Advancing hull split to maximize yield and quality of walnuts

Dr Katherine Evans
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Authors: **M.D. Lang and K.J. Evans**

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Project Leader: Kathy Evans

Perennial Horticulture Centre
Tasmanian Institute of Agricultural Research
(TIAR), University of Tasmania (UTas)
13 St Johns Avenue
New Town TAS 7008
Phone: + 61(3) 6233 6878

Key Personnel: Michael Lang

Perennial Horticulture Centre
TIAR, UTas
Private Bag 3523
Burnie TAS 7320
Phone: + 61 (3) 6233 6878

Leigh Titmus

Walnuts Australia
349 Forth Road
Forth TAS 7310
Phone + 61 (3) 6428 3555

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TABLE OF CONTENTS

1. MEDIA SUMMARY

The Australian walnut industry is undergoing rapid expansion with the potential to supply high quality nuts to local and export markets. Optimizing the quality of nuts is essential to maximise these opportunities. Major factors that determine nut quality are the colour of the edible kernel and whether the shell of the nut is clean and undamaged. Thus, research was conducted to investigate factors that affect nut quality and to help walnut growers determine when walnuts are ready to be harvested.

In a preliminary study to determine when nuts are nearing harvest, the timing of kernel maturity was similar for cultivars Howard, Lara and Vina in NSW, whereas Lara kernels matured earlier than Vina kernels in Tasmania. In NSW, the progression of hull maturity was sooner in Vina than Lara, whereas in Tasmania the progression of these cultivars was similar. A possible reason why these cultivars matured differently may have been the variation in climate between growing regions. Future research should provide further insight into the nature of these events.

An important discovery was that delaying harvest of mature nuts significantly reduced the quality of nuts, by reducing the number of nuts with extra-light kernels, and increasing the number of nuts with yellow stained kernels. Also, it was found that leaving nuts on the ground for seven days or more prior to harvest also affected the quality of the kernel. In California, yellow stained kernels are associated with nuts that have been shaded when they are developing throughout the growing year; however, in our research yellow stained kernels were associated with nuts that were left on the ground prior to harvest, suggesting that conditions on the ground under trees may contribute to the development of this condition.

Nuts need to be removed as soon as possible after kernels are mature to maintain their quality. This study found that foliar sprays of a naturally occurring plant hormone, ethephon, reduced the time delay between hull and kernel maturity, and therefore may help walnut growers maintain the quality of walnuts. However, the concentration of ethephon in kernels was higher than anticipated in some walnuts. Climate can influence the uptake and decomposition of ethephon, hence, further research in a range of environmental conditions is warranted.

2. TECHNICAL SUMMARY

The Australian walnut industry is undergoing rapid expansion with the potential to supply high quality nuts to local and export markets. Optimizing the quality of nuts is essential to maximise these opportunities. Major factors that determine nut quality are the colour of the edible kernel and low external damage to the nut. Thus, trials were conducted in orchards at Goolgowi, NSW, and Swansea, Tasmania, to investigate the temporal development of fruit maturity and harvest delays on kernel quality, to examine the efficacy and crop safety of foliar applied ethephon, and to determine the concentration of ethephon in edible walnut kernels.

The timing of kernel maturity, or packing tissue brown (PTB), was similar in NSW, with 90%, or more, of Howard, Lara and Vina fruits at PTB between 60 and 63 days after 1st January. In Tasmania, the conclusion of PTB in Lara was prior to Vina, and was within 80 days of 1st January. In NSW, the progression of hull maturity, or hull-dehiscence, was sooner in Vina whereas in Tasmania the progression of hull-dehiscence in Vina was similar to that observed for Lara. The difference in climate between growing regions may have influenced the temporal development of fruits in this study. Further monitoring of the temporal development of fruit maturity will identify seasonal variation and improve this initial description for a single growing season.

Delaying harvest of mature nuts significantly reduced kernel quality of nuts. In Chandler, between 57 and 63% of nuts had extra-light kernels if harvest was delayed by 7 days; in comparison, 77% of nuts had extra-light kernels if harvest was not delayed. Similarly, delaying harvest significantly reduced the incidence of Howard and Vina nuts with extra-light kernels. Delaying the harvest of ground located nuts by 7 days significantly increased the incidence of nuts with yellow pellicles i.e. from less than 6% of nuts if harvest was not delayed to 24, 28 and 51% of Howard, Vina and Chandler nuts, respectively, if harvest was delayed. Yellow pellicle has been associated with the progressive shading of nuts in the growing year; however, in Tasmania yellow pellicles were associated with nuts that were on the ground prior to harvest and suggests that abiotic conditions under trees may contribute to the development of this condition.

In this study, nearly all Chandler fruits treated with ethephon at 432 g/ha were hullable within 8 days of treatment; furthermore, with ethephon applied at 756 g/ha nearly all Lara fruit and 90% of Serr fruit were hullable after 10 and 14 days respectively. Ethephon did not significantly reduce kernel quality in Chandler, Serr and Lara within 8, 10 and 14 days of treatment respectively; furthermore, ethephon sprays were not considered to be detrimental to the health of trees. However, studies to determine the decline of ethephon in walnuts found that the concentration of ethephon in ethephon-treated walnuts were lower, and peaked earlier, at NSW in comparison to Tasmania.

Weather prior to application of foliar plant growth regulators (PGRs) may influence the uptake of PGRs, whereas environmental conditions after the application of PGRs, such as ethephon, can affect the decomposition of ethephon and hence, the release of its active form, ethylene. Further research is required in Australia to confirm the efficacy and residue decline of ethephon in a greater range of cultivars and environmental conditions, and to determine if ongoing ethephon treatment influences kernel quality and crop health.

3. INTRODUCTION

The area under walnut cultivation in Australia has increased rapidly, with the recent planting of 650 and 1,600 hectares of orchards in Tasmania and NSW, respectively (Walnuts Australia, 2009). When these trees reach maturity the orchards are predicted to produce 10,000 tonnes of in-shell walnuts each year. Australia currently imports 8,500 tonnes of in-shell equivalent walnuts per annum (Walnuts Australia, 2009) with consumption predicted to increase at approximately 4% per year (Walnut Annual Industry Report, 2007-08). In addition to import substitution, the developing Australian industry has the opportunity to supply the European and Middle East markets with fresh, high quality walnuts during their off season.

Optimizing the quality of the nut is essential to maximise import and export opportunities. Major factors in determining nut quality are: 1) light colour of the edible kernel, 2) low internal damage from mould, and 3) low external damage, such as adhering hull tissue to shells (Olsen et al., 1998). All of these factors are adversely affected by delays in harvest.

Walnut harvest requires both the kernel and the hull, the outer layer of the fruit, to be mature. Kernels are mature and lightest in colour when the packing tissue surrounding the kernel is brown in colour, whereas the splitting and separation of the hull from the nut indicates hull maturity. Cultivar and climatic differences influence the rate at which kernels and hulls mature. In warm climates, the kernel of early maturing cultivars can mature up to three weeks before hull maturity (Olsen et al., 1998; Beede and Stanfield, 2001). In the Riverina district of NSW in 2008-09, kernel maturity was 30 days, or more, earlier than hull maturity in early maturing cultivars (Derek Goullet, Walnuts Australia, pers. comm. 2009). As a consequence of the delay in hull maturity, significant decline in kernel quality occurred in Riverina orchards in 2008-09. Unlike warm climates, hull and kernel maturity are thought to be less distinct from each other in cool-climate Tasmania. Furthermore, a large proportion of nuts across cultivars within an orchard mature simultaneously, placing heavy demands on equipment and labour in large orchards; hence, manipulation of hull maturity can prevent delays in harvest that lead to deterioration in kernel quality.

Ethephon, which breaks down to the active metabolite ethylene (Domir and Foy, 1978), is a plant growth regulator applied pre-harvest to facilitate fruit abscission for harvest. In California, ethephon treatment in walnuts is applied when the kernels of all nuts are mature, or after kernel maturity but approximately 10 days prior to harvest (Olsen et al., 1998); with either timing, ethephon allows earlier than normal harvest while maintaining the quality of the kernel. However, the response to ethephon application can depend on the walnut cultivar, time of application, seasonal conditions and management practices such as spray coverage.

In Australia, preliminary field trials to determine the efficacy of ethephon on hull maturity and nut drop were conducted in Tasmania in 2004-05 and 2005-06. In these trials, Ethrel® 480 Growth Regulator (Bayer CropScience Australia), a commercial ethephon formulation, was applied at 30 ml, 60 ml and 90 ml per 100 l and at a spray volume of 1 000 l/ha at kernel maturity in the early-mid maturing cultivars, Vina and Howard. At these rates, ethephon significantly increased hull maturity of fruit in cultivar Vina, from 85% in water-treatment controls to at least 92% with ethephon treatment, within 8 days of application (Michael Lang, Walnuts Australia, non-published data).

Similarly, hull split in cultivar Howard was significantly increased within 7 days of ethephon treatment.

Knowledge of the timing of hull and kernel maturity in warm- and cool-climate regions of Australia is poorly understood. Furthermore, knowledge of the efficacy and crop safety of foliar applied ethephon, and residue decline in edible walnut kernels, for walnut production in Australia is lacking. Thus, the objectives of this study were to 1) determine the temporal development of kernel and hull maturity, 2) investigate the effect of harvest delays on kernel quality, and 3) examine the efficacy and crop safety of foliar applied ethephon on walnuts in Australia. Furthermore, the concentration of ethephon residues in walnuts was studied (see Appendix) to facilitate the possible registration of ethephon in walnuts in Australia.

4. MATERIALS AND METHODS

4.1 Terminology

At maturity the walnut consists of the hull, shell and edible kernel (Pinney et al., 1998). In this report, the term "fruit" refers to all three parts, whereas the shell and kernel is termed the "nut".

Walnut harvest commences when both the kernel and hull are mature. Maturation of the kernel is attained when the packing tissue around the kernel halves has turned brown, whereas hull maturity is defined by the cracking and separation of 95% or more of the hull from the shell (Olson et al., 1998). In this report, kernel and hull maturity of fruits are termed "packing tissue brown" and "hull-dehiscence" respectively.

4.2 Site descriptions

All trials were conducted in commercial hedgerow orchards at Goolgowi, NSW (34°04'11"S, 145°42'47"E) or at Swansea, Tasmania (42°03'55"S, 148°03'04"E). Trial plots were selected from an area of the orchard with uniform tree growth. Trees were *Juglans regia* cultivars grafted onto *J. hindsii* rootstocks, with canopies of approximately 75 m^3 and 50 m^3 in NSW and Tasmania respectively. The cultural management of experimental sites was identical to that of commercial orchards.

4.3 Temporal development of fruit maturity

A total of five surveys, three and two in NSW and Tasmania respectively, were conducted to determine the temporal development of kernel and hull maturity. The cultivars surveyed were Howard, Lara and Vina in NSW, and Lara and Vina in Tasmania. Each survey was conducted in plots of 50 trees, made up of two adjacent tree-rows of 25 trees each, replicated four times.

Two to three weeks prior to the anticipated date for all fruit to reach packing tissue brown (PTB), ten fruit per plot were removed and assessed for the presence or absence of PTB. The ten fruits consisted of one fruit per single-tree, from each of ten randomly selected trees. Fruits were selected from lateral shoots only in the lower third of the tree canopy. Further assessments were conducted until 90%, or more, of fruits were at PTB.

From PTB until hull-dehiscence, fifty fruit per plot, consisting of ten fruits from each of five randomly selected trees, were removed at 4-7 day intervals and assessed for the presence or absence of hull-dehiscence. Fruits were selected from the lower third of the tree canopy, from both terminal and lateral shoots. The presence of hull-dehiscence was recorded if 95%, or more, of the hull was removed after fruits were rolled by hand for 5 s, with gentle downward pressure, on a steel grating mesh platform (Expamet Gridwalk WK2517, Melsteel Pty. Ltd., 132-134 Abbott Road, Hallam, Victoria, 3803).

4.4 Effect of delayed harvest on nut quality

One trial was conducted in Tasmania to determine the effect of delays in harvest on the quality of mature kernels in Chandler, Howard and Vina. For this trial, nuts that had no hull adhering to shells were termed "hulled" nuts. The trial design was a randomized complete block of single tree plots replicated five times. Factors examined were the location of "hulled" nuts (ground or tree) at the time of harvest and the length of time from hull-dehiscence to harvest (0, 7, 14, 21 or 28 days).

At Swansea, cultivars are grown in distinct areas, or blocks, within the orchard; hence, Chandler, Howard and Vina "hulled" nuts were collected from one row of trees from each "cultivar block" prior to transferring them to the trial in another walnut block adjacent to where nuts were collected. Walnut trees within the "trial block" were already harvested and were in full-leaf for the duration of the trial.

Prior to collecting "hulled" nuts from cultivar blocks, prematurely dropped nuts were removed from beneath tree canopies to remove the possibility of degraded nuts being included in the trial. Trees within the row of each cultivar block were then shaken with a mechanical tree shaker to promote nut-drop from trees. After shaking, dropped nuts were raked from the centre of the tree-row into inter-rows to form a single-row of nuts on either side of the tree-row. Samples of 450 "hulled" nuts, replicated five times, were then randomly selected from these fallen fruit from the one row of trees. Each 450 sample of nuts was divided into two samples of 200 nuts and one sample of 50 nuts. The sample of 50 nuts was placed into a 1 kg breathable poly-mesh bag and commercially dried until 8 to 9% moisture content (Day 0 sample). Each sample of 200 nuts was placed into a 10 kg breathable poly-mesh bags and the bag assigned to the ground or tree of a single-tree plot in the trial site. Poly-mesh bags with "ground" located nuts were placed underneath tree canopies so that all nuts were in contact with the ground; "tree" located nuts were placed within the tree canopy, 1-2 m above ground level, so that nuts were in a single layer within the poly-mesh bag. Samples of 50 nuts each were then randomly selected from each "ground" and "tree" poly-mesh bag, at 7 day intervals from 7 to 28 days after nuts were located within and below the tree canopy. Nuts were placed into 1 kg breathable poly-mesh bags, and commercially dried until 8 to 9% moisture content.

For all samples, drying commenced within 12 hours of sample collection and continued for a period of 24-36 hours. After drying, all nuts were sized and weighed, and kernels weighed and assessed for the presence of extra light coloured kernels. The definition of extra-light kernel colour was described in "USDA grades and standards for shelled walnuts" and "walnut size and colour poster" (California Walnuts® http://www.walnuts.org/walnuts/index.cfm/industry-resources/resources/). Kernels were also assessed for the presence or absence of yellow "stained" pellicle that surrounds the kernel.

4.5 Efficacy and crop safety of ethephon

A total of three trials, two in NSW and one in Tasmania, were conducted to determine the effect of foliar applied ethephon on hull maturity and crop safety. The trials were randomized complete block designs with five replicates of single-tree plots of Serr and Lara in NSW and Chandler in Tasmania. Single-tree buffers were located between plots to prevent ethephon contamination, from spray-drift, between treatments.

When 100% of fruits were at PTB, three rates of the ethephon formulation, Ethrel 720® (Bayer CropScience Pty Ltd, 391-393 Tooronga Rd., East Hawthorn), were applied (Tables 2-4). Treatments were applied once only, with a calibrated air-sheer backpack mister (Stihl SR 400) at a spray volume of 1,500 l/ha.

The efficacy of ethephon was assessed at 6 to 8 day intervals, for up to 22 days after application of treatments. At each assessment, 25 fruits per tree were randomly selected

from the lower third of the tree canopy from both terminal and lateral shoots. After removal, the presence or absence of hull-dehiscence was assessed using methods described previously. Removed nuts were then commercially dried to between 8 and 9% moisture content.

The health of trees after ethephon treatment was assessed by visually measuring the percentage area of premature yellowed leaves within the tree canopy (Horsfall and Barrett, 1945). Crop safety assessments were conducted concurrently with efficacy assessments.

After drying, nuts were weighed prior to the removal of the kernel from the shell. Kernels were then weighed and the presence of extra light coloured kernels recorded, as described previously.

4.6 Data analysis

In the "temporal development of fruit maturity" study the number of calendar days from 01-Jan-10 was regressed on the mean percentage of fruits at kernel maturity and the mean percentage of fruits at hull-dehiscence, using simple linear regression methods, to determine whether there was a statistically significant and linear relationship between variables.

In the "efficacy and crop safety of ethephon" and "effect of delayed harvest on nut quality" trials, the mean percentages of fruits at hull-dehiscence, yellow leaves within the tree canopy, extra light coloured kernels and yellow stained pellicles, and the mean weights of nuts and kernels were subjected to ANOVA to determine whether there was a statistically significant difference among treatment means. The method used to discriminate among treatment means was Fisher's LSD procedure.

All comparisons are reported at the $P = 0.05$ significance level.

5. RESULTS

5.1 Temporal development of fruit maturity

The progression of kernel maturity, or packing tissue brown (PTB), in Howard, Lara and Vina at Goolgowi, NSW, was similar with 90%, or more, of fruits at PTB between 60 and 63 days after 1st January (Figure 1). The onset of PTB was similar in all three cultivars but progression of hull maturity, or hull-dehiscence, was sooner in Vina.

At Swansea, TAS, the progression of PTB in Lara and Vina were distinct from each other, with the conclusion of PTB in Lara within 80 days of 1st January (Figure 1). In contrast, progression of hull-dehiscence in Vina was similar to that observed for Lara.

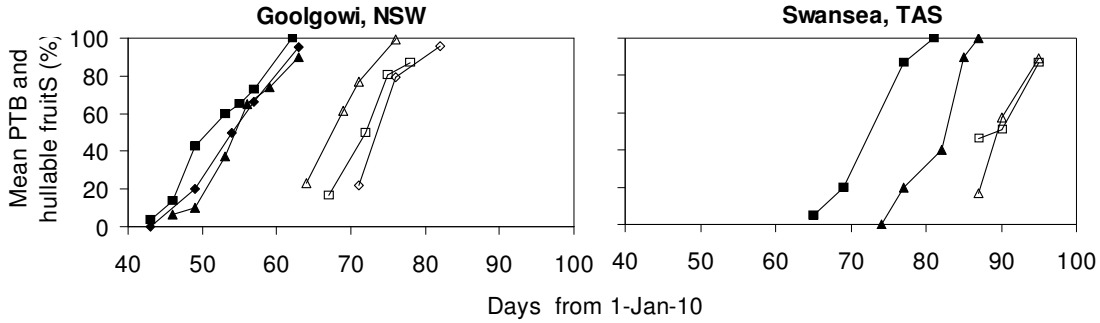

Fig. 1. Temporal progression of observed kernel maturity (PTB) (closed symbols) and hullable fruits (open symbols) on non-ethephon treated Howard (diamonds), Lara (squares) and Vina (triangles) fruits at Goolgowi, NSW, and Swansea, TAS. Each data point represents the mean of four replicates.

The predicted time for cultivars to reach 95% PTB and 80 % hullable fruits was 20-22 days sooner at Goolgowi than at Swansea (Table 1). The time between predicted 95% PTB and predicted harvest, defined as when 80% of nuts had the hull separated from the shell, was less in Vina than Lara i.e. 7-9 days and 13-15 days respectively.

Table 1. Number of days (predicted), from 1st January 2010, for 95% of fruits to have mature kernels (PTB) and 80% of fruits to have hull dehiscence (hullable), and the number of days (predicted) for fruits to go from 95% PTB to 80% hullable (drop-time) at Goolgowi, NSW, and at Swansea, TAS.

Location	Cultivar	Predicted 95% PTB (days) [ZY]	Predicted 80% hullable (days) [ZY]	Predicted drop-time (days) [ZY]
Goolgowi, NSW	Lara	61	76	15
	Vina	65	72	9
	Howard	63	-	-
Swansea, TAS	Lara	81	94	13
	Vina	87	94	7

[Z] Predicted values were derived from simple linear regression models of the observed values (y) against the number of days from 1-Jan-10 (t) for each cultivar.

[Y] A dash indicates that the linear regression was not significant at $P < 0.05$; hence, a predicted value was not calculated.

5.2 Effect of delayed harvest on nut quality

Ground located Vina nuts had significantly fewer extra-light colored kernels in comparison to nuts that were located in trees i.e. 28% and 36% respectively (Table 2). Similarly, fewer Howard nuts had extra-light kernels when located on the ground. The location of nuts prior to harvest did not significantly affect kernel colour in Chandler.

Delaying harvest significantly reduced the mean percentage of extra-light kernels in nuts that were located on the ground. In Chandler, between 57 and 63% of nuts had extra-light kernels if harvest was delayed; in comparison, 77% of nuts had extra-light kernels if harvest was not delayed (Table 3). Similarly, delaying harvest for 7 days or more significantly reduced the incidence of Howard and Vina nuts with extra-light kernels in comparison to nuts that were harvested at hull-maturity.

Delaying harvest significantly increased the presence of yellow pellicles in nuts that were located on the ground prior to harvest. Less than 6% of nuts had yellow pellicles if harvest was not delayed (Table 3); in contrast, 24, 28 and 51 % of Howard, Vina and Chandler nuts, respectively, had yellow pellicles if harvest was delayed by 7 days. Further delays in harvest increased the incidence of nuts with yellow pellicles in all cultivars.

Table 2. Mean percentage of nuts, pooled from all sample dates, with "extra light" and "yellow" coloured kernels according to location (ground or tree) for three walnut cultivars at Swansea, Tas. Within each column, means accompanied by the same letter form a group of means within which there are no statistically significant differences at *P* = 0.05.

Location	Chandler				Howard				Vina			
	Extra-light (%)		Yellow (%)		Extra-light (%)		Yellow (%)		Extra-light (%)		Yellow (%)	
Tree	65.7	a	29.0	a	51.6	a	10.8	a	36.0	a	11.3	a
Ground	60.7	a	65.8	b	45.1	b	34.0	b	27.9	b	35.6	b

Table 3. Mean percentage of "hulled" nuts with "extra light" and "yellow" coloured kernels for nuts located on the "ground" for 0, 7, 14, 21 and 28 days after kernel maturity for three walnut cultivars at Swansea, Tas. Within each column, means accompanied by the same letter form a group of means within which there are no statistically significant differences at *P* = 0.05.

Days	Chandler				Howard				Vina			
	Extra-light (%)		Yellow (%)		Extra-light (%)		Yellow (%)		Extra-light (%)		Yellow (%)	
0	77.3	a	6.4	a	57.8	a	6.2	a	46.0	a	4.8	a
7	62.4	b	50.7	b	46.0	b	24.0	b	29.7	b	28.4	b
14	56.8	b	67.6	c	44.8	b	32.8	c	31.2	b	32.8	b
21	62.8	b	71.3	c	43.2	b	30.8	bc	25.4	b	26.2	b
28	60.8	b	73.4	c	46.2	b	48.3	d	25.2	b	54.9	c

5.3 Efficacy and crop safety of ethephon

5.3.1 Efficacy

Ethephon increased hull-dehiscence in early-season (Serr), mid-season (Lara) and mid- to late-season (Chandler) harvested cultivars, in contrast to non-treatment.

In Serr (NSW trial), at least 45% of fruits were hullable 8 days after treatment; in comparison, only 26% of non-treated fruits were hullable (Table 4). With the two highest ethephon rates, more than 90% of fruits were hullable within 14 days. All fruits were hullable 22 days after treatment.

In Lara (NSW trial), nearly all fruits treated with the two highest rates of ethephon were hullable within 10 days; in contrast, only 50% of non-treated fruits were hullable (Table 5). Within 16 days a near 100% of ethephon treated fruits were hullable.

In Chandler (Tas. trial), 91% of non-treated fruits were hullable 6 days after PTB; however, significantly more fruits were hullable with ethephon-treatment (Table 6).

Table 4. Mean percentage of hullable Serr fruits at 8, 14 and 22 days after application (DAA) of Ethrel® 720 (720 g/L ethephon), and with non-treatment at Goolgowi, NSW. Treatments were applied on 17-Feb-10 at kernel maturity with an air sheer backpack mister (Stihl SR 400) at a spray volume of 1 500 l/ha. Within each column, means accompanied by the same letter form a group of means within which there are no statistically significant differences at *P* = 0.05.

Treatment	Product rate (ml/100 l)	Active ingredient (g/ha)	Hullable fruits (%)					
			8 DAA		14 DAA		22 DAA	
Non-treated	-	-	25.6	a	70.4	a	100.0	a
Ethrel® 720	40	432	44.8	b	74.4	a	100.0	a
Ethrel® 720	70	756	56.8	b	89.6	b	100.0	a
Ethrel® 720	100	1080	77.6	c	91.2	b	100.0	a

Table 5. Mean percentage of hullable Lara fruits at 10 and 16 days after application (DAA) of Ethrel® 720 (720 g/L ethephon), and with non-treatment, at Goolgowi, NSW. Treatments were applied on 3-Mar-10 at kernel maturity with an air sheer backpack mister (Stihl SR 400) at a spray volume of 1 500 l/ha. Within each column, means accompanied by the same letter form a group of means within which there are no statistically significant differences at *P* = 0.05.

Treatment	Product rate (ml/100 l)	Active ingredient (g/ha)	Hullable fruits (%)			
			10 DAA		16 DAA	
Non-treated	-	-	49.6	a	86.4	a
Ethrel® 720	40	432	86.4	b	98.4	b
Ethrel® 720	70	756	99.2	c	100.0	b
Ethrel® 720	100	1080	100.0	c	100.0	b

Table 6. Mean percentage of hullable Chandler fruits at 8 days after application (DAA) of Ethrel® 720 (720 g/L ethephon), and with non-treatment, at Swansea, TAS. Treatments were on 15-Apr-10 at kernel maturity with an air sheer backpack mister (Stihl SR 400) at a spray volume of 1 500 l/ha. Within each column, means accompanied by the same letter form a group of means within which there are no statistically significant differences at *P* = 0.05.

Treatment	Product rate (ml/100 l)	Active ingredient (g/ha)	Hullable fruits (%)	
			6 DAA	
Non-treated	-	-	91.2	a
Ethrel® 720	40	432	99.2	b
Ethrel® 720	70	756	100.0	b
Ethrel® 720	100	1080	100.0	b

5.3.2 Crop safety and kernel colour

The incidence of Serr fruits with extra-light kernels was not reduced within 14 days of ethephon treatment, in comparison to non-treatment, with between 62-70% of fruits with extra-light kernels (Table 7). After 22 days, significantly fewer Serr nuts had extra-light kernels after treatment with ethephon at the highest rate.

In Lara, significantly fewer nuts had extra-light kernels 16 days after treatment with ethephon, in comparison to non-treatment, with less than 5% of fruits with extra-light kernels (Table 8). Less than 18% of nuts had extra-light kernels, irrespective of ethephon treatment or non-treatment.

No significant difference occurred between treatments in Chandler (Tas. trial), with 97-98% of nuts having extra-light kernels 8 days after kernel maturity (data not presented).

The nut and kernel weight of Serr, Lara and Chandler was not adversely affected with ethephon treatment (data not presented). Less than 4.8% of leaves within tree canopies turned yellow prematurely with ethephon treatment (data not presented).

Table 7. Mean percentage of Serr fruits with extra-light kernels at 8, 14 and 22 days after application (DAA) of Ethrel® 720 (720 g/L ethephon), and with non-treatment, at Goolgowi, NSW. Treatments were applied on 17-Feb-10 at kernel maturity with an air sheer backpack mister (Stihl SR 400) at 1 500 l/ha. Within each column, means accompanied by the same letter form a group of means within which there are no statistically significant differences at *P* = 0.05.

Treatment	Product rate (ml/100 l)	Active ingredient (g/ha)	Extra-light kernels (%)					
			8 DAA		14 DAA		22 DAA	
Non-treated	-	-	63.0	a	66.6	a	49.7	b
Ethrel® 720	40	432	66.8	a	69.4	a	46.6	b
Ethrel® 720	70	756	69.9	a	63.6	a	47.6	b
Ethrel® 720	100	1080	64.6	a	62.1	a	31.6	a

Table 8. Mean percentage of Lara fruits with extra-light kernels at 10 and 16 days after application (DAA) of Ethrel® 720 (720 g/L ethephon), and with non-treatment, at Goolgowi, NSW. Treatments were applied on 3-Mar-10 at kernel maturity with an air sheer backpack mister (Stihl SR 400) at 1 500 l/ha. Within each column, means accompanied by the same letter form a group of means within which there are no statistically significant differences at *P* = 0.05.

Treatment	Product rate (ml/100 l)	Active ingredient (g/ha)	Extra-light kernel (%)			
			10 DAA		16 DAA	
Non-treated	-	-	13.7	a	17.8	a
Ethrel® 720	40	432	6.5	a	4.4	b
Ethrel® 720	70	756	2.1	a	3.6	b
Ethrel® 720	100	1080	11.1	a	0.8	b

6. DISCUSSION

This study provides an initial description of the temporal development of kernel and hull maturity in several walnut cultivars in Australia, with hull and kernel maturity occurring earlier in NSW than in Tasmania. The timing of kernel maturity was similar for three cultivars in NSW, whereas Lara kernels matured earlier than Vina kernels in Tasmania. In contrast to kernel maturity, hull maturity in Vina occurred earlier than Howard in NSW, which concurs with the development of hull maturity in California (Hendricks et al., 1998). In Tasmania, hull maturity was similar in Vina and Lara; previously it was generally considered that in Tasmania hull maturity of Vina was earlier than Lara (Rodney Jones, Walnuts Australia, pers. comm. 2010). In California, the onset of kernel and hull maturity is influenced by varietal and climatic differences (Olson et al., 1998); hence, the difference in climate between growing regions i.e. semi-arid and temperate in NSW and Tasmania respectively, may have influenced the temporal development of fruits in this study. Further monitoring of the temporal development of fruit maturity will identify seasonal variation and improve this initial description for a single growing season.

This is the first Australian report of the effects of delaying walnut harvest on the quality of mature kernels. In summary, delaying harvest significantly reduced kernel quality of Chandler, Howard and Vina nuts by reducing the number of nuts with extra-light kernels, and increasing the number of nuts with yellow stained pellicles. In California, the greatest loss of kernel colour of walnuts on the orchard floor occurs within the first nine hours of harvest (Olsen et al., 1998); furthermore, prolonged exposure of walnuts to damp soil reduces nut quality by staining the shell of the nut, and increases the susceptibility of nuts to moulds, in comparison to those nuts that remain on the tree. In California, nuts with observable symptoms of yellow pellicle, similar to those observed in Tasmania, have been observed in Chandler (Lampinen et al., 2007); however, in California, these symptoms have been associated with the progressive shading of nuts, from full sunlight early in the season through to shading within the tree canopy later in the season. In this study, “ground” and “tree” located nuts were randomly selected from nuts that had been shaken from tree canopies; hence, the progressive shading of nuts was the same for nuts that were selected for the ground or tree treatment. However, yellow stained pellicles were associated with nuts that were located on the ground prior to harvest and suggests that abiotic or biotic conditions underneath tree canopies may contribute to the development of yellow pellicles of walnuts in Tasmania.

In this study, a significant increase in hull-dehiscence was observed with the foliar application of ethephon. With walnuts treated with ethephon at the highest rate designated in APVMA permit 11649 i.e. 432 g/ha of ethephon, nearly all Chandler fruit were hullable within 8 days of treatment. Furthermore, with ethephon treatment at 756 g/ha nearly all Lara fruit and 90% of Serr fruit were hullable after 10 and 14 days respectively. Ethephon applications did not significantly reduce kernel quality in Serr and Lara, as long as nuts were harvested within 10 and 14 days of treatment. Similarly, ethephon did not reduce kernel quality in Chandler; however, nuts were harvested after only 8 days in this cultivar. While the use of ethephon led to a slight yellowing of leaves, the severity of symptoms was not considered to be detrimental to the health of trees.

In California, ethephon treatment advanced hull maturity and nut removal in cultivars Serr (Beade et al. 1998, 1999, 2000; Beade and Stanfield, 2001), Ashley (Kotwal et al.

1979), Marchetti (Sibbett and Martin, 1975) and Payne (Carnill and Martin, 1975). However, Beede et al. (2000) suggested that the response to ethephon application depended, in part, on seasonal conditions. Weather prior to application of foliar plant growth regulators (PGRs) may influence cuticle development and thus PGRs uptake, whereas environmental conditions after the application of PGRs, such as ethephon, can affect the decomposition of ethephon and hence, the release of its active form, ethylene (Stover and Greene, 2005) For example, while ethephon is used to enhance fruit abscission in sweet and sour cherry, Olien and Bukovac (1978) reported that at 16^{o}C and lower there was little response in the decomposition of ethephon. Hence, further research is required in Australia to confirm the efficacy of ethephon in a greater range of cultivars and environmental conditions, and to determine if ongoing ethephon treatment influences kernel quality and crop health.

7. TECHNOLOGY TRANSFER

A key component of this project was the transfer of knowledge directly to Walnuts Australia staff. To facilitate this, orchard managers were involved in the development of all trials in this project. A summary of research findings were then reported to Walnuts Australia staff at a meeting conducted during post-harvest "orchard walks" in the Riverina region, NSW. To deliver project findings to a wider grower audience, conclusions from the project are to be published in the 2010 summer edition of the industry journal, *Australian Nutgrower*.

Data of ethephon concentrations in edible walnut kernels, collected during the residue decline studies, were provided to the Australian Pesticides and Veterinary Medicines Authority (APVMA) for the extension of APVMA permit 11649 (see Appendix). APVMA permit 11649 enabled the use of ethephon for the aid and promotion of uniform nut-drop of walnuts in NSW and Tasmanian orchards.

A further component of the project was to foster interest and capacity building in agricultural research. As such, Alix du Boucheron, a visiting Agricultural Science student from the University of Bordeaux, France, assisted in nut drop and kernel quality field trials, and assisted in the assessment of kernel quality, during the Tasmanian walnut harvest.

8. RECOMMENDATIONS

Further research is required across the range of walnut cultivars and climates in which walnuts are grown commercially in Australia to:

1. Monitor the temporal development of fruit maturity

This study provides an initial description of the temporal development of kernel and hull maturity in Howard, Lara and Vina in NSW and Tasmania. However, further monitoring of the temporal development of fruit maturity will enhance the description provided in this report.

2. Determine the cause of the loss of kernel quality with delayed harvest

This study found that delaying harvest significantly reduced kernel quality of walnuts by reducing the number of nuts with extra-light kernels, and by increasing the number of nuts with yellow stained pellicles. However, the cause of loss of kernel colour attributed to a delayed harvest, and the abiotic or biotic conditions that may have contributed to the development of yellow pellicles of walnuts, remains unknown.

3. Confirm the efficacy of ethephon in a greater range of cultivars

In this study, foliar application of ethephon significantly increased hull-dehiscence in Serr, Lara and Chandler. In California, ethephon has advanced hull maturity and nut removal in a greater range of cultivars than reported here; this suggests that in addition to those cultivars examined in this study, hull maturity and nut removal of other cultivars grown in Australia may respond to ethephon treatment.

4. Determine if ongoing ethephon treatment influences tree and crop health.

This study did not consider that ethephon treatment was detrimental to the health of trees; furthermore, kernel quality was not reduced as long as the harvesting of nuts was not delayed. However, further research is required to determine if the use of ethephon over multiple years has any detrimental effect on kernel quality and crop heath.

5. Determine the effect of temperature on ethephon residues in walnuts (as per findings reported in the Appendix).

This study determined that the concentration of ethephon in ethephon-treated walnuts were lower, and peaked earlier, at NSW in comparison to Tasmania. Daily mean temperatures were lower in Tasmania in comparison to NSW. As temperatures can influence the uptake and decomposition of foliar applied plant growth regulators, further research is required to determine the effect of temperature on ethephon residues in walnuts.

9. ACKNOWLEDGMENTS

The research undertaken in this project would not have been possible without the advice and support of Steve Sibbett from the University of California, the assistance of Walnuts Australia staff, namely Derek Goullet, Matt Hockings, Eric Snaidero, Lorraine Richardson, Carl Rademeyer, Bec Goullet, Rodney 'Kouj' Jones and Julie Sulcs, the help provided by Alix du Boucheron from the University of Bordeaux, France, and Jake Eyles from the Tasmanian Institute of Agricultural Research, and the support of Walnuts Australia and Horticulture Australia Limited.

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11. APPENDIX – DETERMINATION OF ETHEPHON RESIDUES IN EDIBLE WALNUT KERNELS

DETERMINATION OF ETHEPHON RESIDUES IN EDIBLE WALNUT KERNELS

Report Authors: Michael D. Lang and Katherine J. Evans

Project Leader
Katherine J. Evans
Perennial Horticulture Centre
Tasmanian Institute of Agricultural Research (TIAR)
University of Tasmania (UTas)
13 St Johns Avenue
New Town TAS 7008
Phone: + 61(3) 6233 6878

Key Personnel
Michael Lang
Perennial Horticulture Centre
TIAR, UTas
Private Bag 3523
Burnie TAS 7320
Phone: + 61 (3) 6230 4522

Andy Huang and Bruce Chen
Symbio Alliance
44 Brandt Street
Eight Mile Plains QLD 4113
Phone: + 61 (3) 6233 6878

This publication reports on studies conducted between 09-Mar-10 and 4-Aug-10 in accordance with aims outlined in **Project WN09000: Advancing hull split to maximise yield and quality of walnuts**.

Date of report: 20 August 2010

TABLE OF CONTENTS

1. ABSTRACT

Two residue chemistry studies were conducted to determine the decline of ethephon in walnuts. Trials were non-replicated and consisted of non-treated and two or three rates of ethephon, with each treatment applied to plots of six trees. Treatments were applied once only, at kernel maturity, with a calibrated air-mist sprayer at a spray volume of 1 000 l/ha. Walnuts from Goolgowi (NSW) that were non-treated, or treated with ethephon at the highest rate specified in APVMA permit 11649, were below the temporary MRL for ethephon immediately after sprays had dried (day 0) and at 3, 7, 14 and 21 days after application. Ethephon concentrations in walnuts from Swansea (Tasmania) were below the temporary MRL at days 0, 13 and 21; however, concentrations on any particular day were not necessarily in the order of the rate of ethephon applied. Further studies are required to determine the decline of ethephon under a range of environmental conditions and ethephon rates.

2. INTRODUCTION

The area under walnut cultivation in Australia has increased rapidly, with the recent planting of 650 and 1 600 hectares of orchards in Tasmania and NSW, respectively (Walnuts Australia, 2009). When these trees reach maturity the orchards are predicted to produce 10 000 tonnes of in-shell walnuts per year. Australia currently imports 8 500 tonnes of in-shell equivalent walnuts per annum, with consumption predicted to increase at approximately 4 % per year (Walnut Industry report, 2007-08). In addition to import substitution, the Australian industry has the opportunity to supply European and Middle East markets with fresh, high quality walnuts.

Optimizing the quality of the nut is essential to maximise import and export opportunities. Major factors in determining nut quality are: 1) light colour of the edible kernel, 2) low internal damage from mould, and 3) low external damage, such as adhering hull tissue to shells (Olsen *et al*., 1998). All of these factors are adversely affected by delays in harvest.

Ethephon, which breaks down to the active metabolite ethylene, is a plant growth regulator applied pre-harvest to facilitate fruit abscission for harvest. In walnuts, ethephon is applied for the promotion of uniform nut-fall. A minor use permit, APVMA permit 11649, allowed the use of ethephon on walnut in NSW and Tasmania. A requirement for the extension of APVMA permit 11649 is the provision of data on the decline of ethephon residues in edible walnut kernel.

The purpose of this study was to collect and analyze ethephon-treated and non-treated walnut samples from orchards in NSW and Tasmania to provide appropriate data of residue chemistry of ethephon in edible walnut kernel.

The study was conducted in accordance with guidelines outlined in MORAG Ag. Part 5A Residues and OECD/OCDE 509: OECD guideline for the testing of chemicals – crop field trial, and reported in accordance with guidelines outlined in Reporting of Residue Trials, Residue Guideline No. 11.

3. MATERIALS AND METHODS

3.1. Site details

Site locations

Table 1: Location of field and laboratory sites

Site	Address
Field site 1	Motspur Park, Tabbita Lane, Goolgowi, NSW, 2652
Field site 2	Walnuts Australia, Springs Road, Swansea, TAS, 7190
Laboratory	Symbio Alliance, 44 Brandt Street, Eight Mile Plains, QLD, 4113

Type of application
Air-mist to plant foliage and fruits

Equipment used
Air-mist sprayer (Stihl® SR 420)

Test system design
Each field site consisted of one non-treated plot and up to three ethephon-treated plots (Table 2). Plots consisted of six-trees, adjacent to each other within a single tree-row. Buffer zones of 20 m and 16 m at field sites 1 and 2, respectively, were placed between plots to prevent contamination from spray drift. Treatments were applied to six-trees to ensure that no more than 25% of the treated area was required for the provision of residue samples.

Field sites were protected by managing weeds that may have affected the integrity of the test crop. Only permitted herbicides were used, and applied to all trees in the test system according to directions on the product label.

Table 2: Detail of the test system at field sites 1 and 2

Site	Cultivar	Planting year	Plot size (no. trees)	No. reps	No. test plots	No. control plots
Field site 1	Vina	2006	6	1	2	1
Field site 2	Chandler	2000	6	1	3	1

3.2. Application details

Test substance
The test substance was Ethrel® 720 Growth Regulator (active constituent: 720 g/L ethephon); Manufacturer, Bayer CropScience Pty Ltd, 391-393 Tooronga Rd., East Hawthorn, VIC, 3123; Batch no. 500073351; Date of Manufacture, 11-Nov-2009.

The test substances were stored at Walnuts Australia, Tabbita Lane, Goolgowi, NSW, 2652, and Walnuts Australia, Springs Road, Swansea, TAS, 7190, in secure, clean and dry areas. Storage temperatures were documented from the day of receipt of the test substance to the day of application at field site 1. Storage temperatures were not documented at field site 2 as treatments were applied immediately after the receipt of the test substance.

Application rates

Products containing 480 g/l ethephon have been replaced by products containing 720 g/l ethephon; hence, this study was conducted using Ethrel® 720 (720 g/l ethephon). Both formulations of ethephon are soluble concentrates. Directions of use in APVMA permit 11649 state an application rate of 30-90 ml of Ethrel® 480 (480 g/l ethephon) per 100 l of water applied at a spray volume of 1 000 l/ha; this equates to 300-900 ml/ha of Ethrel® 420, or 144-432 g/ha of ethephon.

The test substance was applied at a target rate equivalent to the highest permitted rate in APVMA permit 11649 i.e. 432 g/ha of ethephon, and at two higher rates i.e. 756 and 1080 g/ha of ethephon (Table 3). The higher rates represent rates that are equivalent to, and above, the registered label rates for ethephon applications in walnuts in the United States i.e. 3 pints per acre of Ethrel® (2 lb/gal ethephon) applied at a spray volume of 100 gal/acre, which equates to 840 g/ha of ethephon at a spray volume of 935 l/ha (Bayer CropScience, USA, www.cdms.net/LDat/ld167002.pdf).

Table 3: Rates of Ethrel® 720 (active constituent: 720 g/l ethephon) applied at field sites 1 and 2. The test substance was applied applied with a Stihl® SR 420 air-mist sprayer at a spray volume of 1 000 l/ha.

Site	Treatment no.	Test Substance	Product rate (ml/100 l)	Target rate (g/ha)
Field site 1	1	Non-treated	na	na
	2	Ethrel® 720	60	432
	4	Ethrel® 720	150	1080
Field site 2	1	Non-treated	na	na
	2	Ethrel® 720	60	432
	3	Ethrel® 720	105	756
	4	Ethrel® 720	150	1080

Date and time of application of the test substance

Application of the test substance was at 100% packing tissue brown (PTB) stage of fruit development at field site 1, and at 93% PTB at field site 2. The date and time of application of the test substance is recorded in Table 4.

Table 4: Date and time of application of the test substance at Field sites 1 and 2.

Test site	Date	Start time (h)	Finish time (h)
Field site 1	9-Mar-2010	0730	0825
Field site 2	9-Apr-2010	1250	1330

Calibration of air-mister output

Calibrations were performed just prior to the application of the test substance to ensure accurate delivery. The calibration consisted of three consecutive checks for spray volume output. Variation in the output of the three replicates was within 3.0 % of the mean output (Table 5). Calculations for the amount of test substance to be applied were based upon mean output calculated from the calibration data.

Table 5: Calibration of a Stihl® SR 420 air-mist sprayer, with nozzle number 6, at field sites 1 and 2. The time taken to dispense 2 l of water was repeated three times at each site. Data were then converted to the time taken to dispense 1 l of water prior to calculation of the mean time, and the variation from the mean time for each replicate.

Replicate	Field site 1		Field site 2	
	Time (s/l)	Variation (%)	Time (s/l)	Variation (%)
1	20.5	0.0	19.9	0.0
2	20.5	0.0	20.4	2.5
3	20.5	0.0	19.3	3.0
Mean	20.5	-	19.9	-

Speed calibrations

Speed calibrations, or pass-times per tree, were performed prior to the first test substance application. The speed calibrations were conducted in an area adjacent to the test plot.

Actual Application Rate

Actual application rates and the remaining test solution after application were recorded. The accuracy of applications against the protocol was then verified after the application of the test substance (Table 6). Applications were within -4% and +1% of the target rate and were considered to be of acceptable accuracy.

Table 6. Target and applied rates of Ethrel® 720 (active constituent: 720 g/l of ethephon) applied at field sites 1 and 2. The test substance was applied with a Stihl® SR 420 air-mist sprayer with nozzle number 6.

Field site and treatment no.	Test substance	Target rates and spray volume			Applied rates and spray volume						Variation from C to G
		Product rate **'A'** (ml/100 l)	Spray Volume **'B'** (l/ha)	Active Ingredient[Z] **'C'** (g/ha)	Product Rate **'D'** (ml/12 l)	Solution prepared **'sp'** (l)	Solution left-over **'sl'** (l)	Spray volume **'E' (sp–sl)** (l/plot)	Spray Volume[Y] **'F'** (l/ha)	Active Ingredient[X] **'G'** (g/ha)	**'H'**
Field site 1											
1	Non-treated	-	-	-	-	-		-	-	-	-
2	Ethrel® 720	60	1000	432	7.2	12	2.8	9.2	958.3	414.0	- 4.2 %
4	Ethrel® 720	150	1000	1080	18.0	12	2.7	9.3	968.8	1046.3	- 3.1 %
Field site 2											
1	Non-treated	-	-	-	-	-		-	-	-	-
2	Ethrel® 720	60	1000	432	7.2	12	2.4	9.6	1000.0	432.0	0
3	Ethrel® 720	105	1000	756	12.6	12	2.3	9.7	1010.4	763.9	+ 1.0 %
4	Ethrel® 720	150	1000	1080	18.0	12	2.5	9.5	989.6	1068.8	- 1.0 %

[Z] Target active ingredient; C = (A / 100) × B × 0.72, where 0.72 is calculated from 720 g/l of ethephon

[Y] Applied spray volume; F = E × (10000 / 96) where 10000 m^2 is 1 ha and 96 m^2 is the plot size

[X] Applied active ingredient; G = (D / 12) × F × 0.72, where 0.72 is calculated from 720 g/l of ethephon

[W] Variation between target and applied active ingredient; H = [(C – G) / C] × 100

3.3. Sampling details

Part of crop sampled
Walnut fruit minus outer hull (the 'walnut').

Method of sampling
One sample of walnut (described below) was collected from each plot after treatment applications had dried i.e. from 1 h after application (termed "0 day"), and at 3, 7, 14 and 21 days after treatment at field site 1, and at 3, 7, 13 and 21 day intervals at field site 2. Each sample was representative of the entire plot and was collected during a separate run through the entire plot.

Immediately after picking, walnuts were uncovered by removing the outer hull of the fruit with a sharp knife. Each sample weighed a minimum of 1 kg of walnuts, minus the outer hull, and was representative of the entire plot. Sampling from the end trees in plots was avoided.

At field site 1 (Goolgowi, NSW), walnuts were sampled from high and low areas in the tree canopy, and from areas exposed and sheltered by foliage, in proportion to walnut distribution.

At field site 2 (Swansea, TAS), walnuts were sampled in proportion to walnut distribution within trees, as described in field site 1, for the 0, 3 and 7 day samples. Between the 7 and 13 day samples, a large proportion of nuts dropped from trees; hence, for the 13 and 21 day intervals, walnuts were sampled from underneath the tree canopy, in proportion to those remaining on the tree.

Sample collection was completed for the non-treated plot before proceeding to the plot treated with the lower rate of ethephon and then the plot treated with the higher rate of ethephon. Proper handling practices were followed, with new gloves applied to hands between treatments to prevent transfer of residue from one sample to another.

All samples were placed in plastic zip-lock "sample" bags and stored within a freezer within 1 h of sampling.

Date of sampling and sample inventory
Each sample bag was identified by a sample tag with the following identifiers; sample ID, treatment no., treatment, target rate of the active ingredient, days after application, sample date and contact details.

3.4. Storage and shipment of samples

Storage
For pre-shipment storage, the samples were held frozen at temperatures less than minus 15^{o}C, except when there was freezer cycling and sample movement that caused a temperature increase lasting less than 24 h (Appendix).

Shipment
Prior to the shipment of samples, each 1 kg sample of walnuts was divided into two 0.5 kg samples and then placed into individual plastic zip-lock sample bags (termed

"0.5 kg sample bags"). Each 0.5 kg sample bag was identified by a sample tag, as previously described, and returned to minus 15^{o}C storage.

On the day of shipment to the Laboratory site one 0.5 kg sample bag for each of the treatments analysed was removed from minus 15^{o}C storage, placed into polystyrene containers and transported to the laboratory by overnight express shipment. Shipments had the addition of water ice blocks, sufficient to maintain sample integrity while in transit to the laboratory.

Walnut 0.5 kg sample bags were shipped from minus 15^{o}C storage to the Laboratory site, for the determination of ethephon residue in kernels, on 17-May-2010 and 18-May-10 from Goolgowi, NSW, and Swansea, TAS, respectively (termed "Analysis 1") and on 27-Jul-10 from Swansea, TAS (termed "Analysis 2").

3.5. Analysis of samples

For details refer to: Huang, A. and Chen, B., (2010), Method Validation Report for the Determination of Ethephon in Walnuts, Symbio Alliance, Eight Mile Plains, QLD.

3.6. Conduct of trials

Field sites: Michael Lang, Tasmanian Institute of Agricultural Research, University of Tasmania, Private Bag 7320 Burnie, TAS, 7310, AUSTRALIA; Ph. 03 6430 4522; Fax. 03 6430 4959; Email Michael.Lang@utas.edu.au

Laboratory site: Andy Huang and Bruce Chen, Symbio Alliance, 44 Brandt Street, Eight Mile Plains, QLD, 4113, AUSTRALIA; Ph. 07 3340 5700; Fax. 07 3219 0333; Email admin@symbioalliance.com.au

4. RESULTS

4.1. Ethephon concentration

At field site 1 (Goolgowi, NSW), ethephon concentration in walnut kernels was below the temporary MRL of 0.5 mg/kg in all non-treated walnuts, and in all walnuts treated with ethephon at the highest rate designated in APVMA permit 11649 i.e. 432 g/ha of ethephon (Fig. 1). Ethephon rapidly degraded from 0.61 mg/kg from 3 days after application (DAA) of the highest rate of Ethrel® 720 i.e. 1080 g/ha of ethephon, with ethephon concentration below the temporary MRL at 7 DAA.

Ethephon concentrations in non-treated walnuts at field site 2 (Swansea, TAS) were below the temporary MRL of 0.5 mg/kg (Fig. 2). In walnuts treated with 432 g/ha of ethephon, the highest rate allowable under guidelines outlined in PER11649, ethephon concentration was below the temporary MRL immediately after spray droplets had dried, or 0 days after application (DAA), and at 21 DAA. With higher than currently permitted rates of ethephon, concentrations below the temporary MRL were detected at 0, 13 and 21 DAA for some treatments. The concentration of ethephon in all treated-walnuts was above the temporary MRL 7 DAA of ethephon i.e. between 1.1 and 3.3 mg/kg of ethephon.

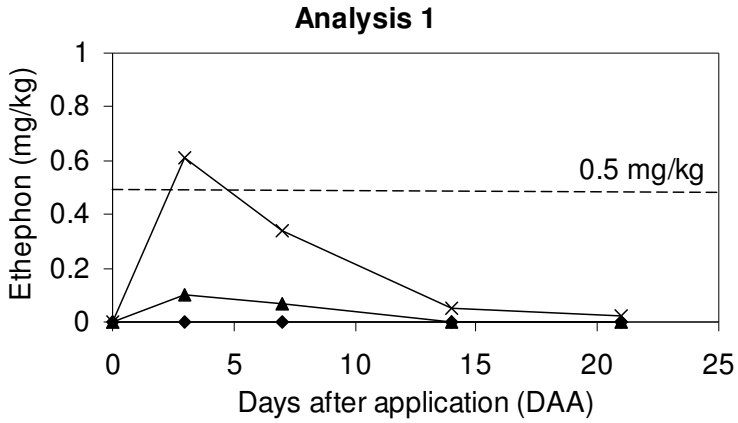

Fig. 1. Concentration of ethephon in walnut kernels at 0, 3, 7, 14 and 21 days after application (DAA) of Ethrel® 720 (active ingredient (a.i.) 720 g/L ethephon) at 432 (triangle) and 1080 g a.i./ha (cross), and in non-treated walnuts (diamond), at field site 1 (Goolgowi, NSW). The test substance was applied on 9-Mar-10 between 0730 and 0825 h with a Stihl® SR 420 air-mist sprayer at a spray volume of 1 000 l/ha. Ethephon concentrations were analysed on 19-May-10 (Analysis 1).

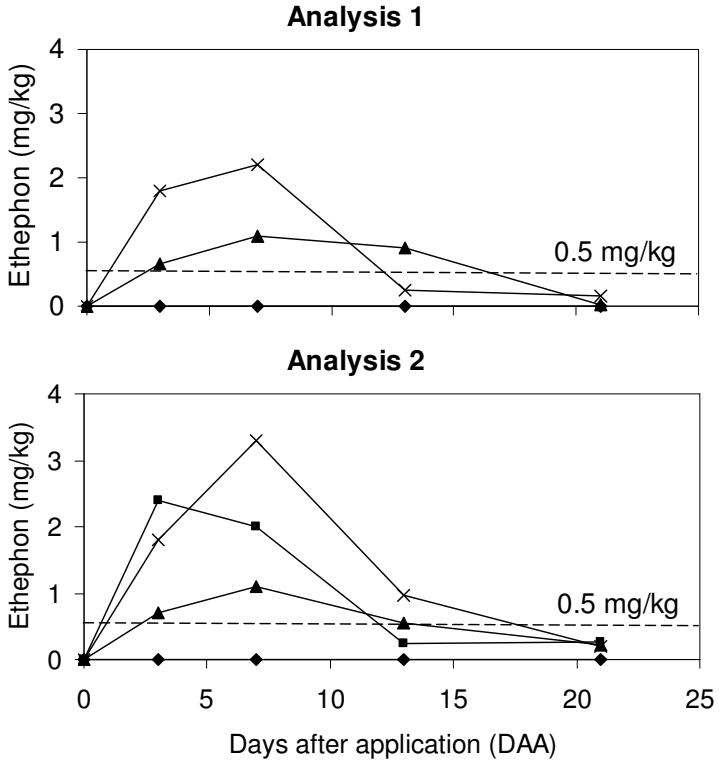

Fig 2. Concentration of ethephon in walnut kernels at 0, 3, 7, 13 and 21 days after application (DAA) of Ethrel® 720 (active ingredient (a.i.) 720 g/L ethephon) at 432 (triangle), 756 (square) and 1080 g a.i./ha (cross), and in non-treated walnuts (diamond), at Field site 2. The test substance was applied on 9-Apr-10 between 1250 and 1330 hrs with a Stihl® SR 420 air-mist sprayer at a spray volume of 1 000 l/ha. Ethephon concentrations were analysed on 19-May-10 (Analysis 1) and 28-Jul-10 (Analysis 2).

4.2 Weather

Mean temperatures were higher at field site 1 (Goolgowi, NSW) in comparison to field site 2 (Swansea, TAS) i.e. 15 to 26°C and 7 to 16°C respectively (Fig. 3). Less than 0.4 mm rainfall occurred within 3 days of applying ethephon at either site (Fig 4). Visual observation of fruit during and after rainfall suggested that ethephon was not "washed-off" fruit (data not presented).

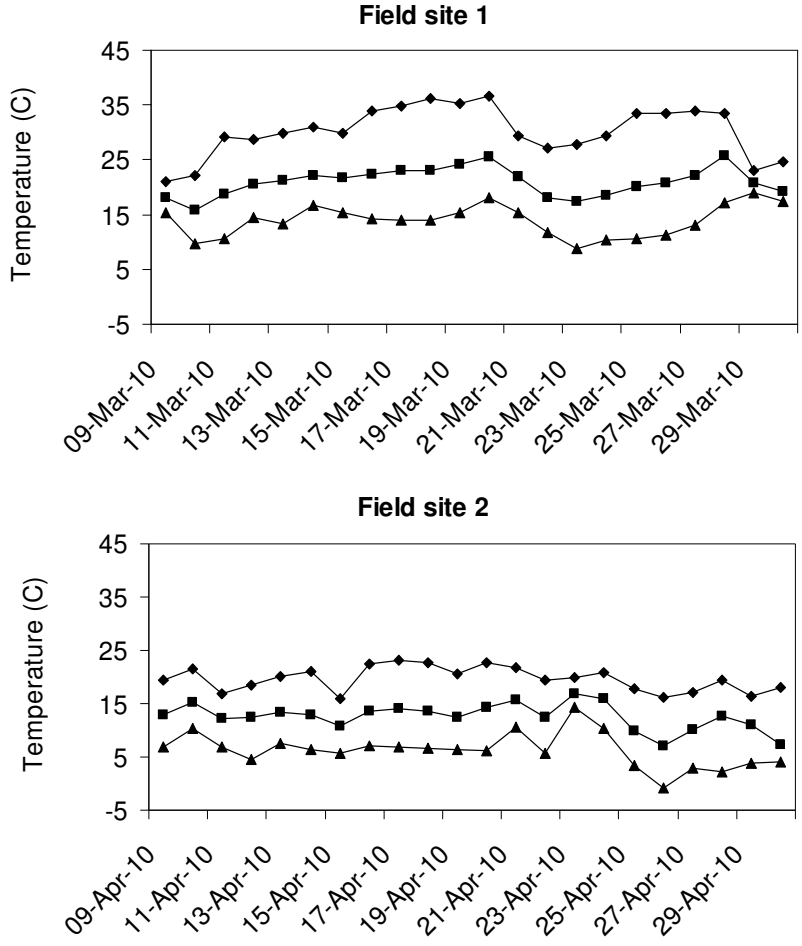

Fig. 3. Maximum (diamond), mean (square) and minimum (triangle) temperatures at field site 1 (Goolgowi, NSW), from the time of applying the test substance (9-Mar-10) to 21 days after application (DAA) (30-Mar-10), and at field site 2 (Swansea, TAS) from the time of application to 21 DAA (9-Apr-10 and 30-Apr-10 respectively).

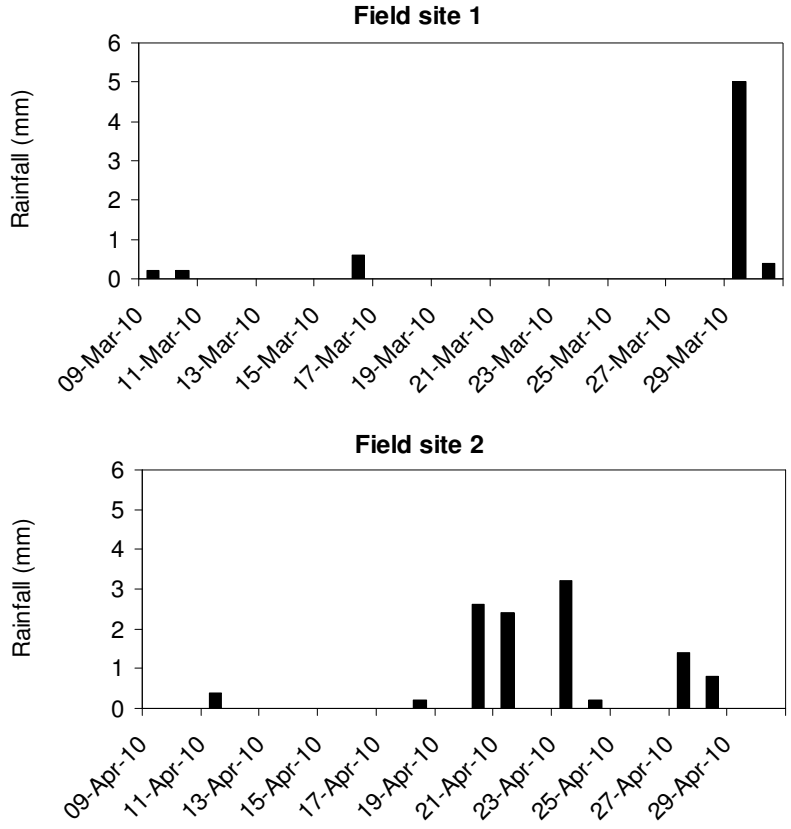

Fig. 4. Daily rainfall at field site 1 (Goolgowi, NSW), from the time of applying the test substance (9-Mar-10) to 21 days after application (DAA) (30-Mar-10), and at field site 2 (Swansea, TAS) from the time of application to 21 DAA (9-Apr-10 and 30-Apr-10 respectively).

5. DISCUSSION

The concentration of ethephon in ethephon-treated walnuts varied between application rates, field sites and sampling and analysis dates. In general, ethephon concentrations were lower, and peaked earlier, at field site 1 ("Goolgowi, NSW") in comparison to field site 2 ("Swansea, TAS"). With walnuts treated with ethephon, at a rate equivalent to the highest rate of ethephon permissible under APVMA permit 11649, concentrations were below the temporary MRL for ethephon in all walnuts at Goolgowi, NSW. In contrast, walnuts at Swansea, TAS, that were treated at the highest permissible rate had ethephon concentrations above the temporary MRL two weeks after treatment; however, concentrations were below the temporary MRL in some treatments where ethephon was applied at rates higher than the permitted rate.

The variation in the temporal development and quantity of ethephon produced between field sites may have, in part, been due to environmental factors. Weather "prior" to application of foliar plant growth regulators (PGRs) may influence cuticle development and thus PGRs uptake (Stover and Greene, 2005); for example, in growth chamber studies, the uptake of 1-naphthalenacetic acid (NAA) increased when

young peach and apple trees were maintained at a temperature of 16°C, in comparison to 21°C, for several weeks prior to application of NAA (Donoho *et al*., 1961). Environmental conditions "after" the application of ethephon can affect the decomposition of ethephon and hence, the release of its active form, ethylene; for example, NAA-induced ethylene production in apple spurs peaked earlier and at higher concentrations when temperatures were maintained at 30°C in comparison to spurs maintained at 20°C, which in turn peaked earlier and higher in comparison to spurs maintained at 10°C (Curry, 1991). Similarly, while ethephon is used to enhance fruit abscission in sweet and sour cherry, Olien and Bukovac (1978) reported that at 16°C and lower there was little response in the decomposition of ethephon to release ethylene. In this study, daily mean temperatures at Swansea were 16°C, or less, and markedly lower than those temperatures experienced at Goolgowi, NSW. Hence, the uptake of ethephon may have been increased, and the decomposition of ethephon limited, in fruits at Swansea, TAS, in comparison to fruits at Goolgowi, NSW; however, research is required to determine the effect of temperature on ethephon residues in walnuts.

6. CONCLUSION

In Australia, APVMA permit 11649 specified a withholding period of 7 days in ethephon treated walnuts prior to harvesting. Similarly, in the United States a withholding period of 5 days is required after ethephon has been applied to walnuts (Bayer CropScience, USA, www.cdms.net/LDat/ld167002.pdf). In the current study, walnuts treated with ethephon were below the temporary MRL of 0.5 mg/kg for ethephon in all walnuts at field site 1 (Goolgowi, NSW) and for many sample dates at field site 2 (Swansea, TAS); however, further residue decline studies are required to determine ethephon concentration in walnuts grown under a greater range of environmental conditions, and in walnuts treated with rates of ethephon above those permitted by APVMA permit 11649.

7. REFERENCES

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8. APPENDIX – STORAGE TEMPERAUTRES

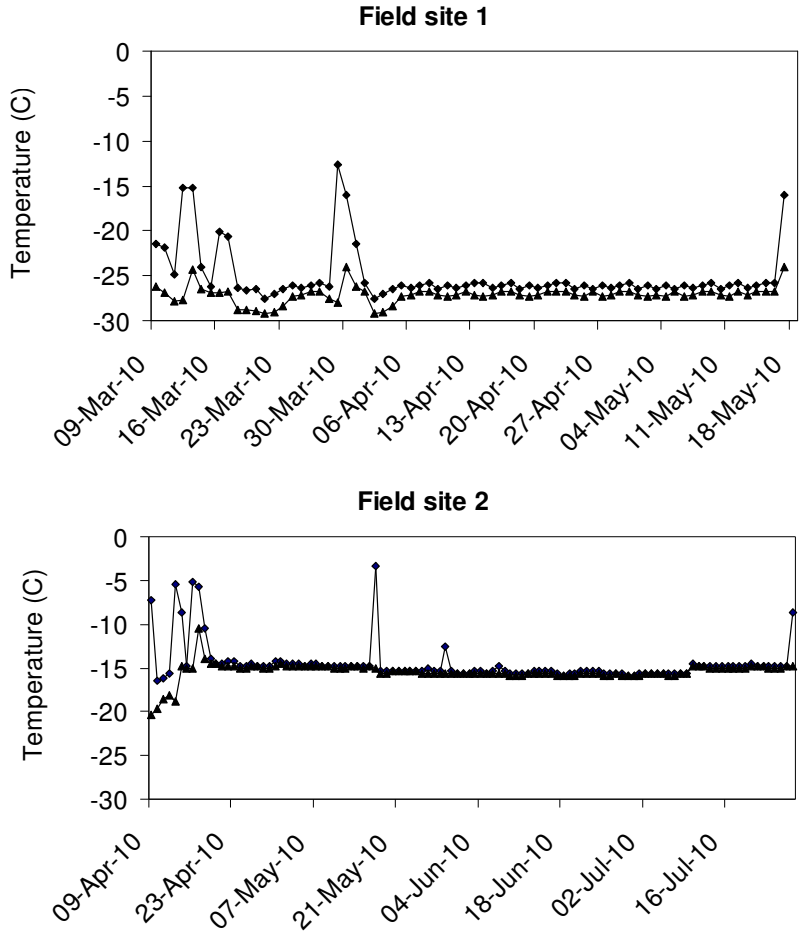

Fig. 5. Maximum (diamond) and minimum (triangle) temperatures of sample storage at field site 1 (Goolgowi, NSW), from the time of applying the test substance (9-Mar-10) to sample shipment for Analysis 1 (17-May-10), and at field site 2 (Swansea, TAS) from the time of application (9-Apr-10) to sample shipment for Analysis 1 and 2 (18-May-10 and 27-Jul-10 respectively).