

**Generation of residue, efficacy and crop
safety data for pesticide minor-use permit
applications – Captan fungicide**

John Duff
The Department of Agriculture, Fisheries and
Forestry, Qld

Project Number: VG11027

VG11027

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**Generation of residue, efficacy and crop
safety data for pesticide minor-use permit
applications – Captan fungicide**

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Purpose of the report

This report provides the necessary crop residue data and permit application to aid the APVMA in issuing a minor use permit for the use of Captan fungicide for the control of Botrytis in leafy lettuce, cucumber, capsicum and chilli grown both in the field and under protected cropping situations

Report Completed –June 2013.

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Media Summary

Horticulture Australia and the Australian Vegetable industry are seeking a permit for captan (various formulations) for the control of grey mould (*Botrytis cinerea*) in cucumber, capsicums, chilli peppers and leafy lettuce crops in protected cropping situations for use in all states. Grey mould is a common fungal disease in capsicum, cucumber, chilli and leafy lettuce crops grown in protected cropping systems. It can affect plants at most stages of production. Affected fruits and other plant parts become water logged and soft and are quickly covered by a thick grey mould. Botrytis can also cause secondary rots on vegetables in storage or transit to market. High humidity conditions within greenhouse structures contribute to the spread of the disease. There are limited products available for hydroponic growers to use for controlling this disease in their growing systems. The main aim of this permit application is to ensure that hydroponic growers of cucumber, capsicums, chilli peppers and leafy lettuce have an effective tool for controlling grey mould in their crops.

This study was conducted to determine the residue levels of captan fungicide in leafy lettuce, cucumber, capsicum and chilli following three foliar applications of a product containing captan 900 g ai/kg as the only active ingredient. Applications were applied at 7 day intervals.

Twelve trials were conducted in major growing regions in Queensland and Northern New South Wales. Trials were conducted in the field and under protected cropping situations. Specimens were collected at 0, 3, 7 and 10 days after the third and final application. Collected specimens were then analysed to determine the level of captan residue.

The analytical study was conducted in two stages, with specimen homogenisation carried out upon specimen receipt; and extraction, purification and instrumental analysis at the analytical facility of AgriSolutions Australia Pty Ltd.

A new permit application has been supplied to Growcom for submission to the APVMA along with a copy of the study report on the field and analytical components of the project which will be used as part of a minor-use permit application to the APVMA.

The outcome of this project is residue data and analysis to support a minor use permit application to be lodged with the APVMA. If approved, an effective fungicide will be available for use by protected cropping vegetable grower for the control of Grey Mould.

Technical summary

Horticulture Australia and the Australian Vegetable industry are seeking a permit for captan (various formulations) for the control of grey mould (*Botrytis cinerea*), which is a common fungal disease in cucumber, capsicums, chilli peppers and leafy lettuce crops in protected cropping situations for use in all states. Grey mould can affect plants at most stages of production. Affected fruits and other plant parts become water logged and soft and are quickly covered by a thick grey mould. Botrytis can also cause secondary rots on vegetables in storage or transit to market. High humidity conditions within greenhouse structures contribute to the establishment and spread of the grey mould. Captan is currently registered for use in a number of fruit crops with edible peel for Botrytis control, with a permit for use in a number of berry crops (PER 13958).

There are limited products available for hydroponic growers to use for controlling disease in their growing systems. The main aim of this permit application is to ensure that hydroponic growers of cucumber, capsicum, chilli and leafy lettuce have an effective tool for controlling grey mould in their crops.

This study was conducted under the OECD Principles of Good Laboratory Practices (GLP) to determine the residue levels of captan in leafy lettuce, cucumber, capsicum and chilli following three foliar applications of a product containing captan 900g ai/kg as the only active ingredient. Applications were applied at 7 day intervals. Twelve trials were conducted in major growing regions in Queensland and New South Wales.

Specific site details and requirements were as per the approved Study Plan and the Standard Operating Procedures (SOPs) of AgriSolutions Australia Pty Ltd. Trials were conducted in field and protected cropping situations. Specimens were collected at 0, 3, 7 and 10 days after the third application according to documented SOPs relevant to the crop being sampled. Specimens were kept frozen after harvest and sent to the AgriSolutions analytical laboratory and analysed as per the study plan with the laboratory report sent tot the Study Director for inclusion in the final Study Report.

The analytical study was conducted in two stages, with specimen homogenisation carried out upon specimen receipt; and extraction, purification and instrumental analysis, at the analytical facility of AgriSolutions Australia Pty Ltd. Specimens were analysed using GC/MS/MS after extraction from the homogenised specimens with acetone. Clean-up was performed by liquid/liquid partitioning and silica SPE cartridge, after which the eluate was evaporated by rotary evaporation. The residue was then redissolved in ethyl acetate prior to instrument analysis.

The data generated from this study is being used as part of a minor-use permit application to be submitted by Growcom to the Australian Pesticides and Veterinary Medicines Authority (APVMA).

Introduction

Protected cropping can create challenges for growers using pesticides. Pesticides are not as easily weathered or washed from the plant surface due to a large majority of crops being grown using a hydroponic style system and the ability to reduce or exclude rain falling onto the crop. As such these pesticides may have a greater residual capability and subsequently greater residues on the harvested produce, which would inevitably affect the withholding period of such pesticides under a protected cropping situation.

Crops grown under protected cropping can also succumb to various diseases due to the higher humidity of such systems, as a result of reduced ventilation and the ability of certain pathogens to quickly develop resistance to the overuse of fungicides in such a closed system. Botrytis is one such pathogen that is favoured by such a growing system.

Limited effective products are available to control grey mould in the crops examined under this permit application with for example iprodione (Rovral®) only being labelled for use against grey mould in Tasmania and Western Australia. Grey mould in cucumber, capsicum, chilli and leafy lettuce grown in protected hydroponic growing systems cause significant economic loss and are currently difficult to manage. Registered fungicide products containing 900g/kg or 800g/kg captan as their only active ingredient are known to provide control of a number of important diseases (including Botrytis) in horticulture, not just in Australia but around the world. Captans' wide spectrum of disease control including grey mould (*Botrytis cinerea*), is utilized widely in horticultural crops including apples, pears, grapes, stone fruit, strawberries, turf, seedling ornamentals and roses with a permit for use in other berry crops (PER 13958).

A study was conducted to determinate the residue level of captan following three foliar applications seven days apart. This study was conducted at 12 different field sites in Queensland and New South Wales. The study plan was conducted by AgriSolutions Australia Pty Ltd as was the analytical component of this study. The study was conducted under the OECD Principles of Good Laboratory Practice (GLP).

This report contains the experimental methods used and presents the results, which make up part of a submission to the APVMA for a new minor use permit for the use of the active ingredient captan and all the registered products containing 900g/kg or 800g/kg captan as their active ingredient.

Materials and Methods

Twelve trials were conducted in major growing regions in Queensland and New South Wales. Trials were conducted in field and protected cropping situations. Specimens were collected at 0, 3, 7 and 10 days after the third application of captan fungicide and analysed to determine the level of residue on the harvested product.

The field phase was conducted at 12 locations as follows:

Site Number	Location	Particulars	Principal Investigator
1	Gatton, QLD	Leafy lettuce (field)	John Duff
2	Gatton, QLD	Leafy lettuce (protected, hydroponic)	John Duff
3	Toowoomba, QLD	Leafy lettuce (field)	John Duff
4	Toowoomba, QLD	Leafy lettuce (protected, hydroponic)	John Duff
5	Gatton, QLD	Cucumber (field)	John Duff
6	Mullumbimby, NSW	Cucumber (protected, hydroponic)	John Duff
7	Buaraba, QLD	Capsicum (field)	John Duff
8	Mullumbimby, NSW	Capsicum (protected, hydroponic)	John Duff
9	Gatton, QLD	Capsicum (field)	John Duff
10	Mullumbimby, NSW	Capsicum (protected, hydroponic)	John Duff
11	Gatton, QLD	Chilli (field)	John Duff
12	Gatton, QLD	Chilli (protected, hydroponic)	John Duff

Field trial timing

Test Site	1	2	3	4
Field Phase Start Date	03-APR-2012	16-MAR-2012	16-MAR-2012	23-MAR-2012
Field Phase End Date	27-APR-2012	09-APR-2012	09-APR-2012	16-APR-2012

Test Site	5	6	7	8
Field Phase Start Date	13-MAR-2012	27-MAR-2012	09-APR-2012	27-MAR-2012
Field Phase End Date	06-APR-2012	20-APR-2012	03-MAR-2012	20-APR-2012

Test Site	9	10	11	12
Field Phase Start Date	02-APR-2012	29-MAY-2012	08-MAY-2012	08-MAY-2012
Field Phase End Date	26-APR-2012	22-JUN-2012	01-JUN-2012	01-JUN-2012

Treatment Information for all sites:

Treatment	Test Item	Rate of Test Item	Rate of Active Ingredient	Application Timing
1	Untreated	N/A	N/A	N/A
2	Captan 900 WG	110g/100L	100 g ai/100L	21, 14 and 7 days before harvest

Equipment

All trials were sprayed to the point of run-off. Two passes were applied along the length of the bench or plots using an LPG powered mini-boom with 4 x T-Jet-60 11003vs, spaced 34cm apart, with a pressure of 30-32psi.

Sampling and storage

Specimens were collected at 0, 3, 7 and 10 days after the third application. A minimum of 12 whole lettuce plants, capsicum fruit or 2kg of chilli fruit were collected and stored in a freezer prior to analysis by AgriSolutions Australia.

Collect whole, saleable lettuce plants from the middle row of the plot, saleable capsicum or chilli fruit avoiding a 1 metre area at each end of the plot. Cut the lettuce plants just above the ground level so as to avoid collecting any soil and remove any withered outer leave, or cut the fruit with secateurs placing the collected specimens into a pre labelled specimen bag. Once the entire sample has been collected, seal the bag, insert into a second bag and seal. Samples were either placed into an esky for transport back to a freezer or taken directly to a freezer for storage.

Samples were stored in a temperature monitored freezer at $\leq -15^{\circ}\text{C}$. Once the last samples were collected all samples were sent to AgriSolutions Australia for processing.

Residue Analysis Method

The method utilised for the analyses of residues of captan, in the leafy lettuce, cucumber, capsicum and chilli specimens obtained during this study was as follows:

DFG Manual of Pesticide Residue Analysis Vol. I – Multiresidue Method S 8

(Updated):

“GAS-CHROMATOGRAPHIC DETERMINATION OF ORGANOHALOGEN, ORGANOPHOSPHORUS AND TRIAZINE COMPOUNDS”. (See pp. 283, Vol. I, & pp. 313, Vol. II)

The captan residues were extracted with acetone then filtered. After dilution with water and sodium chloride solution, the compounds were partitioned into dichloromethane. The organic phase was dried and evaporated, and the residue redissolved in dichloromethane. The extract was then cleaned up on a silica SPE cartridge, evaporated, and reconstituted in ethyl acetate. Final determination was by gas chromatography with mass spectrometer detection (GC/MS/MS). The quantitative determination was carried out by external standardisation.

Modifications to the Analytical Method

AgriSolutions Australia Pty Ltd used the following modifications to Method S 8, during analysis of samples for captan:

- 1) *Method S 8, Section 6.1:* Weight of homogenised sample was decreased from 100 g to 20 g, and extraction volume was decreased from 200 mL to 100 mL.

- 2) *Method S 8, Section 6.1:* Filtered extracts were combined with rinsing solvent in a 200 mL volumetric flask and made up to the mark with acetone. An aliquot (*see Table 14.1 below*) of this mother solution was mixed with 100 mL of water and 25 mL of saturated sodium chloride solution. Residues were then partitioned into two 20 mL portions of dichloromethane. The combined dichloromethane portions were then evaporated by rotary evaporator. This allowed either a dilution/concentration factor in the final extract as follows:

Sample Type	Aliquot Volume	Final Extract Volume	Dilution/Concentration Factor
10ppb Recoveries	50mL	2mL	5x C.F.
10ppm Recoveries	2mL	4mL	10x D.F.
UTC Specimens	50mL	2mL	5x C.F.
Treated Specimens	2mL	4mL	10x D.F.

- 3) *Method S 8, Section 6.2:* Sample extract dried with anhydrous sodium sulphate in flask, then filtered through PTFE syringe filter rather than fluted filter paper.
- 4) *Method S 8, Section 6.2:* Sample clean-up performed using silica SPE (1000mg/6 mL) cartridge, rather than silica gel packed column. An additional 1 cm layer of anhydrous sodium sulphate was added to the top of the cartridge and conditioned with 12 mL of dichloromethane/toluene/acetone [10:2:2] (v/v). The elution volume was reduced from 140 mL to 24 mL of dichloromethane/toluene/ acetone [10:2:2] (v/v).
- 5) *Method S 8, Section 6.2:* The evaporated sample was re-dissolved in ethyl acetate (*see Table 14.1*) with ultrasonication, and transferred to an autosampler mini-vial.
- 6) *Method S 8, Section 6.3:* ThermoFinnigan TraceGC gas-chromatograph with a PolarisQ ion-trap mass-spectrometer detector (GC/MS/MS) replacing Hewlett-Packard 5755G gas-chromatograph fitted with an Electron Capture Detector (GC-ECD). For details on this change, see Section 2.14.3 below.

Results

Residue results for analyses of captan in leafy lettuce (field): Site 1, Gatton, QLD

Specimen Number	Specimen Type	Sampling Timing	Captan (mg/kg)
VG11027/S1/T1/UTC	Leafy lettuce	Commercial harvest	<LOQ
VG11027/S1/T2/0DALA	Leafy lettuce	0 DALA	3.74
VG11027/S1/T2/3DALA	Leafy lettuce	3 DALA	3.93
VG11027/S1/T2/7DALA	Leafy lettuce	7 DALA	2.84
VG11027/S1/T2/10DALA	Leafy lettuce	10 DALA	1.41

Residue results for analyses of captan in leafy lettuce (protected hydroponic): Site 2, Gatton, QLD

Specimen Number	Specimen Type	Sampling Timing	Captan (mg/kg)
VG11027/S2/T1/UTC	Leafy lettuce	Commercial harvest	<LOQ
VG11027/S2/T2/0DALA	Leafy lettuce	0 DALA	4.75
VG11027/S2/T2/3DALA	Leafy lettuce	3 DALA	2.86
VG11027/S2/T2/7DALA	Leafy lettuce	7 DALA	2.44
VG11027/S2/T2/10DALA	Leafy lettuce	10 DALA	1.22

Residue results for analyses of captan in leafy lettuce (field): Site 3, Toowoomba, QLD

Specimen Number	Specimen Type	Sampling Timing	Captan (mg/kg)
VG11027/S3/T1/UTC	Leafy lettuce	Commercial harvest	<LOQ
VG11027/S3/T2/0DALA	Leafy lettuce	0 DALA	2.55
VG11027/S3/T2/3DALA	Leafy lettuce	3 DALA	1.68
VG11027/S3/T2/7DALA	Leafy lettuce	7 DALA	1.64
VG11027/S3/T2/10DALA	Leafy lettuce	10 DALA	0.86

Residue results for analyses of captan in leafy lettuce (protected hydroponic): Site 4, Toowoomba, QLD

Specimen Number	Specimen Type	Sampling Timing	Captan (mg/kg)
VG11027/S4/T1/UTC	Leafy lettuce	Commercial harvest	<LOQ
VG11027/S4/T2/0DALA	Leafy lettuce	0 DALA	3.20
VG11027/S4/T2/3DALA	Leafy lettuce	3 DALA	4.02
VG11027/S4/T2/7DALA	Leafy lettuce	7 DALA	1.90
VG11027/S4/T2/10DALA	Leafy lettuce	10 DALA	1.19

Residue results for analyses of captan in cucumber (field): Site 5, Gatton, QLD

Specimen Number	Specimen Type	Sampling Timing	Captan (mg/kg)
VG11027/S5/T1/UTC	Cucumber fruit	Commercial harvest	<LOQ
VG11027/S5/T2/0DALA	Cucumber fruit	0 DALA	3.75
VG11027/S5/T2/3DALA	Cucumber fruit	3 DALA	3.27
VG11027/S5/T2/7DALA	Cucumber fruit	7 DALA	2.78
VG11027/S5/T2/10DALA	Cucumber fruit	10 DALA	2.35

Residue results for analyses of captan in cucumber (protected hydroponic): Site 6, Mullumbimby, NSW

Specimen Number	Specimen Type	Sampling Timing	Captan (mg/kg)
VG11027/S6/T1/UTC	Cucumber fruit	Commercial harvest	<LOQ
VG11027/S6/T2/0DALA	Cucumber fruit	0 DALA	5.27
VG11027/S6/T2/3DALA	Cucumber fruit	3 DALA	4.03
VG11027/S6/T2/7DALA	Cucumber fruit	7 DALA	2.20
VG11027/S6/T2/10DALA	Cucumber fruit	10 DALA	1.73

Residue results for analyses of captan in capsicum (field): Site 7, Buaraba, QLD

Specimen Number	Specimen Type	Sampling Timing	Captan (mg/kg)
VG11027/S7/T1/UTC	Capsicum fruit	Commercial harvest	<LOQ
VG11027/S7/T2/0DALA	Capsicum fruit	0 DALA	4.06
VG11027/S7/T2/3DALA	Capsicum fruit	3 DALA	5.38
VG11027/S7/T2/7DALA	Capsicum fruit	7 DALA	3.11
VG11027/S7/T2/10DALA	Capsicum fruit	10 DALA	1.78

Residue results for analyses of captan in capsicum (protected hydroponic): Site 8, Mullumbimby, NSW

Specimen Number	Specimen Type	Sampling Timing	Captan (mg/kg)
VG11027/S8/T1/UTC	Capsicum	Commercial harvest	<LOQ
VG11027/S8/T2/0DALA	Capsicum	0 DALA	5.38
VG11027/S8/T2/3DALA	Capsicum	3 DALA	3.29
VG11027/S8/T2/7DALA	Capsicum	7 DALA	2.59
VG11027/S8/T2/10DALA	Capsicum	10 DALA	1.20

Residue results for analyses of captan in capsicum (field): Site 9, Gatton, QLD

Specimen Number	Specimen Type	Sampling Timing	Captan (mg/kg)
VG11027/S9/T1/UTC	Capsicum fruit	Commercial harvest	<LOQ
VG11027/S9/T2/0DALA	Capsicum fruit	0 DALA	2.96
VG11027/S9/T2/3DALA	Capsicum fruit	3 DALA	2.28
VG11027/S9/T2/7DALA	Capsicum fruit	7 DALA	1.91
VG11027/S9/T2/10DALA	Capsicum fruit	10 DALA	1.03

Residue results for analyses of captan in capsicum (protected hydroponic): Site 10, Mullumbimby, NSW

Specimen Number	Specimen Type	Sampling Timing	Captan (mg/kg)
VG11027/S10/T1/UTC	Capsicum fruit	Commercial harvest	<LOQ
VG11027/S10/T2/0DALA	Capsicum fruit	0 DALA	3.75
VG11027/S10/T2/3DALA	Capsicum fruit	3 DALA	5.00
VG11027/S10/T2/7DALA	Capsicum fruit	7 DALA	2.77
VG11027/S10/T2/10DALA	Capsicum fruit	10 DALA	1.71

Residue results for analyses of captan in chilli (field): Site 11, Gatton, QLD

Specimen Number	Specimen Type	Sampling Timing	Captan (mg/kg)
VG11027/S11/T1/UTC	Chilli fruit	Commercial harvest	<LOQ
VG11027/S11/T2/0DALA	Chilli fruit	0 DALA	5.74
VG11027/S11/T2/3DALA	Chilli fruit	3 DALA	4.59
VG11027/S11/T2/7DALA	Chilli fruit	7 DALA	3.65
VG11027/S11/T2/10DALA	Chilli fruit	10 DALA	2.81

Residue results for analyses of captan in chilli (protected hydroponic): Site 12, Gatton, QLD

Specimen Number	Specimen Type	Sampling Timing	Captan (mg/kg)
VG11027/S12/T1/UTC	Chilli fruit	Commercial harvest	<LOQ
VG11027/S12/T2/0DALA	Chilli fruit	0 DALA	4.45
VG11027/S12/T2/3DALA	Chilli fruit	3 DALA	3.35
VG11027/S12/T2/7DALA	Chilli fruit	7 DALA	2.48
VG11027/S12/T2/10DALA	Chilli fruit	10 DALA	1.78

Discussion

All trials were undertaken as per the individual study plans and were carried out using GLP compliant methods. Captan is not registered in cucumber, sweet pepper, chilli pepper or leafy lettuce in Australia, however it is registered in other edible peel crops including pome fruit, stone fruit, grapes and strawberries. There are no phytotoxicity precautions on the label for all crops at the proposed rates in this application. Twelve residue trials have been conducted to generate the residue data to support this permit application and no phytotoxicity was observed in any of the residue trials.

Relevant captan MRLs that could be used to extrapolate to cucumber, sweet pepper, chilli pepper and leafy lettuce include the following:

Aust – Pome fruits and stone fruits - 10 mg/kg.

Aust – Grapes and strawberries - 10 mg/kg.

USA – Sweet peppers, cucumber, leafy lettuce - 0.05 mg/kg.

EU – Cucumbers, lettuce and other fruiting vegetables - 0.02 mg/kg

Codex - Cucumber - 3 mg/kg

Codex - Tomato - 5 mg/kg

A permit application has now been prepared for submission by Growcom to the APVMA and sent to the minor use coordinator, with the relevant fee and complete GLP compliant residue data.

AgriSolutions final report is compliant to the OECD Principles of Good Laboratory Practice (GLP). The report was submitted to HAL as a separate document together with the project final report.

Technology Transfer

The data generated from the study will be included in submissions to the Australian Pesticides and Veterinary Medicines Authority. A minor use permit application for the use of captan on leafy lettuce, cucumbers, capsicum and chilli in both the field and protected cropping has been prepared and forwarded to the HAL Minor Use Coordinator. The data may also be used for additional submissions for permit applications, pesticide label extensions or for inclusion in complete pesticide registration applications.

Recommendations

It is recommended that the new permit application that has been prepared by DAFF QLD and AgriSolutions Australia, be submitted by Growcom on behalf of the vegetable industry to support the issue of a new permit for use to control grey mould (*Botrytis cinerea*), in leafy lettuce, cucumbers, capsicums and chilli in the field and under protected cropping situations.

It is also recommended that the confidential GLP report be offered to registrants of captan for them to use in label extensions of their products.

It is recommended that Growcom submit the permit application and relevant residue data report to the APVMA on behalf of the vegetable industry.