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Invasion pathway risk analysis for the glassy-winged sharpshooter (*Homalodisca vitripennis*): survival and reproductive success following simulated air transportation

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Abstract The glassy-winged sharpshooter, *Homalodisca vitripennis*, is an insect that transmits the economically important plant pathogen *Xylella fastidiosa*. *Homalodisca vitripennis* has been widely studied in North America and the characteristics that make the insect highly successful in new geographic areas are well understood. However, there is a paucity of information on the invasion pathways for long-distance dispersal that have allowed the insect to spread from mainland North America to Hawaii, Easter Island, French Polynesia and the Cook Islands. One potential means of introduction is via motile insects transported in the cargo holds of aircraft. Laboratory studies were undertaken in California to determine the viability of *H. vitripennis* after 24 h of a temperature, humidity and light regime simulating pressurised cargo hold conditions. At all temperatures tested (5, 11 or 22 °C) female adults were capable of surviving whether or not they had access to food and, to a much lower extent, were able to reproduce successfully when re-introduced on a suitable oviposition host plant. This research increases our understanding of the level of risk associated with long-distance air transport as pathway of entry for *H. vitripennis* into countries currently free of the pest.

Keywords Incursion · Air transport · GWSS · Pierce's disease

Introduction

The glassy-winged sharpshooter, *Homalodisca vitripennis* (Germar) (Hemiptera: Cicadellidae), poses a

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serious biosecurity risk to countries that have climates suitable for supporting year round populations of this pest (Hoddle 2004). The insect transmits *Xylella fastidiosa*, a xylem-limited bacterial plant pathogen that forms aggregations in plant water transport vessels and causes disease in a wide range of hosts (e.g., grapes, almonds, and oleanders). *Homalodisca vitripennis* acquires the pathogen during feeding and is a highly efficient vector due to frequent host switching and strong flight and dispersal capability.

Homalodisca vitripennis is native to the southeastern USA and is found in Texas, Georgia, and Florida, and the natural range extends into northeast Mexico (Young 1958; Triapitsyn and Phillips 2000). *Homalodisca vitripennis* has spread with unintentional human assistance to new geographic areas, beginning with California in the late 1980s (Sorensen and Gill 1996) followed by French Polynesia in 1999 (Secretariat of the Pacific Community 2002), Hawaii in 2004 (Bautista et al. 2005), the Cook Islands in 2007 (The Ministry of Agriculture and Forestry 2007) and Easter Island (Petit et al. 2008). The pathogen *X. fastidiosa* is native to the Americas (Purcell 1997) with a range that now includes Canada (Goodwin and Zhang 1997), the USA, Peru, Argentina, Brazil, Paraguay, Central America (Hopkins and Purcell 2002) and Costa Rica (Montero-Astua et al. 2008). The bacterium is invasive in Taiwan (Leu and Su 1993), Kosovo (Hopkins and Purcell 2002), Italy (European Food Safety Authority 2013), and Iran (Amanifar et al. 2014). There are two incursion scenarios in which *H. vitripennis* is of concern: the first is if it establishes in a country or region where *X. fastidiosa* already exists and proves a more efficient vector than native cicadellid species, and the second is if it enters a country that was previously free of *X. fastidiosa* carrying the pathogen, thus introducing the bacterium to a new region where neither the bacterium or *H. vitripennis* previously existed.

Homalodisca vitripennis has several adaptations in order to gain sufficient sustenance from the xylem fluid on which it feeds, which is composed of up to 99 % water in some plant species (Andersen et al. 1992). One of these adaptations is a high consumption rate with daily feeding averaging 100–300 times the dry body weight of the insect (Brodbeck et al. 1993). Adult *H. vitripennis* have been reported to consume 0.21−0.27 mL per hour on some host species (e.g., plum and peach) (Andersen et al. 1989).

The insect's high feeding rate has meant that the isolated location of countries such as New Zealand has been considered an advantage (Biosecurity New Zealand no date) due to the extended travel time the 'hitchhiker' would have to survive without feeding. Direct flights from southern California to Australia or New Zealand take between 12 and 15 h. However, a single stopover can extend this flight time to 17 or more hours (Webjet 2014). Dead *H. vitripennis* adults have been found in aircraft in Cairns, Australia, that have flown from Tahiti and unofficial reports suggest *H. vitripennis* adults have survived the flight from Tahiti to Japan (Grandgirard et al. 2006). Studies have indicated that *H. vitripennis* may be able to survive much longer than was previously thought (over 24 h) without a food source (Phillips 2004; Son et al. 2009), although exactly how long appears to be dependent on temperature.

Long distance transportation of fertilised gravid *H. vitripennis* females is of particular importance because a single female can potentially lay over 100 eggs over her lifetime (>100 days) if a suitable host plant is located (Sisterson 2008). It has been suggested that the establishment of *H. vitripennis* in southern California resulted from just one or two introduction events comprised of very few females, a hypothesis supported by evidence of a genetic bottleneck (Wu et al. 2006; Stenger et al. 2010).

The aircraft pathway for 'hitchhiking' insect pests has been investigated before, both with a focus on interception data (Caton et al. 2006; Liebhold et al. 2006) and experimental testing of the aircraft conditions that insects will be exposed to (Russell 1987), though there appears to be little literature on leafhoppers. Live *H. vitripennis* can fly into aircraft cargo holds or luggage storage bins while planes are on the tarmac. Such a scenario was observed at Fa'a'ā International Airport in Tahiti where planes would be loaded at night under large spotlights that attracted hundreds of adult *H. vitripennis*. Flying insects would hit light fixtures and drop into storage bins that were loaded into the cargo holds of planes (Hoddle pers. obs.). Planes used for inter-island flights in French Polynesia during the day attracted *H. vitripennis* adults if they had yellow logos or propeller tips. Adults were attracted to these yellow areas on planes and observed flying from these yellow areas into the plane cabins and cargo holds (Hoddle pers. obs.). Petit et al. (2008) found that the first detections in the outer islands of

French Polynesia were next to airports or seaports, observations that support air transport as an important invasion pathway. Under the high-density pest conditions that existed prior to the successful biocontrol of *H. vitripennis* in French Polynesia (Grandgirard et al. 2008), adult insects could easily be flown long-distance to new geographic regions.

The cargo hold of an aircraft provides a specific set of environmental conditions that an insect must survive. The temperature can vary between the different holds of the aircraft but is generally between 1.7 and 21 °C (Air Transport Association of America 1991) although if live animals such as pets are being carried, the temperature in the hold must be maintained between 7.2 and 29 °C (US Department of Transportation 2005; Syverson et al. 2008). The mean humidity within the cargo hold, which is pressurized and dark, tends to be low (approximately 30 %) but can rise with animal transportation or low cargo hold temperatures with humidity levels as high as 91 % being periodically recorded (Thornton 2000; JAL-CARGO 2010). Due to restrictions on the transportation of live plant material it is unlikely that there would be any form of food for *H. vitripennis* to utilise in the cargo hold of an aircraft during flights.

A study by Phillips (2004) recorded *H. vitripennis* mortality at 12 and 24 h intervals without a food source and at high temperatures (21–33 °C) and found 25 % mortality after 24 h. A second study investigated adult *H. vitripennis* mortality rates at temperatures ranging from below freezing to 40.1 °C (Son et al. 2009). Mortality was recorded daily with 100 % mortality after 2 days at 34.9 °C and after 3 days at −2.1 and 23.4 °C. These studies were conducted with a light source (Phillips 2004; Son et al. 2009). More importantly from an incursion pathway perspective, earlier studies have not considered the effect of temperature on the length of time *H. vitripennis* can survive without food at an interval of less than 12 h and whether reproductive ability is maintained after this period of stress. The present study employed insect observations at hourly intervals to see whether temperature affects *H. vitripennis* survival and whether reproductive ability is maintained after this fasting period. Work presented here aimed to gain a better understanding of the risk of air transportation of live adult *H. vitripennis* to new regions from countries in which the insect is already established. The actual risk of an incursion event occurring by air will be affected by success at several discrete phases: (1) pre-boarding, (2) in-flight, (3) de-boarding and host finding, (4) and post-flight reproduction. As described above, *H. vitripennis* can readily approach and enter planes, even without human assistance; so satisfying phase i. Phases ii and iv were addressed in this study but further studies are required to quantify risks associated with phase iii.

Materials and methods

Study insects and plants

Field collected male and female *H. vitripennis* adults and nymphs were maintained together in 47.5 × 47.5 × 47.5 cm insect cages (BD44545F, BugDorm store, Taiwan) held in naturally lit greenhouse colonies at the University of California, Riverside USA. Insect colonies were provided fresh basil (*Ocimum basilicum*), bell pepper (*Capsicum annuum*) and okra (*Abelmoschus esculentus*) plants every 2 weeks and plants were hand watered daily. Mixed-age adults were tested between the months of August and October in 2010 (females only; four replicates; n = 240) and again in 2011 (both sexes; three replicates; n = 296), for a total of 536 insects. In 2011 four replicates of newly-emerged adults (molted from fifth instar nymphs, the ultimate immature life stage, less than 2 weeks prior; both sexes; n = 128) were also tested.

For each age cohort and replicate, adult insects were aspirated from the colony and placed into 4 cm-diameter clip cages (one insect per cage) before being subjected to one of the two diet treatments, (1) food: grape seedlings (*Vitis vinifera* cv Pinot Noir) approximately 10 cm tall onto which the clip cage was attached to a foliage-free section of stem, and (2) no food: plant-free control. Three temperature treatments (5, 11 and 22 °C [±2 °C]) were established, each in a separate environment chamber. The experiment was designed as a split-plot with a 2 × 3 factorial treatment structure comprising the two diets crossed with three storage temperature treatments. Within each replicate, the three storage temperature treatments were classified as whole plots and the two diet treatments were classified as sub-plots. For each diet treatment, equal numbers of insects were placed in each of the three chambers.

Humidity averaged 61 % (±17 %), which is within the insect's optimal humidity range and typical of the summer in Riverside, California, humidity conditions that they were exposed to prior to the study (Weather currents 2011). This was also within the relative humidity range experienced in aircraft cargo holds (Thornton 2000) representing a best-case scenario for *H. vitripennis* survival (i.e. a cargo hold that carried many animals so had a relative humidity that was optimal for the insect). Humidity was maintained by placing saturated sodium chloride solution in the environment chambers and temperature and humidity were recorded every half hour using a data logger (Onset Pro Series H0-032-08). Environmental chambers were kept dark to simulate cargo hold conditions.

Insects for a given replicate were transferred from the colony cages to the clip cages and placed in environmental chambers within an hour. Study insects were briefly removed from the environmental chamber every hour to assess survival over a 24 h period. Seven temporal replicates were conducted for adults of mixed ages and a further four replicates of newly molted adults. After 24 h, any surviving insects were aspirated into an individual 2 L bottle cage with mesh windows that contained a single sweet basil plant for feeding and oviposition and held at 24 °C (±10 °C) in a greenhouse. Plants were watered every 2 days. Cages were monitored daily and the dates of egg deposition and hatching were recorded for females as well longevity of the original insects. Monitoring continued until the experimental insect died or for a maximum of 50 days.

Statistical analysis

For the mixed-age cohort and the newly-emerged cohort, the effect of diet, storage temperature, and their interaction on the proportion of insects alive after 24 h (short-term survival) was tested using a generalised linear mixed model (GLMM) with logit link function and binomial error distribution in GenStat (VSN International 2010). The effects of replicate and chamber-within-replicate were classified as random effects in the model with fixed effects of temperature, diet, and their interaction. In 2010 only females were studied whilst both sexes and cohorts were included in the 2011 study, allowing the effect of sex on short-term survival to be tested in 2011. Significance of the fixed effects was established using approximate F statistics at the 5 % probability level, unless otherwise stated. Treatment means were compared on the logit scale using average least significant differences (l.s.d.) and back-transformed to proportions for presentation. For insects that died in the first 24 h, the effect of the diet and storage temperature, and their interaction on the number of hours until death was tested using a linear mixed-effects model (REML). Significance of the fixed effects was established using approximate F statistics at the 5 % probability level, unless otherwise stated. Treatment means were compared using average least significant differences. A similar method was used to analyse the number of days until death for those insects that survived beyond the first 24 h (long-term survival). The effects of diet, storage temperature and their interaction on the proportion of insects still alive after the first 48 h; and those still alive after 50 days were tested in the same way as short-term survival.

Results

Short-term survival

The proportion of insects that survived for the first full 24 h ranged from 0.21 to 0.93 for all temperatures and diet treatments (Table 1). No effect of sex was detected ($F_{1150} = 0.03$, $P = 0.88$) in the 2011 study (when both sexes were tested) and so data from 2010 (in which only females were tested) was combined with that from 2011 for subsequent analysis.

Mixed-age cohort

For the mixed-age cohort, the effect of diet on the proportion of insects surviving differed depending on the storage temperature ($F_{2397} = 4.95$, $P = 0.008$). At 5° insect survival was not significantly affected by diet, whereas at temperatures of 11 and 22 °C survival was significantly higher when food was available (Table 1). Death also occurred faster at higher temperatures for mixed-aged insects without food than at the lower temperatures (Fig. 1). For insects that died within the first 24 h there was evidence of overall diet and storage temperature effects on the number of hours before death (diet: $F_{1127.8} = 5.77$, $P = 0.018$; temperature: $F_{29.4} = 3.93$, $P = 0.057$) whilst the interaction was not significant. The availability of

Table 1 Effects of storage temperature and diet on short-term (24 h) survival and the number of hours until death of a mixed-age *Homalodisca vitripennis* cohort

	Storage temperature									NF mean	F mean
	5°			11°			22°				
	NF	F	5° mean	NF	F	11° mean	NF	F	22° mean		
Insect numbers	69	67		69	67		69	67			
Predicted mean survival[#]	1.15	2.06		−0.18	2.54		−1.35	1.40			
Survival proportion[†]	0.76 b	0.89 ab		0.45 c	0.93 a		0.21 d	0.80 b			
Predicted mean hours until death ± s.e. (n)	18.24 ± 1.7 (18)	17.85 ± 2.2 (9)	18.04 a	18.37 ± 1.4 (37)	11.24 ± 2.5 (6)	14.81 b	14.75 ± 1.3 (53)	12.10 ± 1.8 (15)	13.24 b	17.1 A (98)	13.73 B (30)

The average l.s.d. for survival (logit scale) for the temperature by diet interaction was 0.93. The number of hours until death was calculated using only those insects that died during the 24 h. The overall effects of temperature and diet were significant for hours until death. Means in the same row followed by different letters or cases are significantly different

NF no food, *F* food

[#] Logit scale

[†] Back transformed

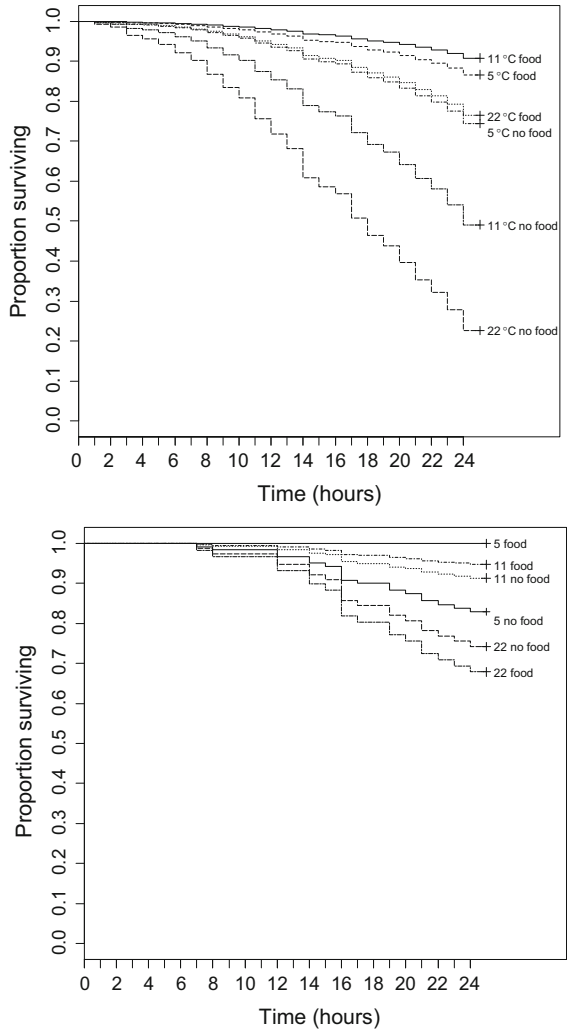

Fig. 1 Survival of *Homalodisca vitripennis* caged with food (food) and without food (no food) over 24 h for the mixed-age cohort (*above*) and newly-emerged cohort (*below*) at storage temperatures of 5, 11 and 22 °C

food reduced the number of hours until death, on average, by 4 h (Table 1). Insects tended to die more quickly at 11 and 22 °C (mean time to death was 14.8 and 13.2 h respectively), compared to 5 °C storage temperature (mean time to death was 18.0 h).

Newly-emerged cohort

Insects with food available, had on average, a higher survival rate than insects denied food for the newly-emerged cohort ($F_{1119} = 5.61$, $P = 0.019$; Table 2). The high survival rates led to insufficient data (in terms of hours before death) being available for formal

Table 2 Effects of storage temperature and diet on short-term (24 h) survival and the number of hours until death of a newly-emerged *Homalodisca vitripennis* cohort

	Storage temperature						NF mean	F mean
	5°		11°		22°			
	NF	F	NF	F	NF	F		
Insect numbers	23	19	23	19	25	19		
Predicted mean survival[#]	1.90	2.91	2.44	2.98	0.36	2.4	1.57	2.77
Survival proportion[†]	0.87	0.95	0.92	0.95	0.59	0.92	0.83 a	0.94 b
Mean hours until death ± s.e. (n)	14.3 ± 1.2 (3)	16.0 (1)	20.0 ± 4 (2)	7.0 (1)	18.0 ± 1.4 (11)	14.0 ± 0 (2)		

The l.s.d. for survival (logit scale) for the diet effect was 1.23. The number of hours until death was calculated using only those insects that died (n) during the 24 h. Means in the same row followed by different letters are significantly different

NF no food, *F* food

[#] Logit scale

[†] Back transformed

statistical analysis; instead descriptive statistics are presented (Table 2).

Long-term survival and reproduction (post 24 h)

Following the initial 24 h temperature study, proportions between 0.34 and 0.98 of the insects from each temperature regime that had been caged without food survived for the next 24 h after being placed on the basil plants (Table 3). For insects stored at 5° there was no significant difference in survival detected between the two diet treatments. For the 11° and 22° storage temperatures insects with access to food had significantly higher survival proportion than those insects without food ($F_{2226.5} = 8.38$, $P < 0.001$; Table 3). Many (68 of 233) of the insects died 24 h after being removed from the controlled environment chambers, however survival was consistently higher for the insects that had had access to food.

Mixed-age cohort

For the mixed-age cohort at 5 °C storage there was no difference detected in the number of days before death between diet treatments, while at both higher storage temperatures insects with access to food survived longer than insects without food ($F_{2119.5} = 14.61$, $P < 0.001$; Table 3). In terms of the probability of survival for 50 days or longer, insects with access to food during the 24 h environment chamber trial generally had a higher survival proportion (0.56) than insects with no access to food (0.22) ($F_{1220} = 17.75$, $P < 0.001$; Table 3), with no effect of storage temperature detected.

Newly-emerged cohort

Insects with food during the 24 h environment chamber trial had a probability of survival of 0.55 for 50 days or longer, which was higher than insects with no access to food ($F_{194.7} = 5.9$, $P = 0.017$; Table 4). No effect of storage temperature was detected. The average number of days before death was greater for insects provided with food in the environment chamber trial compared to insects without food ($F_{149.9} = 28.82$, $P < 0.001$, Table 4).

Very few females reproduced following the 24 h temperature study (Table 5). Of all eggs laid, 45 % were deposited by adults stored at 5 °C, 39 % from adults stored at 11 °C and 16 % from adults stored at 22 °C. For the newly-emerged cohort, only one female with access to food and one female without food oviposited from all temperature treatments. Nymphs emerged from both egg masses. In contrast, reproduction was observed from insects with food from all three temperature regimes in the mixed-age cohort. Those provided with food from 5 °C that oviposited laid 6.3 (±1.2) eggs on average, those from 11 °C laid 5.6 (±1.8) eggs on average, while those from 22 °C laid 5 (±4) eggs on average. Insects without food only succeeded in reproducing from the 5 °C group. Nymphs emerged from all egg

Table 3 Effects of 24 h storage temperature and diet treatments on long-term survival (≥50 days) of mixed-age *Homalodisca vitripennis* following re-introduction to a feeding host

	Storage temperature						NF mean	F mean
	5°		11°		22°			
	NF	F	NF	F	NF	F		
Predicted post treatment 24 h mean survival[#]	0.30	0.89	−0.42	2.37	−0.66	3.94		
Post treatment 24 h mean survival proportion[†]	0.57 c	0.71 bc	0.40 c	0.91ab	0.34 c	0.98 a		
Insect numbers ≥50 days	46	50	28	52	15	42		
Predicted mean ≥50 day survival[#]	−0.7	0.07	−1.8	0.24	−1.4	0.42	−1.29	0.24
50 day or greater survival proportion[†]	0.32	0.52	0.15	0.56	0.20	0.60	0.22 a	0.56 b
Predicted mean days until death (n)	6.6 a (31)	4.8 a (24)	3.0 a (23)	16.9 b (23)	0.4 a (11)	23.2 b (17)		

The mean number of days until death was calculated using only those insects that died in 50 days or less (n). The average l.s.d. for 24 h survival (logit scale) for the temperature by diet interaction was 1.73. The diet effect average l.s.d. for the 50 days or longer survival was 0.67 (logit scale). The average l.s.d. for the number of days until death was 8.63. Means in the same row followed by different letters are significantly different

NF no food, *F* food

[#] Logit scale

[†] Back transformed

Table 4 Effects of 24 h storage temperature and diet treatments on survival of newly-emerged *Homalodisca vitripennis* following re-introduction to a feeding host

	Storage temperature						NF mean	F mean
	5°		11°		22°			
	NF	F	NF	F	NF	F		
Proportion of insects that survived 24 h	0	0.90	0	0.95	0	0.64		
Insect numbers	20	18	21	18	14	17		
Predicted mean survival[#]	−1.05	0.55	−0.42	0.27	−0.91	−0.21	−0.80	0.21
Survival proportion[†]	0.26	0.63	0.40	0.57	0.29	0.45	0.31 a	0.55 b
Predicted mean days until death (n)	10.1 (15)	35.6 (7)	7.9 (13)	19.5 (8)	5.3 (10)	23.8 (9)	7.9 a	27.1 b

The mean number of survival days was calculated only with insects that died in 50 days or less, n is in parentheses. The diet effect l.s.d. for the probability of survival for 50 days or longer was 0.82 (logit scale). The l.s.d. for the number of days until death was 6.9. Means in the same row followed by different letters significantly different

NF no food, *F* food

[#] Logit scale

[†] Back transformed

masses laid by the mixed-age cohort regardless of treatment. The mixed-age cohort oviposited earlier (mean between 9 and 32 days) than the newly-emerged cohort (between 30 and 66 days) but produced fewer eggs (Table 5).

Discussion

The high survival rates observed at all temperature regimes were unexpected. Of those insects that died in the 24 h study, the lowest mean number of survival

Table 5 Reproduction by *Homalodisca vitripennis* and total number of survivors that survived the initial temperature treatments

	Mixed-age cohort						Newly-emerged cohort					
	5 °C		11 °C		22 °C		5 °C		11 °C		22 °C	
	NF	F	NF	F	NF	F	NF	F	NF	F	NF	F
Number of females that oviposited	3	2	0	5	0	3	0	1	1	0	0	0
Number of females survived	39	46	25	49	12	42	9	7	10	8	8	8
Mean days to oviposition	26.6 ± 13.4	32.0 ± 12.0	0	9.0 ± 2.1	0	23.7 ± 13.4	0	30	66	0	0	0
Mean eggs per female ± s.e.	6.3 ± 1.2	6.0 ± 2.0	0	5.6 ± 1.8	0	5.0 ± 4.0	0	8	6	0	0	0

Means are followed by standard errors unless there was only one ovipositing female

NF no food, *F* food

hours for any treatment was 7 h, long enough to survive a flight such as that from the Cook Islands or French Polynesia to Australia or New Zealand (3–5 h [Webjet 2014]). Lower survival rates were observed at 22 °C than at 11 or 5 °C over the 24 h study for both cohorts. The majority of insects without food survived for over 12 h at 5 °C. Low temperatures may cause insects to enter into a form of quiescence that promotes survival when feeding ceases. Son et al. (2010) predicted that *H. vitripennis* adults cease feeding at low temperatures (below 10 °C). It is possible the insects did not need to feed at 5 °C as they were maintaining essential functions only, a hypothesis supported by the observation that insects with food were never seen with their stylet inserted in the grape plant to indicate feeding at this temperature. The risk of an incursion via the aviation industry could be reduced by maintaining cargo holds at the highest acceptable temperature for the duration of the entire travel period (i.e., at gate, taxiing, and flight time). This may stress the insects and reduce the chance of *H. vitripennis* survival, though may not be practical or cost efficient depending on the goods being transported.

In the first 24 h following the temperature study, a low survival rate was seen for those insects that had been caged without a food source at the 11° and 22° storage temperatures. This may be because the insects were too weak following fasting to move themselves onto the plant and begin feeding. The number of survival days following the temperature treatment was lower for insects with no food than those with food for the majority of temperatures and cohorts. This was likely due to the lack of nutrition during the 24 h temperature study, perhaps weakening the insects and inducing early mortality. In terms of the four phases of risk of incursion by air, this finding suggests that most insects will not locate a suitable host plant following air transit. This is a positive biosecurity outcome as it reduces the chance of establishment success should a gravid female arrive in a new country by aircraft. Notwithstanding this fact, host plants of *H. vitripennis* including citrus, *Vitis* spp. and *Ocimum* spp. are cosmopolitan as crop and ornamental species so likely to be present in many recipient locations including in close proximity to arriving aircraft. Accordingly, even insects weakened by starvation during transport will have a finite chance of locating hosts. For insects transported with food plants, the risks are greater.

The survival under temperature and feeding stress findings suggest *H. vitripennis* could arrive in new geographic areas alive, and may encounter host plants. However successful reproduction is essential for establishment. Oviposition was not recorded for insects without food in the 22 °C group which may be due to temperature and feeding stress. The average number of eggs deposited per female at 5 and 11 °C did not exceed eight. This is low-egg masses usually contain 10–20 eggs (Contra Costa County California no date)— an outcome that is positive in biosecurity terms. In other studies, some females never matured eggs and the reason for this is unknown (Setamou and Jones 2005; Sisterson 2008; Pilkington et al. 2014). Although all female insects used for the study had the opportunity to mate prior to caging, there is the possibility that some were unmated. In an invasion situation this may not be an issue as a male and female may arrive and mate, or females may live long enough to mate with their sons, or mating could occur between siblings from the same egg mass. Nevertheless, it is feasible that a single *H. vitripennis* individual could be responsible for establishment of the pest. A single female, once mated, has been shown to oviposit up to

967 eggs over her lifetime (Krugner 2010) suggesting that a viable population could establish under favourable environmental conditions.

The average pre-oviposition period for surviving *H. vitripennis* females in this study ranged between 9 and 66 days, similar to those recorded for Riverside populations (an average of 28.2 days for the F_0 generation and 62.3 days for the F_2 generation) in other studies (Krugner 2010). At least 15 % of the insects that survived the temperature treatment survived for 50 days or more indicating that if a gravid female survives the flight, it is possible that she will have the opportunity to oviposit before dying with the assumption that the female will be strong enough to leave the airplane and find a nutritionally adequate host plant in the new region.

In an invasion scenario, the insect that arrives may be a newly-emerged adult or an adult greater than 3 months old and it is important to understand the risks posed by each. Survival rates were much higher for the newly-emerged cohort than the mixed-age cohort during the 24 h temperature study. Newly-emerged adults are likely to be better able to tolerate transit stresses imposed on them due to their age. Conversely, the post-stress reproductive success of newly-emerged adults was much lower than that of the mixed-age cohort (which was assumed to contain older insects). This may be a function of egg maturation rate (Sisterson 2008) and it is possible eggs of the newly-emerged cohort were not yet mature enough for oviposition. This is supported by the high number of days (30 and 66) before oviposition for the two newly-emerged adults that did reproduce. Oviposition was observed for a maximum of 70 days due to time constraints. It seems that although a young adult stands a better chance of surviving air flights to new regions, older insects may pose a greater threat as successful reproduction could be more likely to occur should these insects escape the plane.

CLIMEX modelling indicates that regions with tropical, semi-tropical, mild-temperate, and moderate Mediterranean climates are likely to be suitable for habitation by *H. vitripennis* (Hoddle 2004). Nelson–Marlborough and Hawkes Bay New Zealand, regions in New South Wales, South Australia, Southern West Australia, and Tasmania in Australia, the Bordeaux region of France, appellations of Galicia, Pais Vasco, Cataluna, Valencia, and Andalucia in Spain and central and southern areas of Italy appear to be vulnerable to invasion by *H. vitripennis* because of hospitable year round climates (Hoddle 2004). Countries such as Italy that have *X. fastidiosa* but are currently free of *H. vitripennis* could find that they have a vector that is much more effective than native vectors should *H. vitripennis* arrive via air transport or other means.

This study shows that cargo holds of aircraft are a viable means of introduction of *H. vitripennis* into other countries. If an adult were trapped in a cargo hold it appears likely it will be alive upon arrival into another country despite a lack of food for the duration of the flight. This is true of any location in which *H. vitripennis* exists, be it North America, French Polynesia or the Cook Islands as even indirect flights from each of these regions rarely exceed 24 h. Air transport should therefore be considered an important invasion pathway. If the insect were a gravid female, oviposition is possible, though less likely than survival. In order to gain more comprehensive understanding further reproductive studies are needed at intervals of several hours to encompass flight lengths from all infested locations. Even so, if all environmental and climatic factors are conducive, one introduction event could lead to the establishment of a population of *H. vitripennis* in a new geographic range.

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