition, pH and temperature on growth and sporulation of *Cercospora cruenta* Sacc. and *Cercospora beticola* Sacc. Phytopathologia Mediterranea 11: 25-29.

General Overview

Cercospora spot of avocado, is one of the most serious pre-harvest avocado diseases in South Africa causing losses of up to 70% on untreated trees. The fungus responsible for this disease is *P. purpurea.* The most commonly chemical control is copper oxychloride although there are other registered fungicides for use against Cercospora spot. A general spray recommendation is not suitable to control this disease in the different avocado production regions in South Africa because the climatic factors are not the same in different regions at a given time. To deal with this problem, Darvas (1982) developed two epidemiological models to predict the onset and development of Cercospora spot. Thus, the main purpose of the study was to investigate the epidemiology of Cercospora spot on avocado in South Africa.

The main objectives of the current study were as follows:

1) To determine if the Darvas 2 model developed by Darvas in the 1980s is still a valid forecasting tool in predicting the timing of the first spray for effective control of *P. purpurea*;

2)   To test the addition of leaf wetness and relative humidity to temperature and rainfall into new prediction models;

3)   To determine the fruit size at which avocado fruit become susceptible to *P. purpurea*;

4) To evaluate the effect of various solid media and a range of temperatures on the growth of *P. purpurea* in artificial culture.

4.1 Major findings

- Rainfall is the most significant parameter for conidia release.
- Temperature also plays a role in conidia release, but to a lesser extent than rainfall.
- Leaf wetness and relative humidity were not factors for conidia release of *P. purpurea* but played a role in disease development.
- The first fungicide spray to control *P. purpurea* on the fruit of avocado should be applied when the fruit is 25 mm in diameter, and not 40 mm as previously stated in the literature by Darvas (1982).
- Conidia are present throughout the season (from the time avocado fruit is pigeon egg size until harvest) because favourable conditions for production of conidia and their release are present for much of summer. Therefore, accurate

timing of the first spray is critical for controlling the disease on avocado fruit.

- For both seasons of the study (2017/18 and 2018/19), it was determined that the critical infection period was in the early month/s of the summer season, i.e., early in October.

- This study determined that the first spray should be applied around mid-October (depending on the geographic region, rainfall and Z values). However, a fixed calendar spray programme for the region to control Cercospora spot would not be effective because the environmental conditions would not be the same between different areas at a given time.

- We also found that fruit exposed to natural infections early in the season, from October to November, developed significantly more *Cercospora* disease symptoms than fruit exposed later in the season. This finding was in agreement with Darvas (1982).

- A new model (the September-to-December model) developed in the 2017/2018 season showed that rainfall played a more significant role in that season, whereas in the 2018/19 season, temperature was a more significant factor.

- A growth study determined that *P. purpurea* fungus grew best on oatmeal agar, and at a temperature of 25°C.

4.2 Implications of findings

- The Darvas 2 model is still an effective forecasting tool, irrespective of climate change. However, whichever model is selected, i.e., either the current Darvas 2 model or one of the new models, it must be used in combination with fruit size monitoring to determine the most accurate and cost-effective time to apply the first fungicide spray.

- Spraying should commence when the Z-value reaches 15.0, with the average fruit size being approximately 25mm in diameter. This is a significant change from the previous norm of 40mm, established by Darvas (1982). Spraying when the fruit was already 40mm in diameter would be too late for good disease control. In addition, there is no published data available to support the 40mm statement.

4.3 Way forward

- Verification of the predictive models will need to be conducted in the field to compare the new forecasting models and the current Darvas 2 model. The predictions generated by the new models should be compared with the current Z-value predictions to identify the best models for the industry to use in the future, especially regarding the timing of the first fungicide spray.

- Due to build-up of pathogen resistance to systemic fungicides, and their negative effects on the environment and consumers (Darvas and Kotzé, 1987), the need for alternative biological controls such as the use of *Bacillus spp.* and *Trichoderma spp.* has increased. Various studies have showed effectiveness of Trichoderma to control foliar diseases (Tronso and Hjeljord, 1998). A study by Galletti *et al.*, (2007) showed that *C. beticola* could be controlled by some *Trichoderma* isolates. Hence, it is suggested that *in vitro* and *in vivo* studies be undertaken to investigate the potential of *Trichoderma* isolates as biological control agents against *P. purpurea.*

4.4 References

Darvas, J.M. and Kotzé, J.M. 1987. Avocado fruit diseases and their control in South Africa. South African Avocado Growers' Association Yearbook 10: 117-119.

Darvas, J.M. 1982. Etiology and control of some fruit diseases of avocado (*Persea americana* Mill.) at Westfalia Estate. D.Sc. thesis, University of Pretoria, Pretoria. South Africa.

Galletti, S., Burzi, P., Cerato, C., Marinello, S. and Sala, E. 2007. *Trichoderma* as a potential biocontrol agent for *Cercospora* leaf spot of sugar beet. Biocontrol 53 (6): 917-930.

Tronsmo, A. and Hjeljord, L.G. 1998. Biological control with *Trichoderma* species. In: Boland, G.S. and Kuykendall, L.D. (Eds), Plant-Microbe Interactions and Biological Control. Marcel Dekker Inc., New York.

Conferences to date (Oral presentations)

Kallideen, R and Schoeman, M.H., 2018. A relook at the epidemiology of Cercospora spot on avocado**.** Professional Development Programme Conference, ARC-VOP, Pretoria, 27 - 29 August 2018.

Kallideen, R and Schoeman, M.H., 2019. A relook at the epidemiology of Cercospora spot on avocado. 51st Congress of the Southern African Society for Plant Pathology (SASPP), Club Mykonos, Langebaan, Western Cape, 20 – 24 January 2019.

Research outputs

Non-peer reviewed articles:

Schoeman M.H and Kallideen, R. 2018. Cercospora spot on avocado – A preliminary report on the relook at the epidemiology of the pathogen. South African Avocado Growers Association Yearbook 41: 84 – 88.

Schoeman, M. H. and Kallideen, R. 2019. Validating spray timing for Cercospora spot. South African Avocado Growers Association Yearbook 42:12 – 18.