TITLE OF REPORT: REPORT FOR THE PROCEEDINGS OF THE 2016 PIERCE'S DISEASE RESEARCH SYMPOSIUM FOR CDFA AGREEMENT NUMBER 15-0286-SA

TITLE OF PROJECT: BROWN MARMORATED STINK BUG RISK AND IMPACTS IN WESTERN VINEYARDS

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Daane, Wiman and Walton will collaborate towards creating a feeding intensity index. Kent Daane, and Frank Zalom will conduct and coordinate regional data collection. Monica Cooper, Lucia Varela, Kent Daane, Vaughn Walton, Nik Wiman and Frank Zalom, will conduct extension outreach activity. Vaughn Walton and Nik Wiman will, conduct and coordinate, statistical analysis, reporting, paper and extension publications.

TIME PERIOD COVERED BY REPORT
The results reported here are from work conducted July 2015 to September 31, 2016.

ABSTRACT AND LAYPERSON SUMMARY OF PROJECT ACCOMPLISHMENTS
Brown Marmorated Stink Bug (BMSB) was found in increasing numbers in vineyards in Oregon from 2013-2016. In California, BMSB was found in areas closely bordering vineyards, but not in any vineyards to date. Temperatures above and below feeding thresholds (Low=6°C, High =26°C) result in cessation of feeding. Older life stages such as adults were found to result in a significant increase in feeding damage on winegrape berries. The feeding activity in relation to berry quality parameters is given for 2015. Increased feeding activity resulted in a significant increase in stylet sheaths per berry. There was a numerical reduction in berry weight and diameter with increasing feeding levels. The number of stylet sheaths in relation to degree days (DD) per day was used to create a feeding factor for BMSB. This feeding factor was significantly correlated with the number of stylet sheaths per berry. Feeding data collected during 2015 and 2016 in Oregon, combined with weather data collected from California will be used to create a BMSB risk index for each of the grapegrowing regions. These findings are preliminary pending additional analysis, and data collection.

INTRODUCTION
Brown Marmorated Stink Bug, *Halyomorpha halys* (Stål) (BMSB, Hemiptera: Pentatomidae) is becoming increasingly prevalent in Oregon and is rapidly becoming an economic concern for western vineyards (Oregon Department of Agriculture 2011, Wiman et al. 2014a). This pest can feed on vegetative tissues, grapes, and can potentially cause contamination of the crop, leading to wine quality losses. Studies funded by a USDA-SCRI CAP grant confirmed spread and increased population levels of *H. halys* in important viticulture regions of Oregon (VMW et al. unpub.). *H. halys* was first found on the west coast in 2004 in Portland (Oregon Department of Agriculture 2011), and the pest is now common in urban and natural areas. Found on high-value specialty crops and non-economic alternate host plants alike, BMSB is increasingly causing agricultural issues for growers (Fig. 1). Since 2012, BMSB has increasingly been encountered by growers and can be found in wine grape vineyards of the Willamette Valley during the harvest period (Wiman et al. 2014a). Winemakers have recently reported finding dead BMSB in fermenting wines and infestation of winery buildings by BMSB.

Immature and adult *H. halys* feed on reproductive plant structures such as fruits, and they may also feed on vegetative tissues, such as leaves and stems, sometimes piercing through bark (Martinson et al. 2013). Fruit feeding by adult BMSB may cause direct crop loss due to berry necrosis. Berry feeding may also result in secondary pathogen infection and provide entry points for spoilage bacteria. Vectoring and facilitation of pathogen proliferation by BMSB is not unrealistic because true bugs (Heteroptera) such as BMSB share feeding behaviors with homopterans implicated as disease vectors in vineyards (Cilia et al 2012, Daugherty 1967, Mitchell 2004, Wiman et al 2014a). BMSB itself is a demonstrated vector of at least one phytoplasma disease (Hiruki 1999, Weintraub and Beanland 2006), while leaf-footed bugs (Heteroptera: Coreidae) and other pentatomids have also been implicated in transmission of pistachio stigmatomycosis (Michailides et al 1998). It is clear that BMSB feeding intensity and associated pathogen infection is directly related to temperature (Wiman et al. 2014b), potentially making this pest more damaging in western production regions than on the east coast.

Brown marmorated stink bug can develop on a wide range of host crops, meaning that it can find refuge or reproduce on non-crop hosts and then spread to cultivated crops such as wine grapes (Nielsen et al. 2008, Nielsen and Hamilton 2009, Leskey et al. 2012a, 2012b, Pfeiffer et al. 2012). However, unlike other pentatomids, BMSB are also capable of completing development on crop plants. As a result, crop damage from nymphs is more common than it is for other stinkbugs. In the Willamette Valley, wine grapes are among the last crops to be harvested and this may increase the potential for late-season infestation and damage by BMSB.

Contamination of grape clusters by BMSB at harvest is a major concern. Adult BMSB have been observed to lodge themselves between the grapes during harvest. Other researchers are evaluating physical removal of BMSB from clusters, as well as removal by chemical cleanup sprays, blowers and electronic sorters. However, some BMSB may remain in grape clusters and release defensive compounds during processing, causing taint in finished wine (E. Tomasino pers comm.). These taints are persistent, and may result in major market losses. Work conducted on Pinot noir has shown that trans-2-decenal, a defense compound produced by BMSB, is a contaminant present in wine that is processed with BMSB.

As in Oregon, many important wine grape growing regions of California are in close proximity to major urban centers where BMSB populations tend to increase and become sources for further spread. Little is known about BMSB seasonal phenology, voltinism, and distribution in these environments. Oregon research has documented rapid colonization and significant increases in populations between seasons, in part because two full generations of BMSB are occurring (NGW, unpublished). In Oregon, BMSB has dispersed from Portland to northern Willamette Valley vineyards within a short period. It is important to survey the wine grape growing regions of Napa, Sonoma, and Lodi because these regions are geographically close to San Francisco and Sacramento, both areas with known BMSB infestation.

Feeding intensity of different life stages of BMSB in vineyards has not been fully determined. To date, most studies have focused on adults, even though nymphs are potentially more damaging. When BMSB egg masses are laid in vineyards, the nymphs are more confined to feed on the vines than the adults, which may fly back and forth between vineyards and borders. Thus, the feeding damage from nymphs may be more concentrated as the nymphs disperse from egg masses to feed on the host plant. No information is available, however, on the impact and severity of feeding by nymphs on grape berries and vines. Spatial distribution of BMSB in vineyards and feeding intensity may reflect environmental suitability. An observation from orchard crops is that the worst BMSB damage tends to occur on the borders (Joseph et al. 2014). Similarly, vineyard borders appear to be more susceptible to BMSB infiltration from surrounding vegetation. Grapevines located close to vineyard borders may provide a better environment for the bugs due to microclimate effects of shading by surrounding vegetation.

This study will help determine the potential for BMSB to cause direct damage to wine grape crops, as well as indirect damage through facilitation and vectoring of spoilage bacteria or vine diseases. Controlled damage studies to assess direct feeding damage by BMSB have been conducted in Oregon (Oregon State University, and New Jersey (Rutgers). These studies showed an increasing number of stylet sheaths in grape berries as the numbers of BMSB test populations increased. Increased numbers of stylet sheaths were associated with decreases of berry counts, premature raisening, and increased berry necrosis but this work focused on adult feeding and was conducted for one-week periods only. Direct crop impact may be more pronounced under more optimal temperature regimes with different varietals, and with longer feeding periods by nymphs to more realistically simulate crop infestation by reproductive BMSB, as is found in vineyards in Oregon and presumably California.

OBJECTIVES

1. Survey key Oregon and California viticulture areas for BMSB presence.
2. Determine BMSB temperature-related field feeding intensity, impact and regional risk index.
3. Provide Extension for identification, distribution, and importance of Brown Marmorated Stink Bug in western vineyards.

RESULTS AND DISCUSSION

1. Survey key Oregon and California viticulture areas for BMSB presence.

Methods. Surveys focused on high-risk regions containing vineyards and wineries in close proximity to high traffic areas such as highways, urban centers, throughways and railroad lines. Initial beat sheet sampling in the aforementioned areas and in California included Sonoma, Napa and Lodi. Pheromone-baited pyramid traps (Khrimian et al. 2014) were used in conjunction with monitoring using beat sheets. The BMSB pheromone traps were placed in the center of each row selected for beat sheet sampling. BMSB were additionally sampled from study vineyards using beat sheet sampling every two weeks starting in August from two rows, once on the vineyard edge and once in the center of the same block. Our goal was to start surveys of California vineyard regions before the reported movement of BMSB into commercial vineyards. The vineyard regions sampled were California's north coast wine grape region (Mendocino, Napa and Sonoma counties), Lodi-Woodbridge wine grape region, and San Joaquin Valley (Fresno County). All vineyard surveys were conducted in concert with other ongoing studies, with outreach to participating farmers on BMSB description and potential presence. At each site, about 100 vines were visually sampled every 2–4 weeks. Specifically, in Mendocino County, 6 vineyard sites around Ukiah and Hopland (4 Chardonnay, 1 Merlot, 1 Grenache) were sampled as part of a leafhopper project. In Napa County, seven vineyard sites (2 Cabernet Sauvignon near St. Helena, 1 Cabernet Sauvignon near Oakville, 1 Chardonnay near Yountville, 1 Merlot near Carneros and 1 Pinot Noir and 1 Chardonnay near Carneros) were sampled as part of a red blotch or vine mealybug study. In Stanislaus and Stockton counties (Lodi Woodbridge wine grape region) three vineyards were sampled (1 Cabernet Sauvignon, 1 Pinot Noir, 1 Chardonnay) and in Fresno County five table grape blocks (2 Thompson seedless, 3 flame seedless were sampled). An additional sampling protocol was followed in three vineyard blocks in Sacramento, Yolo and Amador counties for all Hemipteran insects, but have yet to find any BMSB at any of these sites. Sampling at these sites has been conducted by visual observations and sweeping of grape foliage and other vegetation present in and adjacent to the vineyards. To date, no BMSB were found during these field visits in California.

Sampling in Oregon included seven vineyards in the northern Willamette Valley. There were no clear differences in between sampling sites and data from all vineyards were pooled for the respective seasons. Work in Oregon is currently being completed for the 2016 season. This was the fourth year of sampling in these vineyards and data is presented as BMSB per pyramid trap over a two-week period (Fig. 1, 2016 data not shown).

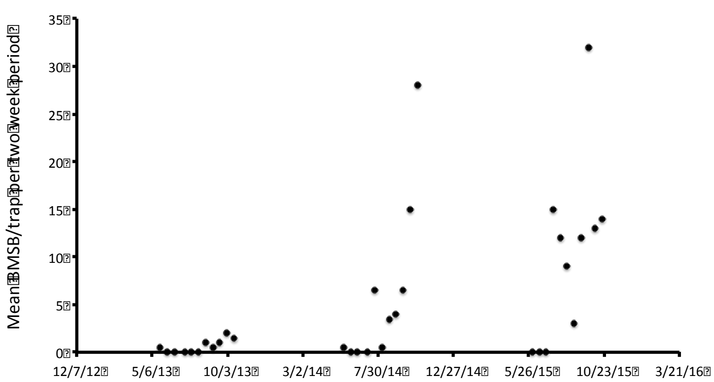

Figure 1. Number of BMSB per trap (7 traps) per two-week period in the northern Willamette Valley, Oregon during 2013-2015.

Results. In all of the seven locations, BMSB was found in low numbers during the early part of summer in Oregon. The number of BMSB per trap and increased to ca. 30 BMSB per trap per two-week period during September through October of 2014 and 2015. The total cumulative number of BMSB trapped per trap during the whole increased from 34 (2013) to 101 (2015) BMSB per trap collected during the respective seasons. Data collection for 2016 is not yet complete for the Oregon trial sites. Preliminary information from 2016 data indicates similar population trends to that of 2015.

In California, at the UC Berkeley lab (Daane Lab) starting in October 2015, we began monitoring the farms and gardens by utilizing traps containing aggregation pheromones, as well as sweep net collections of the landscape. In Fresno County, we have sampled five Hmong farming operations, each about 3-7 acres in size. Sampling consisted of utilizing a d-vac to collect insects from three different crops (egg plant, long beans, peppers, tomatoes, peas, bitter melon, squash) at each site ever other week. From these samples, no BMSB were found, but Say's stink bug (*Chlorochroa sayi*) and Bagrada bug (*Bagrada hilaris*) were collected. There was one report of a possible BMSB find from a home garden in Napa county during 2016, but the ID of the specimen needs to be confirmed.

UC Davis (Zalom Laboratory) BMSB sampling was initiated in Fall 2015 by making visual observations and collections of stink bugs from community gardens and vineyards in Sacramento, Yolo, San Joaquin and Amador counties. BMSB have previously been captured in the cities of Sacramento, Davis (Yolo county) and Stockton (San Joaquin county), but none have been captured in agricultural situations to date. We continued more intensive sampling of community gardens in Sacramento and Davis, and have also sampled community gardens in Galt (Sacramento county) and Lodi (San Joaquin county). Six species of stink bugs were collected from these gardens including *Eushistus conspersus*, *Thyanta pallidovirens*, *Chlorochroa uhleri*, *Chlorochroa ligata*, *Murgantia histronica*, and *Nezara viridula*, but BMSB was only found in community gardens in Sacramento where it was also observed feeding on grapes that were growing there. We have yet to sample gardens elsewhere in these counties, but we have met with University of California Cooperative Extension Farm Advisor Dr. Jhalendra Rijal to discuss plans for collaboratively sampling community gardens and landscape plantings in the vicinity of previous finds in Stockton and Modesto (Stanislaus county) in the coming year. We intend to use findings of BMSB breeding populations at such sites as an indicator of where we might target sampling in nearby vineyards. The Zalom lab has obtained a permit to maintain a BMSB colony that we initiated during 2015 with bugs collected from community gardens in Sacramento, and is presently using the colony in various behavior and control studies.

2. Determine BMSB temperature-related field feeding intensity, impact and regional risk index.

Methods. *Feeding intensity*. In Oregon, we deployed portable electronic feeding monitors (Wiman et al. 2014b) during 2016 in order to determine in-vineyard feeding intensity. Portable feeding monitors consisting of an open circuit enclosed onto a section of the grape vine will be located within 20 meters of the pheromone traps. Four electronic feeding monitors were placed in each of the two rows in a partially shaded vineyard border, and a fully sun-exposed location within the center of each vineyard. Each feeding monitor was used to determine feeding frequency, duration and time. Each portable feeding monitor logged feeding for five individual BMSB. The insects were replaced once per week. The relative risk and intensity of BMSB feeding damage were determined by creating a feeding index of insect-days (Ruppel 1983) for each of the vineyard regions using standard methods as described by Wiman et al. (2014b). Additionally, these feeding patterns were verified by counting the number of stylet sheaths and plant damage within the monitored feeding area.

Results. Data from this work showed clear feeding activity patterns on a daily basis (Fig. 2 a, b) with a decrease in feeding at temperatures below and above 6 and 26°C respectively. These data support the estimated lower (3-6°C) and upper (26-29°C) threshold ranges of temperature-related feeding activity of BMSB (Wiman et al 2014).

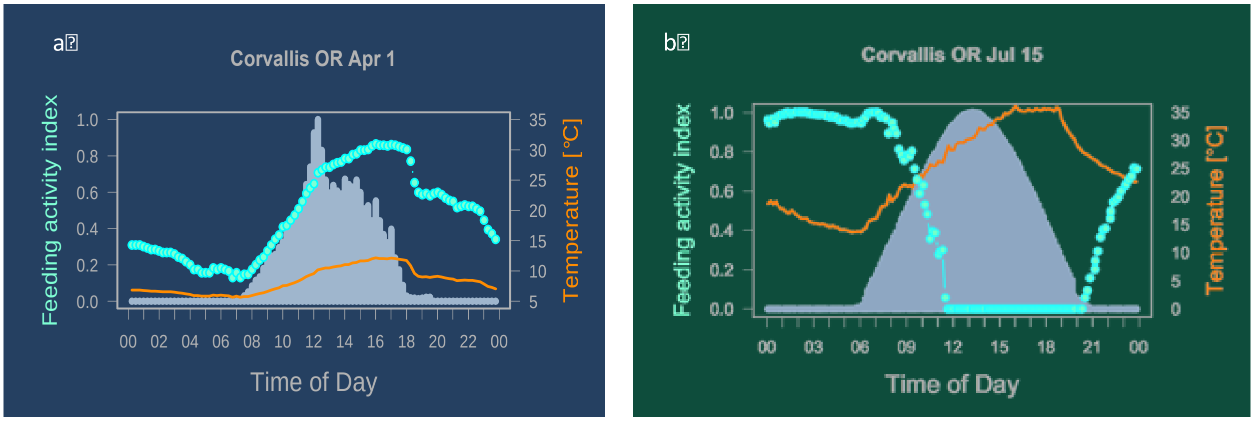

Figure 2. Adult *Halyomorpha halys* feeding activity on Pinot noir winegrapes during cool (a) and warm (b) days in Corvallis, Oregon during 2016 using electric feeding monitors (adapted from Wiman et al 2014b).

Feeding impact. Feeding exclusion sleeves (48.0 cm x 39.5 cm, Premier Paint Roller, Richmond Hill, NY, item 60597) were placed over wine grape clusters in a commercial vineyard with known BMSB infestation in the northern Willamette Valley. The trial was maintained for a four and three-week period respectively from August 21 to September 21, 2015, and August 22 to September 21, 2016. There were four treatments: 1) no BMSB; 2) a partial egg mass with 10 hatching eggs; 3) three BMSB nymphs; 4) three adult BMSB. All treatments were enclosed in a single sleeve. Ten replicates of each treatment were established in a randomized block design. Forty sleeves (ten of each treatment) were placed in a partially shaded vineyard border row, and forty sleeves were placed in a fully sun-exposed vineyard row in each vineyard (80 sleeves total). BMSB insects were exposed to clusters during the period when BMSB are typically found in vineyards in the Willamette Valley. Dead insects were replaced every week with BMSB of the same life stage during the exposure period. At the end of the experimental period, all clusters were removed and taken to the laboratory for further inspection. Feeding activity of BMSB was determined by counting the number of stylet sheaths per berry at the end of the exposure period. Additional key quality parameters were determined, including berry weight, pH, sugar, raisening, cracking and presence or absence of spoilage bacteria such as botrytis using the slip-skin method (Crisosto et al. 2002). These data together with weather data (five dataloggers per vineyard location), feeding intensity and direct impact on crop can be used to develop a relative risk model for BMSB damage in different vineyard regions (Ruppel 1983, Froissart et al. 2010, Wiman et al. 2014a, 2014b).

Results. During 2015 there were significantly higher temperatures recorded in locations that received higher temperature exposure levels compared to 2016 (Fig. 3a, b). Mean temperatures ranged from 12.3-23.8°C during the experimental periods. Temperatures ranged from 23.5-28.2°C on days when there were full sun exposure to virtually indistinguishable on cloudy days. These trends were however not found during 2016 where the mean sunny (18.0°C) and shady (18.4°C) regimes were statistically similar ($F_{2,53}$=0.01, p=0.99).

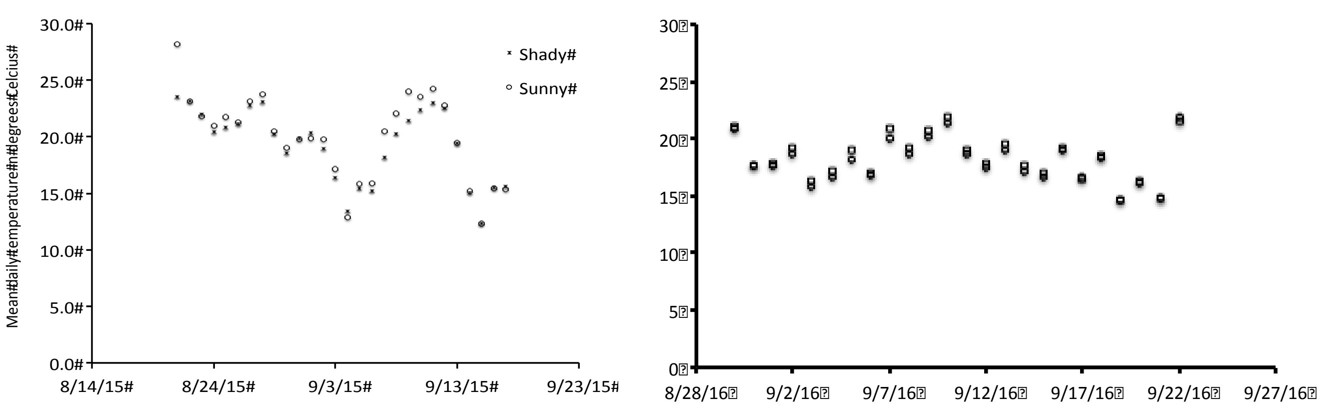

Figure 3a. Mean daily-recorded temperatures from each of shady and sun-exposed locations on Pinot noir preceding the harvest period on vines in Corvallis, Oregon during 2015 and 2016.

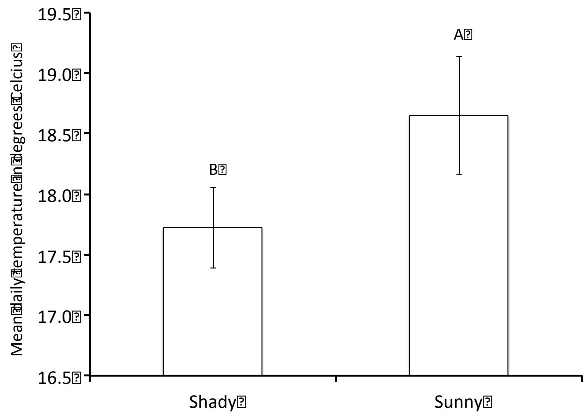

Figure 3b. Mean temperatures recorded in each of two sun-exposed locations on Pinot noir vines in Corvallis Oregon during 2015 for a one-month period. Significantly different letters indicates different temperatures.

During 2015 there were significantly higher levels of stylet sheaths between sunny and shady locations in vines ($F_{1, 4074}$=45.079, p<0.01; Fig. 4), and there were higher levels of stylet sheaths in treatments with adults compared to immature BMSB life stages. Feeding activity of BMSB still needs to be determined for 2016, but the trends found during 2015 appeared consistent with those found during 2016.

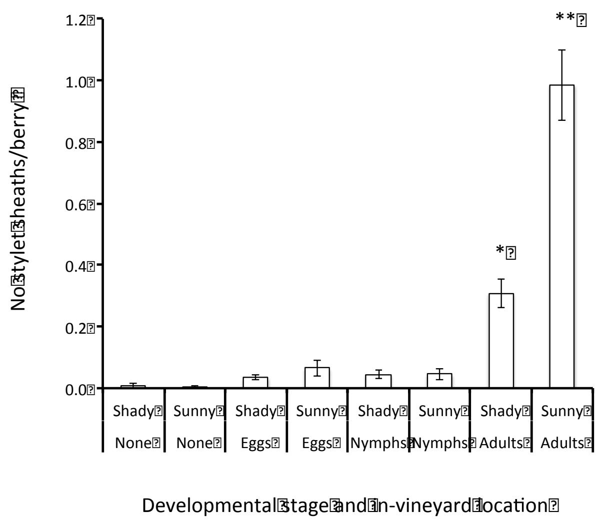

Figure 4. Number of stylet sheaths per berry on Pinot noir in Corvallis, Oregon during 2015. Bars with no, one and two asterisks (*) are significantly different from other bars.

In order to determine if there were differences in BMSB feeding days (Insect days, Ruppel 1983) between sunny and shaded locations during 2015, we determined the mortality rates over the four-week period of the feeding trial and there were however no clear differences in cumulative mortality rates between locations that BMSB were placed on vines. For 2015, the winegrape quality parameters (Table 1-2) showed statistically lower berry and cluster weights, lower berry diameter, less berries per cluster, and more stylet sheaths per berry (Table 1) between climate regimes. There was a numerical decrease in berry and cluster weights, lower berry diameter with increasing age of BMSB life stage, as well as warmer temperatures (Table 2).

Table 1. Mean berry characteristics of Pinot noir grapes and *Halyomorpha halys* feeding activity (±SE) for temperature regimes (N = 40) and life stage treatments (N = 20) during 2015 in Corvallis, Oregon.

Group	Berry Weight (Grams)	Cluster Weight (Grams)	Berry Diameter (mm)	Berries/ Cluster	Stylet Sheaths/Berry	°Brix
Shady	1.3±0.03a	90.0±4.9a	12.1±0.1a	72.4±3.9a	6.8±3.0b	21.6±0.04a
Sunny	1.1±0.03b	56.7±4.9b	11.6±0.1b	50.2±3.9b	12.8±3.0a	22.0±0.04a
Control	1.2±0.005a	75.9±5.8a	-	-	0.009±0.006b	21.5±0.3a
Eggs	1.1±0.002a	69.7±6.5a	-	-	0.047±0.012b	22.0±0.3a
Nymphs	1.2±0.003a	80.8±9.2a	-	-	0.060±0.018b	21.6±0.2a
Adults	1.2±0.013a	67.1±9.4a	-	-	0.781±0.177a	22.1±0.5a

Table 2. Mean grape berry characteristics of Pinot noir and *Halyomorpha halys* feeding activity (±SE) for temperature regimes and life stage treatments (N = 10) during 2015 in Corvallis, Oregon.

Treatment	Cluster Weight (Grams)	Stylet Sheaths/Berry	°Brix
Shady Control	87.1±5.2a	0.013±0.013c	21.3±0.4a
Shady Egg	89.0±6.3a	0.028±0.012c	22.2±0.5a
Shady Nymph	94.0±14.4a	0.072±0.031c	21.9±0.2a
Shady Adult	89.9±14.6a	0.335±0.092b	21.1±0.6a
Sunny Control	64.8±9.4a	0.006±0.004c	21.7±0.5a
Sunny Egg	50.3±7.4a	0.066±0.200c	21.9±0.5a
Sunny Nymph	67.6±10.5a	0.047±0.018c	21.3±0.4a
Sunny Adult	44.3±6.8a	1.226±0.282a	23.0±0.5a

BMSB feeding was correlated based on BMSB life stage and temperature (Ruppel, 1983). For life stages, a factor of 1 was attributed to control treatments, 5.22 for eggs, 6.67 for nymphs and 86.78 for adults. These factors were obtained by dividing the number of stylet sheaths found for each life stage by the number of stylet sheaths found in the control treatments (0.009) over the two seasons. We assume, based on the electronic feeding monitors, that no stylet sheaths are found in situations where temperatures are below 6 and above 26°C respectively. These zero values of feeding were used in the fitting of a non-parametric curve in order to describe the lower and upper thresholds of BMSB feeding. The effect of temperature was determined by estimating the number of degree-days (DD) per day for each temperature regime. The DD/day were estimated using the lower and upper thresholds of 14 and 34ºC (Nielsen et al., 2008), respectively. Based on the relative number of DD/day in each regime, the corresponding factor was attributed to each of the regimes. The life stage factor was multiplied by DD/day to create a feeding factor (Table 3).

Table 3. *Halyomorpha halys* (BMSB) feeding factor based on life stage, and number of Degree-Days/day (DD/day). *Halyomorpha halys* feeding activity was acquired using an electronic feeding monitor as well as over two seasons on Pinot noir during 2015 and 2016 in Corvallis, Oregon.

BMSB life stage	Year	Temperature regime	Numerical BMSB life stage factor	DD/day	Feeding factor
None	2015	Shady	1	7.07	7.07
None	2015	Sunny	1	10.03	10.03
None	2016	Shady	1	7.115	7.115
None	2016	Sunny	1	8.59	8.59
Eggs	2015	Shady	5.22	7.07	36.9054
Eggs	2015	Sunny	5.22	10.03	52.3566
Eggs	2016	Shady	5.22	7.115	37.1403
Eggs	2016	Sunny	5.22	8.59	44.8398
Nymphs	2015	Shady	6.67	7.07	47.1569
Nymphs	2015	Sunny	6.67	10.03	66.9001
Nymphs	2016	Shady	6.67	7.115	47.45705
Nymphs	2016	Sunny	6.67	8.59	57.2953
Adults	Feeding monitor	Cold	86.78	0	0
Adults	2015	Shady	86.78	7.07	613.5346
Adults	2015	Sunny	86.78	10.03	870.4034
Adults	2016	Shady	86.78	7.115	617.4397
Adults	2016	Sunny	86.78	8.59	745.4402
Adults	Feeding Monitor	Hot 1	86.78	10.88	944.1664
Adults	Feeding Monitor	Hot 2	86.78	12.33	1069.9974

For the BMSB feeding correlation, the regression of stylet sheaths/berry on the feeding factor resulted in a significant fit using the function y =(0.0000089)*x*(x-(143.717))*((1028.8)-x)*exp(1/(-0.12867) ($R^2 = 0.71$; $F = 6.33$; df = 1, 4; $p < 0.003$, Fig. 5).

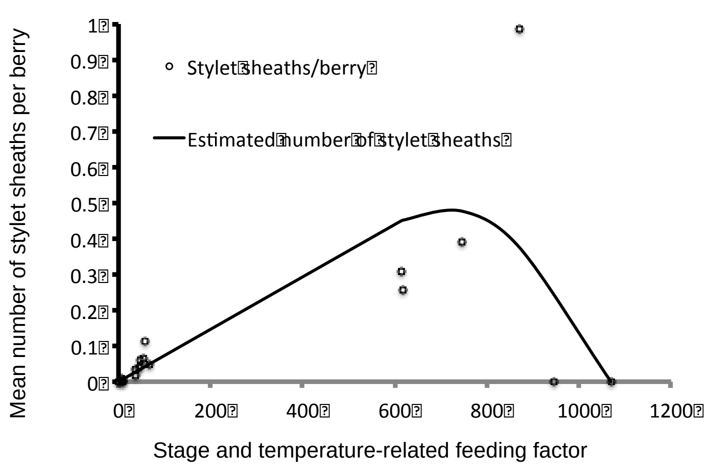

Figure 5. *Halyomorpha halys* (BMSB) stylet sheats per berry over feeding factor. The feeding factor was estimated based on life stage, and number of Degree-Days/day (DD/day). *Halyomorpha halys* feeding activity was acquired using an electronic feeding monitor during 2016 and also on Pinot noir winegrape during 2015 and 2016 in Corvallis, Oregon.

3. Provide Extension for identification, distribution, and importance of Brown Marmorated Stink Bug in western vineyards.

Methods. Because BMSB may first be seen in small organic gardens and ornamental trees, we also began outreach or surveys of small organic farms (Napa and Sonoma counties) and Southeast Asian vegetable farms (Fresno County). In the north coast region, we have partnered with Master Gardener groups in Napa and Sonoma to gain access to home gardens in which we may find desirable host source plants. Additionally, contacts have been made, in partnership with the Napa Agricultural Commissioner, allowing us access to survey and sample small-diversified farms. No BMSB have yet to be found at these sites.

Results. In Oregon, we presented results of earlier and work for this grant to growers in five locations, McMinnville, Oregon (63 attendees), Milton Freewater, Oregon, (30 attendees), Roseburg, Oregon, (50 attendees), Medford, Oregon, (48 attendees) and Rickreal Oregon (211 attendees). Several extension meetings were held in the San Joaquin Valley and coastal winegrape regions as represented by the sampled regions mentioned above.

Conclusions. In California's north coast wine grape region, Lodi-Woodbridge wine grape region, and San Joaquin Valley (Fresno County), vineyards and small vegetable farms no BMSB were found. While this is only the initial study, BMSB have been found in the Lodi Woodbridge region in ornamental trees, but have yet to be found near the vineyards sampled. During 2016, there was a report of BMSB found in Napa in a home garden. In Oregon, BMSB were found in increasing numbers from 2013-2016 (2016 data not shown) in each of the seven vineyards sampled.

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