Assessment of Feeding Acceptance and Injury of Kerman Pistachios, *Pistacia vera*, by Brown Marmorated Stink Bug (Hemiptera: Pentatomidae)

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Abstract

In the United States, California (CA) is the primary commercial producer of pistachio nuts, *Pistacia vera* L. (Anacardiaceae). The brown marmorated stink bug (BMSB), *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae), an invasive and polyphagous insect pest from Asia, has established in urban areas in several pistachio-growing counties in CA. Breeding BMSB populations have not been detected in commercial pistachio acreage. However, the detection of BMSB in Kern and Fresno counties, major Kerman pistachio producing areas in CA, underscored key knowledge gaps on BMSB ecology in CA and motivated investigations on the susceptibility of pistachio nuts to BMSB feeding. Laboratory feeding trials conducted in quarantine under permit indicated that adult BMSB stylets can penetrate developing pistachio shells and associated feeding was correlated with kernel necrosis for nuts collected mid to late season (June to August 2016). Feeding damage estimates indicated that higher levels of kernel injury were associated with female BMSB when compared to feeding by male BMSB. These results suggest that there is probable risk of feeding damage to field grown pistachios from BMSB. The implications of this study for BMSB pest management in the CA pistachio system and future research directions are discussed.

Key words: Kerman pistachios, brown marmorated stink bug, negative binomial regression, correlation analysis, feeding injury risk assessment

The brown marmorated stink bug (BMSB), Halyomorpha halys (Stål) (Hemiptera: Pentatomidae), is a polyphagous insect native to Asia (i.e., China, Japan, Korea, and Taiwan) that feeds on fruit, nut, and vegetable crops (Lee et al. 2013). BMSB established in the mid-Atlantic states of the United States during the 1990s (Rice et al. 2014). Since these initial detections, BMSB has spread to more than 42 US states, including California (CA), and more than 170 agricultural and ornamental host plants have been recorded (Rice et al. 2014, Lara et al. 2016, NIC 2016). BMSB movement within the United States has most likely been facilitated by short-distance flights and accidental long-distance transportation by humans (Holtz and Kamminga 2010, Lee and Leskey 2015, Wiman et al. 2015). The ongoing spread of BMSB and its polyphagous feeding habits have raised concerns over potential economic damage that BMSB populations may generate across a wide diversity of U.S. agricultural systems. For example, in 2010, BMSB caused more than \$37 million in losses to the apple industry in Mid-Atlantic States (Leskey et al. 2012a).

In CA, BMSB is recognized as a pest of known economic importance (NIC 2017). CA agricultural commodities at risk from BMSB include a wide variety of fruit, tree nut, and vegetables crops which collectively are valued at more than \$27 billion per year (USDA-ERS 2016). In CA, BMSB was first detected in 2002 (Lara et al. 2016), and reproductive BMSB populations have been confirmed for nine counties spanning the San Joaquin Valley, a major agricultural production area. BMSB detections have been documented in 21 additional CA counties, but breeding BMSB populations have not been confirmed (Fig. 1). In addition to the threat BMSB poses to CA agriculture, established BMSB populations generate nuisance problems for residents in urban areas of northern and southern CA (Arrington 2015, Lara et al. 2016) when BMSB adults form overwintering aggregations in human-made structures (Lee et al. 2013, Rice et al. 2014, Lara et al. 2016). With respect to agricultural areas, BMSB has been detected on commercial kiwifruit acreage in Butte County (2015) (UC IPM 2016), commercial almond acreage in Sutter County (2016) (J. Nay, personal observation), and commercial peach production in Stanislaus County (Rijal and Duncan 2017). Complementary laboratory and field studies that clarify the potential economic threat from BMSB to agricultural crops grown in CA are needed.

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Fig. 1. Overlap of pistachio-growing areas with the current known distribution of brown marmorated stink bug in California; counties with no brown marmorated stink bug (BMSB) detection or established populations are shown in white.

Pistachio nuts, *Pistacia vera* L. (Anacardiaceae), have been identified as being at risk from BMSB feeding in CA (Lara et al. 2016). Reproductive urban populations of this pest have established in pistachio-producing counties in CA (Fig. 1). CA pistachios are grown on approximately 89,000 hectares spread across 22 counties. The economic value of the CA pistachio industry is estimated to be \$1.5 billion, and production of 'Kerman' pistachio nuts accounts for 98% of all pistachio nuts grown in the United States (Kallsen et al. 2009, ACP 2015, CDFA 2015). Around 94% of CA's total pistachio production is concentrated in the San Joaquin Valley, with Kern (31% of production area), Fresno (21%), Madera (19%), Tulare (13%), and Kings (9%) counties accounting for the highest production (ACP 2015).

Potential feeding damage to Kerman pistachio nuts is a significant industry concern and is warranted by evidence of direct feeding injury by BMSB to other nut-producing host plants in the United States. For example, hazelnuts grown in Oregon are damaged when feeding BMSB adults penetrate the hard shell and feed on the kernel during different stages of development (Hedstrom et al. 2014). Subsequent feeding causes inferior quality nuts because they fail to develop kernels (blanks), are malformed, or have corking (i.e., lesion) damage (Hedstrom et al. 2014). While BMSB has not been reported to occur in key commercial pistachio production regions from China, i.e., Xinjiang province (EI 2016), a complete reproductive/feeding host plant list for BMSB in the native range has not been compiled, and host plant information is still coming from invaded ranges (Yu and Zhang 2007, Lee et al. 2013, Bergmann et al. 2016). For example, BMSB has been recorded feeding on the fruits of ornamental *Pistacia chinensis* in CA (Ingels 2016) and has been reported to feed on *Rhus typhina*, another confamilial, native to eastern North America (Yu and Zhang 2007).

Specifically, BMSB feeding damage to pistachio nuts was considered possible as other resident hemipteran species are already problematic in the CA pistachio system. This hemipteran complex includes leaf-footed bugs (*Leptoglossus* spp. [Coreidae]), plants bugs (Miridae), and pentatomids including, *Thyanta pallidovirens* (Stål), *Chinavia hilaris* (Say), and *Chlorochroa uhleri* (Stål), all of which can feed directly on pistachio nuts, resulting in injury to developing pistachio kernels (Daane et al. 2005, Ferguson et al. 2005). Hemipteran species occurring in the pistachio system have also been implicated in facilitating nut infection by disease-causing pathogens at feeding wounds, a process that could lead to further nut crop losses (Michailides and Morgan 2004, 2016).

Baseline management of the pistachio-hemipteran pest complex along with other insect and mite pests can cost the industry approximately \$19 million (U.S.) per year (Beede et al. 2008, ACP 2015). While native resident pests can be managed on commercial pistachio with pesticides (UC IPM 2014a,b), overreliance on chemical control for BMSB (and other hemipteran pests in the pistachio system) could have negative impacts on natural enemy communities leading to resurgence and secondary pest outbreaks, and potentially resistance development (Leskey et al. 2012b, Rice et al. 2014, Kuhar and Kamminga 2017). Studies examining the spatial movement patterns of BMSB populations have demonstrated that established BMSB populations can display seasonal preference for host plants (Bakken et al. 2015, Venugopal et al. 2015). Pesticide management strategies are affected by influxes of migrating BMSB populations into crops, and area-wide management is recommended for managing invasive BMSB populations in U.S. agricultural systems (Krawczyk and Hull 2012, Quarles 2014, Cira et al. 2017, Leskey 2017, Morehead and Kuhar 2017).

In CA, the distribution of BMSB populations is still largely confined to urban areas, and thus, field research assessing the efficacy of management programs that would set pest control guidelines for crops, similar to what has been developed in the east coast of the United States (Jentsch 2015, Leskey 2017), is still in development in CA. Nevertheless, the recognized threat from established BMSB population in CA to specialty crops, like pistachios, is a valid concern for growers and orchard pest managers. The economic risk posed by the incipient BMSB invasion prompted proactive research to assess the risk of crop feeding injury to the CA pistachio industry. Pistachio feeding from other species of hemipterans in CA has been assessed experimentally, and results suggest that after mid-June the hardened pistachio shell can restrict kernel injury from feeding insects (Daane et al. 2005, 2016).

However, at the time the studies reported here were conducted, information concerning the susceptibility of developing pistachio kernels to BMSB feeding was not known. Consequently, we evaluated under permit in quarantine, through a series of laboratory trials using excised pistachio rachises, the ability of adult female and male BMSB to feed on developing pistachio nuts collected during the mid-to-late portion of the growing season. This period was chosen as it is likely to coincide with the availability of susceptible pistachio clusters to potential BMSB feeding. Experiments simulated no-choice feeding scenarios, a situation adult BMSB may experience should they migrate into commercial pistachio production areas in the San Joaquin Valley from overwintering areas in urban environments. Two key questions framed these feeding trial studies. First, can BMSB feed on Kerman pistachio nuts? Second, can BMSB feeding injury be associated with symptoms of kernel damage such as necrosis? The results from guarantine laboratory experiments and discussion of their application to BMSB biology and management are presented here.

Materials and Methods

Estimated BMSB Phenology and Pistachio Rachis Collection

Assessing possible damage of pistachio nuts to BMSB should occur when migrating BMSB adults entering orchards would be likely to encounter developing nuts. Specifically for BMSB, 538 degreedays are required for completion of egg-adult development and there is a 148 degree-day preoviposition period for females (Nielsen et al. 2008). Also, the lower and upper developmental thresholds estimated from temperature-dependent development models are 14.17°C and 35.76°C, respectively (Nielsen et al. 2008). In CA, detection of first generation BMSB eggs has occurred as early as 12 April 2016 (Sacramento) and nymphs, second instar or older, have been collected from pheromone-baited traps as early as May 2016 (Sacramento and Los Angeles; both cities are in CA) (Lara, unpublished; C. Ingels and C. Pickett, pers. comm.). These parameter values were integrated into degree-day models using the single-sine method and horizontal cutoff for daily degree-day accumulation. Compiled daily temperature data for 2015, 2016 and 2017 were accessed through http://ipm.ucanr.edu/WEATHER/ for weather stations (station ID number in parentheses) in Kern (CIMIS #125), Madera (NCDC #5233), Fresno (CIMIS #80), Kings (Touchstone #27), and Tulare (CIMIS #203) counties assuming a scenario of BMSB establishment in these counties. Degree-day model projections indicated that the first generation of BMSB adults in these areas could be expected in late June through early July. The predicted appearance of adults based on degree-day calculation was assumed to be the period when BMSB flight activity and possible migration into pistachio orchards could occur.

Consequently, rachis collection in the San Joaquin Valley for use in experiments was synchronized with the transitional period corresponding to the second to third phenological phases of the Kerman pistachio-growing season, a period that aligns with the anticipated phenology of adult BMSB detailed above. Pistachio phenology in CA has the following three phases: phase one (April-May) includes flower pollination, fruit set, and shell (endocarp) enlargement. Phase two (May-June) consists of shell hardening and the onset of kernel enlargement. Subsequently, during phase three (June-September), the expanding kernel fills the nut causing shells to split, and finally the hull slips from the shell (Ferguson et al. 2005). While some insects feed upon and injure developing pistachio nuts during phase one (this could occur if parental BMSB coming out of winter diapause infiltrated pistachio orchards earlier in the growing season), pistachio trees can compensate to some degree for these early-season losses by reducing natural nut drop in favor of fruit set (Daane et al. 2005). For this reason and because the focus of experiments was on assessing feeding injury from BMSB to kernels already in development, sampling pistachio nuts from the first phase of development were not included in this study. Therefore, for BMSB feeding trials, in-season Kerman pistachio rachises were collected from an organic orchard in Madera, CA during the period of 8 June 2016 to 25 August 2016, and rachis collections for experiments ended before the start of pistachio harvest in early September 2016.

Experimental Setup

Excised rachises, hydrated with moistened paper towels in sealed plastic bags, were transported to the Quarantine and Insectary Facility at the University of California Riverside. In quarantine, all pistachio nuts were inspected under a stereomicroscope to confirm fruits were free of external damage that could be incorrectly assigned to BMSB feeding following exposure in test arenas. Rachises were exposed to adult BMSB within 48 h of collection.

Selected rachises had an average of 16 pistachio nuts with a minimum and maximum of 8 and 27 nuts, respectively. A total of seven sets of pistachio rachises, each set with 10 rachises, were used for BMSB feeding trials conducted semimonthly during June to August 2016. As part of these feeding trials, BMSB were subjected to no-choice feeding scenarios where the only food options were pistachio nuts and stem tissue to which nuts were attached. Rachises that were not exposed to BMSB were used as control treatments to determine whether visible feeding damage to the pistachio exterior (hull) and kernels observed on nuts exposed to BMSB adults could be correlated with feeding attempts. For each feeding trial, an upper limit of 10 rachises with 30 or fewer nuts per rachis was selected for presentation to adult BMSB. The size of each rachis was not fixed to allow for natural variation in nut density variation which could occur in the field and may lead to differences in accumulated feeding

attempts between nuts on different-sized clusters. The influence of this covariate on feeding injury estimates was included in statistical modeling (see *Results*).

For each set of 10 rachises, six rachises were assigned randomly to one of three BMSB exposure treatments, thus providing a total of two replicates per treatment during each trial run. BMSB exposure treatments consisted of four reproductive adults, corresponding to four males only ('treatment 1'), four females only ('treatment 2'), or a mixed adult combination (i.e., two male and two female BMSB for a total of four adults; 'treatment 3'). The number and representation of both BMSB sexes was adapted from a hazelnut field study by Hedstrom et al. (2014). All adult BMSB were sourced from laboratory colonies maintained in guarantine under California Department of Food and Agriculture permit no. 3020 and reared on a diet consisting of apples, carrots, green beans, avocados, seedless grapes, butterfly bush (Buddleja davidii), and empress tree (Paulownia tomentosa). Nondiapausing adults of mixed age were sourced from adult maintenance cages where the daily oviposition of egg masses indicated cohorts were in a reproductive state (eggs from adult cages were harvested daily and used to maintain BMSB colonies). The remaining four rachises not exposed to BMSB adults in feeding trials were assigned to control treatments to measure naturally-occurring nut deterioration in the absence of BMSB.

The experimental holding unit used for all treatments was a cylindrical plastic container, 13×13 cm (Tri-State Plastic, Inc., Latonia, Kentucky), lined with coffee filter paper. Organza fabric attached to the 10-cm circular opening of the lid facilitated air circulation and prevented insect escape. An individual rachis was hydrated by inserting the stem through a lid attached to a waterfilled plastic cup (59 ml) (First Street, Los Angeles, CA) and placed into an exposure cage. A second water-filled plastic cup containing a cotton roll (Primo Dental Products, New York, NY) was added to all experimental units to provide direct water access to BMSB adults, including control treatments lacking BMSB. All feeding trials were conducted in a temperature controlled room at 23°C with 40% RH and 16:8 (L:D) h photoperiod. This photoperiod is above the 13.5–14 h threshold needed by BMSB females for ovarian development (Lee et al. 2013).

Data Collection

All feeding trials were run for a 5-d exposure period to measure cumulative feeding attempts. For replicates that received BMSB adults, observations were made daily (i.e., every 24 h) to record the total number and sex of feeding BMSB adults and the location of feeding attempts (i.e., stem tissue or directly on the pistachio nut). Egg masses deposited by female BMSB were removed daily from treatments. Collected egg masses were transferred to labeled Petri dishes and percentage hatch rates recorded. The general purpose in recording egg masses from mixed-age females under laboratory conditions was to document the possibility (yes/no) that oviposition by nondiapausing females in a reproductive state reared on a mixed food diet continued to oviposit when exposed to pistachio nuts only.

After 5 d, all rachises were removed from experimental containers and inspected for evidence of necrotic damage to kernels and feeding attempts by BMSB. To quantify feeding by BMSB, the external surface area of each pistachio was examined under a stereomicroscope and the total number of salivary sheaths on each nut was recorded by treatment. BMSB produces a gel-like salivary sheath surrounding plant tissue and its stylets to minimize the loss of plant juices. The sheath substance hardens and remains on plant tissue even after BMSB removes its mouthparts (Peiffer and Felton 2014). Therefore, the presence of salivary sheaths can be used as direct evidence of attempted feeding. After sheath counting, pistachio nut shells were split and internal contents were examined under a stereomicroscope. Necrotic damage to kernels, evident as dark-brown spotting, was scored '1' if present and '0' if absent.

Under this experimental framework, four separate data sets were generated: 1) Daily counts of feeding attempts per exposed nut across treatments, 2) the hatch rates of egg masses laid by experimental females from treatments 2 and 3, 3) cumulative counts of BMSB-feeding sheaths on individual pistachio nuts, and 4) kernel damage scoring of nuts exposed and not exposed (i.e., control replicates) to BMSB.

Statistical Analyses

Daily feeding observations were pooled across the 5-d observation window to compare aggregate BMSB feeding attempts on nuts and stems between males and females using negative binomial regression models with the log link function in PROC GENMOD in SAS 9.3 (SAS Institute 2011). In these analyses, the negative binomial distribution was selected to account for overdispersion detected in the feeding count data. Statistical comparisons were made separately between BMSB treatment 1 (male) and 2 (female) and between males and females from treatment 3 using the 'estimate' statement in PROC GENMOD.

The effects from BMSB adult exposure treatments (i.e., male only, female only or a combination of both sexes), the size of developing kernel embryos at the time of collection from trees (i.e., either small/absent, or present and having grown in size) (see Ferguson and Kallsen 2016), and the number of nuts offered to BMSB on the extent of attempted nut feeding, indicated by the cumulative number of feeding sheaths, were analyzed using negative binomial regression and the log link function in PROC GENMOD in SAS 9.3 (SAS Institute 2011). The statistical significance of each predictor at the 0.05 level was evaluated using a Type 3 analysis which has a comparable application as the Type III sums of squares from general linear models (Johnston 1993). Proper specification of the final negative binomial model form was confirmed with a goodness-of-fit chi-squared test and again with a likelihood ratio test comparing a full model with a reduced intercept-only model with no predictors.

The strength of the association between the incidence of kernel necrosis and evidence of BMSB feeding across all treatments was measured by setting both factors as binary variables, i.e., '1' if damage was present and '0' if damage was absent, and the tetrachoric correlation option available in PROC FREQ in SAS 9.3 was used to account for the dichotomy of these observed variables (SAS Institute 2011). Additionally, the association between kernel necrosis and the different BMSB treatment levels was measured using a chi-square test of independence in PROC FREQ in SAS 9.3. The chi-square test evaluated the null hypothesis that there was homogeneity of proportions of kernel damage across all BMSB treatments. The previously used statistical tests do not provide direct separation of feeding incidence differences that could exist between groups comprised of feeding from males and females. Therefore, the odds ratio of kernel damage associated with male and female only BMSB groups was assessed directly using binomial logistic regression in PROC LOGISTIC in SAS 9.3 (SAS Institute 2011). Data corresponding to mixed adult groups (treatment 3) were not included in this third comparison. Specifically, the general logistic regression model estimated the probability that a pistachio kernel would display damage depending on exposure to either male or female BMSB.

Egg mass hatch data tabulated as nymph (first instar) emergence counts were used to calculate average percent hatch rates as [no. of emerged BMSB nymphs/total no. of eggs in egg mass] × 100. Further post hoc statistical analyses were used to compare levels of feeding (using PROC GENMOD) and kernel injury (using PROC FREQ) in SAS 9.3 (SAS Institute 2011) between female groups (n = 15) that produced egg masses with female groups that did not (n = 11) from mixed sex treatments.

Results

BMSB feeding was evident in the form of salivary sheaths deposited by adults on the exterior surface of the pistachio exocarp as well as visible penetration marks on the shell interior made by piercing stylets (Figs. 2, 3). Additional confirmation of feeding was provided by daily observations made during the course of feeding trials. Analysis of feeding observations pooled across the 5-d observation period revealed BMSB feeding behavior patterns (Figs. 4-6). Females from treatment 2 fed more on pistachio rachises (i.e., pistachio nuts and stems combined) than males in treatment 1 (Wald χ^2 = 6.55, df = 1, P = 0.01) (Fig. 4). After accounting for the sex of BMSB, the number of feeding events was not statistically different between pistachio nuts or stem tissue (Wald $\chi^2 = 0.97$, df = 1, P = 0.32) (Fig. 4). For the combined sex treatment, females generally fed more on rachises than males, but this difference was not statically significant (Wald $\chi^2 = 1.41$, df = 1, P = 0.24) (Fig. 5). Similarly, the number of recorded feeding events for females and males did not differ between pistachio nuts or stem tissue (Wald $\chi^2 = 0.07$, df = 1, P = 0.80) (Fig. 5). Overall, observed aggregate feeding (on nuts and stems) was significantly higher for females (treatment 2) than both males (treatment 1) (Wald $\chi^2 = 5.41$, df = 1, P = 0.02) and mixed adult groups (treatment 3) (Wald $\chi^2 = 6.21$, df = 1, P = 0.01) but was not different between males and mixed adult groups (Wald $\chi^2 = 0.09$, df = 1, P = 0.76) (Fig. 6).

Analyses of feeding sheath counts (Table 1) using negative binomial regression generated the following model,

$$log(feeding sheaths) = 1.48 - (0.55) \times 1 - (0.37) \times 2 + (0.18) \times 3 + (0.25) \times 4$$
(1)

where x_1, x_2, x_3 , and x_4 correspond to binary predictor variables along with estimated coefficients. Negative binomial regression results revealed that feeding attempts by BMSB on pistachio nuts depended significantly on the sex of adults (Wald $\chi^2 = 38.19$, df = 2, P < 0.0001, x_1 and x_2 , coded as 0-0 for females, 1-0 for males, and 0-1 for mixed adult groups; presence of a developing kernel, x_3 (1 for 'absence', -1 for 'presence') (Wald $\chi^2 = 25.63$, df = 1, P < 0.0001); and the number of pistachios nuts available for feeding, x_4 , sorted among two bin classes, 1 for '8-17 nuts' and -1 for '18-27 nuts' (Wald $\chi^2 = 21.48$, df = 1, P < 0.0001). Across all other factors, the mean number of feeding sheaths counted was statistically higher for female groups compared to either male groups (Wald $\chi^2 = 36.94$, df = 1, P < 0.0001) or mixed sex groups (Wald χ^2 = 16.53, df = 1, P < 0.0001) (Table 1). Mixed adult groups produced a statistically significantly higher count of feeding sheaths than male-only groups (Wald $\chi^2 = 4.06$, df = 1, P = 0.04) (Table 1).

Interestingly, this second set of analyses on feeding sheath prevalence showed that pistachios sampled with no enlarged kernel embryos (i.e., small or absent) exhibited a significantly higher number of salivary sheaths marks (Wald $\chi^2 = 24.99$, df = 1, P < 0.0001) than pistachios with kernel embryos in later stages of development (Table 1). The former set of small kernel embryos correspond to the tail-end of the second phenological phase for developing nuts. Also, pistachio rachises with 8–17 nuts accumulated higher numbers of feeding sheaths per nut compared to rachises with 18–27 nuts, and this remained a consistent pattern



Fig. 2. (a) Brown marmorated stink bug (BMSB) adult inserting stylets into developing pistachio nut and (b) BMSB salivary sheath (indicated by black arrow) marking feeding site on Kerman pistachio exocarp.



Fig. 3. (a) Healthy pistachio kernel (in shell) and (b) developing kernel exposed to brown marmorated stink bug (BMSB) adults showing evidence of necrotic damage with arrow pointing to the successful penetration of stylets through the shell.



Fig. 4. Mean number (\pm SE) of brown marmorated stink bug (BMSB) adults feeding attempts on pistachio nut and stem tissue, across daily observations for male-only (n = 26 replicates) and female-only (n = 26 replicates) treatments. Different letters indicate significant statistical differences.



Fig. 5. Mean number (\pm SE) of male and female brown marmorated stink bug (BMSB) in the mixed group treatment (n = 22 replicates) observed feeding daily on pistachio nut and stem tissue. Different letters indicate significant statistical differences.

across all other factors (Table 1). Finally, the goodness-of-fit chisquared test (Pearson $\chi^2 = 1198.5$, df = 1276, P = 0.94) failed to reject the null hypothesis that the negative binomial distribution fit the empirical count data. Similarly, the likelihood ratio test (LR = $-2 \times (1960.88 - 2024.28)$, df = 4, P = 0) rejected the null hypothesis that predictor coefficients were equal to zero. Therefore, the full negative binomial regression model significantly explained more of the observed variation in sheath counts than the reduced model with no predictors.



Fig. 6. Mean number (\pm SE) of males, females, and mixed brown marmorated stink bug (BMSB) groups observed feeding daily on pistachio nut and stem tissue combined. Different letters indicate significant statistical differences.

The third set of analyses concerning kernel damage revealed there was a 0.70 correlation between pistachio nuts that were identified with exterior feeding sheaths and internal kernel necrosis (Table 1). The chi-square test of independence based on a 2 × 3 contingency table comparing presence-absence of kernel damage directly with three BMSB-exposure treatments was statistically significant (Pearson $\chi^2 = 28.81$, df = 2, P < 0.0001), indicating that observed levels of kernel damage were not uniform among BMSBexposure treatments. Separating the initial correlation analyses by sex showed that correlation between attempted feeding and necrosis was 0.83 and 0.61 for females and males, respectively. Furthermore, the logistic regression generated the following model,

$$\pi = \frac{\exp(-0.52 + 0.83b_1)}{1 + \exp(-0.52 + 0.83b_1)}$$
(2)

where π is the estimated probability of kernel damage given b_1 , a binary variable with a value of 0 or 1 corresponding to male and female groups, respectively. Estimated log-odds values were -0.52 and 0.83, and both coefficients were statistically significant at the 0.05 level. Specifically, -0.52 is the log-odds of a kernel having damage versus no damage if the exposure is limited to male BMSB. A value of 0.83 is the log-odds of a kernel having damage versus no damage if the exposure is limited to female BMSB. Therefore, based on equation 2 and the experimental conditions of this study, the probabilities of kernel damage from male and female BMSB are 0.37 and 0.58, respectively. Finally, in control treatments, less than 3.5% of all pistachio nuts (27 out of 775 nuts) with a kernel displayed symptoms of damage, but evidence of previous insect feeding was not apparent on the exterior/interior of the shell or directly on the kernel.

Finally, a total of 53 BMSB egg masses laid by nondiapausing females of mixed age were collected during the course of this study: 16 egg masses (433 eggs; mean clutch size = 29 ± 1.96 [SE]) were from mixed sex groups and 37 egg masses (968 eggs; mean clutch size 27 ± 1.16 [SE]) were from all female treatment groups. Mean percent hatch for these egg masses was $75\% \pm 3.48\%$ (SE) and $77\% \pm 3.54\%$ (SE) for mixed sex groups and female only groups, respectively. There was no statistically significant difference in

	Factor 1: sex treatments ¹ ($n = 1281$)		
	Male	Female	Mixed sex
Feeding sheaths	2.22 ± 0.13^{a} Factor 2: rachis rating ^{1,2} (<i>n</i> = 1281)	4.28 ± 0.44^{b}	2.67 ± 0.17^{a}
	8–17 nuts	18–27 nuts	
Feeding sheaths	3.83 ± 0.21^{a}	2.16 ± 0.11^{b}	
	Factor 3: kernel rating ^{1, 3} ($n = 1281$)		
	Absent	Present	
Feeding sheaths	5.30 ± 0.43^{a}	2.53 ± 0.11^{b}	
	Factor 4: kernel injury ^{1, 4} ($n = 720$)		
	Male	Female	
Kernel necrosis	0.37 ± 0.03^{a}	0.57 ± 0.03^{b}	

Table 1. Mean number (± SE) of brown marmorated stink bug (BMSB) feeding sheaths and measured levels of kernel necrosis across four factors from BMSB feeding for male- and female-only treatments and mixed sex BMSB groups

¹Different letters within rows indicate statistical significance at the 0.05 level.

²Nut cluster density was expressed as two equal-sized bin classes.

³Kernel rating refers to the size of a developing kernel at the time of collection (i.e., either small/absent or in later stages of nut filling).

⁴Kernel injury was determined from the presence of necrotic kernel tissue and compared between female and male treatment groups.

feeding attempts ($\dot{L}\beta = 0.05, \chi^2 = 0.71, P = 0.39$), and kernel injury ($\chi^2 = 3.01$, df = 1, P = 0.08), between females that produced no egg masses and those that did during the pistachio exposure period, signaling the validity of results for interpretation in fully developed models (above) that did not account for this categorical variable.

Discussion

BMSB is established in CA, and the types of damage or risk this pest poses to pistachio production are unknown (Daane et al. 2016). Therefore, the purpose of this research was to evaluate the ability of BMSB adults to feed on developing pistachios collected over June-August during the second to third phenological stage of nut development, the time predicted from degree-day models as to when BMSB could likely infiltrate pistachio orchards. Results from this study revealed that under laboratory conditions, BMSB can readily feed on stem tissue and penetrate the shell of Kerman pistachios nuts representative of the mid-to-late portion of the growing season (i.e., early June-late August) in CA. This is a key transitional period when the onset of shell hardening is believed to reduce the susceptibility of pistachios to most other insect damage (Daane et al. 2016). Therefore, the assumption that the pistachio shell, can act as a physical feeding barrier that protects maturing kernels from direct BMSB adult feeding injury, even before the shell naturally splits to expose the kernel, is not supported by the results from this study.

Furthermore, the incidence of insect damage during this post fruit-set period is important because pistachio trees cannot compensate for insect damage through natural nut drop, as in the early portion of the growing season, and nuts are less likely to develop external symptoms of feeding injury (e.g., epicarp lesions) (Daane et al. 2016). Thus, monitoring hemipteran feeding activity during this period would be difficult because damage accumulated from early-season feeding attempts is not a reliable indicator of current feeding pressure and damaged nuts would remain on the cluster until harvest (Daane et al. 2016). These unmarketable nuts would need to be culled resulting in an economic loss to growers. Consequently, this experimental evaluation of BMSB feeding on mid-to-late season pistachios nuts is pertinent for understanding whether or not BMSB poses a threat to CA pistachios.

Our results demonstrated that in a quarantine laboratory, BMSB can feed on and damage pistachio nuts. Interestingly, attempted feeding on pistachio nuts by BMSB adults from both sexes was significantly higher on pistachio nuts either lacking or with small kernel embryos (late phase two of the growing season) compared to pistachio nuts with advanced stages of kernel embryo development (phase three of the growing season) (see Ferguson and Kallsen 2016). In the case of pistachios, this significant outcome indicates that rather than being deterred by failure to find an acceptable food item, BMSB adults may invest additional probing effort searching a seemingly empty nut, which could reduce feeding and probing times on nuts with kernels later in the season. This finding is relevant because intuitively decreased probing of kernels with nuts may decrease the likelihood for pistachio nut infestation by fungal pathogens (Michailides and Morgan 2016). However, this potential disease phenomenon associated with BMSB probing behavior needs further study for pistachios and other commercial nut crop systems at risk from BMSB feeding (e.g., hazelnuts, almonds).

Additionally, measured levels of attempted feeding by BMSB adults were higher on rachises with a relatively lower density of nuts compared to rachises with a higher density of nuts. This result implies that BMSB may have potential to exert higher feeding pressure on low nut density rachises because there are relatively fewer nuts available for feeding. This could be relevant to perennial crop systems with alternate bearing, like pistachios, where percent damage could become greater on some trees due to reduced crop density. This assertion requires validation under field conditions where tree architecture, nut cluster density variation, and selection of available nut clusters by BMSB may influence measured outcomes. In addition to nut density, binomial regression analyses showed that attempted feeding on pistachio nuts by females was higher than males. This result may be attributable to the fact that females have higher nutritional needs for egg production in comparison to males (Nielsen et al. 2008).

Also, a strong correlation existed between BMSB exposure and kernel injury, and this type of injury was not evident in control replicates where nuts were not exposed to BMSB. Logistic regression analyses suggest that the probability of kernel necrosis in treatments with BMSB exposure, as indicated above, was significantly higher for female groups compared to male groups. This differential risk of injury could be attributed to the higher feeding attempts from BMSB females. This conclusion was equally supported by correlation analyses. While the presence of both sexes is expected to occur under field conditions, the detection of differential injury associated with BMSB sex is relevant for understanding how behavioral feeding differences between males and females may translate to quantifiable differences in accumulated crop injury. Furthermore, this result can be used as a basis to standardize the representation of both sexes in BMSB field studies for at-risk fruit and nut crop systems where adults are used to determine the full range of crop injury symptoms throughout the growing season. Injury differences attributed to the sex of test specimens have not been previously detected in experimental field feeding trials with BMSB, either because only males were used (Hedstrom et al. 2014) or this factor was not directly controlled (Wiman et al. 2015).

Future field trials with BMSB (adults and nymphs) in mesh bags enclosing nut clusters, for example, during the early, mid and late portion of the pistachio-growing season in CA would help clarify the feeding-injury relationship in greater detail for this crop. Although we were not able to assess under field conditions whether attempted feeding during phase one by BMSB adults or nymphs could induce nut drop (due to the fact that developing nut clusters were excised and this restricted the observation window and any tree-mediated level response), interrupt kernel embryo development (Ferguson and Polito 2016), facilitate the transmission/spread of disease-causing nut pathogens, the types of injury symptoms inflicted on nuts by BMSB that cause these problems were observed on pistachios nuts under quarantine conditions. Similar feeding damage symptoms leading to these types of economic damage are known for other hemipteran pests whose presence in the CA pistachio system is a management concern to commercial growers (Rice et al. 1985; UC IPM 2014a,b; Daane et al. 2016, Michailides and Morgan 2016).

In this regard, the confirmed feeding ability of BMSB on mid-tolate season pistachios, the manifestation of injury on BMSB-exposed excised nuts (including the lack of injury on pistachio rachis controls), the ability of BMSB females to continue laying egg masses during periods of exposure only to pistachios, and previously documented evidence of BMSB harboring and vectoring disease-causing pathogens (Brust and Rane 2013), suggest that BMSB poses a risk to commercial pistachio nut production in CA. Future studies are needed to clarify whether BMSB can use pistachio as a reproductive host (see Acebes-Doria et al. 2016) and whether commercial orchards with neighboring potential feeding/reproductive host plants would provide overwintering sites for adults, thus allowing early-season crop access to BMSB, as is the case with other pentatomids that overwinter in the CA pistachio system (UC IPM 2014a).

Exposure of phase two and three Kerman pistachios to BMSB could occur if first-generation adults (i.e., the progeny produced by overwintering adults) migrate into pistachio orchards from source populations in neighboring urban areas during late spring and summer months. Active BMSB adults not in winter diapause are capable of long-distance flight with an average of 5 km per day and a recorded maximum flight potential of up to 67 km and 75 km per day set by male and female BMSB, respectively (Wiman et al. 2015). Therefore, in CA, BMSB migrations into pistachio-growing areas from urban areas or other invaded adjacent crop systems could occur and this risk would continue to increase as established BMSB populations become more common along the urban-agricultural interface. Furthermore, research on the current distribution of BMSB in Europe suggests this pest can establish in new climatic areas that do not conform to projections based solely on BMSB occurrence data from the home range of this pest in Asia (Zhu et al. 2016). It is unclear whether ecological shifts in the distribution of BMSB populations as those observed in Europe could occur in CA (and other parts of the United States) and whether these changes may expand the risk of BMSB establishment to new areas previously thought not to be at risk of invasion.

Laboratory experiments with BMSB populations indicate that cold tolerance among BMSB populations established in the northeast United States can vary depending on the source location of sampled populations (Cira et al. 2016). This finding suggests BMSB may be able to adapt to prevailing climatic conditions in invaded areas. However, cold tolerance for BMSB may not be a key factor limiting distribution in all areas of the United States (Cira et al. 2016, Rathburn and Irwin 2017), such as the San Joaquin Valley, which experiences relatively mild winters. More importantly for CA agriculture, assessment of responses by BMSB to heat stress to which it would be exposed to over summer periods during the months of June to September is needed. Heat stress has been shown to have a significant effect on the pestiferousness of the olive fruit fly, *Bactrocera oleae* (Diptera: Tephritidae), in olive production areas in the San Joaquin Valley (Johnson et al. 2011).

However, monitoring data indicate that BMSB can generally tolerate the Mediterranean climate of CA and that its distribution has subsequently expanded into pistachio-growing counties since first detection in 2002 (Lara et al. 2016; Fig. 1). Furthermore, preliminary laboratory studies suggest that BMSB can complete egg-adult development under summer temperature profiles, compiled from historical weather data for Kern County (Lara et al., unpublished). These preliminary results suggest that hot summer temperatures alone might not be sufficient to restrict the establishment of BMSB in key pistachio-growing areas like Kern County. Establishment of BMSB in other parts of the world with a Mediterranean climate also suggests that heat stress may not be entirely restrictive (see Bariselli et al. 2016). Laboratory studies are needed to better understand the potential impact of high summer temperatures representative of the San Joaquin Valley on BMSB development, survivorship, and fecundity rates. Future complementary lab and field studies may also shed insight on the temporal preference BMSB may display for host plants in CA urban and agricultural settings (see Bakken et al. 2015, Venugopal et al. 2015, Acebes-Doria et al. 2017) and the suitability of these crops to support reproductive BMSB populations. In turn, these data could provide further practical insight on the anticipated pest status on crops predicted to be at risk from BMSB, such as pistachios, in the San Joaquin Valley.

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