AV504
Production, formulation and application of biological control agents for avocado anthracnose

Dr Lindy Coates
Queensland Department of Primary Industries

Know-how for Horticulture™

AV504

This report is published by the Horticultural Research and Development Corporation to pass on information concerning horticultural research and development undertaken for the avocado industry.

The research contained in this report was funded by the Horticultural Research and Development Corporation with the financial support of the avocado industry.

Cover Price $20.00

HRDC ISBN 1 86423 463 6

Published and Distributed by:

Horticultural Research and Development Corporation
Level 6
7 Merriwa Street
Gordon NSW 2072

Telephone: (02) 9418 2200
Fax: (02) 9418 1352

DPI
QUEENSLAND
DEPARTMENT OF
PRIMARY INDUSTRIES

FINAL REPORT

PROJECT NUMBER: AV504

Production, formulation and application of biological control agents for avocado anthracnose

July 1995 - June 1996

HRDC

AUSTRALIAN
AVOCADO
GROWERS'
FEDERATION INC

ACKNOWLEDGMENTS

The project team gratefully acknowledge the financial support of the following organisations:

- The Australian Avocado Growers' Federation
- The Horticultural Research and Development Corporation
- The Department of Primary Industries, Queensland

We would also like to sincerely thank Dr Marcelle Stirling, Mr Ken Pegg, Ms Rosemary Kopittke and the avocado growers involved with field trials at Duranbah, Bellthorpe, Glasshouse and Woombye.

The Project Team:

Dr Lindy Coates	Senior Plant Pathologist, DPI
Mr Tony Cooke	District Experimentalist, DPI
Ms Kathy Cannon	Temporary Laboratory Technician, DPI
Ms Jan Dean	Temporary Laboratory Technician, DPI

TABLE OF CONTENTS

1. SUMMARY

Industry Summary

The aim of this project was to optimise the production, formulation and application of selected biocontrol agents for the control of avocado anthracnose. Biocontrol agents to be used in this project were selected in a previous project (HRDC Project No. AV207).

In the first year of the project, *Bacillus* sp. 359 was evaluated in field trials which were established at three different sites in south-eastern Queensland and northern New South Wales. The isolate was applied (with or without a nutrient base) at monthly intervals from fruit set to harvest, and was compared to the standard copper treatment. None of the treatments tested, including copper, *significantly* reduced anthracnose at any of the field sites. This was attributed to:

- the high degree of tree-to-tree variation in fruit disease levels exhibited at each of the field sites,
- high rainfall during fruit development, and
- poor survival of *Bacillus* sp. 359 on avocado leaf surfaces.

A laboratory trial was also conducted to examine the effect of ethylene-ripening on post harvest disease levels in avocado fruit. "Fuerte" avocado fruit ripened with ethylene developed far less disease (both anthracnose and stem end rot) than those ripened in air at the same temperature. This was most likely due to the rapid ripening of ethylene-treated fruit compared to air-ripened fruit, as disease development is not only related to fruit ripeness stage, but also to length of time after harvest.

A feasibility study for the commercialisation of anthracnose biocontrol agents was commissioned by HRDC for the AAGF during the first year of the project. The consultant's report concluded that there were serious constraints to commercialising this technology at the present time. As a result of this study, the project was terminated as of June 30, 1996. Further evaluation of biocontrol agents for avocado anthracnose was therefore discontinued at this point in time.

Technical Summary

Bacillus sp. 359, previously shown to be an effective biocontrol agent for the suppression of *Colletotrichum gloeosporioides*, was evaluated in a series of field trials on avocado cvs Hass and Fuerte. Monthly spray applications of 359, with or without a nutrient base, were made from fruit set to harvest and were compared to the standard copper treatment. None of the treatments tested, including copper, *significantly* reduced anthracnose at any of the field sites. This was attributed to

- the high degree of tree-to tree variation in fruit disease levels exhibited at each of the field sites,
- high rainfall during fruit development, and
- poor survival of 359 on avocado leaf surfaces.

A laboratory trial was also conducted to examine the effect of ethylene-ripening on postharvest disease levels in avocado fruit. "Fuerte" avocado fruit ripened with ethylene developed far less disease (both anthracnose and stem end rot) than those ripened in air at the same temperature. This was most likely due to the rapid ripening of ethylene-treated fruit compared to air-ripened fruit, as disease development is not only related to fruit ripeness stage, but also to length of time after harvest.

2. RECOMMENDATIONS

Extension/adoption by industry

Due to the discontinuation of the project after the first year, further development of biocontrol agents for avocado anthracnose will not take place at the present time. Aside from commercialisation constraints, inconsistent antagonist performance in the field has emerged as a problem which must be addressed before a product can be developed.

At the present time, however, immediate benefits could be gained by further promoting the practice of controlled ripening for avocado fruit. The reduction in both anthracnose and stem end rot levels as a result of this practice should not be underestimated.

Directions for future research

If funds should become available in the future to continue biocontrol research, antagonist survival and application methods will need to be investigated more fully. The causes of high tree-to-tree variability in fruit disease levels should also be investigated, as this may be the key to achieving good control of anthracnose. The role of nutrition (eg calcium), canopy management, tree health and fruit maturity in this variability should be determined.

A recent report prepared by Dr Ian Muirhead for the avocado industry has summarised many of the options available for future anthracnose research.

Financial/commercial benefits

This aspect has already been dealt with in considerable detail as a result of the Hassall Report commissioned by HRDC on behalf of the AAGF.

3. TECHNICAL REPORT

Introduction

Anthracnose caused by *Colletotrichum gloeosporioides* and *C. acutatum* continues to be the most serious disease of avocado fruit in Australia. Major losses due to anthracnose occur after harvest during marketing, and serious pre-harvest losses also occur in some cultivars such as Fuerte (Fitzell, 1987).

Constraints on chemical use in horticulture, particularly in relation to pesticides applied postharvest, continue to increase. Recent examples of postharvest fungicide withdrawals in Australia include Benlate^{R} (benomyl) and Ronilan^{R} (vinclozolin). While these restrictions have not yet affected the avocado industry, it is unlikely that pesticides used on avocado will not suffer some form of restriction on use in the future. Non-chemical alternatives for disease control, such as biological control, need to be explored.

In a previous project (AV207), bacteria and yeasts isolated from avocado fruit and leaf surfaces were evaluated for potential as biological control agents for anthracnose. A small group of isolates with considerable antagonistic activity against the anthracnose pathogens were selected for further study (Coates *et al.*, 1995; Stirling *et al.*, 1995).

The aim of the current project was to further evaluate the most promising of these isolates with a view to producing a formulation which could be used on a large scale for the control of anthracnose. During the first year of the project, a consultants' firm was commissioned by HRDC to investigate strategies for the commercialisation of anthracnose biocontrol technology. The consultant's report concluded that there were serious constraints to commercialising this technology at the present time. As a result, project AV504 was terminated as of June 30 1996. Results presented in this report therefore comprise only one year of research.

Materials and Methods

Field evaluation of *Bacillus* sp. 359

Field trials to evaluate the performance of *Bacillus* 359 in three different growing regions were established at the following sites:

1. Duranbah, northern NSW,
2. Bellthorpe, south-eastern Qld.,
3. Woombye, south-eastern Qld.,

At the Duranbah and Bellthorpe sites, the following treatments were applied to "Hass" avocado trees as monthly sprays from fruit set to harvest:

1. Water (control)
2. *Bacillus* 359 in water
3. Diluted culture media (control)
4. *Bacillus* 359 in diluted culture media
5. Copper hydroxide (Kocide^{R} 2 gL^{-1})
6. Integrated treatment (alternating sprays of copper hydroxide and 359)

The first spray was applied in late October 1995, and the final spray in early June 1996. Due to frequent showery weather during fruit development, it was not always possible to apply treatments on the scheduled date. For this reason, spray intervals were sometimes more or less than four weeks (see Table 1 for actual spray dates).

At the Woombye sites, the following treatments were applied to both "Fuerte" and "Hass" avocado trees in late October 1995:

1. Water (control)
2. *Bacillus* 359 in water
3. Copper hydroxide (Kocide^{R} 2gL^{-1})

A severe hail storm in November caused major fruit damage and losses at this site. A new trial was therefore established in the Glasshouse Mountains region, with the first spray being applied in late November. As for the Woombye site, both "Fuerte" and "Hass" trees were selected for treatment at the Glasshouse site.

A randomised block design was used at each field site. At Duranbah, 4 x ½ tree replications were used for each treatment. At Bellthorpe and Glasshouse, 4 x 1/3 tree replications were used for each treatment.

Ordinary tap-water which had been allowed to sit outdoors in clear plastic containers for 48 hours prior to each scheduled spray date was used in all trials. This procedure, through exposure to sunlight, causes chlorine breakdown. If chlorine is not removed from the water source, survival of the antagonist may possibly be reduced. Water used in previous field trials conducted under Project AV207 was autoclaved to remove chlorine. While this procedure effectively removes chlorine, it is not commercially feasible.

Bacillus 359 was cultured in yeast-glucose-peptone broth for 36 hours. For the "359 in water" treatment, 2 x 600-800 mL cultures (depending on the volume to be sprayed onto trees) were centrifuged and then resuspended in 600-800 mL of water. For the "359 in diluted media" and the "359 integrated" treatments, 1 x 600-800 mL culture was centrifuged and added to an uncentrifuged 600-800 mL culture. Antagonist preparations were then diluted 1 in 10 with water, and Tween-80 was added at a rate of 0.05% (v/v). Viable cell concentrations were determined by serial dilution and plating onto yeast-glucose-peptone agar.

For the "integrated" treatment, alternating monthly sprays of *Bacillus* 359 and copper hydroxide were applied. In order to monitor antagonist survival, leaf samples were taken from all treatments (except copper) immediately *after* every second spray and also immediately *before* the following spray (which was approximately 30 days later). The methods used for processing leaves were described in detail in the AV207 Final Report. Leaf samples were also taken from the water, diluted media and copper treatments at the Duranbah field site in December 1995, March and June 1996. The objective of this sampling was to gather more data on the effect of copper on natural microflora populations in avocado orchards. A plate-dilution frequency / most probable number method (Andrews and Kenerley, 1978; Meynell and Meynell, 1970) was used to estimate numbers of bacteria, yeasts and filamentous fungi on leaf surfaces.

Fruit were harvested from each site at commercial maturity. At the Glasshouse site, "Fuerte" fruit were harvested in April and "Hass" fruit in May. At Duranbah and Bellthorpe, "Hass" fruit were harvested in July. Once harvested, fruit were transported to the laboratory where they were weighed, sized and assessed for injury (insect and mechanical) and field disease symptoms. Fruit were then stored at 24^0C and assessed daily for firmness. When judged to be ripe, "Hass" fruit were cut into quarters, peeled and examined for symptoms of anthracnose and stem end rot. "Fuerte" fruit were examined externally for disease. The estimated fruit surface area affected by each of the diseases was recorded.

Incidence data was arc-sine transformed prior to analysis. All data was analysed by analysis of variance using Ran-B (DPI statistical analysis program). Treatment means were compared using the LSD test at $P = 0.05$.

Disease assessment of avocado fruit from sprayed and unsprayed orchards at Duranbah

A sampling study was conducted to compare postharvest disease levels in adjacent unsprayed and sprayed avocado orchards at Duranbah, Northern NSW. Three orchards were included in the study, two of which had not been sprayed with pesticides for several years. Trees sampled in the unsprayed orchards were approximately 10-15 years old, and those sampled in the sprayed orchard varied from 5 - 25 years old. It should be noted that the sprayed orchard was the same one used in the field evaluation of *Bacillus* 359. On each of the unsprayed orchards, five "Hass" trees were randomly selected. On the sprayed orchard, five sprayed and five unsprayed "Hass" trees were selected. Approximately twenty fruit were harvested from each tree and transported to the laboratory where they were held at 24^0C. Fruit were assessed daily for firmness, and when judged to be ripe, were rated for anthracnose and stem end rot as previously described. In addition to these fruit, a sample of copper-sprayed fruit from the field biocontrol trial was included in the evaluation.

Disease development in ethylene-ripened avocado fruit

Freshly harvested "Fuerte" avocado fruit were surface-sterilised with 70% ethanol and inoculated with *C. gloeosporioides* (5 x 10^5 spores/mL) in marked areas on the fruit surface. Fruit were incubated at 25^0C under high humidity for 24 h, and then half of the fruit were transferred to an ethylene ripening room (100 ppm C_2H_4 at 18^0C) for 48 h. The remaining half of fruit were stored in normal air at 18^0C for 48 h. For each treatment (ie. ethylene or non-ethylene treated) there were 8 replicates of ten fruit. All fruit were then held at 24^0C and assessed daily for firmness. When fruit were judged to be ripe, the diameter of anthracnose lesions within inoculated areas was measured. Natural infection levels (anthracnose and stem end rot) were also assessed and expressed as the percentage surface area affected by each disease. Data was analysed by analysis of variance, and treatment means compared using the LSD test at $P = 0.05$.

Results and Discussion

Field evaluation of *Bacillus* 359

The concentration of *Bacillus* 359 in spray suspensions applied to avocado trees is shown in Table 1. On average, the concentration of the '359 in water' treatment was marginally lower than that of the '359 in diluted media' treatment at both the Duranbah and Bellthorpe field sites. This is most likely due to the method of preparing antagonist suspensions (ie more centrifuging required for the '359 in water' treatment). The mean concentration of 359 in spray suspensions ranged from 9.3 x 10^7 to 2.5 x 10^8 cfu/mL across all treatments and growing regions (Table 1).

Table 1. Field evaluation of *Bacillus* 359: concentration of 359 in spray suspensions

(a) Duranbah

Spray date	Antagonist concentration cfu/mL		
	359 in water	**359 in diluted culture media**	**359 integrated treatment[1]**
20/10/95	5.1 x 10^7	6.9 x 10^7	-
23/11/95	5.8 x 10^7	7.1 x 10^7	7.6 x 10^7
14/12/95	5.3 x 10^7	3.0 x 10^8	-
17/1/96	1.9 x 10^8	1.6 x 10^8	1.2 x 10^8
7/2/96	1.5 x 10^7	5.6 x 10^8	-
6/3/96	1.1 x 10^8	3.6 x 10^8	1.2 x 10^8
3/4/96	2.3 x 10^8	4.1 x 10^8	-
20/5/96	1.3 x 10^7	1.5 x 10^8	2.3 x 10^8
12/6/96	1.2 x 10^8	1.9 x 10^8	-
MEAN OF ALL DATES	**9.3 x 10^7**	**2.5 x 10^8**	**1.4x 10^8**

(b) Bellthorpe

Spray date	Antagonist concentration cfu/mL		
	359 in water	359 in diluted culture media	359 integrated treatment[1]
26/10/95	1.8×10^8	1.7×10^8	5.3×10^8
24/11/95	1.4×10^8	4.2×10^8	-
20/12/95	6.2×10^7	9.9×10^7	1.0×10^8
29/1/96	2.9×10^6	2.7×10^8	-
14/2/96	1.8×10^8	2.4×10^8	2.7×10^8
14/3/96	7.0×10^7	4.8×10^7	-
10/4/96	1.4×10^8	8.3×10^7	1.1×10^8
10/5/96	1.1×10^8	6.2×10^7	-
6/6/96	8.4×10^6	4.5×10^7	1.2×10^8
MEAN OF ALL DATES	**9.9×10^7**	**1.6×10^8**	**2.3×10^8**

(c) Glasshouse

Spray date	Antagonist concentration cfu/mL	
	359 in water - Fuerte	359 in water - Hass
24/11/95	3.3×10^8	3.9×10^8
20/12/95	1.0×10^8	1.3×10^8
29/1/96	3.4×10^6	1.9×10^6
14/2/96	9.6×10^7	5.0×10^7
14/3/96	7.8×10^7	1.2×10^8
10/4/96	6.7×10^7	7.7×10^7
10/5/96	-[2]	1.0×10^8
MEAN OF ALL DATES	**1.1×10^8**	**1.2×10^8**

[1] **359 only applied every second month (copper applied every other month)**

[2] **fruit harvested 22/4/96.**

Table 2 shows the survival of *Bacillus* 359 at each field site over the duration of the trial. Overall, survival of 359 was greatest at the Duranbah site. Numbers of 359 always declined over the four week period following spraying. At Duranbah, numbers of 359 usually remained above 10^4 cfu/g, although in a few cases declined to very low levels. This was particularly the case in the '359 integrated' treatment, probably because of the presence of copper residues on leaf surfaces.

Survival of *Bacillus* 359 at the Bellthorpe and Glasshouse field sites was generally very poor. Of particular concern were the low numbers of 359 recorded immediately after spraying at several spray dates. In some cases, the occurrence of rainfall very soon after treatment application may have been responsible for the poor establishment of 359 populations of leaf surfaces. This may have also affected the copper treatment.

At each of the three field sites, there were no significant treatment effects on anthracnose levels in ripe fruit (Tables 3(a), 4(a) and 5(a)). Even the copper treatment did not significantly reduce anthracnose and was usually no lower in anthracnose than the 'water only' treatment. There were also no significant treatment effects on stem end rot, although the copper and "359 integrated" treatments tended to have the lowest stem end rot values at both the Duranbah and Bellthorpe field sites. Stem end rot levels were very low in both 'Fuerte' and 'Hass' avocado fruit harvested from the Glasshouse site. Surprisingly, anthracnose levels were much lower in 'Fuerte' than 'Hass" fruit at the Glasshouse site.

Only at the Duranbah site were there any significant treatment effects on the percentage of fruit with 5% of less disease (Table 3(a)). At this site, the copper and "359 integrated" treatments had significantly more fruit with 5% or less disease than the other 359 treatments. Given the poor performance of 359 alone, it seems likely that the reduction is disease observed in the '359 integrated' treatment is largely due to the copper component of the treatment.

At the Duranbah site, algal spot was observed on the leaves and fruit which did not receive copper sprays (Table 3(a)). Algal spot was particularly severe on trees sprayed with 'diluted media' only or '359 in diluted media'. Algal spot was not observed at the Bellthorpe and Glasshouse field sites.

Tables 3(b), 4(b) and 5(b) show the effect of orchard position on disease, fruit size and ripening time. At the Bellthorpe site (Table 4(b)), anthracnose levels in fruit harvested from block 1 were much greater than fruit harvested from the other three blocks. Fruit from block 1 were on average larger and more rapid to ripen than those from other blocks. There was also considerable variation in 'Hass' fruit harvested from the Glasshouse site in terms of anthracnose, stem end rot and ripening rate (Table 5 (b)).

The effect of copper sprays on natural populations of phylloplane microorganisms at the Duranbah field site is shown in Table 6. Copper sprays consistently reduced the number of bacteria and yeasts on avocado leaf surfaces throughout the year, which is consistent with the findings of Stirling (1996). Copper reduced numbers of filamentous fungi only at the March and June sampling dates. The 'diluted media' treatment on the other hand increased numbers of bacteria, yeasts and filamentous fungi on avocado leaves in the majority of cases. This parallels previous findings in Project AV207.

Table 2. Field evaluation of *Bacillus* 359: survival of 359 on avocado leaf surfaces

(a) Duranbah

Sampling date	Sample type	Mean number of 359 colonies (log_{10}cfu/g fresh weight ± s.e)		
		359 in water	359 in diluted culture media	359 integrated
23/11/95	Post-spray	5.26 ± 0.1	6.67 ± 0.2	5.30 ± 0.1
14/12/95	Pre-spray	4.56 ± 0.5	4.67 ± 0.2	3.84 ± 1.4
17/1/96	Post-spray	5.03 ± 0.1	5.95 ± 0.1	5.19 ± 0.1
7/2/96	Pre-spray	4.49 ± 0.2	4.96 ± 0.2	2.57 ± 0.9
6/3/96	Post-spray	5.80 ± 0.4	7.01 ± 0.1	7.06 ± 0.2
3/4/96	Pre-spray	1.10 ± 1.1	5.56 ± 0.2	4.15 ± 1.4
20/5/96	Post-spray	6.29 ± 0.1	6.33 ± 0.8	6.05 ± 0.3
12/6/96	Pre-spray	5.71 ± 0.1	4.10 ± 1.4	0

(b) Bellthorpe

Sampling date	Sample type	Mean number of 359 colonies (log_{10}cfu/g fresh weight ± s.e)		
		359 in water	359 in diluted culture media	359 integrated
26/10/95	Post-spray	1.85 ± 1.1	4.19 ± 0.4	4.31 ± 0.2
24/11/95	Pre-spray	0	0	0
20/12/95	Post-spray	4.79 ± 0.1	5.95 ± 0.3	4.81 ± 0.1
29/1/96	Pre-spray	0	0	0
14/2/96	Post-spray	4.45 ± 0.2	5.01 ± 0.2	3.99 ± 0.2
14/3/96	Pre-spray	1.32 ± 1.3	1.2 ± 1.2	1.3 ± 1.1
10/4/96	Post-spray	6.27 ± 0.3	5.87 ± 0.2	6.63 ± 0.3
10/5/96	Pre-spray	3.30 ± 1.3	4.49 ± 0.3	2.77 ± 1.0

(c) Glasshouse

Sampling date	Sample type	Mean number of 359 colonies (log_{10}cfu/g fresh weight ± s.e)	
		359 in water - Fuerte	359 in water - Hass
20/12/95	Post-spray	4.08 ± 0.3	5.67 ± 0.4
29/1/96	Pre-spray	0	0
14/2/96	Post-spray	3.89 ± 0.3	4.53 ± 0.5
14/3/96	Pre-spray	1.04 ± 1.0	0
10/4/96	Post-spray	3.62 ± 0.1	5.14 ± 0.1
10/5/96	Pre-spray	no sample taken	4.88 ± 0.3

Table 3. Field evaluation of *Bacillus* 359: the effect of monthly pre-harvest spray applications of 359 on postharvest disease levels in "Hass" avocados harvested from the Duranbah field site.

(a) effect of treatment

	Water (control)	359 in water	Diluted culture media (control)	359 in diluted culture media	359 integrated	Copper
Anthracnose (% area)	25.5 a[1]	31.7 a	36.4 a	31.9 a	29.9 a	25.0 a
Stem end rot (% area)	13.8 a	12.4 a	4.5 a	8.8 a	2.2 a	2.2 a
% fruit with 5% or less disease	0.48[2] ab (21.0)	0.30 b (8.4)	0.44 ab (18.4)	0.29 b (8.1)	0.64 a (35.4)	0.68 a (39.7)
Ripening time (days)	12.7 a	12.2 a	12.3 a	13.3 a	11.9 a	12.3 a
Fruit weight (g)	247.6 a	240.9 a	254.6 a	247.1 a	243.8 a	244.7 a
Fruit diameter (mm)	72.1 a	71.6 a	73.2 a	72.0 a	73.0 a	72.8 a
Insect injury (% area)	1.0 a	0.4 a	0.8 a	0.3 a	0.5 a	0.2 a
Physical injury (% area)	3.3 a	3.4 a	2.9 a	2.3 a	1.9 a	2.8 a
Algal spot (% area)	15.0 a	10.8 ab	21.4 a	18.2 a	1.1 b	0.1 b
No. of fruit harvested	83	62	93	95	65	96

[1] **Means followed by the same letter *within rows* do not differ significantly at P = 0.05**

2 Arc-sine transformed means. Equivalent means shown in parentheses

(b) effect of orchard position

	Block 1	Block 2	Block 3	Block 4	Significance level
Anthracnose (% area)	20.7	35.0	36.0	28.6	NS[1]
Stem end rot (% area)	5.2	5.1	11.8	7.1	NS
Ripening time (days)	13.1	12.6	11.5	12.5	NS
Fruit weight (g)	233.1	239.8	259.6	253.3	NS
Fruit diameter (mm)	70.6	71.8	74.3	73.2	0.025

[1] **NS = not significant**

Table 4. Field evaluation of *Bacillus* 359: the effect of monthly pre-harvest spray applications of 359 on postharvest disease levels in "Hass" avocados harvested from the Bellthorpe field site.

(a) effect of treatment

	Water (control)	359 in water	Diluted culture media (control)	359 in diluted culture media	359 integrated	Copper
Anthracnose (% area)	6.3 a[1]	13.3 a	17.1 a	17.9 a	7.0 a	7.8 a
Stem end rot (% area)	8.0 a	8.5 a	6.5 a	7.8 a	3.8 a	3.6 a
% fruit with 5% or less disease	0.69[2] a (40.3)	0.59 a (31.4)	0.62 a (34.2)	0.59 a (30.7)	0.76 a (47.6)	0.97 a (68.5)
Ripening time (days)	11.7 a	12.2 a	11.8 a	12.1 a	12.5 a	13.1 a
Fruit weight (g)	256.2 a	240.3 a	247.1 a	245.5 a	260.0 a	244.7 a
Fruit diameter (mm)	71.7 a	73.2 a	71.0 a	70.6 a	72.5 a	70.5 a
Insect injury (% area)	0.1 a	0.2 a	0.4 a	0.1 a	0.1 a	0.1 a
Physical injury (% area)	2.5 abc	2.3 bcd	3.2 a	2.8 ab	1.6 d	1.5 d
No. of fruit harvested	230	238	171	155	183	225

[1] **Means followed by the same letter *within rows* do not differ significantly at P = 0.05**

2 Arc-sine transformed means. Equivalent means shown in parentheses

(b) effect of orchard position

	Block 1	Block 2	Block 3	Block 4	Significance level
Anthracnose (% area)	28.9	6.1	4.4	7.0	> 0.01
Stem end rot (% area)	5.5	5.3	6.2	8.5	NS[1]
Ripening time (days)	11.0	12.7	13.0	12.1	> 0.01
Fruit weight (g)	282.6	229.5	243.9	239.7	> 0.01
Fruit diameter (mm)	76.2	68.6	69.9	71.6	> 0.01

[1] **NS = not significant**

Table 5. Field evaluation of *Bacillus* 359: the effect of monthly pre-harvest spray applications of 359 on postharvest disease levels in "Fuerte" and "Hass" avocados harvested from the Glasshouse field site.

(a) effect of treatment

	Fuerte			Hass		
	Water (control)	359 in water	Copper	Water (control)	359 in water	Copper
Anthracnose (% area)	1.9 a[1]	2.3 a	0.4 a	14.6 a	19.7 a	18.2 a
Stem end rot (% area)	0.4 a	0.2 a	0.1 a	1.2 a	1.0 a	1.2 a
% fruit with 5% or less disease	1.23[2] a (88.8)	1.10 a (79.7)	1.42 a (97.7)	0.92 a (63.6)	0.87 a (58.6)	0.97 a (68.5)
Ripening time (days)	12.4 a	12.2 a	12.4 a	10.3 a	10.5 a	11.1 a
Fruit weight (g)	243.1 a	254.0 a	237.1 a	224.2 a	213.2 a	219.1 a
Fruit diameter (mm)	68.7 a	69.4 a	68.7 a	68.9 a	67.5 a	67.6 a
Insect injury (% area)	0.7 a	1.0 a	0.3 a	0.9 a	0.7 a	0.3 a
Physical injury (% area)	2.6 a	1.1 b	1.0 b	3.0 a	3.2 a	1.8 b
No. of fruit harvested	85	117	85	230	144	174

[1] **Means followed by the same letter *within rows* do not differ significantly at P = 0.05**

2 Arc-sine transformed means. Equivalent means shown in parentheses

(b) effect of orchard position - Fuerte

	Block 1	Block 2	Block 3	Block 4	Significance level
Anthracnose (% area)	2.1	0.6	3.1	0.3	NS[1]
Stem end rot (% area)	0.6	0.2	0.2	0	NS
Ripening time (days)	12.4	11.1	14.2	11.7	NS
Fruit weight (g)	250.1	245.7	258.2	224.9	NS
Fruit diameter (mm)	69.6	69.7	70.0	66.5	0.040

(c) effect of orchard position - Hass

	Block 1	Block 2	Block 3	Block 4	Significance level
Anthracnose (% area)	29.8	13.9	6.0	20.2	0.028
Stem end rot (% area)	1.0	0.3	0.5	2.8	0.034
Ripening time (days)	11.9	10.4	9.5	10.7	0.021
Fruit weight (g)	207.3	202.5	231.7	233.9	NS
Fruit diameter (mm)	66.1	66.7	69.0	70.2	NS

[1] **NS = not significant**

Table 6. Field evaluation of *Bacillus* 359: the effect of copper oxychloride sprays on natural populations of phylloplane microorganisms on "Hass" avocado leaf surfaces (Duranbah field site)

Treatment	Mean no. of microorganisms (log_{10} cfu/g fresh weight ± s.e.)								
	14.12.95			6.3.96			12.6.96		
	Bacteria	Yeasts	Fil. fungi	Bacteria	Yeasts	Fil. fungi	Bacteria	Yeasts	Fil. fungi
Water	4.90 ± 0.3	4.55 ± 0.3	3.97 ± 0.3	5.97 ± 0.2	5.13 ± 0.2	5.60 ± 0.1	6.87 ± 0.1	5.05 ± 0.3	5.51 ± 0.2
Diluted media	5.59 ± 0.3	4.72 ± 0.4	5.14 ± 0.2	6.33 ± 0.2	5.89 ± 0.2	5.59 ± 0.1	6.66 ± 0.2	5.46 ± 0.4	5.60 ± 0.3
Copper	4.10 ± 0.3	1.89 ± 1.0	4.86 ± 0.3	5.32 ± 0.3	4.60 ± 0.3	4.63 ± 0.3	6.49 ± 0.3	4.96 ± 0.2	5.14 ± 0.2

Disease assessment of 'Hass' avocado fruit from sprayed and unsprayed orchards at Duranbah

Levels of anthracnose in "Hass" avocado fruit from neighbouring unsprayed and sprayed orchards are shown in Table 7(a) - (e). Anthracnose levels were found to be around three times higher in the sprayed orchard than in both of the unsprayed orchards. This difference was not simply the effect of tree age, as similar levels of anthracnose were observed in both old (Table 7(c)) and young (Table 7(e)) trees on the sprayed orchard. While it is not possible to draw direct conclusions form this sampling study because of the possible variation in a number of factors between the three orchards, the study does highlight *possible* inadequacies with the copper spray treatment. Poor copper coverage seems an unlikely reason for the high anthracnose levels on orchard 3 since the young trees on this orchard were hand-sprayed to the point of run-off at intervals of four weeks from fruit set to harvest.

The study also highlights the high degree of variability in anthracnose levels between individual trees. The reasons for this variability need to be understood before effective control measures for anthracnose can be developed.

Table 7. Disease assessment of "Hass" avocado fruit from sprayed and unsprayed orchards at Duranbah.

(a) Orchard 1: Trees 10-15 years old, unsprayed with pesticides for a number of years.
Number of trees sampled: Five (selected from throughout the entire orchard).
Harvest date: 1 July 1996.

	Tree 1	Tree 2	Tree 3	Tree 4	Tree 5	Average
Number of fruit sampled	17	19	18	18	19	
Anthracnose (mean % area)	1.4	27.4	13.8	9.4	2.5	10.9
Stem end rot (mean % area)	0.2	2.6	2.2	2.8	1.6	2.1
% fruit with spotting bug damage	11.8	15.8	11.1	38.9	21.0	19.7

(b) Orchard 2: Trees 10-15 years old, unsprayed with pesticides for a number of years.
Number of trees sampled: Five (selected from throughout the entire orchard).
Harvest date: 1 July 1996.

	Tree 1	Tree 2	Tree 3	Tree 4	Tree 5	Average
Number of fruit sampled	17	19	17	17	18	
Anthracnose (mean % area)	22.6	12.5	11.2	4.9	2.1	10.7
Stem end rot (mean % area)	0.8	2.7	3.6	0	0.4	1.5
% fruit with spotting bug damage	11.8	10.5	0	17.6	5.6	9.1

(c) Orchard 3: Trees over 25 years old, sprayed regularly with pesticides
Number of trees sampled: Five (fruit were harvested from a single row.).
Harvest date: 1 July 1996.

	Tree 1	Tree 2	Tree 3	Tree 4	Tree 5	Average
Number of fruit sampled	19	18	20	19	18	
Anthracnose (mean % area)	57.3	34.2	10.5	6.6	45.7	30.9
Stem end rot (mean % area)	2.7	1.1	3.2	1.3	2.7	2.2
% fruit with spotting bug damage	0	5.6	5.0	0	0	2.1

(d) Orchard 3: Trees 5-10 years old, had not received pesticides for 1995-1996 season.
Number of trees sampled: Five (fruit were harvested from a single row of trees).
Harvest date: 1 July 1996.

	Tree 1	Tree 2	Tree 3	Tree 4	Tree 5	Average
Number of fruit sampled	20	18	20	18	19	
Anthracnose (mean % area)	41.3	26.7	33.8	56.4	47.4	41.1
Stem end rot (mean % area)	1.9	5.5	3.5	7.4	0.6	3.8
% fruit with spotting bug damage	0	0	0	0	0	0

(e) Orchard 3: Trees 5-10 years old, and were part of the biocontrol field trial.
Trees were hand-sprayed regularly with copper fungicide during the 1995-1996 season.
Number of trees sampled: Four (randomly located in trial plot).
Harvest date: 1 July 1996.

	Tree 1	Tree 2	Tree 3	Tree 4	Tree 5	Average
Number of fruit sampled	20	20	20	21	--	
Anthracnose (mean % area)	15.2	39.0	35.1	15.6	--	26.2
Stem end rot (mean % area)	1.8	4.0	0.3	0.4	--	1.6
% fruit with spotting bug damage	0	0	0	0	--	0

Disease development in ethylene-ripened avocado fruit

Table 8 compares disease levels and ripening times in ethylene and air-ripened 'Fuerte' avocado fruit. In all cases the differences between ethylene and air-ripened fruit were significant. With ethylene treatment, fruit ripened faster, more uniformly and with less disease.

Table 8. Postharvest disease levels in ethylene-ripened "Fuerte" avocado fruit

	Air-ripened	Ethylene-ripened
Lesion diameter (mm) in inoculated area	4.2 a[1]	0.4 b
Natural anthracnose infection (% area affected)	5.1 a	1.1 b
Natural stem end rot infection (% area affected)	3.2 a	0.2 b
Mean ripening time (days)	8.3 a	6.1 b

1 Means followed by the same letter *within rows* do not differ significantly at P = 0.05.

Conclusions

- None of the treatments tested, including copper, significantly reduced anthracnose in any of the field trials conducted. The large tree-to-tree variation in fruit disease levels may partly account for the lack of treatment effect in these trials. Frequent rainfall during fruit development was probably also an important factor in the poor performance of treatments, as was inadequate survival of antagonists on avocado leaf and fruit surfaces. These factors could help explain why *Bacillus* 359 consistently performs in the laboratory (on detached avocado *and* mango fruit) but not in the field. Clearly more effort must be directed towards improving application methods and survival capacity of this antagonist.

- Controlled ripening of 'Fuerte' avocado fruit with ethylene resulted in greatly reduced anthracnose and stem end rot levels, as well as more rapid and uniform fruit ripening.

References

Andrews, J.H. and Kenerley, C.M. 1978. The effects of a pesticide program on non-target epiphytic microbial populations of apple leaves. *Canadian Journal of Microbiology,* **24:** 1058-72

Coates, L.M., Stirling, A.M., Cooke, A.W. and Cannon, K.T. 1995. Biological control of avocado anthracnose. *Proceedings of the Australian Avocado Growers' Federation Conference,* Fremantle, April 30 - 3 May, 1995.

Fitzell, R.D. 1987. Epidemiology of anthracnose disease of avocados. *South African Avocado Growers' Association Yearbook* **10:** 113-16.

Meynell, G.G. and Meynell, E. 1970. *Theory and Practice in Experimental Bacteriology.* Second Edition. Cambridge University Press.

Stirling, A.M. 1996. The role of epiphytic microorganisms in the suppression of *Colletotrichum gloeosporioides* on avocado. PhD thesis. The University of Queensland.

Stirling, A.M., Coates, L.M., Pegg. K.G. and Hayward, A.C. 1995. Isolation and selection of bacteria and yeasts antagonistic to preharvest infection of avocado by *Colletotrichum gloeosporioides. Australian Journal of Agricultural Research,* **46:** 985-95.