

## **PART ONE**

### **CERCOSPORA SPOT DISEASE OF AVOCADOS**

#### **1 - INTRODUCTION**

In South Africa, Cercospora spot disease of avocados was first described from the Tzaneen area by Brodrick, Pretorius and Freaan (1974) and at that time it was thought to be caused by a Phomopsis species. Growers named it "black spot". According to Mr. W.E. Maddison, Section Manager of Westfalia Estate, who has over 30 years of practical experience with avocados, the disease was first observed in the mid-sixties as a problem of increasing economic importance.

According to Brodrick et al. (1974) the disease spread alarmingly throughout the Northern Transvaal following the heavy rains during the 1971/72 season and today it is found in all major avocado centres of the Lowveld.

#### **2 - LITERATURE REVIEW**

Cercospora spot of avocados was first described in Florida by Stevens (1922), who reported that the disease was responsible for a large percentage of inferior quality fruit. Subsequently, Zentmyer (1953) referred to it as the most important disease of avocados in Florida. Cercospora spot is also prevalent in Martinique and Cameroon (Gustafson, 1976) and in Mexico, where it is the second most common disease of avocados (Turu, 1969).

Various descriptions of the symptoms of the disease have been published. Stevens (1922) described Cercospora spot or blotch disease of avocados as a surface spotting of fruit from seedling trees which first appears when the fruit is less than semi-developed, but is more pronounced during late maturity. Fully developed spots are 3-6 mm in diameter, slightly irregular, usually black in colour and often with white areas of sporulation in the centre. The spots may be scattered on infected fruit or they may coalesce to form irregular black patches. Normally the disease is confined to the rind of the fruit, although the flesh may be invaded during the advanced stages of disease development. The interior of the spots consists of brown, spongy tissues made up of dead collapsed rind cells interwoven with dark mycelium of the fungus. According to Zentmyer (1953), Cercospora spot is manifested as small, brown, slightly sunken lesions with a definite margin, but irregular in shape, scattered on the fruit. The spots later develop cracks through which other fungi may penetrate the fruit. Small angular spots are caused by the pathogen on leaves (Ruehle, 1943b; Zentmyer, 1953). In South Africa, Cercospora spot is characterised by minute, raised, shiny, black spots, 1-3 mm in diameter which are frequently associated with cracking and corking of the lenticels.

Stevens (1922) found Cercospora spot disease only on seedling avocados and never on grafted cultivars. Ruehle (1943b) reported that the most susceptible cultivars in Florida were Waldin, Booth 7, Booth 8, Taylor, Linda, Lula, Nabal, Trapp and Wagner, while Collinson, Fuchsia and Pollock were moderately susceptible.

Traditionally, Cercospora purpurea Cke has been implicated as the causal organism of Cercospora spot (Cooke, 1878). However, in the revision by Deighton (1976), C. purpurea was renamed Pseudocercospora purpurea (Cke) Deighton. P. purpurea is characterised by dark to black, globular to irregular stromata, 15 - 125 µm in diameter in fruit spots. Fascicles are fairly to extremely dense, divergent to compact. Conidiophores are pale to medium dark olivaceous brown, dark in mass, uniform in width and colour, multi-septate, not or rarely branched, slightly geniculate, straight to undulate with a small spore scar at the rounded tip, 3 - 4,5 x 20-200 µm. Some isolates show only short divergent conidiophores and others only long ones, appearing especially long when conidia are persistent. Conidia are obclavato-cylindric, pale olivaceous, with long obconically truncate bases; obtuse to subacute tips, indistinctly 1 - 9 septate, straight to curved, 2 - 4,5x 20 - 100 µm. Hosts include Persea americana Mill., P. carolinensis Nees. and P. palustris Sarg. (Chupp, 1953).

Saccardo (1901) stated that Cercospora perseae Ellis & Martin is a synonym of Pseudocercospora (Cercospora) purpurea. According to Chupp (1953) this is incorrect. The type of C. perseae has effuse fruiting with conidiophores in a true coremium while P. purpurea has distinct spots and conidiophores in divergent fascicles.

Stevens (1922) believed that another spore form of P. purpurea exists and that this is probably produced in pycnidia on the bark of dead twigs. Some of his cultures produced small black bodies, similar to immature pycnidia a number of months after having been inoculated on sterilised avocado twigs. However, these bodies never contained spores.

P. purpurea grows readily on ordinary laboratory media and produces a typical growth which is first greyish in colour and later becomes brown or blackish-brown. Round, raised, tufted grey colonies which are hemispherical in outline are produced on Cornmeal Agar. The surface growth is composed of short, thickly tufted hyphae and the colony has a tough or leathery consistency (Stevens, 1922).

A number of reports have been published on the chemical control of Cercospora spot. Stevens (1922) recommended preventative sprays with two or three applications of Bordeaux mixture. The first application was to be made after fruit set, followed by one or two sprays at three-weekly intervals. He further pointed out that the control of Cercospora spot is more important than the control of anthracnose since elimination of Cercospora spot would result in less wound openings for the anthracnose organism to enter. Ruehle (1940; 1941; 1943a; 1953) evaluated various Bordeaux and cuprous oxide mixtures for the control of Cercospora spot on different avocado cultivars. Three applications sprayed at monthly intervals resulted in a tenfold and more decrease in the percentage infected fruit, while a fivefold decrease was observed following two applications. McMillan (1976) reported that Cercospora spot in Florida could be controlled by timely applications of copper sprays to developing leaves and fruit. An application of copper in early May, followed by another in early June gave effective control on cultivars maturing in summer and autumn. On cultivars maturing in winter a third application in mid-July was necessary for effective control of the disease. McMillan (1976) further stated that Cercospora spot can be controlled by monthly field sprays with benomyl at 1,7 – 2,2 kg/ha. In South Africa, Brodrick et al. (1974) reported that promising results were obtained with three, four and five applications of an unspecified organic systemic fungicide.

Brodrick (1978) discussed various methods for fungicidal control experiments on avocado fruit diseases. He recommended that fruit on the tree be enclosed in paper bags which are tied around the fruit pedicel for a certain period of time to investigate natural infection. This technique was also employed successfully by Kotzé (1963) and Viljoen, Steyn and Kotzé (1972) to establish infection periods for other fruit diseases.

### 3 - MATERIALS AND METHODS

The importance of *Cercospora* spot was determined by recording the percentage losses in export fruit for the past six years (1976-1981) at Westfalia Estate. In this packhouse survey, fruit with more than five mature *Cercospora* spots was classified unsuitable for export. Fuerte, Edranol, Hass and Ryan avocado cultivars were also included in the study to obtain information on the relative susceptibility of these cultivars to the disease. It was conducted throughout the picking season in order to study the seasonal distribution of *Cercospora* spot. All the fruit used in the investigation was commercially sprayed. With benomyl at a concentration of 0,025 percent active ingredient (a.i.) twice every summer season, the first spray being applied in November and the second in January. In total about 150 000 fruits were examined.

To confirm the positive identification of the causal organism, artificial inoculations were made to reproduce typical *Cercospora* spot symptoms under controlled conditions (Koch's postulates). Axenic cultures of *P. purpurea* were grown on sterile avocado pieces placed on Water Agar in Petri dishes. The agar consisted of 10g technical agar added to one litre distilled water and sterilised at 121°C for 15 minutes. Sterile avocado pieces were obtained by thorough flaming of the surface of hard fruit and then cutting it aseptically into pieces. Conidia from sporulating *P. purpurea* colonies were suspended in sterile distilled water and applied to Fuerte fruit with an atomizer. Fruit on the tree was closed in paper bags prior to inoculation to prevent natural infection and was then closed in polythene bags for five days immediately after inoculation. Finally the polythene bags were removed and the fruit was enclosed in paper bags again until harvest. At harvest time symptoms were analysed and re-isolations of the fungus were made on Potato Dextrose Agar containing 39g Merck PDA suspended in one litre water. Notes were made on the appearance and development of symptoms under natural orchard conditions and on fruit inoculated artificially.

Observations were made on the pathogen and its cultural characteristics during the laboratory work. Cultures of the fungus were submitted to the Commonwealth Mycological Institute in England to confirm its identity.

During the isolation studies of *P. purpurea* and associated organisms, a number of artificial media were evaluated, viz. Czapek-Dox Agar, V-8 Juice Agar, Malt Extract Agar, Cornmeal Agar and Potato Dextrose Agar (PDA). In later work, mainly PDA was used. In the isolation procedure the surface of the fruit was sterilised with 96 percent ethanol for five seconds and a thin layer was cut from the epidermis over the disease spots and small pieces of the sub-epidermal brownish tissues were transferred to PDA in Petri dishes. Cultures were placed under near ultra violet light (Philips TL 40 W/08 RS) at ambient temperature to induce sporulation. Isolations were made from both young and old lesions (50 fruits each) as well as from sunken and raised *Cercospora*

spots (50 fruits each).

To investigate the distribution of *Cercospora* spots on the various aspects of the trees, four Fuerte trees were chosen which were not shaded from any direction, at block 34A of Westfalia Section. An average of about 75 fruits were picked from each aspect of each tree on 26 May, 1982 and the mean number of *Cercospora* spots was established. In the same block, trees with different root rot severity ratings were also selected. The root rot disease index is a rating scale from 0 (healthy) to 10 (dead). On 5 May, 1982 an average of 75 fruits were picked from four trees in each disease category between 0 and 7 and the mean number of *Cercospora* spots per fruit was established and correlated to the disease index of the trees.

A Hirst spore trap was operated from October, 1977 until April, 1979 in a 14 year old Fuerte orchard at block 34 of Westfalia Section, to monitor the number of air-borne *P. purpurea* conidia. It was placed 1,5m above the ground and it was adjusted so as to draw in air at a rate of nine litres per minute. As trapping surface, microscope slides covered with vaseline on one side were used. Slides were changed and examined daily. Three stains were initially used to facilitate the identification of spores, viz.

Methylene Blue, Rose Bengal and Malachite Green. Later in the study, Methylene Blue dissolved in water at 0,25 percent, was used as a standard stain. Conidia were identified by taking measurements and considering the number of septae as well as the degree of staining. Conidia which measured between 2 - 4,5µm in width and 20-100µm in length, having no more than nine septae and exhibiting more pronounced apical and basal cell staining were classified as *P. purpurea* conidia. In orchard 34 of Westfalia Section, close to the spore trap, a small weather station was established. Temperature, relative humidity and rainfall were recorded and correlated with the spore trap results.

Experiments on the critical infection period of avocado fruit by *Cercospora* spot disease were conducted at block 35 of Westfalia Section during the 1977/78 season, at block 34A of Westfalia Section during the 1978/79 season and at block 14 of Westfalia Section during the 1981/82 season. According to the method of Kotzé (1963) and Viljoen *et al.* (1972) trees were selected at random and fruits were closed in brown paper bags on the trees. Initially all fruits were bagged and then exposed to natural *Cercospora* spot infection according to a pre-determined time schedule and then closed again to prevent contaminating infections. In the 1977/78 summer the experiment was started with fruit exposures from October until March in a cumulative way on a monthly basis. The rainfall experienced during that period was 1 503 mm and assessment of results took place on 20 June, 1978. The experiment in 1978/79 started in November and ran until March with both monthly and cumulative time exposures. The rainfall during these five months was 773 mm and results were evaluated on 5 June, 1979. The 1981/82 experiment also included monthly and cumulative time exposures from November until March and the rainfall during this period was 612 mm and the results were analysed on 13 May, 1982. There were four single tree replicates in each treatment and 200 fruits were closed in paper bags on each tree at the commencement of the experiments.

The following chemicals were evaluated in experiments on the control of *Cercospora* spot disease at Westfalia Estate:

benomyl, in a 50% a.i. WP form  
thiophanate-methyl, in a 65% a.i. WP form  
thiabendazole (TBZ), in a 45% a.i. flowable formulation  
fosetyl-Al, in a 80% a.i. WP form  
glyodin, in a 30% a.i. EC form  
captafol, in a 80% a.i. WP form  
captan, in a 50% a.i. WP form  
copper oxychloride, in a 80% a.i. WP form  
copper hydroxide, in a 77% a.i. WP form  
etaconazole, in a 10% a.i. EC form  
propiconazole, in a 10% a.i. EC form  
prochloraz, in a 40% a.i. EC form  
bitertanol, in a 19,5% a.i. EC form  
PP 296, experimental fungicide  
B 77, experimental fungicide  
Nu Film 17 (pinolene), film forming agent  
Agridex, experimental additive  
Plyac, experimental additive  
Solvaid, experimental additive  
Biofilm, experimental additive

The chemical control experiments were initiated in 1976/77 season, when three systemic fungicides were tested, namely benomyl, thiophanate-methyl and fosetyl-Al. The site of the experiment was at block 34A of Westfalia Section, with four single tree replicates in each treatment and trees in all treatments being sprayed twice with high volume ground sprayers to run-off point. The first spray was applied on 19 November, 1976 and the second on 3 February, 1977.

In another intensive investigation during the 1977/78 season, the optimum timing and the number of benomyl sprays needed for the best *Cercospora* spot control was tested. The site of the experiment was again at block 34A of Westfalia Section, where four single tree replicates selected at random were used in each treatment. The concentration of benomyl was 0,025% a.i. with 0,02% Nu Film 17. Results were evaluated twice, first at the peak of the Fuerte picking season, on 12 April, 1978 and again on 7 June, 1978. 100 Fruits were harvested from each tree in each treatment of each assessment and evaluated for the severity of *Cercospora* spot infection.

In the 1978/79 season the two-spray treatments were further investigated. Applications were made in mid November and mid-January. The effect of Biofilm and Solvaid experimental additives were compared against the standard Nu Film 17, in mixtures with benomyl. Experimental fungicides etaconazole and propiconazole were also tested. To one of the benomyl treatments TBZ was added in the second spray. There were eight single tree replications in each treatment and 100 fruits were harvested for evaluation from each tree. Assessment of the results was carried out on 25 May, 1979.

In the 1979/80 season the *Cercospora* spot control experiment was continued in block 34A of Westfalia Section. Plyac and Solvaid experimental additives were tested against Nu Film 17. New experimental fungicides were also evaluated, these included: PP 296, B 77 and glyodin. Other fungicides used in the experiment were: copper oxychloride,

copper hydroxide, captafol, fosetyl-Al and benomyl. Two sprays were applied for all treatments, the first in mid-November and the second in mid-January, with the exception of treatments 9 and 11 in which a third spray was applied in mid-December. The number of single tree replicates was eight in each treatment and 100 fruits from each tree were used for evaluation. Assessment of results was made on 3 May, 1980.

Chemical control experiments were continued in the 1980/ 81 season and captafol was tested at various concentrations and in spray programmes with benomyl and copper oxychloride with the addition of Nu Film 17. Eight randomly selected trees at block 34A of Westfalia Section were used in each treatment and in all treatments trees were sprayed twice (mid-November and mid-January). An average of 50 fruits were harvested and evaluated from each tree in each treatment on 14 April, 1981.

In the 1981/82 season the site of the Cercospora spot control experiment was transferred to block 34B of Westfalia Section. Fuerte trees in this block were six years of age at the commencement of the experiment. Six randomly selected trees were used in each treatment and the test fungicides included benomyl, captafol, captan, copper oxychloride, copper hydroxide and prochloraz. In all treatments trees were sprayed in mid-November and mid-January. An average of 60 fruits were harvested from each tree in each treatment and evaluated on 8 April, 1982.

Data collection in the experiments was based on methods recommended by Brodrick (1978). Each fruit was examined for the presence of Cercospora spot immediately after harvest, the number of spots was established on an evaluation scale with five categories. The number of spots in category one was zero, in category two 1 to 5, in category three 6 to 10, in category four 11 to 20 and in category five 21 or more. Fruit in categories one and two were regarded as marketable whereas fruit in categories three, four and five were classified as non-marketable. Analyses of the results were carried out by using standard statistical methods and a level of probability for significance of 95 percent.

## **4 - RESULTS**

### **4.1 LOSSES CAUSED BY CERCOSPORA SPOT AT WESTFALIA ESTATE**

The importance of Cercospora spot on the commercially sprayed avocados at Westfalia Estate is illustrated in Table 1. The disease caused most damage to Fuerte and Ryan cultivars, while losses of Edranol and Hass were significantly less.

The difference in losses between the seasons followed the rainfall pattern measured from the beginning of October until the end of February. When losses of Fuerte fruits were correlated with the rainfall figures, it was found that a significant ( $r = 0,829749$ ) correlation exists between Cercospora spot damage and rainfall and this correlation was best described with a linear regression model of  $y = 4,47 + 0,01 x$  (Fig. 1) .

The correlation between rainfall and Cercospora spot damage to Ryan best fitted the linear regression model of  $y = -3,43 + 0,007 x$ , with a significant correlation coefficient of  $r = 0,899659$  (Fig. 2).

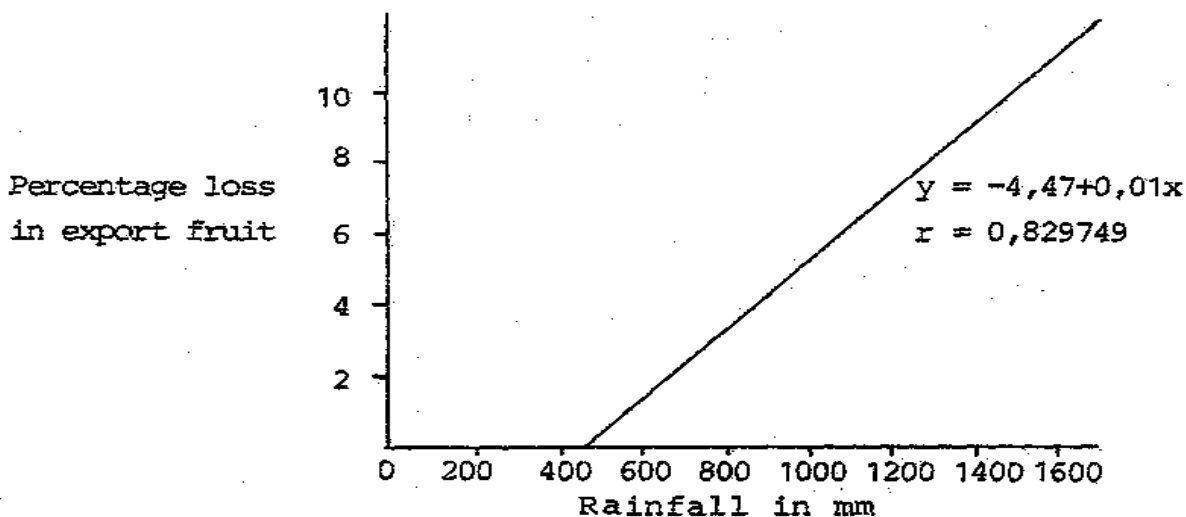


FIG. 1. - The correlation between rainfall and Cercospora spot losses on commercially sprayed Fuerte at Westfalia Estate.

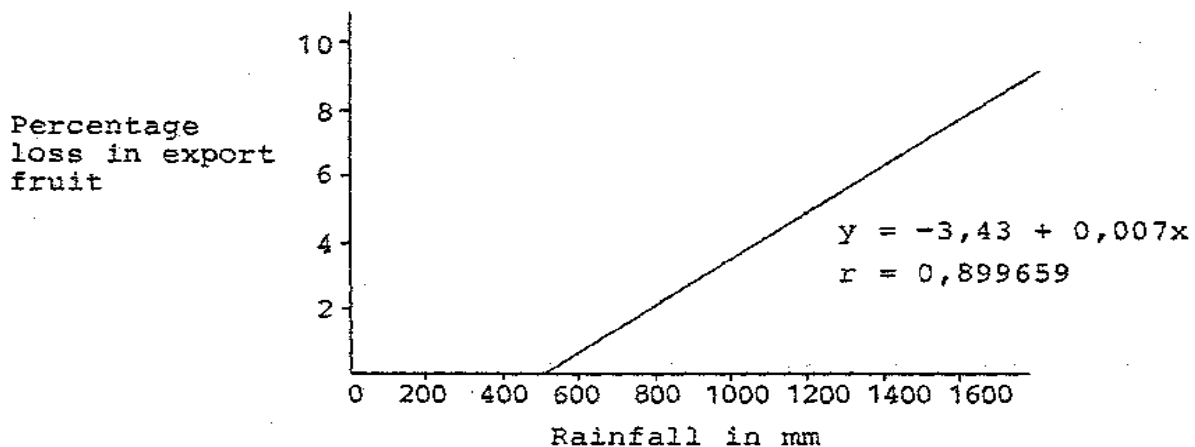


FIG. 2. - The correlation between rainfall and Cercospora spot losses on commercially sprayed Ryan at Westfalia Estate.

The statistical analysis of the losses in export fruit showed an increase in the severity of Cercospora spot at the beginning of the harvest season and a slight decrease towards the end of the picking season. The non-linear regression model to describe Cercospora spot incidence on Fuerte from February to August is  $y = -2,25 + 4,44x - 0,46x^2$ , with a highly significant correlation coefficient of  $R = 0,961865$  (Fig. 3).

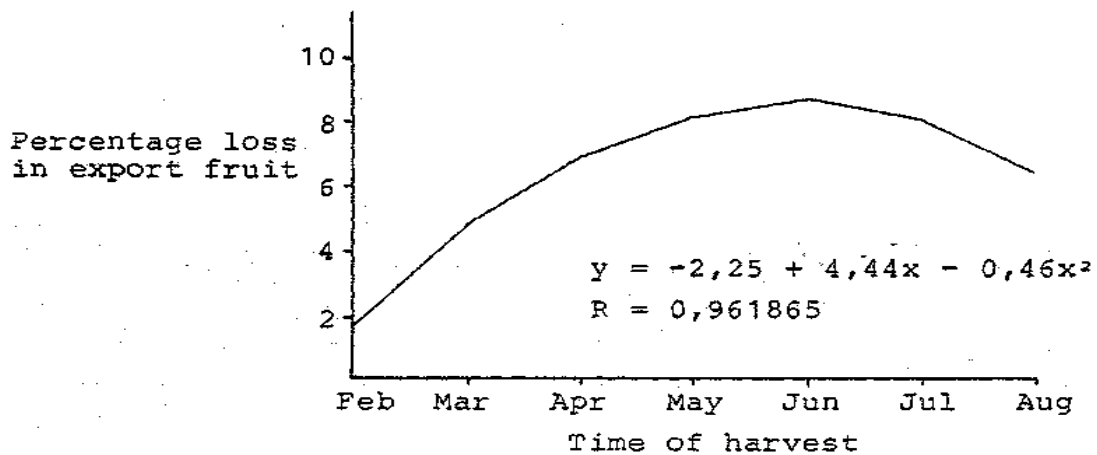


FIG. 3. - The losses in export fruit of commercially sprayed Fuerte in relation to harvest time at Westfalia Estate.

The increase in losses of Ryan export fruit for a five month harvest period fitted the regression line of  $y = 0,58 + 1,69x$ , with a non-significant correlation coefficient of  $r = 0,697568$  (Fig. 4).

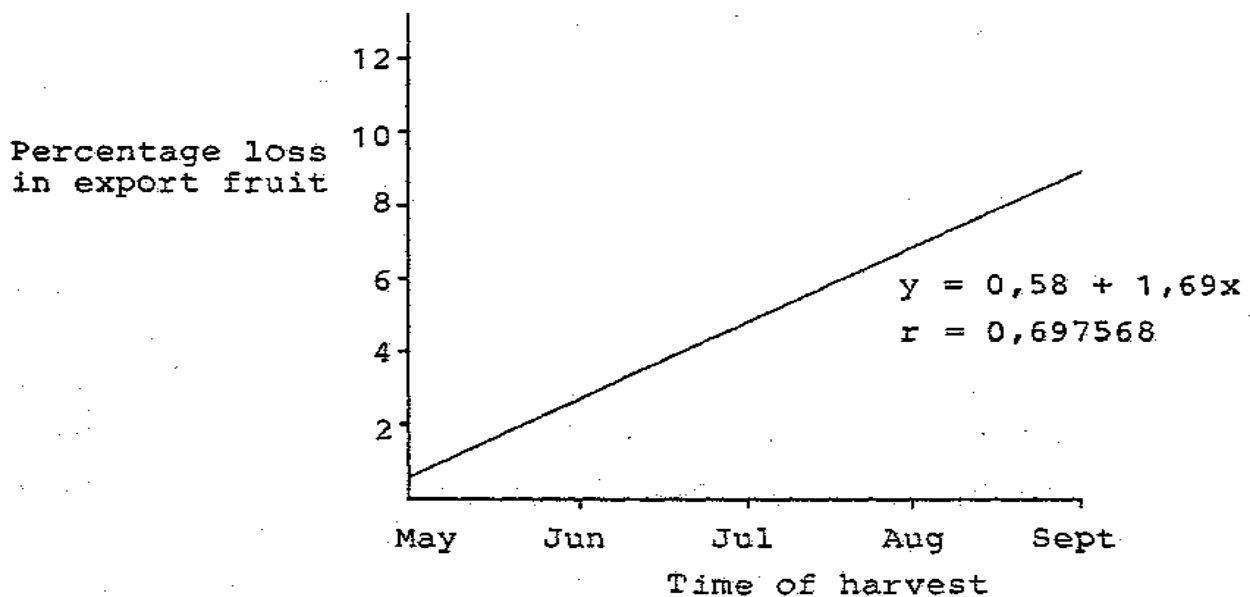


FIG. 4. - The losses in export fruit of commercially sprayed Ryan in relation to harvest time at Westfalia Estate.



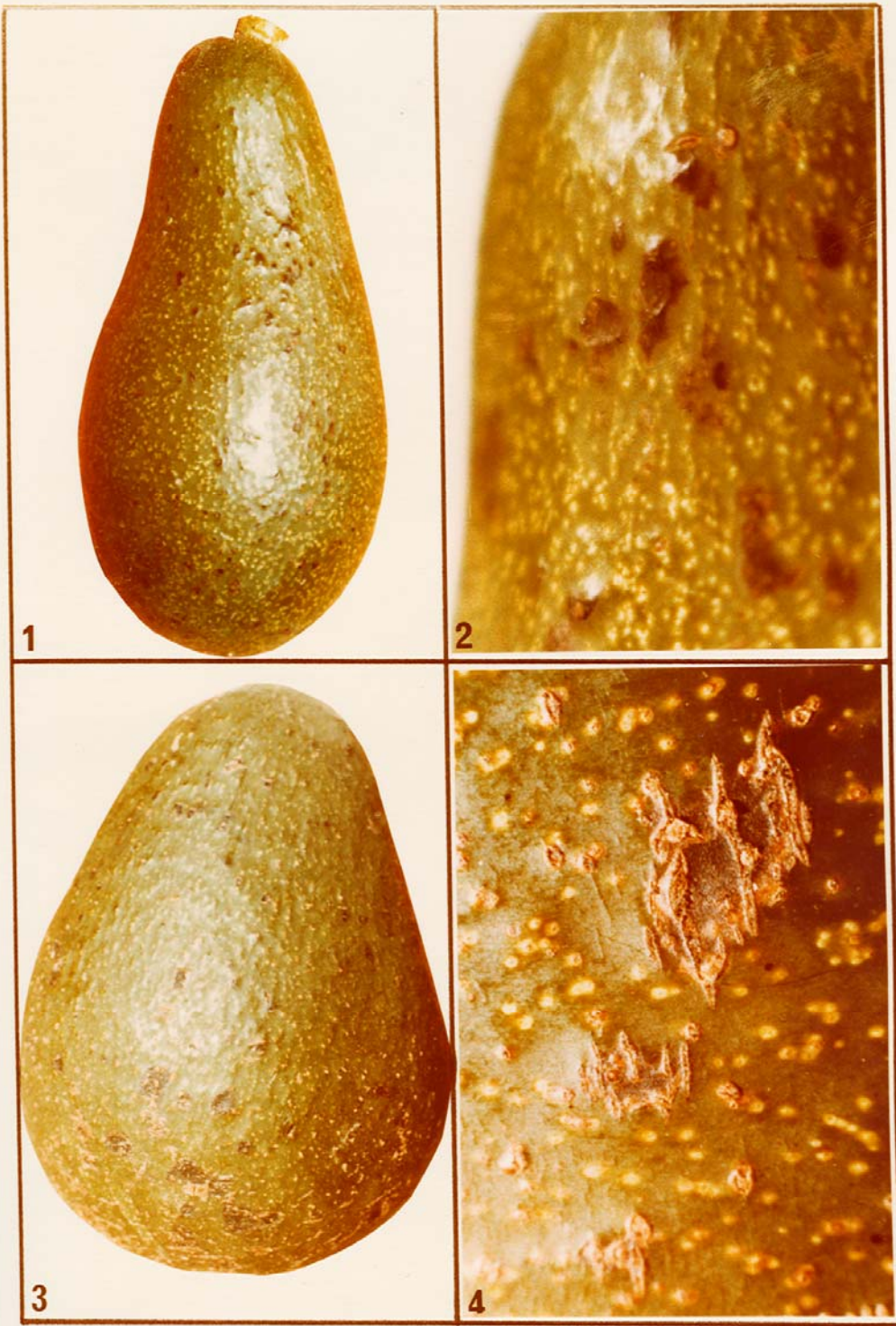
TABLE 1. - Percentage losses caused by *Cercospora* spot disease on commercially sprayed avocados at Westfalia Estate.

Cultivar	Season	February	March	April	May	June	July	August	September	Mean percent loss (N = 150 000 fruit)
Fuerte	1975/76	4,07	4,95	10,60	7,69	7,63	-	-	-	6,98
	1976/77	0,18	1,32	6,89	9,92	14,00	15,33	-	-	7,94
	1977/78	-	2,58	7,80	8,73	12,70	10,00	-	-	8,36
	1978/79	-	0,38	0,95	0,80	1,68	5,50	6,00	-	2,55
	1979/80	-	1,55	1,48	4,42	6,60	2,29	5,19	-	3,58
	1980/81	-	13,77	15,16	15,49	7,40	8,10	-	-	11,98
	Mean	2,12	4,09	7,14	7,84	8,33	8,24	5,59	-	6,89a
Edranol	1975/76	-	-	0	0,14	1,16	0,25	-	-	0,38
	1976/77	-	-	-	0	0,39	0	-	-	0,13
	1977/78	-	-	-	0	0,04	0	0,51	0	0,11
	1978/79	-	-	-	0	0	0	0	0	0
	1979/80	-	-	0,62	0	1,30	0,32	0,37	0,07	0,44
	1980/81	-	-	-	-	0,06	0,46	0	-	0,13
	Mean	-	-	0,31	0,02	0,49	0,17	0,22	0,03	0,19b
Hass	1975/76	-	-	-	-	-	0	0	2,00	0,66
	1976/77	-	-	-	-	0	0	0	-	0
	1977/78	-	-	-	-	-	-	0,38	0,40	0,39
	1978/79	-	-	-	-	-	-	0	-	0
	1979/80	-	-	-	-	-	-	0	0,25	0,12
	1980/81	-	-	-	-	-	0	0	-	0
	Mean	-	-	-	-	0	0	0,06	0,88	0,19b
Ryan	1975/76	-	-	-	4,00	-	1,00	4,00	12,33	5,33
	1976/77	-	-	-	-	-	7,52	7,50	-	7,51
	1977/78	-	-	-	-	-	-	6,66	-	6,66
	1978/79	-	-	-	-	-	0	2,27	-	1,13
	1979/80	-	-	-	-	-	-	3,80	-	3,80
	1980/81	-	-	-	-	-	6,60	1,60	-	4,10
	Mean	-	-	-	4,00	-	3,78	4,30	12,33	4,75a

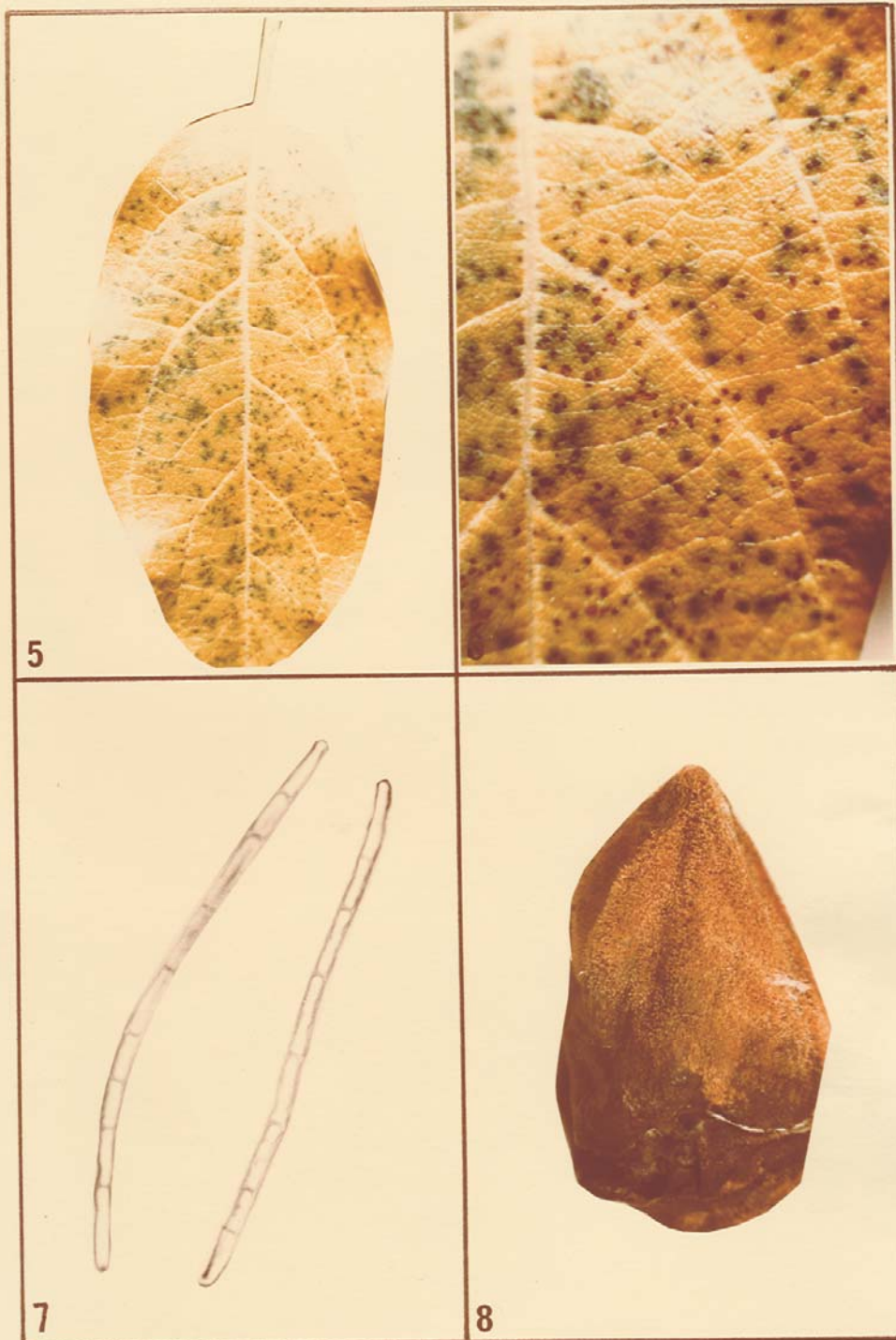
Means with letters a and b differ statistically at 0,05 level (Duncan's multiple range test)

## 4.2 OBSERVATIONS ON THE SYMPTOMS AND THE PATHOGEN

Fuerte fruit was artificially inoculated with the pathogen and it was reisolated from the spots, thereby fulfilling the requirements of Koch's postulates. Observations on symptoms made during the course of this study at Westfalia Estate correspond with descriptions given by earlier workers. The colour of the spots may vary from light brown to black, depending on the stage of development of the disease and the amount of corky cells in cracks associated with the spots. At first, the green epidermis of the fruit becomes slightly darker at the site of the infection and it turns darker as the fruit matures. It would appear that the disease initially causes a swelling of the tissues around the infection site causing the spot to rise above the level of the epidermis (Photos 1 and 2). However, at a later stage when epidermal cells are killed and the tissues dry out, the spot becomes sunken and horizontal cracks appear, mainly on the perimeter of the spot. These cracks may serve as entrance sites for other fruit rotting fungi, particularly *Colletotrichum gloeosporioides* (Photos 3 and 4).



Photos : 1 : Cercospora spot on Fuerte - raised type  
 2 : Cercospora spot on Fuerte - raised type (close-up)  
 3 : Cercospora spot on Fuerte - sunken type  
 4 : Cercospora spot on Fuerte - sunken type (close-up)



Photos : 5 : Cercospora spot on Fuerte leaf

6 : Cercospora spot on Fuerte leaf (close-up)

7 : Conidia of Pseudocercospora purpurea (1600 X)

8 : Fuerte fruit mummified by Thyronectria pseudotrichia

Typical leaf symptoms of *Cercospora* spot infection were commonly seen on older attached leaves of Fuerte and Ryan cultivars (Photos 5 and 6).

Several isolates of the pathogen were sent to the Commonwealth Mycological Institute in England where taxonomists confirmed the identity of the fungus as *Pseudocercospora purpurea* (Cke) Deighton.

From a large number of isolations and sporulation studies it was established that most of the fresh *P. purpurea* isolations produce conidia on artificial media if cultures are kept under continuous near UV light. Sporulation begins about 10 days after isolation and a fair amount of conidia are produced for about 10 days, after which the fungus becomes sterile and previously produced conidia disappear. Such cultures regain the ability to sporulate if transferred to sterile, freshly cut avocado pieces, but only for a limited period. Sporulation under fluorescent light alternated with dark periods resulted in a weak sporulation by the fungus. No sporulation was observed in cultures grown in complete darkness.

The size, form and the number of septae of the conidia derived from colonies cultured on avocado pieces were identical to those collected in the orchard, using the spore trap (Photo 7). Some conidia of the cultures of *P. purpurea* grown on artificial media (PDA) exceeded the maximum length of 100 µm given by Chupp (1953) and in some conidia the number of septae was 13 instead of the described maximum of 9.

In some isolations, besides the *Pseudocercospora* conidial form, spermogonia-like bodies with abundant spermatia were found. They developed in a few direct isolations from fruit-to-PDA and from-fruit-to-sterile avocado.

### 4.3 DISTRIBUTION OF CERCOSPORA SPOT

The distribution of *Cercospora* spots on fruit in the various aspects of the trees is presented in Table 2.

TABLE 2. - The distribution of *Cercospora* spots in various aspects of Fuerte avocado trees in 1981/82 season.

Aspect of tree	Mean number of <i>Cercospora</i> spot/fruit (N = 1200 fruit)
North	0,51 a
South	0,12 a
East	0,06 a
West	0,16 a

Means with the letter a do not differ statistically at  $p = 0,05$  level

Although the number of *Cercospora* spots on fruit in the warm, sunny northern and

western aspects of the trees appeared to be more prominent than on the southern and eastern sides, the difference was not statistically significant.

The distribution of Cercospora spots on fruit harvested from various disease rating trees is analysed in Fig. 5.

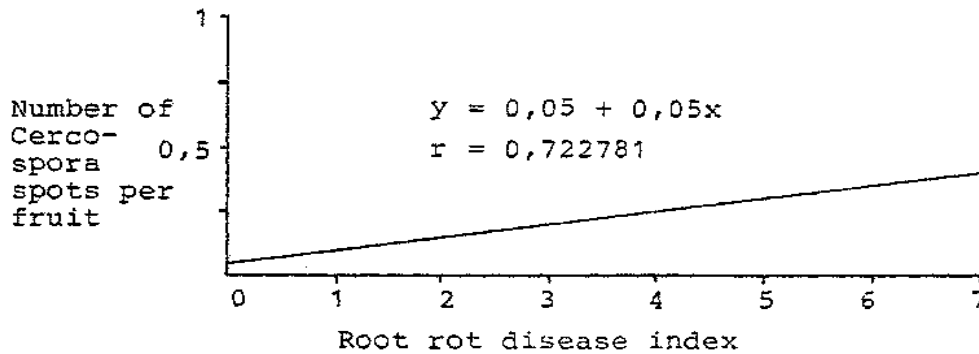


FIG. 5. - The correlation between root rot severity of Fuerte trees and the number of Cercospora spots on the fruit.

TABLE 3. - Percentage incidence of P. purpurea and other fungi in young and old Cercospora spots of Fuerte fruit.

Organisms	Percentage incidence	
	Young spots in Apr. '78	Old spots in Dec. '78
<u>Pseudocercospora purpurea</u>	48,0	14,0
<u>Colletotrichum gloeosporioides</u>	7,5	24,5
<u>Phoma</u> spp.	0,5	2,5
<u>Cladosporium</u> spp.	0,5	2,5
<u>Pestalotiopsis</u> spp.	0,5	0
<u>Phomopsis</u> spp.	0	2,5
<u>Thyronectria pseudotrichia</u>	0	1,5
<u>Fusarium decemcellulare</u>	0	0,5
<u>Drechslera</u> spp.	0	0,5
Unidentified fungi	0,5	6,0
Sterile isolations	37,0	38,5

A significant correlation was found between the number of Cercospora spots on fruit and the root rot disease severity index of Fuerte trees. The linear regression model to determine the correlation is  $y = 0,05 + 0,05x$ , with  $r = 0,722781$ .



The incidence of P. purpurea and associated organisms was studied in young and old Cercospora spot lesions. In both cases spots were mature (Table 3).

Pseudocercospora purpurea was more readily isolated from young spots than from old lesions. Furthermore, there were more secondary organisms present in old spots, with a marked increase in the incidence of Colletotrichum gloeosporioides. The incidence of unidentified fungi was also higher in old lesions, while the number of sterile isolations from the two spot groups was practically the same.

A comparison was also made of the incidence of P. purpurea in sunken and raised Cercospora spot lesions. Isolations were made from both types of spots at the same time, in April, 1978 (Table 4).

TABLE 4. - Percentage incidence of P. purpurea and associated organisms in sunken and raised Cercospora spot on mature Fuerte fruit.

Organisms	Percentage incidence	
	Raised spots	Sunken spots
<u>Pseudocercospora purpurea</u>	41,0	50,5
<u>Colletotrichum gloeosporioides</u>	2,5	24,5
<u>Phoma</u> spp.	0	1,5
<u>Cladosporium</u> spp.	2,0	2,5
<u>Pestalotiopsis</u> spp.	1,5	2,0
Unidentified fungi	0	1,5
Sterile isolations	53,0	17,5

The incidence of P. purpurea did not differ much in the two types of lesions. The occurrence of C. gloeosporioides, however, was considerably higher in sunken lesions. The latter also contained more secondary fungi, while a greater percentage of sterile tissue was present in raised spots.

#### 4.4 SPORE TRAPPING OF P. PURPUREA CONIDIA

Spore trap results and weather data are presented in Table 5.

With the multiple regression analysis of the weekly number of conidia caught in the spore trap and the weekly rainfall and mean temperature values, a significant correlation was found. The equation for the regression line is  $Z$  (number of conidia) =  $-8,99 + 3,22x$  (temperature °C) +  $0,18y$  (rainfall mm), with a correlation coefficient of  $R = 0,543507$ . However, when this weekly conidia catch was compared to either rainfall or temperature respectively, no significant correlation was found. The equation of the linear regression line between conidia and temperature is  $y = -48,84 + 3,05x$ , with  $r =$

0,340300 and between conidia and rainfall is  $y = 15,31 + 0,15x$ , with  $r = 0,341572$ . The number of P. purpurea conidia first reached significant proportions with the onset of the rainy, warm summer period, which in this instance, started in November. Large numbers of P. purpurea conidia were detected in the orchard even as late as the last week of March, when commercial harvesting of the Fuertes had already started.

TABLE 5. - The effect of climatic factors on the incidence of air-borne conidia of P. purpurea during the summer of 1978/79.

Date	Number of <u>P. purpurea</u> conidia	Mean temperature °C	Mean relative humidity %	Rainfall mm
1978				
October 1st week	5	20,8	72,0	7,5
2nd week	9	20,6	69,5	8,5
3rd week	3	19,9	70,5	31,3
4th week	6	20,1	61,3	5,8
November 1st week	6	20,4	70,5	86,9
2nd week	24	22,5	64,8	52,4
3rd week	16	20,9	68,6	17,3
4th week	20	23,1	64,8	15,8
December 1st week	31	22,9	67,0	23,0
2nd week	15	21,9	63,3	40,8
3rd week	8	23,6	60,5	0
4th week	20	24,4	57,8	43,3
1979				
January 1st week	29	21,3	64,0	22,0
2nd week	26	24,0	61,2	19,3
3rd week	20	23,9	63,5	59,7
4th week	34	22,7	66,0	61,3
February 1st week	27	23,5	62,8	14,4
2nd week	3	25,9	59,6	0,2
3rd week	65	25,1	65,7	28,4
4th week	16	23,5	67,4	73,2
March 1st week	45	21,7	72,0	137,8
2nd week	7	22,1	61,4	4,0
3rd week	14	25,0	59,0	0,2
4th week	39	23,1	62,3	19,6

The daily analysis of typical spore trap results along with the weather data is presented in Table 6 to illustrate the role of the climatic factors in spore production.

TABLE 6. - The effect of climatic factors on the incidence of air-borne conidia of P. purpurea on a daily basis from 27 January until 6 February, 1979.

Date	Number of <u>P. purpurea</u> conidia	Mean temperature °C	Mean relative humidity %	Rainfall mm
1979 January 27	1	24,3	59,3	0
28	1	24,0	64,2	0
29	3	26,0	62,5	0
30	7	21,8	77,4	12,5
31	18	20,0	81,8	47,5
February 1	10	21,0	67,9	14,1
2	4	21,3	68,1	0,2
3	8	22,8	60,4	0,1
4	1	23,5	61,2	0
5	2	24,0	60,9	0
6	0	25,5	59,4	0

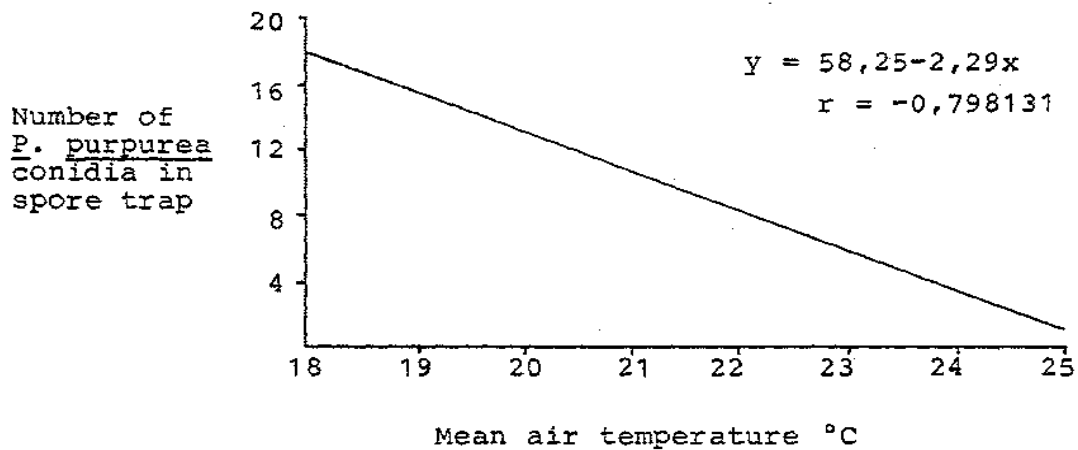


FIG. 6. - The effect of air temperature on the number of trapped P. purpurea conidia analysed on a daily data basis.



The multiple regression analysis of the data of the daily spore trapping in Table 6 indicated a significant correlation between the number of conidia and the rainfall and temperature figures. The resulting equation is  $z$  (number of conidia) =  $24,87 - 0,93x$  (temperature °C) +  $0,25y$  (rainfall mm), with  $R = 0,875452$ . If analysed separately, the correlation between the number of conidia and temperature is illustrated with a linear regression model of  $y = 58,25 - 2,29x$ , with a significant correlation coefficient of  $r = -0,798131$  (Fig. 6).

The correlation between the number of trapped conidia of P. purpurea and the mean relative humidity was also significant  $r = 0,798686$  and the linear regression model to describe the correlation is  $y = -32,66 + 0,57x$  (Fig. 7).

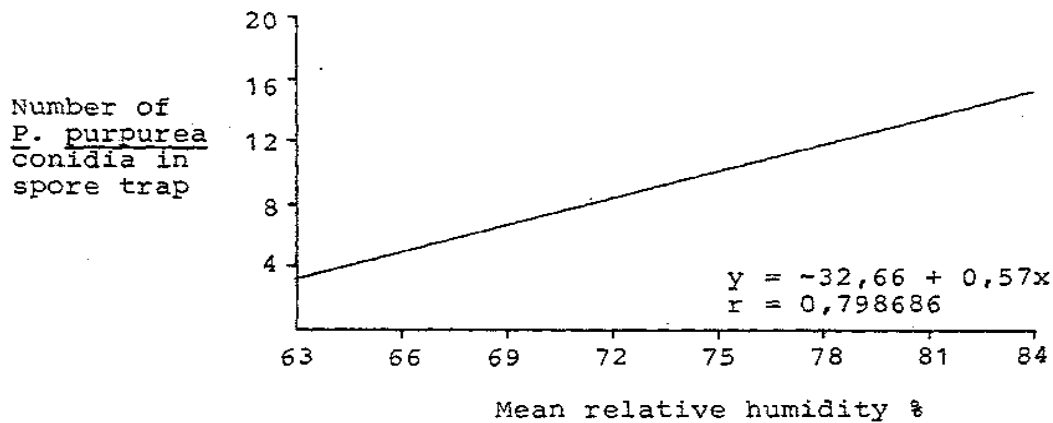


FIG. 7. - The effect of RH on the number of trapped P. purpurea conidia analysed on a daily data basis.

The most significant correlation ( $r = 0,905787$ ) was found between the number of P. purpurea and rainfall. The linear regression model fitted to the data is  $y = 2,71 + 0,33x$  (Fig. 8).

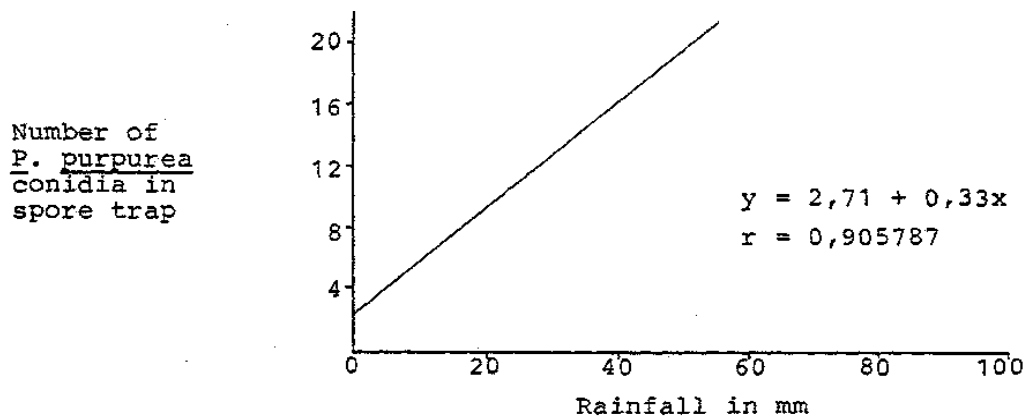


FIG. 8. - The effect of rainfall on the number of trapped P. purpurea conidia analysed on a daily data basis.

It is interesting to note that most of the *P. purpurea* conidia were trapped in the early morning from about 01h00 to 06h00.

#### 4.5 CRITICAL INFECTION PERIODS FOR CERCOSPORA SPOT

A number of experiments were conducted to detect the critical period for fruit infection by *P. purpurea* under natural orchard conditions. During the 1977/78 summer the first experiment was undertaken with Fuerte fruit exposures from October until March in a cumulative way on a monthly basis. Treatments in this experiment included the exposing of fruit from the early summer period (October) and these exposure times were gradually extended by a month at a time up to March. The linear regression fitted to the number of Cercospora spots on the fruit and the number of months (exposure period) is  $y = 2,80 + 0,74x$ , with  $r = 0,403114$ . This indicates a non-significant increase of Cercospora spots on the fruit with the increase in the length of the exposure time. If exposure time is decreased on a monthly interval from the full season's exposure to the end of summer (March), the correlation is significant  $r = -0,859188$ . The increase in Cercospora spot incidence on the fruit in relation to the length of the exposure time is described with the linear regression model of  $y = 6,11 - 0,79x$  (Fig. 9).

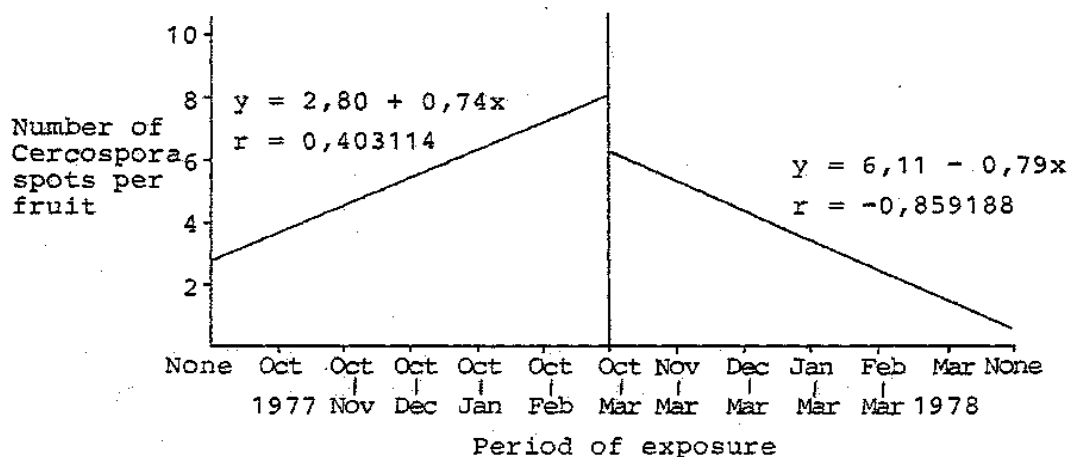


FIG. 9. - The effect of the length of the exposure time on the natural Cercospora spot infection in 1977/78.

The experiment for the detection of critical infection periods in the 1978/79 season was initiated in November and fruit was exposed at a monthly interval on a one monthly basis and also on a monthly cumulative basis. A good correlation,  $r = -0,899350$  was found between the number of Cercospora spots on the fruit exposed on a monthly basis and timing of these exposures. According to this correlation the infection was more severe on fruit exposed to natural infection early in the season and it is described as  $y = 6,33 - 0,88x$ . A significant,  $r = -0,872669$  increase in disease incidence was also found

for the monthly cumulative exposures. The linear regression model for this increase is  $y = 7,46 - 1,17x$  (Fig. 10).

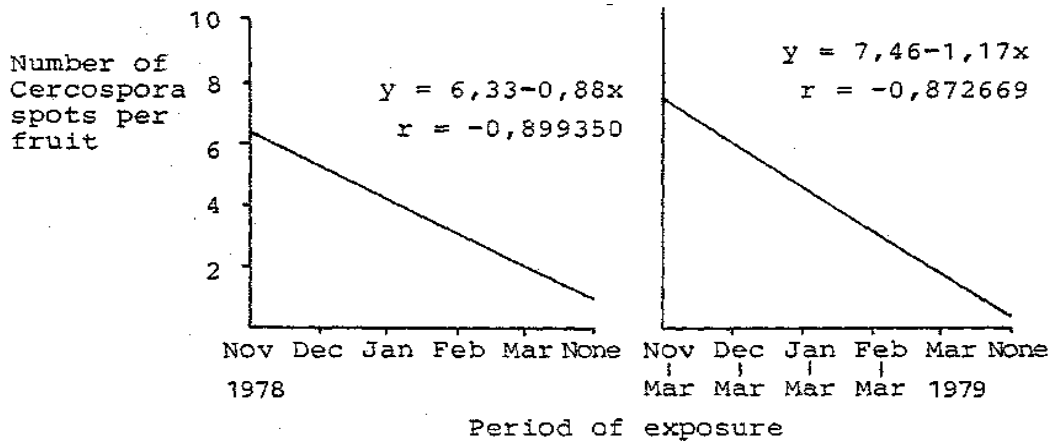


FIG. 10. - The effect of the length and timing of the exposure periods on the natural Cercospora spot infection in 1978/79.

Data of the 1978/79 fruit exposure experiment was also used to analyse correlations between the severity of Cercospora spot disease and rainfall and spore trap figures. The correlation between rainfall and Cercospora spot infection in the monthly exposure is  $y = 1,65 + 0,009x$ , with  $r = 0,319655$  and the linear regression model for the correlation between rainfall and the monthly cumulative exposures is  $y = -0,10 + 0,008x$ , with  $r = 0,885196$  (Fig. 11).

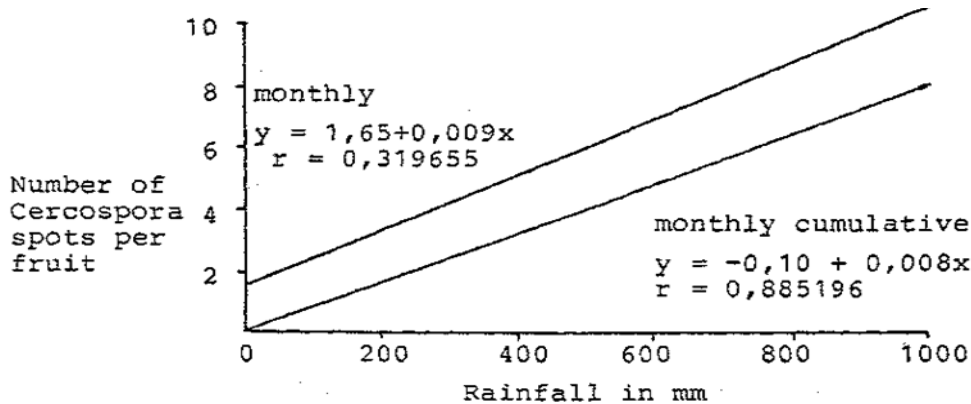


FIG. 11. - The correlation between Cercospora spot infection and rainfall in 1978/79.

The incidence of Cercospora spot infection in the 1978/79 fruit exposure experiment was correlated with the number of P. purpurea conidia in the spore trap on the monthly

basis and the following regression line was obtained  $y = 2,84 + 0,003x$ , with  $r = 0,089442$ . The linear regression model for the correlation between infection and the number of conidia on the monthly cumulative basis is  $y = 0,06 + 0,01x$ , with  $r = 0,853691$  (Fig. 12).

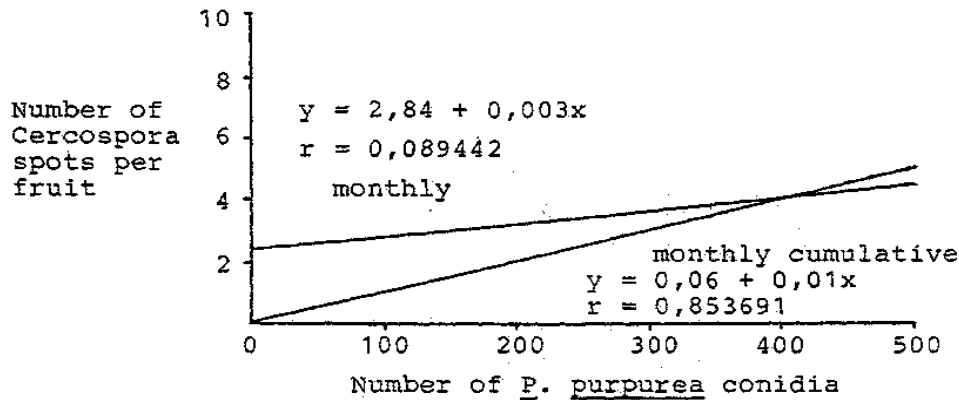


FIG. 12. - The correlation between Cercospora spot infection and the number of trapped P. purpurea conidia in 1978/79.

The third experiment to study critical infection periods under orchard conditions was conducted in 1981/82. The exposure periods were monthly and monthly cumulative. Cercospora spot infection correlated significantly with the exposure period on the monthly basis indicating an increase with early summer exposures. The linear regression model to describe the correlation is  $y = 4,48 - 0,69x$ , with  $r = -0,711963$ . The correlation between infection and the monthly cumulative exposures is also significant,  $r = -0,734053$  and the equation is  $y = 5,04 - 0,74x$  (Fig. 13).

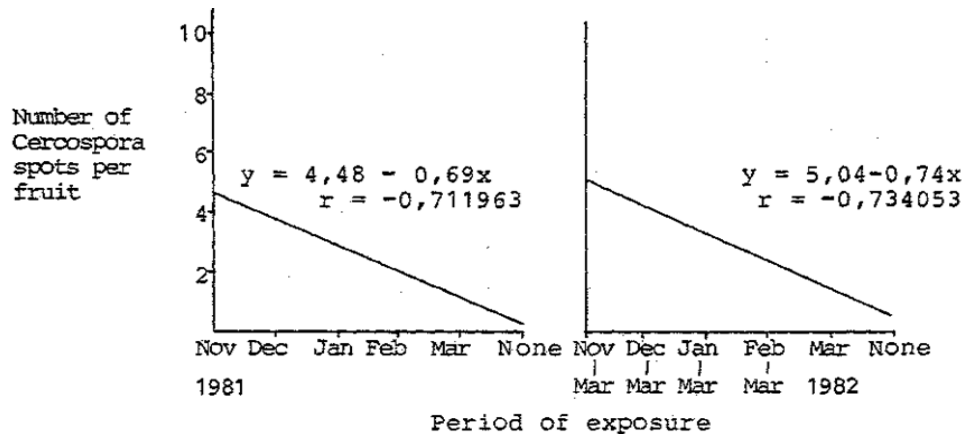


FIG. 13. - The effect of the length and timing of the exposure periods on the natural Cercospora spot infection in 1981/82.

Cercospora spot infections recorded in the fruit exposure experiment of 1981/82 were also correlated with rainfall figures. The linear regression for the correlation between infection and rainfall in the monthly exposure is  $y = -1,55 + 0,01x$ , with a non-significant  $r = 0,701418$ . The correlation between infection and rainfall in the monthly cumulative exposures is  $y = 0,21 + 0,006x$ , with a significant correlation coefficient of  $r = 0,786247$  (Fig. 14).

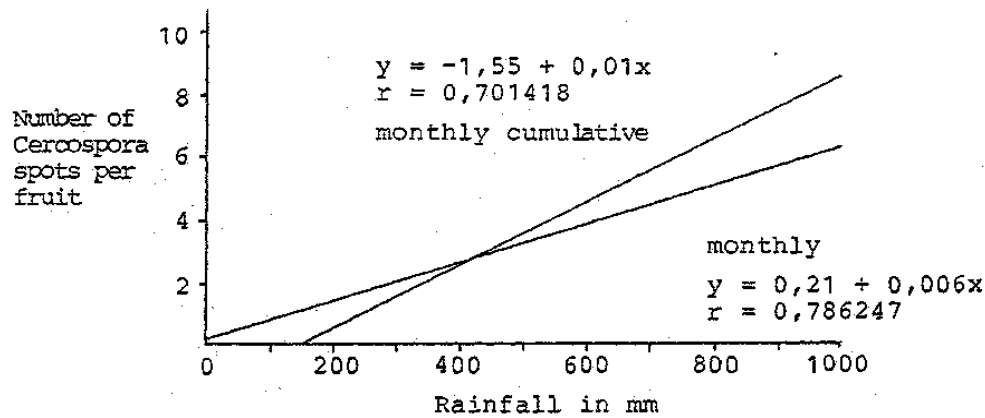


FIG. 14. - The correlation between Cercospora spot infection and rainfall in 1981/82.

TABLE 7. - Control of Cercospora spot on Fuerte sprayed twice in the 1976/77 season.

Treatment number	Treatments	Mean number of Cerc. spots/ fruit (N = 4800)	
		Assessed on 22 Apr. 1977	Assessed on 15 June 1977
1	Fosetyl-Al 0,3% a.i.	2,5b	4,3b
2	Benomyl 0,02 % a.i.	0,9b	1,2c
3	Benomyl 0,025% a.i.	0,6b	1,3c
4	Thiophanate-methyl 0,05% a.i.	1,1b	3,3b
5	Thiophanate-methyl 0,07% a.i.	1,0b	2,1bc
6	Control	5,2a	8,3a

Means with letters a, b and c differ statistically at 0,05 level (Duncan's multiple range test)

#### 4.6 CHEMICAL CONTROL OF CERCOSPORA SPOT

Results of the 1976/77 season's chemical control experiment are presented in Table 7.

In the assessment made in April, all three chemicals at the various concentrations showed an equally significant control of Cercospora spot disease. Due to the increase in disease severity, differences between the treatments in June became significant and here benomyl proved to be the most effective at both rates. Thiophanate-methyl at the low rate and fosetyl-AI were statistically inferior to benomyl, but they significantly reduced disease in comparison with the control.

The experiment in 1977/78 investigated the optimum timing of benomyl sprays and results are given in Table 8. In this benomyl timing experiment the severity of Cercospora spot infection increased greatly during the two months which elapsed between assessments. The disease was generally better controlled with frequent, short interval sprays. Late applications slowed down the increase of disease incidence between assessments. Good control was achieved by two benomyl sprays when one was applied in the first week of November and the second around mid-January (treatment No. 14). The first spray was applied prior to an appreciable amount of rain at the end of November, while the second was in the middle of the very rainy January. Another two-spray treatment (No. 15) applied about four weeks later was inferior at the time of the first assessment, but showed a good residual effect in the second assessment.

TABLE 8. - The effect of the number and timing of benomyl sprays on Cercospora spot of Fuerte in 1977/78.

Treatment number	No. of applications	Time interval weeks	Date of applications	Mean number of Cerc. spots per fruit (N = 12 000)	
				Assessed on 12 Apr. 1978	Assessed on 7 June 1978
1	-	-	Control	5,1ab <sup>bc</sup>	19,5a <sup>c</sup>
2	1	-	6 Oct. 1977	4,0abc <sup>abc</sup>	10,4bc <sup>ab</sup>
3	2	8	6 Oct. 1977; 2 Dec. 1977	2,3abc <sup>abc</sup>	13,9ab <sup>bc</sup>
4	3	8	6 Oct. 1977; 2 Dec. 1977; 2 Febr. 1978	1,9abc <sup>abc</sup>	7,3bc <sup>ab</sup>
5	4	8	6 Oct. 1977; 2 Dec. 1977; 2 Febr. 1978; 6 Apr. 1978	1,0bc <sup>ab</sup>	3,5c <sup>a</sup>
6	3	8	2 Dec. 1977; 2 Febr. 1978; 6 Apr. 1978	0,3c <sup>a</sup>	3,4c <sup>a</sup>
7	2	8	2 Febr. 1978; 6 Apr. 1978	3,0abc <sup>abc</sup>	6,4bc <sup>ab</sup>
8	1	-	6 Apr. 1978	3,4abc <sup>abc</sup>	10,3bc <sup>ab</sup>
9	1	-	11 Nov. 1977	0,9bc <sup>ab</sup>	9,0bc <sup>ab</sup>
10	2	9	11 Nov. 1977; 19 Jan. 1978	0,9bc <sup>ab</sup>	6,8bc <sup>ab</sup>
11	3	9	11 Nov. 1977; 19 Jan. 1978; 27 Mar. 1978	2,7abc <sup>abc</sup>	6,5bc <sup>ab</sup>
12	2	9	19 Jan. 1978; 27 Mar. 1978	6,0a <sup>c</sup>	12,3bc <sup>ab</sup>
13	1	-	2 Febr. 1978	5,6a <sup>c</sup>	10,7bc <sup>ab</sup>
14	2	10	2 Nov. 1977; 19 Jan. 1978	0,5c <sup>a</sup>	6,1bc <sup>ab</sup>
15	2	10	28 Nov. 1977; 15 Febr. 1978	2,7abc <sup>abc</sup>	5,4c <sup>a</sup>

Means with letters a, b and c differ statistically at 0,05 level (Duncan's multiple range test)

In the 1978/79 experiment new additives and fungicides were tested against *Cercospora* spot, all in two-spray treatments and results are presented in Table 9. The various stickers added to the benomyl mixture did not significantly influence the efficiency of the fungicide against the disease. At low rates, propaconazole gave significantly less effective control than the benomyl and Nu Film 17 combination, while etaconazole and propaconazole at the high rates significantly increased the disease incidence compared to the control. TBZ was added to benomyl with the aim of enhancing post-harvest *Dothiorella* fruit rot control but it did not significantly improve *Cercospora* spot control compared to the benomyl plus Nu Film 17 mixture.

TABLE 9. - The evaluation of fungicides and additives for the control of *Cercospora* spot on Fuerte sprayed twice in 1978/79.

Treatment number	Treatments	Mean number of Cerc. spots per fruit (N = 7 200)
1	Benomyl 0,025% a.i. + Nu Film 17 0,02%	1,3c
2	Benomyl 0,025% a.i. + Biofilm 0,05%	1,9bc
3	Benomyl 0,025% a.i. + Solvaid 0,03%	2,1bc
4	Benomyl 0,025% a.i. + TBZ 0,05% a.i. + Nu Film	1,2c
5	Etaconazole 0,025% a.i. + Nu Film 17 0,02%	7,0a
6	Propiconazole 0,025% a.i. + Nu Film 17 0,02%	8,1a
7	Etaconazole 0,005% a.i. + Nu Film 17 0,02%	2,5bc
8	Propiconazole 0,005% a.i. + Nu Film 17 0,02%	3,0b
9	Control.	4,3b

Means with letters a, b and c differ statistically at 0,05 level (Duncan's multiple range test)

Additives were tested again in the 1979/80 season together with some new fungicides (Table 10). The best *Cercospora* spot control was obtained by using captafol at a concentration of 0,16 percent a.i. in two and three-spray applications. There were no appreciable differences between benomyl in combination with various additives and results were consistently good. Cu-hydroxide and Cu-oxychloride tended to be less effective than benomyl, however, the differences were not significant. Treatments which failed to check the disease were fosetyl-Al, PP 296, B77, glyodin and bitertanol.

In the 1980/81 experiment captafol was tested at various concentrations and in spray programmes with other fungicides and compared to the standard benomyl spray (Table 11). The statistical analyses of the data obtained from the experiment showed that all chemical treatments ensured an effective reduction of *Cercospora* spot. There were no significant differences between the various chemical treatments.

Results of the experiment in 1981/82 on *Cercospora* spot control are presented in Table 12. All treatments in the experiment controlled the disease significantly, except captain

which failed to reduce it to a statistically lower level when the chemical was sprayed alone.

TABLE 10. - The effect of two- and three-spray treatments on Cercospora spot control of Fuerte in 1979/80.

Treatment number	Number of applications	Treatments	Mean number of Cerc. spots/fruit (N=10 400)
1	2	Benomyl 0,025% a.i. + Nu Film 17 0,02%	2,6 cd
2	2	Benomyl 0,025% a.i. + Plyac 0,03%	2,6 cd
3	2	Benomyl 0,025% a.i. + Solvaid 0,03%	2,7 cd
4	2	B 77 150 ppm + Glyodin 0,125% + Nu Film 17	7,1 b
5	2	Bitertanol 0,01% a.i. + Nu Film 17 0,02%	7,8 b
6	2	PP 296 0,08% a.i. + Nu Film 17 0,02%	9,2 ab
7	2	Cu-hydroxide 0,15% a.i. + Nu Film 17 0,02%	3,9 c
8	2	Cu-oxychloride 0,25% a.i. + Nu Film 17 0,02%	3,1 cd
9	3	Cu-oxychloride 0,25% a.i. + Nu Film 17 0,02%	3,6 c
10	2	Captafol 0,16% a.i. + Nu Film 17 0,02%	0,6 d
11	3	Captafol 0,16% a.i. + Nu Film 17 0,02%	0,7 d
12	2	Fosetyl-Al 0,3% a.i. + Nu Film 17 0,02%	11,4 a
13	-	Control	9,6 ab

Means with letters a,b, c and d differ statistically at 0,05 level (Duncan's multiple range test)

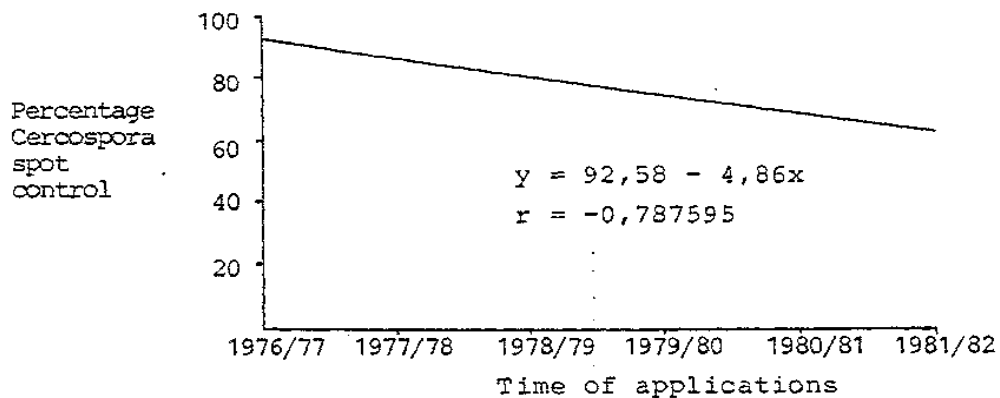


Fig. 15. - The control of Cercospora spot disease by benomyl in experiments over the past six years.

Lastly, the percentage disease control by benomyl has been calculated for the past six



years by using data from Table 7 (assessment in April, treatment Nos. 2 and 6), Table 8 (assessment in April, treatment Nos. 14 and 1), Table 9 (treatment Nos. 1 and 9), Table 10 (treatment Nos. 1 and 13), Table 11 (treatment Nos. 1 and 8), and Table 12 (treatment Nos. 1 and 10) and statistically analysed results are presented in Fig. 15. There was a statistically significant decline in the efficacy of benomyl measured in terms of percentage disease control. This reduction is described by the linear regression line of  $y = 92,58 - 4,86x$ , with a correlation coefficient of  $r = -0,787595$ .

TABLE 11. - The effect of fungicide treatments on Cercospora spot of Fuerte in 1980/81.

Treatment number	Time of applications	Treatments	Mean number of Cerc. spots/fruit (N=2 000)
1	Nov. 1980 Jan. 1981	Benomyl 0,025% a.i. + Nu Film 17 0,02%	6,3 b
2	Nov. 1980 Jan. 1981	Captafol 0,16% a.i. + Nu Film 17 0,02%	5,3 b
3	Nov. 1980 Jan. 1981	Captafol 0,16% a.i. + Nu Film 17 0,02% Benomyl 0,025% a.i. + Nu Film 17 0,02%	4,6 b
4	Nov. 1980 Jan. 1981	Captafol 0,16% a.i. + Nu Film 17 0,02% Cu-oxychloride 0,25% a.i. + Nu Film 17 0,02%	7,5 b
5	Nov. 1980 Jan. 1981	Captafol 0,08% a.i. + Nu Film 17 0,02%	5,6 b
6	Nov. 1980 Jan. 1981	Captafol 0,08% a.i. + Nu Film 17 0,02% Benomyl 0,025% a.i. + Nu Film 17 0,02%	4,1 b
7	Nov. 1980 Jan. 1981	Captafol 0,08% a.i. + Nu Film 17 0,02% Cu-oxychloride 0,25% a.i. + Nu Film 17 0,02%	4,5 b
8	-	Control	15,9 a

Means with letters a and b differ statistically at 0,05 level (Duncan's multiple range test)

## 5. DISCUSSION

Cercospora spot is an important disease of avocados in areas where high rainfall and favourable temperature are found (Stevens, 1922; Turu, 1969; Brodrick *et al.*, 1974; Gustafson, 1976). It is present in most of the avocado growing areas of the Transvaal Lowveld in South Africa and is undoubtedly the most important pre-harvest avocado fruit disease at Westfalia Estate. Losses in export fruit are appreciable (up to 12%) even on commercially sprayed fruit (Table 1) and the disease is significantly more severe on unsprayed fruit (Tables 7, 8, 9, 10, 11 and 12), (up to 69%). Fuerte and Ryan cultivars

are considerably more susceptible to infections by the pathogen than Edranol and Hass (Table 1). Significant correlations were found between losses on Fuerte and Ryan in relation to the amount of rain, the higher the rainfall, the more severe are the Cercospora spot losses (Figs. 1 and 2). Regression analysis between losses and harvest time revealed that there was a highly significant increase during the harvest period of Fuerte which decreased slightly towards the end of the dry winter season (Fig. 3). The increase in losses with late harvest was non-significant in the case of Ryan (Fig. 4).

TABLE 12. - Control of Cercospora spot of Fuerte with two-spray treatments in 1981/82.

Treatment number	Time of applications	Treatments	Mean number of Cerc. spots/fruit (N=3 600)
1	Nov. 1981 Jan. 1982	Benomyl 0,025% a.i. + Nu Film 17 0,02%	0,9 b
2	Nov. 1981 Jan. 1982	Cu-oxychloride 0,25% a.i. + Nu Film 17 0,02%	0,4 b
3	Nov. 1981 Jan. 1982	Cu-hydroxide 0,23% a.i. + Nu Film 17 0,02%	0,4 b
4	Nov. 1981 Jan. 1982	Captan 0,1% a.i. + Nu Film 17 0,02%	2,4 ab
5	Nov. 1981 Jan. 1982	Prochloraz 0,04% a.i. + Nu Film 17 0,02%	1,0 b
6	Nov. 1981 Jan. 1982	Captafol 0,08% a.i. + Nu Film 17 0,02% Cu-oxychloride 0,25% a.i. + Nu Film 17 0,02%	0,5 b
7	Nov. 1981 Jan. 1982	Captafol 0,08% a.i. + Nu Film 17 0,02% Cu-hydroxide 0,23% a.i. + Nu Film 17 0,02%	0,7 b
8	Nov. 1981 Jan. 1982	Captafol 0,08% a.i. + Nu Film 17 0,02% Captan 0,1% a.i. + Nu Film 17 0,02%	1,3 b
9	Nov. 1981 Jan. 1982	Captafol 0,08% a.i. + Nu Film 17 0,02% Cu-oxychl. 0,25% + Bitertanol 0,0125% + Agridex 0,1%	0,5 b
10	-	Control	3,2 a

Means with letters a and b differ statistically at 0,05 level (Duncan's multiple range test)

The majority of the symptom descriptions (Stevens, 1922; Zentmyer, 1953; Brodrick *et al.*, 1974) agree with observations made at Westfalia Estate. Differences from earlier descriptions of disease symptoms on Fuerte include the recognition of development stages of the disease. A characteristic swelling of the epidermal tissues at the infection site is the cause of the raised form which is the early development stage. It appears that this is the stage that Brodrick *et al.* (1974) described. Cercospora spot lesions become sunken in the later stage of disease development and probably these mature spot

symptoms compare with those described by Zentmyer (1953). Cracks are formed only in older spots and they do not necessarily develop in lenticels. It is true, however, that the pathogen may infect the fruit through cracks in lenticels caused by other factors.

The causal organism was identified as Pseudocercospora purpurea (Cke) Deighton and this was confirmed by taxonomists at the Commonwealth Mycological Institute in England. Typical conidia of P. purpurea were readily produced in fresh isolations on artificial media and on sterile avocado pieces. Cultures which became sterile, regained the ability to produce conidia on sterile avocado if kept under near UV light. This finding is in contrast to all previous reports on the *in vitro* sporulation of the fungus and it is the first reported case of inducing sporulation of P. purpurea under laboratory conditions. This is also the first confirmed record of the occurrence of Cercospora spot disease and of the fungus, Pseudocercospora purpurea in South Africa. The fact that the pathogen is a very slow growing organism which becomes sterile easily in cultures on artificial media is probably the reason why it was not previously identified and why the disease in South Africa is referred to as fruit spotting caused by a sterile fungus (Gorter, 1977). Black fruiting bodies found in older cultures of P. purpurea by Stevens (1922) were also observed in this study but differed from his observations in that these fruiting bodies sporulated and appeared to be spermatogonia and not pycnidia. Their role in the Cercospora spot disease is unknown. There is no known sexual stage of the fungus and no evidence of its existence was found in this study. Since the pathogen can be isolated from fruit and leaf spots all year round in the conidial form, it appears to be independent of a sexual overwintering stage.

The disease is distributed throughout the tree on Fuerte, though there was a non-significant tendency for more Cercospora spots to occur on fruit from the northern and western aspects (Table 2). The incidence of the disease increased significantly with the increase in root rot severity of Fuerte trees (Fig. 5). This may point to the secondary invading behaviour of the pathogen. Fruit produced on trees with less foliage are more exposed to factors which enhance lenticel cracking and consequently a higher Cercospora spot infection may occur through the minute wounds in the cracks. The age of spots has a marked influence on the success of isolation of the pathogen, it being much more easily recovered from young lesions (Table 3). The incidence of secondary invaders, mainly Colletotrichum gloeosporioides was also high in old spots. A similar phenomenon has been described by Kotzé (1963) and is well known in sub-tropical fruit. Although it is suggested in this study that sunken spots are older than raised spots, the success of recovery of P. purpurea from the two types of lesion of the same age on Fuerte fruit was nearly equal. However, the incidence of secondary organisms was again higher in sunken spots where cracks were present (Table 4). The above findings confirm the statements of Ruehle (1943b) and Zentmyer (1953) that anthracnose is often associated with Cercospora spot.

Spore trapping was attempted in this study for the first time in the history of Cercospora spot investigations. Virtually the whole first year's effort (1977/78 season) was confined to finding appropriate techniques and gaining sufficient experience in the identification of trapped conidia. Of the three stains tested Methylene Blue proved to be the most suitable. It stained conidia of P. purpurea by giving a moderate blueish cast which was more dense in the basal and apical cells of the conidia. Other Cercospora-like conidia

caught in the spore trap stained differently and of course shape, measurements and the number of septae in the conidia were also necessary for the positive identification. A significant correlation was found between the number of conidia trapped weekly and the weekly rainfall and mean air temperature values, but the correlation was not significant if rainfall and temperature were analysed separately in relation to the conidia figures (Table 5). In Table 6, not only the multiple regression analysis of the daily number of conidia and temperature and rain correlated significantly, but also the correlations between the number of conidia and temperature (Fig. 6), relative humidity (Fig. 7) and rainfall (Fig. 8) respectively were also significant. The negative correlation of the air temperature to the number of conidia can be explained by the actual cooling effect of rain. High relative humidity is coupled to an increase in the number of conidia and this again is a function of the rainfall. Relative humidity evidently plays an important role in the spore release mechanism of the fungus, since most conidia were caught in the early mornings when high humidity prevailed. Rain is the most important factor that influenced the production of *P. purpurea* conidia, showing the highest level of significance (Fig. 8) and repeatedly exhibited a significant effect on Cercospora spot incidence (Figs. 1 and 2). Equations were obtained which may be used to forecast the number of conidia expected to be produced and released into a given orchard's atmosphere and which can be used as an indication of high risk infection periods. This could be of value in the chemical control of the disease by enabling one to choose the optimum timing of the first spray. It is, however, of little importance in the timing of the follow-up sprays because the time period favourable for spore production is too long and there are many high risk periods at Westfalia Estate.

Experiments on the critical infection period by Cercospora spot disease firstly proved that long exposures of Fuerte fruit to natural infection resulted in a higher disease incidence (Fig. 9). Exposures on a monthly basis from November until March showed that a significantly more severe infection occurred on fruit exposed early in the summer season (Fig. 10). Poor correlation was found between rainfall and disease incidence in the monthly exposures, but the correlation was significant between rainfall and disease in the monthly cumulative exposures (Fig. 11). The same conclusion is made from correlations between disease incidence and spore trap results analysed on monthly and monthly cumulative basis (Fig. 12).

In a third experiment on the detection of the critical infection period, the monthly exposures again pointed to the importance of the time of the infection in disease incidence by being significantly more prominent on fruit exposed early in the growing season, provided infection is taking place (Fig. 13). Comparisons between the incidence of the disease and rainfall again proved that time between infection and symptom development plays a decisive role (Fig. 14).

With regard to the critical infection period, it can be concluded that Cercospora spot disease severity is determined by two major factors. Firstly, the high risk infection periods or the availability of conidia and weather conditions favourable for infection and secondly, the time or latent phase which must elapse between infection and symptom development. The latent phase appears to be about three months in duration as deduced from the above experiments and artificial inoculation experiments undertaken at Westfalia Estate to establish Koch's postulates. This also agrees with the statement

of Stevens (1922) that late season's fruit is less susceptible to *Cercospora* spot disease.

In the preliminary chemical control experiments, benomyl gave the best results at a rate of 0,025 percent a.i. applied twice in the summer season (Table 7). Further experiments proved that two-spray benomyl treatments provided acceptable control and that the timing with November and January applications was near to optimum (Table 8). It is unclear why some of the treatments yielded unexpected results. For example, the three-spray application of benomyl in treatment No. 11 was found to be inferior to the two-spray treatment of No. 10 at the time of the first assessment.

It appears that sprays relatively early in the summer season (November) are more effective than late sprays in controlling the disease. This underlines earlier findings that the critical period for *Cercospora* spot disease is the early rainy season. The addition of the additive Nu Film 17 to benomyl was found to be insignificantly more effective than the mixing of Biofilm and Solvaid additives (Table 9). TBZ in the second spray of a benomyl treatment, aimed mainly at post-harvest disease control did not show efficacy against *Cercospora* spot disease. Etaconazole and propiconazole were ineffective. Benomyl with the commercially used Nu Film 17 and benomyl with Plyac and Solvaid additives were equally effective in the 1979/80 season's experiment (Table 10). The fungicide that gave best results was captafol, while benomyl, Cu-oxychloride and Cu-hydroxide also provided significant control compared with the unsprayed fruit. B 77, glyodin, bitertanol, PP 296 and fosetyl-Al were ineffective.

The discovery of effective treatments with non-benzimidazole type fungicides led to the extensive testing of captafol and Cu-formulations. It was proved that the two-spray treatments of captafol at the 0,08 percent a.i. rate is as effective as the 0,16 percent a.i. concentration and that it gave good control both alone and in spray programmes with Cu-oxychloride (Table 11). This was confirmed again by results of the 1981/82 season's experiment (Table 12). Cu-oxychloride and Cu-hydroxide used alone and in spray programmes with captafol equalled the efficacy of benomyl treatments. There was a non-significantly higher disease incidence on prochloraz sprayed fruit and captan failed to control the disease. Bitertanol was included in the second spray of a captafol plus Cu-oxychloride spray programme (treatment No. 9) to investigate its effect on post-harvest diseases and it showed no control against *Cercospora* spot.

With regard to the chemical control of the disease at Westfalia Estate, it must be added that benomyl has been used exclusively for the past 10 years in most of the producing avocado orchards. This raised the question of resistance of *P. purpurea* to benomyl. The matter was not fully investigated from the resistance point of view, but the finding on the progressively poorer control by benomyl (Fig. 15) may suggest an increased tolerance of the fungus to the chemical. For this reason, Westfalia Estate has adopted the policy of alternating fungicides with different modes of action in controlling the disease. The chemicals presently recommended to replace benomyl under such conditions are captafol and the Cu-formulations.