

LOW OVERNIGHT TEMPERATURES ASSOCIATED WITH A DELAY IN 'HASS' AVOCADO (*PERSEA AMERICANA*) FEMALE FLOWER OPENING, LEADING TO NOCTURNAL FLOWERING

David Pattemore^{1,2*}, Max N. Buxton¹, Brian T. Cutting¹, Heather McBrydie¹, Mark Goodwin¹, Arnon Dag³

¹The New Zealand Institute for Plant & Food Research Limited, Ruakura Research Centre, Hamilton 3210, New Zealand

²School of Biological Sciences, University of Auckland, Auckland, New Zealand

³Gilat Research Center, Agricultural Research Organization, 85280, Israel

Abstract—Avocado (*Persea americana*) has synchronously protogynous flowers: flowers open first in female phase before closing and opening the next day in male phase. Cultivars are grouped based on whether the flowers typically first open in female phase in the morning (type A), or in the afternoon (type B). However, it is known that environmental factors can alter the timing of flower opening, with cold temperatures being shown to affect the timing of flowering. The aim of this study was to investigate how low spring temperatures in New Zealand affect the flowering cycle of commercial avocado cultivars, focusing primarily on the receptive female phase of 'Hass', a type A cultivar. Time-lapse photography was used to assess flower opening times of 'Hass' over three years. Decreasing minimum overnight temperatures were associated with a delay in the timing of 'Hass' female flower phases and resulted in nocturnal flowering of both male and female phase flowers. We recorded insects visiting female flowers at night, and some nocturnal flower visitors collected were carrying avocado pollen. Our study suggests that nocturnal pollination needs to be considered for avocados grown in temperate regions. Furthermore, as the timing of the female phase of 'Hass' varied significantly with overnight temperature, the activity patterns of potential pollinators need to be considered to ensure adequate pollinator activity across the range of times in which 'Hass' flowers are receptive.

Keywords: *Pollination, pollinators, floral biology, phenology, honey bees, flies*

INTRODUCTION

Avocado (*Persea americana*) is an evergreen subtropical fruit tree native to Central America and Mexico and is now grown commercially in tropical and temperate regions globally (Knight Jr 2002). Although self-compatible, avocado flowers show several adaptations for maximising cross-pollination. Flowers are protogynous, opening first as functionally female (pistillate) before closing and opening on the following day as functionally male (staminate), thus preventing self-pollination within a flower (Ish-Am & Eisikowitch, 1993; Evans et al. 2010). Opportunity for geitonogamy is further reduced through synchronous flower opening within a tree and within cultivars, with cultivars grouped in two broad types based on the timing of flower phases. Type 'A' cultivars typically open as female in the morning, then close before opening as male in the afternoon of the following day (e.g. 'Hass'), while type 'B' cultivars open first as female in the afternoon and then close before opening as male in the morning of the following day (e.g. 'Bacon', 'Zutano', and 'Fuerte') (Alcaraz et al. 2011). This dichogamy promotes cross-pollination between the two different cultivar 'types', with one releasing pollen at the same time as the other cultivar is functionally female. While self-

pollination can produce fruit, there is evidence of preferential retention of cross-pollinated fruits leading to a high proportion of these fruits at harvest (Gazit & Gafni 1986; Goldring et al. 1987; Degani et al. 1997). Therefore, cultivar types are usually inter-planted to maximise opportunities for cross-pollination in commercial orchards.

Flowering in avocado is known to be sensitive to environmental conditions (Davenport, 1986), with low temperatures being shown to delay and/or lengthen typical flowering cycles (Ish-Am & Eisikowitch, 1991), omit the female stage of flowering (Ish-Am & Eisikowitch, 1991), and/or reduce yield (Sedgley 1977; Sedgley & Grant 1983). Ish-Am & Eisikowitch (1991) reported that this delay in flowering did occasionally lead to flowers remaining open overnight, but only for type B flowers and type A staminate flowers. In regions where temperatures are cooler, a clear understanding of how temperature can influence the timing of the receptive female phase is important for determining which pollinators may be of particular importance and for developing management strategies to improve pollination.

With relatively sticky pollen (Gazit 1976), avocado flowers require an insect vector for pollination, rather than being able to rely on wind pollination. In most regions, including New Zealand, commercial honey bee (*Apis mellifera*) hives are introduced into orchards to provide this service (Ish-Am & Eisikowitch, 1993; Avocado Industry Council, 2006). However, honey bees are not the only insects

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*Corresponding author: David.Pattemore@plantandfood.co.nz

to visit avocado flowers (Read et al. 2017), and some species, such as bumble bees (*Bombus* spp.), can achieve greater levels of cross-pollination than honey bees for avocado (Ish-Am et al. 1998). As different insect species vary in their daily activity patterns, complementarity in these activity patterns may play an important role in optimising fruit set, especially if the timing of flowering also varies during the day (Read et al. 2017).

Avocado growers in New Zealand experience climatic conditions that are considered marginal for avocado production, with relatively cold and variable spring weather (Appendix I). The implications of this for the timing of avocado flowering and the probability of fruit set are poorly understood. The aim of this study was to assess correlations between overnight temperatures and the timing of the 'Hass' avocado flower phases in New Zealand, with a focus on the receptive female phase. This study also aimed to record which nocturnal insects visited the flowers and to determine if these visitors carried avocado pollen, in order to assess the potential for a new paradigm of nocturnal pollination of avocado.

MATERIALS AND METHODS

Time-lapse images of flowering

Data were collected between late October to early December over three years (2011, 2012 and 2013) from three avocado orchards in the Bay of Plenty and Waikato regions of New Zealand: Katikati in 2011 and 2013 (37°30'50"S 175°58'30"E), Aongatete in 2012 (37°36'32"S 175°55'14"E) and Maungatautari in 2011 (37°58'21"S 175°34'40"E). Nikon D5100 Digital SLR cameras were used to take time-lapse images of 'Hass' avocado flowers (every 5 minutes in 2011, and every 10 minutes in 2012 & 2013), for multiple consecutive days as they cycled through their flowering sequence. Cameras were powered by paired 36-amp hour, 12-volt hour deep cycle marine batteries, with voltage transformed to 220V AC and distributed via 30 m power cables. Batteries were changed every 2-3 days. This power setup was required to keep the cameras operating long enough to capture full sequences of flowering, but then also limited the range and number of trees that could be photographed at any given time. Due to electrical faults and weather conditions the system shut down on occasion before the regular battery replacement and camera check was performed, leading to lost opportunities to capture full flowering cycles.

All cameras were set up to take images of clusters of flowers within an inflorescence over several days and were moved to new inflorescences when the majority of flowers in view had finished. Inflorescences were selected based on a visual assessment of the number of flower buds that were close to opening in the female phase. Only complete sequences of flower opening and closing in either male or female phase were used for analyses.

In 2011, four cameras were used to collect time-lapse images of flowers between 31 October and 2 December. 35 inflorescences on 14 'Hass' trees were filmed, with between 1 and 14 flowers filmed per inflorescence. In 2012, three cameras were used between 24 September and 21 November.

26 inflorescences on 5 'Hass' trees were filmed, with between 1 and 16 flowers filmed per inflorescence. In both 2011 and 2012, only inflorescences on the northern aspect of focal trees were used to keep aspect constant. Although multiple trees were filmed in these years, the logistics of obtaining the images resulted in very low replication per tree in the final dataset. As all 'Hass' trees are clones of single original tree, variation between trees is less important than variation between sites and years. So, the effect of tree identity was not analysed further in this study. Temperature was recorded with HortPlus temperature and humidity loggers placed within a Henshall radiation screen (Henshall 1989) and positioned near the centre of each study block.

In 2013, three cameras were used to collect time-lapse images of flowers between 7 October and 15 November, with cameras placed on the northern, western and south-eastern sides of the tree to assess whether flower opening differed between sides of a tree. A single 'Hass' tree was photographed in 2013, with a total of 16 inflorescences filmed, with between 2 and 17 flowers filmed per inflorescence. Temperature measurements were made at the four quadrants around the tree (north, south, east and west) using thermistors (3 * 50mm tubes) mounted in Henshall screens. All temperature sensors were scanned at 60-second intervals and the average was recorded each hour using a Campbell CR10 data logger.

Images were reviewed and the flower stage was recorded for each flower in view that changed state during the entire time-lapse sequence. Flower stage was assigned based on a predetermined phenology scale of five stages (Fig. 1). We report here on the timing of Stage 1, when flowers first showed signs of opening, which was hypothesised to be most likely correlated to overnight temperatures; Stage 2, when the stigma was first accessible; and Stage 5, when the stigma was no longer accessible on the closing female flower.

All occasions when a flower was recorded reaching Stage 1, Stage 2, or Stage 5 were identified. For the first image of a flower at Stage 1, Stage 2 or Stage 5, the date and time (in minutes since that day's sunrise) were recorded and matched with the minimum temperature recorded the previous night by the relevant sensor (e.g. the southern sensor was used for the south-eastern flowers). All occasions of a flower remaining open overnight in either male or female phase were recorded.

We used binomial generalised linear mixed models in R version 3.2.4 (package lme4), to test if there was a significant association between overnight minimum temperatures and whether the female flowers remained open overnight or not. The response variable was a binary variable indicating whether the flower was open overnight or not, and the main fixed effect variable was the minimum overnight temperature. We ran two models, one with all data from the three years and including year as a random effect, and the second as a generalised linear model just on the data from 2013 with the side of the tree also included as a fixed effect. P-values were generated using the Satterthwaite method of denominator synthesis, implemented within the package lmerTest (this method results in non-integer degrees of freedom). The significance of the 'overnight temperature' term was determined with a likelihood ratio test between models with and without this term. Due to small sample sizes (18 individual flowers in



FIGURE 1. Avocado female flower stages. A = Stage 0, unopened flower bud; B = Stage 1, female flowers show first opening movement; C = Stage 2, stigma first accessible on flower; D = Stage 3, female flower fully open; E = Stage 4, female flower showing first closing movements; F = Stage 5, stigma no longer accessible on closing female flower

2011, 29 in 2012, and 75 in 2013), GLMMs run on the dataset with the timing of each stage as response variables did not converge. So, for these data we ran an analysis of variance on the datasets for each of Stages 1, 2 and 5, with year as a blocking factor, using the command 'aov' in R version 3.2.4 with $\alpha = 0.05$. For each year we then separately assessed the significance of Pearson product-moment correlation coefficients between the previous night's minimum temperature and the mean time (minutes since sunrise) to reach each of Stage 1, Stage 2 and Stage 5, using the command 'cor.test' in R. We then followed this same process for each of three sides of the tree in 2013. To reduce the probability of type I errors due to the multiple comparisons, we used a Bonferroni-corrected alpha of 0.006.

Nocturnal flower visitors

Nocturnally active insects were collected from two 'Hass' avocado orchards in the Bay of Plenty Region, New Zealand. Collections were made on 10 November 2012 at the Maungatautari site, 29 and 30 October 2013 at the Katikati site, and 6 and 8 November 2014 at a second Katikati site (37°30'41.06"S 175°57'14.44"E) on evenings when both female- and male-phase 'Hass' flowers remained open after dark and insects were active. Both orchards also had type 'B' pollenisers planted at a ratio of about 1:8 (polleniser: 'Hass'). Sampling took place between approximately 2100 h and 0000 h.

When possible, insects were caught directly into plastic containers (~50 mL volume) from avocado flowers which they were visiting. Some samples were captured using nets, including insects that were active in proximity to, but not interacting directly with, avocado flowers, as the sampling method with head lamps frequently disrupted insect flower visitors causing them to fly off. Sample containers were immediately placed onto ice to slow and euthanize insects quickly, minimizing opportunity for attached avocado pollen to become dislodged or consumed. Samples were then stored at -20°C until processing, where each sampled insect was inspected under a stereomicroscope and adhered avocado pollen was identified morphologically and counted. Insect specimens were subsequently preserved and identified to the lowest taxonomic level possible.

RESULTS

In total, the timing of the flowering cycles of 122 female-phase and 165 male-phase flowers were documented between 2011 – 2013. Overnight minimum temperature significantly predicted whether female 'Hass' flowers stayed open overnight the following night or not, both when considering the entire dataset ($P = 0.00027$, Table 1) or when modelling the data in 2013 including the sides of the tree ($P = 0.00013$, Table 2), although the side of the tree was not a significant factor (Table 2).

TABLE 1. Output from fitting a binomial Generalised Linear Mixed Model to data from 2011–2013 relating whether female ‘Hass’ flowers remained open overnight to the overnight minimum temperature from the previous night (fixed effect), taking into account variation by year (random effect).

Variable	Estimate	Std. Error	Z value	P value	ΔAIC	X ² value	P-value
(intercept)	1.83497	0.99982	1.835	0.06646			
Overnight temperature	-0.30948	0.09119	-3.394	0.00069	11.21	13.248	0.00027

TABLE 2. Output from fitting a binomial Generalised Linear Model to data from 2013 relating whether a female ‘Hass’ flower remained open overnight to the overnight minimum temperature from the previous night and the side of the tree.

Variable	Estimate	Std. Error	Z value	P value	ΔAIC	X ² value	P-value
(intercept)	2.99829	1.09005	2.751	0.005949			
Overnight temperature	-0.39092	0.11569	-3.379	0.000727	12.65	14.651	0.00013
SE side of tree	0.02378	0.70802	0.034	0.973205			
W side of tree	0.42807	0.66913	0.640	0.522341			

Overnight minimum temperatures were significantly correlated with the timing of Stage 1 ($F = 66.45$, $P < 0.0001$), Stage 2 ($F = 64.41$, $P < 0.0001$) and Stage 5 ($F = 27.51$, $P < 0.0001$), and Year had a significant effect for Stage 1 ($F = 3.65$, $P = 0.029$) and Stage 2 ($F = 3.49$, $P = 0.034$), but not Stage 5 ($F = 1.48$, $P = 0.23$).

The timing of Stages 1 (Fig. 2A), 2 (Fig. 2C) and 5 (Fig. 2E) were correlated with overnight minimum temperature in both 2011 ($P < 0.0001$, $P = 0.00036$ and $P < 0.0001$, respectively) and 2013 (all $P < 0.0001$). In 2013 this correlation was apparent for both Stage 1 and Stage 2 on the northern ($P = 0.0045$ and $P = 0.001$ respectively) and western ($P = 0.0001$ and $P < 0.0001$) sides of the tree (Figs. 2B & 2D).

Using the Bonferroni-corrected alpha of 0.006, no significant correlation between temperature and flower stage timing was detected in 2012 for Stage 1 ($P = 0.024$, Fig. 2A), Stage 2 ($P = 0.044$, Fig. 2C), or Stage 5 ($P = 0.9277$, Fig. 2E), or in 2013 on the south-eastern side of the tree for Stage 1 and 2 ($P = 0.0065$ and $P = 0.1032$ respectively, Figs. 2B & 2D), or Stage 5 in 2013 for any of the sides, north, west or south-east ($P = 0.0162$, $P = 0.0444$ and $P = 0.2113$ respectively, Fig. 2F). These results show that the timing of Stage 1 and Stage 2 was delayed by approximately 30 minutes for every 1°C degree drop in previous overnight minimum temperature, with the latest mean Stage 2 recorded 9 – 10 h after sunrise following nights with minimum temperatures below 8°C (Fig. 2A – D).

Declining overnight temperature had a stronger effect on the timing of Stage 5 when the flowers were no longer able to be pollinated, with an approximate 70-minute delay for every 1°C drop in overnight minimum temperature (Fig. 2E), although the data showed strong bimodality, with a cluster of data points in all years showing Stage 5 occurring 23 – 26 h after sunrise (i.e. the following morning). These data points represent female ‘Hass’ flowers closing the following morning after a nocturnal flowering event.

The proportion of female phase flowers remaining open overnight increased with decreasing overnight minimum temperatures (Fig. 3). When minimum overnight temperatures dropped to 4–6°C, c. 50% of the flowers remained open overnight; when minimum overnight temperatures were 13–15°C, only c. 10% of the flowers remained open overnight (Fig. 3). In contrast, over 80% of male phase flowers remained open overnight when overnight temperatures ranged between 7°C and 15°C, and only dropped to 60% when overnight temperatures were 4–6°C (Fig. 4).

The nocturnal female flowering indicated in the time-lapse data was confirmed through night-time visits to these orchards following low-temperature events. Furthermore, it was observed through both orchard visits and through the time-lapse images that male phase ‘Hass’ avocado flowers remained open overnight on almost all nights studied, including during the warmest conditions recorded in this study.

A total of 161 individual arthropods were caught from avocado flowers at night, representing eight different orders: Araneae, Blattodea, Coleoptera, Diptera, Heteroptera, Hymenoptera, Lepidoptera and Neuroptera (Table 3, Appendix 2), with the largest diversity of flower visitors in Coleoptera, Diptera and Lepidoptera (Fig. 5A). Coleoptera, Diptera and Neuroptera individuals carried more pollen grains on average and at greater frequencies than individuals from other orders (Table 3, Figs. 5B & 5C), while despite having the highest species richness the average number of pollen grains carried by Lepidoptera was low. Of the Diptera, Anisopodids (Wood gnats) and Tipulids (Crane flies) had multiple individuals carrying relatively high numbers of pollen grains (Table 3), as did the Noctuid moth *Rhapsa scotosialis*, the Coleopteran *Costelytra zealandica* (Grass Grub beetle), and the Neuropteran *Micromus tasmaniae* (Tasmanian lacewing; Table 3). One resting *Apis mellifera* (Hymenoptera) individual was also collected and carried 120 pollen grains but was not included in the tables or analyses because it was not active when caught.

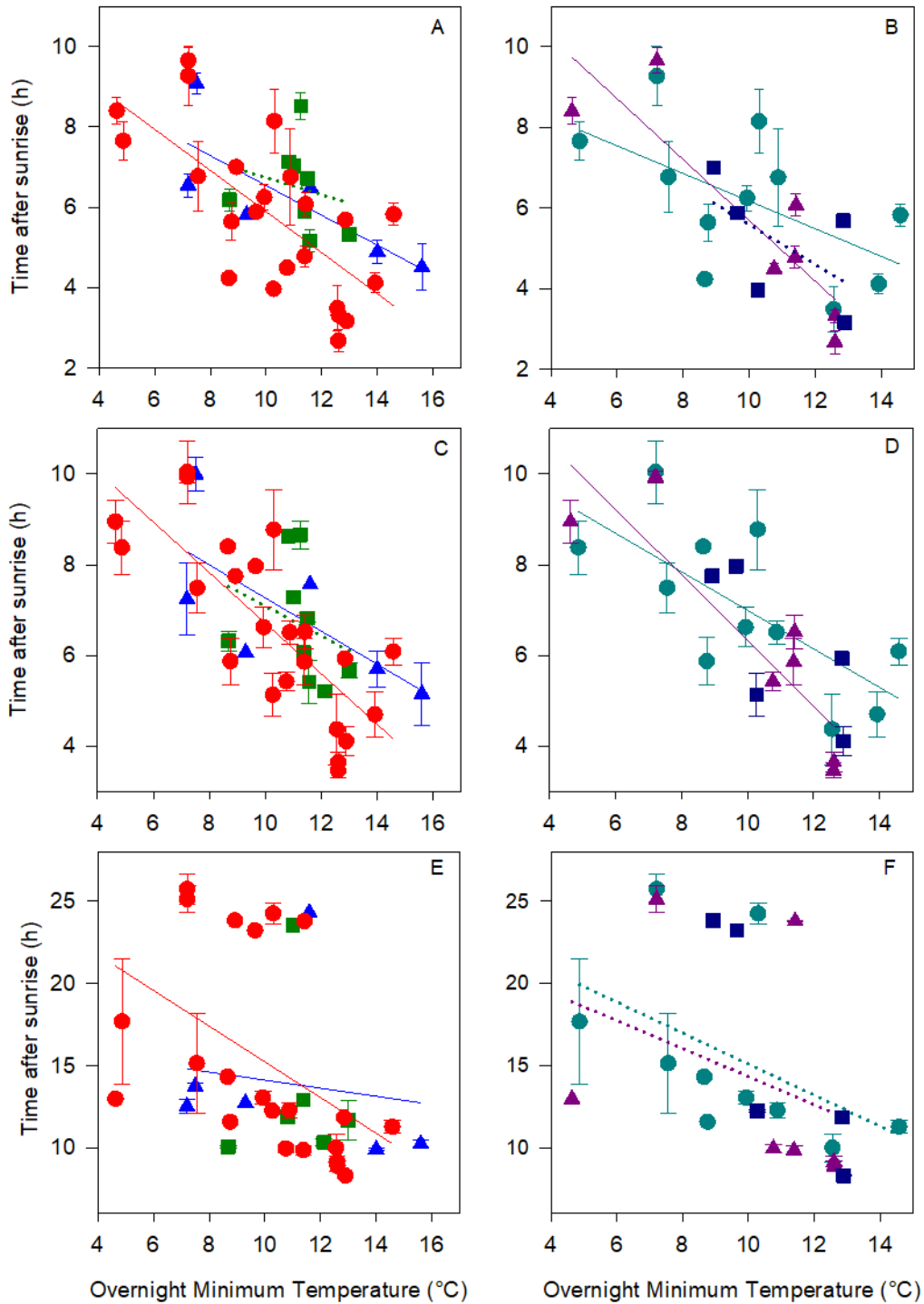


FIGURE 2: The relationship between overnight minimum temperatures and the time to avocado flowering Stages 1 (A), 2 (C) and 5 (E) for 2011, 2012 and 2013, and the time to Stages 1 (B), 2 (D) and 5 (F) in regards to the location of the flowers on the tree (2013 only). Datapoints show times averaged by day of observations (mean +/- SEM). Sunset occurred between 13-16 hours after sunrise during this study. The cluster of points between 23-26 hours in Fig. 2E & 2F indicate flowers closing at or after dawn the following day. Significant correlations ($P < 0.006$) are indicated by solid lines; dotted lines indicate correlations that had P -values between 0.05 – 0.006.

DISCUSSION

This study demonstrates that low overnight minimum temperatures are associated with the delay in the female flower phase in ‘Hass’ avocados such that the flowers remain open overnight. Furthermore, a greater proportion of flowers remained open as functionally female overnight as the prior

nights’ minimum temperatures decreased. Over the temperature ranges recorded in this study, male phase ‘Hass’ flowers remained open almost every night, similar to findings in previous studies (Ish-Am & Eisikowitch 1991), which resulted in our study in significant nocturnal male-female phase overlap within the single cultivar following cold nights.

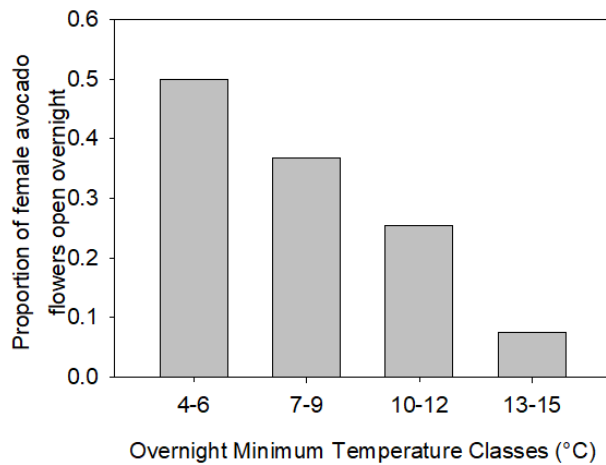


FIGURE 3: The relationship between overnight minimum temperature ranges and the proportion of female 'Hass' avocado flowers that remained open overnight. Temperatures are grouped into classes, each encompassing a range of 3°C.

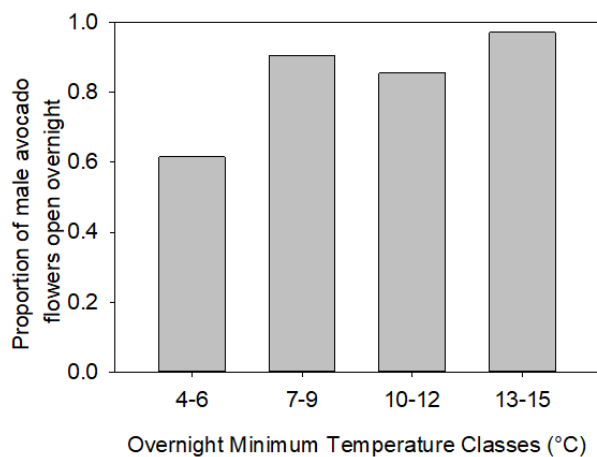


FIGURE 4: The relationship between overnight minimum temperature ranges and the proportion of male 'Hass' avocado flowers that remained open overnight. Temperatures are grouped into classes, each encompassing a range of 3°C.

To obtain sufficient replication of sequences of the full cycle of flowering required a camera system powered by a central battery bank, which limited how far apart cameras could be placed. Our approach in 2011 of shifting the cameras and battery bank frequently resulted in just 18 full sequences from 11 trees. In subsequent years we focused more on obtaining full sequences of flowering. While this prevented us from assessing the influence of tree identity in this study, tree identity is less important in commercial horticulture with crops like 'Hass' avocado where every tree is a clone of the original parent 'Hass' tree. As the influence of the location of the orchard and the year are likely to have a much stronger influence on the pattern of flowering than tree identity, further work is needed to validate the relationship we have documented here in other regions of the world where 'Hass' are grown.

This nocturnal flowering suggests nocturnal pollination could be important in temperate regions with strong climatic fluctuations during the flowering period. The question

remains whether these nocturnal female phase flowers are capable of being pollinated, fertilised and producing viable seed or fruit. Of particular importance is whether pollen deposited on stigmas at night are able to germinate and fertilise the ovules. Pollen growth and ovule fertilisation in many food crops appears to be sensitive to temperature stress, and these processes are often considered to be the most vulnerable in plant stress tolerance (Zinn et al. 2010). Temperature extremes have been shown to alter typical flower and fruit development in avocado (Gazit 1976; Sedgley & Annells 1981; Whiley & Winston 1987; Loupassaki et al. 1997; Alcaraz et al. 2011). While low temperatures have been shown to negatively affect pollen germination and pollen tube growth rates in a number of crops (e.g. Hedhly et al. 2005; Pham et al. 2015; Milatović et al. 2016), the results for avocado have not been as clear with some studies showing a negative effect in particular laboratory conditions (Loupassaki et al. 1997), while others report a difference in the effect of temperature on pollen tube growth between cultivars but no strong positive or negative trend across the temperature ranges tested (Alcaraz et al. 2011).

However, while cold overnight temperature may trigger a process that leads to nocturnal flowering, the temperatures may actually be significantly warmer the following night when the female flowers remain open. In this study, the weather conditions on the nights insects were collected from female flowers at night were mild (~10°C -15°C minimum), rather than cold (<10°C). Further work is required to understand the variation in the activity patterns of these nocturnal flower visitors with temperature. Likewise, more work is required to understand how pollen germination and pollen tube growth are affected by nocturnal conditions and whether that might inhibit fertilisation. While pollen tube growth can be slowed by low temperatures initially, warmer temperatures in the following day may lead to a resumption in pollen tube growth.

Pollination of flowers can take place only when the female flowers are receptive and there is pollen available for transfer. When female-phase 'Hass' flowers start to open in the late afternoon and are thus only receptive during evening, overnight and early the next morning, this will reduce or eliminate pollination by honey bees (*Apis mellifera*), whose activity peaks in the middle of the day or early afternoon (Corbet et al. 1993). It is therefore important that future research should determine whether these late-opening flowers can set fruit successfully in the absence of honey bee visitation, as growers primarily rely on honey bees to manage pollination. It will also be important to understand what proportion of the flowering season is dominated by these colder temperatures in each growing region, and what potential pollinators may be visiting these flowers. If fruit set can occur at these times and the plants encounter these conditions on a significant proportion of the days during flowering, it will be important to ensure that there is adequate pollinator activity across the range of times that these 'Hass' flowers are receptive, especially in the late afternoon, dusk, overnight and dawn.

Eight different invertebrate orders were captured from avocado flowers at night. Coleoptera, Diptera and Lepidoptera were the most frequently caught floral visitors, but it was coleopteran, dipteran and neuropteran individuals

TABLE 3: Arthropod visitors to avocado flowers carrying at least one avocado pollen grain, the total number caught ('Caught'), the number of individuals that were carrying avocado pollen ('Carrying'), and the mean number of avocado pollen grains carried with standard errors when the number carrying pollen is greater than 1 ('mean \pm SEM'). Only species found to carry pollen are represented in this table; a full list of floral visitors is available in Appendix 2.

Order	Family	Species	Caught	Carrying	Mean \pm SEM
Araneae	Unidentified	sp.	1	1	1
Coleoptera	Scarabaeidae	<i>Costelytra zealandica</i>	14	10	6.5 \pm 2.53
	Cerambycidae	<i>Oemona hirta</i>	1	1	5
	Chrysomelidae	<i>Diachus auratus</i>	1	1	30
		<i>Eucolaspis</i> sp.	1	1	17
Diptera	Anisopodidae	<i>Sylvicola</i> sp.	8	6	30.4 \pm 2.53
	Bibionidae	<i>Dilophus nigrostigma</i>	1	1	26
		sp.	1	1	171
	Chironomidae	<i>Chironomus</i> sp.	2	2	3.5 \pm 0.5
	Drosophilidae	<i>Drosophila</i> sp.	1	1	13
	Mycetophilidae	<i>Anomalomyia</i> sp.	1	1	3
		<i>Macrocera scoparia</i>	1	1	6
	Tipulidae	sp.	13	8	19.5 \pm 6.56
Heteroptera	Miridae	<i>Diomocorus</i> sp.	15	3	0.4 \pm 0.24
Hymenoptera	Ichneumonidae	<i>Netelea ephippiata</i>	1	1	4
Lepidoptera	Geometridae	<i>Cleora scriptaria</i>	1	1	11
		<i>Declana floccosa</i>	2	2	1.5 \pm 1.5
		<i>Pseudocoremia suavis</i>	8	2	4 \pm 3.86
	Noctuidae	<i>Rhaphsa scotosialis</i>	10	5	11.4 \pm 7.89
	Tineidae	<i>Opogona omoscopa</i>	3	1	0.3 \pm 0.00
	Unidentified	sp. A	2	1	0.5 \pm 0.5
Neuroptera	Hemerobiidae	<i>Micromus tasmaniae</i>	12	5	7.1 \pm 5.11

that carried the greatest number of pollen grains on average. This is an important distinction to make, as not all floral visitors behave as pollinators: visitation does not necessarily infer pollination (King et al. 2013; Popic et al. 2013; although see Vázquez et al. 2005). Species such as *Costelytra zealandica* (Coleoptera), *Micromus tasmaniae* (Neuroptera), along with Tipulidae and *Sylvicola* species (Diptera) may be especially important, as these were both frequently caught and often carried a high number of pollen grains. Compared with diurnal pollination, nocturnal pollination is poorly understood and relatively little research in New Zealand has tested assumptions that nocturnal floral visitors can act as pollinators (Buxton et al. 2018, but see Pattemore & Wilcove 2011). It is clear from this study that future work is required into the nature of all these nocturnal interactions before their role as nocturnal pollinators can be accurately gauged.

Avocado is characterised by high fruit and flower abscission, resulting in extremely low fruit set (approximately 0.3%), although artificial hand pollination has been shown to increase fruit set up to 5.2-8%, suggesting that pollination may be a limiting factor in avocado orchards (Evans et al. 2010; Ish-Am & Lahav 2011; Garner & Lovatt 2015). No study published to date has assessed the fruit set rate of nocturnal flowers, and it remains unknown if any of these flowers are retained throughout the fruit development process. Avocado often experience strong biennial bearing and increases in fruit set rates in typically low bearing years could help to ameliorate this pattern (Dixon et al 2006, Evans et al. 2010). This new information about the timing of the 'Hass' female-phase flowering and a potential new community of pollinators provides an opportunity to investigate new ways to improve avocado pollination and increase fruit set especially in low bearing years.

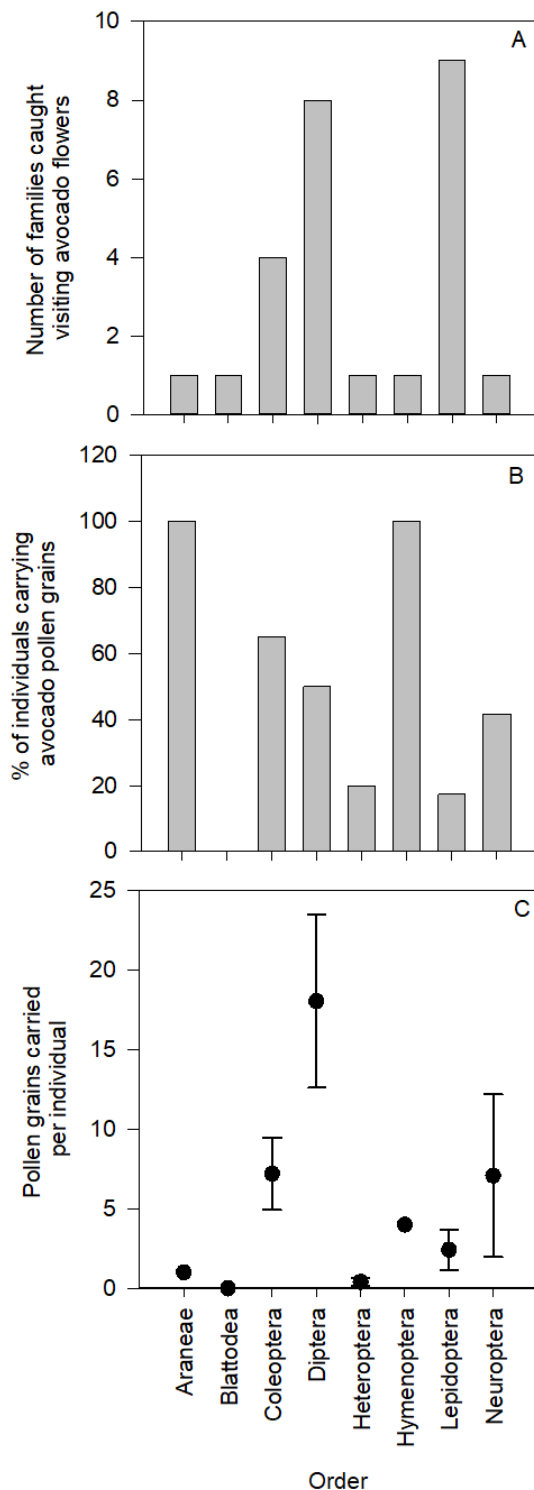


FIGURE 5: Summary of arthropod flower visitors caught while visiting open 'Hass' avocado flowers at night, showing the number of families of arthropods caught in each order (A), the percentage of individuals of each order that were found to be carrying avocado pollen grains (B), and the mean (\pm SEM) number of pollen grains found on each individual of each order (C).

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APPENDICES

Additional supporting information may be found in the online version of this article:

APPENDIX I. Graph showing daily maximum and minimum temperatures during the avocado flowering season in 2011, 2012 and 2013.

APPENDIX II. Full list of nocturnal flower visitors caught on avocado flowers, including those not found to carry avocado pollen.

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