Screening of olive cultivars for tolerance to *Fusicladium oleagineum* in South Africa

By

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DECLARATION

I, Zakhele Cyril Msimango declare that the dissertation, which I hereby submit for the degree Masters Institutionis Agrariae (M Inst Agrar): Horticultural Science at the University of Pretoria is my own work and has not previously been submitted by me for a degree at any other tertiary institution.

Signature: .....

Date: .....

DEDICATION

"This work is dedicated to my parents, Gerald Sipho (late father) and Merriam Mfoko Msimango (mother), who inspired me to never give up pursuing my dream to the utmost regardless of the thorny situations encountered."

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ABSTRACT

Olive leaf spot (OLS) caused by *Fusicladium oleagineum*, is one of the major fungal diseases affecting olive orchards in South Africa and worldwide. Fungicides (copper containing sprays) successfully control OLS, when disease risk is low, but heavy infestation impairs effectiveness of chemical control and could lead to severe yield loss. Regular chemical treatment may prevent disease build-up in the orchard, but with cost and chemical residue implications. This study was aimed at evaluating commercial olive cultivars ('Mission'; 'Manzanilla de Seville'; 'Frantoio'; 'Nandi'; 'Nocellara del Belice'; 'Coratina'; 'Barouni' and 'Leccino') for resistance against OLS.

A growth chamber trial was conducted at Bien Donne experimental farm of the Agricultural Research Council. Only one temperature treatment (16°C) was applied during inoculation, while a relative humidity of above 80% was maintained. Plants were kept for 48 hours at 99% RH after inoculation and then moved to a shade net area for disease development at a temperature of 25±5°C. The results were used for categorising the eight olive cultivars evaluated in this study according to their OLS susceptibility.

'Frantoio' was categorised as highly tolerant, while 'Nandi' and 'Leccino' were found to be moderately tolerant and 'Nocellara del Belice' fairly tolerant. 'Coratina' was found to be the most susceptible cultivar. 'Mission' and 'Manzanilla de Seville' were found to be fairly susceptible. The findings of this study provide a basis for preliminary recommendations of OLS tolerant cultivars and selections for commercial olive production, as well as for further evaluation of OLS tolerance of olive cultivars and selections both in a controlled environment (glasshouse/ growth chamber), and under field conditions.

As the results of this study were obtained over one season only (2011/12), it is recommended that the evaluation of OLS tolerance of the eight cultivars included in this study should be repeated both under controlled conditions, as well as under field conditions in the major olive production regions of South Africa, to verify the preliminary results.

***Keywords**:* cultivars, evaluation, fungal disease, *Fusicladium oleagineum,* germination, infection, inoculum, *Olea oleagineum* L., olive, olive leaf spot, tentative screening.

CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ACRONYMS AND ABBREVIATIONS

ANOVA	Analysis of Variance
ARC	Agricultural Research Council
CRBD	Complete Randomised Block Design
LSD	Least significant difference
OLE	Olive Leaf Extract
OLS	Olive Leaf Spot
OSDP	Olive Sector Development Plan
RH	Relative Humidity
SAOI	South African Olive Industry
spp.	species
W/w	Weight/Weight

CHAPTER 1

INTRODUCTION

1.1 OVERVIEW OF THE GLOBAL AND THE SOUTH AFRICAN OLIVE INDUSTRY

The olive (*Olea europaea* sub-species *oleaceae*) is native to the coastal areas of the eastern Mediterranean Basin. The olive is of major agricultural importance in the Mediterranean region as the source of olive oil and table olives (Palgrave 1977). Olive trees require a Mediterranean climate which is characterised by cold (mean monthly temperature ≥-3°C), wet but short winters (April to August) and long, warm to hot, dry, sunny summer with a maximum temperature of 30°C between September to March weather conditions (Costa 1998).

Temperature is the most critical climatic factor governing olive production, because olive trees have a chilling requirement for fruit bud initiation and differentiation. These conditions are an average temperature of 12°C in May to July (southern hemisphere), with a maximum daily temperature lower than 21°C as a minimum requirement for vegetative growth (Costa 1998). High temperatures, 27°C, during late winter or spring appear to have a beneficial effect on flowering (Costa 1998), with fruit growth, oil accumulation and colour development promoted at 25°C (Flanagan & Hildenbrand 2003).

Olives are grown in various countries worldwide such as Algeria, Argentina, Australia, Chile, Croatia, Cyprus, France, Greece, Italy, Israel, Jordan, Morocco, New Zealand, Portugal, Slovenia, South Africa, Spain, Syria, Turkey, Tunisia, and the United States. Spain, Italy, and Greece are the leading production regions which represents more than three-quarters of the total olive oil production in the world (Table 1.1) (Costa 1998, Flanagan & Hildenbrand 2003).

From 2000 to 2008, South Africa's olive plantings comprised between 3000 and 4000ha (Olive Directory 2010). Currently the area planted to olives is expanding rapidly, due to the fast growth of the industry, with the Karoo region of the Western Cape becoming the primary olive production area in South African, as it has favourable climatic conditions for olive growing (OSDP 2013,

Rubidge 2013). In comparison, approximately 10 million ha are under cultivation in the rest of the olive producing countries in the world (Costa 1998).

Spain was reported as leading producing and consuming country of table olive and olive oil with 58950 t (19.7%) globally, (IOC 2013). South African local production is estimated at 2000 t which is equivalent to 2 million litres of olive oil (OSDP 2013). However, recent statistics show an increase in local consumption, which is estimated at 7500 t of oil and 2000 t of table olives, indicating that South Africa is now a net importer of olive product i.e. demand exceeds supply (OSDP 2013).

Table 1.1: The main producing and consuming countries of table olives and olive oil in the world (Olive-Directory 2010)

Country	Production in tons (2009)	Production % (2009)	Consumption (2005)	Annual per capita consumption (kg)
World	2,907,985	100%	100%	0.43
Spain	1,199,200	41.2%	20%	13.62
Italy	587,700	20.2%	30%	12.35
Greece	332,600	11.4%	9%	23.7
Syria	168,163	5.8%	3%	7
Tunisia	150,000	5.2%	2%	11.1
Turkey	143,600	4.9%	2%	1.2
Morocco	95,300	3.3%	2%	1.8
Portugal	53,300	1.8%	2%	7.1
France	6,300	0.2%	4%	1.34
United States	2,700	0.1%	8%	0.56
Others	169,122	5.8%	18%	1.18

Olive trees were brought to South Africa in the 1660's, but commercial olive production commenced only in 1925 (Costa 1998). Since 1925, expansion of the South African olive industry (SAOI) was suppressed by the limited local market (minimum consumption) (Costa 1998, Flanagan & Hildenbrand 2003).

In the 1970's, the South African olive industry expanded due to several factors, including increased awareness of the health benefits of olive oil, increased international exposure and exposure to Mediterranean cuisine, as well as increased living standards (Costa 1998), due to modernisation. Since then the olive industry in South Africa has been concentrated around Paarl in the Western Cape Province (Figure 1.1) (Costa 1998, OSDP 2013), but a remarkable growth of newly established orchards were, recently noted and a tree census is currently underway (OSDP 2013).

Market reports show that imports of olive products into South Africa have been fluctuating year on year since 2004 and an increase in imports of more than 40% (7500 t) olive oil and 275% of table olives was observed during the 2010 industry review. Since then a dramatic increase (up to 140%) in local olive oil production occurred, whilst table olives showed slow growth with 3000 t of production and about 17% import (Olive Directory 2010).

The South African Olive Industry has rapidly grown throughout the country since the 1990's, when new olive plantations were established in low summer rainfall areas (such as the Oudtshoorn/ Swartberg area in the Klein Karoo and Vaalharts in Northern Cape), as well as some higher rainfall areas (parts of Mpumalanga, e.g. Lydenburg) (OSDP 2013), replacing other agricultural enterprises such as maize, ostrich and cash crops (Costa 1998) (Figure 1.1).

Recent research showed that olive production in South Africa has increased by at least 20% p.a. since 1990 (i.e. doubling in size every four to five years) which indicates that the olive industry is one of the fastest growing sub-sectors in agriculture (Olive-go-wild 2008). However, this significant growth and expansion in South Africa occurs mainly in regions with very diverse climatic conditions which are favourable for the occurrence of fungal diseases, such as olive leaf spot (OLS).

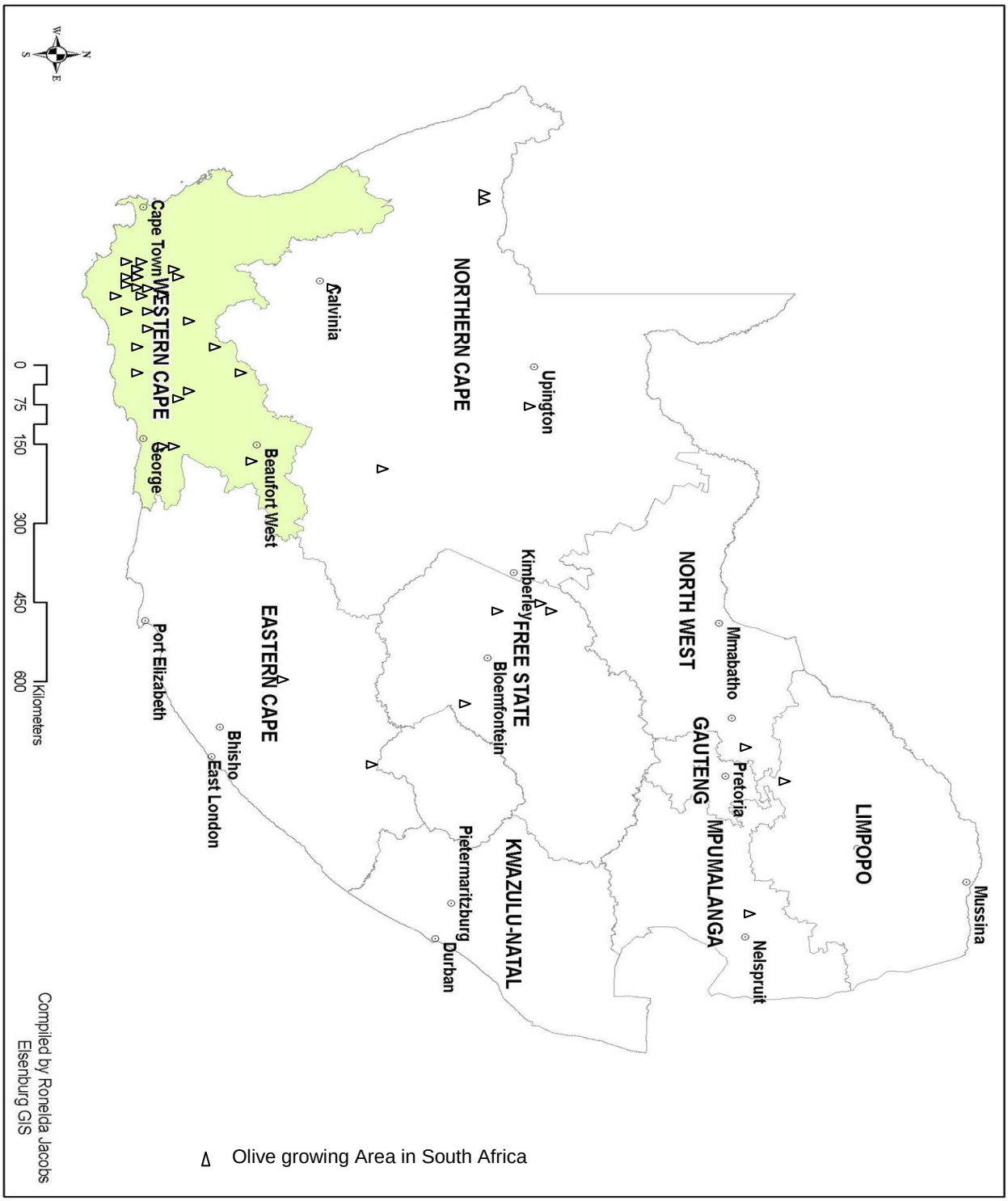

Figure 1.1: Olive growing areas in South Africa and Western Cape as leading growing area (Costa 1998), map adapted by Jacobs (2013).

Germination of the spores of *F. oleagineum* (syn. *Spilocaea oleagina,* formerly *Cycloconium oleaginum*) causing OLS, requires very high humidity (RH) ≥80%, with temperatures in the range of 0 - 27°C, but it is restricted at temperatures above 30°C (Obanor *et al*. 2010). The optimal temperature range for spore germination, infection and mycelia growth is 16 to 21°C (Graniti 1993, Viruega & Trapero 2002). Conidia develop only in the presence of free moisture or when the relative humidity is above 70% (Obanor *et al*. 2008, 2010).

New OLS infections are associated with rainfall and mostly occur during autumn and in winter. Late winter to spring infections may stay latent (remaining symptomless) during summer. Latent lesions grow again in summer during favourable temperature and humidity conditions, which serves as a source of inoculum for infection in the next season (Obanor 2006).

In Table 1.2 the average monthly temperatures, rainfall and relative humidity for established and new olive growing regions of South Africa are indicated for three years (2011, 2012 and 2013). In Table 1.3 the long term average monthly temperatures, rainfall and relative humidity values for these regions are given.

The Paarl region (the major olive production area in South Africa) and Riebeeck-West are characterized by a Mediterranean-type climate. These two production areas are in the winter rainfall region of South Africa. The peak rainfall occurs from April to September, but summer rainfall events also often occur. The average temperature for both these regions is within the optimal range for OLS infection from October to April. The average relative humidity during the period May to September (above 70%) is within the optimal range for OLS infection. Therefore both the temperature, rainfall and RH conditions in these regions are favourable for OLS infection during several months of the year. A need for alternative, but environmentally friendly, control measures is highly important to all olive growing areas of South Africa even though disease occurrences are not reported.

Barkley-West (Northern Cape) and Oudtshoorn (Klein-Karoo) are some of the new olive production areas in South Africa. They both receive summer rainfall and are very dry during winter. The peak rainfall occurs from December to March, whereas winter rainfall events rarely occur. The average temperature for these regions is within the optimal range for OLS infection from April to October (Barkley-West) and April to December (Oudtshoorn) respectively. However long-term average RH data indicate values below optimal requirements for OLS infection (Table 1.3). This indicates that there is a lower risk of OLS disease incidence in these regions.

There was some commercial olive orchards in the Lydenburg area (in the 1980's and 1990's) but these enterprises were discontinued due to unknown reasons. It is assumed that olive production in this region was not economical due to high disease incidence caused by the climatic conditions as explained above. Another possible explanation might be that insufficient flower bud initiation was obtained, due to the high temperatures prevailing from May to July. According to Costa (1998) an average temperature of 12ºC from May to July, as well as a minimum daily temperature below 21ºC, are required for olive flower bud initiation and in the Lydenburg area temperatures during these months exceed these values.

Conditions in regions with warm temperatures (between 26ºC and 30ºC) and RH above 70 % favour disease incidence of olive (Obanor *et al*. 2008), which causes growers to depend on chemical sprays for disease control (Sistani *et al*. 2009). Disease incidence negatively affects yield, quality and orchard lifespan. Regular chemical treatment may prevent disease build-up in the orchard but with cost and chemical residue implications. Thus the need for using cultivars tolerant to commercially important fungal diseases, such as OLS was seen as a solution.

The optimal range of relative humidity was exceeded for the period of three successive years (Table 1.2) which favours the development of olive scab. Thus the temperature, rainfall and relative humidity conditions in these regions are favourable for OLS infection.

A need for alternative, but environmentally friendly, control measures is highly important to all olive growing areas of South Africa even though disease occurrences are not reported.

Table 1.2 Average monthly temperatures, rainfall and relative humidity for established and new olive growing regions of South Africa for three years 2011, 2012 and 2013 (Agromet Stellenbosch).

Region	Month	Tx			Tn			T			Rain			RHx			RHn		
		2011	2012	2013	2011	2012	2013	2011	2012	2013	2011	2012	2013	2011	2012	2013	2011	2012	2013
Barkley West-Ulco	1	30	35	36	18	19	20	24	27	27	219	28	57	91	66	65	42	18	17
	2	30	32	35	18	18	18	23	24	26	145	91	69	92	82	69	38	29	17
	3	30	32	32	17	18	18	23	25	25	164	26	26	90	65	65	37	22	22
	4	24	27	27	12	11	11	17	19	19	73	1	1	92	70	70	40	24	24
	5	22	25	25	8	10	10	14	17	17	57	13	13	89	61	61	36	21	21
	6	18	21	21	3	4	4	10	12	12	65	0	0	84	62	62	33	21	21
	7	18	21	21	2	6	6	9	13	13	0	4	4	77	63	63	25	23	23
	8	23	22	22	5	5	5	14	13	13	0	0	0	66	62	62	19	16	16
	9	28	26	27	10	8	8	19	17	18	0	3	0	47	60	42	13	16	10
	10	10	31	30	30	13	12	21	22	22	0	23	4	45	57	45	11	14	12
	11	11	34	34	32	17	16	23	26	25	12	14	23	51	53	56	12	12	11
	12	12	32	32	33	17	17	24	24	24	66	60	94	75	80	76	19	25	24
Oudtshoorn: Rooirivier	1	34	35	32	17	17	15	25	25	23	4	20	4	76	79	80	24	19	23
	2	34	32	33	18	15	15	25	23	23	92	5	22	87	78	80	29	24	20
	3	32	30	31	16	14	14	23	22	22	18	25	44	82	83	80	26	27	25
	4	28	25	25	13	10	9	20	17	17	0	32	19	82	88	86	28	30	27
	5	17	22	24	6	7	7	11	14	15	7	9	17	87	86	83	34	29	25
	6	18	18	19	5	4	4	11	11	11	44	39	23	91	93	86	40	42	30
	7	18	17	20	4	3	4	10	10	12	27	75	8	91	91	87	38	39	32
	8	21	19	20	5	4	4	12	11	11	43	33	36	86	90	82	30	32	28
	9	25	23	23	7	6	5	15	14	13	4	2	6	86	86	84	21	25	22
	10	26	24	26	10	9	10	17	16	18	6	33	55	79	88	82	23	33	25
	11	27	30	29	10	11	12	18	20	20	21	1	35	83	78	83	22	19	24
	12	31	32	32	12	16	15	21	24	22	5	28	8	82	83	78	22	28	23
Paarl: BELLEVUE	1	35	35	33	18	18	17	25	25	24	6	3	13	77	76	77	24	24	24
	2	36	32	32	19	16	16	26	23	23	0	4	30	76	81	81	26	25	27
	3	33	32	31	16	18	16	23	23	22	9	26	20	82	73	79	26	26	28
	4	28	27	27	13	12	11	19	19	18	42	73	99	83	82	88	28	32	29
	5	22	22	24	10	8	10	15	14	15	120	63	105	89	93	89	41	40	37
	6	19	19	19	8	8	7	13	12	12	165	155	204	92	93	93	48	46	44
	7	22	18	20	8	7	8	14	12	13	39	156	135	83	91	90	34	45	45
	8	21	18	19	8	6	7	13	11	12	96	166	300	87	94	93	36	44	44
	9	23	21	20	9	8	7	15	14	12	61	127	117	90	92	93	36	41	43
	10	25	25	26	11	11	11	17	17	17	26	35	43	86	82	85	29	34	32
	11	27	30	28	11	12	14	18	20	20	46	17	116	83	84	85	29	26	31
	12	29	34	30	14	18	15	21	25	22	21	3	0	81	78	74	26	27	29
Riebeeck-West: Riebeeksrivier	1	31	31	29	18	17	15	23	23	21	18	1	14	78	82	84	30	30	31
	2	32	28	28	18	15	15	24	21	21	4	10	29	81	86	87	30	31	33
	3	28	28	27	16	16	16	22	21	21	14	24	26	83	86	81	30	33	33
	4	22	23	22	13	13	13	18	18	18	41	58	98	81	80	78	36	36	38
	5	19	18	20	12	11	12	15	14	16	117	60	95	84	88	79	48	49	43
	6	16	16	15	9	9	9	12	12	12	147	136	194	91	89	89	57	55	55
	7	17	14	16	11	8	9	14	11	12	44	124	99	75	90	90	44	55	55
	8	17	14	15	9	7	8	13	10	11	91	148	239	82	94	90	44	59	56
	9	19	17	15	9	9	8	14	12	11	40	77	103	90	93	93	48	52	56
	10	22	20	21	11	11	11	16	15	16	34	51	52	87	88	89	36	45	38
	11	23	25	25	11	13	13	16	18	19	39	44	44	86	86	87	34	30	37
	12	25	29	29	13	17	16	19	22	22	13	9	2	87	83	82	33	33	30
Lydenburg: Mpumalanga	1	30	30	30	20	19	19	6	8	8	94	8	93	50	92	49	--	48	--
	2	30	32	31	19	20	19	2	3	6	93	3	93	41	92	46	--	41	--
	3	35	36	29	21	22	17	28	29	4	20	18	93	96	95	46	82	76	--
	4	33	34	27	18	16	13	26	25	3	40	12	92	96	95	41	67	79	--
	5	32	36	27	15	18	10	24	27	1	5	1	90	96	93	34	60	50	--
	6	29	31	26	13	10	7	21	21	0	4	0	89	95	93	29	81	53	--
	7	29	32	25	12	12	8	21	22	0	10	0	90	96	94	34	71	50	--
	8	34	35	26	15	14	9	25	25	0	9	0	87	95	94	31	69	55	--
	9	29	26	29	12	13	12	1	2	1	84	89	86	28	42	32	20	19	--
	10	28	27	27	15	15	14	4	5	3	90	91	90	39	49	42	--	--	--
	11	29	28	29	17	16	17	2	4	3	91	91	89	46	48	44	--	--	--
	12	29	29	27	18	18	18	5	5	5	91	92	93	48	50	57	--	--	--

Source: ARC Infruitec-Nievoorbij: Agromet - ISCW, Stellenbosch.

Description
- Tx: Average Maximum Temperature (°C)
- Tn: Average Minimum Temperature (°C)
- T: Average Temperature [Calculated From Hourly Data] (°C)
- Rain: Average Total Rainfall [Calculated From Hourly Data] (mm)
- RHn: Average Minimum Relative Humidity (%)
- RHx: Average Maximum Relative Humidity (%)

Table 1.3: Long-term average monthly temperatures, rainfall and relative humidity for established and new olive growing regions of South Africa for the maximum of eleven years between 2003 and 2013 (Agromet, Stellenbosch).

Long-term average					Tx	Tn	T	Rain	RHx	RHn	RH
Region	Start	End	Month	Years							
Barkley West-Ulco	2003/04/01	2013/12/31	1	11	33	19	25	110	77	27	52
			2	11	32	18	25	90	81	30	55
			3	11	30	16	22	68	80	30	56
			4	11	26	11	18	37	81	30	57
			5	11	22	7	14	20	73	26	50
			6	11	20	4	12	13	73	27	49
			7	11	20	4	12	2	66	22	43
			8	11	23	6	15	3	60	17	36
			9	11	28	10	19	10	49	14	28
			10	11	30	13	22	20	59	16	33
			11	11	33	16	24	36	60	15	34
			12	11	34	18	25	59	70	19	41
Oudtshoorn: Rooirivier	2007/10/01	2013/12/31	1	6	33	16	24	7	80	23	51
			2	6	33	16	24	32	82	25	54
			3	6	31	15	22	17	81	25	54
			4	6	26	11	18	14	85	28	58
			5	6	23	8	15	12	85	29	58
			6	6	19	5	11	36	90	37	67
			7	6	19	4	11	29	87	34	64
			8	6	21	5	12	26	84	27	57
			9	6	22	7	14	4	76	24	52
			10	6	24	10	17	30	70	27	56
			11	6	29	12	20	22	82	23	52
			12	6	31	14	22	17	81	24	53
Paarl: BELLEVUE	2005/01/01	2013/12/31	1	9	34	17	25	7	78	25	52
			2	9	34	17	24	13	81	27	54
			3	9	33	16	23	15	79	26	54
			4	9	28	13	19	49	83	28	59
			5	9	23	10	15	128	89	40	71
			6	9	20	8	13	153	91	45	75
			7	9	21	7	13	127	88	41	71
			8	9	20	7	13	130	90	39	72
			9	9	22	8	14	73	90	39	69
			10	9	27	11	18	30	84	30	59
			11	9	28	13	20	64	83	29	58
			12	9	32	16	23	17	79	27	53
Riebeeck-West: Riebeeksrivier	2007/02/01	2013/12/31	1	7	29	16	22	10	84	31	58
			2	7	29	16	22	18	84	31	59
			3	7	28	16	22	15	80	30	56
			4	7	24	14	18	54	79	35	57
			5	7	19	12	15	121	87	48	69
			6	7	16	10	13	164	87	52	73
			7	7	16	9	13	125	84	47	67
			8	7	16	9	12	121	89	49	71
			9	7	17	9	12	77	91	49	72
			10	7	22	11	16	38	87	38	64
			11	7	24	12	18	70	87	35	62
			12	7	28	15	21	23	84	32	59
Lydenburg: Mpumalanga	2000/04/01	2013/12/31	1	14	29	19	24	161	96	52	78
			2	14	30	19	24	109	95	48	76
			3	14	29	17	22	90	96	48	76
			4	14	27	14	20	57	96	49	76
			5	14	26	10	17	19	93	37	70
			6	14	24	7	15	8	92	33	68
			7	14	24	7	14	5	90	30	63
			8	14	26	9	17	9	89	32	62
			9	14	28	12	19	24	90	34	62
			10	14	28	15	21	80	93	44	70
			11	14	28	17	22	139	95	51	75
			12	14	29	18	23	145	95	53	77

Source: ARC Infruitec-Nievoorbij: Agromet - ISCW, Stellenbosch.

Description:
- Tx: Average Maximum Temperature (°C)
- Tn: Average Minimum Temperature (°C)
- T: Average Temperature [Calculated From Hourly Data] (°C)
- Rain: Average Total Rainfall [Calculated From Hourly Data] (mm)
- RHn: Average Minimum Relative Humidity (%)
- RHx: Average Maximum Relative Humidity (%)
- RH: Average Relative Humidity [Calculated From Hourly Data]

A wide spectrum of cultivars are available in South Africa that have been imported from other countries, but due to a lack of local breeding programmes, there are no cultivars available which are ideally suited to South African conditions. As a result, imported cultivars need to be evaluated in terms of their degree of tolerance to disease under South African conditions.

According to Costa (1998), Olive-Go-Wild (2008) and Olive Directory (2010) the following well-known olive cultivars have been grown for many years in South Africa:

- 'Leccino', 'Nocellara' and 'Arberquina' - produce oil with soft, subtle herbaceous flavours.
- 'Frantoio' - a typical Tuscan variety, with strong green overtones.
- 'Coratina' - produces rather bitter oil.
- 'Flavolosa' - specifically selected for oil production, produces intensely fruity oil.
- 'Mission' - a dual-purpose (table and oil) olive that is more suited to table olive production, with the olive producing delicate oil that is best consumed within 6 to 9 months.
- 'Manzanilla de Sevilla' - a table olive cultivar that is more suited for green processing, but for which there is little demand in the market, due to its size.
- 'Barouni' - a table olive that is not widely planted in South Africa, but which meets demand for large green "Queen" sized olives.
- 'Kalamata' - a table olive cultivar that makes very delicate oil.
- 'Ascolano' - produces oil with a very fruity aroma.

South Africa has developed and launched a novel table olive cultivar called 'Nandi', which can be processed into either green or black olives. It displays a good size and has an excellent flesh-to-pip ratio. Tolerance to OLS must either be confirmed or evaluated for all these cultivars, as some have been tentatively grouped according to their known resistance to OLS (Table 1.4).

Table 1.4: Tentative grouping of commercial olive cultivars and selections according to the cultivar's level of susceptibility to OLS (Costa, pers. com. 2011)

tentative grouping						
Olive Leaf Spot (OLS) (*Spilocaea oleagina*)						
highly susceptible						
	susceptible					
		fairly susceptible				
			medium			
				fairly tolerant		
					tolerant	
						highly resistant
			Olive cultivar			
Souri						
Mv97/3	Mission	Frantoio	Man de Sev.			
Haas	Meski			Arberquina	Arbosana	Zorzalena
					Koroneiki	DA12I

1.2 OLIVE LEAF SPOT FUNGAL DISEASE

In South Africa two major fungal diseases affect olive orchards, namely anthracnose (*Colletotrichum gloeosporioides*), which causes rapid fruit spoilage and cankers on shoots and olive leaf spot ('peacock's spot') (*Fusicladium oleagineum*), which causes sooty spots and yellowing of leaves, later resulting in leaf drop and death of shoots (Costa 1998). Soil-borne root diseases (*Phytophthora* spp., *Verticillium* wilts, *Phoma* spp.) which are exacerbated by poor irrigation scheduling (localised over-irrigation and excess free water at the tree stem) are also problematic to olive trees (Costa 1998, Sistani *et al*. 2009).

All olive-growing regions worldwide experience similar problems caused by the destructive fungal diseases, OLS and others of similar types such as *Verticilium* wilt (Graniti 1993, Ciccaresa *et al*. 2002, Costa 1998, Sistani *et al*. 2009, Sanei & Razavi 2010, Obanor *et al*. 2010, 2011). Olive leaf spot was first recognised in Europe in 1845 (Viruega *et al*. 2011) with its causal agent *F. oleagineum* (Obanor 2006). In a warm, dry climate OLS can stay latent for more than a year without causing any significant problems, as it requires cool, moist weather for infection and further development (Graniti 1993, Obanor *et al*. 2005a, 2008, 2010).

The pathogen feeds on leaf sap causing leaf damage as depicted in Figure 1.2, also referred to as ‘Peacock’s eye’ spot appearance on the olive leaf blade (Graniti 1993). Infection also occurs on whole fruit and fruit pedicles, and can result in abscission (Obanor 2006). OLS is regarded as a disease which may cause substantial losses (estimated to 40 to 45% of annual yield) (Viruega *et al*. 2011).

Figure 1.2: Olive leaf spot or 'Peacock's eye' spot on olive leaf blades

1.2.1 Symptoms of Olive Leaf Spot

The symptoms of OLS, caused by the pathogen *F. oleagineum* have been found to appear mainly on the leaves (Obanor *et al*. 2008, Sergeeva *et al*. 2009), usually appearing first on the upper leaf surface (MacDonald *et al*. 2000, Agosteo & Scolaro 2002). Numerous configurations of lesions can occur on the same leaf due to heavy infestations. Lesions start to appear as small sooty blotches of 2 to 6 mm in diameter across the leaf blade. A few weeks later these blotches become muddy green to black spots that expand up to 15 mm in diameter (MacDonald *et al*. 2000).

The spots then turn dark brown, expanding and coalescing with the adjacent infection to cover a large proportion of the leaf area (as indicated in Figure 1.2). Some lesions expand to form a yellow halo that is similar to the spots on a peacock's feather, from which the names 'Peacock's spot' and 'bird's eye spot' are derived (Graniti 1993, Obanor *et al*. 2008, Sergeeva *et al*. 2009).

Research conducted in Spain and Italy has shown that young leaves and new shoots frequently become a susceptible site of infection (Viruega & Trapero 2002). In late winter and early spring new infections develop, leading to leaf abscission during summer, leaving partially defoliated shoots (Figure 1.3a), with some healthy leaves on the tree (Costa 1998, Obanor *et al*. 2008 & 2010). Disease infestations result in poor vegetative growth of newly developing leaves, which serves as a site of infection when conditions are favourable, as well as leading to dieback of twigs and a delay of fruit ripening (Obanor *et al*. 2010, 2011).

The delay in ripening reduces yield and negatively impacts olive orchard productivity (MacDonald *et al*. 2000). Any remaining latent infection after winter sporulation, when encountering heat spells in summer; tend to form old lesions, so called "summer spots" (Figure 1.3b). The "summer spots" become brittle and whitish in colour, occasionally producing conidia on the leaf (MacDonald *et al*. 2000, Agosteo & Scolaro 2002).

Heavy disease infestation eventually reduces productivity and the life-span of an olive orchard (Graniti 1993, MacDonald *et al*. 2000). Olive leaf spot is often more severe in the lower part of trees with a dense canopy. Graniti (1993) observed that not all infected leaves fall after infection, with the remaining infected leaves becoming a source of infection during the next season.

A

B

Figure 1.3: (A) Severe olive leaf spot infection causing twig dieback on a healthy susceptible cultivar of “Haas” and (B) old lesions or summer spots on the leaf blade where epidermal cells are damaged by olive scab

1.2.2 The life cycle of OLS in relation to the phenological cycle of the olive tree

The *F. oleagineum* pathogen, a causal agent of OLS disease, is described as a biotroph fungus as it establishing a long-term feeding relationship with the living cells of a host, without killing it, as part of the infection process depends on environmental condition for visible infection. The optimal temperature range for spore germination, infection and mycelia growth is 16 to 21ºC (Graniti 1993, Viruega & Trapero 2002). Conidia germinate only in the presence of free water or when the relative humidity is above 70% (Obanor *et al*. 2008, 2010). Temperatures above 30ºC tend to inhibit spore germination, with the fungus being inactive in areas where the summers are warm and dry (Obanor *et al*. 2008). Figure 1.4 is a schematic representation of the life cycle of OLS as proposed by Obanor (2006). Severely infected leaves (and sometimes fruit) on the ground allow over-wintering of the fungus.

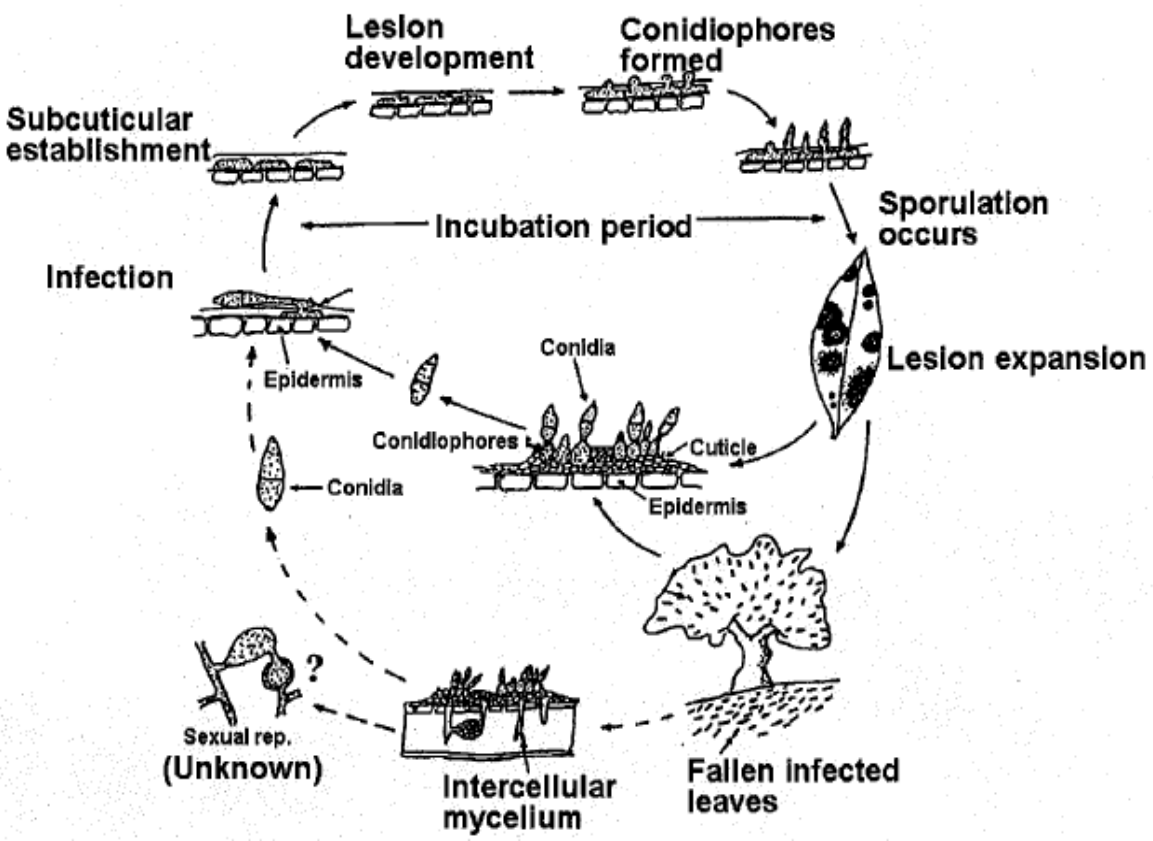

Figure 1.4: A schematic representation of the life cycle of olive leaf spot, as proposed by Obanor (2006)

The pathogen is generally known to survive unfavourable conditions, e.g. dry, hot weather in fallen leaves, as well as in infected leaves on the tree. The conidia formed on the leaves on the tree can survive for several months; even though once they have separated from the conidiophores they lose their ability to germinate within a week (they need to find a host within 48h) (Viruega & Trapero 1999).

After a period of humid, warm weather (optimum of 21ºC) new batches of conidia are readily produced on the foliar spots. Obanor (2006) observed that young leaves are very susceptible to infection during spring in New Zealand, and that foliage in the lower part of tree was more frequently infected. This is consistent with the pathogen's requirement for high humidity to develop with a requirement of ≥70% humidity with unbroken optimal temperature for 48 h for spores to germinate (Obanor *et al*. 2010).

New infections are associated with rainfall and mostly occur during autumn and in winter in the winter rainfall areas. Late winter to spring infections become ineffective and symptom development is delayed, or the infection stays latent (remaining symptomless) during summer. Latent lesions (summer spot) which appear as dead cell on the leaf surface (Figure 1.5), developing in summer during favourable temperature and humidity conditions, serve as a source of inoculum for infection in the following season.

By summer, most infected leaves have fallen from the tree, leaving partially defoliated shoots (Figure 1.6), with healthy foliage remaining (Viruega *et al*. 2011). Obanor (2006) reports that not all infected leaves fall from the tree and that the fungus survives in those infected leaves which remain on the tree. The margins of the lesions enlarge in the autumn with new spores developing in these lesions (Viruega & Trapero 2002, Obanor 2006).

Figure1.5: Light microscope image showing an olive leaf blade with summer spot (white spot) and dead cells under the upper epidermal cells.

Research findings show that summer spores can take several years to cause economic loss (Viruega & Trapero 2002). Rain and free water (from irrigation) containing conidia spread the spore to the newly developing leaves in the lower parts of trees, which are the most common sites of infection (Graniti 1993, Viruega & Trapero 2002). Recent studies also show that animals, birds and insects can also transmit the disease (SAOI 2012). Due to the aforementioned reasons, lateral spreading of disease between trees is limited to adjacent trees (Graniti 1993).

Figure 1.6: Partially defoliated and defoliated shoots with healthy foliage that remained after winter infection and die back caused by severe disease infestations on olive tree twigs as indicated by the arrows.

It was also found that the disease infestation in densely planted orchards is high when conditions are favourable. Specifically in Mediterranean climatic-type regions with cool and moist weather conditions, it is difficult to control disease infection during autumn, winter and spring (Graniti 1993, Costa 1998, Obanor *et al*. 2008, Viruega *et al*. 2011). Whereas, latent lesions (summer spots) develop in summer during favourable conditions and serve as a source inoculum for infection of the upper epidermis in the next season as infection occurs in the palisade cells.

Martin (1994) reported that in hot climatic regions, trees produce few or no new leaves in mid-summer, but they resume growth again in the autumn, resulting in two peaks of leaf production in spring. These new leaves are most susceptible to OLS disease. The leaves that are infected tend to abscise prematurely, resulting in major defoliation of the tree. Flower development is also negatively affected and yield is substantially reduced (Obanor 2006), as a result of defoliation and eventual death of twigs, as illustrated in Figure 1.6.

1.3 CONTROL OF OLIVE LEAF SPOT

Although an olive tree is easily infected by pests and disease when conditions are favourable, infection occurs on a limited scale causing fewer problems than with most other fruit tree species (Sistani *et al*. 2009). Olives have fewer natural enemies than other crops (e.g. apples and pears) and chemical treatments (very often copper containing sprays) are still used as standard control measure against fungal diseases in olive orchards. Fungicides (copper containing sprays) successfully control OLS, when disease risk is low (Teviotdale *et al*. 1989a & b; Sistani *et al*. 2009).

However, olives and olive oil apparently retain chemical residues of fungicides, affecting oil aroma (Teviotdale *et al*. 1989a, Sistani *et al*. 2009). Therefore, copper treatments should never be applied within 14 days before harvest (Roca *et al*. 2007). According to Graniti (1993) and MacDonald *et al*. (2000) studies carried out in Italy and New Zealand showed that heavy infestations impair the effectiveness of chemical control and could lead to severe yield loss. This could be caused by late appearance of visible symptoms after disease onset. Furthermore, severe defoliation causes a delay in ripening and a decrease in oil yield. Regular chemical treatment may prevent disease build-up in the orchard, but with costs and chemical residue implications (Costa 1998, Sistani *et al*. 2009).

Currently olive trees showing ‘Peacock’s spot’ symptoms on the leaves are immediately treated with copper containing sprays to eliminate disease build-up. Late applications of copper sprays were reportedly more effective in the Western Cape province before the winter rains began and again during early spring, as the wet weather continued (Costa 1998), but aligned with the required safety period before harvest.

The research finding shows that nutrition and other cultivation practices affect tolerance of olive trees to OLS. A calcium deficiency and high nitrogen level in the soil tend to promote susceptibility of trees to OLS (Obanor 2006). Olive orchards that were inter-planted with cereals or fodder crops, in which the soil moisture and organic matter were depleted, were found to suffer more damage from the OLS disease compared to trees in orchards without any cover crop (Obanor 2006). The severe damage on the trees in orchards with a cover crop could be caused by high nitrogen content in the soil from fodder crop or cereal grain fertilizer.

As a result of increasing labour costs of harvesting olives, the worldwide industry including South Africa is moving towards easily mechanised planting systems, namely ultra-high density plantings. These new systems, however, favour disease occurrence with susceptible cultivars easily becoming infected by OLS. The development of resistant cultivars is a priority in terms of preventing fungal disease infection.

An intensive search for cultivars that are tolerant to the commercially important diseases namely OLS, anthrachnose, *Verticilium wilt,* Cercospora spp. and Phytophthora spp., has already begun throughout the olive growing regions of the world, which are subject to diverse weather conditions. Some genotypes are already known to be tolerant to OLS, with others having varying degrees of tolerance. These cultivars need to be evaluated for OLS susceptibility/tolerance under South African conditions.

1.4 RESEARCH AIM AND OBJECTIVES

The proposed study aimed at evaluating commercial olive cultivars and selections for resistance against OLS. The research was aimed to address the feasibility of using tolerant olive cultivars and selections as a biological control measure against OLS, which could provide high quality olive products, which are residue (pesticide) free and to expand growth opportunities in the olive industry of South Africa through the use of new olive cultivars. The findings of the project were expected to provide a basis for recommendation of OLS tolerant cultivars and selections for commercial olive production.

In addition, the information accumulated in the study should also help to show how olives can reliably be selected for specific temperature ranges in order that the disease OLS might effectively be controlled. In order to achieve the research aims, the study had the following objectives:

- To identify the most suitable tolerant cultivars/selections to the fungal disease olive leaf spot (OLS) which is caused by *F. oleagineum* under optimum temperature and RH conditions for infection by *F. oleagineum*
- To review the tolerance of existing commercial cultivars against OLS which could be used as standard comparisons for new cultivars/selections evaluated for this disease in future

1.5 HYPOTHESIS

The commercial cultivars and new selections are tolerant to OLS under optimal temperature and relative humidity conditions for disease infection.

CHAPTER 2

MATERIALS AND METHODS

2. MATERIALS AND METHODS

A trial was conducted to assess the tolerance of commercial olive cultivars to OLS and the timeframe for evaluation is given in Table 2.1 below.

Table 2.1: Timeframe of all processes involved during trials conducted to screen olive cultivars for tolerance to olive leaf spot (OLS).

1. Selection of well-known commercial cultivars used in SA Olive Industry (May 2011) to conduct entire trial
2. In the next six months (May - October 2011) development of nursery plant material in the glasshouse began
3. Inoculation of olive plant material under controlled favourable conditions in the growth chamber (October 2011) and repeated in April 2012 in the growth chamber trial
4. Data collection to evaluation cultivars' tolerance /susceptibility to OLS disease (Nov. 2011 to Jan 2013) began after six weeks of inoculation
5. Screening cultivar tolerance and susceptibility to OLS disease and make recommendations to be used as the basis for further comparisons to screen new selections for the SA industry and possibly worldwide (Jan – July 2013)

2.1 EXPERIMENTAL SITES, EXPERIMENTAL DESIGN AND ESTABLISHMENT OF EXPERIMENTAL PLANTS BEFORE INOCULATION

2.1.1 Glasshouse trial

A glasshouse trial was conducted at Elsenburg, Department of Agriculture: Western Cape Province (GPS Coordinates: 33.845259"S, 18.834722"E), in collaboration with the Agricultural Research Council (ARC) Infruitec-Nietvoorbij. Eight commercial cultivars ('Mission', 'Manzanilla de Sevilla', 'Frantoio', 'Nandi', 'Nocellara del Belice', 'Coratina', 'Barouni' and 'Leccino'), were selected for inclusion in the trial, based on a tentative grouping of cultivars by Costa (2011 Pers. Comm., unpublished) according to their susceptibility to OLS. In May 2011, rooted one year old olive trees of eight commercial cultivars were obtained from a nursery. The original experimental design was a split plot with glass house (temperature) as the main plot and cultivar as the split plot in a completely randomized block design. In each glasshouse 6 plants per cultivar were randomly allocated (Figure 2.1) during the beginning of the trial (May 2011).

T1		
B	C	D
F	K	G
J	L	B
C	D	F
K	G	J
L	B	C
D	F	K
G	J	L
D	F	K
L	B	C
K	G	J
C	D	F
J	L	B
F	K	G
B	C	D
F	J	C
K	B	L
D	G	B
L	C	K
J	D	C
F	G	J
B	D	F
C	K	L

T2		
G	J	L
D	F	K
L	B	C
K	G	J
C	D	F
J	L	B
F	K	G
B	C	D
F	K	G
J	L	B
C	D	F
K	G	J
L	B	C
D	F	K
G	J	L
K	B	L
D	G	B
L	C	K
J	D	C
F	G	J
B	D	F
C	K	L
G	J	L

T3		
F	J	C
K	B	L
D	G	B
L	C	K
J	D	C
F	G	J
B	D	F
C	K	L
D	F	K
L	B	C
K	G	J
C	D	F
J	L	B
F	K	G
B	C	D
G	J	L
B	F	K
D	F	K
L	B	C
K	G	J
C	D	F
J	L	B
F	K	G
B	C	D

cultivars:

B	Frantoio
C	Manzanila de Sevilla
D	Leccino
F	Coratina
G	Mission
J	Baroni
K	Nandi
L	Nocellara DB

Figure 2.1: Experimental layout of the glasshouse trial at Elsenburg for screening eight olive cultivars for susceptibility to olive leaf spot, a complete randomised block design with temperature treatments (T1=15ºC, T2=20ºC and T3=25ºC) was used

In May (2011), the plants were immediately transplanted into larger plastic pots (13 cm diameter, 8L volume) containing a mixture of medium to fine composted bulk and coarse river sand (50:50% w/w), and 10% perlite to increase water retention. The growing medium was mixed with a slow release nutrient organic fertilizer containing seaweed, fishmeal and humic acid Neutrog fertilizer (Seamungus) (Neutrog Africa (Pty) Ltd, Philadelphia, South Africa). Plants were first established (May) in the glasshouses at a temperature of 22±5ºC and RH above 70%, to allow them to acclimatise to their changed environment.

The plants were kept in the glasshouse (May - October 2011) until inoculation and natural daylight prevailed. Each plant (one year old and of the same size) received the same volume of irrigation water (500 ml) two days per week.

After observing mineral deficiency symptoms, iron chelate was manually applied (drenched) with irrigation water (5g/440 ml per plant) to correct any iron deficiency in the plants. Where pests, such as Olive Psyllid (*Euphyllura olivina* Costa) and leaf miners occurred, a chlorpherapyr/ Pyrrole, (BASF South Africa (Pty) Ltd, Cape Town) spray of 3ml per 5l water was applied which does not affect fungus development. Not all of the commercial cultivars included in the trial were being attacked by insect pests, however all trees received the same treatment at the same time. Trees with long extended shoots and water shoots were cut back to half their length (eight nodes from the main stem) to encourage new growth, because sufficient presence of healthy, young leaves was required for inoculation with the disease.

For the first 6 months (May - October 2011) the trees was able to develop new growth, which was residue free. Before inoculation (end October 2011), the temperature in the glasshouses were adjusted to 15ºC (T1), 20ºC (T2) and 25ºC (T3) and RH of above 70% for 24 h for the plants to acclimatise to the environment. These three temperature treatments (T1=15ºC, T2=20ºC and T3=25ºC) were continued after inoculation, while a relative humidity (RH) of above 70 % was maintained in all three glasshouses. Each glasshouse was equipped with a MT669 data logger (Major Tech tools, Johannesburg, South Africa) to accurately monitor temperature and RH.

2.1.2 Growth chamber trial

Six months after establishing the plants in the glasshouse, within 20 days after inoculation, the cooling (air-conditioning) system began to malfunction. As a result temperature treatments were impossible to control and it was decided to move the plants to a new site (growth chamber) where the trial was started again using the same experimental plants.

The growth chamber trial was conducted at Bien Donne experimental farm of the ARC (GPS coordinates: 33.843056”S, 18.977369”E). The same procedures, as mentioned above for the glasshouse trial, were followed to establish plant material before inoculation.

Due to limited growth chamber facilities available, only one temperature (16ºC) was used. The experimental layout was a completely randomised block design with cultivars as treatments (6 plants per cultivar). Before inoculation (end May 2012) the temperature in the growth chamber was adjusted to 16ºC and a RH of above 80% was maintained in the growth chamber for 24 h for the plants to acclimatise to the environment. After inoculation plants were kept for 48 h at 16°C and maximum RH (≥80%) and then moved to a shade net area for continued disease development after this incubation period.

2.2 INOCULUM PRODUCTION

The inoculum used in this trial was obtained from naturally infected olive trees growing in different areas around the Stellenbosch region. Infected leaves were collected from the orchard a day before the inoculation process and stored at 4ºC overnight to ensure the viability of the spores. According to Viruega *et al*. (2011) the *F. oleagineum* pathogen is difficult to culture *in vitro*, and they found that when culturing using olive leaf extract (OLE) agar, the pathogen produced few or no conidia.

Infected (sporulated) leaves were suspended in 300ml distilled water and the solution was shaken at 180 rpm for 5 minutes, to dislodge the conidiophores. After filtration through Myra cloth the inoculum solution was kept in a refrigerator (at 4ºC) for 30 minutes. A spore count was done using a haemocytometer under the microscope (16 mm magnification lenses).

2.3 INOCULATION PROCESS AND APPLICATION OF TEMPERATURE TREATMENTS

2.3.1 Glasshouse trial

In October 2011, for each temperature treatment, six plants per cultivar were artificially inoculated by applying a conidial suspension with a concentration dosage of 1.7×10^5 spores per ml using a manual spray. The plants were sprayed, repeatedly with the conidial suspension on the leaf surface up to incipient run-off to ensure good leaf coverage with conidial spores. Control (non-inoculated) plant material was covered with plastic bags to protect them from inoculum.

Plants were visually monitored for lesion development on a weekly basis and disease assessments were scheduled for 6, 8, and 10 weeks after inoculation. Within 20 days after inoculation, the cooling (air conditioning) system started to malfunction and as a result temperature treatments were impossible to control (temperature spikes).

High temperatures were found to supressed disease development. Sunburn was detected on the plant leaves. Huge temperature variations occurred and the maximum temperature peaked at 34ºC during midday, which had a negative impact on spore germination. The problem was aggravated by hot summer spells during mid-December 2011 to February 2012. The expected results were not obtained. This problem led to the decision to move the experimental site to Bien Donne Experimental Farm of the ARC Infruitec-Nietvoorbij.

2.3.2 Growth Chamber trial

In mid-May 2012, six plants per cultivar were artificially inoculated using the same procedure as described in Chapter 2.3.1, on the same plants which were used in the glasshouse after they recovered. The temperature and RH were accurately monitored using MT669 data loggers and additional mercury thermometers. Airflow from the fans was curtained off by means of double layered nets that were hung in front of each fan, to decrease the airflow speed, as well as to prevent damage to young shoots and twigs, whilst temperature remain constant.

2.4 DATA COLLECTION

Plants were monitored 14 days (2 weeks) after inoculation for lesion development; thereafter disease assessments were done every second week until week 17 after inoculation.

The following measurements were taken for assessing disease incidence.

- The presence of lesions on marked leaves of experimental plants
- The disease severity on the leaf surface

Disease assessment was done using Palti's method (Palti 1949). On each cultivar, four shoots evenly distributed around the crown were labelled. On these four shoots per plant, a position 5 - 10 cm below the shoot growing tip was marked with tape and the leaves occurring in this marked zone were monitored for lesion development. All the leaves in this marked zone were examined and appraised individually for presence of lesions. The leaf size at assessment varied between 1 cm and 5 cm in length, depending on leaf age. Each leaf was roughly divided into four Cartesian planes for the appraisal of the necrotic areas.

On each leaf, the diameter of the lesion/s present was measured (in millimetres) for scoring. The number of spots counted per grid was used to rank the evaluated genotypes for tolerance against OLS. Data plant leaves were numerically scored according to a six class scale (categorised as *a, b, c, d, e* and *f*) considering the severity of leaf symptoms and the intensity of defoliation.
The presence of leaf lesions was used as a measure of disease severity to group the leaves into the following categories.

a. the leaf is free from disease (*healthy*)
b. less than ¼ of the leaf surface affected (*very light infection, <24%*)
c. full ¼ of the leaf surface affected (*light infection, 25%*)
d. up to ½ of the leaf surface affected (*moderate infection, 26-50%*)
e. up to ¾ of the leaf surface affected (*severe infection, 50-75%*)
f. over ¾ of the leaf surface affected (*very severe infection, >75%*)

The degree of infection on each leaf was categorised from (*a*) to (*f*) and the number of leaves in each category was recorded separately for each type of infection. The degree of infection for each cultivar was calculated by summing the number of spots per leaf in each category. The sum of the total values obtained for all categories of leaves was then divided by the total number of examined leaves per cultivar to estimate percentage. The degree of infection calculated for each cultivar was used to rank the evaluated genotypes for tolerance to OLS disease.

A further test confirming infection was done by dipping leaves in a 5% sodium hydroxide (NaOH) solution for 2 - 3 min at room temperature. The 5% NaOH solution causes blemishes beneath the cuticle on the upper epidermal cells of infected leaves to become more visible, enabling visual detection of disease infection (mycelium) (Lops *et al*. 1993, Lopez-Doncel & Trapero 1999) and mycelia was checked under the microscope.

2.5 STATISTICAL PROCEDURES

The experimental design was a randomized complete block with eight cultivars (‘Barouni’, ‘Coratinna’, ‘Frantoio’, ‘Leccino’, ‘Manzanilla’, ‘Mission’, ‘Nandi’ and ‘Nocellara del Belice’) randomly allocated within each of the thee block replicates. Severity of Olive leaf spot disease symptoms was assessed at 6, 8, 10, 12 and 17 weeks after inoculation. At each observation time 100 leaves from each experimental unit was scored on a 6-point scale for presence and severity of leaf symptoms. Percentage disease incidence was calculated as the number of leaves with disease symptoms as a percentage of the total leaves. Percentage disease severity was calculated using the midpoint of each severity range category for individual leaves and then averaged per experimental unit.

Univariate analysis of variance was performed on percentage disease incidence and severity, for each assessment time separately, using GLM (General Linear Models) Procedure were carried out using SAS statistical software version 9.2 (SAS Institute Inc. 1999).

Observations over time were also combined in a split-plot analysis of variance with week as a sub-plot factor (Little 1972). A Shapiro-Wilk test was performed on the standardized residuals from the model to test for normality (Shapiro 1965). In cases where there was significant deviation from normality outliers were removed when the standardized residual for an observation deviated with more than three standard deviations from the model value (Glass *et.al.* 1972). Fisher's t-least significant difference was calculated at the 5% level to compare treatment means (Ott 1998). A probability level of 5% was considered significant for all significance tests.

Logistic regression was conducted on severity scores on the last day of scoring (week 17) only, using the Logistic Procedure of SAS statistical software version 9.2 (SAS Institute Inc. 1999). Logistic regression analysis was used to investigate the relationship between the ordinal severity scores (classes) and cultivars. The purpose was to obtain probabilities (odds) for each cultivar relative to the severity score category estimates (class intercepts) (Hosmer & Lemeshow 2004). Logistic regression results are illustrated using a figure that shows the relative level of infection for each cultivar to the severity score category estimates.

CHAPTER 3

RESULTS

3.1 GLASSHOUSE TRIAL

The experimental plants that were established in the three glasshouses in March 2011 and exposed to a temperature of 22±5ºC and an RH of above 70% for eight months (March 2011 to October 2011), successfully developed new growth, which was assumed to be free of chemical residues.

Disease assessment was scheduled to be done at 20, 40 and 60 days (six, eight, ten and seventeen weeks) after inoculation, but within 20 days after inoculation the cooling (air-conditioning) system began to malfunction. As a result temperature treatments were impossible to control. Sunburn was detected on the plant leaves. Large temperature variations occurred and the maximum temperature peaked at 34ºC during midday which is detrimental for spore development but favours vegetative growth.

At 20 days after inoculation, no symptoms of infection were detected. It was expected that symptoms would develop, especially on cultivars that were susceptible, based on the tentative grouping (Chapter 1) of cultivars/selections according to susceptibility to OLS proposed by Costa (2011, unpublished). No visible symptoms of OLS infection were detected up until 14 weeks after inoculation. Thereafter, an unknown fungal disease causing white spots was observed on some leaves of ‘Nandi’.

The absence of OLS infection was ascribed to the fact that optimal conditions for infection could not be maintained in the glasshouses. The optimal temperature range for spore germination, infection, and mycelia growth of *F. oleaginium* fungal disease is 16 to 21ºC (Graniti 1993, Viruega & Trapero 2002).

By 14 weeks after inoculation, the glasshouse trial did not produce the results required to meet the expected study outcomes. Emergency repair work required to the glasshouses' control systems, to ensure that the temperature and RH conditions required for executing the trial was not possible, due to the time-consuming and very strict procurement procedures. Consequently it was decided to move the experimental site to Bien Donne Experimental Farm of the ARC Infruitec-Nietvoorbij.

3.2 GROWTH CHAMBER TRIAL

3.2.1 Visual assessment of OLS disease severity

When disease evaluation in the growth chamber began (June 2012), 20 days after inoculation (on May 2012), no visible symptoms of OLS were detected on the leaves. During the sixth week after inoculation (early July 2012), circular black blotchy lesions became visible on the leaf surfaces. It was observed that symptoms were more visible on older leaves (from 10 weeks of age) compared to young leaves, which corresponds to the findings of Wilson & Miller (1949) cited by Obanor *et al* (2011). The findings of Wilson & Miller (1949) cited by Obanor *et al.* (2011) showed that as the leaves become older, the conidia germination decreases but the dispersed spores appear as sooty mould on the surface. It is assumed that these symptoms tended to appear after the fungus caused internal damage to the leaves (observed as necrotic tissue as depicted in Chapter 1, Figure 1.2). In this study it was also found that the sooty blotch symptom became conspicuous while affected leaves were still on the tree.

Scoring of leaves affected by OLS was executed until leaves became detached from the plants in this trial. During week 17 detached leaves were evaluated and scored as part of this study, because according to Obanor *et al.* (2008a) conidia of this disease can germinate on detached olive leaves when provided with free moisture and at temperatures ranging from 5 to 25ºC, with an optimum of 20ºC.

The level of OLS infection of each cultivar is presented in Figure 3.1(*a*) to (f) from the first week of scoring (6 weeks after inoculation) until the end of the trial (17 weeks after inoculation). The results presented in the six graphs in Fig. 3.1 were obtained after a GLM procedure (t-tests) which was obtained by using SAS®9.2 statistical software, on the collected data's of W6, W8, W10, W12 and until W17. The performance of each cultivar within a specific category (level of infection) throughout the evaluation period is depicted. During week 6 to week 12 after May inoculation, all cultivars were observed to have low levels of infection for all the categories (Figure 3.1a).

In the category ≤25% infection (Figure. 3.1b), cv. 'Nandi' had the highest level of infection (20%) from week 6 until week 12, thereafter no new infection was further recorded for this cultivar throughout the trial. From week 12 to 17 after inoculation, an increased level of infection was found for all other cultivars, in all categories (Figure 3.1(a) to (f)). Based on the final visual assessment results at 17 weeks after inoculation in the category <25% infection, 'Coratina' was considered susceptible and 'Frantoio' tolerant.

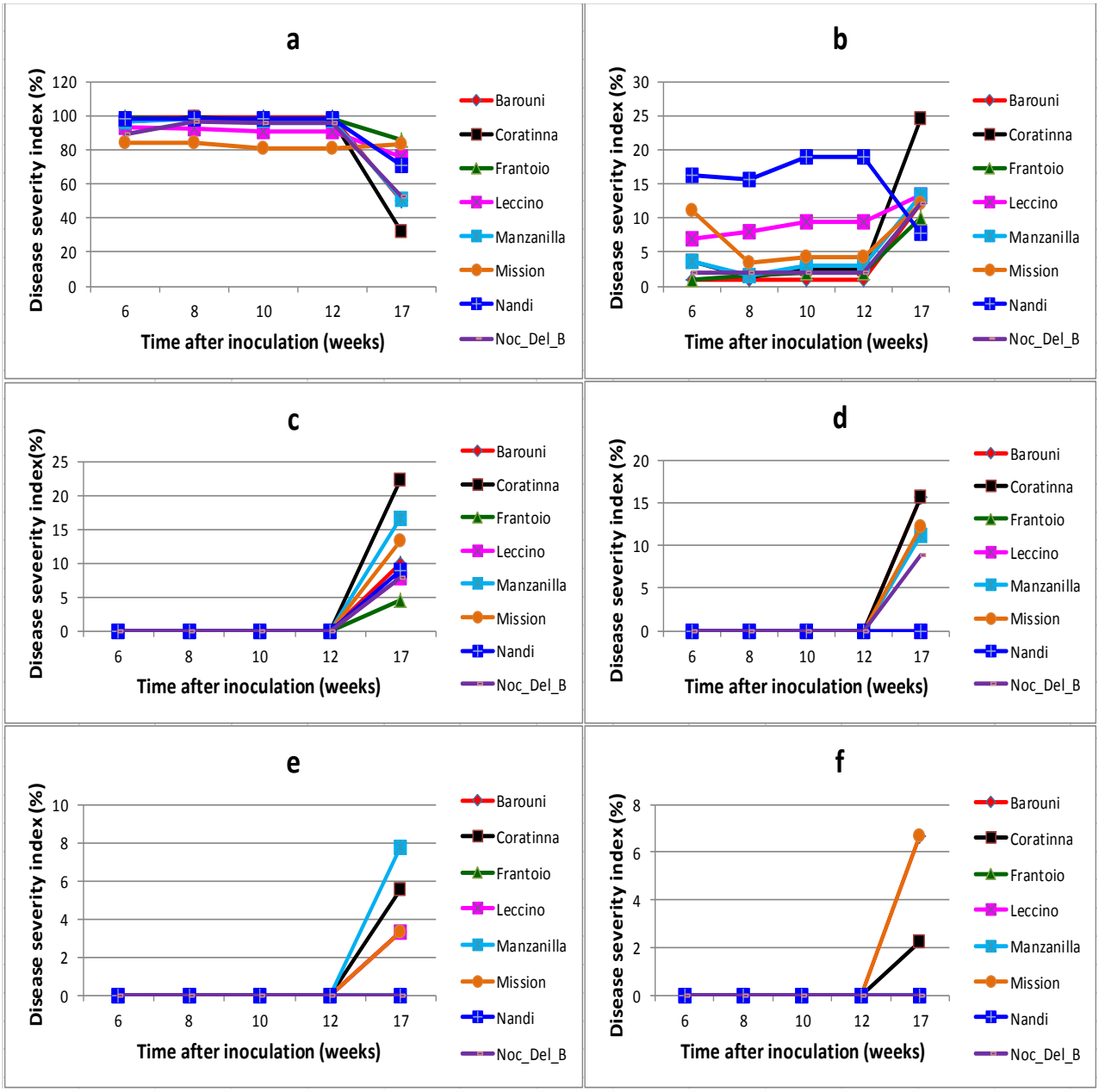

Figure 3.1: The tolerance of the eight cultivars to OLS disease during the growth chamber trial (2011/12 season) observed in (*a*) healthy leaves and (*b*) ≤25% infection of the leaf surface, (*c*) ≤35% infection of the leaf surface and (*d*) ≤50% infection of the leaf surface, (*e*) ≤75% infection of the leaf surface and (*f*) High infection level of the leaf surface.

In the next level of infection (category ≤35%, Figure 3.1c) 'Coratina' was recorded to have the highest level of infection (22%), compared to 'Manzanilla' (17%) and 'Mission' (13%). In the next level of infection (category <50%, Figure 3.1d), only 'Coratina' (16%), 'Mission' (12%) and 'Manzanilla' (11%) had disease symptoms while 'Nocellara del Belice' had less than 10% infection.

In the next level of infection (category ≤75%, Figure 3.1e) 'Manzanilla' (8%) had a higher level of infection compare to 'Coratina' (6%) and 'Mission' (3%). At the maximum level of infection, (≥75%, Figure 3.1f), 'Mission' had the highest level of infection (7%) whilst 'Coratina' had 2% and none of the other cultivars were observed to have this level of infection. It was observed that this high level of leaf infection resulted in defoliation, as heavily infected leaves were observed on the ground. It was noted that most of the infected leaves appeared to fall prematurely when infection was high.

Table 3.1 depicts the OLS severity calculated for the eight olive cultivars evaluated at 17 weeks after inoculation. At present, there is no standard minimum value (threshold value) for OLS infection that can be used for comparison between cultivars. It still needs to be established through research.

In the category <25% infection, cvs 'Frantoio' and 'Nandi' had the lowest level of infection with 85% and 83% healthy leaves. Only the cv. 'Coratina' had a significantly higher level of infection (24%) compared to the other cultivars evaluated. In the next category (≤35% infection) 'Coratina' again had the highest level of infection (20%), followed by 'Manzanilla' (17%) and 'Mission' (13%). In the category ≤50% infection, the level of infection of cvs 'Barouni', 'Coratina', 'Mission' and 'Manzanilla' were significantly higher than those of the other four cultivars evaluated.

There were no significant differences in the ≤75% infection category between cultivars. In the category high infection (≥75%), cvs 'Mission' and 'Barouni' had the highest levels of infection (7% at maximum infection level).

Table 3.1: Mean OLS severity calculated for eight olive cultivars 17 weeks after inoculation with *F. oleagineum* in the growth chamber trial at Bien Donne (2011/12 seasons).

	Level of infection coverage per 100 leaves					
Cultivars	**healthy leaf** *(significant difference)*	**≤25% Infection** *(significant difference)*	**≤35% Infection** *(significant difference)*	**≤50% Infection** *(significant difference)*	**≥75% Infection*** *(no significant difference)*	**High Infection** *(significant difference)*
Barouni	52 *bcd*	12 *b*	10 *bc*	16 *a*	3 *a*	7 *a*
Coratina	32 *d*	24 *a*	20 *a*	16 *a*	6 *a*	2 *ab*
Frantoio	86 *a*	10 *b*	4 *c*	0 *b*	0 *a*	0 *b*
Leccino	76 *ab*	13 *b*	8 *bc*	0 *b*	3 *a*	0 *b*
Manzanilla	51 *bcd*	13 *b*	17 *ab*	11 *a*	8 *a*	0 *b*
Mission	53 *bcd*	12 *b*	13 *abc*	12 *a*	3 *a*	7 *a*
Nandi	83 *a*	8 *b*	9 *bc*	0 *b*	0 *a*	0 *b*
Noc. del Belice	71 *abc*	12 *b*	8 *bc*	9 *ab*	0 *a*	0 *b*

* Means within a column which have the same letter are not significantly different. This was tested at 0.05 level of significance (P-value=0.05). t-tests (or LSD means) results for each level of infection showing the OLS severity among eight olive cultivars (2011/12 season) during W17 obtained from a RCBD performed in SAS statistical software.

Apart from the visual symptoms associated with OLS that were identified during the assessment of leaves of experimental plants, the “white spot” symptom that was found in the glasshouse trial, was also found in the growth chamber trial (Fig. 3.2). Only a few leaves were affected by these symptoms. However, laboratory tests showed no link between “white spot” and OLS diseases infection. Isolations were made to identify the nature of this disease but no fungi were associated with the lesion, further future research needed to similar infection.

It was observed that cv. Nandi had the highest percentage of this symptom, as indicated in Figure 3.2, but no damage and leaf drop were detected. The white spot symptom remained the same throughout the trial. This symptom was observed only on plants falling into the first two categories of infection, healthy leaves and (<25% infection).

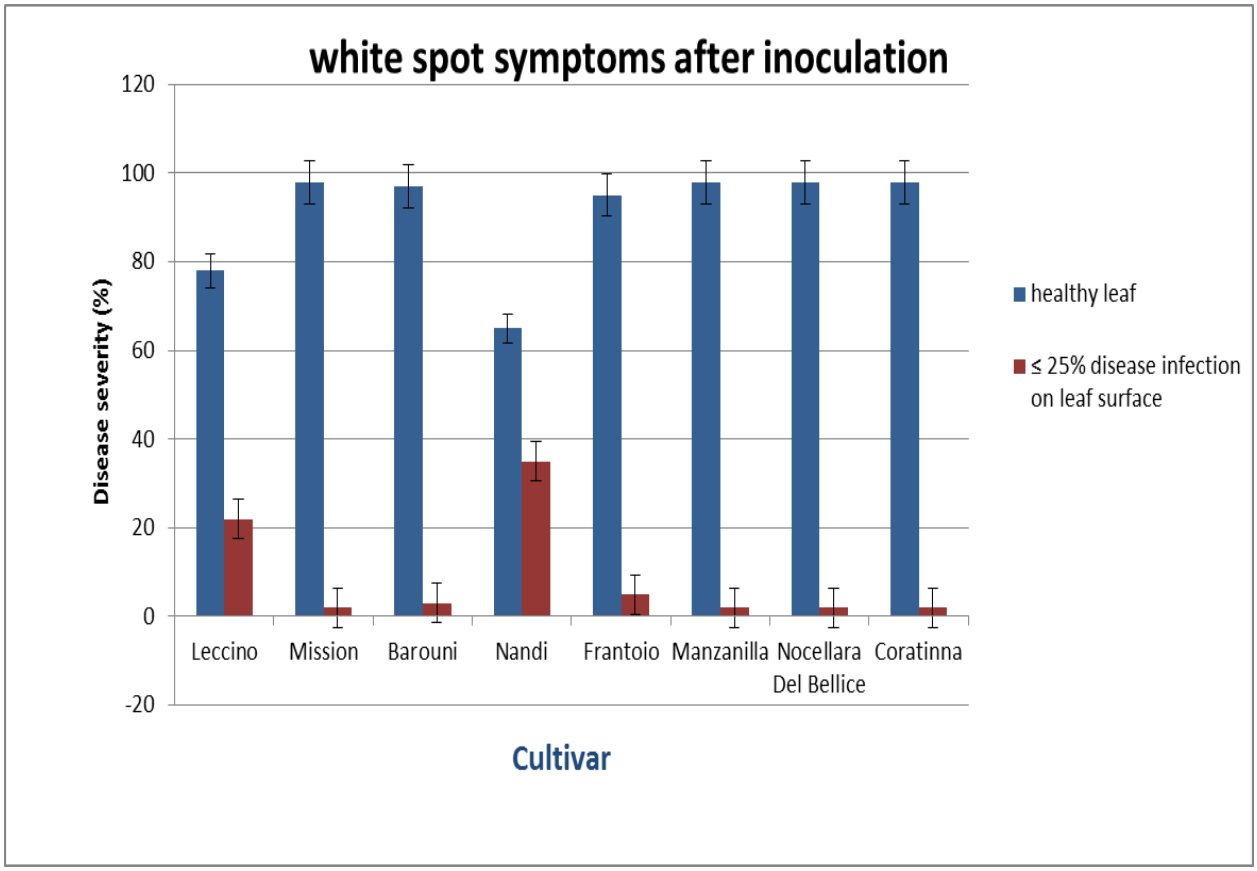

Figure 3.2: Severity of white spot symptom on eight cultivars during the growth chamber trial at Bien Donne (2011/12 season).

3.2.2 Laboratory test to confirm results of visual assessment

During the 17th week after inoculation, laboratory tests were executed on 100 infected leaves of each commercial cultivar. The laboratory test was performed to expose visually undetectable symptoms and confirm infection level on the leaves. The scoring of leaves, during the laboratory test, was used to rank each cultivar for tolerance against OLS disease. The 5% NaOH solution caused blemishes beneath the cuticle on the upper epidermal cells of infected leaves (Figure 3.3) indicating the presence of mycelia and enabling visual detection of disease infection. In Figure 3.3 a fine whitish hair-like network (hyphae of the mycelium) is visible inside the leaf tissue as detected under a light microscope (16 mm magnification lens) using lactophenol blue solution.

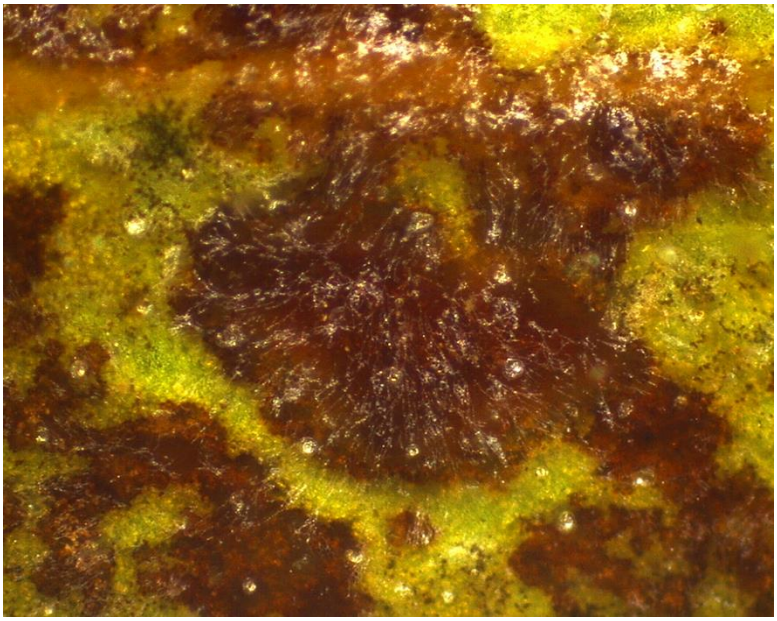

Figure 3.3: Blemishes beneath the cuticle on the upper epidermal cells of a heavily infected leaf after dipping leaf into 5% NaOH solution.

The class intercepts that were obtained from the Logistic regression analysis together with the least significant difference (LSD) means of the infection locality from the ANOVA were used to assess the level of susceptibility of each cultivar. Cultivars ‘Frantoio’, ‘Nandi’, ‘Leccino’ and ‘Nocellara Del Belice’ which intercepted in the same block below the healthy line (black line) showed significant tolerance to OLS disease infection, whilst ‘Coratina’, ‘Barouni’, ‘Mission’ and ‘Manzanilla’ intercepting above the healthy line showed susceptibility.

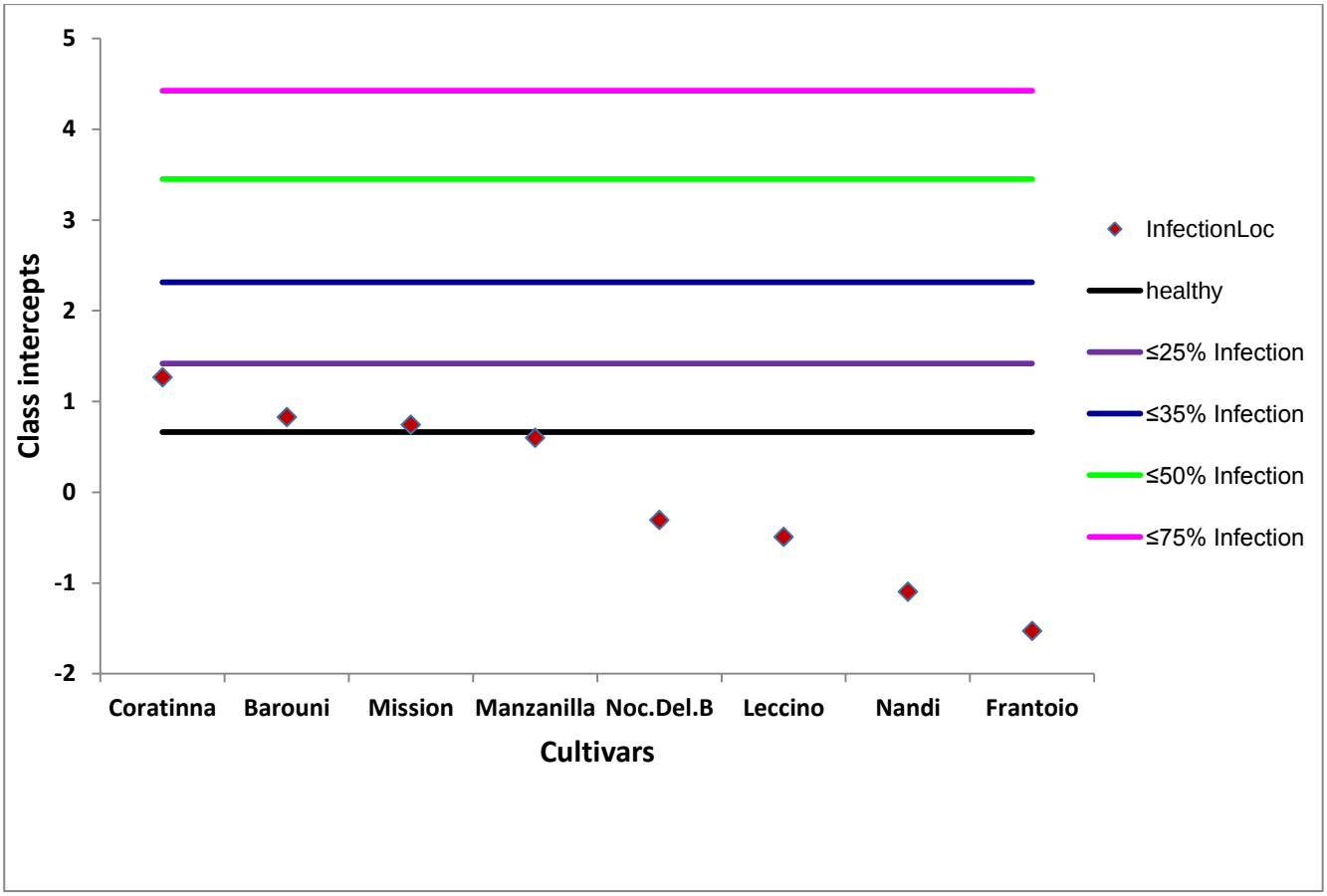

Figure 3.4: Infection location (InfectionLoc) identifying the level of susceptibility for eight olive cultivars using the logistic regression analysis during Week 17 for the trial at Bienne Donne (2011/12 season).

3.3. CATEGORISING THE EIGHT OLIVE CULTIVARS ACCORDING TO OLS SUSCEPTIBILITY

In the growth chamber trial all cultivars tested were infected but to different levels. The results of this trial were used for the final categorising of the eight olive cultivars evaluated in this study according to their OLS susceptibility as indicated in Table 3.2 below. The tentative grouping of olive cultivars that was described in Chapter 1 was also considered when evaluating and grouping cultivars with their degree of tolerance. 'Frantoio' was categorised as highly tolerant, while 'Nandi' and 'Leccino' were found to be moderately tolerant and 'Nocellara del Belice' fairly tolerant. 'Coratina' was found to be the most susceptible cultivar (highly susceptible). Two of the well-established and important cultivars grown in the South African olive industry, 'Mission' and 'Manzanilla de Seville', were also found to be fairly susceptible.

Table 3.2: Grouping of eight olive cultivars evaluated in a growth chamber trial at Bien Donne (2011and 2012 seasons) into categories according to tolerance to OLS caused by *F. oleaginium*

	Category I: Tolerant			**Category II: Susceptible**		
Cultivar(s)	**Highly** *(>80%)*	**Moderate** *(≤75%)*	**Fairly** **(≤***65-75%)*	**Fairly** *(45-65%)*	**Moderate** *(35-45%)*	**Highly** *(<35%)*
Frantoio	x					
Nandi		x				
Leccino		x				
Nocellara del Belice			x			
Barouni				x		
Manzanilla					x	
Mission				x		
*Coratina						x

* Highly susceptible
Descriptions (s):

Category I: Tolerant
Highly tolerant (>80%) is defined as infection with one tiny (rusty) leaf spot on the blade or midrib (pedicle) of the leaf, which can usually only be detected by means of 5%NaOH solution. A moderate tolerance rating (75-85%) refers to olive leaf spots of not more than two small visible spots (diameter ≤2mm) Fairly tolerant (65-75%) refers to one large spot, not exceeding 5mm in diameter.

Category II: Susceptible
Prominent and large spot lesions (with a diameter of <10mm) are present on the leaf blade. The number of lesions detected on the leaf determines the degree of susceptibility in terms of fairly, moderate or highly rating.

CHAPTER 4

DISCUSSION & RECOMMENDATIONS

4.1 GENERAL DISCUSSION

Olive leaf spot (OLS), also called 'Peacock spot', causes sooty spots and yellowing of leaves resulting in leaf drop, shoot die-back and leads to dramatic decreases in yield. This disease is one of the major economically important diseases affecting olive orchards worldwide, including South Africa. Chemical treatments (copper containing sprays) are used as a standard control measure of this disease.

Therefore, olives and olive oil could retain chemical residues of fungicides, affecting oil aroma. Fungicides successfully control OLS, when disease incidence is low, but heavy infestation impairs effectiveness of chemical control and could lead to severe yield loss. Regular chemical treatment may prevent disease build-up in the orchard, but with cost and chemical residue implications.

The need for alternative control measures against OLS disease has led to an intensive search for cultivars which are tolerant to commercially important diseases, such as OLS, anthracnose, *Verticillium* wilt, diseases caused by *Cercospora* spp. and *Phytophthora* spp. This need resulted in this study, which was focused on OLS and was aimed at evaluating commercial olive cultivars and selections for tolerance against OLS.

The findings of the study provide a basis for preliminary recommendations of cultivars and selections tolerant to OLS disease for commercial olive production, as well as for further evaluation of OLS tolerant cultivars and selections under controlled conditions (glasshouse/growth chamber), as well as under field conditions in various olive producing regions of South Africa. The information obtained can be used to develop a rapid screening technique for the assessment of new selections and germplasm in the field, where evaluated cultivars can be used as basic source of comparison of disease occurrence.

4.1.1 Glasshouse trial

The glasshouse trial did not produce the results required to meet the expected study outcomes. The absence of OLS infection is ascribed to the fact that optimal conditions for infection could not be maintained in the glasshouses, due to malfunctioning of the temperature control system. The optimal temperature range for spore germination, infection, and mycelial growth of *F. oleaginium* is 16 to 21ºC (Graniti 1993, Viruega & Trapero 2002), coupled with the presence of free water or relative humidity above 70% (Obanor *et al*. 2008, 2010). Temperatures above 30ºC tend to inhibit spore germination, with the fungus being inactive in areas where the summers are warm and dry (Obanor *et al*. 2008).

The high temperature (27ºC and above, with the maximum temperature peaking at 34ºC during hot spells, between November - February 2011/2012) combined with low RH (less than 50%), could have caused spores to remain dormant, and the restricted spore development could have led to spores dying. Therefore, a new experimental site (Bien Donne-ARC Infruitec-Nietvoorbij) was used to carry out a growth chamber trial to pursue the primary objectives of this study.

The study outcome in the glasshouse trial provided evidence that maximum temperatures in excess of 30ºC were probably detrimental to conidial development and germination. An optimum temperature of 16ºC to 20ºC was found to be ideal for disease development in olive trees cultivated under controlled conditions in the growth chamber trial.

4.1.2 Growth chamber trial

After a month of inoculation in the growth chamber, there were no visible symptoms of OLS on the leaves. During the twelfth week after inoculation OLS symptoms began to be visible as black circular spots on the leaf surface. The affected area on the leaf blade formed chlorotic circles around necrotic blotchy lesions. The infection became more prominent and conspicuous while infected leaves were still attached to the trees.

Furthermore it was found in this study that the symptom development is easier to observe on the old leaves (at least 10 weeks of age) than on young leaves. These findings linked to those of Obanor *et al.* (2011) who showed that the age of olive leaves significantly affects conidia germination and development. Disease infection seemed to occur at an early stage while the leaves are tender and less cutinised but as they get older, spore germination decreases and more spores are produced for survival. Similar results have been reported for some other pathogens (Bentes & Matsuoka 2002). The black circular spots are an indication of production of conidia to overcome unfavourable surrounding climatic conditions.

Results from this current study gave evidence that disease onset and infection occurs at an early stage while leaves are still young, although visual symptoms appear at a later stage (10 weeks after inoculation). Disease severity was correlated with leaf age with the highest severity observed on old leaves (12 weeks) when leaves showed necrotic symptoms (senescing appearance).

A laboratory test using 5% NaOH solution confirmed infection on the leaves. Apart from the visual symptoms associated with OLS that were detected during visual assessment of leaves, the “white spot” symptom that was found in the glasshouse trial was also found in the growth chamber trial and ‘Nandi’ had the highest incidence of this symptom. Further investigation is needed to find its morphology and causal agent. However the occurrence of the white spot symptom did not continue and there was no significant damage to the plants.

The results of the growth chamber trial were used for final categorising of the eight olive cultivars evaluated in this study according to their OLS susceptibility (refer Table 3.2). ‘Coratina’ was found to be the most susceptible cultivar (moderate to highly susceptible). Two of the well-established and important cultivars grown in the South African olive industry, ‘Mission’ and ‘Manzanilla de Seville’ were also found be moderately susceptible.

'Frantoio' was categorised as highly tolerant, while 'Nandi' and 'Leccino' were found to be moderately tolerant and 'Nocellara del Belice' was fairly/slightly tolerant. This agrees with the findings by Costa (1998) who stated that all eight cultivars evaluated in this study are susceptible to OLS under South African conditions.

This study's results contradict the results reported by Surico (1997, as cited by Costa, 1998) that cv. 'Coratina' is fairly resistant. 'Coratina', 'Mission', 'Manzanilla' and 'Barouni' were found to be the most susceptible cultivars under SA conditions in this study (Chapter 3, Table 3.3).

In California, Sutter (1994) found that 'Manzanilla', 'Barouni' and 'Mission' were amongst the most tolerant cultivars against OLS disease. The results of this study also contradict the findings of Mekuria *et al* (2001) that 'Manzanilla' was semi resistant under field conditions. This could related to cultivar's gene adaptability to a specific region (e.g. 'Manzanilla de Seville' and 'Manzanilla de Jean' (Spain), with suffix "de Sevilla, etc." means country of its origin).

A possible clarification by Obano *et al.* (2008) could be that either the tree was able to adapt differently to a specific climate conditions or a particular variety differs in levels of epicuticular wax and thus different level of chemicals in various cultivars could have contributed to resistance against the disease. In a similar study conducted in Spain, Viruega and Trapero (1999) found that under field conditions disease occurrence varied between seasons, consequently further studies are underway.

4.2 GROUPING OF CULTIVARS ACCORDING TO THEIR OLS SUSCEPTIBILITY

Apart from the work of Costa (1998) and the tentative grouping of commercial olive cultivars and selections according to their levels of susceptibility to OLS (Costa, Pers. Com. 2011)[1], no other published research results regarding OLS susceptibility of olive cultivars under South African conditions are available.

[1] Carlo Costa of Agricultural Research Councel (ARC) Infruitec-Nietvoorbij, Stellenbosch, South Africa

In a similar study conducted in Spain, Viruega and Trapero (1999) found that under field conditions disease occurrence varied between seasons. The growth chamber trial produced positive results to meet the study objectives and supports the hypothesis of this study that most of olive cultivars can get infected by olive scab, but to different degrees of infection.

The findings of the study provide a basis for further evaluation of OLS tolerance of cultivars and selections, both in a controlled environment and under field conditions, as well as for preliminary recommendations of OLS tolerant cultivars and selections for commercial olive production. The choice of cultivar presumably depends to a large extent on the climatic conditions of a specific area. However, further investigation is needed to assess the risk of OLS disease occurrence for established, as well as new potential olive growing regions of South Africa, as they have different climate conditions.

The Paarl area, where the South African olive industry is concentrated at present, is characterised by favourable climatic conditions for the occurrence of OLS. Winter temperatures can fall to a minimum of less than 4ºC, with a maximum of approximately 18ºC. Average temperatures can range between a minimum of 10ºC to a maximum of 15ºC and 84 % maximum relative humidity during the autumn/winter season, as observed in the 2011, 2012 and 2013 seasons and this favours disease occurrence in this area, which is influenced by very wet weather conditions.

Therefore, based on OLS tolerance, ‘Frantoio’ (highly tolerant), ‘Nandi’ and ‘Leccino’ (moderately tolerant) and ‘Nocellara del Belice’ (fairly tolerant) are considered well adapted cultivars for commercial production in the Paarl area. ‘Coratina’ (moderate to highly susceptible), as well as ‘Mission’ and ‘Manzanilla de Seville’ (fairly susceptible) are expected to be more prone to be infection by OLS in the Paarl area, with the consequent negative effects on growth and yield.

When comparing the latter area (Paarl) to some new olive planting areas such as Riebeek-West (newly important olive producing area) which is characterised by high relative humidity conditions (above 70% RH during winter), disease occurrence is likely to be a problem in this winter rainfall area.

In specific summer rainfall areas the incidence of OLS disease could be less of problem, due to the fact that dry (little rain, low relative humidity), warm weather conditions with temperature above 27ºC occur in summer, which supresses spores germination. Oudtshoorn and Prince Albert in the Klein Karoo, as well as Vaalharts and Barkley-West in the Northern Cape are characterised by drier and warmer conditions, which are less favourable for the occurrence of OLS compared to the Paarl area. Therefore, based on OLS tolerance, 'Coratina' (moderate to highly susceptible), as well as 'Mission' and 'Manzanilla de Seville' (fairly susceptible) are expected to be suitable cultivars for commercial production in these areas, provided that other climatic requirements for commercial production are met.

Summer rainfall areas that experience warm winter conditions and high temperatures in summer could be detrimental to OLS disease development. Dry weather conditions (Karoo) and very warm conditions are detrimental to the disease build-up and development, and thus under these conditions the disease may be kept in a latent stage for a long time until a future outbreak.

In this case alternative control measures are important, and tolerant cultivars could be used to manage OLS disease. Previous research stated that disease severity increases with increased temperature from 5ºC to 15ºC and then decreases from 15ºC to 25ºC, whereas spores die above 27ºC (Viruega *et al*. 2011). Currently, tolerant cultivars could contribute to minimising chemical input and reduce the disease incidence when the weather is wet, temperatures are favourable (10ºC to 21ºC) and there is an abundant source of spores (maximum primary inoculum).

With reference to OLS tolerance cv. ‘Coratina’ was found to be a moderately to highly susceptible cultivar and ‘Mission’ and ‘Manzanilla de Seville’ were moderately susceptible. These cultivars are among the most important cultivars in the SA olive industry. However for high density planting systems, using susceptible cultivars could require more chemical inputs to control fungal diseases such as OLS. Therefore it is recommended that OLS tolerant cultivars are rather used for high density planting systems.

Based on OLS tolerance, ‘Frantoio’ (highly tolerant), ‘Nandi’ and ‘Leccino’ (moderately tolerant) and ‘Nocellara del Belice’ (fairly tolerant) are considered well adapted cultivars for commercial production for all olive growing regions of South Africa. These cultivars are recommended for use in breeding of new selections for OLS tolerance. The results of the growth chamber trial in this study were obtained over one season only (2011/12). It is therefore recommended that the evaluation of OLS tolerance of the eight cultivars included in this study should be repeated over more than one season, both under controlled conditions (glasshouse or growth chamber), as well as under field conditions in the major olive production regions of South Africa, to verify the preliminary results.

CHAPTER 5

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