

## **Final Report**

# **Generation of pesticide data – Sigastus Weevil**

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Peracto Pty Ltd

**Project code:**

MC17007

**Project:**

Generation of pesticide data – Sigastus Weevil MC17007

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## Summary

Macadamia Seed Weevil (*Kuschelorrhynchus macadamiae*) formerly known as Sigastus Weevil, is a major pest in macadamias with up to 30% of crop lost if not treated. Indoxacarb (Avatar<sup>®</sup> Insecticide) has been previously identified as the best integrative approach for control of Macadamia Seed Weevil in macadamias due to its unique mode of action. The aim of this project was to undertake replicated field trials to provide data to support the continued use of Avatar<sup>®</sup> Insecticide for control of Macadamia Seed Weevil under PER86827, or potential label registration in the future. The minor use permit was issued by the Australian Pesticides and Veterinary Medicines Authority (APVMA) on 13 September 2018 until 30 September 2021. Residue and crop safety data was generated for use of indoxacarb (Avatar<sup>®</sup> Insecticide) in macadamias; whilst efficacy and crop safety data were generated for use of indoxacarb (Avatar<sup>®</sup> Insecticide) and Acephate (PER81463) in macadamias. These trials were conducted under commercial conditions to satisfy the Australian Pesticides and Veterinary Authority (APVMA) regulatory requirements.

One residue study was conducted with two trial sites located in NSW and QLD to determine indoxacarb residue in macadamias. Residue data was generated on the macadamia husk and kernel for the label and double label rate of indoxacarb. At the current with holding period (WHP) of 6 weeks (41 or 42 days), residue results ranged less than the limit of detection at 0.005 mg/kg for kernels, including the double label rate and were between 0.005 and 1.734 mg/kg for husks. No phytotoxicity or crop safety issues were observed in these trials. The residue data generated are below the temporary MRL of 3 mg/kg in macadamia kernels and supports the renewal of PER86827-Indoxacarb / Macadamia / Macadamia Seed Weevil.

Two efficacy trials were conducted in 2018-19, as replicated field trials, to evaluate the crop safety and efficacy of Avatar<sup>®</sup> Insecticide (300 g/kg indoxacarb) and DC-163 (200 g/L DC-163) compared to Acephate (Lancer), for the control of Macadamia Seed Weevil (*Kuschelorrhynchus macadamiae*) [KUSCMA] in macadamia cv. 816. DC-163 (200 g/L DC-163) was an experimental product included as a potential alternative to Avatar<sup>®</sup> Insecticide.

Treatments included Avatar<sup>®</sup> Insecticide at 3.75, 7.5 and 15 g ai/100 L and DC-163 at 2 g ai/100 L and were compared to Lancer 970 (970 g/kg acephate) and an untreated control.

For efficacy trial 1, Avatar<sup>®</sup> Insecticide at the proposed rate of 7.5g ai/100L provided a significant reduction in the incidence of Macadamia Seed Weevil damage to nuts. At 47DAAA there was a numerical reduction in the incidence of damage to nuts with Avatar<sup>®</sup> Insecticide applied at 7.5g ai/100L but this was not statistically significant. After 47DAAA the number of Macadamia Seed Weevil larvae declined as the nuts matured. Under the conditions of this trial, no phytotoxic effects were observed with Avatar<sup>®</sup> Insecticide at rates up to 15 g ai/100 L or DC-163 to macadamia cv.816.

For efficacy trial 2, Avatar<sup>®</sup> Insecticide at the proposed rate of 7.5g ai/100L provided a significant reduction in the incidence of Macadamia Seed Weevil damage to nuts at 27, 47 and 63DAAA and also density of Macadamia Seed Weevil larvae at 63 DAAAA. At 27 and 47DAAA there was a numerical reduction in the density of Macadamia Seed Weevil Larvae with Avatar<sup>®</sup> Insecticide applied at 7.5g ai/100L but this was not statistically significant. After 63DAAA the number of Macadamia Seed Weevil larvae and the incidence of damage declined as the nuts matured. Under the conditions of this trial, no phytotoxic effects were observed with Avatar<sup>®</sup> Insecticide at rates up to 15 g ai/100 L or DC-163 to macadamia cv.816.

Acephate did not provide a significant reduction in either Macadamia Seed Weevil larvae density or damage incidence to nuts in either of the efficacy trials and was less effective than Avatar<sup>®</sup> Insecticide for control of Macadamia Seed Weevil.

The results of the residue, crop safety and efficacy trials support the renewal of the minor-use permit for indoxacarb in macadamias.

## Keywords

Macadamia Seed Weevil, macadamias, indoxacarb

## Introduction

Macadamia Seed Weevil is a major pest in macadamias with up to 30% of crop lost if not treated. Indoxacarb (Avatar<sup>®</sup> Insecticide) has a specific mode of action that will reduce the impact on beneficial insects and bee populations and has been previously identified as the best integrative approach for control of Macadamia Seed Weevil in macadamias. The aim of this project was to undertake replicated field trials to provide data to support the continued use of Avatar<sup>®</sup> Insecticide for control of Macadamia Seed Weevil under PER86827, or potential label registration in the future. The minor use permit was issued by the Australian Pesticides and Veterinary Medicines Authority (APVMA) on 13 September 2018 until 30 September 2021.

## Methodology

Trials were conducted in commercial macadamia orchards in Northern NSW and Southern Queensland. Trials were conducted in the 2018-19 season.

### Field Trials – GLP Residue

One residue study was conducted with two trial sites located in NSW and QLD to determine indoxacarb residue in the macadamias. Residue trials were carried out as un-replicated small plot trials in commercial macadamia orchards. 2 trials were conducted, one with 2 treatments and one with 3 treatments. There were 2 application timings and 4 sample timings. Site's 1 and 2 were started at a growth stage that was too early and the kernel and shell were unable to be separated. These sites were cancelled. Site's 3 and 4 replaced site's 1 and 2 and all field applications occurred at the same scheduled timings in the original study plan however sampling was adjusted to commercial harvest rather than first commercial harvest.

Table 1: GLP Residue Trials

Peracto Study Number	Trials required	Trial #	Trial Locations	Conditions	Trial Timings
HMC17007	Indoxacarb GLP residue study	2	Queensland: Bundaberg New South Wales: Lindendale	Field trials, outdoor	Field Phase: Jan – Mar 19  Analytical Phase: May – Aug 19

### Field Component

Study plans were prepared for the study as per OECD GLP Guidelines for residue studies. The draft Study Plan was distributed to the HIA Project Reference Group and the Quality Assurance section of Peracto Pty Ltd for comment. After all comments were considered, the final Study Plan was prepared and given to the Study Director for signing and then sent to the Sponsor representative for signing. A copy of the signed Study Plan was then sent to all relevant personnel involved in the Study.

Field sites were selected in commercial crops grown under Good Agricultural Practice at locations where the nominated crop is commonly grown. Specific site details and requirements were as per the approved Study Plan and Standard Operating Procedures (SOPs) of Peracto Pty Ltd. Each field site within the study selected a different variety of macadamia (741 and 816). Test plots were an un-replicated plot design and marked on an area as determined in the Study Plan with sufficient separation between treated and untreated plots. Plots were clearly identified as detailed in the Study Plan.

Treatments were applied using calibrated hand spray equipment incorporating a nozzle configuration appropriate for the particular crop and application method which simulated best commercial practice. The application number and frequency followed the original schedules listed in the Study Plan at all sites. Sampling was carried out by hand as described in the Study Plan at all sites. The appropriate documentation was completed, with relevant SOPs adhered to that were relevant to the crop and plant portion to be sampled. The macadamia nuts (in shell and husk) were processed. Samples were labelled as described in the Study Plan and then immediately packed into an appropriate vessel and returned to the test site freezer. Frozen samples were dispatched upon completion of the study to the Analytical Laboratory. All stages of the process were monitored by the Peracto QA team with appropriate reports being presented in the final report.

For study HMC17007, the nominal treatment information and sample timings for macadamia trials were as follows:

Table 2: Targeted Application and Sample Timings Trial Site 3 (Queensland)

Trt.	Test Item	Rate of Test Item (g/100 L)	Rate of Active (g a.i./100 L)	Application Timing	Sample Timing	Specimen Type
T1	Untreated Control	N/A	N/A	N/A	Commercial Harvest <sup>1</sup>	Macadamia nut in husk
T2	Avatar® Insecticide + Agral Spray Adjuvant <sup>1</sup>	25	7.5	A and B	0 DAAB 14 DAAB 28 DAAB CH (42 DAAB)	Macadamia nut in husk
T3	Avatar® Insecticide + Agral Spray Adjuvant <sup>1</sup>	50	15	A and B	0 DAAB 14 DAAB 28 DAAB CH (42 DAAB)	Macadamia nut in husk <sup>2</sup>

DAAB = Days After Application B; CH = Commercial Harvest

A = 52 ± 1 days before commercial harvest; B = 42 ± 1 days before commercial harvest

Note <sup>1</sup>: Commercial Harvest is to coincide with 42 DAAB of the treated plots

Table 3: Targeted Application and Sample Timings Site 4 (New South Wales)

Trt.	Test Item	Rate of Test Item (g/100 L)	Rate of Active (g a.i./100 L)	Application Timing	Sample Timing	Specimen Type
T1	Untreated Control	N/A	N/A	N/A	Commercial Harvest <sup>1</sup>	Macadamia nut in husk
T2	Avatar® Insecticide + Agral Spray Adjuvant <sup>1</sup>	25	7.5	A and B	0 DAAB 14 DAAB 28 DAAB CH (42 DAAB)	Macadamia nut in husk

DAAB = Days After Application B; CH = Commercial Harvest

A = 52 ± 1 days before commercial harvest; B = 42 ± 1 days before commercial harvest

Note <sup>1</sup>: Commercial Harvest is to coincide with 42 DAAB of the treated plots

### Analytical Component

AWRI performed processing to separate the husk, shell and kernel. Analysis was originally to be conducted on all three portions and presented as a “whole portion” however AWRI encountered problems processing macadamia shell. The requirements for analysis were then adjusted to husk and kernel only following discussions with Hort Innovation and the APVMA.

Samples were sent frozen from the field test sites to the Australian Wine Research Institute (AWRI) as per the Study Plan, where the laboratory supervised the analytical component of the project. The laboratory acknowledged receipt of the residue samples, and the samples were analysed as per the Study Plan and the appropriate analytical method. The laboratory report was sent to the Study Director for inclusion in a composite Study Report.

#### Analytical Method:

Indoxacarb residues were determined on macadamia husk and kernels according to analytical methods and standard operating procedures developed by the Australian Wine Research Institute (AWRI):

A summary of the method is presented below:

A 3 g subsample of sample homogenate was weighed into a 50 mL centrifuge and 0.05 mL of the surrogate standard solution (12.5 µg/mL d5-atrazine) added. 12 mL of milli-Q water was added and the samples left to hydrate for at least 15 minutes. 15 mL of acetonitrile (1% acetic acid) was added and the tube shaken for approximately two (2) minutes then cooled in a laboratory freezer for fifteen (15) minutes. Magnesium, sulphate (6g) and sodium acetate (1.5 g) was added with 2 glass beads and the sample tube shaken for a further one (1) minute. The extract was centrifuged, and a 6 mL aliquot of supernatant was taken and added to a 15 mL dispersive solid-phase extraction (high fat dSPE) tube containing 400 mg primary-secondary amine and 1200 mg magnesium



sulphate. The sample tube was shaken for a minimum of one (1) minute then centrifuged. A 1 mL aliquot was taken from the centrifuged dSPE tube and evaporated to dryness in a TurboVap then reconstituted using 0.1 mL methanol, and 0.1 mL 25% methanol/0.1% formic acid/0.01% EDTA solution. The final extract was added to a 2 mL amber vial containing a 0.3 mL insert then analysed using an Agilent 1290 liquid chromatograph (LC) with 6460C tandem mass spectrometer (MS/MS). Samples with residues determined above the quantitation range (> 1.00 mg/kg) were diluted using the extract generated in the 15 mL dSPE tube and the blank unfortified extract (this ensures the concentration of the surrogate standard remains constant). The extract was diluted directly into a glass culture tube to give a total volume of 1 mL then evaporated and reconstituted as above.

#### Efficacy / Crop Safety Trials

Two efficacy trials were conducted, in Northern NSW in 2018 - 19, as replicated field trials to evaluate the crop safety and efficacy of Avatar 300 WG (300 g/kg indoxacarb) and DC-163 (200 g/L DC-163) for the control of Macadamia Seed Weevil (*Kuschelorrhynchus macadamiae*) in macadamia cv. 816.

Treatments included Avatar at 3.75, 7.5 and 15 g ai/100 L and DC-163 at 2 g ai/100 L and were compared to Lancer 970 (970 g/kg acephate) and an untreated control.

Two foliar applications, in a spray volume of 1600 L/ha, generating a coarse spray quality, were made at a 27-day interval, commencing when 10% of nuts had reached final size. The crop safety and efficacy of Avatar and DC-163 were evaluated at 27 days after application A (27DAAA), 47, 63 and 76DAAA as per the table below.

Table 4: Targeted Application and Assessment timing (trial 1 and 2)

No.	Product	Rate		Application schedule	Assessments
		Product (mL or g/100 L)	Active ingredient (g ai/100 L)		
1	Untreated control	Nil	Nil	N/A	Crop safety and efficacy assessments at 4 timings
2	Avatar 300 WG*	12.5 g	3.75	Two dilute foliar applications to the point of run-off at a 27-day interval, commencing at beginning of pest activity.	
3	Avatar 300 WG*	25 g	7.5		
4	Avatar 300 WG*	50 g	15		
5	DC-163*	10 mL	2.0		
6	Lancer 970 WP	80 g	77.6		

\* = Tank mixed with Agral Spray Adjuvant at 10 mL/100 L

## Results

### MC17007 Residue Study

GLP Study Title: Determination of residues of indoxacarb in macadamias following two applications of Avatar<sup>®</sup> Insecticide

The study was conducted at two field sites as follows:

Table 5: Actual trial timings for MC17007 GLP residue study

Study Commencement Date	11/09/18		
Test Sites	1 & 2	3	4
Locations	N/A – site's cancelled	Bundaberg, Queensland	Lindendale, New South Wales
Field Phase Start Dates	20/11/18 & 26/11/18	16/01/19	21/01/19
Field Phase Completion Date	N/A – site's cancelled	11/03/19	13/03/19

Site's 1 and 2 were started at a growth stage that was too early and the kernel and shell were unable to be separated. These sites were cancelled with site's 3 and 4 replacing site's 1 and 2. All field applications occurred at the same scheduled timings in the original study plan however sampling was adjusted to commercial harvest rather than first commercial harvest.

The macadamias treated in Queensland received two applications of Avatar<sup>®</sup> Insecticide at nominal rates of 7.5 or 15 g a.i./100L; the actual application rates were 7.3 and 14.7 g a.i./100L. Agral Spray adjuvant was applied at a rate of 25 mL/100L to treatment 2 and 3 at each application timing.

The macadamias treated in New South Wales received two applications of Avatar<sup>®</sup> Insecticide at a nominal rate of 7.5 g a.i./100L; the actual application rate was 7.3 g a.i./100L. Agral Spray adjuvant was applied at a rate of 25 mL/100L to treatment 2 at each application timing.

Whole macadamias in husks were collected from the treated plots at 0, 14, 28 and 41 or 42 (commercial harvest) days after application B whilst whole macadamias in husks were collected from the untreated control to coincide with commercial harvest.

Residues of indoxacarb in macadamias for sites 3 and 4 are on the following pages (table 6 and 7). Residue data was generated on the macadamia husk and kernel for the label and 2x rate of indoxacarb. At the current WHP of 6 weeks (41 or 42 days), residue results ranged less than the limit of detection at 0.005 mg/kg for kernels, including the double label rate and were between 0.005 and 1.734 mg/kg for husks. No phytotoxicity or crop safety issues were observed in these trials. The residue data generated are below the temporary MRL of 3 mg/kg in macadamia kernels and supports the renewal of PER86827.

Table 6: Residue results for Site 3 – Queensland – Macadamias

Specimen ID	Specimen Type	Treatment Number	Nominal Sample Timing	Test Item	Rate of Active (g a.i./100L)	Moisture Content (%)	Indoxacarb Residues "Wet Weight" (mg/kg)	Indoxacarb Residues "Dry Weight" (mg/kg)
TN015-H	Husk	T2	0 DAAB	Avatar <sup>®</sup> Insecticide + Agral Spray Adjuvant	7.5	69.80	0.792	2.621
TN016-H	Husk	T3	0 DAAB	Avatar <sup>®</sup> Insecticide + Agral Spray Adjuvant	15	69.02 (69.02)	1.983 (1.899)	6.399 (6.128)
TN017-H	Husk	T2	14 DAAB	Avatar <sup>®</sup> Insecticide + Agral Spray Adjuvant	7.5	70.08	0.888	2.969
TN018-H	Husk	T3	14 DAAB	Avatar <sup>®</sup> Insecticide + Agral Spray Adjuvant	15	67.05	1.569	4.760
TN019-H	Husk	T2	28 DAAB	Avatar <sup>®</sup> Insecticide + Agral Spray Adjuvant	7.5	70.64	0.456	1.554
TN020-H	Husk	T3	28 DAAB	Avatar <sup>®</sup> Insecticide + Agral Spray Adjuvant	15	66.62	0.958	2.869
TN021-H	Husk	T1	CH	Avatar <sup>®</sup> Insecticide + Agral Spray Adjuvant	N/A	69.40	< LOD	< LOD
TN022-H	Husk	T2	CH (42 DAAB)	Avatar <sup>®</sup> Insecticide + Agral Spray Adjuvant	7.5	67.64	0.763	2.357
TN023-H	Husk	T3	CH (42 DAAB)	Avatar <sup>®</sup> Insecticide + Agral Spray Adjuvant	15	69.68	1.734	5.719
TN015-K	Kernel	T2	0 DAAB	Avatar <sup>®</sup> Insecticide + Agral Spray Adjuvant	7.5	-	< LOD	-
TN016-K	Kernel	T3	0 DAAB	Avatar <sup>®</sup> Insecticide + Agral Spray Adjuvant	15	-	< LOQ (< LOQ)	-
TN017-K	Kernel	T2	14 DAAB	Avatar <sup>®</sup> Insecticide + Agral Spray Adjuvant	7.5	-	< LOD	-
TN018-K	Kernel	T3	14 DAAB	Avatar <sup>®</sup> Insecticide + Agral Spray Adjuvant	15	-	< LOQ	-
TN019-K	Kernel	T2	28 DAAB	Avatar <sup>®</sup> Insecticide + Agral Spray Adjuvant	7.5	-	< LOD	-
TN020-K	Kernel	T3	28 DAAB	Avatar <sup>®</sup> Insecticide + Agral Spray Adjuvant	15	-	< LOQ	-
TN021-K	Kernel	T1	CH	Avatar <sup>®</sup> Insecticide + Agral Spray Adjuvant	N/A	-	< LOD	-
TN022-K	Kernel	T2	CH (42 DAAB)	Avatar <sup>®</sup> Insecticide + Agral Spray Adjuvant	7.5	-	< LOD	-
TN023-K	Kernel	T3	CH (42 DAAB)	Avatar <sup>®</sup> Insecticide + Agral Spray Adjuvant	15	-	< LOD	-

DAAB = Days After Application B; CH = Commercial Harvest

Note: Results in parenthesis are duplicate analysis, <sup>1</sup>LOD = Limit of Detection = 0.005 mg.kg; LOQ = Limit of Quantitation = 0.010 mg.kg

Table 7: Residue results for Site4 – New South Wales - Macadamias

Specimen ID	Specimen Type	Treatment Number	Nominal Sample Timing	Test Item	Rate of Active (g a.i./100L)	Moisture Content (%)	Indoxacarb Residues "Wet Weight" (mg/kg)	Indoxacarb Residues "Dry Weight" (mg/kg)
TN024-H	Husk	T2	0 DAAB	Avatar <sup>®</sup> Insecticide + Agral Spray Adjuvant	7.5	70.63 (70.63)	1.510 (1.375)	5.141 (4.682)
TN025-H	Husk	T2	14 DAAB	Avatar <sup>®</sup> Insecticide + Agral Spray Adjuvant	7.5	71.55	0.785	2.758
TN026-H	Husk	T2	28 DAAB	Avatar <sup>®</sup> Insecticide + Agral Spray Adjuvant	7.5	70.72	0.564	1.925
TN027-H	Husk	T1	CH	Avatar <sup>®</sup> Insecticide + Agral Spray Adjuvant	N/A	69.53	< LOD	< LOD
TN028-H	Husk	T2	CH (42 DAAB)	Avatar <sup>®</sup> Insecticide + Agral Spray Adjuvant	7.5	68.54	0.293	0.933
TN024-K	Kernel	T2	0 DAAB	Avatar <sup>®</sup> Insecticide + Agral Spray Adjuvant	7.5	-	< LOD	-
TN025-K	Kernel	T2	14 DAAB	Avatar <sup>®</sup> Insecticide + Agral Spray Adjuvant	7.5	-	< LOD	-
TN026-K	Kernel	T2	28 DAAB	Avatar <sup>®</sup> Insecticide + Agral Spray Adjuvant	7.5	-	< LOD	-
TN027-K	Kernel	T1	CH	Avatar <sup>®</sup> Insecticide + Agral Spray Adjuvant	N/A	-	< LOD	-
TN028-K	Kernel	T2	CH (42 DAAB)	Avatar <sup>®</sup> Insecticide + Agral Spray Adjuvant	7.5	-	< LOD	-

DAAB = Days After Application B; CH = Commercial Harvest

Note: Results in parenthesis are duplicate analysis

<sup>1</sup>LOD = Limit of Detection = 0.005 mg.kg; LOQ = Limit of Quantitation = 0.010 mg.kg

### MC17007 Efficacy Trial 1

Full details of this trial can be found in the report titled: To determine the efficacy and crop safety of indoxacarb (Avatar® Insecticide), DC-163 and the industry standard acephate (PER81463) for the control of Macadamia Seed Weevil in macadamia cv. 816 Tregeagle, New South Wales, 2018-19. Below is a brief summary of the trial.

The trial consisted of one (1) field site as follows:

Table 8: Actual trial timings for Efficacy trial No. 1

<b>Location</b>	Tregeagle, NSW
<b>Field Phase Start Date</b>	25/10/18
<b>Field Phase Completion Date</b>	09/01/19

Table 9: Chronology of events:

Date	Days after application timing (DAA#)	Crop stage		Event
		BBCH scale	Description	
25/10/18	0DAAA	71	10 % of fruit had reached final size	Treatment application A
21/11/18	27DAAA	73	30 % of fruit had reached final size	Crop safety and efficacy assessment Treatment application B
11/12/18	47DAAA	75	50% of fruit had reached final size	Crop safety and efficacy assessment
27/12/18	63DAAA	77	70% of fruit had reached final size	Crop safety and efficacy assessment
09/01/19	76DAAA	79	90% of fruit had reached final size	Crop safety and efficacy assessment

**Results and discussion from Efficacy Trial No 1**

Table 10: Macadamia Seed Weevil Damage.

No.	Treatment	Rate (g ai/100L)	Macadamia Seed Weevil Damage Incidence (%) on nuts		
			27DAAA	47DAAA	63DAAA
1	Untreated control	--	62.2 ab	60.2	68.8
2	Avatar 300 WG *	3.75	10.0 d	61.2	56.5
3	Avatar 300 WG *	7.5	17.7 cd	35.8	49.2
4	Avatar 300 WG *	15	3.3 d	43.6	93.6
5	DC-163 *	2.0	37.6 bc	20.6	62.4
6	Lancer 970 WP	77.6	76.9 a	63.9	89.6
<i>P</i> -value			0.0001	0.1824	0.5669
LSD ( <i>P</i> ≤ 0.05)			tA	NSD	NSD

Means followed by same letter or symbol do not significantly differ (*P* = 0.05, LSD).

Mean comparisons performed only when AOV Treatment *P*(*F*) is significant at mean comparison OSL.

\* = Tank mixed with Agral 600 at 10 mL/100L

DAA# = Days after application timing

NSD = No significant difference due to a *P*-value > 0.05

tA = Original plot means are presented with analysis of variance and letters of separation from data transformed using  $y = \text{Arcsine square root percent } (x)$

Table 11: Macadamia Seed Weevil Larvae Counts.

No.	Treatment	Rate (g ai/100L)	Density of Macadamia Seed Weevil Larvae (number per plot)			
			27DAAA	47DAAA	63DAAA	76DAA
1	Untreated control	--	6.5 b	10.5	2.5	0.0
2	Avatar 300 WG *	3.75	0.0 c	12.5	6.5	0.0
3	Avatar 300 WG *	7.5	3.5 bc	11.3	6.0	0.3
4	Avatar 300 WG *	15	1.0 bc	6.0	9.7	0.7
5	DC-163 *	2.0	5.0 bc	2.3	4.7	0.3
6	Lancer 970 WP	77.6	20.0 a	13.8	3.0	0.0
<i>P</i> -value			0.0031	0.6443	0.7401	0.4819
LSD ( <i>P</i> ≤ 0.05)			tA	NSD	NSD (tS)	NSD (tA)

Means followed by same letter or symbol do not significantly differ (*P* = 0.05, LSD).

Mean comparisons performed only when AOV Treatment *P*(*F*) is significant at mean comparison OSL.

\* = Tank mixed with Agral 600 at 10 mL/100L

DAA# = Days after application timing

NSD = No significant difference due to a *P*-value > 0.05

tA = Original plot means are presented with analysis of variance and letters of separation from data transformed using  $y = \text{Arcsine square root percent } (x)$

tS = Original plot means are presented with analysis of variance and letters of separation from data transformed using  $y = \text{SQRT } (x + 0.5)$

Table 11: Crop Safety.

No.	Treatment	Rate (g ai/100L)	Crop Safety (% Crop Damage)			
			27DAAA	47DAAA	63DAAA	76DAAA
1	Untreated control	--	0	0	0	0
2	Avatar 300 WG *	3.75	0	0	0	0
3	Avatar 300 WG *	7.5	0	0	0	0
4	Avatar 300 WG *	15	0	0	0	0
5	DC-163 *	2.0	0	0	0	0
6	Lancer 970 WP	77.6	0	0	0	0
<b>P-value</b>			1.000	1.000	1.000	1.000
<b>LSD (<math>P \leq 0.05</math>)</b>			NSD	NSD	NSD	NSD

Mean comparisons performed only when AOV Treatment P(F) is significant at mean comparison OSL.

\* = Tank mixed with Agral 600 at 10 mL/100L

DAA# = Days after application timing

NSD = No significant difference due to a P-value > 0.05

Avatar 300 WG at the proposed rate of 7.5g ai/100L provided a significant reduction in the incidence of Macadamia Seed Weevil damage to nuts at 27 days after application. At 47DAAA there was a numerical reduction in the incidence of damage to nuts with Avatar applied at 7.5g ai/100L but this was not statistically significant. After 47DAA the number of Macadamia Seed Weevil larvae and the incidence of damage declined as the nuts matured.

Acephate (Lancer 970 WP) did not provide a significant reduction in either Macadamia Seed Weevil larvae density or damage incidence to nuts.

Under the conditions of this trial, no phytotoxic effects were observed with Avatar at rates up to 15 g ai/100 L or DC-163 to macadamia cv.816.

### MC17007 Efficacy Trial 2

Title: To determine efficacy and crop safety of indoxacarb (Avatar<sup>®</sup> Insecticide), DC-163 and the industry standard acephate (PER81463) for the control of Macadamia Seed Weevil in macadamia cv. 816, Alstonville, New South Wales, 2018-19

The trial consisted of one (1) field site as follows:

Table 12: Actual trial timings for Efficacy trial No. 2

<b>Location</b>	Alstonville, NSW
<b>Field Phase Start Date</b>	25/10/18
<b>Field Phase Completion Date</b>	09/01/19

Table 13: Chronology of events:

Date	Days after application timing (DAA#)	Crop stage		Event
		BBCH scale	Description	
25/10/18	0DAAA	71	10 % of fruit had reached final size	Treatment application A
21/11/18	27DAAA	73	30 % of fruit had reached final size	Crop safety and efficacy assessment Treatment application B
11/12/18	47DAAA	75	50% of fruit had reached final size	Crop safety and efficacy assessment
27/12/18	63DAAA	77	70% of fruit had reached final size	Crop safety and efficacy assessment
09/01/19	76DAAA	79	90% of fruit had reached final size	Crop safety and efficacy assessment

### Results and discussion from Efficacy Trial No 2

Table 14: Macadamia Seed Weevil Damage.

No.	Treatment	Rate (g ai/100L)	Macadamia Seed Weevil Damage Incidence (%) on nuts		
			27DAAA	47DAAA	63DAAA
1	Untreated control	--	22.3 a	74.0 a	99.3 a
2	Avatar 300 WG *	3.75	9.9 ab	64.4 ab	68.8 bc
3	Avatar 300 WG *	7.5	4.3 b	11.4 c	8.3 e
4	Avatar 300 WG *	15	4.9 b	40.2 bc	34.8 de
5	DC-163 *	2.0	18.2 a	24.6 c	41.1 cd
6	Lancer 970 WP	77.6	14.1 ab	74.5 a	91.5 ab
<i>P</i> -value			0.0479	0.0032	0.0001
LSD ( $P \leq 0.05$ )			tS	29.44	tA

Means followed by same letter or symbol do not significantly differ ( $P=0.05$ , LSD).

Mean comparisons performed only when AOV Treatment P(F) is significant at mean comparison OSL.

\* = Tank mixed with Agral 600 at 10 mL/100L

DAA# = Days after application timing

NSD = No significant difference due to a  $P$ -value  $> 0.05$

tA = Original plot means are presented with analysis of variance and letters of separation from data transformed using  $y = \text{Arcsine square root percent } (x)$

tS = Original plot means are presented with analysis of variance and letters of separation from data transformed using  $y = \text{SQRT } (x + 0.5)$



Table 15: Macadamia Seed Weevil Larvae Counts.

No.	Treatment	Rate (g ai/100L)	Density of Macadamia Seed Weevil Larvae (number per plot)			
			27DAAA	47DAAA	63DAAA	76DAA
1	Untreated control	--	1.3	18.3	17.7 a	0.7
2	Avatar 300 WG *	3.75	0.5	12.5	1.3 bc	0.0
3	Avatar 300 WG *	7.5	0.3	2.0	0.0 c	0.0
4	Avatar 300 WG *	15	0.3	6.0	2.0 bc	0.5
5	DC-163 *	2.0	1.0	9.7	0.3 c	0.3
6	Lancer 970 WP	77.6	0.5	13.8	9.3 ab	0.3
<b>P-value</b>			0.5702	0.2616	0.0094	0.719
<b>LSD (P ≤ 0.05)</b>			NSD (tA)	NSD	tL	NSD

Means followed by same letter or symbol do not significantly differ (P=.05, LSD).

Mean comparisons performed only when AOV Treatment P(F) is significant at mean comparison OSL.

\* = Tank mixed with Agral 600 at 10 mL/100L

DAA# = Days after application timing

NSD = No significant difference due to a P-value > 0.05

tA = Original plot means are presented with analysis of variance and letters of separation from data transformed using  $y = \text{Arcsine square root percent } (x)$

tL = Original plot means are presented with analysis of variance and letters of separation from data transformed using  $y = \text{Log } (x + 1)$

Table 16: Crop Safety.

No.	Treatment	Rate (g ai/100L)	Crop Safety (% Crop Damage)			
			27 DAAA	47 DAAA	63 DAAA	76 DAAA
1	Untreated control	--	1.0	1.0	1.0	1.0
2	Avatar 300 WG *	3.75	1.0	1.0	1.0	1.0
3	Avatar 300 WG *	7.5	1.0	1.0	1.0	1.0
4	Avatar 300 WG *	15	1.0	1.0	1.0	1.0
5	DC-163 *	2.0	1.0	1.0	1.0	1.0
6	Lancer 970 WP	77.6	1.0	1.0	1.0	1.0
<b>P-value</b>			1.000	1.000	1.000	1.000
<b>LSD (P ≤ 0.05)</b>			NSD	NSD	NSD	NSD

Mean comparisons performed only when AOV Treatment P(F) is significant at mean comparison OSL.

\* = Tank mixed with Agral 600 at 10 mL/100L

DAA# = Days after application timing

NSD = No significant difference due to a P-value > 0.05

Avatar 300 WG at the proposed rate of 7.5g ai/100L provided a significant reduction in the incidence of Macadamia Seed Weevil damage to nuts at 27, 47 and 63DAAA and also density of Macadamia Seed Weevil larvae at 63 DAAAA. At 47DAAA there was a numerical reduction in the larvae density with Avatar applied at 7.5g ai/100L but this was not statistically significant. After 63DAAA the number of Macadamia Seed Weevil larvae and the incidence of damage declined as the nuts matured.

Acephate (Lancer 970 WP) did not provide a significant reduction in either Macadamia Seed Weevil larvae density or damage incidence to nuts.

Under the conditions of this trial, no phytotoxic effects were observed with Avatar at rates up to 15 g ai/100 L or DC-163 to macadamia cv.816

## Outputs

### 1. Residue trials were conducted in 2018-19 season.

- **Study Plan (GLP Compliant)**

A residue study plan was prepared;

- **Analytical Report (GLP Compliant)**

An analytical phase report was received from the Australian Wine Research Institute (AWRI);

- **Final GLP Report (GLP Compliant)**

A final GLP report incorporating the field trial details and analytical report was prepared;

### 2. Efficacy Trials were conducted in the 2018 – 19 season.

- **Study Plan**

An efficacy study plan was prepared;

- **Final Efficacy Reports**

Two final efficacy reports were prepared

### 3. An APVMA Item 21 permit application prepared for submission with the data generated from this project.

- **Permit Application**

Reports from residue, crop safety and efficacy studies undertaken in MC17007 were submitted with an Item 21 permit application to the APVMA as detailed below

Target	Active Ingredient	Applicant	APVMA Number:	Submission Date	Status
Macadamia Seed Weevil <i>(Kushelorrhynchus macadamiae)</i>	Indoxacarb	Hort Innovation	DC21-82805070 Application No: 122144	Submitted by Hort Innovation with efficacy and residue reports generated in MC17007  14/10/19	APVMA assessment due for completion by 15 April 2020

## Outcomes

The purpose of this project was to generate residue, efficacy and crop safety data for use of indoxacarb (Avatar<sup>®</sup> Insecticide ) for the control of Macadamia Seed Weevil in macadamias to support the renewal and continuation of the current permit PER86827 (Avatar Insecticide / Macadamia / Macadamia Seed Weevil) and also to support the indoxacarb tMRL. Hort Innovation will also be provided the data to the registrant in support any potential label extension in future.

Residue data was generated on the macadamia husk and kernel for the label and double rate of indoxacarb. At the current WHP of 6 weeks (41 or 42 days), residue results ranged less than the limit of detection at 0.005 mg/kg for kernels, including the 2x rate and were between 0.005 and 1.734 mg/kg for husks. No phytotoxicity or crop safety issues were observed in these trials. The residue data generated are below the temporary MRL of 3 mg/kg in macadamia kernels and supports the renewal of PER86827.

The two efficacy and crop safety trials showed that Avatar<sup>®</sup> Insecticide significantly reduced the incidence of damage to nuts by Macadamia Seed Weevil and also reduced Macadamia Seed Weevil larvae density and was safe to the crop.

## Monitoring and evaluation

Not Applicable

## Recommendations

None to report

## Refereed scientific publications

None to report

## References

None to report

## Intellectual property, commercialisation and confidentiality

No project IP, project outputs, commercialisation or confidentiality issues to report

## Acknowledgements

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## Appendices

None to report