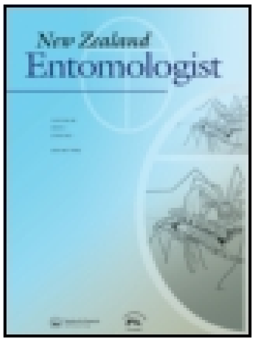

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ABSTRACT

The brown marmorated stinkbug (BMSB), *Halyomorpha halys* Stål (Hemiptera: Pentatomidae), is native to Asia and is characterised by its polyphagous feeding habits and high hitchhiking potential. In invaded areas, such as the eastern USA, economic damage to agricultural crops by BMSB has been significant. In northern Italy, where BMSB is invasive, feeding damage has been recorded in commercial kiwifruit orchards. In New Zealand, a major kiwifruit producer, BMSB originating from the USA, Italy and China (the native range of kiwifruit) have been intercepted. These BMSB interceptions pose a high biosecurity risk to key agricultural industries in New Zealand, including kiwifruit. However, information on the ability of BMSB to feed on key commercial kiwifruit varieties, and the types of damage it may cause to this crop, is lacking. To address this issue, *Actinidia chinensis* var. 'SunGold' (G3) and *Actinidia deliciosa* var. 'Green' (Hayward), were exposed to adult BMSB under no-choice and choice feeding trials. Across kiwifruit cultivars (i.e. Green and SunGold) and experimental setups (i.e. choice and no-choice), mixed adult groups (i.e. males and females feeding together) caused significantly more damage than individual females and males. After accounting for adult density, there was no experimental evidence that BMSB exhibited a feeding preference for either SunGold or Green varieties. However, there were variety differences for the development of BMSB feeding injury, with lower incidence of damage recorded for SunGold.

KEYWORDS

BMSB; feeding injury; invasive species; kiwifruit; pentatomid

Introduction

Brown marmorated stink bug (BMSB), *Halyomorpha halys* Stål (Hemiptera: Pentatomidae), is a polyphagous stink bug species native to East Asia (i.e. China, Japan, Taiwan and Korea) (Rice et al. 2014). Adult BMSB have strong flight dispersal and hitchhiking capabilities (Lee & Leskey 2015; Wiman et al. 2015a). These dispersion traits in combination with a polyphagous feeding habit have assisted BMSB in spreading and colonising new areas with favourable climate, food and lack of natural enemies (Rice et al. 2014). Invasive BMSB populations are well established in parts of North America (i.e. USA in the 1990s and Canada in 2012) and Europe (e.g. Switzerland [detected 2004], Liechtenstein [2004], France [2012], Greece [2011], Italy [2012], Hungary [2013], Russia [2013], Romania [2014], Georgia [2015] and Abkhazia [detected after 2013]) where it causes

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nuisance problems in residential areas and damage to agricultural crops (Rice et al. 2014; Haye et al. 2015; Macavei et al. 2015; Gapon 2016; Maistrello et al. 2016). In 2017, BMSB established in Chile (Faúndez & Rider 2017) and this outcome continues to confirm predictions that this insect has the ability to colonise suitable climatic regions outside of its native range, including the Southern Hemisphere (Zhu et al. 2012).

Kriticos et al. (2017) and Zhu et al. (2012) identified parts of Australasia (e.g. the majority of southern and eastern coastal areas of Australia, and most of the North Island in New Zealand, including the Bay of Plenty) as being climate-suitable for BMSB establishment. Government biosecurity agencies have led efforts aimed at reducing the risk of accidental BMSB introductions into New Zealand and Australia through direct pathways such as maritime shipping routes and aerial transport of BMSB-infested materials (KVH 2013, 2016a, 2017a; MPI 2017; AU-DAWR 2017a). However, despite attempts at incursion mitigation, a well-defined window of opportunity for BMSB establishment exists in austral spring and summer. In New Zealand, for example, more than 152 interception events, consisting of one or more BMSB adults, occurred over September 2016–April 2017 (KVH 2017b). During this critical period, BMSB have been introduced into Australasia from the Northern Hemisphere in a state of diapause. However, it is possible that BMSB adults that manage to escape from points of entry would encounter favourable climate conditions that could break diapause and allow small founder BMSB populations to establish (Duthie 2015).

The potential for BMSB establishment in New Zealand poses a substantial threat to agricultural and ornamental host plants of economic importance, of which kiwifruit has been identified as an at-risk crop (KVH 2016b; AU-DAWR 2017a, 2017b). Kiwifruit has been reported as a BMSB feeding host in China, the largest producer of kiwifruit in the world and part of the native range of both species (Yang et al. 2009; Ferguson 2015). In New Zealand, kiwifruit is a major horticultural export crop with a market value exceeding NZ$1.7 billion (SNZ 2016) and the majority of kiwifruit production (> 80% of c. 12,000 ha) is concentrated in the Bay of Plenty (KVH 2016c; Zespri 2017). The introduction of BMSB into major New Zealand kiwifruit production areas is possible given that 69% of BMSB interceptions from 2016–2017 occurred in Auckland where international air and shipping ports are situated and commercial kiwifruit orchards are located, accounting for > 400 producing ha in that region (KVH 2016c; Zespri 2017). Once established locally, BMSB adults have high dispersal potential, with laboratory studies suggesting some individuals may be capable of flying c. 70 km per day (Wiman et al. 2015a). Accidental human-assisted dispersal from Auckland to Te Puke in the Bay of Plenty would require movement of only c. 200 km.

Interestingly, New Zealand has a resident fauna of native and exotic pentatomids (Larivière 1995), but none of these species have been documented as kiwifruit pests, nor are there records of specialised natural enemies occurring in kiwifruit orchards that can attack BMSB (see Todd et al. 2011). BMSB has the potential to be the first exotic pentatomid kiwifruit pest that could establish in New Zealand and subsequently benefit from enemy free space (see Zhang et al. 2017). This risk to kiwifruit could be further amplified because insecticides have limited efficacy for reducing crop damage by BMSB (Kuhar & Kamminga 2017; Morehead & Kuhar 2017) and this pest could cause economic losses and disrupt existing pest management programmes similar to that seen in other fruit crops (Leskey et al. 2012; BOPRC 2017; KVH 2017c).

BMSB feeding damage is suspected in kiwifruit orchards in northern Italy and South Korea (KVH 2016a; Palmer 2017), and BMSB has been detected in a commercial kiwifruit orchard in northern California, USA (Lara et al. 2016). In this context, BMSB establishment in Chile (Faúndez & Rider 2017) is concerning because Chile is the third largest global exporter of commercial kiwifruit (primarily *A. deliciosa*) (Cruzat 2014). However, despite anecdotal reports of BMSB inhabiting kiwifruit orchards in the native and invaded ranges, there are no data quantifying BMSB feeding damage on commercial kiwifruit varieties such as *Actinidia chinensis* var. *deliciosa* 'Hayward' marketed as Zespri® Green and *Actinidia chinensis* var. *chinensis* 'Zesy002' (Gold3) marketed as Zespri® SunGold. The majority of China's kiwifruit production consists of local varieties for domestic consumption (Ferguson 2015) so it is not clear whether commercial varieties, like those developed in New Zealand

and representative of global export markets, would be susceptible to BMSB feeding. Hill et al. (2007, 2010) noted *Actinidia* germplasm present in New Zealand displayed varying levels of resistance to non-native armoured scale insects. Consequently, some kiwifruit varieties may be less preferred by BMSB as feeding hosts or tolerant to BMSB feeding injury and this possibility may warrant potential future investigation (see Hedstrom et al. 2014; Joseph et al. 2014; Wiman et al. 2015b).

The primary objective of this study was to assess under quarantine laboratory conditions the acceptance, preference and feeding damage levels from adult BMSB to kiwifruit varieties, Green and SunGold, in vine-condition representative of late-season fruit that is exported. Adult BMSB were used in this study because this stage is highly mobile, it has been intercepted at ports of entry in New Zealand, and adults have strong piercing sucking mouthparts capable of penetrating deep into plant tissue and causing significant feeding injury (Hedstrom et al. 2014; Peiffer & Felton 2014; Wiman et al. 2015b). The results of choice and no-choice feeding trials exposing adult BMSB to Green and SunGold kiwifruit are presented here.

Methods

Experimental setup

Zespri® Green and Zespri® SunGold kiwifruit, picked on the vine condition the week of 15 February 2016 from the Bay of Plenty, New Zealand, were phytosanitary inspected, certified and air-freighted to California, and received at University of California, Riverside on 24 February 2016 for use in experiments. This timeframe coincided with the availability of on the vine kiwifruit, a concern for potential early-season feeding by BMSB, and active (non-diapausing) BMSB colonies in California. To maintain approximate vine-condition quality, shipped fruit were packaged in sets of four to six inside high-grade 12″ × 16″ ethylene absorption bags (PeakFresh® USA, Lake Forest, CA) with activated 1-unit Tyvek-enclosed food grade silica packets (Desi Pak®, Belen, NM). Packaged fruit were maintained in a walk-in temperature-controlled chamber at 4 °C until used in adult BMSB feeding trials.

Feeding trials consisted of no-choice and choice exposure of kiwifruit to BMSB and were designed to measure feeding ability and kiwifruit preference, respectively, at each of four BMSB densities: 1. no BMSB (i.e. the control treatment provided a baseline measure of fruit deterioration under experimental conditions in the absence of BMSB); 2. one single adult BMSB female; 3. one single adult BMSB male; and 4. a mixed group of four BMSB adults (two female and two male). The maximum number and representation of both BMSB sexes and length of exposure was adapted from previous BMSB fruit exposure work that assessed feeding damage (Hedstrom et al. 2014; Lara et al. unpubl. data). Reproductive adult BMSB were sourced from laboratory colonies maintained in quarantine at the University of California, Riverside Insectary & Quarantine facility (UCR-IQF) under California Department of Food and Agriculture permit number 3020. All BMSB used in feeding trials were mature adults with no previous exposure to kiwifruit.

For no-choice and choice trials, single Green and/or SunGold kiwifruit were placed in a 13 cm × 13 cm cylinder container (Tri-State Plastic, Inc., Latonia, KY) lined with coffee filter paper, accompanied with a 59 mL plastic water cup (First Street™, Los Angeles, CA) with a perforated lid which accommodated a moistened cotton wick for BMSB adults to drink from. Choice test arenas included one Green and one SunGold kiwifruit and the water supply. Fruit were randomly assigned to one of four BMSB exposure treatments listed above. The experimental setup represented a 2 × 4 factorial design (eight treatment combinations) for no-choice and choice trials with each treatment combination replicated 10 times.

All feeding trials lasted 7 days and were conducted over a 10 week period, from 25 February 2016 to 2 May 2016 at 23 °C with 40% RH and 16:8 L:D photoperiod. This photoperiod is above the 13.5–14 h threshold needed to maintain BMSB adults in an active state (Lee et al. 2013). During weeks 5–10 of the study, flesh firmness for kiwifruit varieties at the start of feeding trials was measured (i.e. mean kg F/cm^2 ± SEM): 6.41 ± 0.84 (Green) and 5.06 ± 0.60 (SunGold), which indicated that fruit were starting to soften under storage conditions. These values are below the range of recommended guidelines for kiwifruit harvesting from vines (Li et al. 2016) so the influence of this covariate (uncontrolled variable) in feeding measurements was accounted for in statistical analyses.

At the end of each feeding trial, kiwifruit were removed from treatment containers and the following data were collected: 1. total number of BMSB salivary sheaths (Figure 1; Peiffer & Felton 2014) on the pericarp were counted under a stereomicroscope and recorded as feeding events; 2. dissolved soluble contents (i.e. degrees Brix) were measured from the mid-centre of each fruit using a digital refractometer (model HI96813, Hanna Instruments, Woonsocket, RI); 3. weight loss (g) was recorded as the difference of weight measurements taken at the beginning and end of each trial using a digital scale (model Accu-124, Fisher Scientific, Arvada, CO); 4. terminal flesh firmness (kg F/cm^2) was measured using a handheld digital penetrometer equipped with a 7.9 mm probe (model TR-FHT-1122, Teren, China); and 5. internal BMSB feeding damage on kiwifruit flesh evident as white corking tissue was recorded. To assess feeding damage all experimental kiwifruit were cut lengthwise to produce eight symmetric fruit wedges. Each wedge side (i.e. interior flesh) was inspected and scored separately as '0' or '1' for absence or presence of corking damage (Figures 2–3), respectively. All scores were summed to generate a total damage score out of a maximum score of 16, indicative of all 16 kiwifruit wedge sides displaying BMSB feeding damage.

Statistical analyses

Total salivary sheath counts for no-choice and choice kiwifruit exposure trial groups were analysed separately using negative binomial regression and the log link function using PROC GENMOD in SAS 9.3 (SAS Institute 2011). Statistical significance of categorical variables (i.e. kiwifruit type,

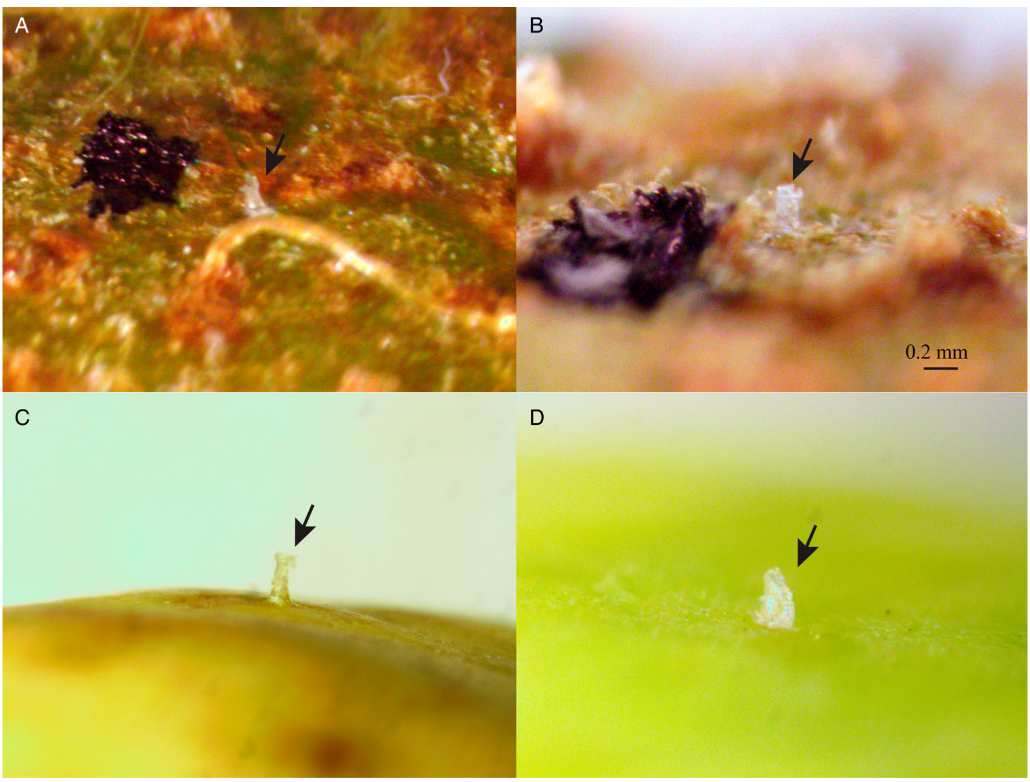

Figure 1. Examples of salivary sheaths (arrows) deposited by brown marmorated stink bug (BMSB) on the external surface of different foodstuffs. **A–B,** Green kiwifruit; **C**, pistachio nut; **D**, table grape.

Figure 2. Comparison of brown marmorated stink bug (BMSB) feeding damage on Green kiwifruit. **A**, External surface of Green kiwifruit not exposed to BMSB (control); **B**, flesh of Green kiwifruit not exposed to BMSB; **C**, external surface of Green kiwifruit exposed to BMSB with black circles (drawn in with an indelible black marker) indicating position of feeding sheaths that were identified using a microscope (see Figure 1); **D**, flesh of Green kiwifruit exposed to BMSB with arrows pointing to internal white corking damage to fruit flesh which was caused by BMSB stylet penetration and presumed feeding.

Green or SunGold) and BMSB treatments (single female, single male, mixed group with two female and two male adult BMSB), and flesh firmness, a covariate factor, was determined using Type 3 analyses. Planned contrasts between BMSB treatments, excluding negative control replicates (no BMSB sheaths were present for this treatment because fruit were not exposed to feeding adults, thereby eliminating the requirement to compare control sheath counts to BMSB-exposed fruit) were conducted for least mean squares (lsmeans) among no-choice ($n = 3$) and choice ($n = 3$) treatment groups. Additionally, goodness of fit chi-square tests were conducted to assess final model fit.

To satisfy normality assumptions, Brix (degrees) and weight loss (g) values were transformed using a Box–Cox power transformation, lambda1 = 0.45 for Brix and lambda2 = −0.14 for weight loss, using PROC TRANSREG in SAS 9.3 (SAS Institute 2011). Transformed values for each response variable were subjected separately to analysis of covariance using PROC GLM in SAS 9.3 (SAS Institute 2011). Statistical significance of kiwifruit variety, sex treatments and flesh firmness was determined using Type III sums of squares. Planned contrasts between sex treatments for Brix ($n = 7$) and kiwifruit weight loss ($n = 4$) were conducted for lsmeans.

Mean kiwifruit damage scores for no-choice trials were analysed using negative binomial regression and the log link function using PROC GENMOD in SAS 9.3 (SAS Institute 2011). Similarly, statistical significance of kiwifruit variety, BMSB treatments and flesh firmness was determined using Type 3 analyses. For analyses of no-choice replicates, all possible treatment pairwise contrasts between sex treatments and kiwifruit varieties ($n = 15$) were performed for lsmeans. For choice trials, the damage scores were analysed separately with chi-square tests of homogeneity (to test equality of

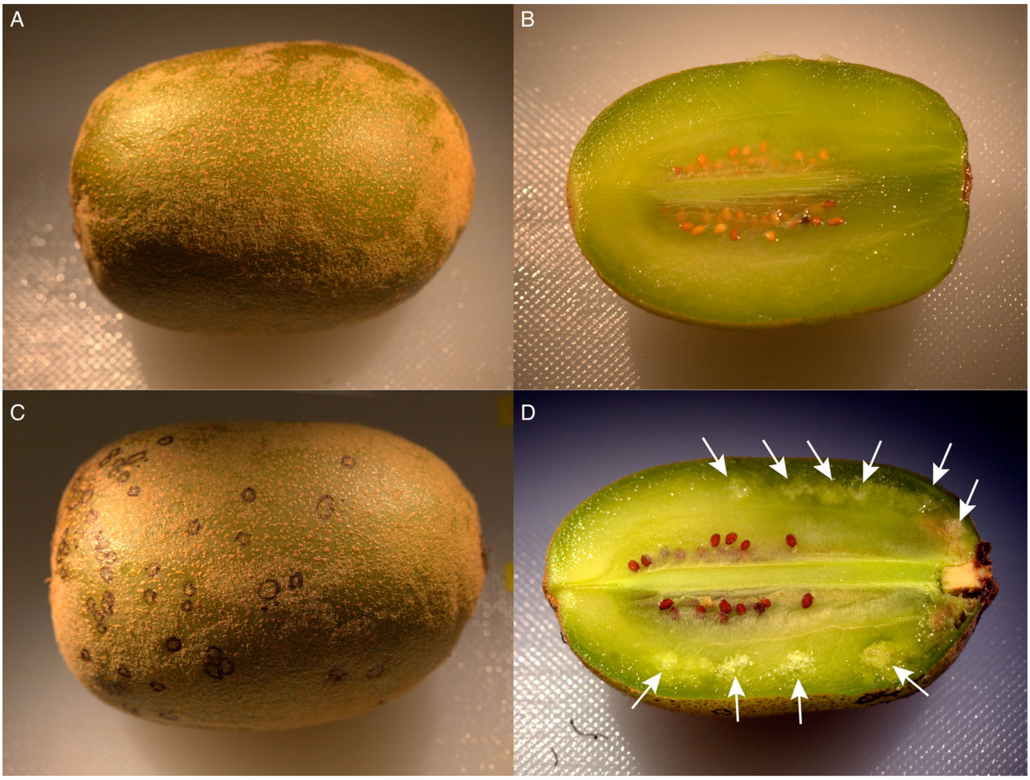

Figure 3. Comparison of brown marmorated stink bug (BMSB) feeding damage on SunGold kiwifruit. **A**, External surface of SunGold kiwifruit not exposed to BMSB (control); **B**, flesh of SunGold kiwifruit not exposed to BMSB; **C**, external surface of SunGold kiwifruit exposed to BMSB with black circles (drawn with an indelible black marker) indicating the location of feeding sheaths that were identified using a microscope (see Figure 1); **D**, flesh of SunGold kiwifruit exposed to BMSB with arrows pointing to internal white corking damage to fruit flesh which was caused by BMSB stylet penetration and presumed feeding.

damage proportions) between kiwifruit varieties within BMSB treatment groups using PROC FREQ in SAS 9.3 for planned contrasts ($n = 3$) (SAS Institute 2011).

Finally, unadjusted P-values were pooled across all multiple comparisons ($n = 35$) and adjusted with the Bonferroni correction using PROC MULTTEST in SAS 9.3 to control the family-wise Type 1 error rate (SAS Institute 2011).

Results

Type 3 analyses of total salivary sheath counts for no-choice trials revealed that BMSB sex treatments ($\chi^2 = 41.02$, d.f. = 2, $P < 0.0001$), kiwifruit variety ($\chi^2 = 11.31$, d.f. = 1, $P < 0.001$), and kiwifruit firmness ($\chi^2 = 4.60$, d.f. = 1, $P = 0.03$) were statistically significant main effects. None of the interaction terms between any of these factors was statistically significant at the 0.05 level (results not shown), thus indicating that fixed main effects could be examined directly. In particular, for every unit increase in fruit firmness, there was an expected 0.07 decrease in the log count of sheaths found on the fruit exterior ($\chi^2 = 5.06$, d.f. = 1, $P = 0.02$). Also, across BMSB treatments, log total salivary sheath counts per kiwifruit were 1.29 times higher on Green (lsmean ± SEM: 3.35 ± 0.14) than SunGold (lsmean ± SEM: 2.58 ± 0.15) kiwifruit, which was statistically significant ($\chi^2 = 12.07$, d.f. = 1, $P < 0.001$). Pairwise comparisons among sex treatments and across kiwifruit varieties in no-choice trials revealed the following pattern: log total salivary sheath counts for mixed BMSB groups were significantly higher than those recorded for either single males or females, but log total sheath counts were not statistically different between single males and females (Figure 4; see Table 1 for Bonferroni adjusted *P*-values). For choice trials, Type 3 analysis revealed that only BMSB treatment was a statistically significant factor ($\chi^2 = 43.63$, d.f. = 2, $P < 0.0001$). Furthermore, pairwise comparisons among sex treatments and across kiwifruit varieties revealed a pattern similar to no-choice trials: log total salivary sheath counts for mixed BMSB groups were significantly higher than either single males or females, but log total salivary sheath counts were not statistically different between single males and females (Figure 5; see Table 1 for reported Bonferroni adjusted *P*-values).

Type III analyses of Brix levels indicated that kiwifruit variety ($F = 133$, d.f. = 1, $P < 0.0001$) and firmness ($F = 58.18$, d.f. = 1, $P < 0.0001$) were statistically significant main effects, but not BMSB treatment ($F = 0.74$, d.f. = 3, $P = 0.53$) (Figure 6). In the final Brix model with only significant terms, transformed Brix levels were 1.18 times higher for SunGold (lsmean ± SEM: 3.93 ± 0.03) than Green (lsmean ± SEM: 3.31 ± 0.03) and this difference was statistically significant ($F = 145$, d.f. = 1, P <0.0001) and independent of whether or not fruit were exposed to BMSB.

Type III analyses for kiwifruit weight loss indicated that BMSB treatment ($F = 2.92$, d.f. = 3, P = 0.04), kiwifruit variety ($F = 49.62$, d.f. = 1, P = <.0001) and firmness ($F = 4.30$, d.f. = 1, $P = 0.04$) were statistically significant main effects. In particular, weight loss across all BMSB treatments was 1.20 times higher for SunGold (lsmean ± SEM: 1.84 ± 0.03) than Green (lsmean ± SEM: 1.52 ± 0.03) kiwifruit. Planned pairwise comparisons across kiwifruit variety revealed that weight loss was not significantly different between control (no BMSB exposure) and BMSB groups as well in the following set of comparisons: between mixed BMSB groups and single BMSB females or males, and between single BMSB females and single males (see Table 1 for reported Bonferroni adjusted *P*-values).

Type 3 analyses for kiwifruit feeding damage indicated that BMSB treatments ($\chi^2 = 20.37$, d.f. = 2, $P < 0.0001$), kiwifruit variety ($\chi^2 = 20.67$, d.f. = 1, $P < 0.0001$) and firmness ($\chi^2 = 10.83$, d.f. = 1, P = 0.001) were statistically significant main effects, with a statistically significant interaction between kiwifruit variety and BMSB treatment ($\chi^2 = 10.32$, d.f. = 2, $P = 0.006$). Planned comparisons of kiwifruit damage (i.e. corking) associated with BMSB exposure in no-choice trials indicated the existence of significant differences across sex, group and fruit varieties (Figure 7; see Table 1 for reported Bonferroni adjusted *P*-values). Choice trials indicated that when BMSB adults were given a choice between SunGold and Green kiwifruit, fruit damage levels were not significantly different between fruit varieties for BMSB single males, single females or mixed adult groups (Figure 8; see Table 1 for reported Bonferroni adjusted *P*-values).

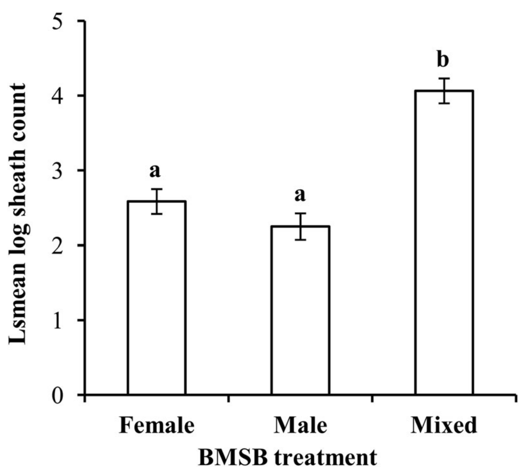

Figure 4. Least squares means (lsmeans) (± SEM) for log brown marmorated stink bug (BMSB) salivary sheath counts across Green and SunGold kiwifruit exposed under a no-choice feeding scenario to different densities of adult BMSB (i.e. one male, one female, or a mix of two males and two females). Different letters indicate statistical significance at the 0.05 level (Bonferroni adjusted *P*-values; see Table 1) between treatment groups.

Table 1. List of pairwise contrasts and adjusted *P*-values for SunGold (SG) and Green (G) kiwifruit exposed to different densities and sexes (single male, single female, or mixed adult sexes) of brown marmorated stink bug (BMSB).

Comparison	Exposure	Response variable	Pairwise contrast	d.f.	Statistic value	Raw *P*-value	Bonferroni adjusted *P*-value
1	No-choice	Weight loss (g)	Control vs All BMSB sex groups	1	$F = 4.92$	0.03	1
2	No-choice	Weight loss (g)	Single female vs Single male	1	$F = 3.21$	0.08	1
3	No-choice	Weight loss (g)	Single female vs Mixed group	1	$F = 2.03$	0.16	1
4	No-choice	Weight loss (g)	Single male vs Mixed group	1	$F = 0.11$	0.74	1
5	No-choice	Brix (degrees)	Control vs All BMSB sex groups	1	$F = 1.17$	0.28	1
6	No-choice	Brix (degrees)	Control vs Single male	1	$F = 1.76$	0.19	1
7	No-choice	Brix (degrees)	Control vs Single female	1	$F = 1.08$	0.30	1
8	No-choice	Brix (degrees)	Control vs Mixed group	1	$F = 0.16$	0.69	1
9	No-choice	Brix (degrees)	Single female vs Single male	1	$F = 0.06$	0.80	1
10	No-choice	Brix (degrees)	Single female vs Mixed group	1	$F = 0.39$	0.53	1
11	No-choice	Brix (degrees)	Single male vs Mixed group	1	$F = 0.71$	0.40	1
12	No-choice	Damage	Single female (G) vs Single male (G)	1	$z = -0.4$	0.69	1
13	No-choice	Damage	Single female (G) vs Mixed group (G)	1	$z = -1.35$	0.18	1
14	No-choice	Damage	Single female (G) vs Single female (SG)	1	$z = 3.16$	0.002	0.06
15	No-choice	Damage	Single female (G) vs Single male (SG)	1	$z = 3.63$	0.0003	0.009**
16	No-choice	Damage	Single female (G) vs Mixed group (SG)	1	$z = -0.07$	0.95	1
17	No-choice	Damage	Single male (G) vs Mixed group (G)	1	$z = -0.82$	0.41	1
18	No-choice	Damage	Single male (G) vs Single female (SG)	1	$z = 3.29$	0.001	0.04*
19	No-choice	Damage	Single male (G) vs Single male (SG)	1	$z = 3.75$	0.0002	0.006**
20	No-choice	Damage	Single male (G) vs Mixed group (SG)	1	$z = 0.31$	0.75	1
21	No-choice	Damage	Mixed group (G) vs Single female (SG)	1	$z = 4.74$	<.0001	<.0001***
22	No-choice	Damage	Mixed group (G) vs Single male (SG)	1	$z = 4.73$	<.0001	<.0001***
23	No-choice	Damage	Mixed group (G) vs Mixed group (SG)	1	$z = 1.69$	0.09	1
24	No-choice	Damage	Single female (SG) vs Single male (SG)	1	$z = 1.01$	0.31	1
25	No-choice	Damage	Single female (SG) vs Mixed group (SG)	1	$z = -3.63$	0.0003	0.01
26	No-choice	Damage	Single male (SG) vs Mixed group (SG)	1	$z = -3.91$	<.0001	0.003
27	No-choice	Sheaths	Single female vs Single male	1	$z = 1.39$	0.16	1
28	No-choice	Sheaths	Single female vs Mixed group	1	$z = -6.25$	<.0001	<.0001***
29	No-choice	Sheaths	Single male vs Mixed group	1	$z = -7.16$	<.0001	<.0001***
30	Choice	Sheaths	Single female vs Single male	1	$z = 1.89$	0.06	1
31	Choice	Sheaths	Single female vs Mixed group	1	$z = -5.8$	<.0001	<.0001***
32	Choice	Sheaths	Single male vs Mixed group	1	$z = -7.59$	<.0001	<.0001***
33	Choice	Damage	Single female: G vs SG	1	$\chi^2 = 5.37$	0.02	0.72
34	Choice	Damage	Single male: G vs SG	1	$\chi^2 = 0.02$	0.89	1
35	Choice	Damage	Mixed group: G vs SG	1	$\chi^2 = 5$	0.02	0.89

Note: *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

Discussion

BMSB establishment in New Zealand poses a serious biosecurity risk to agricultural commodities such as kiwifruit (Zhu et al. 2012; KVH 2016a; Kriticos et al. 2017). However, kiwifruit varieties are known to differ in their susceptibility to hemipteran feeding (Hill et al. 2007, 2010, 2011) and field evidence of BMSB feeding damage to commercially-grown SunGold and Green kiwifruit varieties is lacking. To address this deficiency, we compared in the laboratory, under quarantine

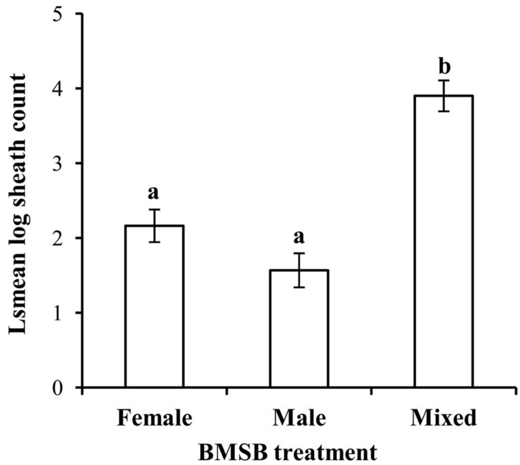

Figure 5. Least squares means (lsmeans) (± SEM) log brown marmorated stink bug (BMSB) salivary sheath counts across Green and SunGold kiwifruit exposed under a choice scenario to different densities of adult BMSB (i.e. one male, one female, or a mix of two males and two females). Different letters indicate statistical significance at the 0.05 level (Bonferroni adjusted *P*-values; see Table 1) between treatment groups.

conditions, the feeding ability of BMSB adults on late-season vine-condition SunGold and Green kiwifruit, two major commercial kiwifruit varieties.

Laboratory feeding trials revealed that BMSB adults will feed on Green and SunGold kiwifruit varieties when they are the only food option available. While salivary sheaths are a reliable indicator of BMSB feeding in the laboratory, their use in the field to monitor BMSB feeding is limited because a microscope is required to locate and count sheaths over the entire kiwifruit surface (Figure 1). Additionally, the presence of sheath structures may reflect past feeding activity and not current field presence because BMSB is a highly mobile pest that feeds and disperses (Bakken et al. 2015; Venugopal et al. 2015). Furthermore, other hemipterans, if present in kiwifruit production areas, could leave similar sheath structures after feeding, thus confounding conclusions that BMSB feeding

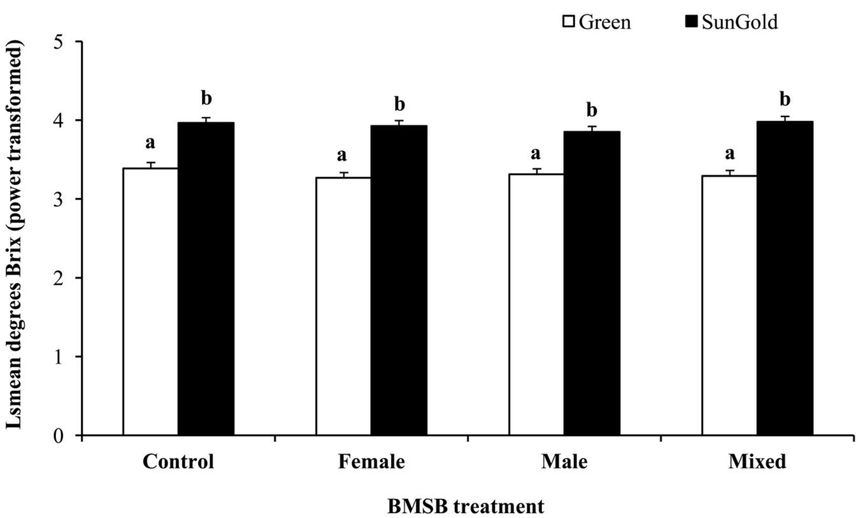

Figure 6. Least squares means (lsmeans) (± SEM) for power transformed degrees Brix for Green and SunGold kiwifruit exposed under a no-choice scenario to different densities of adult BMSB (i.e. none, one male, one female, or a mix of two males and two females). Different letters indicate statistical significance at the 0.05 level (Bonferroni adjusted *P*-values; see Table 1) between treatment groups.

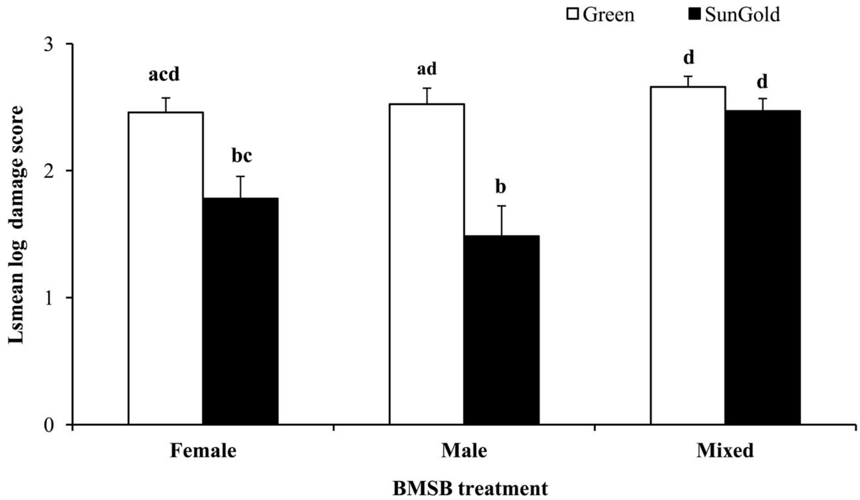

Figure 7. Least squares means (lsmeans) (± SEM) for log damage scores for Green and SunGold kiwifruit exposed under a no-choice scenario to different densities of adult BMSB (i.e. one male, one female, or a mix of two males and two females). Different letters indicate statistical significance at the 0.05 level (Bonferroni adjusted *P*-values; see Table 1) between pairwise treatments combinations.

damage was observed on kiwifruit (Peiffer & Felton 2014). This latter situation is one reason why it was unclear whether hemipteran feeding damage found in commercial kiwifruit orchards in Italy and Korea, if assessed by the presence of feeding sheaths, is exclusively attributable to BMSB (KVH 2016a). Pest managers should be aware of this possibility when sampling kiwifruit for hemipteran feeding damage in commercial orchards.

Most importantly, this study confirmed that feeding attempts by single BMSB females, males and mixed adult groups can translate to noticeable and quantifiable internal feeding damage which was detected as the incipient development of corking tissue on both Green and SunGold kiwifruit flesh (Figures 2–3). Results presented here suggest a higher incidence of feeding damage on Green kiwifruit compared to SunGold may occur under no-choice conditions in the laboratory (Figure 7). In

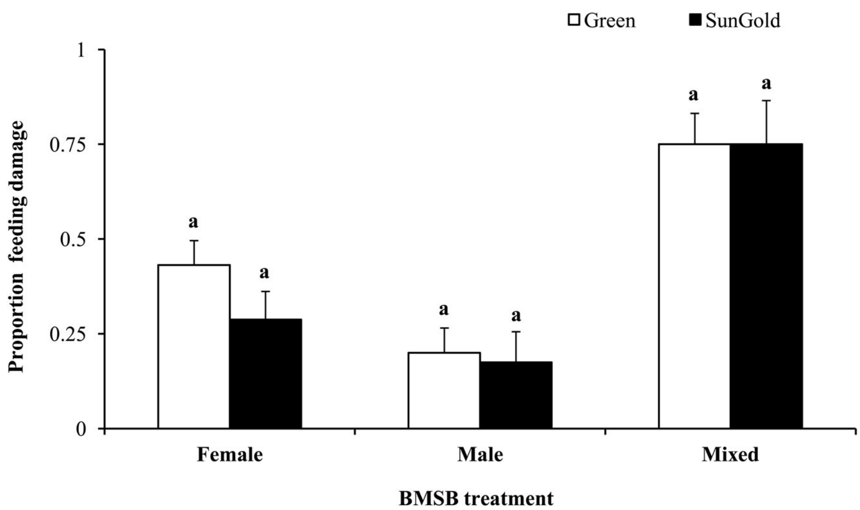

Figure 8. Mean (± SEM) proportion of feeding damage for Green and SunGold kiwifruit exposed under a choice scenario to different densities of adult BMSB (i.e. one male, one female, or a mix of two males and two females). Different letters within BMSB treatment indicate statistical significance at the 0.05 level for Bonferroni adjusted *P*-values (see Table 1) between kiwifruit types.

no-choice trials, across all experimental BMSB densities, adults fed more on Green kiwifruit than on SunGold and, not surprisingly, this translated to a higher incidence of observed damage on the former (Figure 4). The underlying factors associated with crop quality and insect preference which may have prompted higher BMSB feeding on Green compared to SunGold kiwifruit under no-choice conditions require further study, but this pattern may change when both feeding hosts are simultaneously available under field conditions (Bakken et al. 2015; Venugopal et al. 2015). Furthermore, BMSB feeding restricted to a 7 day window did not translate into statistically significant differences in Brix (Figure 6) or weight differences for either Green or SunGold kiwifruit. It is important to note that these observations under laboratory conditions do not preclude the possibility that sugar concentrations, weight changes (indicative of size and grade) or other crop quality characteristics (e.g. fruit set, maturation rates) could be affected significantly in the field during the growing season under prolonged BMSB exposure and consequent feeding pressure.

Interestingly, when given a choice between two kiwifruit varieties, Green versus SunGold, BMSB adults did not exhibit a statistically significant level of preference that could be detected in the number of feeding attempts (Figure 5) or damage levels (Figure 8). This pattern was consistent among all BMSB treatment densities. This consistency in feeding behaviour implies that both varieties may have equal desirability as feeding hosts when encountered simultaneously by BMSB adults in relatively close proximity in a confined space. Furthermore, after accounting for BMSB density in no-choice experiments, there was no evidence suggesting that significantly more feeding attempts occurred on one particular kiwifruit variety. Nevertheless, no-choice trials revealed that, for mixed groups, the relative risk of damage for SunGold was 0.88 times that of Green kiwifruit, a marginal but significant difference, because SunGold is gaining traction in global markets as a high-value variety (Dwiartama 2017).

In this study, we evaluated use of on the vine and subsequently cold-stored SunGold and Green kiwifruit, assuming conservatively late season access of BMSB populations to commercial kiwifruit varieties just prior to harvest. However, these quarantine evaluations do not fully account for the threat this invasive pest poses to kiwifruit over the entire growing season. The risk of BMSB feeding preferences and subsequent damage to developing fruit throughout the growing season may be assessed through field studies in commercial orchards conducted in invaded (e.g. Italy and California) and native ranges (e.g. Korea and China).

In addition, to better assess the establishment likelihood of BMSB in New Zealand, live diapausing BMSB adults that are intercepted at ports of entry (e.g. in Auckland) during the high risk period (i.e. September–April) could be subjected in quarantine to simulated (in growth chambers) or natural (in quarantine greenhouses) spring/summer climate conditions for Auckland (see Duthie 2015). These studies would measure the duration of exposure required by diapausing BMSB adults arriving from the Northern Hemisphere to terminate diapause allowing them to transition into an active reproducing stage under spring/summer environmental conditions that typify the receiving area. Similar quarantine studies could determine if climatic conditions in Auckland and other parts of New Zealand (e.g. horticultural production areas in the Bay of Plenty and Hawkes Bay) would initiate and terminate winter diapause for BMSB adults. These data on initiation and termination of diapause in BMSB would help with better understanding establishment risks posed by hitchhiking individuals that are in diapause and regional risks to agricultural commodities, such as kiwifruit, should founding BMSB populations spread.

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Disclosure statement

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