Evaluation of key chemicals for pest management in the olive industry

Dr Robert Spooner-Hart
University of Western Sydney

Project Number: OL13002

OL13002

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FINAL REPORT

HAL PROJECT OL13002

EVALUATION OF KEY CHEMICALS FOR PEST MANAGEMENT IN THE OLIVE INDUSTRY

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PURPOSE OF THE REPORT

This report describes the assessment of the efficacy of a number of pesticides for use against key Australian olive pests, olive lace bug and olive anthracnose, via field and laboratory trials. It also provides efficacy data which can support submissions to the APVMA for their registration or permitted use.

This project has been funded by HAL and RIRDC using olive industry voluntary contributions and levy funds, voluntary contributions from Nufarm Australia and matched funds from the Australian Government.

March 2014

TABLE OF CONTENTS

SECTION	TOPIC	Page
TABLE OF CONTENTS		1
LIST OF TABLES		4
LIST OF FIGURES		7
MEDIA SUMMARY		8
TECHNICAL SUMMARY		9
INTRODUCTION		11
PART 1. EVALUATION OF INSECTICIDES FOR CONTROL OF OLIVE LACE BUG		12
2009–10 SEASON	FIELD TRIAL 1. Evaluation of insecticides Actara® (thiomethoxycam) and Lebaycid® (fenthion) foliar sprays for control for control of olive lace bug	14
2010–11 SEASON	FIELD TRIAL 2. Evaluation of insecticides Actara® (thiomethoxycam), Shield Systemic Insecticide™ (clothianidin) and Lebaycid® (fenthion) foliar sprays for control for control of olive lace bug	18
2011–2012 SEASON	FIELD TRIAL 3. Evaluation of insecticides Actara® (thiomethoxycam), Samurai Systemic Insecticide™ (clothianidin) and Lebaycid® (fenthion) foliar sprays for control for control of olive lace bug	21
	FIELD TRIAL 4. Evaluation of insecticides Actara® (thiomethoxycam), Samurai Systemic Insecticide™ (clothianidin) and Lebaycid® (fenthion) foliar sprays for control for control of olive lace bug	25
2013 SEASON	FIELD TRIAL 5. Evaluation of insecticides foliar sprays Sumi-Alpha® Flex (esfenvalerate) and Lebaycid® (fenthion) foliar sprays for control of olive lace bug	29
PART 2. EVALUATION OF FUNGICIDES FOR CONTROL OF OLIVE		32

ANTHRACNOSE

2009–2010 SEASON	FIELD TRIAL 1. Evaluation of fungicides Aero® Fungicide (pyraclostrobin + metiram), Nufarm Tri-base Blue® flowable copper fungicide, NorShield 750 WP copper fungicide and copper oxychloride for control of olive anthracnose	36
2010–2011 SEASON	FIELD TRIAL 2. Evaluation of fungicides Aero® Fungicide (pyraclostrobin + metiram), Nufarm Tri-base Blue® flowable copper fungicide, NorShield 750 WP copper fungicide and copper oxychloride for control of olive anthracnose	40
	FIELD TRIAL 3. Evaluation of fungicides Aero® Fungicide (pyraclostrobin + metiram), Amistar® (azoxystrobin), Nufarm Tri-base Blue® flowable copper fungicide and NorShield 750 WP copper fungicide for control of olive anthracnose	45
2011–2012 SEASON	FIELD TRIAL 4. Evaluation of fungicides Aero® Fungicide (pyraclostrobin + metiram), Amistar® (azoxystrobin), Nufarm Tri-base Blue® flowable copper fungicide and NorShield 750 WP copper fungicide for control of olive anthracnose	49
	FIELD TRIAL 5. Evaluation of fungicides Aero® Fungicide (pyraclostrobin + metiram), Amistar® (azoxystrobin), Nufarm Tri-base Blue® flowable copper fungicide and NorShield 750 WP copper fungicide for control of olive anthracnose	53
DETACHED FRUIT ASSAYS		57
	Detached olive fruit assay 1. O_{12-2}	60
	Detached olive fruit assay 2. O_{12-3}	62
	Detached olive fruit assay 3. O_{12-4}	65
	Detached olive fruit assay 4. O_{13-1}	67
	Relative cultivar susceptibility	72
SOFT NOSE		74

DISCUSSION	OLIVE LACE BUG	75
	ANTHRACNOSE	77
TECHNOLOGY TRANSFER		79
RECOMMENDATIONS		81
ACKNOWLEDGEMENTS		84
BIBLIOGRAPHY OF LITERATURE CITED		85
APPENDICES	Appendix 1. Letter sent to pesticide companies introducing the project and seeking expressions of interest	87
	Appendix 2. Analysis of olive oil from fungicide treatments in Anthracnose Trial 1, Lovedale 2010	88
	Appendix 3. Analysis of olive oil from fungicide treatments in Anthracnose Trial 2, Coonalpyn 2011	90
	Appendix 4. Analysis of olive oil from fungicide treatments in Anthracnose Trial 3, Boort 2011	92
	Appendix 5. Molecular characterisation of *Colletotrichum* spp. isolates associated with olive fruit rot (anthracnose)	94
	Appendix 6. University of Western Sydney media release on the RIRDC/HAL project	97
	Appendix 7. NSW DPI media release on the RIRDC/HAL project published in Agriculture Today and The Land Newspaper (Fairfax Press)	98
	Appendix 8. Raw Data from investigations	99

LIST OF TABLES

TABLE NO.	NAME	Page
1	Description of field trials evaluating insecticides against olive lace bug	13
2	Treatments, Lovedale trial; 1st spray 9 October 2009, 2nd spray 28 October 2009	14
3	Treatments, Pine Mountain trial	18
4	Pre-treatment and post-treatment counts, number of infested twigs with olive lace bug, Pine Mountain trial	20
5	Treatments, Hilldale trial	21
6	Pre-treatment and post-treatment counts, number of infested twigs with olive lace bug, Hilldale trial	23
7	Treatments, Yelarbon trial	25
8	Pre-treatment and post-treatment counts, number of infested twigs with olive lace bug, Yelarbon trial	27
9	Treatments, Coominya trial	29
10	Pre-treatment and post-treatment counts, number of infested twigs with olive lace bug, Coominya trial	30
11	Description of field trials evaluating fungicides against olive anthracnose	34
12	Treatments, Lovedale trial	37
13	Treatments, Coonalpyn trial 1	41
14	Percentage levels of infection in fruit sampled from the trial site, Coonalpyn 2011	43
15	Fungicide treatments applied in Field Trials 3 &4	46
16	Mean weight of 200 fruit from anthracnose fungicide treatments, Boort	47
17	Area under disease progress stairs for fungicide treatments, Boort trial 2011–12	51
18	Predicted back-transformed means from the proportions analysis at each timepoint. Numbers in columns followed by	52

different letters are significantly different ($P \leq 0.05$)

19 Fungicide treatments applied in Coonalpyn trial 2 53

20 Area under disease progress stairs for fungicide treatments, Coonalpyn trial 2 55

21 Predicted back-transformed means from the proportions analysis at each timepoint 56

22 Summary of treatments and cultivars used in detached olive fruit assays 58

23 Area under disease progress stairs back-transformed means for fungicide treatments in detached fruit assay $O_{12\text{-}2}$ 60

24 Analyses of deviance (AOD) for proportional analysis at each timepoint (ignoring Nil treatment) for detached fruit assay $O_{12\text{-}2}$ 61

25 Predicted back-transformed means from the proportions analysis at each timepoint for detached fruit assay $O_{12\text{-}2}$ 61

26 Area under disease progress stairs statistics (ignoring Nil treatment) for fungicide treatments in detached fruit assay $O_{12\text{-}3}$ 62

27 Area under disease progress stairs back-transformed means for fungicide treatments in detached fruit assay $O_{12\text{-}3}$ 62

28 Analyses of deviance (AOD) for proportional analysis at each timepoint (ignoring Nil treatment) for detached fruit assay $O_{12\text{-}3}$ 63

29 Predicted back-transformed means from the proportional analysis at each timepoint for detached fruit assay $O_{12\text{-}3}$ 64

30 Area under disease progress stairs back-transformed means for fungicide treatments in detached fruit assay $O_{12\text{-}4}$ for cv. Arbequina 65

31 Analyses of deviance (AOD) for proportional analysis at each timepoint (ignoring Nil treatment) for detached fruit assay $O_{12\text{-}4}$ 65

32 Predicted back-transformed means from the proportional analysis at each timepoint for detached fruit assay $O_{12\text{-}3}$ 66

33 Area under disease progress stairs Wald type F-statistics (ignoring Nil treatment) for fungicide treatments in detached fruit assay $O_{13\text{-}1}$ 67

34	Area under disease progress stairs back-transformed means for fungicide treatments in detached fruit assay $O_{13\text{-}1}$	68
35	Analyses of deviance (AOD) tables for proportional analysis at each timepoint (ignoring Nil treatment) for detached fruit assay $O_{13\text{-}1}$	69
36	Proportional analysis: Predicted means (logit scale and AVSED) and back-transformed means from the proportional analysis at each timepoint for detached fruit assay $O_{13\text{-}1}$	70
37	Relative cultivar susceptibility to *Colletotrichum acutatum* determined from AUDPS mean values of pathogen only treatment in Trial $O_{13\text{-}1}$	72
38	Relative cultivar susceptibility to *Colletotrichum acutatum* determined from proportion of infected fruit of pathogen only treatment in Trial $O_{13\text{-}1}$	72
39	Summary of efficacy of insecticides against olive lace bug from field trials	75

LIST OF FIGURES

FIGURE NO.	NAME	Page
1	Trial site, Lovedale NSW 2009–2010	16
2	Percentage infested twigs per tree (bars) before treatment, 8 and 19 days after the first treatment and 8 days after the second treatment with their standard error of mean, Lovedale trial	17
3	Adult lace bugs (and associated damage) on leaves prior to application of insecticide treatments	23
4	Trial site, Yelarbon NSW 2010–11	27
5	Trial site, Coominya Qld 2013–2014	31
6	Trial site, Lovedale NSW 2009–2010	36
7	Mean % anthracnose infection in cultivar Manzanillo fruit of three fungicide treatments and Water Control, following 2 applications. Bars represent SE of the mean	39
8	Trial site, Coonalpyn SA 2010–11	40
9	Mean % anthracnose infection in cultivar Manzanillo fruit in four fungicide treatments and Water Control, following 3 applications of each between 22 December 2010 and 7 April 2011, 10 days after the last application	42
10	Flooding in part of the Boort olive grove, January 2011	46
11	Nufarm Tri-base Blue® flowable copper fungicide fresh residues on fruit and leaves	50
12	NorShield 750 WP copper fungicide spray on leaves and fruit immediately following spraying	50
13	Scoring fruit samples for anthracnose infection	54

MEDIA SUMMARY

The project *Evaluation of key chemicals for pest management in the olive industry* arose in response to a major review of pests and diseases of olives in Australia and the availability of acceptable pesticides for their sustainable management, that identified olive lace bug and the fruit rot disease anthracnose as the two serious problems without effective approved chemicals. Olive lace bug is an Australian native species which severely damages olive tree foliage, causing leaf loss and twig and branch dieback. Anthracnose is a fruit rot caused by cosmopolitan fungi which damage fruit, reducing yield and olive oil quality. The project aimed to identify suitable chemicals against these two key pests, and conduct trials to generate efficacy data, to assist in their registration with the Australian pesticides regulatory authority, the APVMA, for legal use in olives.

A total of six olive lace bug insecticide efficacy field trials were conducted during the project in NSW and Queensland, where this pest is a major problem. Two products, Actara® (thiomethoxycam) and Samurai Systemic Insecticide™ (clothianidin), were shown to be highly efficacious against olive lace bug when applied as a single foliar spray, and were as good as or better than the industry standard, Lebaycid® (fenthion). Lebaycid® currently has a restricted use pattern in olives as well as in many other crops, and its future is under threat. A further investigation in Queensland with Sumi-Alpha® Flex (esfenvalerate), an insecticide from a different chemical group to Actara® and Samurai Systemic Insecticide™, also showed good efficacy against olive lace bug.

A total of five anthracnose fungicide efficacy field trials were conducted during the project in the Hunter Valley NSW, Coonalpyn SA and Boort Vic. They assessed the efficacy of formulations of copper-based protectant fungicides (copper oxychloride, copper oxide, tribasic copper sulphate) and two curative fungicides, Amistar® (azoxystrobin) and Aero® Fungicide (pyraclostrobin + metiram). In addition, a series of four laboratory-based assays using olive fruit further assessed fungicide efficacy against the anthracnose pathogen. Overall, the field and laboratory trials demonstrated that Amistar® and Aero® were generally superior to the copper products, and Aero® (which contains two fungicides with different modes of action) was more consistent in its performance than Amistar®. Copper products, nevertheless, still have a role in anthracnose management, and in addition they can be used to manage a number of other olive diseases.

The laboratory assays also showed that some olive varieties, such as Barnea, were more susceptible to anthracnose than other commonly grown varieties Manzanillo, Picual and Arbequina. This finding supports previous anecdotal information from Australia.

The project also aimed to conduct preliminary investigations on the importance of the minor nutrients calcium and boron on occurrence of the fruit disorder soft nose, but we did not find a confirmed case of this problem so were unable to conduct the work. However, based on adverse conditions (including flooding) experienced in some of our trial groves, it appears that the conditions responsible for this disorder are quite complex and not only associated with imbalances of these nutrients.

TECHNICAL SUMMARY

This document reports on investigations conducted on key pests and diseases of olives, namely olive lace bug, *Froggattia olivinia*, and anthracnose, *Colletrotrichum* spp. The project aimed to assess two neonicotinoid insecticides, Actara® (thiomethoxycam) and Shield Systemic Insecticide™ (clothianidin)/Samurai Systemic Insecticide™ (clothianidin) against olive lace bug via field trials, as alternatives to the organophosphates dimethoate and fenthion. It also aimed to evaluate the efficacy of applications of strobilurin fungicides and new formulations of copper against anthracnose. It addressed two key objectives of the Australian olive industry's Strategic Plan, namely, sustainable production and product quality.

A total of six olive lace bug insecticide efficacy field trials were conducted during the project, within the centre of origin for this species, NSW and Queensland. The two neonicotinoid products Actara® (thiomethoxycam) and Shield Systemic Insecticide™/Samurai Systemic Insecticide™ (clothianidin), showed high efficacy against olive lace bug when applied as a single foliar spray, and in three of the trials performed significantly better than the industry standard, Lebaycid® (fenthion). Mean efficacy of Actara® treatments was 92.6%, clothianidin (1 trial of Shield Systemic Insecticide™ and 2 of Samurai Systemic Insecticide™) had a mean efficacy of 96.8%, compared with the industry standard, Lebaycid® 74.0%. A preliminary investigation evaluating the synthetic pyrethroid Sumi-Alpha® Flex (esfenvalerate) showed it also had good efficacy (91.5%) against olive lace bug.

Based on these trials a single application of the neonicotinoids, especially Samurai Systemic Insecticide™ applied as a foliar spray at a concentration of 40g/100L to run-off appears to be adequate for controlling populations of olive lace bug, even under high pest pressure.

A total of five anthracnose fungicide efficacy field trials were conducted during the project. These were conducted in the Hunter Valley NSW (1 trial), Coonalpyn SA (2 trials) and Boort Vic (2 trials). They assessed efficacy of formulations of copper-based fungicides (copper oxychloride, copper oxide, tribasic copper sulphate (Nufarm Tri-base Blue® flowable copper fungicide) and two strobilurin fungicides, Amistar® (azoxystrobin) and Aero® Fungicide (pyraclostrobin + metiram). All chemical treatments were demonstrated to reduce anthracnose disease in more than one of the five trials when compared with treatments sprayed with water only.

In addition, a series of four laboratory-based detached olive fruit assays further assessed fungicide efficacy and determined relative susceptibility of fruit from a number of different olive cultivars to anthracnose caused by *Colletotrichum acutatum*. These trials had the advantage of being conducted under standardised environmental conditions and with uniform fungal inocula.

Overall, the field and laboratory trials determined that strobilurin fungicides provided superior and more consistent efficacy to olive anthracnose when compared with the copper products. Aero® was more consistent in its efficacy than Amistar®. This trend was more obvious in trials with the cultivar Barnea but was reproduced in certain trials with the cultivars Manzanillo, Picual and Arbequina. The use of copper products for disease control in olives still has merit given they sometimes gave rise to a significant disease reduction and they also have potential to control other foliar

pathogens causing diseases such as peacock spot and leaf mould. Furthermore, protectant chemicals with multi-site activity such as copper and metiram reduce the risk of resistance to the strobilurins developing in populations of *Colletotrichum* spp.

When the relative susceptibility of the olive cultivars to anthracnose was compared, it was clear that the cultivar Barnea was far more susceptible than any others tested. This confirmed anecdotal observations by many members of the Australian olive industry. Interestingly, the cultivar Picual was demonstrated to be very susceptible when challenged with an isolate of *C. acutatum* in a detached fruit assay, which contrasted with a report from a similar Spanish study where it was designated as resistant. Our results need to be confirmed in further studies.

We did not find a confirmed case of the fruit disorder soft nose during the project, so did not conduct any trials with minor nutrients (calcium and boron) purported to be implicated in expression of soft nose symptoms. Interestingly, the high rainfall and associated waterlogging experienced in one season during this project would have reduced availability and uptake of these divalent cations, suggesting that soft nose may have a different or more complex etiology.

INTRODUCTION

Background to the project

In 2008, Peter Dal Santo, AgAware Consulting Pty Ltd, collated information on minor use of pesticides for the Australian Olive Industry (AOA) (Dal Santo 2008), in preparation for a Strategic Agrichemical Review Process (SARP). In his report, he concluded that information on pest incidence, pesticide use, residues and IPM techniques gathered from Australia and overseas could be used to plan for future pesticide requirements to fit with the philosophy of AOA. From a pesticide access perspective, he reported "the APVMA classifies olives as a member of the 'Assorted tropical and sub-tropical fruit (edible peel) group' and a minor crop. Therefore access to minor-use permits by the olive industry should be relatively straightforward" (Dal Santo 2008).

The information in Dal Santo's (2008) report was used as the key input for the olive SARP, with a meeting conducted on 2 October 2008 in Melbourne, facilitated by Dal Santo. The SARP identified that there were two key pests/pathogens in olives with insufficient effective chemical control options, namely olive lace bug and anthracnose. The current project arose directly as a result of the olive industry SARP.

In September 2010, Dal Santo and Spooner-Hart contacted the major pesticide companies in Australia: Bayer CropScience, Chemtura, Dow AgroSciences, Dupont, Farmoz, Nufarm / BASF, Organic Crop Protectants, Sipcam, Pacific Australia Pty Ltd, Sumitomo and Syngenta to introduce the project and to seek expressions of interest from them in providing products for evaluation against olive lace bug and anthracnose. The letter is provided in Appendix 1.

Based on the pesticide companies' feedback and discussions with the AOA's Chemical Permits Committee and the APVMA, we finally selected the insecticides Actara® (thiomethoxycam) (Bayer); and Shield Systemic Insecticide™ (clothianidin) (later changed to a different formulation, Samurai Systemic Insecticide™ (clothianidin) (Sumitomo); and the fungicides Nufarm Tri-base Blue® flowable copper fungicide® (Nufarm), Cabrio Top® (later renamed Aero® Fungicide (pyraclostrobin + metiram)®) (Nufarm); and Amistar® (azoxystrobin) (Syngenta). More details of the chemicals used in the trials are provided in the relevant sections in this report.

One of the key objectives of the AOA was to involve growers in the project work as an educational activity. As a result, we contacted growers for their interest in collaboration, and a prior history of their anthracnose and/or olive lace bug in their groves, initially through the industry journal Olives Australia (Spooner-Hart & Sergeeva 2010) and subsequently at the AOA annual conferences.

PART 1. EVALUATION OF INSECTICIDES FOR CONTROL OF OLIVE LACE BUG

Introduction

The olive lace bug, *Froggattia olivinia* Froggatt (Hemiptera: Tingidae) (OLB), is an Australian sap-sucking insect endemic to eastern New South Wales (NSW) and SE Queensland (SE Qld). It was first described feeding on olives in 1901 (Froggatt 1901) and is now a serious pest in many parts of eastern Australia (Hely et al. 1982; Spooner-Hart et al. 2002, 2007, 2010; Hall et al. 2012). It has spread from its original distribution, probably with movement of plant material, to all mainland states including Western Australia (Hardie et al. 2004; Botha & Learmonth 2011). *F. olivinia* feeds on mesophyll cells, and is responsible for chlorotic spotting and yellowing of leaves, and in heavy infestations leaf drop and dieback. There are five nymphal instars which can complete their life cycle in as little as 5–6 weeks, depending on climatic conditions (Spooner-Hart et al. 2002). In many parts of Australia, there appears to be 3–5 generations per season. At the time of commencement of this project, the chemicals permitted for use against olive lace bug were fenthion, dimethoate and Natrasoap®. While Natrasoap (potassium salts of fatty acids) is a treatment option for organic olive growers, it is not a highly efficacious or economic option for olive lace bug control for conventional growers. Fenthion and dimethoate were seen as not long-term options, because of their withdrawal from use overseas (particularly the EU whose member countries grow the majority of the world's olives) and impending reviews of these agrichemicals by the APVMA. Hence, alternative efficacious products were needed by the Australian olive industry against this increasingly important pest.

A total of six olive lace bug insecticide efficacy field trials were conducted during the project. These were conducted in SE Queensland (Ipswich region 1 site, Lockyer Valley 1 site and Goondiwindi region 1 site), and the lower Hunter Valley (1 site) and upper Hunter Valley (1 site) (Table 1). These are locations where lace bug is endemic and has a history of causing serious damage.

Insecticides evaluated for efficacy against olive lace bug

Actara® active constituent thiamethoxam (chemical group 4A) 250 g/kg water dispensible granule, Syngenta Australia Pty Ltd. Actara® is a second generation neonicotinoid insecticide, registered in Australia for use against sucking insects in a range of crops.

Lebaycid® active constituent fenthion (chemical group 1A) 550 g/L emulsifiable concentrate, Bayer CropScience. At the commencement of this project, Lebaycid® was registered for use against a wide range of insect pests in various crop situations, and had permitted minor use in olives against olive lace bug and several other pests (PER 8560, 2008-9), which continued through the project with subsequent permits (PER 11782, 2009–2011; PER 12857, 2011–2012 surrendered). It currently has permitted use in olives (PER13868) until end October 2014. Lebaycid® was used as the industry standard in all trials.

Samurai Systemic Insecticide™ (active constituent clothianidin (chemical group 4A) 500 g/kg water dispersible granule), Sumitomo Chemical Australia Pty Ltd.

Samurai Systemic Insecticide™ is registered for use against a range of insect pests in horticultural crops.

Shield Systemic Insecticide™ (active constituent clothianidin 200 g/L suspension concentrate), Sumitomo Chemical Australia Pty Ltd., registered for use against several insect pests in a range of cropping situations, sometimes as a foliar spray. While the project commenced with Shield Systemic Insecticide™ as the formulation of clothianidin recommended by the manufacturer, this recommendation was changed to Samurai Systemic Insecticide™ in 2010.

Sumi-Alpha® Flex (active constituent esfenvalerate (chemical group 3A) 50 g/L emulsifiable concentrate) Sumitomo Chemical Australia Pty Ltd, registered for use against a range of insect pests in field crops, pasture and vegetable crops.

Table 1. Description of field trials evaluating insecticides against olive lace bug

Season	Location	Insecticides tested
2009–10 Oct–Nov 2009	Lovedale, Hunter Valley NSW	Actara® (thiomethoxycam), Lebaycid® (fenthion)
2010–11 Oct 2010	Pine Mountain, near Ipswich SE Qld	Actara® (thiomethoxycam), Shield Systemic Insecticide™ (clothianidin), Lebaycid® (fenthion)
2011–2012 Oct 2011	Hilldale, Hunter Valley NSW	Actara® (thiomethoxycam), Samurai Systemic Insecticide™ (clothianidin), Lebaycid® (fenthion)
2011–2012 Nov–Dec 2011	Yelarbon, NSW near Goondiwindi North central NSW	Actara® (thiomethoxycam), Samurai Systemic Insecticide™ (clothianidin), Lebaycid® (fenthion)
2013–2014 Oct 2013	Coominya, Lockyer Valley SE Qld	Sumi-Alfa Flex, Lebaycid® (fenthion)

2009–10 SEASON

FIELD TRIAL 1. Evaluation of insecticides Actara® (thiomethoxycam) and Lebaycid® (fenthion) foliar sprays for control for control of olive lace bug

Location:

Swish Wine
113 Wilderness Road Lovedale
Hunter Valley NSW 2320 (32.46° S, 150.77° E)

The olive grove was planted in 1998, with a mix of olive varieties. One side of the grove was adjacent to a forest, one side to a vineyard, one to the road and the other to other olive trees. The experimental block was planted with the cultivar (cv.) Corregiola at a grid of 6 × 6 m (~304 trees/ha). Trees had recently been lightly pruned, and were approximately 3.5 m high (Figure 1).

Experimental design

Two factorial experiment with insecticidal option (3 treatments) being the fixed factor and block (5 blocks) being the random factor (i.e. 3 × 5). Treatments were organized as randomised complete blocks.

Description of plots and blocks:

The experiment had five blocks, each comprising three trees. Each treatment was applied to a total of five trees. Total number of trees in the experiment was 15.

Each individual tree represented an experimental plot.

Treatments

Table 2. Treatments, Lovedale trial; 1st spray 9 October 2009, 2nd spray 28 October 2009

Treatment number	Insecticide	Rate g or mL/100 L	Active ingredient	g a.i./100L
1	Actara®[a]	30.0	250g/kg thiamethoxam	7.50
2	Lebaycid®[b]	75	550g/L fenthion	41.25
3	Water			

[a] Rate recommended Syngenta Australia Pty Ltd
[b] Rate on APVMA Permit

Spray application

Treatments were applied using a Hardi wheelbarrow sprayer fitted with 100 L tank, a 2.5 kW Honda petrol engine and an extension wand with 1 solid cone nozzle 1.8 mm diameter. Sprays were apply at the pressure of 8 bars to the point of run-off, which equated to 3.0 L/tree (= 912 L/ha). Treatments were applied on October 9, 2009 between 06.30 and 09.00 and on October 28, 2009 between 11.00 and 13.00. There was no wind and temperatures were in the mid 20°Cs at the times of spray.

Assessments

Assessments were conducted at the commencement of the trial on 8 October, 8 days after the first spray on 17 October, 19 days after the first spray on 28 October and 8 days after the second spray on 5 November 2009.

One tree per replicate was assessed at four cardinal points. At each point, 10 twigs 20–30 cm long were assessed for presence or absence of lace bug. At the assessments before the sprays, as well as 8 and 19 days after the first sprays adult lace bugs were observed on the majority of twigs while young nymphs were observed on the majority of twigs 8 days after the second spray.

Statistical analysis

Data were analysed using general linear model of analysis of variance (ANOVA) (SPSS® for Windows™ Version 17). Each variable was visually tested for normality using P-P plot and Levene's test was used for testing the assumption of equality of error variance. Data at all assessment times approached normal distribution and assumption of equality of variance was met so Ryan's Q-test was used to separate treatment means where the F-test showed significant differences between treatments.

Corrected efficacy

Corrected efficacy was calculated using Henderson-Tilton's formula:

Corrected % = (1 - (n in Co after treatment * n in T before treatment/ n in Co before treatment * n in T after treatment))*100

Where: n = Insect population, T = treated, Co = control

Results

1st assessment 08 Oct 2009, pre-treatment

There were no significant differences in the number of twigs infested with lace bug between trees assigned to each treatment ($F_{2, 8} = 0.156$, $P = 0.858$) (Figure 2).

2nd assessment 17 Oct 2009, 8 days post-treatment

There were significant differences between treatments in number of twigs infested with lace bug ($F_{2, 8} = 5.962$, $P = 0.026$ (Figure 2)). Both insecticidal treatments significantly reduced lace bug infestation in comparison to water sprayed Control. There were no significant differences between insecticidal treatments. Corrected efficacy calculated using the Henderson-Tilton formula for Lebaycid® (fenthion) was 67.9% and for Actara® (thiomethoxycam) was 56.2%.

3rd assessment 28 Oct 2009, 19 days post-treatment

There were no significant differences between treatments in number of twigs infested with lace bug ($F_{2, 8} = 0.636$, $P = 0.557$ (Figure 2)). Corrected efficacy calculated using Henderson-Tilton formula for Lebaycid® (fenthion) was 18.2% and for Actara® (thiomethoxycam) was 25.6%.

4th assessment 5 November 2009, days post 2nd treatment

There were significant differences between treatments in the number of twigs infested with lace bug ($F_{2, 8} = 6.243$, $P = 0.023$ (Figure 2)). Both insecticidal treatments significantly reduced lace bug infestation in comparison to water sprayed Control. There were no significant differences between insecticidal treatments. Corrected efficacy calculated using Henderson-Tilton formula for Lebaycid® (fenthion) was 100% and for Actara® (thiomethoxycam) 92.4%.

Figure 1. Trial site, Lovedale NSW 2009–2010

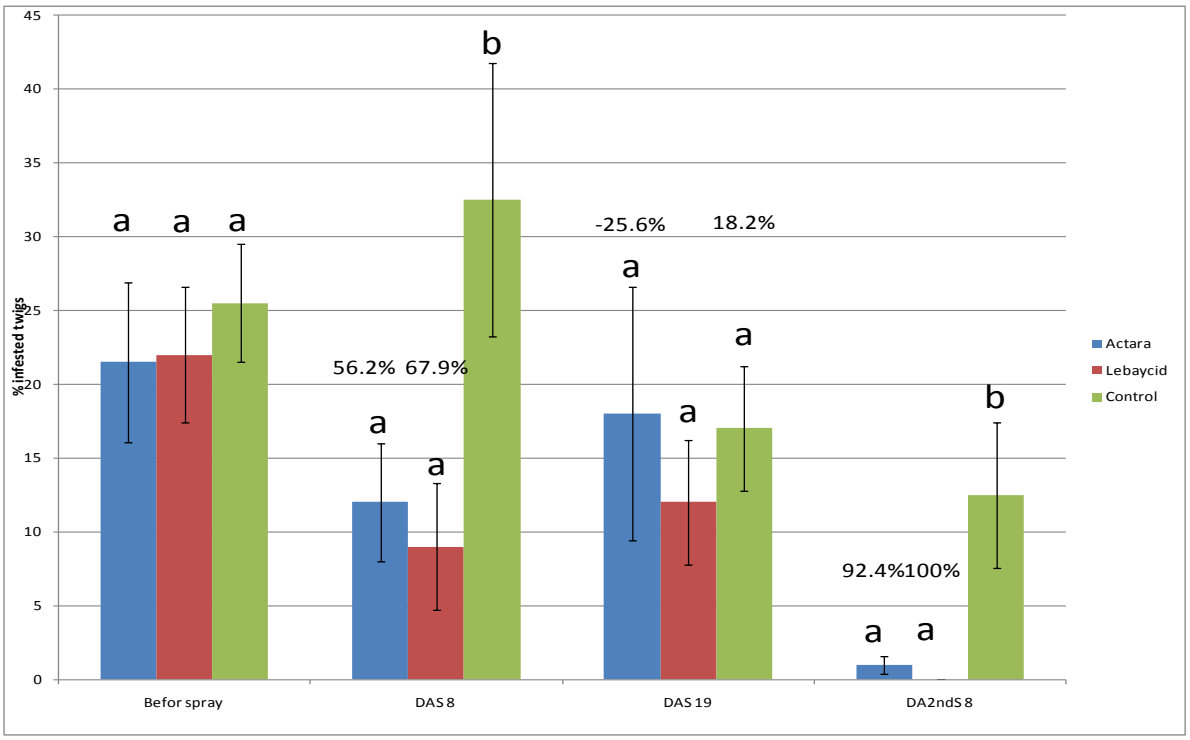


Figure 2. Percentage infested twigs per tree (bars) before treatment, 8 and 19 days after the first treatment and 8 days after the second treatment with their standard error of mean, Lovedale trial

There are no significant differences between treatments marked with the same letter within each assessment time.

Percentage figures above bars represent corrected efficacy, calculated using Henderson-Tilton formula.

2010–11 SEASON

FIELD TRIAL 2. Evaluation of insecticides Actara® (thiomethoxycam), Shield Systemic Insecticide™ (clothianidin) and Lebaycid® (fenthion) foliar sprays for control for control of olive lace bug

Location

Len Mahon
Pine Mountain Road
Pine Mountain, near Ipswich Queensland 4306 (27.53° S, 152.72° E)

Experimental design

Two factorial experiment with insecticidal options (4 treatments) being the fixed factor and block (5 blocks) being the random factor (i.e. 4 × 5). Treatments were organized as randomised complete blocks.

Description of plots and blocks

The grove was small (approx. 10 ha), but with a number of olive groves in the vicinity. It comprised a mixed planting of cultivars, but the experimental block was comprised of only cv. UC 13. The trees were 10 years old and approximately 2.5 m high, and lightly foliaged, as a result of persistent olive lace bug attack. Trees were planted at 5 × 5 m spacing (~420 trees/ha). Each treatment was applied to individual trees, with at least two trees either side acting as buffer trees. The olive lace bug infestation was high, with adults and all stage nymphal instars present prior to the treatment applications. At the post-treatment count, the remaining population was comprised mainly of adults with some late-instar nymphs.

Treatments

Table 3. Treatments, Pine Mountain trial

Treatment number	Insecticide	Rate g or mL/100 L	Active ingredient	g a.i./100L
1	Actara®	30.0	250g/kg thiamethoxam	7.50
2	Shield Systemic Insecticide™ [a]	60.0	200g/L clothianidin	12.00
3	Lebaycid®	75.0	550g/L fenthion	41.25
4	Water			

[a] Rate recommended by Sumitomo Chemical Australia

Assessments

Assessments were conducted at the commencement of the trial, just prior to spraying on 30 September 2010 and 11 days after the single spray application for all treatments (i.e. 11 October 2010).

One tree per replicate was assessed at four cardinal points. At each point five twigs, 20–30 cm long, were assessed for presence or absence of lace bug. Presence of any lace bug (and of any stage) was scored 1, absence was scored 0. The total number of twigs assessed per tree was 20.

Spray application

A pressurised backpack sprayer with a tank capacity of 8 L was used to apply treatments. Treatments were applied at 310 kPa pressure using a hand wand with a single adjustable hollow cone nozzle. The nozzle had an output of 600 mL/min. Every tree was sprayed to run-off, which equated to 120 sec, and thus, a volume of ~1.2 L of spray applied per tree or at a rate of approximately 504 L/ha. Weather at the time of application (09.00–11.00) was 17–18° C, cloudy with slight breeze.

Statistical analysis

Data were analyzed using SPSS® for Windows™ Version 12 (SPSS Inc. 2003). All data were analysed using the general linear model of analysis of variance (ANOVA). Each variable was visually tested for normality using Q-Q plot and Levene's test was used for testing the assumption of equality of error variance. When the assumptions of normal distribution or of equality of error variance were not met, appropriate transformations were used. Tukey B test was used if data met assumption of equality of error variance and Games-Howell test was used if the assumption was not met.

Results

The results of the pre-treatment and post-treatment counts are presented in Table 4.

1st assessment, pre-treatment

There were no significant differences in the number of twigs infested with lace bug between trees assigned to each treatment (mean 18.1 ± 0.340; $F_{3,19} = 1.667$, $P = 0.456$).

2nd assessment, 12 days post-treatment

Post-treatment count data were log (X+1) transformed prior to analysis. There was a significant difference between treatments $F_{3,19} = 41.221$, $P < 0.001$) with Shield Systemic Insecticide™ (clothianidin) and Actara® (thiomethoxycam) significantly superior to Lebaycid® (fenthion) and all treatments significantly superior to the Water Control.

Table 4. Pre-treatment and post-treatment counts, number of infested twigs with olive lace bug, Pine Mountain trial

TREATMENT	PRE-TREATMENT COUNT (SE)	POST-TREATMENT COUNT (SE)
Actara® (thiomethoxycam)	18.20 (0.49)	0.68 c (0.37)
Shield Systemic Insecticide™ (clothianidin)	18.00 (0.71)	0.14 c (0.14)
Lebaycid® (fenthion)	17.40 (1.08)	6.14 b (1.90)
Water	18.80 (0.20)	19.40 a (0.25)

Numbers in columns followed by different letters are significantly different (Tukey B test ($P \leq 0.05$))

Conclusions

All three insecticidal treatments significantly reduced infestation of adult lace bug after a single spray (corrected efficacy Shield Systemic Insecticide™ (clothianidin) 99.3%, Actara® (thiomethoxycam) 96.3%, Lebaycid® (fenthion) 65.8%), but the first two were superior to the industry standard, Lebaycid® (fenthion). It can be concluded that Actara® (thiomethoxycam) and Shield Systemic Insecticide™ (clothianidin) have potential to be used for control of lace bug on olives.

2011–2012 SEASON

FIELD TRIAL 3. Evaluation of insecticides Actara® (thiomethoxycam), Samurai Systemic Insecticide™ (clothianidin) and Lebaycid® (fenthion) foliar sprays for control for control of olive lace bug

Location:

Neville Schofield
Hilldale Olives
Hilldale NSW 2420 (32.51° S, 151.65° E)

Experimental design

Two factorial trial with insecticidal options (4 treatments) being the fixed factor and block (4 blocks) being the random factor (i.e. 4 × 4). Treatments were organized as randomised complete blocks.

Description of plots and blocks

The grove comprised a small (10 ha) mixed olive cultivar planting on a northerly slope and, open on three sides and with forest on the fourth side. The experimental site was comprised of grafted trees of cv. Kalamata, 11 years old and approx. 2.5 m high with an open structure (light-medium foliage). They were spaced approx. 5.5 m apart in rows 6 m apart (~300 trees/ha). The experimental site had 4 blocks, and treatments were individual trees, with at least one unsprayed tree on either side acting as a buffer. Olive lace bug infestation was moderate, and both nymphal stages and adults were present throughout the trial.

Treatments

Table 5. Treatments, Hilldale trial

Treatment number	Insecticide	Rate g or mL/100 L	Active ingredient	g a.i./100L
1	Actara®	30.0	250g/kg thiamethoxam	7.50
2	Samurai Systemic Insecticide™ [a]	40.0	500g/kg clothianidin	20.00
3	Lebaycid®	75.0	550g/L fenthion	41.25
4	Water			

[a] Rate recommended by Sumitomo Chemical Australia

Assessments

Assessments were conducted at the commencement of the trial, just prior to spraying on 4 October 2011 and 7 days after the single spray application for all treatments (i.e. 11 October 2011).

One tree per replicate was assessed at four cardinal points. At each point five twigs, 20–30 cm long, were assessed for presence or absence of lace bug. Presence of any lace bug (and of any stage) was scored 1, absence was scored 0. The total number of twigs assessed per tree was 20.

Spray applications

A diaphragm pump with a tank capacity of 50 L was used to apply treatments. Treatments were applied at 400 kPa pressure using a hand wand with a single adjustable hollow cone nozzle. The nozzle had an output of 650 mL/min. Every tree was sprayed to run-off, which equated to 120 sec, and thus, a volume of ~1.3 L of spray was applied per tree or at a rate of approximately 390 L/ha. Weather at the time of application (13.00–15.00) was 18° C, with no breeze.

Statistical analysis

Data were analyzed using SPSS® for Windows™ Version 12 (SPSS Inc. 2003). All was analysed using the general linear model of analysis of variance (ANOVA). Each variable was visually tested for normality using Q-Q plot and Levene's test was used for testing the assumption of equality of error variance. When the assumptions of normal distribution or of equality of error variance are not met, appropriate transformations were used. Tukey B test was used if data met assumption of equality of error variance and Games-Howell test was used if the assumption was not met.

Results

1st assessment, pre-treatment

There were no significant differences in the number of twigs infested with lace bug between trees assigned to each treatment (mean 9.10 ± 0.63; $F_{3,15} = 0.279$, $P = 0.840$) (Table 6).

Figure 3. Adult lace bugs (and associated damage) on leaves prior to application of insecticide treatments

Table 6. **Pre-treatment and post-treatment counts, number of infested twigs with olive lace bug, Hilldale trial**

TREATMENT	PRE-TREATMENT COUNT (SE)	POST-TREATMENT COUNT (SE)
Actara® (thiomethoxycam)	8.00 (1.47)	1.00 b (0.707)
Samurai Systemic Insecticide™ (clothianidin)	9.50 (1.85)	0.00 b (0.00)
Lebaycid® (fenthion)	9.50 (0.87)	1.25 b (0.75)
Water	9.25 (1.03)	15.25 a (2.06)

Numbers in columns followed by different letters are significantly different (Tukey B test pre and Games-Howell test post treatment ($P \leq 0.05$))

2nd assessment, 7 days post-treatment

A summary of the post-treatment counts are presented in Table 6. Post-treatment count data were log (X+1) transformed prior to analysis. There was a significant difference between treatments ($F_{3,15} = 211.417$, $P < 0.001$) with all insecticide treatments significantly superior to the Water Control, but not different from each other.

Corrected efficacy calculated using Henderson-Tilton formula for Samurai Systemic Insecticide™ (clothianidin) was 100.0%, for Actara® (thiomethoxycam) was 92.42% and for Lebaycid® (fenthion) was 92.02%.

Conclusions

All three insecticidal treatments significantly reduced infestation of lace bug after a single spray (corrected efficacy Samurai Systemic Insecticide™ (clothianidin) 100%, Actara® (thiomethoxycam) 92.4%, Lebaycid® (fenthion) 92.0%). It can be concluded that Actara® (thiomethoxycam) and Shield Systemic Insecticide™ (clothianidin) have potential to be used for control of lace bug in olives.

FIELD TRIAL 4. Evaluation of insecticides Actara® (thiomethoxycam), Samurai Systemic Insecticide™ (clothianidin) and Lebaycid® (fenthion) foliar sprays for control for control of olive lace bug

Location:

James Tomich
Twin Rivers Olives
Twin Rivers NSW 2410 (28.65° S, 150.73° E)
via Yelarbon Qld (Yelarbon trial)

Experimental design

Two factorial experiment with insecticidal options (4 treatments) being the fixed factor and block (4 blocks) being the random factor (i.e. 4 × 4). Treatments were organized as randomised complete blocks.

Description of plots and blocks

The olive grove is a former managed investment scheme, being brought back into production, and comprises 180,000 trees over 500 ha, on the border of NSW and Qld. The trees were 10–13 years old, and mixed cultivars Frantoio, Manzanillo and Corregiola, with a number of trees missing or dead. The trial was conducted in a large block of cv. Frantoio in the middle of the grove. Trees were approximately 3.0–3.5 m high and lightly foliaged, as a result of olive lace bug attack and probably prior lack of water. The experiment had four blocks, each comprising an area of approximately 1000 m^2, with varying numbers of trees. Olive lace bug infestation was very high. Treatment replicates were individual trees, separated by a minimum of 15 m and at least one buffer tree.

Treatments

Table 7. Treatments, Yelarbon trial

Treatment number	Insecticide	Rate g or mL/100 L	Active ingredient	g a.i./100L
1	Actara®	30.0	250g/kg thiamethoxam	7.50
2	Samurai Systemic Insecticide™	40.0	500g/L clothianidin	8.00
3	Lebaycid®	75.0	550g/L fenthion	41.25
4	Water			

Assessments

Assessments were conducted at the commencement of the trial, just prior to spraying on 30 November 2011 and 12 days after the single spray application for all treatments (i.e. 12 December 2011).

One tree per replicate was assessed at four cardinal points. At each point five twigs, 20–30 cm long, were assessed for presence or absence of lace bug. Presence of any lace bug (of any stage) was scored 1, absence was scored 0. The total number of twigs assessed per tree was 20. All stages of olive lace bug (including 1st instar nymphs) were present throughout the trial period.

Spray applications

A diaphragm pump with 50L spray tank was used to apply treatments. Treatments were applied at with a single hollow cone nozzle using hand wand with a single adjustable hollow cone nozzle. The nozzle had an output of 1.2 L/min. Every tree was sprayed to run-off, which equated to 80 sec, and thus, a volume of ~1.6 L of spray applied per tree or at a rate of 625 L/ha (assuming an original planting density of 390 trees/ha). Weather at the time of application (13.00–15.00 AEDT) was 27° C, with no wind.

Statistical analysis

Data were analyzed using SPSS® for Windows™ Version 12 (SPSS Inc. 2003) using the general linear model of analysis of variance (ANOVA). Each variable was visually tested for normality using Q-Q plot and Levene's test was used for testing the assumption of equality of error variance. When the assumptions of normal distribution or of equality of error variance are not met, appropriate transformations were used. Tukey B test was used if data met assumption of equality of error variance and the Games-Howell test was used if the assumption was not met.

Results

There were no significant differences in the number of twigs infested with lace bug between trees assigned to each treatment (mean 18.94 ± 0.266; $F_{3,15} = 0.295$, $P = 0.828$) (Table 8).

2nd assessment, 7 days post-treatment

A summary of the post-treatment counts are presented in Table 8. There was a significant difference between treatments ($F_{3,15} = 201.118$, $P < 0.001$), with insecticide efficacy falling into three groups. Samurai Systemic Insecticide™ (clothianidin) and Actara® (thiomethoxycam) were superior to all other treatments, but not different from each other. Lebaycid® (fenthion) was superior to the Water Control only.

Corrected efficacy calculated using Henderson-Tilton formula for Samurai Systemic Insecticide™ (clothianidin) was 91.15%, for Actara® (thiomethoxycam) was 88.77% and for Lebaycid® (fenthion) was 38.04%.

Table 8. **Pre-treatment and post-treatment counts, number of infested twigs with olive lace bug, Yelarbon trial**

TREATMENT	PRE-TREATMENT COUNT (SE)	POST-TREATMENT COUNT (SE)
Actara® (thiomethoxycam)	19.25 (0.48)	2.25 a (0.63)
Samurai Systemic Insecticide™ (clothianidin)	19.00 (0.41)	1.75 a (0.48)
Lebaycid® (fenthion)	19.00) (0.71)	12.25 b (0.48)
Water	18.50 (0.65)	19.25 c (0.75)

Numbers in columns followed by different letters are significantly different (Tukey B test ($P \leq 0.05$))

Figure 4. Trial site, Yelarbon NSW-Qld border 2010–11

Conclusions

All three insecticidal treatments significantly reduced a heavy infestation of olive lace bug after a single spray (corrected efficacy Samurai Systemic Insecticide™ (clothianidin) 91.2%, Actara® (thiomethoxycam) 88.8%, Lebaycid® (fenthion) 38.0%). However, the first two products were greatly superior to the industry standard, Lebaycid® (fenthion). It can be concluded that Actara® (thiomethoxycam) and Samurai Systemic Insecticide™ (clothianidin) have potential to be used for control of lace bug in olives.

2013 SEASON

FIELD TRIAL 5. Evaluation of insecticides foliar sprays Sumi-Alpha® Flex (esfenvalerate) and Lebaycid® (fenthion) foliar sprays for control of olive lace bug

In 2013, two sites were identified in South-East Queensland to conduct efficacy trials with Sumi-Alpha® Flex (esfenvalerate) against olive lace bug, following discussion between the AOA's Chemical Permits Committee and Sumitomo Chemical Australia Pty Ltd, the manufacturers. A rate of 30 mL/100L was determined as the trial rate. Unfortunately, after conducting a pre-treatment count at one site at Leyburn, Qld 4365, the spray unit broke down and was unavailable for several weeks, by which time the lace bug population was in decline. We abandoned this site, and continued with the other site at Coominya.

Location

Aaron Prior
Comvita Australia Pty Ltd
Bischoffs Road Coominya Qld 4311 (27.38° S, 152.50° E)

Experimental design

Two factorial experiment with insecticide treatment options (2, see description of blocks) being the fixed factor, and block (5 blocks) being the random factor (i.e. 2 × 5). Treatments were organized as randomised complete blocks.

Description of plots and blocks

The experimental block is part of a large grove growing olives for olive leaf extract. As leaves, not fruit are harvested, plants were grown at super-high density, and formed a hedgerow approximately 1.5–2.0 m high and 1.5 m wide, with rows approx. 5 m apart (Figure 5). Trees in the experimental block were 13 years old and of mixed cultivars; there was low to medium olive lace bug pressure. There were insufficient treatment sites (10) in the block with adequate olive lace bug infestation to enable application of a third treatment (Lebaycid® (fenthion), industry standard), as initially planned. So only two treatments were applied, but with five replicates of each (Table 9).

Table 9. Treatments, Coominya trial

Treatment number	Insecticide	Rate g or mL/100 L	Active ingredient	g a.i./100L
1	Sumi-Alpha® Flex (esfenvalerate)[a]	30.0	50g/L esfenvalerate	1.5
2	Water			

[a] Rate recommended by Sumitomo Chemical Australia

Assessments

Assessments were conducted at the commencement of the trial, just prior to spraying on 30 September 2013 and 7 days after the single spray application for all treatments (i.e. 7 October 2013).

Treatments 2.5 m of row were measured and marked at both ends of each, leaving a minimum of 3 m as a buffer. Twenty twigs 10–15 cm long were randomly selected within each treatment replicate. Presence of any lace bug (and of any stage) was scored 1, absence was scored 0. The total number of twigs assessed per replicate was 20.

Spray application

A Croplands diaphragm pump with 50 L spray tank was used to apply treatments. Treatments were applied at a pressure of 300 kPa using a hand wand with a single hollow cone nozzle. The nozzle had an output of 503 mL/min. The time taken to spray 2.5 m of hedgerow to run-off was 3 min, so the application rate was ~1.5 L per 2.5 m row. The application rate equated to ~1200 L/ha. Weather at the time of application (13.00-15.00) was 29°C, with no wind.

Statistical analysis

Data was analyzed using SPSS® for Windows™ Version 12 (SPSS Inc. 2003). All was analysed using the general linear model of analysis of variance (ANOVA). Each variable was visually tested for normality using Q-Q plot and Levene's test was used for testing the assumption of equality of error variance. When the assumptions of normal distribution or of equality of error variance are not met, appropriate transformations will be used. As there were only two treatments, no *post-hoc* assessments were required.

Table 10. **Pre-treatment and post-treatment counts, number of infested twigs with olive lace bug, Coominya trial**

TREATMENT	PRE-TREATMENT COUNT (SE)	POST-TREATMENT COUNT (SE)
Sumi-Alpha® Flex (esfenvalerate)	10.00 (0.91)	1.00 b (0.71)
Water	11.00 (2.04)	13.00 a (1.87)

Numbers in columns followed by different letters are significantly different ($P \leq 0.05$)

There were no significant differences in the number of twigs infested with lace bug between trees assigned to each treatment (mean 10.50 ±1.05; $F_{1,7} = 0.200$, $P = 0.670$) (Table 10).

2nd assessment, 7 days post-treatment

A summary of the post-treatment counts are presented in Table 10. Sumi-Alpha® Flex (esfenvalerate) was significantly superior to the Water Control ($F_{1,7} = 36.000$, $P = 0.001$).

Corrected efficacy calculated using Henderson-Tilton formula for Sumi-Alpha® Flex (esfenvalerate) was 91.54%.

Figure 5. Trial site, Coominya Qld 2013-2014

Conclusions

Sumi-Alpha® Flex (esfenvalerate) significantly reduced an infestation of olive lace bug after a single spray compared to a Water Control, although there was no comparative treatment with the industry standard, Lebaycid® (fenthion). It can be concluded that Sumi-Alpha® Flex (esfenvalerate) has potential to be used for control of lace bug in olives.

PART 2. EVALUATION OF FUNGICIDES FOR CONTROL OF OLIVE ANTHRACNOSE

Introduction

Anthracnose is considered the most important disease of olive fruit worldwide (Talhinhas et al. 2005; Morel et al. 2008). It is caused by various species of the fungal pathogen *Colletotrichum* spp. In Australia, anthracnose has been reported or diagnosed in all olive growing states, including Tasmania, but is more prevalent in the summer-dominant rainfall regions (Sergeeva et al. 2011). As with other crops, anthracnose fungi infect the fruit cuticle and remain latent until they mature, causing yield losses from pre- and post-harvest rots, most conspicuously as a 'soapy fruit' rot (Talhinhas et al. 2011). Anthracnose in olives also increases free fatty acid levels contributing to reduced oil quality (Iannota et al. 1999; Carvalho et al. 2004).

Colletotrichum species can also infect olive flowers (Sergeeva et al. 2008a; Morel et al. 2009) causing their abortion or that of developing fruit. They can also cause leaf spots (Sergeeva et al. 2008b) and infect other vegetative organs such as shoots, leaves and branches. These and mummified fruit remaining on trees from the previous season act as sources for infection (Morel & Trapero 2012). Secondary infection of ripening fruit occurs during periods of wet weather. In Spain, disease severity has been modelled as a function of temperature (10–25^{o}C; infection greatest at 17^{o} C) and wetness duration (increasing from 0–48h) (Morel et al., 2012). Higher olive planting densities also favour faster disease progress through the grove (Morel et al. 2012). Finally, cultivars have been shown to vary in their susceptibility to anthracnose disease (Morel et al. 2008; Morel & Trapero 2009). Relative susceptibility of different cultivars grown in Australia has not been previously studied, hence a preliminary investigation was undertaken in this project, comparing results with those published in European reports cited above. In particular, our study included cv. Barnea which was not tested in the European studies but is widely grown in Australia and observed to be very susceptible to anthracnose.

The genus *Colletotrichum* has undergone significant revisions in recent years based upon morphological and molecular characters. *C. acutatum* has been divided into several species that include newly resolved species *C. simmondsii* (Shivas & Tan 2009) and *C. clavatum* (Faedda et al. 2011) that also cause olive anthracnose. This latter and newly described species was reported as the primary cause of anthracnose of Italian olives. Similarly, *C. gloeosporioides* has undergone a major revision (Weir et al. 2012) based upon multi-gene phylogenies (8 nuclear gene regions) and some Australian olive isolates have been designated as *C. kahawae* subsp. *ciggaro* and *C. theobromicola*. With these revisions and renaming of species and sub-species there are still cases where apparently clear differences in pathogenicity of isolates are not reflected genetically. In simple terms, these phylogenetic divisions delineating newly named species tell us something of the broad parentage of isolates but not always much of their biology. This will only be resolved with more studies that gather both genetic and biological (particularly pathogenicity) data for individual isolates. Therefore in light of these recent advances in *Colletotrichum* spp. taxonomy it was desirable that fungal isolates collected during this project and those used in detached fruit assays be genetically characterised to determine their species or sub-species identity.

At the commencement of the project, the Australian olive industry had limited access to chemical controls for anthracnose. This component of the project aimed to compare the efficacy of different copper formulations currently being used by the Australian olive industry as well as testing chemicals from the strobilurin group of fungicides with systemic (azoxystrobin) and/or curative properties (azoxystrobin & pyraclostrobin). The pyraclostrobin was formulated with the dithiocarbamate fungicide metiram in the product, Aero® Fungicide. Curative and systemic properties of a fungicide are desirable for the Australian olive industry since they may potentially be used to reduce both abortion of flowers and young fruit, and secondary spread during ripening.

A total of five anthracnose fungicide efficacy field trials were conducted during the project. These were conducted in the Hunter Valley NSW (1 trial), Coonalpyn SA (2 trials) and Boort Vic (2 trials) (see Table 11).

In addition, a series of four laboratory-based detached olive fruit assays further assessed fungicide efficacy and determined relative susceptibility of fruit from different olive cultivars to anthracnose caused by *Colletotrichum acutatum*.

Table 11. Description of field trials evaluating fungicides against olive anthracnose

Season	Location	Fungicides tested
2009–2010 Feb–March 2010	Lovedale, Hunter Valley NSW	Aero® Fungicide (pyraclostrobin + metiram), Nufarm Tri-base Blue® flowable copper fungicide, Copper oxychloride, NorShield 750 WP copper fungicide
2010–2011 Dec 2010–June 2011	Coonalpyn SA	Aero® Fungicide (pyraclostrobin + metiram), Nufarm Tri-base Blue® flowable copper fungicide, Copper oxychloride, NorShield 750 WP copper fungicide
2010–2011 Dec 2010–May 2011	Boort Vic	Aero® Fungicide (pyraclostrobin + metiram), Amistar® (azoxystrobin), Nufarm Tri-base Blue® flowable copper fungicide, NorShield 750 WP copper fungicide
2011–2012 Nov–Dec 2011	Coonalpyn SA	Aero® Fungicide (pyraclostrobin + metiram), Amistar® (azoxystrobin), Nufarm Tri-base Blue® flowable copper fungicide, NorShield 750 WP copper fungicide
2011–2012	Boort Vic	Aero® Fungicide (pyraclostrobin + metiram), Amistar® (azoxystrobin), Nufarm Tri-base Blue® flowable copper fungicide, NorShield 750 WP copper fungicide

Fungicides evaluated against anthracnose

Aero® Fungicide (active constituent pyraclostrobin (chemical group 11) 50 g/kg, and metiram (chemical group M3) 550 g/kg) water dispensible granule, Nufarm Australia Pty Ltd. Aero® Fungicide is registered in Australia for use against a number of fungal pathogens, including anthracnose, in a range of horticultural crops.

Amistar® (active constituent azoxystrobin (chemical group 11) 250 g/L) suspension concentrate, Syngenta Australia Pty Ltd. At the commencement of this project, Amistar® was registered in Australia for use against a number of fungal pathogens, including anthracnose, in a range of horticultural crops, did not have permitted minor use in olives against anthracnose because a previous permit (PER 8276 had expired in June 2006). However, during the project subsequent permits were granted (PER

12610, Dec 2010–June 2011; PER 13174, Nov 2011–March 2013), and an extant permit PER 14550 expires in March 2015.

Barmac copper oxychloride fungicide (active constituent copper (Cu) present as copper oxychloride 500 g/kg) Barmac Industries Pty Ltd, was registered for use against a range of fungal and bacterial diseases in fruit, vegetable and ornamental crops. Copper oxychloride fungicides have had permitted minor use in olives against anthracnose since 2001 via permits PER 3839, PER 6463, PER 8586, and in the extant permit PER 11360.

Nufarm Tri-base Blue® flowable copper fungicide (active constituent copper (Cu) present as tribasic copper sulphate 190g/L), Nufarm Australia Pty Ltd. Nufarm Tri-base Blue® is registered for a range of fungal and bacterial diseases on fruit, nut, vegetable crops and ornamentals. At the commencement of this project, this product did not have permitted minor use in olives against anthracnose although permits for copper oxychloride and copper hydroxide had been previously granted. Subsequently, as a result of its assessment in this project, it was included in PER 11360 which permits the three formulations of copper, and which expires in March 2017.

NorShield 750 WP copper fungicide (active constituent copper (Cu) present as cuprous oxide 750 g/kg) Yara NiproPty Ltd, is registered for use against a range of diseases in horticultural crops. At the commencement of this project, this product did not have permitted minor use in olives against anthracnose although permits for copper oxychloride and copper hydroxide had been previously granted. Subsequently, fungicides containing cuprous oxide were included in PER 11360. NorShield 750 WP copper fungicide is no longer available, although products with similar specifications are registered by the APVMA.

2009-2010 SEASON

FIELD TRIAL 1. Evaluation of fungicides Aero® Fungicide (pyraclostrobin + metiram), Nufarm Tri-base Blue® flowable copper fungicide, NorShield 750 WP copper fungicide and copper oxychloride for control of olive anthracnose

Location

Swish Wine
113 Wilderness Road Lovedale
Hunter Valley NSW 2320 (32.46° S, 150.77° E)

Experiment site

The experimental grove comprised 160 trees: 10 year old cv. Manzanillo, as part of a much larger, multi-cultivar planting. The trees had been pruned in the previous season, and were approximately 2.5 m in height, and in light bearing (Figure 6). Only trees with sufficient set fruit were included in the trial.

Figure 6. Trial site, Lovedale NSW 2009–2010

Experimental design and treatments

A two factorial experimental design was used with fungicides (4 treatments) being the fixed factor and block (4 blocks) being the random factor (i.e. 4 × 4). Treatments were organized as randomised complete blocks.

The experiment comprised four blocks, each with 24 trees although many of the trees had insufficient fruit to be included in the trial. One tree containing sufficient fruit was used as the assessment tree for each treatment (i.e. a total four trees for each treatment). Thus, there were a total of 16 experimental trees. These trees were well buffered by non-sprayed trees, to reduce the risk of spray cross-contamination.

Fungicide application rates and treatment codes are listed in Table 12. Sprays were applied from early-mid fruiting until harvest (full black stage). Treatments were applied using a Hardi wheelbarrow sprayer fitted with 100 L tank, a 2.5 kW Honda petrol engine and an extension wand with 1 solid cone nozzle 1.8 mm diameter. Sprays were applied at the pressure of 800 kPa to the point of run-off at 3.2 L/tree (= 896 L/ha). Treatments were applied on 17 February 2010 between 07.30 and 10.00 (AEDT), and on 15 March 2010 between 07.30 and 09.30 (AEDT). There was no wind and temperatures were in the mid 20° Cs at the time of spraying.

Table 12. Treatments, Lovedale trial

Treatment Number	Fungicide	Rate g or mL/100 L	Active ingredient	g a.i./100 L
1	Nufarm Tri-base Blue® flowable copper fungicide	400	Tribasic copper sulphate	76.0
2	Aero® Fungicide	200	Metiram Pyraclostrobin	110 10.0
3	Barmac copper oxychloride fungicide	400	Copper oxychloride	200
4	NorShield 750 WP copper fungicide	112	Cuprous oxide	84.1
5	Water			

Assessments

Fruit samples were collected in the grove at the commencement of the trial and 14 days after the last spray. Fifty fruit were randomly collected from trees in the experimental grove at the time of initiating the trial (10 February 2010), to determine background incidence of latent anthracnose infection. Sampled fruit were returned to the UWS plant pathology laboratory, where they were placed in a humid environment

and kept under conditions of 25° C for 4 days, then examined under an illuminated Maggi lamp at 10× magnification for presence of anthracnose.

An additional sample of 50 fruit was collected from non-experimental trees in the trial site on 15 March, 2010, prior to application of the second set of treatments to assess the background level of infection in non-sprayed trees.

The trial was terminated on 31 March 2010, as fruit were fully ripe and ready for harvest. All fruit were harvested from all treatment trees and placed separately into labelled plastic bags. The bags were placed into a cooled esky, returned to the UWS laboratory, and placed in a refrigerator overnight. The next morning, a random sample of up to 100 fruit (depending on the number of fruit in each replicate) from each replicate was removed and visually examined for presence/absence of visible signs of anthracnose. The percentage of the fruit with symptoms of anthracnose was calculated for each replicate.

In addition, a representative sample of fruit from each of the treatments was sent to the Oil Testing Service, NSW DPI Wagga Wagga for oil extraction and analysis, as anthracnose negatively impacts on the quality of oil produced from infected fruit.

Statistical analysis

Data of fruit infection at harvest was analysed using general linear model of analysis of variance (ANOVA; SPSS® for Windows™ Version 18). The data were visually tested for normality using a Q-Q plot and Levene's test was used for testing the assumption of equality of error variance. Data at all assessment times approached normal distribution and the assumption of equality of variance was met, so Tukey B test was used to compare treatment means. Significance was assessed at $P \leq 0.05$.

Results

No anthracnose was recorded from any fruit sampled at the commencement of the trial or before the second spray, suggesting that latent infection was very low. At harvest, there was a very high level of incidence of anthracnose visible on fruit in the nil chemical control treatment trees, with many fruit falling.

Treatment means of percentage infected fruit (± SE) at harvest are presented in Figure 7. There were significant differences between treatments in percentage of infected fruit ($F_{3, 12} = 5.255$, $P = 0.15$). The treatments fell into three groups. Aero® Fungicide (pyraclostrobin + metiram) significantly reduced fruit infection compared to copper oxychloride and the Water Control. Nufarm Tri-base Blue® flowable copper fungicide had significantly less percentage infected fruit than the Water Control. There were no other differences between treatments.

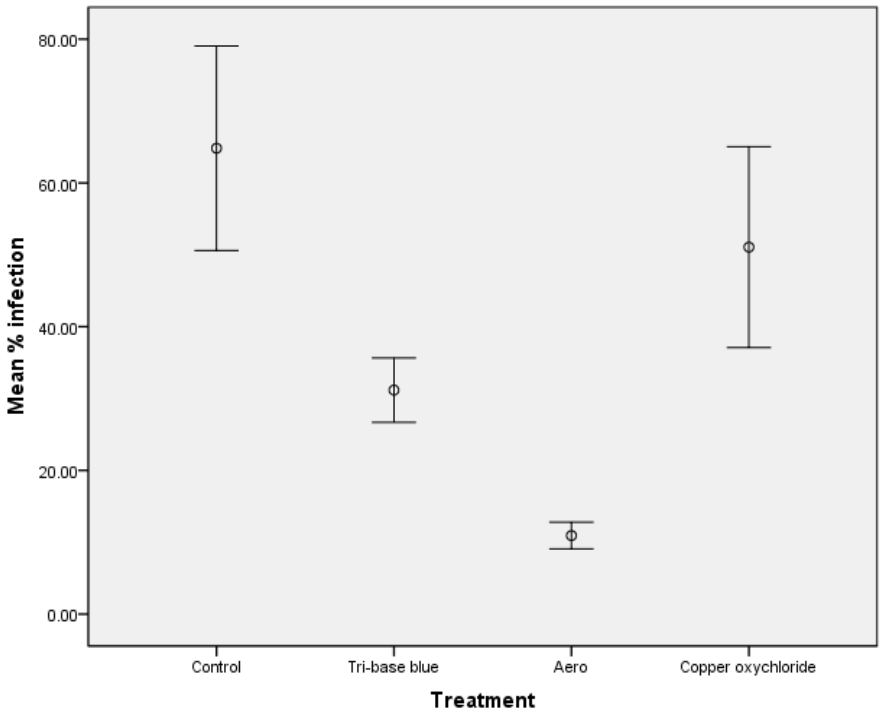


Figure 7. Mean % anthracnose infection in cultivar Manzanillo fruit of three fungicide treatments and Water Control, following 2 applications. Bars represent SE of the mean

Oil quality

The data on olive oil quality from the different treatments are provided in Appendix 2. Oil from the Aero® Fungicide (pyraclostrobin + metiram) treatment had by far the lowest free fatty acid levels at 1.7, compared with Nufarm Tri-base Blue® flowable copper fungicide at 14.6 and the Water Control at 27.9. These differences are consistent with the observed relative percentages of fruit infestation. However, all of these values are well in excess of the requirement for Extra Virgin Olive Oil (EVOO). With regard to Peroxide Value, the levels were similar in all treatments, and were within the acceptable range for EVOO.

Conclusions

Two applications of Aero® Fungicide (pyraclostrobin + metiram) resulted in significantly less infected fruit than the industry standard, copper oxychloride. Nufarm Tri-base Blue® flowable copper fungicide had significantly less infected fruit than the Water Control, demonstrating its efficacy against the anthracnose pathogen. In this trial, the industry standard, copper oxychloride, did not significantly reduce fruit infection compared with the Water Control. Higher levels of anthracnose present (thus, the treatments) impacted negatively on oil quality. These results confirm the substantial reduction in oil quality which can result from anthracnose infection of fruit in the field, even when fungicides are applied. This trial also demonstrates that two applications of copper-based fungicides may be insufficient to substantially reduce anthracnose infection in olive fruit in years with severe pathogen pressure, where fruit losses in untreated trees can approach 100%.

2010-2011 SEASON

FIELD TRIAL 2. Evaluation of fungicides Aero® Fungicide (pyraclostrobin + metiram), Nufarm Tri-base Blue® flowable copper fungicide, NorShield 750 WP copper fungicide and copper oxychloride for control of olive anthracnose

Location

Justin Brown
Coonalpyn Olives
Coonalpyn South Australia (35.68° S, 139.85° E)

Experiment site

The experimental grove comprised 160 trees (cv. Manzanillo) that were 10 years old and planted in a single row. They formed part of a larger, multi-cultivar planting of 210 ha. Trees were planted as an equivalent of 380/ha. There had been a history of anthracnose infection in the experimental grove, and there was evidence of mummified fruit on trees and on the ground from the previous season's infected fruit. These were expected to provide sufficient fungal inoculum and disease pressure for the experiment. At the time of the trial's commencement trees were approximately 3.0–3.5 m in height and had recently set fruit at medium-good bearing. Only trees with sufficient set fruit were included in the trial (Figure 8).

Figure 8. Trial site, Coonalpyn SA 2010–11

Experimental design & treatments

The experiment had four blocks, with three trees comprising a treatment, and treatments were organized into randomised complete blocks. Fruit were sampled from the middle tree in each treatment set, the others being buffers to minimise risk of fungicide spray cross-contamination.

Fungicide application rates and treatment codes are listed in Table 13. Treatments were applied using a hand wand from a 2.5 kW Honda petrol engine and an extension wand with a flow-jet hollow cone nozzle. Treatments were applied on 22 December 2010 between 08.00 and 11.00 (AEDT), 20 February between 08.00 and 11.00 (AEDT) and 7 April 2011 between 08.30 and 11.00. There was little wind and temperatures were between 13–23° C at the time of spraying. Sprays were applied at a pressure of 550 kPa at the rate of 1.5 L/min, to the point of run-off, and the rate was 2.7, 2.9 and 2.9 L/tree, respectively (viz. 1026 and ~1100 L/ha, respectively).

Table 13. Treatments, Coonalpyn trial 1

Treatment Number	Fungicide	Rate g or mL/100 L	Active ingredient	g a.i./100 L
1	Nufarm Tri-base Blue® flowable copper fungicide	400	Tribasic copper sulphate	76.0
2	Aero® Fungicide	200	Metiram Pyraclostrobin	110.0 10.0
3	Copper oxychloride	400	Copper oxychloride	200
4	NorShield 750 WP copper fungicide	105	Cuprous oxide	78.8
5	Water			

Assessments

Fruit were sampled from all treatments at harvest and placed separately into labelled plastic bags on 17 June 2011. The bags were placed into an esky, and returned to the UWS laboratory, and placed in a refrigerated room (10° C) overnight. The following day, a random sample of up to 100 fruit (depending on the number of fruit in each replicate) from each replicate was removed and visually examined as previously described for the presence of anthracnose symptoms. Fruit were scored for presence/absence of visible signs of anthracnose, and the percentage of the fruit with symptoms of anthracnose was calculated for each replicate.

Samples (non-replicated) from each treatment were also taken three times during the trial, 12 February, 5 April and 10 June 2011 to monitor levels of fruit infection during the trial period.

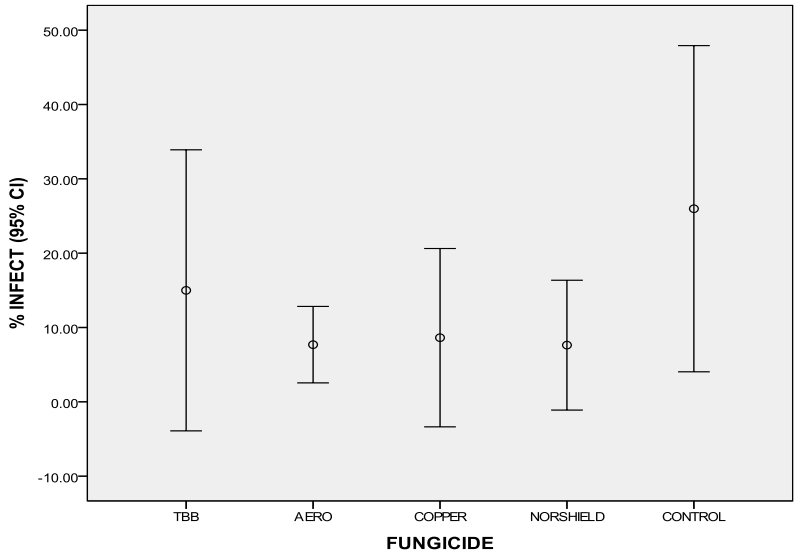
In addition, a representative sample of fruit from each of the treatments from the final sampling date was sent to Modern Olives Laboratory Services, Lara Victoria for oil extraction and analysis.

Statistical analysis

Data of fruit infection at harvest was analysed using general linear model of analysis of variance (ANOVA) (SPSS® for Windows™ Version 18). The data was visually tested for normality using Q-Q plot and Levene's test was used for testing the assumption of equality of error variance. Data at all assessment times approached normal distribution and the assumption of equality of variance was met. Tukey B test was used to compare treatment means. Significance was assessed at $P \leq 0.05$.

Results

There was no significant difference between treatments in percentage of infected fruit at harvest, although there was a trend ($P_{4,16} = 0.07$) towards the control having a higher proportion of fruit infected than other treatments. Treatment means of percentage infected fruit (± SE) are presented in Figure 9.



Bars represent 95% confidence limits

Figure 9. Mean % anthracnose infection in cultivar Manzanillo fruit in four fungicide treatments and Water Control, following 3 applications of each between 22 December 2010 and 7 April 2011, 10 days after the last application

The percentages of infected fruit from the three samples taken during the trial period are presented in Table 14. Infestations varied between sample dates, with young infected fruit aborting, resulting in a much lower level of field infection in the April sample in all treatments. By early-June, however, ideal conditions for anthracnose

(rain and cool weather) combined with an overwhelming inoculum levels from secondary infection resulted in very high scores in fruit from all treatments (44–93%).

Table 14. Percentage levels of infection in fruit sampled from the trial site, Coonalpyn 2011

DATE OF SAMPLE	TREATMENT	% INFECTION
12-2-2011	Nufarm Tri-base Blue® flowable copper fungicide	36
	Aero® Fungicide (pyraclostrobin + metiram)	16
	Copper oxychloride	4
	NorShield 750 WP copper fungicide	4
	Control	20
5-4-2011	Nufarm Tri-base Blue® flowable copper fungicide	2
	Aero® Fungicide (pyraclostrobin + metiram)	2
	Copper oxychloride	0
	NorShield 750 WP copper fungicide	0
	Control	0
10-6-2011	Nufarm Tri-base Blue® flowable copper fungicide	93
	Aero® Fungicide (pyraclostrobin + metiram)	44
	Copper oxychloride	74
	NorShield 750 WP copper fungicide	93
	Control	89

Oil quality

The data on olive oil quality from the different treatments are provided in Appendix 3. Oil from the Aero® Fungicide (pyraclostrobin + metiram) treatment had by far the lowest Free Fatty Acid (FFA) levels at 0.44, which was the only FFA value below the

limit for Extra Virgin Olive Oil (EVOO). Oil from the Nufarm Tri-base Blue® flowable copper fungicide treatment had a FFA value of 0.81, which was at the maximum permitted limit for EVOO. Oil from all of the other treatments had FFA value in excess of the requirements for being classified as EVOO. With regard to Peroxide Value, however, the levels were similar in all treatments, and were well within the acceptable range for EVOO.

Conclusions

The lack of significant difference between fungicide treatments, even when applied three times during the season is likely to be due to high disease pressure and the large variation in fruit infection between the single sample trees, confounding any potential for chemical efficacy. This is particularly evident in the Water Control and Nufarm Tri-base Blue® flowable copper fungicide treatments, where confidence limits range approximately 40–50% of the mean. Interestingly, the Aero® Fungicide (pyraclostrobin + metiram) treatment resulted in more consistent infection levels and the confidence limits are much tighter (Figure 9).

This season was the worst historic year for anthracnose in olives in South Australia, and many commercial crops were unable to be harvested because of anthracnose severity and wet soils. The conclusion from this result is that in severe anthracnose years, especially with susceptible cultivars, no fungicide treatments may effectively manage this disease.

FIELD TRIAL 3. Evaluation of fungicides Aero® Fungicide (pyraclostrobin + metiram), Amistar® (azoxystrobin), Nufarm Tri-base Blue® flowable copper fungicide and NorShield 750 WP copper fungicide for control of olive anthracnose

Location

Boundary Bend Olives
Boort Victoria 3537 (36.10° S, 143.72° E)

Experimental site

The experimental grove comprised of 12 year old trees of cv. Barnea, as part of a much larger, multi-cultivar planting (2,716 hectares). There had been a history of anthracnose infection in the experimental grove area, and there was evidence of mummified fruit on trees and the ground from the previous season's infected fruit. Therefore it was expected there would be carry-over fungal inoculum. The trees were approximately 4 m in height, and had recently flowered and set fruit.

Experimental design and treatments

The experiment comprised of four blocks with a row of trees forming each treatment replicate thereby providing a two factorial experimental design with fungicides (5 treatments) being the fixed factor and block (4 blocks) being the random factor (i.e. 5 × 4). Treatments were organized as randomised complete blocks. Each treatment row was separated from other treatments by a minimum of two rows of buffer trees. Trees were planted at a density of 380 trees/ha.

Fungicide application rates and treatment codes are listed in Table 15.

It was anticipated that samples would be collected at harvest. However, there was extensive rain and flooding on the grove (see Figure 10), and the trial site was inaccessible for more than 8–10 weeks from January to March, a critical time for the trial treatments to be applied. It was during this time that anthracnose became rampant, and most of the olive fruit, especially in the lower half of trees, became infected and abscised. Continuing heavy rain also prevented or delayed application of subsequent timed treatments on the trial site.

Table 15. Fungicide treatments applied in Field Trials 3 & 4

Treatment Number	Fungicide	Rate g or mL/100 L	Active ingredient	g a.i./100 L
1	Nufarm Tri-base Blue® flowable copper fungicide	400	Tribasic copper sulphate	76.0
2	Aero® Fungicide (pyraclostrobin + metiram)	200	Metiram Pyraclostrobin	110 10.0
3	Amistar® (azoxystrobin)	80	Azoxystrobin	20.0
4	NorShield 750 WP copper fungicide	112	Cuprous oxide	84.1
5	Water			

Figure 10. Flooding in part of the Boort olive grove, January 2011

Assessment

When possible, treatments were applied with an airblast sprayer at a rate of 1000 L/ha. Three applications of the treatments were made: on 23 December 2010, 21 January 2011 and 12 April 2011. At harvest, on 23 May 2011, few fruit remained on any trees, and most of the remaining fruit showed symptoms of infection or were becoming desiccated. As it was impossible to collect meaningful fruit infestation or yield data as occurred at other trial sites, it was determined that the best option would be to randomly collect a sample of fruit from a minimum of 20 trees in the middle of each treatment block, and weigh a subsample of 200 fruit.

In addition, a representative sample of fruit from each of the treatments was taken at harvest and sent to Modern Olives Laboratory Services, Lara Victoria for oil extraction and analysis.

Results

As reported above, no fruit infection data were obtained for this trial, because of waterlogging and flooding at the field site.

However, the mean weights of the subsamples of 200 fruit from each treatment are presented in Table 16. The mean weight of 200 fruit was highest for Aero® Fungicide (pyraclostrobin + metiram), followed by Nufarm Tri-base Blue® flowable copper fungicide, with Aero® Fungicide (pyraclostrobin + metiram) ranking first in three of the four blocks. However, there were no significant differences in fruit weight ($F_{4,16}$ = 1.2760, P = 0.323) between any of the treatments (Table 16).

Table 16. Mean weight of 200 fruit from anthracnose fungicide treatments, Boort

TREATMENT	MEAN WEIGHT OF 200 FRUIT (g) (SE)
Aero® Fungicide (pyraclostrobin + metiram)	503.8 (47.1)
Amistar® (azoxystrobin)	472.8 (15.7)
NorShield 750 WP copper fungicide	455.0 (44.0)
Nufarm Tri-base Blue® flowable copper fungicide	457.0 (23.6)
Control	404.3 (11.7)

Oil quality

The data on olive oil quality from the different treatments are provided in Appendix 4. Oil from the Aero® Fungicide (pyraclostrobin + metiram) treatment had by far the lowest free fatty acid (FFA) levels at 0.44, which was the only FFA value below the limit for Extra Virgin Olive Oil (EVOO). Oil from the Nufarm Tri-base Blue® flowable copper fungicide treatment had a FFA value of 0.81, which was at the maximum permitted limit for EVOO. Oil from all of the other treatments had FFA value in excess of the requirements for being classified as EVOO. With regard to Peroxide Value, however, the levels were similar in all treatments, and were well within the acceptable range for EVOO.

Conclusions

As previously discussed for Trial 2 in Coonalpyn SA, this season was the worst historic year for anthracnose in olives in Victoria, with only a proportion of the grove at Boort able to be harvested, and even when harvest took place, the oil quality was poor, primarily because of anthracnose infection. Many trees also suffered from waterlogging and a substantial number did not recover. We were unable to apply the fungicide treatments for much of the trial period and consequently were unable to score treatments for infection levels, and oil quality was very poor. This result confirms our conclusion from the Coonalpyn trial that in a severe anthracnose year, with high inoculum levels and weather conditions conducive to infection and spread, particularly in susceptible olive cultivars, it is unlikely that applications of any fungicides, no matter how efficacious, will be able to manage the disease.

2011-2012 SEASON

FIELD TRIAL 4. Evaluation of fungicides Aero® Fungicide (pyraclostrobin + metiram), Amistar® (azoxystrobin), Nufarm Tri-base Blue® flowable copper fungicide and NorShield 750 WP copper fungicide for control of olive anthracnose

Location
Boundary Bend Olives
Boort, Victoria 3537 (36.10° S, 143.72° E)

Experimental site

The experimental grove comprised 13 year old cv. Barnea, as part of a much larger, multi-cultivar planting (2,716 hectares). As with Trial 3, there had been a history of anthracnose infection in the experimental grove area with evidence of mummified fruit on trees and the ground from the previous season's infected fruit. Therefore it was expected there would be carry-over fungal inoculum. The trees were approximately 4 m in height, and had recently flowered and set fruit.

Experimental design and treatments

This was a two factorial experiment with fungicide treatment options (5 treatments) being the fixed factor, and block (6 blocks) being the random factor (i.e. 25× 6). Treatments were organized as randomised complete blocks. The experiment comprised of six blocks in three rows of trees. Three consecutive trees formed a treatment replicate. All treatments were separated by at least three trees within the row and a buffer row between treatment rows. Trees were planted at a density of 380 trees/ha.

The fungicide application rates and treatment codes were as listed in Table 15 since this was a repeat of the previous year's problematic trial (Field Trial 3). However, in this case, treatments were applied with a Silvan diaphragm pump and 100L spray tank, connected to a hand wand with a single hollow cone ceramic nozzle. It was calibrated to provide an output of 2.3 L/min at each time treatments were applied. Treatments were applied at young fruit set on 24 November 2011 between 14.00 and 18.00 (temp. 21° C, no wind), and at fruit colouring on 10 April 2012 between 11.00–14.00 (temp. 17° C, very light breeze). The weather between November and April was generally hot and dry, and there was no indication of anthracnose infections so there was no requirement for treatment applications between these times. Trees were sprayed to run-off, which was estimated to be 2.9 L/min (~1100 L/ha). Chemical deposits of two copper formulations on leaves and fruit are displayed in Figures 11 and 12.

Assessments

Two hundred fruit were randomly picked from each treatment and scored for visible anthracnose infection at the completion of the trial (9 May 2012). Fruit were then transported back to the NSW DPI Plant Health Diagnostic Laboratory, Menangle and incubated in moist chambers for a further 6 days (= 7 days total) and rescored.

Figure 11. Nufarm Tri-base Blue® flowable copper fungicide fresh residues on fruit and leaves

Figure 12. NorShield 750 WP copper fungicide spray on leaves and fruit immediately following spraying

Statistical analyses

Two analyses of the data were collected. These were:

- analysis of Area Under the Disease Progress Stairs (AUDPS) measure (Simko & Piepho 2011)
- analysis of binomial proportions at each timepoint (viz. 0 and 7 days)

Each analysis involved fitting a mixed model, comprising fixed and random effects. A square root transformation of the AUDPS was applied to reduce variance heterogeneity.

The mixed model analysis was performed using the ASReml package (Gilmour et al., 2009) and all other data manipulation or reporting of the analysis results was performed within the R statistical software environment (R Development Core Team 2012).

Results

AUDPS analysis

There was a significant difference between treatments ($F_{4,20} = 12.35$; $P < 0.001$). There were significant pairwise differences between both Aero® Fungicide (pyraclostrobin + metiram) and Amistar® (azoxystrobin) and the Nufarm Tri-base Blue® flowable copper fungicide and Control treatments (Table 17).

Table 17. Area under disease progress stairs for fungicide treatments, Boort trial 2011-12

TREATMENT	AREA UNDER STAIRS (Square root means)
Aero® Fungicide (pyraclostrobin + metiram)	13.6 c (3.7)
Amistar® (azoxystrobin)	15.4 c (3.9)
NorShield 750 WP copper fungicide	37.5 bc (6.1)
Nufarm Tri-base Blue® flowable copper fungicide	70.2 b (8.4)
Control	174.9 a (13.2)

Numbers in columns followed by different letters are significantly different (LSD square root means = 3.3)

Proportional Analysis

There were significant differences between treatments at both 0 and 7 days ($F_{4,22}$ = 5.222, P = 0.004; $F_{4,20}$ = 11.620, P < 0.001, respectively). For both sampling dates all treatments except NorShield 750 WP copper fungicide were significantly superior to the Control. At day 7, there were other significant differences between treatments, with Aero® Fungicide (pyraclostrobin + metiram) and Amistar® (azoxystrobin) superior to Nufarm Tri-base Blue® flowable copper fungicide. There were no other differences between treatments.

Table 18. Predicted back-transformed means from the proportions analysis at each timepoint. Numbers in columns followed by different letters are significantly different ($P \leq 0.05$)

TREATMENT	PROPORTION OF INFECTED FRUIT	
	DAY 0	DAY 7
Aero® Fungicide (pyraclostrobin + metiram)	0.001 a	0.009 a
Amistar® (azoxystrobin)	0.006 ab	0.012 a
NorShield 750 WP copper fungicide	0.010 ab	0.023 ab
Nufarm Tri-base Blue® flowable copper fungicide	0.013 bc	0.044 b
Control	0.032 c	0.104 c

Conclusions

All chemical treatments significantly reduced the proportion of infected fruit compared with the Water Only Control. Aero® Fungicide (pyraclostrobin + metiram) and Amistar® (azoxystrobin) treatments were mostly superior to the copper products.

FIELD TRIAL 5. Evaluation of fungicides Aero® Fungicide (pyraclostrobin + metiram), Amistar® (azoxystrobin), Nufarm Tri-base Blue® flowable copper fungicide and NorShield 750 WP copper fungicide for control of olive anthracnose

Location

Steve Head
Coonalpyn Olives
Coonalpyn South Australia (35.68° S, 139.85° E)

Experimental site

The trial comprised of 11 year old trees cv. Manzanillo that formed part of a larger, multi-cultivar planting of 260 ha. As with previous trials, there had been a history of anthracnose infection in the experimental grove. However, this trial was conducted in a separate block to that used in Field Trial 2.

Experimental design and treatments

The trial area was divided into five blocks in two rows of trees. An individual tree formed each treatment unit or replicate. Trees with adequate set fruit were selected for fungicide treatment and were always separated by buffer trees. Fungicides treatments were as listed in Table 19 and were randomised within blocks.

Table 19. Fungicide treatments applied in Coonalpyn trial 2

Treatment Number	Fungicide	Rate g or mL/100 L	Active ingredient	g a.i./100 L
1	Nufarm Tri-base Blue® flowable copper fungicide	400	Tribasic copper sulphate	76.0
2	Aero®	200	Metiram Pyraclostrobin	110 10.0
3	Amistar®	80	Azoxystrobin	20.0
4	NorShield 750 WP copper fungicide	112	Cuprous oxide	84.1
5	Water			

Assessments

One hundred fruit were randomly picked from each treatment and scored for visible anthracnose infection at the completion of the trial (11 May 2012, Figure 13). Fruit

were then transported back to the NSW DPI Plant Health Diagnostic Laboratory, Menangle and incubated in moist chambers for a further 4 days (= 5 days) and rescored.

Statistical analysis

Two analyses of the data were collected. These were:

- analysis of Area Under the Disease Progress Stairs (AUDPS) measure (Simko & Piepho 2011)
- analysis of binomial proportions at each timepoint (viz. 0 and 5 days)

Each analysis involved fitting a mixed model, comprising fixed and random effects. A square root transformation of the AUDPS was applied to reduce variance heterogeneity.

The mixed model analysis was performed using the ASReml package (Gilmour et al. 2009) and all other data manipulation or reporting of the analysis results was performed within the R statistical software environment (R Development Core Team 2012).

Figure 13. Scoring fruit samples for anthracnose infection

Results

AUDPS analysis

There was a significant difference between treatments ($F_{4,16} = 6.684$; $P = 0.002$). All fungicide treatments were superior to the Control but were not different from each other (Table 20).

Table 20. Area under disease progress stairs for fungicide treatments, Coonalpyn trial 2

TREATMENT	AREA UNDER STAIRS (Square root means)
Aero® Fungicide (pyraclostrobin + metiram)	0.8 a (0.9)
Amistar® (azoxystrobin)	2.7 a (1.6)
NorShield 750 WP copper fungicide	9.3 a (3.0)
Nufarm Tri-base Blue® flowable copper fungicide	4.9 a (2.2)
Control	37.6 b (6.1)

Numbers in columns followed by different letters are significantly different ($P \leq 0.05$; LSD square root means = 2.4)

Proportional Analysis

Fungicide treatments were not significant at Day 0 ($F_{4,20} = 1.527$, $P = 0.232$), but there were significant differences between treatments at Day 5 ($F_{4,17} = 7.191$, $P = 0.001$) (Table 21). Aero® Fungicide (pyraclostrobin + metiram) was superior to all treatments except Amistar® (azoxystrobin) and Nufarm Tri-base Blue® flowable copper fungicide, and all fungicides were superior to the Control (Table 21).

Table 21. Predicted back-transformed means from the proportions analysis at each timepoint

TREATMENT	PROPORTION OF INFECTED FRUIT	
	DAY 0	DAY 5
Aero® Fungicide (pyraclostrobin + metiram)	0.002 a	0.004 a
Amistar® (azoxystrobin)	0.004 a	0.009 ab
NorShield 750 WP copper fungicide	0.010 a	0.030 b
Nufarm Tri-base Blue® flowable copper fungicide	0.002 a	0.019 ab
Control	0.014 a	0.087 c

Letters following numbers in each column indicate significant differences at 5% level ($P \leq 0.05$) between treatments (at each timepoint)

Conclusions

Although there were no apparent differences between treatments in anthracnose infection at harvest, incubation and expression of latent infection resolved significant differences. The AUDPS analysis suggests that all the chemical treatments significantly reduced the proportion of diseased fruit while the proportional analysis of the incubated fruit resolved that Aero® Fungicide (pyraclostrobin + metiram) was superior to NorShield 750 WP copper fungicide and the Control.

DETACHED FRUIT ASSAYS

Introduction

Detached fruit assays were used to determine efficacy and persistence of chemical treatments to anthracnose. These assays also provided a convenient means to objectively compare susceptibility of different olive cultivars to anthracnose since they were conducted under controlled environment conditions and with equal pathogen inoculum concentrations.

Materials and Methods

Apparently healthy, ripening olive fruit samples were collected from unsprayed trees on olive groves in Boort, Victoria and Coonalpyn, South Australia and transported to the NSW DPI Plant Health Diagnostic Laboratory, Menangle. Assay methods broadly followed those described by Moral and Trapero (2009) with slight modifications described below.

Detached fruit were sorted into uniform sub-samples of 100 fruit. They were surface-sterilised for 60 seconds in 0.1% sodium hypochlorite, rinsed in sterile water and air-dried in trays. Fruit were then sprayed to runoff with an agrichemical solution (Table 21) using a hand-held atomiser, ensuring the surface was evenly covered with chemical, and set out to dry on a laboratory bench. An aqueous suspension of *C. acutatum* conidia (10^6 conidia/mL) was prepared. A haemocytometer and light microscope were used to estimate spore concentrations and the sprayer was calibrated. The spore suspension (10mL) was then sprayed on fruit with a hand-held atomiser giving in an equivalent of 10^5 conidia/fruit.

Treated fruit were transferred into plastic dishes with forceps. Each dish had a 5×5 grid of walled compartments. Each treatment therefore comprised of 25 fruit, and was replicated four times. Dishes were randomised, placed into incubation trays (22 × 16 × 10 cm) and incubated under fluorescent lights (12 h, 40 $\mu molm^2S^{-1}$, $23^oC \pm 2^oC$). A moistened paper blotter was included in each tray and dishes were inserted into sealable plastic bags to maintain 100% RH.

Fruit were visually assessed for anthracnose symptoms (sunken lesions with orange spore masses) after time intervals (typically at 4–7, 14, 21 days after treatments were applied). Numbers of fruit displaying anthracnose symptoms were recorded and carefully removed from dishes with forceps. Fruit with lesions but no obvious sporulation were examined by light microscopy to confirm conidia, where present, were consistent with *Colletotrichum* spp.

A summary of the four detached fruit assays and treatments is provided in Table 22.

Table 22. Summary of treatments and cultivars used in detached olive fruit assays

		Agrichemical Treatment								
Chemical	Cultivar	Water Control	Water + Pathogen*	Aero® Fungicide (pyraclostrobin + metiram) ® 2 g/L + Pathogen*	Amistar® (azoxystrobin)® 0.8 mL/L + Pathogen*	NorShield 750 WP copper fungicide 1.6 g/L + Pathogen*	Nufarm Tri-base Blue® flowable copper fungicide ® 2.8 mL/L + Pathogen*	Curex® 3[#] 5m L/L + Pathogen*	Cabrio® (pyraclostrobin) 0.4 mL/L + Pathogen *	Polyram® 1.5 g/L + Pathogen*
Experiment										
O_{12-2}	Barnea	+	+	+	+	+	+	-	-	-
O_{12-3}	Barnea Manzanillo	+	+	+	+	+	+	+	-	-
O_{12-4}	Arbequina	+	+	+	+	+	+	-	-	-
O_{13-1}	Barnea, Manzanillo Picual Arbequina	+	+	+	+	+	+	-	+	+

*Pathogen = *C. acutatum* (@10^5 conidia/fruit)

[#] Curex® 3 is a low dose formulation of tribasic copper sulphate + amino acids and was supplied by Nufarm Australia Pty Ltd

Statistical analyses

Data for each trial comprised repeated measures of the cumulative number of fruit diseased in each replicate (out of a total 25 fruit).

Two analyses were conducted for each trial, namely analysis of AUDPS measure and analysis of binomial proportions at each timepoint (as described for Field Trials 4 and 5).

Each analysis involved fitting a mixed model, comprising fixed and random effects. For each AUDPS analysis, the mixed model comprised fixed effects of treatment (and cultivar and cultivar by treatment for O_{12-3}) and random effects of replicate (and cultivar and treatment by replicate for O_{12-3}). A square root transformation of the AUDPS was applied to reduce variance heterogeneity. For the analysis of binomial proportions at each time-point, the mixed model also included random effects of tray (to allow for over-dispersion). Finally, for the combined analysis, the mixed model also included interactions of cultivar and treatment with day, and random effects of replicate by day and tray by time. For the O_{12-3} analysis, an alternative parameterisation was used as well, where the fixed effects included only cultivar and treatment within each cultivar, in order to test for differences between treatments for each cultivar in turn.

Wald type F statistics are presented for each analysis (with a Kenward-Rogers adjustment as implemented in ASReml). For detached fruit assays, the F-statistic excluded the Nil treatment. P-values for pairwise differences between treatments are presented, corrected to control the false discovery rate (FDR) using the method of Benjamini and Hochberg (1995) (rather than to control the family-wise error rate, as in standard multiple comparison approaches such as the Bonferroni or Duncan's multiple range test). For the proportional analyses, p-values were calculated using Wald type t-statistics, except where either mean was 0% or 100%, in which case the p-value was calculated using a simple test of proportions (using the prop-test function in R).

All mixed model analysis was performed using the ASReml package (Gilmour et al. 2009) and all other data manipulation or reporting of the analysis results was performed within the R statistical software environment (R Development Core Team 2012).

Relative susceptibility of olive cultivars to anthracnose was estimated from the proportion of diseased fruit in Pathogen Only treatments in the various experiments.

Detached olive fruit assay 1. $O_{12\text{-}2}$

AUDPS analysis

There was a significant difference between treatments ($F_{4,18} = 14.9$; $P < 0.001$). All fungicide treatments were different to the Control but were not different from each other (Table 23).

Table 23. Area under disease progress stairs back-transformed means for fungicide treatments in detached fruit assay $O_{12\text{-}2}$

TREATMENT	AREA UNDER STAIRS (Square root means)
Aero® Fungicide (pyraclostrobin + metiram)	73.1 a (8.5)
Amistar® (azoxystrobin)	206.5 c (14.4)
NorShield 750 WP copper fungicide	128.1 b (11.3)
Nufarm Tri-base Blue® flowable copper fungicide	234.1 c (15.3)
Control 1 (Pathogen only)	261.1 c (16.2)
Control 2 untreated fruit	129.8 b (11.4)

Numbers in columns followed by different letters are significantly different ($P \leq 0.05$; LSD square root means = 2.4)

Proportional Analysis $O_{12\text{-}2}$

F-statistics for treatments were significant on days 14 and 21 (Table 24). Significant pairwise differences between Aero® Fungicide (pyraclostrobin + metiram) and Nufarm Tri-base Blue® flowable copper fungicide and Pathogen only treatments were observed on day 7 (Table 25), as well as between other treatments and Nufarm Tri-base Blue® flowable copper fungicide, Amistar® (azoxystrobin) and Pathogen Only treatments on days 14 and 21.

Table 24. Analyses of deviance (AOD) for proportional analysis at each timepoint (ignoring Nil treatment) for detached fruit assay $O_{12\text{-}2}$

	DF	F.con	*P*
Treatment (day 7)	4,15	2.588	0.079
Treatment (day 14)	4,18	7.603	0.001
Treatment (day 21)	4,18	11.940	<0.001

Table 25. Predicted back-transformed means from the proportions analysis at each timepoint for detached fruit assay $O_{12\text{-}2}$

TREATMENT	Day 7	Day 14	Day 21
Nil	0.03	0.23 ab	0.51 ab
Pathogen only	0.10	0.47 c	0.93 c
Aero® Fungicide (pyraclostrobin + metiram)	0.00	0.08 a	0.34 a
Amistar® (azoxystrobin)	0.02	0.27 bc	0.91 c
NorShield 750 WP copper fungicide	0.02	0.22 ab	0.52 ab
Nufarm Tri-base Blue® flowable copper fungicide	0.11	0.47 c	0.77 bc

Different letters after numbers in each column indicate significant differences between treatments ($P \leq 0.05$).

Conclusions

Both analyses demonstrated that the Aero® Fungicide (pyraclostrobin + metiram) treatment was superior to the Pathogen Only Control and mostly to all other treatments. Amistar® (azoxystrobin) and the copper treatments were largely not significantly effective in controlling anthracnose in this trial. The Nil treatment suggested that there was a high proportion (approximately 50%) of fruit with latent anthracnose infection that developed over the 21 day incubation period.

Detached olive fruit assay 2. $O_{12\text{-}3}$

AUDPS Analysis $O_{12\text{-}3}$

There were both significant F-statistics for treatments and cultivars, but the F statistic for treatment × cultivar was not significant (Table 26). Within each cultivar, the F statistic for treatments within cv. Barnea was significant but not for cv. Manzanillo (Table 26). The only significant pairwise differences for cv. Manzanillo were between the Nil and other treatments (except Aero® Fungicide (pyraclostrobin + metiram)). For cv. Barnea, significant pairwise differences occurred between both Nil and Aero® Fungicide (pyraclostrobin + metiram) and the remaining treatments, as well as between Amistar® (azoxystrobin) and both Curex® 3 and Pathogen Only treatments (Table 27).

Table 26. Area under disease progress stairs statistics (ignoring Nil treatment) for fungicide treatments in detached fruit assay $O_{12\text{-}3}$

	DF	F.con	*P*
Treatment	5,21	7.318	<0.001
Cultivar	1,21	497.200	<0.001
Treatment × Cultivar	5,21	2.209	0.092
Barnea	5,42	8.196	<0.001
Manzanillo	5,42	1.331	0.270

Table 27. Area under disease progress stairs back-transformed means for fungicide treatments in detached fruit assay $O_{12\text{-}3}$

TREATMENT	Manzanillo	Barnea
Nil	7.0 a (2.6)	83.1 a (9.1)
Pathogen only	39.1 b (6.3)	200.8 c (14.2)
Aero® Fungicide (pyraclostrobin + metiram)	20.1 ab (4.5)	93.9 a (9.7)
Amistar® (azoxystrobin)	23.8 b (4.9)	133.9 b (11.6)
NorShield 750 WP copper fungicide	28.3 b (5.3)	172.2 bc (13.1)
Nufarm Tri-base Blue® flowable copper fungicide	38.0 b (6.2)	154.8 bc (12.4)
Curex® 3	30.4 b (5.5)	196.7 c (14.0)
5% LSD	(NA)	

Different letters after numbers in each column indicate significant differences between treatments ($P \leq 0.05$) (for each cultivar). (Square root means shown in brackets.)

Proportional Analysis $O_{12\text{-}3}$

F-statistics for treatments and cultivars were significant at all timepoints, whilst the F-statistic for treatment × cultivar was not significant at any timepoint (Table 28). F-statistics for treatments within cv. Barnea were significant at all timepoints, whereas no F-statistics for treatments within cv. Manzanillo were significant at any timepoint.

The only significant pairwise differences for cv. Manzanillo were at day 14, between Nil and NorShield 750 WP copper fungicide , Nufarm Tri-base Blue® flowable copper fungicide and Curex® 3, and between Aero® Fungicide (pyraclostrobin + metiram) and Pathogen Only treatments (Table 28). For cv. Barnea at day 4, the only significant pairwise difference was between the pathogen only treatment and both Amistar® (azoxystrobin) and Nil treatments. At day 7, there were significant pairwise differences between Nil, Aero® Fungicide (pyraclostrobin + metiram) and Amistar® (azoxystrobin) treatments with each of Pathogen Only, NorShield 750 WP copper fungicide and Curex® 3 treatments. These differences largely persisted at 10 and 14 days, except that pairwise differences between Amistar® (azoxystrobin) and both Aero® Fungicide (pyraclostrobin + metiram) and Nil treatments became significant.

Table 28. Analyses of deviance (AOD) for proportional analysis at each timepoint (ignoring Nil treatment) for detached fruit assay $O_{12\text{-}3}$

	DF	**F.con**	***P***
Day 4			
Treatment	5,37	3.0590	0.021
Cultivar	1,37	12.2800	0.001
Treatment × Cultivar	5,37	0.3211	0.897
Barnea	5,37	3.0190	0.022
Manzanillo	5,37	0.3613	0.872
Day 7			
Treatment	5,23	8.6400	<0.001
Cultivar	1,42	78.2900	<0.001
Treatment × Cultivar	5,20	1.3960	0.267
Barnea	5,20	9.2960	<0.001
Manzanillo	5,20	0.7425	0.601
Day 10			
Treatment	5,32	6.0660	<0.001
Cultivar	1,42	227.2000	<0.001
Treatment × Cultivar	5,23	0.8401	0.535
Barnea	5,23	5.8900	0.001
Manzanillo	5,23	1.0160	0.431
Day 14			
Treatment	5,20	9.2600	<0.001
Cultivar	1,42	281.3000	<0.001
Treatment × Cultivar	5,42	0.8668	0.511
Barnea	5,42	8.5430	<0.001
Manzanillo	5,42	1.5830	0.186

Table 29. Predicted back-transformed means from the proportional analysis at each timepoint for detached fruit assay $O_{12\text{-}3}$

TREATMENT	Day 4		Day 7		Day 10		Day 14	
	Manz.	**Barnea**	**Manz.**	**Barnea**	**Manz.**	**Barnea**	**Manz.**	**Barnea**
Nil	0.00 a	0.09 a	0.01 a	0.17 a	0.03 a	0.26 a	0.06 a	0.41 a
Pathogen only	0.00 a	0.28 b	0.00 a	0.51 bc	0.11 a	0.67 cd	0.32 c	0.83 c
Aero® Fungicide (pyraclostrobin + metiram)	0.02 a	0.13 ab	0.02 a	0.18 a	0.04 a	0.33 ab	0.14 ab	0.42 a
Amistar® (azoxystrobin)	0.00 a	0.09 a	0.01 a	0.27 a	0.07 a	0.48 bc	0.18 abc	0.66 b
NorShield 750 WP copper fungicide	0.00 a	0.14 ab	0.00 a	0.53 c	0.10 a	0.63 cd	0.21 bc	0.69 bc
Nufarm Tri-base Blue® flowable copper fungicide	0.04 a	0.14 ab	0.05 a	0.33 ab	0.06 a	0.49 bc	0.27 bc	0.78 bc
Curex® 3	0.00 a	0.15 ab	0.02 a	0.62 c	0.05 a	0.70 d	0.25 bc	0.78 bc

Manz = cv. Manzanillo; Different letters after numbers in each column indicate significant differences between treatments ($P \leq 0.05$) (for each cultivar).

Conclusions

Aero® Fungicide (pyraclostrobin + metiram) and Amistar® (azoxystrobin) both significantly reduced anthracnose expression relative to the inoculated control treatment over 14 days incubation in fruit of cv. Barnea using the two statistical analyses methods. Additionally, in the proportional analysis, Aero® Fungicide (pyraclostrobin + metiram) significantly reduced disease in cv. Manzanillo at 14 days incubation. Both these chemicals were also generally superior to one or all of the copper products

Background levels of anthracnose in the nil control varied between the two cultivars with a much greater proportion of cv. Barnea fruit developing disease symptoms over the incubation period than cv. Manzanillo.

Detached olive fruit assay 3. $O_{12\text{-}4}$

AUDPS Analysis $O_{12\text{-}4}$

The overall F-statistic for treatments was not significant ($F_{5,21} = 1.556$, $P = 0.216$), although pairwise differences between the Nil treatment and other treatments were significant (Table 30).

Table 30. Area under disease progress stairs back-transformed means for fungicide treatments in detached fruit assay $O_{12\text{-}4}$ for cv. Arbequina

Treatment	AUDPS
Nil	5.2 a (2.3)
Pathogen only	86.6 b (9.3)
Aero® Fungicide (pyraclostrobin + metiram)	52.8 b (7.3)
Amistar® (azoxystrobin)	114.7 b (10.7)
NorShield 750 WP copper fungicide	71.5 b (8.5)
Nufarm Tri-base Blue® flowable copper fungicide	116.4 b (10.8)
Curex® 3	107.0 b (10.3)
5% LSD	(3.3)

Different letters after numbers indicate significant differences between treatments ($P \leq 0.05$). (Square root means and 5% LSD shown in brackets.)

Proportional Analysis $O_{12\text{-}4}$

F-statistics for treatment effects were either just significant or non-significant at each timepoint (Table 31). There were no significant pairwise differences at day 7 or 14. At day 21, there were significant pairwise differences between Nil and other treatments (Table 32).

Table 31. Analyses of deviance (AOD) for proportional analysis at each timepoint (ignoring Nil treatment) for detached fruit assay $O_{12\text{-}4}$

	DF	F.con	*P*
Treatment (day 7)	5,21	2.719	0.048
Treatment (day 14)	5,19	1.780	0.165
Treatment (day 21)	5,18	2.031	0.122

Table 32. Predicted back-transformed means from the proportional analysis at each timepoint for detached fruit assay $O_{12\text{-}3}$

TREATMENT	Day 7	Day 14	Day 21
Nil	0.02 a	0.02 a	0.02 a
Pathogen only	0.08 a	0.13 a	0.29 bc
Aero® Fungicide (pyraclostrobin + metiram)	0.06 a	0.08 a	0.18 b
Amistar® (azoxystrobin)	0.17 a	0.22 a	0.30 bc
NorShield 750 WP copper fungicide	0.04 a	0.08 a	0.30 bc
Nufarm Tri-base Blue® flowable copper fungicide	0.12 a	0.19 a	0.36 bc
Curex® 3	0.05 a	0.20 a	0.40 c

Different letters after numbers in each column indicate significant differences between treatments ($P \leq 0.05$).

Conclusions

None of the chemical treatments significantly reduced the level of disease expression in cv. Arbequina. However, it should be noted that there was a relatively low infection response to inoculation with *C. acutatum* in this trial suggesting that this cultivar is less susceptible to anthracnose disease than cv. Barnea used in previous experiments.

Detached olive fruit assay 4. $O_{13\text{-}1}$

AUDPS Analysis $O_{13\text{-}1}$

There are highly significant treatment effects and treatment × cultivar interactions (Tables 33 and 34). That is, the efficacy of the fungicide treatments, as measured by the AUDPS, varied between cultivars. For instance, the differences between treatments are clearly greater in absolute value for cv. Barnea (with a range of 65–23 = ~42 in treatment means) compared to other treatments (with ranges of at most 20 for cv. Picual). Differences between treatments were also highly significant for each of the four cultivars in turn.

Table 33. Area under disease progress stairs Wald type F-statistics (ignoring Nil treatment) for fungicide treatments in detached fruit assay $O_{13\text{-}1}$

	DF	**F.con**	***P***
Treatment	5,18	81.49	<0.001
Cultivar	3,54	786.90	<0.001
Treatment × Cultivar	15,54	14.67	<0.001
Barnea	5,71.6	75.63	<0.001
Picual	5,71.6	29.80	<0.001
Manzanillo	5,71.6	16.89	<0.001
Arbequina	5,71.6	12.15	<0.001

Table 34. Area under disease progress stairs back-transformed means for fungicide treatments in detached fruit assay $O_{13\text{-}1}$

TREATMENT	Barnea	Picual	Manzanillo	Arbequina
Pathogen Only	451.5 d	344.8 c	108.5 bc	101.5 cd
Nufarm Tri-base Blue® flowable copper fungicide	399.0 c	271.2 b	124.3 c	127.8 d
Amistar® (azoxystrobin)	309.7 b	281.7 b	42.0 a	98.0 cd
Cabrio® (pyraclostrobin)	159.2 a	234.5 a	14.0 a	15.8 a
Polyram® (metiram)	372.7 c	378.0 c	138.2 c	71.8 abc
Aero® Fungicide (pyraclostrobin + metiram)	285.2 b	208.2 a	84.0 b	43.8 ab
5% LSD	33.0			

Different letters after numbers in each column indicate significant differences between treatments, for each cultivar ($P \leq 0.05$)

Proportional Analysis $O_{13\text{-}1}$

Treatment, cultivar and treatment × cultivar effects were all highly significant at each timepoint (Table 35). In most cases, differences between treatments were significant for individual cultivars, especially at day 14 and day 21 (Table 36).

Table 35. Analyses of deviance (AOD) tables for proportional analysis at each timepoint (ignoring Nil treatment) for detached fruit assay $O_{13\text{-}1}$

	DF	F.con	*P*
Day 7			
Treatment	5,69	15.230	<0.001
Cultivar	3,69	51.090	<0.001
Treatment × Cultivar Trt × Cult	15,69	2.794	0.002
Barnea: treat	5,69	15.930	<0.001
Picual: treat	5,69	4.373	0.002
Manzanillo: treat	5,69	1.708	0.144
Arbequina: treat	5,69	1.606	0.170
Day 14			
Treatment	5,69	28.960	<0.001
Cultivar	3,69	155.900	<0.001
Treatment × Cultivar Trt × Cult	15,69	5.201	<0.001
Barnea: treat	5,69	18.240	<0.001
Picual: treat	5,69	16.590	<0.001
Manzanillo: treat	5,69	5.495	<0.001
Arbequina: treat	5,69	4.240	0.002
Day 21			
Treatment	5,69	18.740	<0.001
Cultivar	3,72	128.600	<0.001
Treatment × Cultivar Trt × Cult	15,69	4.823	<0.001
Barnea: treat	5,69	14.430	<0.001
Picual: treat	5,69	1.061	0.390
Manzanillo: treat	5,69	9.699	<0.001
Arbequina: treat	5,69	8.019	<0.001

Table 36. Proportional analysis: Predicted means (logit scale and AVSED) and back-transformed means from the proportional analysis at each timepoint for detached fruit assay $O_{13\text{-}1}$

	Back-transformed			
	Barnea	**Picual**	**Manzanillo**	**Arbequina**
7 days				
Pathogen Only	0.59 d	0.08 b	0.09 b	0.09 a
Nufarm Tri-base Blue® flowable copper fungicide	0.37 c	0.16 ab	0.02 ab	0.06 a
Amistar® (azoxystrobin)	0.22 b	0.18 ab	0.00 a	0.03 a
Cabrio® (pyraclostrobin)	0.06 a	0.12 b	0.00 a	0.00 a
Polyram® (metiram)	0.38 c	0.28 a	0.04 ab	0.03 a
Aero® Fungicide (pyraclostrobin + metiram)	0.09 a	0.07 b	0.01 ab	0.01 a
14 days				
Pathogen Only	0.99 d	0.89 a	0.24 b	0.19 c
Nufarm Tri-base Blue® flowable copper fungicide	0.92 d	0.60 c	0.25 b	0.22 c
Amistar® (azoxystrobin)	0.70 bc	0.57 c	0.07 ac	0.15 bc
Cabrio® (pyraclostrobin)	0.32 a	0.46 bc	0.01 c	0.02 a
Polyram® (metiram)	0.79 c	0.88 a	0.28 b	0.11 abc
Aero® Fungicide (pyraclostrobin + metiram)	0.61 b	0.37 b	0.15 ab	0.06 ab
21 days				
Pathogen Only	1.00 d	1.00 a	0.29 cd	0.30 bcd
Nufarm Tri-base Blue® flowable copper fungicide	0.99 cd	0.79 b	0.44 ad	0.45 d
Amistar® (azoxystrobin)	0.85 b	0.86 b	0.17 bc	0.38 cd
Cabrio® (pyraclostrobin)	0.53 a	0.76 b	0.07 b	0.07 a
Polyram® (metiram)	0.96 cd	1.00 a	0.47 a	0.27 bc
Aero® Fungicide (pyraclostrobin + metiram)	0.93 bc	0.75 b	0.32 ad	0.18 b

Different letters after numbers in each column indicate significant differences between treatments, for each cultivar ($P \leq 0.05$)

Conclusion

Again the most useful efficacy data came from the cv. Barnea where at 7 days incubation, all the chemical treatments significantly reduced disease expression compared with the inoculated control. This experiment attempted to directly compare the two strobilurin chemicals (azoxystrobin and pyraclostrobin) without the addition of metiram that is contained in the Aero® Fungicide (pyraclostrobin + metiram) formulation. The product Cabrio® (a.i. pyraclostrobin) sometimes outperformed all chemicals tested or was equivalent to the other strobilurin formulations. Alternatively, metiram alone was generally not demonstrated to be efficacious. Overall, this experiment confirmed efficacy of the strobilurins and general superiority over the protectant chemicals.

Relative cultivar susceptibility

There were clear differences between cultivars for the AUDPS analysis (Table 37).

Table 37. Relative cultivar susceptibility to *Colletotrichum acutatum* determined from AUDPS mean values of pathogen only treatment in Trial $O_{13\text{-}1}$

Cultivar	Mean AUDPS	SE
Barnea	451.5 a	11.8
Picual	344.8 b	11.8
Manzanillo	108.5 c	11.8
Arbequina	101.5 c	11.8
5% LSD	33.03	

Letters after numbers indicate significant differences between cultivars ($P \leq 0.05$)

Proportional analysis $O_{13\text{-}1}$

There were significant differences between cultivars at each timepoint, with cvs Barnea and Picual having higher susceptibilities than cvs Manzanillo and Arbequina (Table 38).

Table 38. Relative cultivar susceptibility to *Colletotrichum acutatum* determined from proportion of infected fruit of pathogen only treatment in Trial $O_{13\text{-}1}$

CULTIVAR	Mean proportion of infect fruit	Approx SE
Day 7		
Barnea	0.59 b	0.050
Picual	0.08 a	0.023
Manzanillo	0.09 a	0.025
Arbequina	0.09 a	0.025
Day 14		
Barnea	0.99 c	0.017
Picual	0.89 b	0.035
Manzanillo	0.24 a	0.040
Arbequina	0.19 a	0.036
Day 21		
Barnea	1.00 b	0.959
Picual	1.00 b	0.959
Manzanillo	0.29 a	0.043
Arbequina	0.30 a	0.044

Letters after numbers at each day assessment indicate significant differences between cultivars ($P \leq 0.05$)

Conclusion

These analyses confirmed that the cv. Barnea is very susceptible to anthracnose infection, particularly when compared with cvs Manzanillo and Arbequina. A surprising result was that cv. Picual was also relatively susceptible to this disease, although it can be seen in Table 34 above that it was slower to develop than in cv. Barnea. These results contrast with a Spanish study (Moral and Trapero, 2009) which suggested that cv. Picual is resistant to anthracnose while cv. Manzanillo is highly susceptible and cv. Arbequina is moderately susceptible. There are many possible reasons for this contrasting result: there could be variation in pathogenicity between fungal isolates used in these studies; the cultivars tested could have significant intrinsic genetic variation in their disease susceptibility; the correct naming of the Australian varieties could have been compromised; and there could be environmental influences from where the fruit were grown that were reflected in their disease susceptibility. Finally, this was a preliminary and laboratory-based study compared with the more extensive use of both field and laboratory data in the European report. A more comprehensive use of similar field and laboratory data as well as different fungal isolates and experiment repetition over time would provide more robust conclusions regarding relative susceptibility to anthracnose of olive cultivars grown in Australia.

SOFT NOSE

A minor component of the project was to investigate the issue of apical end rot, or soft nose in olives, as this had been reported by several growers as a problem which may dispose fruit to anthracnose attack. In this disorder, the apical end of the fruit shrivels, mostly close to maturity. While the cause is unknown, it is thought likely a result of sudden changes in changes in watering regimes, or with calcium and boron (Sergeeva et al. 2011; Spooner-Hart et al. 2007) However, despite raising this issue with industry on a number of occasions in the press and at conferences/field days, we did not have any confirmed cases of soft nose, nor were any received at the NSW DPI diagnostic service during the period of the project. This work, therefore, did not occur. On the other hand, it appears that olive growers are becoming more experienced with maintaining effective fertiliser and irrigation regimes, with improved monitoring systems including regular leaf analyses, especially in the larger, commercial groves.

Interestingly, the high rainfall and associated waterlogging experienced in one season during this project would have reduced availability and uptake of these divalent cations, suggesting that soft nose may have a different or more complex etiology.

DISCUSSION

OLIVE LACE BUG

Based on the series of field trials from six locations over three years, the two neonicotinoid products Actara® (thiomethoxycam) and Shield Systemic Insecticide™ (clothianidin)/Samurai Systemic Insecticide™ (clothianidin), showed high efficacy against olive lace bug when applied as a single foliar spray (Table 39). Mean efficacy of four Actara® (thiomethoxycam) treatments was 92.56%, clothianidin (1 trial of Shield Systemic Insecticide™ (clothianidin) and 2 of Samurai Systemic Insecticide TM) had a mean efficacy of 96.8%, compared with the industry standard, Lebaycid® (fenthion) at 73.97%. These data are also supported by the statistical analyses which showed that the neonicotinoid insecticides Actara® (thiomethoxycam) and Samurai Systemic Insecticide TM (clothianidin) performed significantly better than Lebaycid® (fenthion), with one spray application.

Table 39. Summary of efficacy of insecticides against olive lace bug from field trials

YEAR	LOCATION	NO. OF SPRAYS	EFFICACY %				
			Actara ®	Shield Systemic Insecticide ™	Samurai Systemic Insecticide ™	Lebaycid ®	Sumi-Alpha® Flex
2009-10	Lovedale NSW	2	92.80			100	
2010-11	Pine Mt Qld	1	96.25	99.25		65.8	
2010-11	Hillvale NSW	1	92.42		100	92.02	
2010-11	Yelarbon NSW	1	88.77		91.15	38.04	
2012-13	Coominya Qld	1					91.54
MEAN (SE)			92.56 (1.53)		96.80* (2.83)	73.97 (14.03)	91.54

* Combined Shield Systemic Insecticide™ and Samurai Systemic Insecticide™ results

It is interesting to note that the only time Lebaycid® (fenthion) performed better than either of the neonicotinoid insecticides was when it was applied twice with an interval of 19 days between sprays. All of the tested insecticides did not perform as well under very high lace bug pressure, probably also associated with poor host-plant health restricting movement of systemic/translocated insecticides (e.g. Yelarbon site). Lebaycid® (fenthion) as a single application performed poorly in two trial sites (Pine Mountain (66%) and Yelarbon (38%). The current permitted use for Lebaycid® (fenthion) (PER 13868) is a maximum of three applications per season, with a minimum retreatment interval of seven days, and a withholding period of nine weeks. Given its performance in these trials, normally at least two applications would be required to effectively control medium-high infestation levels of olive lace bug. However, based on these trials it appears that one application of the neonicotinoids, especially Samurai Systemic Insecticide™ (clothianidin), at the recommended dose appears to be sufficient for controlling populations, even under high bug pressure. In summary, OLB 3 and 4 showed that the neonicotinoid insecticides Actara® (thiomethoxycam) and Samurai

Systemic Insecticide™ (clothianidin) performed significantly better than Lebaycid® (fenthion), with one spray application.

Most of the lace bug trials were conducted in October. At that time, there were mixed stages present (particularly adults and late nymphal instars), indicating that the first cohort had just completed its development. At Yelarbon, in December, there appeared to be overlapping generations with early instars as well as adults present, indicating that the second cohort had emerged. Mixed generations are more difficult to manage, as adults are reasonably motile within groves and more tolerant of sprays, whereas nymphs commonly reside on the undersides of leaves (Spooner-Hart et al. 2002).

The relative doses of the three neonicotinoid formulations were different, and this probably reflected their relative efficacies. All were applied at the concentration recommended by the manufacturer, namely 30g, 60g and 40g /100L water for Actara® (thiomethoxycam), Shield Systemic Insecticide™ (clothianidin) and Samurai Systemic Insecticide™ (clothianidin), respectively. This resulted in a dose of the active constituents/100L being 7.5 g thiamethoxam (Actara®); 12.0 g clothianidin (Shield Systemic Insecticide™) and 20.0 g clothianidin (Samurai Systemic Insecticide™).

Only one trial was undertaken to assess the efficacy of Sumi-Alpha® Flex (esfenvalerate). While this product was not part of the project protocols, this work was conducted because of a recommendation from the AOA Chemical Permits Committee. They recommended this product be evaluated because it was cheap, was thought to be effective against infestations of lace bug with overlapping generations, and was likely to have a shorter withholding period than either Lebaycid® (fenthion) or the neonicotinoid products. Because of insufficient treatment blocks at the trial site, the industry standard, Lebaycid® (fenthion), was not able to be used as a comparator. Nevertheless, a single foliar application of Sumi-Alpha® Flex (esfenvalerate) achieved a calculated efficacy of 91.5% compared to the Water Only Control, demonstrating it could be an effective alternative chemical for controlling lace bug.

It is recommended that the olive industry pursue a permit for use of Samurai Systemic Insecticide™ (clothianidin) in olives for olive lace bug (and possibly other minor heteropteran pests such as Rutherglen bug, *Nysius* spp. and green vegetable bug, *Nezara viridula*). Apart from its confirmed efficacy against olive lace bug, it is likely to be registered in Australia as a control for the tephritid fruit flies Queensland fruit fly, *Bactrocera tryoni* and Mediterranean fruit fly, *Ceratitis capitata* (Kevin Bodnaruk, pers. comm.). Both of these fruit fly species have been recorded attacking olives, and previous permits allowed use of Lebaycid® (fenthion) and dimethoate for their control. There are no chemicals currently permitted for use against fruit flies in olives. The industry may wish to discuss the possible registration of Samurai Systemic Insecticide™ (clothianidin) with its manufacturer Sumitomo Australia Pty Ltd, including any additional data which may be required. It should also be noted that a separate HAL-funded project (OL13004) has generated some residue data for both Actara® (thiomethoxycam) and Samurai Systemic Insecticide TM (clothianidin) in olives.

The industry needs to further consider if they see a role for Sumi-Alpha® Flex (esfenvalerate) in olive lace bug management. While it is cheap and appears to be efficacious, it has a number of detrimental characteristics, particularly its broad spectrum activity. In these considerations, discussions should take place with HAL and the manufacturer, Sumitomo Chemical Australia Pty Ltd.

In October 2011, the APVMA suspended approvals for products containing dimethoate in a range of crops, including olives. Dimethoate had previously been permitted use in olives against olive lace bug, fruit fly and green vegetable bug. There is an extant permit for dimethoate use in olives (PER 13999), which expires in October 2014. The AOA with support from the project successfully applied for a permit for fenthion (PER 12857) in August 2011; a subsequent permit (PER 13559) was surrendered, and replaced with PER 13868, which severely limits fenthion use and greatly extends the withholding period to 60 days. However, fenthion is under review and could be suspended at any time. This makes generation of data (especially residue data) for systemic insecticide alternatives (such as Actara® (thiomethoxycam) and/or Samurai Systemic Insecticide™ (clothianidin)) for OLB control a critical issue.

While this project focussed on the assessment of insecticides for control of olive lace bug, it is clear that an integrated approach needs to be adopted, and the Australian olive industry is committed to minimising pesticide use in olive production, and has expressed its support for Integrated Pest and Disease Management (IPDM) programs. However, although *F. olivinia* is an important native pest species, little is known about its biology. While this project has demonstrated that a number of registered insecticides are efficacious against olive lace bug in the field, the timing of applications for effective management is problematic, especially for growers without effective monitoring systems. In addition, limited information is available on natural enemies of *F. olivinia*, and on its ecology in its native habitat. There is some evidence that some olive cultivars are more tolerant of olive lace bug attack, that lace bug develops at different rates on different cultivars and that trees under stress are likely to be more severely attacked and damaged by olive lace bug. Elucidation of these and other aspects of the lace bug biology will greatly assist in its future management.

ANTHRACNOSE

The fungicide Aero® Fungicide (pyraclostrobin + metiram) which is formulated with a combination of the strobilurin (pyraclostrobin) and a dithiocarbamate (metiram) provided consistent and often superior control of olive anthracnose in field trials and laboratory bioassays. The other strobilurin fungicide, Amistar® (azoxystrobin), had less consistent efficacy in detached fruit assays but was equal to Aero® Fungicide (pyraclostrobin + metiram) in two field experiments. Therefore, it would also be a suitable candidate for maintaining as a permit or possible registration. Given the potential for resistance development it would be advisable to develop a use-pattern where it is alternated or combined with a chemical with a different mode of action

Copper products were less effective in controlling anthracnose infection in the highly susceptible cultivar, Barnea. However, they did provide intermediate and statistically significant control over the Water Controls in cv. Manzanillo in two field trials and in cv. Picual in one detached fruit assay. Therefore, they could be used in wet seasons as protectant fungicides and would add value for anthracnose resistance management. Copper products may also have use in the control of other fungal diseases such as peacock spot and leaf mould and help to prevent the spread of bacterial diseases such as olive knot and bacterial shoot blight. In cases where there was a superior copper formulation, this was normally Nufarm Tri-base Blue® flowable copper fungicide.

As a result of our early field efficacy trial results with copper fungicides, we queried the wording of the Conditions of Use in PER 8586, for copper oxychloride (viz.“ Apply in autumn before winter rain, and again as fruit colour changes”) as being able to provide a sufficient level of efficacy. This wording was probably taken from the Directions of Use for two copper oxychloride fungicide products registered for use in olives, Runge Agrichems Copper Oxychloride-WP, and Ospray Copper Oxychloride-WP. As a result, the wording of the subsequent permit for copper fungicides, PER11360 (March 2009-March 2017), was changed to read (“Copper sprays are best applied prior to the onset of conditions conducive to disease (i.e. warm, humid, wet weather)”).

The project has identified differences in olive cultivar susceptibility to anthracnose, best observed in the detached fruit bioassays that were conducted under controlled environmental conditions and with uniform fungal inoculum. However, the range of cultivars tested was limited, and the experiments should be repeated to provide more confidence in the conclusions. This is particularly true for cv. Picual, where the data we generated was different to reports from Spanish researchers. Obtaining field data on cultivar susceptibility could also be undertaken to verify results from detached fruit assays as was done in the Spanish study.

It is recommended that the industry support further research that develops a disease forecasting model for olive anthracnose that would optimise fungicide application timing. The environmental conditions that favour the development of olive anthracnose have been determined in Europe and can be modelled to predict when disease is likely to be expressed and spread. However, such models are empirical and would need to be validated in any given production area. For example, previous work on onion downy mildew in the NSW Riverina between 1992–4 by Dr Tesoriero determined that the Downcast model, that had been developed in Canada, over-predicted disease incidence primarily due to the fact that short rainfall periods followed by a return to sunny conditions with lower relative humidity were not conducive for disease. Notwithstanding microclimates within the olive tree canopy, a similar effect could apply in certain years and in similar Mediterranean climates. In the downy mildew case, no chemical applications were required in one year of the project thereby saving significant funds and time for growers who followed the researchers’ advice.

The project identified a range of strains of the *Colletotrichum* species complex in the *C. acutatum* and *C. gloeosporioides* groups. Further work is required to understand their relative virulence, any biological significance such as modes of survival, persistence between seasons, ability to infect different plant organs and susceptibility to fungicides.

TECHNOLOGY TRANSFER

A number of technology transfer activities occurred during the project. These included five refereed scientific papers, three industry journal articles, two international conference presentations, two national industry conference presentations, two field days, and numerous field visits and grower communications. These outputs are detailed below.

Published Papers

Sergeeva V, Spooner-Hart R 2011. Diseases and disorders associated with environmental stress in sustainable olive orchards in Australia *in* Tous J et al. (eds.) *Acta Horticulturae* 924:145–150.

Spooner-Hart R 2011. Evaluation of key chemicals for pest management in the olive industry- a project update. *The Olive Press* Spring edition 16:3.

Spooner-Hart R, Sergeeva V 2010. Anthracnose rears its ugly head this season. *The Olive Press* 15:3, 23–4.

Sergeeva V, Spooner-Hart R 2010. Anthracnose and Queensland fruit fly in olives *The Olive Press* 15:2, 23–24.

Sergeeva V, Spooner-Hart R 2010. Olive diseases and disorders in Australia. *Bulletin of the IOBC/WPRS Working Group Integrated Protection of Olive Crops* 59:29–32.

Sergeeva V, Spooner-Hart R 2009. Anthracnose and cercosporiose on olives in Australia - an update. *Australian and New Zealand Olivegrower and Processor* 65:31–34.

Other communications

Conference Papers

Spooner-Hart R 2013. Management options for olive lace bug. AOA National Conference Hobart, Tasmania, 7–8 Oct. 2013.

Tesoriero L, Spooner-Hart R, Collins D 2013. Managing olive anthracnose. AOA National Conference Hobart, Tasmania, 7–8 Oct. 2013.

Spooner-Hart R, Tesoriero L 2012. Olive lace bug. AOA National Conference Adelaide, South Australia, 31 Oct–1 Nov. 2012.

Tesoriero L, Spooner-Hart R, Collins D 2012 Olive anthracnose. AOA National Conference Adelaide, South Australia, 31 Oct–1 Nov. 2012.

Spooner-Hart R 2011. Evaluation of chemicals for management of key pests in the Australian olive industry. AOA National Conference Wangaratta, Victoria, 25–26 Oct. 2011.

Industry and other media publicity

University of Western Sydney 2010. Managing olive pests. *Research Directions*, University of Western Sydney, January 2010. (Appendix 7)

NSW DPI 2012. New olive varieties found less susceptible to anthracnose. *Agriculture Today*, NSW DPI. (Appendix 8)

Field Days

- Robert Spooner-Hart conducted an industry field day at Casino NSW on 26 June 2010, where he gave a presentation on *Major olive pests and disease problems in Northern NSW and their management*, with particular focus on anthracnose, olive lace bug and green vegetable bug. He also visited several groves in the Summerland area, and discussed pest and disease management strategies with the growers.

- Len Tesoriero and Robert Spooner-Hart conducted a field day for Tasmanian olive growers at Richmond on 9 October 2013, sponsored by the Tasmanian Olive Growers' Association. The field day focussed on anthracnose (suspected but to that time not confirmed on olives in that state) and management of olive pests and diseases, where the presenters particularly discussed the evaluation of pesticides in the current project. Following the field day, Drs Tesoriero and Spooner-Hart visited and inspected the four largest olive groves in Tasmania. We observed symptoms at two groves probably associated with olive lace bug damage, and confirmed for the first time the presence of anthracnose in fruit sampled from a grove in Tasmania.

- Robert Spooner-Hart participated in a field day in the Canberra region 29 November 2013, organised by the Olive Alliance where he conducted the session on *Pest management of olive lace bug and the concepts of Integrated Pest Management*, during which he outlined the current status of chemicals permitted for use in olives.

Other project technology transfer activities

- Regular and frequent email and phone communication with Mike Baker (Chair) and other members of the AOA Chemical Permits Committee, to discuss aspects of chemical availability and use in the olive industry.

- We also received and responded to numerous emails and telephone calls from growers throughout Australia about a range of olive pest and disease problems (not only those associated with olive lace bug and anthracnose).

RECOMMENDATIONS •

A number of important findings and outcomes from this project have been reported above. However, it was not possible to investigate these further within the limits of a three year project. The following recommendations are made in this context:

- It is recommended that the olive industry pursue a permit for use of Samurai Systemic Insecticide™ (clothianidin) in olives for olive lace bug (and possibly other minor heteropteran pests such as Rutherglen/coon bug, *Nysius* spp., and green vegetable bug, *Nezara viridula*). Apart from its confirmed efficacy against olive lace bug, it is likely to be registered in Australia as a control for the tephritid fruit flies Queensland fruit fly, *Bactrocera tryoni* and Mediterranean fruit fly, *Ceratitis capitata* (Kevin Bodnaruk, pers. comm.). The data presented in this report should be used to provide evidence of product efficacy.

- The industry may wish to discuss the possible registration of Samurai Systemic Insecticide TM (clothianidin) with its manufacturer Sumitomo Australia Pty Ltd, including any additional data which may be required. It should also be noted that a separate project (OL13004) has generated some residue data for both Actara® (thiomethoxycam) and Samurai Systemic Insecticide™ (clothianidin).

- The industry needs to further consider if they see a role for Sumi-Alpha® Flex (esfenvalerate) in olive lace bug management. If they do, then further discussions need to take place with Sumitomo Australia, and trials will need to be conducted to generate appropriate residue data to accompany application for a permit. The authors, however, caution against this approach because of the old chemistry and ecosystem-disruptive nature of this product.

- There are some newer chemistries which have been recently developed for phytophagous hemipterans. Some of these are currently being evaluated against macadamia lace bug and are showing good efficacy (Ruth Huwyer, NSW DPI, pers. comm.). It is recommended that these chemicals be evaluated, initially in laboratory and potted plant bioassays. Spooner-Hart has already been in discussion with Bayer with a view to conducting these investigations in the near future. A small amount of current funding (say, $10000) could be redirected to support this work.

- This project has highlighted the paucity of information on the biology and ecology of olive lace bug. It is recommended that the industry support research on these important aspects of this native pest, so that a more integrated approach can be taken for its management.

- It is recommended that the olive industry pursue a permit (and possibly registration, in collaboration with Nufarm Australia) for use of Aero® Fungicide (pyraclostrobin + metiram) in olives for anthracnose (and possibly other fungal diseases such as peacock spot and leaf mould). This recommendation is based on its consistent and superior performance in field trials and laboratory bioassays, and the reduced risk of resistance associated with its dual active constituents. The development of strobilurin resistance in strains of a range of fungal pathogens is well documented. If Aero® Fungicide (pyraclostrobin + metiram) were to become the permitted fungicide of choice in olives, there would be little need to maintain Amistar® (azoxystrobin) as a

second option. The data presented in this report should be used to provide evidence of product efficacy in a submission to the APVMA.

- Copper products were of less value in controlling anthracnose infection in the highly susceptible cultivar, Barnea. However, they did provide intermediate and statistically significant control over the Water Controls in cv. Manzanillo in two field trials and in cv. Picual in one detached fruit assay. Therefore they could be used in wet seasons as protectant fungicides and would have some value for anthracnose resistance management. Copper products may also have use in the control of other fungal diseases such as peacock spot and leaf mould and help to prevent the spread of bacterial diseases such as olive knot and bacterial shoot blight. Further research is required to obtain objective data on the efficacy for their use in a spray program where they are potentially alternated with strobilurin fungicides.

- It is recommended that the AOA approach the APVMA to change the Directions of Use labels for the two copper oxychloride products currently registered for anthracnose and other olive fungal diseases to the wording in the extant permit PER11360.

- Although not an initial objective of the project, we identified differences in olive cultivar susceptibility response to anthracnose, best observed in the detached fruit bioassays that were conducted under controlled environmental conditions and with uniform fungal inocula. However, the range of cultivars tested was limited, and the experiments need to be repeated to provide more confidence in conclusions regarding cultivar susceptibility to anthracnose. This is particularly true for cv. Picual, where the data we generated is different to reports from Spanish researchers. Obtaining field data on cultivar susceptibility could also be undertaken to verify results from detached fruit assays, as was done in the Spanish study.

- It is therefore recommended that the industry support further research that develops a disease forecasting model for olive anthracnose that would optimise fungicide application timing. The environmental conditions that favour the development of olive anthracnose have been determined in Europe and can be modelled to predict when disease is likely to be expressed and spread. However, such models are empirical and would need to be validated in any given production area. For example, previous work on onion downy mildew in the NSW Riverina between 1992–4 by Dr Tesoriero determined that the Downcast model, that had been developed in Canada, over-predicted disease incidence primarily due to the fact that short rainfall periods followed by a return to sunny conditions with lower relative humidity were not conducive for disease development. Notwithstanding microclimates within the olive tree canopy, a similar effect could apply in certain years and in similar Mediterranean climates. In the downy mildew case, no chemical applications were required in one year of the project, thereby saving significant funds and time for growers who followed the researchers' advice.

- The project identified a range of strains of the *Colletotrichum* species complex associated with anthracnose disease of olives. At this stage, we do not know the relative importance of *C. acutatum* and *C. gloeosporioides* groups. Further work is required to understand their relative virulence, any biological significance such as

modes of survival, persistence between seasons, ability to infect different plant organs and susceptibility to fungicides.

- In the event that soft nose is reported as a significant problem, it is recommended that the work envisaged in this project be undertaken, but as a specific project, investigating this disorder's apparent complex etiology.

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Dr Vera Sergeeva for her contribution to the project for the first 2.5 years

Oleg Nicetic for his contribution to the project (including statistical support) for the first 2 years of the project

Damian Collins, NSW DPI, for statistical advice and support, particularly of the fungicide field trials and laboratory bioassays against anthracnose

The numerous growers who assisted in the trial work and/or provided their groves for trial work. Of particular note are:

Staff of Boundary Bend Boort, Victoria: Leandro Ravetti, Simon Robb and John Mackley

Jim and Lisa Rowntree, Coonalpyn, South Australia

Steve Head, Coonalpyn, South Australia.

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APPENDICES

Appendix 1. Letter sent to pesticide companies introducing the project and seeking expressions of interest

Locked Bag 1797
Penrith South DC NSW 1797 Australia

University of Western Sydney
Bringing knowledge to life

Centre for Plants & the Environment

24 September, 2009

Dear Colleague,

We are participants in a newly commenced 3 year RIRDC project "EVALUATION OF KEY CHEMICALS FOR PEST MANAGEMENT IN THE OLIVE INDUSTRY"

The industry-identified target organisms are:

- olive lace bug, *Froggattia olivinia,*
- fruit rots, in particular anthracnose *Colletotrichum acutatum* and *C. gloeosporioides*, but also cercosporiose, *Pseudocercospora cladosporioides.*

The Australian olive industry currently has a very limited number of legal pesticide choices available for control of these problems, and is particularly interested in products compatible with IPDM.

The project aims to identify acceptable pesticides for the olive industry for these key insects and diseases (based on efficacy, environmental safety and the manufacturer's/distributor's interest in participating in this market). It will also conduct field trials to generate the data on efficacy and residues, where required, for submission to APVMA for their registration or permitted use.

At this time, we are seeking expressions of interest from pesticide companies in providing products for evaluation in this project.

Please contact either Robert Spooner-Hart or Peter Dal Santo at the contact addresses below.

We look forward to your positive response,

Yours sincerely,

Robert Spooner-Hart and Peter Dal Santo

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Appendix 2. Analysis of olive oil from fungicide treatments in Anthracnose Trial 1, Lovedale 2010

NSW GOVERNMENT | Industry & Investment

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Oil Testing Service Customer Service
Phone: 02 6938 1957
Fax: 02 6938 1649
Email: wagga.csu@industry.nsw.gov.au

Client Name: Robert Spooner-Hart
Company: University of Western Sydney
Address: Locked Bag 1797
PENRITH SOUTH NSW 2750

Phone: 02 4570 1429
Fax: 02 4570 1103
Email: r.spooner-hart@uws.edu.au
Mobile: 0414 953 129

REPORT OF CHEMICAL ANALYSIS

Test Report No: RO10/1100

Sample(s) arrived on:	7 April 2010	
Report Completion Date:	9 April 2010	
Number of Samples:	THREE	
Sample Description:	Olive Fruit	
Sample Identification:	RO10/1100-1	Red
	RO10/1100-2	Blue
	RO10/1100-3	White

Requested tests:	**Analytical Method Used**
NATA Endorsed Tests	
Fatty Acids Profile	IOC COI/T.20/Doc No. 24-2001
Free Fatty Acids	AOCS Ca 5a-40
Non NATA Tests	
*** Oil Content by Solvent Extraction	WWAI 2-1607
*** Oil Content by Cold Press Extraction	WWAI 2-1614
*** Peroxide Value	ISO 3960:2007

*** *NATA Accreditation does not cover the performance of this service.*

Results of R010/1100

	10/1100-1	10/1100-2	10/1100-3	IOC* limits	Units
Client ID	Red	Blue	White		
Oil Content by Cold Press Extraction					
In whole fruit as received	1	7	21	N/A	%
At 50% moisture	2	8	15	N/A	%
Moisture Content					
In whole fruit as received	59.6	54.8	28.8	N/A	%
Oil Content by Solvent Extraction					
In whole fruit as received	15.8	17.8	30.0	N/A	%
At 50% moisture	19.6	19.7	21.1	N/A	%
Fatty Acids Profile					
Myristic acid C14:0	< 0.1	< 0.1	< 0.1	≤ 0.05	% of total fatty acids
Palmitic acid C16:0	15.9	15.5	15.0	7.5- 20.0	% of total fatty acids
Palmitoleic acid C16:1	2.7	2.8	2.7	0.3-3.5	% of total fatty acids
Heptadecanoic acid C17:0	0.1	0.1	0.1	≤ 0.3	% of total fatty acids
Heptadecenoic acid C17:1	0.3	0.3	0.2	≤ 0.3	% of total fatty acids
Stearic acid C18:0	3.8	3.3	3.2	0.5 - 5.0	% of total fatty acids
Oleic acid C18:1	64.8	64.4	66.1	55.0 - 83.0	% of total fatty acids
Linoleic acid C18:2	10.9	11.9	11.1	3.5 - 21.0	% of total fatty acids
Linolenic acid C18:3	0.8	1.0	0.9	≤ 1.0	% of total fatty acids
Arachidic acid C20:0	0.4	0.4	0.4	≤ 0.6	% of total fatty acids
Eicosenoic acid C20:1	0.2	0.2	0.2	≤ 0.4	% of total fatty acids
Behenic acid C22:0	0.1	0.1	0.1	≤ 0.2	% of total fatty acids
Lignoceric acid C24:0	< 0.1	< 0.1	< 0.1	≤ 0.2	% of total fatty acids
Total	100	100	100		
Saturation Ratio					
Polyunsaturated: (C18:3+C18:2)	12	13	12	N/A	% of total fatty acids
Monounsaturated: (C16:1+C17:1+C18:1+C20:1)	68	68	69	N/A	% of total fatty acids
Saturated: (C14:0+C16:0+C17:0+C18:0+C20:0+C22:0+C24:0)	20	19	19	N/A	% of total fatty acids

Myristic acid (C14:0) shown to 1 decimal place as that is the limit of reporting.

Free Fatty Acids	1.7	14.6	27.9	≤ 0.8	% as oleic acid
Peroxide Value	4	4	3	≤ 20	mEq O_2/kg oil

** Please note that the underlined result is outside IOC limits for classification of Extra Virgin Olive Oil

F Boshuizen
Technical Officer

Report Date: 9 April 2010

This report supersedes any previous report with this Report Number (see top of this page).
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NATA Accredited Laboratory Number 14949

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Appendix 3. Analysis of olive oil from fungicide treatments in Anthracnose Trial 2, Coonalpyn 2011

modern olives laboratory services
PO BOX 92 LARA VIC 3212 TEL: 03 5272 9570 FAX: 03 5272 9599 EMAIL: lab@modernolives.com.au

Report Date: 24/06/2011

Analysis Report Nº 11/466

	Submitter Details	
Name:	Robert Spooner-Hart	
Company:	University of Western Sydney	ABN:
Address:	Locked Bag 1797 Penrith South DC NSW 1797	
Phone:	02 4570 1429	Fax: 02 4570 1314
Email:	r.spooner-hart@uws.edu.au	

SAMPLES

Number:	**5**	Date sampled:	-
Type:	**Olives**	Date received:	**22/06/2011**
Sampling:	The laboratory is not responsible for the sampling. The samples are analysed as received.		

SAMPLE REFERENCES

Identification of sample	Laboratory reference code	Identification of sample	Laboratory reference code
Treatment 1 – Blue	**11/466-01**	**Treatment 4 – Pink**	**11/466-04**
Treatment 2 - Red	**11/466-02**	**Treatment 5 - White**	**11/466-05**
Treatment 3 - Yellow	**11/466-03**		

NATA
This document is issued in accordance with NATA's accreditation requirements.
Accredited for compliance with ISO/IEC 17025
Accreditation No 15594

International Olive Council Chemical Testing Laboratory Recognised

AOA Accredited Laboratory

AOCS Approved chemist
AOCS Your Global Fats and Oils Connection

modern olives laboratory services

PO BOX 92 LARA VIC 3212 TEL: 03 5272 9570 FAX: 03 5272 9599 EMAIL: lab@modernolives.com.au

RESULTS

Quality parameters

Lab ref	FFA	PV	K232	K270	Δ K
11/466-01	0.81	4.0	-	-	-
11/466-02	0.44	5.3	-	-	-
11/466-03	1.06	5.0	-	-	-
11/466-04	0.93	4.9	-	-	-
11/466-05	1.32	5.3	-	-	-
IOC limits	≤ 0.8	≤ 20	≤ 2.50	≤ 0.22	≤ 0.01

Claudia Guillaume
Laboratory Manager

ANALYSIS REQUIRED, METHODS, LIMIT OF REPORTING AND UNITS.

Description	Ref	Analytical Reference	Results, units reported as	U*
(*) Free fatty acids	FFA	AOCS Ca 5a-40	g % of oleic acid	0.02
(*) Peroxides value	PV	AOCS Cd 8-53	meq O2 / kg oil	0.52

(*)NATA accreditation covers the performance of these tests.

* The uncertainty (U) is an expanded uncertainty using a coverage factor of 2, which gives a level of confidence of approximately 95%.
[1] This value corresponds to oleic acid, being the big uncertainty of them.

Ref: International Olive Council (IOC) limits are only for extra virgin olive oil category.
Please note that the underlined results do not comply with the category.

Appendix 4. Analysis of olive oil from fungicide treatments in Anthracnose Trial 3, Boort 2011

modern olives
laboratory services
PO BOX 92 LARA VIC 3212 TEL: 03 5272 9570 FAX: 03 5272 9599 EMAIL: lab@modernolives.com.au

Report Date: 26/05/2011

Analysis Report Nº 11/332

	Submitter Details	
Name:	Leandro Ravetti	
Company:	Modern Olives	ABN:
Address:	PO BOX 92 Lara VIC 3212	
Phone:	03 5272 9500	Fax: 03 5272 9599
Email:	l.ravetti@modernolives.com.au	

SAMPLES

Number:	**5**	Date sampled:	**25/05/2011**
Type:	**Olive Oil**	Date received:	**26/05/2011**
Sampling:	The laboratory is not responsible for the sampling. The samples are analysed as received.		

SAMPLE REFERENCES

Identification of sample	Laboratory reference code	Identification of sample	Laboratory reference code
Control	11/332-01	Norshield	11/332-04
Aero	11/332-02	Tri-base	11/332-05
Amistar	11/332-03		

modern olives
laboratory services
PO BOX 92 LARA VIC 3212 TEL: 03 5272 9570 FAX: 03 5272 9599 EMAIL: lab@modernolives.com.au

RESULTS

Quality parameters

Lab ref	FFA	PV	PPH
11/332-01	4.7	-	-
11/332-02	2.3	-	-
11/332-03	4.8	-	-
11/332-04	4.5	-	-
11/332-05	2.1	-	-
IOC limits	≤ 0.8	≤ 20	-

Claudia Guillaume
Laboratory Manager

ANALYSIS REQUIRED, METHODS, LIMIT OF REPORTING AND UNITS.

Description	Ref	Analytical Reference	Results, units reported as	U*
(*) Free fatty acids	FFA	AOCS Ca 5a-40	g % of oleic acid	0.02

(*)NATA accreditation covers the performance of these tests.

* The uncertainty (U) is an expanded uncertainty using a coverage factor of 2, which gives a level of confidence of approximately 95%.
[1] This value corresponds to oleic acid, being the big uncertainty of them.

Ref: International Olive Council (IOC) and DGF limits are only for extra virgin olive oil category.
Please note that the underline result does not comply with the category.

NOTES: This report is only valid for the samples detailed above. This report shall not be reproduced except in full, without approval of the laboratory.
Test results and findings may be provided to authorised staff and used for statistical and certification purposes in accordance with company policies. The source of the information will remain confidential unless otherwise required by Law or regulatory policies.

Appendix 5. Molecular characterisation of *Colletotrichum* spp. isolates associated with olive fruit rot (anthracnose)

Isolates of *Colletotrichum* spp. collected during this project and a reference collection held at the University of Western Sydney were characterised from their β-tubulin and /or ITS rDNA gene sequences and compared with published sequences from Australia and overseas. A summary of these isolates, their species identity and the GenBank accession number of sequences alignments for their closest match are listed in the Appendix 5 Table below.

Appendix 5 Table. Confirmation of *Colletotrichum* spp. by analyses of sequencing ITS rDNA and β-tubulin from olives

Lab number	ITS	β-Tubulin
11-568 A	AJ301924.1 AF272781.1 DQ286132.1[b] EU670080.1 AM991137.1 FJ788419.1[a]	FJ788419.1[a]
11-563 B	AJ301924.1 AF272781.1 DQ286132.1[b] EU670080.1 AM991137.1 FJ788419.1[a]	FJ788419.1[a]
11-563 C	EF622200.1 AJ301924.1 AF272781.1 DQ286132.1[b] EU670080.1 AM991137.1 FJ788419.1[a]	FJ788419.1[a]
11-563 D	EF622200.1 AJ301924.1	FJ788419.1[a]

Lab number	ITS	β-Tubulin
	AF272781.1 DQ286132.1[b] EU670080.1 AM991137.1 FJ788419.1[a]	
11-563 F	EF622200.1 FJ788419.1[a] AJ301924.1 AF272781.1 DQ286132.1[b] EU670080.1 AM991137.1 FJ788419.1[a]	
12-617 R2T1 DAM2588	EF622200.1 AJ301924.1 AF272781.1	FJ788419.1[a]
12-617 R3T1 DAM2589	EF622200.1 AJ301924.1 AM991137.1 AF272781.1 FJ788419.1[a]	FJ788419.1[a]
12-121 DAM2587 Centre colony	EF622200.1 AJ301924.1 AF272781.1 DQ286132.1[b] EU670080.1 AM991137.1 FJ788419.1[a]	FJ788419.1[a]
12-121 DAM2587	EF622200.1	FJ788419.1[a]

Lab number	ITS	β-Tubulin
Edge colony	AM991137.1 AJ301924.1 DQ286132.1[b] EU670080.1	
12-121 DAM2587		
12-058 DAM2586	AJ301982.1 AJ301981.1 AJ301949.1	AY376556.1
11-600 DAM2562	EF622200.1 AJ301924.1 AF272781.1 DQ286132.1[b] EU670080.1 AM991137.1 FJ788419.1[a]	FJ788419.1[a]
11-600 DAM2561	EF622200.1	FJ788419.1[a]
11-600 DAM2560	EF622200.1 AM991137.1	FJ788419.1[a]
13-881	JN121183[c]	FJ788419.1[a]

[a] voucher culture ex Simmonds

[b] ATCC56816

[c] UWS14

[d] UWS166

Appendix 6. University of Western Sydney media release on the RIRDC/HAL project

University of Western Sydney
Bringing knowledge to life

RESEARCH DIRECTIONS

Managing Olive Plant Pests

Associate Professor Robert Spooner-Hart from the Centre for Plants and the Environment is researching which environmentally friendly pesticides should be used by Australia's olive industry to control pests and diseases. This research has been funded by The Rural Industries Research and Development Corporation.

'Currently in Australia there are a limited number of environmentally-friendly pesticides available to control pests and disease within the olive industry' explains Prof. Spooner-Hart. 'Research has been conducted on how to control some of the pests (e.g. the black scale) that affect olives however, many insects (including the olive lace bug) have not been studied and we don't have effective controls for them. It's not only pests that are affecting the olive industry disease is becoming more common owing to a lack of effective protection available for olive trees and fruit. New research is particularly needed to study the selection and timing of pesticide and fungicide applications in olive groves, to maintain a productive olive industry which uses effective pesticides which are safe to humans and the environment'.

This research will be conducted through laboratory bioassays and field trials to generate data for the Australian Pesticides and Veterinary Medicine Authority (APVMA). A number of sites throughout Australia's olive production areas will be used to test different pesticides and their effectiveness on olive lace bug and olive fruit rots. Different types of pesticides will be trialled over three seasons in order to gather the most comprehensive data. Once the trials are complete, the data will be analysed and provided to the APVMA, to seek registration of the most suitable pesticides.

Effective pest and disease control measures are necessary to ensure a healthy Australian olive crop every year. This research aims to provide evidence that will assist the olive industry to operate in a way that is commercially viable, efficient and ecologically sustainable – with all the attendant health and economic benefits that will flow from such an approach.

Project Title: Evaluation of key chemicals for pest management in the olive industry
Funding has been set at: $157,574
Contact Details: r.spooner-hart@uws.edu.au
http://www.uws.edu.au/centre_for_plants_and_the_environment
January 2010

Office of Research Services www.uws.edu.au/researchservices

Appendix 7. NSW DPI media release on the RIRDC/HAL project published in Agriculture Today and The Land Newspaper (Fairfax Press)

New olive varieties found less susceptible to anthracnose | NSW Department of Primary Industries

Part of NSW Department of Primary Industries

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Home » About us and our services » News and events » Agriculture Today » August 2012

NEWS AND EVENTS

- Media contacts
- Ministerial Media Releases
- News releases
- Agriculture Today
- Newsletters
- Events

New olive varieties found less susceptible to anthracnose

From the August 2012 edition of *Agriculture Today*.

Testing olives for the success of different chemical controls against anthracnose disease in the new plant health laboratories at the Elizabeth Macarthur Agricultural Institute. (Photo: Alf Manciagli.)

The fungal fruit rot disease anthracnose has led to significant losses and downgrading of Australian olive oil quality.

Wet weather associated with recent La Nina years is to blame but in the search for controls for anthracnose new olive varieties have been identified that are not only higher producing but importantly, less susceptible to the disease.

At Elizabeth Macarthur Agricultural Institute (EMAI) near Camden, researcher Len Tesoriero is helping an entomologist colleague from University of Western Sydney associate professor Robert Spooner-Hart, to develop controls for this disease with funding from the Australian Olive Industry Association and the Rural Industries Research and Development Corporation.

"The fungus causing anthracnose disease not only causes fruit to rot and drop as they ripen, but also increases undesirable oxidation of the oil resulting in a downgrading from extra virgin status," Dr Tesoriero said.

"Trials in olive groves in major production areas where anthracnose has been prevalent have identified safe and effective chemical controls, while comparison of different olive varieties has determined their relative disease susceptibility.

"This information will allow the industry to prioritise chemical registrations for olive diseases and provide growers with objective risk management information to plan future plantings."

The Australian olive industry has expanded rapidly over the past decade.

One property in north-western Victoria alone has over 15 million trees.

Dr Tesoriero said such large-scale production requires a sophisticated and skilled management team to maintain trees that produce quality olives consistently.

"Irrespective of the size of the enterprise, Australian olive producers need access to pest and disease controls that are effective, economical, don't leave undesirable chemical residues in oil and are safe to workers and the environment," he said.

Locally produced olive oil is a high standard and has won numerous international awards.

Domestic olive oil production has reduced Australia's reliance on imports of sometimes dubious quality, while significant quantities are being exported to countries like the US.

The project is also examining controls for olive lace bug, a native Australian insect that attacks olives and causes leaf damage that weakens trees.

Contact Len Tesoriero, (02) 4640 6217, len.tesoriero@dpi.nsw.gov.au

Read more

- New EMAI biosecurity laboratories ready for anything
- Big chill, a blower and mature flies

- Ron Aggs

This article appears in the August 2012 edition of *Agriculture Today*.

AgricultureToday

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http://www.dpi.nsw.gov.au/aboutus/news/agriculture-today/august-2012/new-olive-varieties-found-less-susceptible-to-anthracnose [6/08/2012 1:21:51 PM]

Appendix 8. Raw Data from Investigations

EFFICACY ASSESSMENT OF INSECTICIDES AGAINST OLIVE LACE BUG

Lovedale Field Trial Olive Lace Bug Assessment 2009

Treatments	
1	Actara®
2	Lebaycid®
3	Water Control

Treatment	**Rep**	**Pretreatment count/40 twigs** 9/10/2009	**Posttreatment count (8 DAT spray1) /40 twigs** 17/10/2009	**Posttreatment count (19 DAT spray 1) /40 twigs** 28/10/2009	**Posttreatment count (8 DAT spray 2) /40 twigs** 5/11/2009
1	1	13	5	14	0
1	2	11	10	17	1
1	3	5	4	2	0
1	4	12	5	2	1
1	5	2	0	1	0
2	1	8	2	5	0
2	2	7	10	11	0
2	3	7	0	3	0
2	4	6	4	4	0
2	5	16	2	1	0
3	1	4	3	3	0
3	2	12	21	13	12
3	3	11	22	5	5
3	4	11	8	7	3
3	5	13	11	6	5

Pine Mountain Field Trial Olive Lace Bug Assessment 2010

Treatments	
1	Actara®
2	Shield Systemic Insecticide™
3	Lebaycid®
4	Water Control

Treatment	Rep	Pretreatment count/20 twigs 30/9/2010	Posttreatment count/20 twigs 11/10/2010
1	1	17	2
1	2	18	0
1	3	18	0
1	4	18	1
1	5	20	1
2	1	18	0
2	2	17	0
2	3	20	1
2	4	16	0
2	5	19	0
3	1	18	6
3	2	16	12
3	3	20	1
3	4	14	4
3	5	19	8
4	1	19	20
4	2	19	20
4	3	19	19
4	4	19	19
4	5	18	19

Hilldale Field Trial Olive Lace Bug Assessment 2011

Treatments	
1	Actara®
2	Samurai Systemic Insecticide™
3	Lebaycid®
4	Water Control

Treatment	**Rep**	**Pretreatment count/20 twigs** 4/10/2011	**Posttreatment count/20 twigs** 11/10/2011
1	1	10	3
1	2	5	1
1	3	6	0
1	4	11	0
2	1	7	0
2	2	6	0
2	3	11	0
2	4	14	0
3	1	12	3
3	2	8	2
3	3	9	0
3	4	9	0
4	1	8	10
4	2	11	14
4	3	7	19
4	4	11	18

Yellarbon Field Trial Olive Lace Bug Assessment 2011

Treatments	
1	Actara®
	Samurai Systemic Insecticide™
2	Lebaycid®
3	Water Control

Treatment	Rep	Pretreatment count/20 twigs 30/11/2011	Posttreatment count/20 twigs 12/12/2011
1	1	20	2
1	2	19	2
1	3	20	4
1	4	18	1
2	1	18	1
2	2	20	3
2	3	19	2
2	4	19	1
3	1	19	12
3	2	17	13
3	3	20	13
3	4	20	11
4	1	17	17
4	2	19	20
4	3	20	20
4	4	18	20

Coominya Field Trial Olive Lace Bug Assessment 2013

Treatments	
1	Sumi-Alpha® Flex
2	Water Control

Treatment	**Rep**	**Pretreatment count/20 twigs** 30/09/2013	**Posttreatment count/20 twigs** 7/10/2013
1	1	8	3
1	2	9	1
1	3	12	0
1	4	11	0
2	1	8	13
2	2	14	12
2	3	15	9
2	4	7	18

EFFICACY ASSESSMENT OF FUNGICIDES AGAINST OLIVE ANTHRACNOSE

Lovedale Field Trial Olive Fruit Assessment (cv. Barnea) 2009-10

Treatments	
1	Aero® 200g/100L
2	Tri-base Blue® 280mL/100L
3	Barmac copper oxychloride 500 g/kg
4	Water Control

Number of diseased mature fruit (%) at harvest

Treatment	Rep	Diseased fruit %
1	1	11.43
1	2	7.81
1	3	8.51
1	4	16.00
2	1	28.26
2	2	40.19
2	3	36.23
3	4	20.00
3	1	51.15
3	2	23.20
3	3	89.29
3	4	40.62
4	1	68.00
4	2	88.50
4	3	78.75
4	4	24.05

Boort Field Trial Olive Fruit Assessment (cv. Barnea) 2012

Treatments	
1	Aero® 200g/100L
2	Amistar® 80mL/100L
3	BB# 80mL/100L
4	NorShield 160g/100L
5	Tri-base Blue® 280mL/100L
6	Nil Control

Experimental chemical, not part of HAL trial

Cumulative number of diseased harvested mature fruit/200
***Incubated in humid chambers**

Treatment	Rep	Harvest	Day 7*
1	1	0	1
1	2	0	4
1	3	0	1
1	4	0	3
1	5	1	1
1	6	0	2
2	1	4	4
2	2	2	5
2	3	0	0
2	4	1	3
2	5	1	4
2	6	0	0
3	1	8	15
3	2	7	9
3	3	1	2
3	4	13	15
3	5	6	17
3	6	5	15
4	1	1	2
4	2	1	3
4	3	2	4
4	4	1	6
4	5	1	2
4	6	7	13
5	1	6	20
5	2	4	9
5	3	0	2
5	4	3	15
5	5	3	11
5	6	1	3
6	1	9	35
6	2	16	31
6	3	1	8
6	4	9	24
6	5	4	8
6	6	5	34

Coonalpyn Field Trial Olive Fruit Assessment (cv. Manzanillo) 2012

Treatments	
1	Aero® 200g/100L
2	Amistar ®80mL/100L
3	NorShield Systemic InsecticideTM 160g/100L
4	Tri-base Blue® 280mL/100L
5	Water Control

Cumulative number of diseased harvested mature fruit/100
***Incubated in humid chambers**

Treatment	Rep	Harvest	Day 4*
1	1	1	1
1	2	0	0
1	3	0	1
1	4	0	0
1	5	0	0
2	1	0	1
2	2	0	0
2	3	0	0
2	4	1	1
2	5	1	3
3	1	2	4
3	2	0	0
3	3	0	0
3	4	3	6
3	5	0	7
4	1	1	4
4	2	0	0
4	3	0	2
4	4	0	4
4	5	0	0
5	1	2	8
5	2	3	10
5	3	1	6
5	4	0	6
5	5	1	15

Olive Experiment O13-1 Detached fruit assay for chemical controls of anthracnose (*Colletotrichum acutatum*) in four olive cultivars.
Complete Randomised Design with 4 replicates of 25 fruit.

Treatment	Inoculum	cv Barnea @ 7 days			
		Rep 1	Rep 2	Rep 3	Rep 4
Nil (water)	5000 spores/fruit	16	15	14	14
Nil (water)	Nil	0	0	0	0
NorShield	5000 spores/fruit	10	9	8	10
Amistar®	5000 spores/fruit	7	3	7	5
Cabrio®	5000 spores/fruit	1	2	1	2
Polyram®	5000 spores/fruit	9	11	7	11
Aero®	5000 spores/fruit	1	3	3	2
		cv Picual @ 7 days			
Nil (water)	5000 spores/fruit	3	2	1	2
Nil (water)	Nil	0	0	0	0
NorShield	5000 spores/fruit	3	3	5	5
Amistar®	5000 spores/fruit	5	4	5	4
Cabrio®	5000 spores/fruit	3	4	2	3
Polyram®	5000 spores/fruit	6	5	9	8
Aero®	5000 spores/fruit	2	2	2	1
		cv Barnea @ 14 days			
Nil (water)	5000 spores/fruit	25	24	25	25
Nil (water)	Nil	0	0	0	0
NorShield	5000 spores/fruit	23	24	23	22
Amistar®	5000 spores/fruit	17	19	17	17
Cabrio®	5000 spores/fruit	8	7	5	12
Polyram®	5000 spores/fruit	20	22	19	18
Aero®	5000 spores/fruit	14	14	17	16
		cv Picual @ 14 days			
Nil (water)	5000 spores/fruit	23	22	22	22
Nil (water)	Nil	0	0	0	0
NorShield	5000 spores/fruit	15	15	15	15
Amistar®	5000 spores/fruit	15	13	15	14
Cabrio®	5000 spores/fruit	10	11	12	13
Polyram®	5000 spores/fruit	20	19	25	24
Aero®	5000 spores/fruit	11	10	8	8
		cv Barnea @ 21 days			
Nil (water)	5000 spores/fruit	25	25	25	25
Nil (water)	Nil	0	0	0	0
NorShield	5000 spores/fruit	25	24	25	25
Amistar®	5000 spores/fruit	23	22	21	19
Cabrio®	5000 spores/fruit	14	13	8	18
Polyram®	5000 spores/fruit	25	25	23	23
Aero®	5000 spores/fruit	23	24	22	24
		cv Picual @ 21 days			
Nil (water)	5000 spores/fruit	25	25	25	25

Nil (water)	Nil	0	0	0	0
NorShield	5000 spores/fruit	20	20	20	19
Amistar®	5000 spores/fruit	22	22	21	21
Cabrio®	5000 spores/fruit	18	19	19	20
Polyram®	5000 spores/fruit	25	25	25	25
Aero®	5000 spores/fruit	19	18	19	19
		cv Manzanillo @ 7 days			
Nil (water)	5000 spores/fruit	4	3	1	1
Nil (water)	Nil	0	0	0	0
NorShield	5000 spores/fruit	1	0	0	1
Amistar®	5000 spores/fruit	0	0	0	0
Cabrio®	5000 spores/fruit	0	0	0	0
Polyram®	5000 spores/fruit	0	1	1	2
Aero®	5000 spores/fruit	0	0	0	1
		cv Arbequina @ 7 days			
Nil (water)	5000 spores/fruit	5	2	1	1
Nil (water)	Nil	0	0	0	0
NorShield	5000 spores/fruit	3	1	1	1
Amistar®	5000 spores/fruit	1	0	2	0
Cabrio®	5000 spores/fruit	0	0	0	0
Polyram®	5000 spores/fruit	1	2	0	0
Aero®	5000 spores/fruit	1	0	0	0
		cv Manzanillo @ 14 days			
Nil (water)	5000 spores/fruit	7	7	6	4
Nil (water)	Nil	0	0	0	0
NorShield	5000 spores/fruit	5	7	7	6
Amistar®	5000 spores/fruit	3	2	2	0
Cabrio®	5000 spores/fruit	0	0	1	0
Polyram®	5000 spores/fruit	4	8	7	9
Aero®	5000 spores/fruit	4	1	5	5
		cv Arbequina @ 14 days			
Nil (water)	5000 spores/fruit	6	5	2	6
Nil (water)	Nil	0	0	0	0
NorShield	5000 spores/fruit	4	5	6	7
Amistar®	5000 spores/fruit	5	3	3	4
Cabrio®	5000 spores/fruit	0	0	1	1
Polyram®	5000 spores/fruit	4	4	1	2
Aero®	5000 spores/fruit	3	1	0	2
		cv Manzanillo @ 21 days			
Nil (water)	5000 spores/fruit	9	10	6	4

Nil (water)	Nil	0	0	0	0
NorShield	5000 spores/fruit	10	13	11	10
Amistar®	5000 spores/fruit	4	5	3	5
Cabrio®	5000 spores/fruit	1	0	4	2
Polyram®	5000 spores/fruit	9	15	11	12
Aero®	5000 spores/fruit	9	3	10	10
		cv Arbequina @ 21 days			
Nil (water)	5000 spores/fruit	10	8	5	7
Nil (water)	Nil	0	0	0	0
NorShield	5000 spores/fruit	9	12	11	13
Amistar®	5000 spores/fruit	10	10	8	10
Cabrio®	5000 spores/fruit	2	2	2	1
Polyram®	5000 spores/fruit	9	6	5	7
Aero®	5000 spores/fruit	8	3	2	5