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Erika A. Maslen for the degree of Master of Science in Horticulture presented on August 10, 2016

Title: The Impact of Temperature on *Halyomorpha halys* (Hemiptera: Pentatomidae) Life Table Parameters and Feeding Pressure

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Halyomorpha halys (Stål) (Hemiptera: Pentatomidae) is an invasive insect pest that has become established and problematic in the Pacific Northwest. Known colloquially as the brown marmorated stink bug (BMSB), *H. halys* causes direct damage through feeding activity on a wide range of agronomic and horticultural crops. The objectives of this research were: 1) to establish ovipositional and survival thresholds of *H. halys*; and 2) to determine the impact of life stage and temperature on feeding activity using wine grapes as a model host plant. In the first study, cohorts of *H. halys* were exposed to seven constant temperatures, from 15 to 32ºC, in growth chambers to determine longevity and fecundity. The lower and upper temperature thresholds for reproduction was 18 and 32ºC, respectively. As temperatures increased, survival of adult females decreased. Highest rates of oviposition occurred at 25ºC, followed in order by 27, 22, 32 and 18ºC. Pre-ovipositional periods were shortest at 30ºC at 7 days, while ovipositional periods were longest at 22ºC at 113 days, with highest mean oviposition at 25ºC at 1.43 ± 0.21 eggs per female per day. Mean female longevity was highest at 18ºC at 76.1 days. In the second study, the impact of life stage and temperature on *H. halys* feeding was determined on wine grapes for four weeks during pre-véraison to determine the relative feeding activity. Both temperature and life stage had a significant effect on feeding activity in wine grapes. The mean number of stylet sheaths, an indicator of feeding activity, was highest at 1.226 ± 0.282 stylet

sheaths per berry in sunny regimes containing adults and lowest at 0.028 ± 0.012 stylet sheaths per berry in shady regimes containing eggs. Average cluster and berry weight, diameter and number of berries per cluster were not significantly different between temperature regimes. The results from these studies demonstrates the relative importance of the environment as indicated by accumulated degree-days and life stage on feeding activity. Feeding activity was higher in older life stages and increased with the accumulation of degree days. The data can be incorporated into control and management strategies, as well as in prediction and risk models.

The Impact of Temperature on *Halyomorpha halys* (Hemiptera: Pentatomidae) Life Table Parameters and Feeding Pressure

by
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I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

Erika A. Maslen, Author

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CHAPTER 1

General Introduction

Erika A. Maslen

Background

Halyomorpha halys (Stål), the brown marmorated stink bug (BMSB), is an invasive insect species (Hemiptera: Pentatomidae) in the United States. A generalist herbivore, *H. halys* will feed on nearly any plant species and part, but is more selective about reproductive host plants (Rice et al., 2014). BMSB is native to China, Japan, Korea, Taiwan and Vietnam and was first found in the US in Allentown, Pennsylvania in 1996 (Day et al., 2009; Hoebeke & Carter, 2003; Lee et al., 2013). BMSB is thought to have been transported on bulk shipping containers and was initially misidentified as a native species (Hamilton, 2009).

Brown marmorated stink bug was not positively identified in Pennsylvania until 2001 (Bernon, 2004), when it was first recorded as a nuisance pest for homeowners. BMSB became an economic pest of specialty crops, causing damage throughout the United States (Rice et al., 2014). *Halyomorpha halys* is polyphagous and has a very wide host range (Poplin et al., 2014). Host plants include over 300 different ornamentals, crops and exotic plant species (Wiman et al., 2014a). An estimated $37 million in apple grower losses occurred in 2010 (USAA, 2010), though economic damage in other crops has not been measured (Kuhar et al., 2012; Leskey et al., 2012b). Over $21 billion worth of crops in the United States have been estimated to be threatened by BMSB feeding damage (ODA, 2013).

As of August 2015, BMSB can be found in 42 states in the US, is established as an agricultural pest in 22 of those states (Leskey, 2015; Venugopal et al., 2016) and can be found in two Canadian provinces (Xu et al., 2014). *Halyomorpha halys* has been reported in Switzerland, New Zealand, France, Germany, Liechtenstein, Italy, Hungary and Greece (Harris, 2010; Haye et al., 2015; Venugopal et al., 2016; Wermelinger et al., 2008). Day length and temperature are two

major environmental factors that impact the distribution and movement of BMSB (Nielsen et al., 2008; Venugopal, 2016; Zhu et al., 2012). Bioclimatic modeling by Zhu et al. (2012) suggests countries with the most suitable *H. halys* establishment conditions include Italy, France, Croatia, Algeria, Cameroon, North American coastal regions, Chile, Brazil, Uruguay and Argentina. In the USA, the states of Oregon, California and Washington have a highly suitable climate for BMSB (Zhu et al., 2012), while Florida, California, North Carolina and New Jersey could see positive BMSB population growth (Nielsen et al., 2016).

Nymphs and adults feed on fruit, stems, leaves and other plant parts, depositing saliva and causing damage (Kuhar et al., 2012). *Halyomorpha halys* is a polyphagous feeder that can damage fruit and vegetables by feeding, rendering crops unmarketable (Leskey et al., 2012b; Panizzi et al., 2000). Agricultural host plants of economic importance in the US include, but are not limited to: apple (*Malus domestica* Mill.), blueberry (*Vaccinium corymbosum* L.), hazelnut (*Corylus avellana* L.), peach (*Prunus persica*. (L.) Batsch), pears (*Pyrus* spp. L.) and soybean (*Glycine max* Willd.) (Hedstrom et al., 2014; Hoebeke & Carter, 2003; Kuhar et al., 2012; Leskey et al., 2012b; Wiman et al., 2015a). The exact feeding method of *H. halys* is unknown, though pentatomids typically feed using the stylet-sheath or lacerate-and flush feeding methods (Hori, 2000). BMSB is thought to use a stylet to pierce plant flesh and feed on phloem in stylet-sheath feeding, or by moving cells with a stylet tip to lacerate cells in lacerate-and-flush feeding (Hori, 2000; Kuhar et al., 2012; Panizzi et al., 2000). A stylet sheath made up of dried proteinaceous saliva is left behind after feeding (Peiffer and Felton, 2014). Stylet sheaths can be counted to indicate feeding activity on crop plants (Wiman et al., 2015a). The watery saliva contains enzymes (Peiffer & Felton, 2014) that break down the plant cells, causing necrosis,

dimpling, discoloration, scars, wounds and bruises (Leskey et al., 2012b). Further damage from feeding occurs as fruit abortion and root rot (Kuhar et al., 2012; Leskey et al., 2012b). Plant wounds may become infected with diseases and pathogens (Welty et al., 2008), exacerbating detrimental impacts by *H. halys*.

Pyrethroid and neonicotinoid insecticides are the most used and effective chemical sprays against *H. halys* (Fiola, 2011b; Lee et al., 2013; Leskey et al., 2012b; Rice et al., 2014). These insecticides are detrimental to natural enemies and only provide short-term crop protection (Poplin et al., 2014; Rice et al., 2014). Reliance on pesticides to control BMSB can lead to increasing pesticide resistance and secondary pest outbreaks, without eliminating damage (Wiman et al., 2014a). Few other chemical classes are recommended for *H. halys* control and frequent applications are required to manage BMSB effectively, making resistance development more likely.

There is a need for additional life table data for BMSB to more accurately estimate invasive potential and model population growth (Harcourt, 1969; Kakde et al., 2014; Nielsen et al., 2008). Survival and reproduction data representing different temperatures are important for predicting population dynamics in different environments. Nielsen et al. (2008) provided BMSB nymph developmental and survival data at multiple temperatures and reproduction at 25ºC. Haye et al. (2014) explored nymph development at naturally fluctuating temperatures and reproduction at 20, 25 and 30ºC of overwintering adults. Several studies have researched laboratory reproduction at 25ºC and estimated nymph developmental periods and adult longevity (Kawada & Kitamura, 1983; Medal et al., 2013). Results from these studies confirm that developmental thresholds are between 15 and 35ºC, with the optimal rearing temperature at 25ºC.

Morphological and Phenological Descriptors

Halyomorpha halys can be distinguished as adults from other native stink bugs in the United States by the white bands on the fourth antennal segment and outer edges of the abdomen (Hoebeke & Carter, 2003). There are several native species that have morphological or behavioral similarities to that of *H. halys*. These include *Brochymena* spp. Amyot & Serville, *Euschistus conspersus* Uhler and *Boisea rubrolineata* Barber (Hoebeke & Carter, 2003; Wiman et al., 2015). BMSB have smooth pronotum, while *Brochymena* pronotum are toothed and rough (ODA, 2013). *Euschistus conspersus* tends to be smaller in size than BMSB and have a green abdomen (Hedstrom et al., 2014), while *B. rubrolineata* are black with red markings.

Adults are 12 to 17 mm long and shield-shaped (Hoebeke & Carter, 2003). Males have a genital capsule on their ventral abdominal segment, while females have none (Rice et al., 2014; Vetek et al., 2014). Adults have a variety of abdominal colorations, from pale white through red and sometimes green, depicting age and sexual maturity (Wiman et al., 2014b). BMSB have a brown and tan mottled dorsal surface with white markings and red eyes (Hamilton, 2009).

Adult females lay egg masses containing an average of 28 eggs, usually between June to September in the US (Bernon, 2004; Kobayashi, 1967; Nielsen & Hamilton, 2009). Females require between 117.65 (Haye et al., 2014) and 147.65 (Nielsen et al., 2008) DD until oviposition can begin. Egg masses can be oviposited every 4.32 days (Nielsen et al., 2008) and a female can lay an average of 240 eggs in its lifetime (Lee et al., 2013; Nielsen et al., 2008). Adults live an average of 111 days (Kawada & Kitamura, 1983; Medal et al., 2013). Eggs are pale green, spherical, 1 mm in diameter and 1.6 mm tall (Hoebeke & Carter, 2003; Nielsen and Hamilton, 2009). Egg masses are usually oviposited on the underside of leaves, but can be found

on any plant surface (Medal et al., 2013). Eggs have been observed developing between 15 and 33ºC and require 53.30 DD to hatch (Nielsen et al., 2008).

When nymphs first molt from each instar stage, they are completely white. As the exoskeleton hardens, red bands and spots appear on the body, darkening with time. Nymphs emerge from the eggs via a circular operculum opened by a black-framed, triangular egg breaker (Rice et al., 2014). First instar nymphs are elliptical and red bodied with black heads, legs, antennae and dorsal markings (Rice et al., 2014). First instars are approximately 2.4 mm long (Hoebeke & Carter, 2003). After emergence the first instars aggregate around the egg mass (Lee et al., 2013). Eggshells serve as the primary food source for first instars (Rice et al., 2014). The first instar stage lasts up to 17 days at 17ºC, or as few as 3 days at 33ºC (Nielsen et al., 2008).

Second instar nymphs are black bodied with white markings. White markings are more prominent on second than first instars, and second instars develop horn-like appendages on the body and head. Second instar nymphs are 3.7 mm long (Hoebeke & Carter, 2003). The second instars stage lasts between 30 days at 17ºC to 7.5 days at 33ºC (Nielsen et al., 2008). Third instar nymphs are 5.5 mm long, while fourth instar nymphs are 8.5 mm (Hoebeke & Carter, 2003). The third and fourth instar stages last between 22 and 23 days, respectively, at 17ºC and 7.5 and 7 days at 33ºC, respectively (Nielsen et al., 2008). Second, third and fourth instar nymphs are morphologically similar, and are mainly distinguished by size.

The final fifth instar nymphs lose pronotum and body appendages from earlier instar stages. Fifth instar nymphs are 12 mm long (Hoebeke & Carter, 2003) and have more abdominal coloration than all other instars. The fifth instar stage lasts about 28 days at 17ºC, 8 days at 27ºC, 8.5 days at 30ºC and 10.5 days at 33ºC, respectively (Nielsen et al., 2008). The number of

degree-day accumulation for BMSB to develop from egg to adult is 537.63 DD (Nielsen et al., 2008) to 588.24 DD (Haye et al., 2014).

Halyomorpha halys overwinters in protected structures or houses, flying to them during the fall (Wermelinger et al., 2008). Adults emerge in late spring to mate and reproduce (Welty et al., 2008). *H. halys* is highly mobile, having the capability of flying up to 45 miles in 24 hours (Wiman et al., 2014b). In the United States, BMSB is bivoltine in western states, producing two generations per summer season (Day et al., 2009; Nielsen et al., 2008; Nielsen & Hamilton, 2009; Niva & Takeda, 2003).

Oregon and California Wine Grape Production

California is the leading wine producing state in the United States, producing 783 million gallons in 2014 (WineAmerica, 2014). Wine grapes are the highest valued fruit crop in the US, generating over $5 billion, with wine sales reaching $37.6 billion in 2014 (Statista, 2014; WineAmerica, 2014). Combined, Oregon and California produce over 90% of the wine and wine grapes in the US.

Oregon produced 58,000 tons of wine grapes on 19,000 acres in 2014, valued at $118.3 million (ODA, 2015). The major growing regions of wine grapes in Oregon are the Willamette Valley, Southern Oregon and Columbia Gorge American Viticulture Areas (AVAs). California produced 3.89 million tons of wine grapes on 565,000 acres in 2014, valued at $5.24 billion (CDFA, 2014; USDA, 2014). The major wine grape growing regions in California are the North Coast, Central Coast, South Coast and Central Valley AVAs.

The dominant arthropod pests for wine grape growers in Oregon are eriophyid, erineum and spider mites, leafhoppers, thrips and mealybugs (Skinkis et al., 2016). California wine grape

pests include mealybugs, spider mites, sharpshooters, leafrollers and thrips (UCIPM, 2015). Thrips, mealybugs and sharpshooters are hemipteran species that are of concern for the wine grape industry.

Growers control thrips by using the insecticides Delegate, Entrust (spinosads) or Surround (kaolin) before bud break. Farmers are encouraged to delay mowing or tilling until after spring, to discourage thrips from moving up higher in the canopy (Skinkis et al., 2016). Mealybugs are currently controlled by spraying Admire, Platinum (neonicotinoids), Applaud (thiadiazine), Movento (tetramic acid) or natural oils before bud break (Skinkis et al., 2016). Sharpshooters species in wine grapes are monitored by placing sticky traps before budbreak in the vineyard and checking them weekly (UCIPM, 2015).

Potential Threat of *Halyomorpha halys* to Oregon and California Agriculture

Halyomorpha halys is not yet a major agricultural pest in Oregon or California, but is already present and causing economic damage in some crops. BMSB has a higher temperature threshold in the US than its native range, coinciding with areas of agricultural importance where they have invaded (Nielsen et al., 2008). Oregon and California's climates are highly suitable for BMSB establishment (Zhu et al., 2012). As *H. halys* populations continue to increase and disperse throughout the United States, more effective control strategies must be established. The feeding impacts of the different life stages are not fully understood, nor are the survival, reproduction and longevity of adults. These factors are highly influenced by temperature, potentially having differing impacts on agricultural crops. BMSB life table and feeding pressure data can help predict invasive potential, population growth and damage. Further research on the timing, feeding damage severity, oviposition rates and impacts by different life stages on wine

grapes and other crops by BMSB is needed to create management systems and economic thresholds of damage against this agricultural pest.

CHAPTER 2

Temperature-Dependent Life Tables of *Halyomorpha halys* (Hemiptera: Pentatomidae)

Erika A. Maslen

Introduction

Halyomorpha halys (Stål) (Hemiptera: Pentatomidae) is an economic pest of over 300 ornamental and agricultural plants in the United States, Asia and parts of Europe (Lee et al., 2013; Smith et al., 2014; Wermelinger, 2008). *Halyomorpha halys* was first found in the United States in 1996, and has since become a destructive and potential agricultural pest and nuisance issue in most states. Little data is available on the reproductive and developmental parameters of *H. halys* exposed to different temperatures. For this reason, researchers have a limited ability to determine risk and develop temperature-related population models.

Second instar nymphs and subsequent life stages of BMSB feed on plant tissues of a wide range of host plants (Wiman et al., 2014a). During feeding, *H. halys* deposits saliva within the plant tissues, causing necrosis and scarring (Peiffer & Felton, 2014; Wiman et al., 2015). Feeding damage can cause economic damage, though economic action thresholds have not been determined. *Halyomorpha halys* has a lack of suitable natural enemies (Wiman et al., 2014b). In addition, *H. halys* has high dispersal and reproductive capabilities, ovipositing an average of 240 eggs a season per female and flying up to 45 miles in a day (Lee et al., 2013; Nielsen et al., 2008; Wiman et al., 2014b). These factors, along with photoperiods, temperature fluctations, overwintering refuges and high population reservoirs are major factors contributing to the economic impact and rapid invasion and establishment of BMSB (Nielsen et al., 2016; Niva &Takeda, 2003; Wiman et al., 2015).

The impact of temperature on the developmental temperature, fecundity, survival and mortality of exotic insect pests, including BMSB, can be used to determine its invasive potential (Nielsen et al., 2008). Haye et al. (2014) and Nielsen et al. (2008) provided some BMSB egg and

nymph developmental periods and degree-day (DD) requirements. Both studies illustrated that at temperatures below 15 or above 35ºC, *H. halys* cannot complete its life cycle. Nielsen et al. (2008) described oviposition at 25ºC. Medal et al. (2013) and Kawada and Kitamura (1983) researched *H. halys* laboratory reproduction at 25ºC. Nymph developmental periods averaged one week per instar stage, adult longevity averaged 111 days and pre-ovipositional periods averaged two weeks (Kawada and Kitamura, 1983; Medal et al., 2013).

Life table studies on other stink bug species, including *Nezara viridula* (Linnaeus), *Eysarcoris ventralis* (Westwood), *Stiretrus anchorago* (Fabricius), *Chinavia impicticornis* (Stål) and *C. ubica* (Rolston) can be used for comparison due to developmental, behavioral and morphological similarities. Kiritani et al. (1963) found that *N. viridula* mean adult female longevity was 41.1 days at 25ºC, similar to *H. halys* longevity of 47.97 days found by Nielsen et al. (2008). Mean fecundity per female for *N. viridula* was 114.4 eggs and is dissimilar to *H. halys* (Nielsen et al., 2008; Table 2.1). Kiritani and Hôkyo (1962) provided nymph developmental periods for *N. viridula* at 25 and 30ºC at 38.7 and 34.8 days, respectively. These data are similar to *H. halys* developmental periods of 42.3 and 33.2 days (Haye et al., 2014) and 44.9 and 33.4 days (Nielsen et al., 2008). *Eysarcoris ventralis* adult longevity (31.3 days), pre-ovipositional (6.88 days) and ovipositional periods (14.44 days) are similar to those of *H. halys* (Table 2.1), with a pre-ovipositional period of 4.32 days (Nielsen et al., 2008; Haye et al., 2014; Prakash & Rao, 1999). *Stiretrus anchorago* nymph longevity and survival at 18.3, 26.7 and 32.2ºC were dissimilar to nymph longevity and survival of *H. halys* (Nielsen et al., 2008; Haye et al., 2014), with *S. anchorago* longevity being much lower (Waddill & Shepard, 1974). *Chinavia impicticornis* and *C. ubica* developmental periods at 26ºC for each instar stage and egg to female

adult period produced similar results to *H. halys* (Nielsen et al., 2008; Haye et al., 2014; Silva et al., 2015). Female adult longevity was 66.8 and 41.4 days for *C. impicticornis* and *C. ubica,* respectively, with 178.9 eggs per female, which were dissimilar to Nielsen et al. (2008) and Haye et al. (2014) findings, with 212 and 69.65 eggs per female.

Life tables are constructed to determine mortality of a population as a response to abiotic or biotic factors. From life tables, generations per season, pest abundance and establishment risk can be estimated. Temperature is the most significant environmental factor influencing survival and reproduction (Fand et al., 2015). The developmental rate of the population is estimated using life tables. These age-specific fecundity and survival data related to temperature are vital components of population and risk models (Kiritani et al., 1963). The goal of this study was to estimate survival and reproduction rates of BMSB adults over a range of constant temperatures. This study will be used to estimate *H. halys* population dynamics and determine risk by creating population models.

Materials and Methods

Collection and Rearing

Halyomorpha halys colonies were established using specimens collected in the Willamette Valley, OR from May to June 2015 using a beat sheet (Model DP1000; BioQuip, Rancho Dominguez, CA). Colonies were continuously supplemented by weekly collections throughout the summer of 2015. The majority of *H. halys* used in this study were collected from English holly (*Ilex aquifolium* L.), western red cedar (*Thuja plicata* (Donn) D. Don), vine maples (*Acer circinatum* Pursh) and lilac (*Syringa vulgaris* L.).

Colonies were reared in 30 x 30 x 30 cm bug dorms (#2840R; BugDorm, Taichung, Taiwan) at 22 ± 1ºC and a photoperiod of 16:8 light:dark (L:D). Each cage contained a 473-mL plastic container (460938; Arrow Plastic Stor, Elk Grove, IL) filled with water and a modified mesh-topped lid to provide a water source and humidity. A diet of organic Spanish peanut (*Arachis hypogaea hypogaea* L.), soybean (*Glycine max max* (L.) Merr.), pumpkin seed (*Cucurbita* spp. L.) and sunflower (*Helianthus annuus* L.) was provided in an open petri dish on the cage floor. Food was replaced weekly. Jalapeño pepper (*Capsicum annuum* L.), empress tree (*Paulownia tomentosa* (Thunb.) Steud.) and green bean (*Phaseolus vulgaris* L.) plants grown in an Oregon State University (OSU) greenhouse and *I. aquifolium* branch clippings with berries from Corvallis, OR were provided for additional nutrition, habitat and oviposition substrate. Plants were grown in 800-mL plastic pots filled with Professional Growing Mix Custom Blend soil (4v; Pro Gro, Portland, OR) and were approximately 0.5 meters tall. Plants and clippings were watered daily and replaced weekly. Dead *H. halys* were removed daily to discourage mold and disease.

Newly emerged adult *H. halys* from the field-collected colonies were separated by sex into separate rearing cages on a daily basis. Twenty-five male and female adults (50 total) aged one to three days were then placed in new rearing cages and placed in temperature-controlled growth chambers (E-30BHO; Percival Scientific, Perry, IA) for the duration of the experiment. Two replicate rearing cages for each temperature were placed within each chamber. There was only one replicate at 15ºC because no egg laying was anticipated at this temperature (Nielsen et al., 2008). Constant temperature treatments were 15, 18, 22, 25, 27, 30 and 32ºC, and a temperature and light recording HOBO data logger (UA-002-08; Onset, Bourne, MA) was

placed inside each chamber to verify temperature. All treatments were maintained at 85% RH and 16:8 L:D conditions. One replicate cage at 32ºC only contained 18 males and females (36 total). Cages were checked daily to record reproduction and mortality. Egg masses were removed from cages and the number of eggs in each mass was counted. Dead *H. halys* were sexed and removed. Checks were performed until all females within a cage had died.

Statistical Analysis

Mortality, Survival and Oviposition

All insects in replicated cages within temperature treatments were treated as cohorts. Adult female survival and reproduction at each temperature excluded missing individuals. The mean overall mortality rate at each temperature was determined by averaging female mortalities per day per cohort. Nonparametric Kruskal-Wallis rank-sum tests were conducted to determine differences in survival between temperature treatments. The impact of temperature on the mean duration of adult female survival and reproduction was analyzed using ANOVA with temperature as the independent factor. Differences in the means were separated using Tukey's HSD after significant ANOVAs. All analyses were conducted using Statistica (Statsoft 7.1; Tulsa, OK).

The intrinsic rate of population increase (r_m) at each temperature was estimated using mean survival and fecundity values for each temperature. The equation $r_m = \log_e R_O/T$ (Price, 1997), where R_o is the net reproductive rate and T is the mean generation time, was used to determine r_m. The equation $R_o = \Sigma\, l_x m_x$ was used to determine net reproductive rate. The upper, lower and optimal thresholds of r_m were estimated by fitting the nonlinear estimation model (Brière et al., 1999) to the r_m values obtained at each experimental temperature.

Oviposition, Maternity and Survival Curves

The time scale for survival and reproduction was converted from calendar days to DD to measure the amount of accumulated heat units required by *H. halys* to reproduce and develop (Zalom et al., 1983). A regression was used on eggs laid per female per day (EFD) to model oviposition rates at each temperature. Data were fitted using the function $v^2 = (a)v^1(v^1-(b))((c)\exp(1/0.235)$, where v^1 is temperature, a, b and c are the constants 0.0007, 14.1698 and 32.6041, respectively.

A Pearson's Chi-square was used on survival and maternity (M_x) models as a goodness-of-fit test. The number of eggs laid per adult female per DD was plotted by summing the eggs laid during thirty-five consecutive 30-DD periods, starting at 575 DD and ending at 100% mortality at 1615 DD. Net maternity over time was fitted using the Cauchy distribution, $F(x_0 \mid Y^2) = 1/\pi\ Y^2/(x- x_0)+Y^2)$, where x_0 is the location and Y is the scale.

Age-specific survival (L_x) data of nymphs from Nielsen et al. (2008) was incorporated with adult survival data and fitted using the Gompertz function and the two-parameter probability density function $F(x \mid a, b) = \exp(-b/a(\exp(ax)-1))$, where a is the shape and b is the rate. The data were fitted using R version 3.2.2. using 'flexsurv' (Jackson, 2015).

Age-specific survival at the end of nymph development (Nielsen et al., 2008) and the beginning of adult development in the current experiment were set to the same value of 28%. Nymph survival rates per DD were estimated from Nielsen et al. (2008).

Results

Mortality, Survival and Oviposition

The mean female mortality rates as a proportion of the total number of deaths per day for 15, 18, 22, 25, 27, 30 and 32ºC were 0.164, 0.267, 0.413, 0.676, 0.633, 0.956, and 0.962 females per day, respectively (Fig. 2.1A and Fig. 2.1B). Temperature significantly impacted mean female adult survival ($F_{6, 290} = 23.4$, $p < 0.001$, Table 2.1). Females lived significantly longer at 15 and 18ºC. Females reared at 22ºC had significantly shorter survival periods compared to the two lower temperatures and statistically lower survival periods were recorded at the four higher temperatures of 25, 27, 30 and 32ºC (Table 2.1, Fig. 2.1A and Fig. 2.1B). Rank-sum tests comparing the effects of temperature on survival of reproducing females ($\chi^2 = 24.6$; df = 1, 6; $p < 0.001$) indicated significant differences. The median adult female survival times at 15, 18, 22, 25, 27, 30 and 32ºC were 89, 71, 60, 40, 41, 28 and 35 days, respectively.

Survival rates were lowest at 30, 32, 27 and 25ºC (Table 2.1, Fig. 2.1.A and Fig. 2.1B). Life span for adult females ranged from 2 to 187 days and for adult males 2 to 169 days. In general, an increase in temperature resulted in a decrease in both female and male mean adult survival periods (Table 2.1, Fig. 2.1A and 2.1B). The shortest survival period (2 days) for female adults was recorded at 18, 30 and 32ºC, respectively. Adult females exposed to 25 and 27ºC had similar longevity, and longevity at 30 and 32ºC was statistically similar. The combined adult lifespan at the higher four temperatures was shorter compared to the lower three temperatures (Fig. 2.1A and 2.1B).

Pre-ovipositional periods were also affected by temperature. The maximum mean fecundity of 2.34 egg masses and 48 eggs per female was recorded at 25ºC. Mean number of EF

was highest (32.97) at 25ºC, followed by 27, 22, 30, 18 and 32ºC at 17.57, 16.93, 11.71, 2.23 and 2.04 EF, respectively.

Fecundity of *H. halys*, as measured by number of eggs per female per day (EFD), was significantly higher at 25 and 30ºC compared to the other temperatures ($F_{5, 379} = 9.6$, $p < 0.001$; Table 2.1). The numerically highest levels of daily oviposition occurred at 25ºC (Fig. 2.2C). Comparable oviposition levels were found at 27 and 30ºC (Fig. 2.2D and E). The statistically lowest levels where oviposition occurred were recorded at 32 and 18ºC (Fig. 2.2F and A). No oviposition occurred at 15ºC. The oviposition period was longest (113 days) at 22ºC (Fig. 2.2B and Table 2.1).

Distribution curves of EFD using the rank-sum test showed differences ($N = 45$, $df = 5$; $p < 0.001$). EFD at 25, 27 and 30ºC were statistically similar and the highest at 1.4 ± 0.2, 1.1 ± 0.2 and 1.4 ± 0.2, respectively. EFD between 27 and 22ºC were statistically similar at 1.1 ± 0.2 and 1.0 ± 0.1, respectively. EFD at 18 and 32ºC were lowest and statistically similar at 0.2 ± 0.0 and 0.3 ± 0.2, respectively.

Oviposition, Survival and Maternity Curves

The best-fit regression on oviposition resulted in the function $v^2 = (0.00000156)x(6.513))((42.0577)-x)\exp(1/0.253))$ ($R^2 = 0.91$, $p = 0.002$). Temperature-specific oviposition (Fig. 2.3) was estimated to be 11.41 EF at 15ºC, went up to 52.44 EF at 18ºC, was 95.14 EF at 22ºC and peaked at 107.23 EF at 25ºC. At 27ºC, EF went down to 101.11 EF, 64.41 EF at 30ºC and 17.95 EF at 32ºC.

Age-specific survival (L_x; Fig. 2.4) of eggs and nymphs was 100% at 0 DD. At 54 DD, 90% of the eggs hatched into first instars, with 80% of the initial population surviving to become

second instars at 107 DD. At 216 DD, 64% of the initial population survived to become third instars, with 49% of the initial population surviving to become fourth instars at 303 DD. At 394 DD, 35% of the initial population survived to become fifth instars, with 28% of the initial population surviving until adulthood at 520 DD. Adults entered the experiment at 589 DD after being aged one to three days and all died by 1615 DD.

The distribution on maternity using the rank-sum test resulted in the function $F(x_0 \mid Y^2) = 1/(2.3\pi(1+(-2.82)^2)$ ($p = 0.004$). Age-specific maternity (M_x; Fig. 2.5) of females started at 646 DD at 0.27 eggs/female (EF), and increased to 2.28 EF at 905 DD. M_x decreased to 0.23 EF at 1025 DD before increasing to 13.03 EF at 1085 DD. EF oscillated between 1.81 and 11.73 EF until 1355 DD before reaching 0 EF at 1402 DD. All females died by 1615 DD.

Discussion

This study provides data for temperature-dependent survival, developmental and reproductive parameters of *H. halys*. Suitable temperatures for female adult survival and oviposition were indicated by relatively higher longevity and reproduction of *H. halys*. Survival and reproductive potentials were summarized by r_m and indicate that maximum population increase using non-linear estimation is 27ºC. Initial data on *H. halys* from Haye et al. (2014) and Nielsen et al. (2008) show that our lower and upper estimated thresholds for population increase are realistic, though current life table parameters are dissimilar to Nielsen et al. (2008) results. Our results indicate lower and upper temperature thresholds for reproduction and survival at 15 and 32ºC, respectively. Low survival and slow developmental rates recorded at 15 and 32ºC indicate that developmental extremes for *H. halys* are near 15 and 32ºC. Our survival curve shows that the largest proportion of a BMSB population is made up of adults, with a majority of

nymphs not surviving until the adult life stage.

Pre-ovipositional periods ranged from 7 days at 30ºC to 23 days at 18ºC. These findings are similar to earlier research that showed pre-ovipositional period ranges from 3 days at 30ºC to 22 days at 15ºC (Nielsen et al., 2008). The ovipositional period ranged from 6 days at 32ºC to 113 days at 22ºC. At 25ºC, the ovipositional period was 64 days, whereas earlier research suggested ovipositional periods of 12 days at the same temperatures (Haye et al., 2014). At 25ºC, total eggs oviposited per female was 141.07 eggs, while Nielsen et al. (2008) showed 212.25 eggs.

Pre-ovipositional periods at 27 and 32ºC increased to 14 and 12 days, respectively, from 10 and 7 days at 25 and 30ºC. These results do not follow predicted trends of pre-ovipositional periods decreasing with temperature and may be due to replicates at 27 and 30ºC started in mid-September, while all other temperatures started in late July or early August. Even though lab-reared adults were maintained in environmentally controlled colonies, some of these insects may have innately sensed natural seasonality and started to overwinter, delaying oviposition. This has been observed in late-season collected adults that went into diapause and in lab colonies.

Little or no reproduction was recorded in the present study and by Nielsen et al. (2008) and Haye et al. (2014) at 15 and 35ºC, which may be close to the lower and upper extremes for *H. halys* development. Further research on these developmental boundaries needs to be completed to further explain reproduction under these temperatures. The next step to test physiological limits of reproduction would be to use larger populations of *H. halys* and fluctuating temperatures representing natural conditions. Fluctuating temperatures may dramatically impact both reproduction and survival for *H. halys*, and current DD models do not

estimate the impact of these fluctuations on population levels.

Temperature trials at 15, 18, 22 and 27ºC had unaccounted female deaths at the end of the experimental periods. This was most likely the result of escape during daily checks, as individuals were observed at times to fly out of the cages. Females could have also stayed hidden in plant materials that were exchanged and escaped this way. *Halyomorpha halys* in this experiment were confined in cages and experienced constant environmental conditions which would not be found in nature. These conditions were set to establish and see the impact of temperature on *H. halys* lifetables.

The current study is one of the few studies on adult *H. halys* survival and reproduction under a range of temperatures. This work is intended to provide data to identify temperature ranges favorable for *H. halys* oviposition and survival. Suitable temperatures were found to be between 18 and 32ºC. More work on environmental factors, such as humidity, photoperiod and a larger range of temperatures contributing to population increase and development is needed to establish accurate life tables and to predict risk.

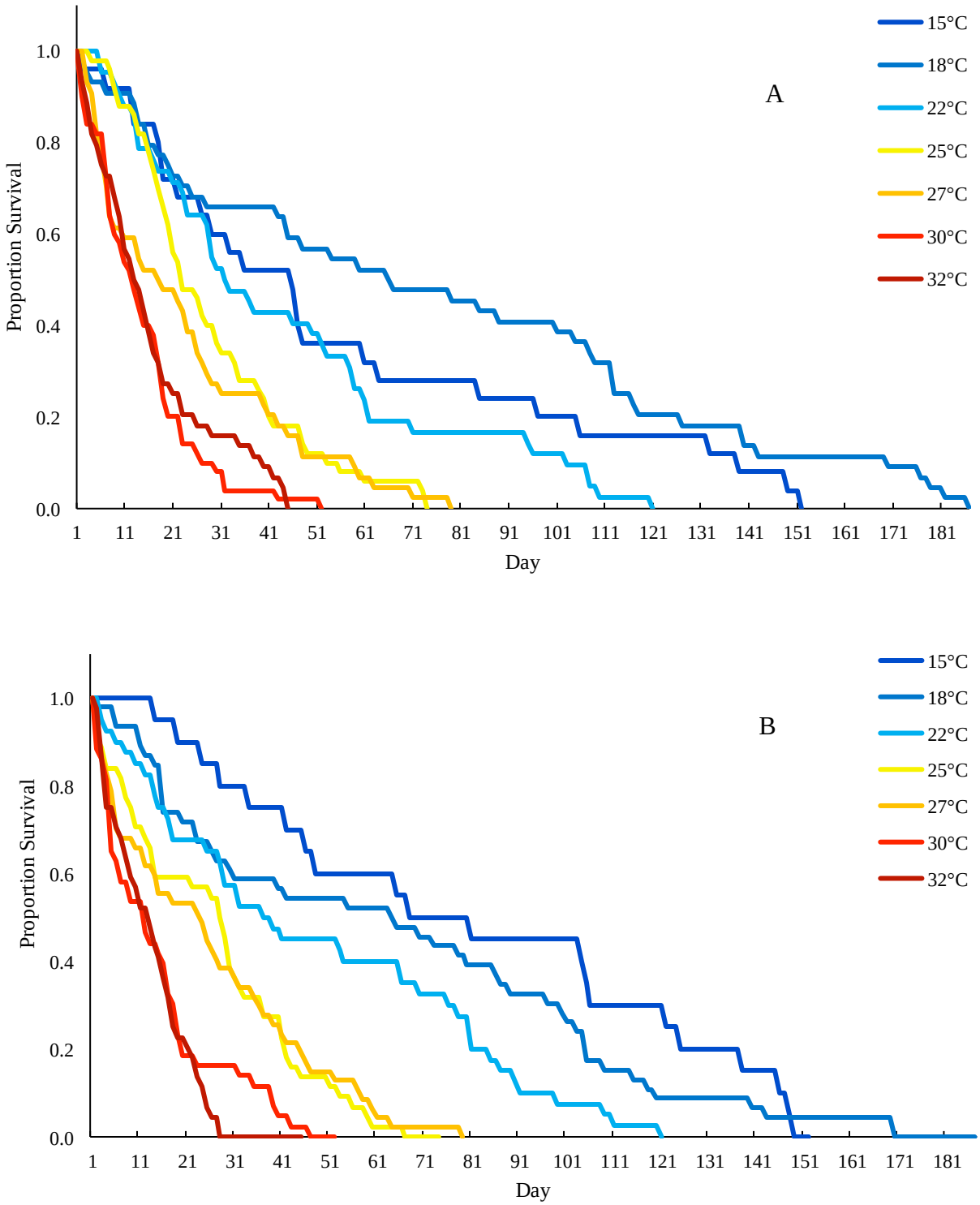

Figure 2.1. Proportion of female (A) and male (B) adult *Halyomorpha halys* survival at seven constant temperatures (ºC).

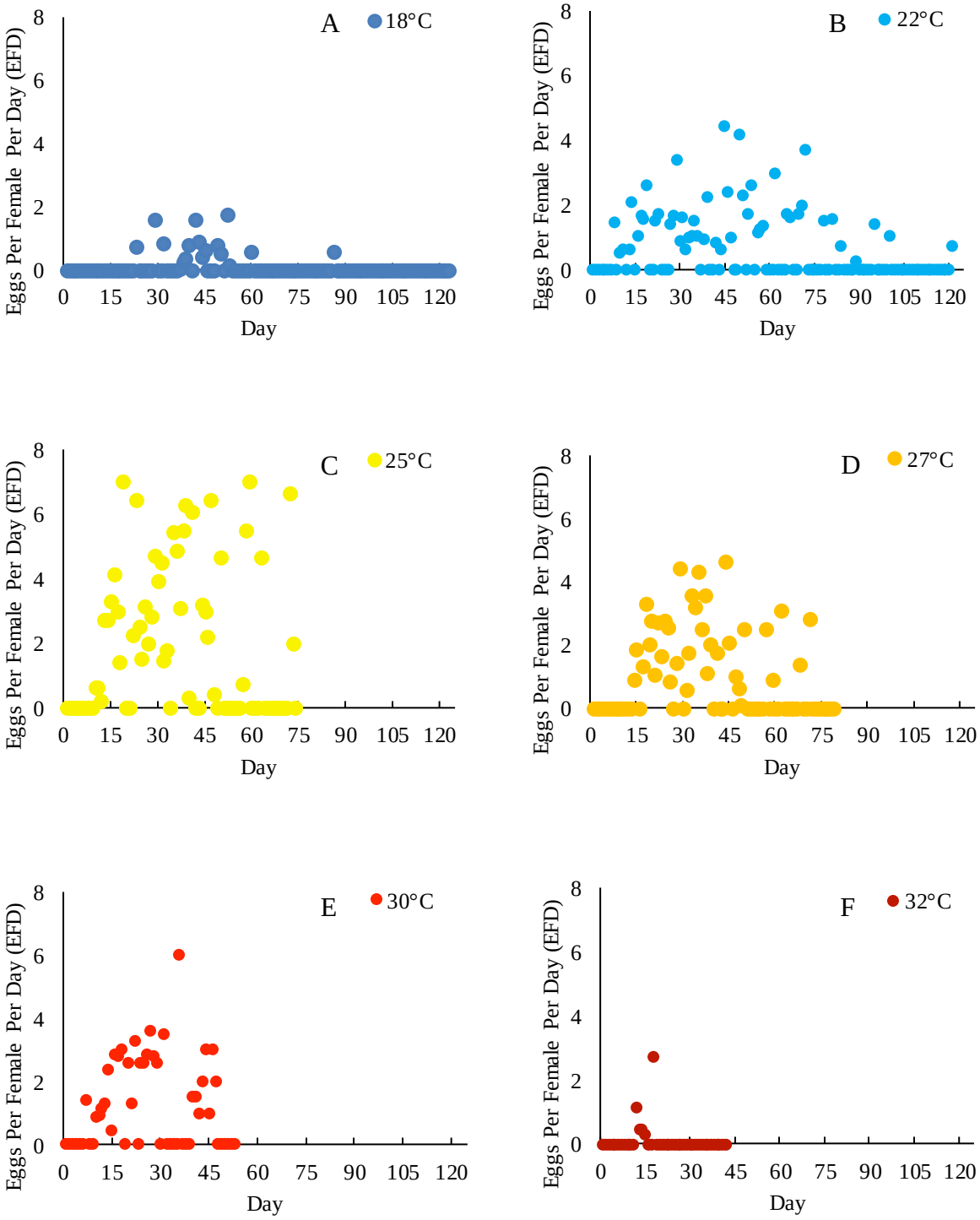

Figure 2.2. Mean eggs per female per day (EFD) of *Halyomorpha halys* at six constant temperatures: 18ºC (A), 22ºC (B), 25ºC (C), 27ºC (D), 30ºC (E) and 32ºC (F).

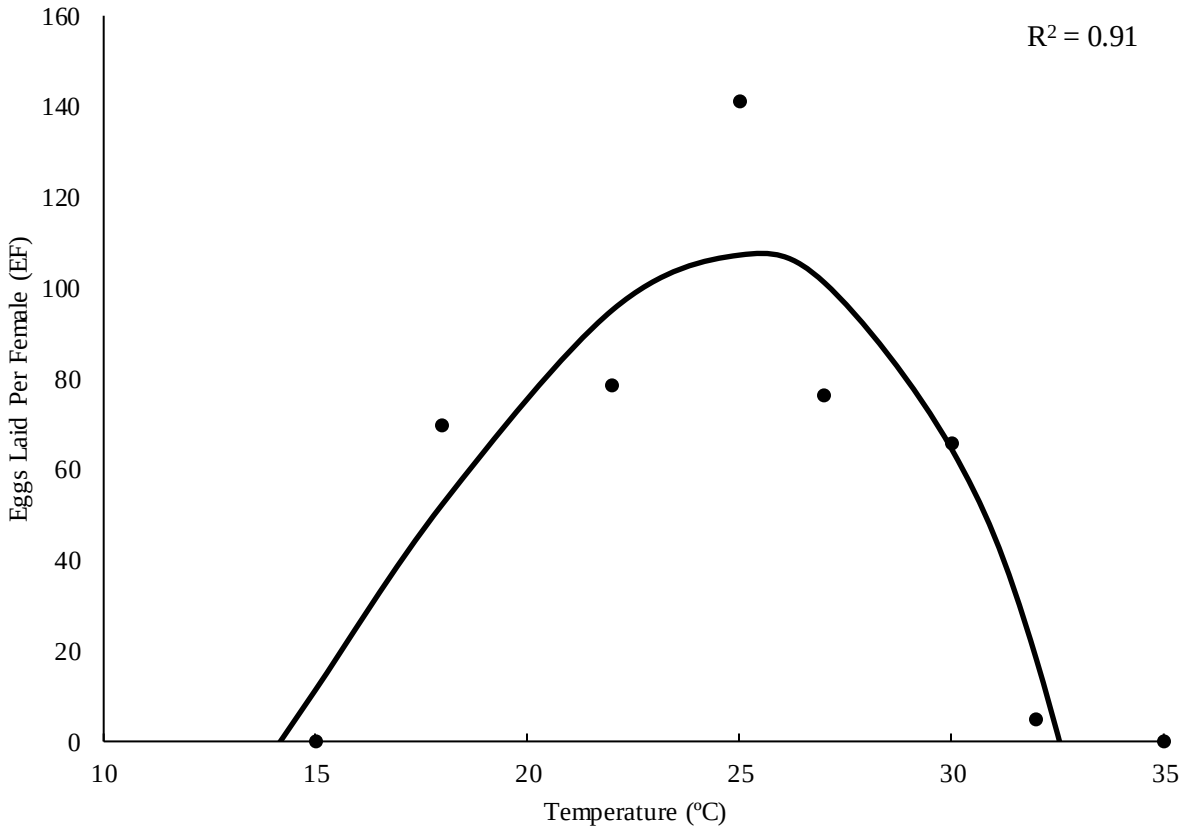

Figure 2.3. Eggs laid per female (EF) of *Halyomorpha halys* as a function of temperature (ºC).

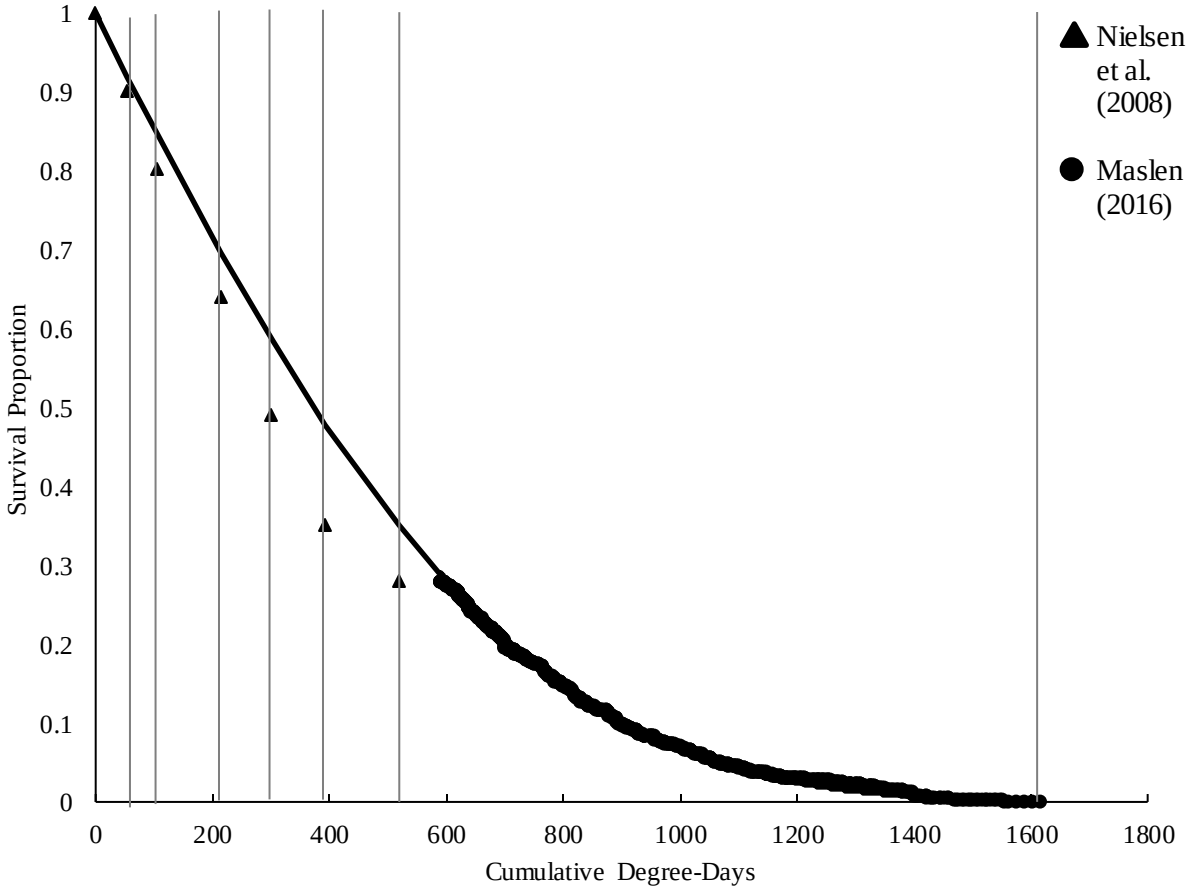

Figure 2.4. Age-specific survival (L_x) of adult *Halyomorpha halys* over physiological time (degree-days). Vertical lines indicate life stages from left to right: first through fifth instar nymphs and adults, until 100% mortality (right line).

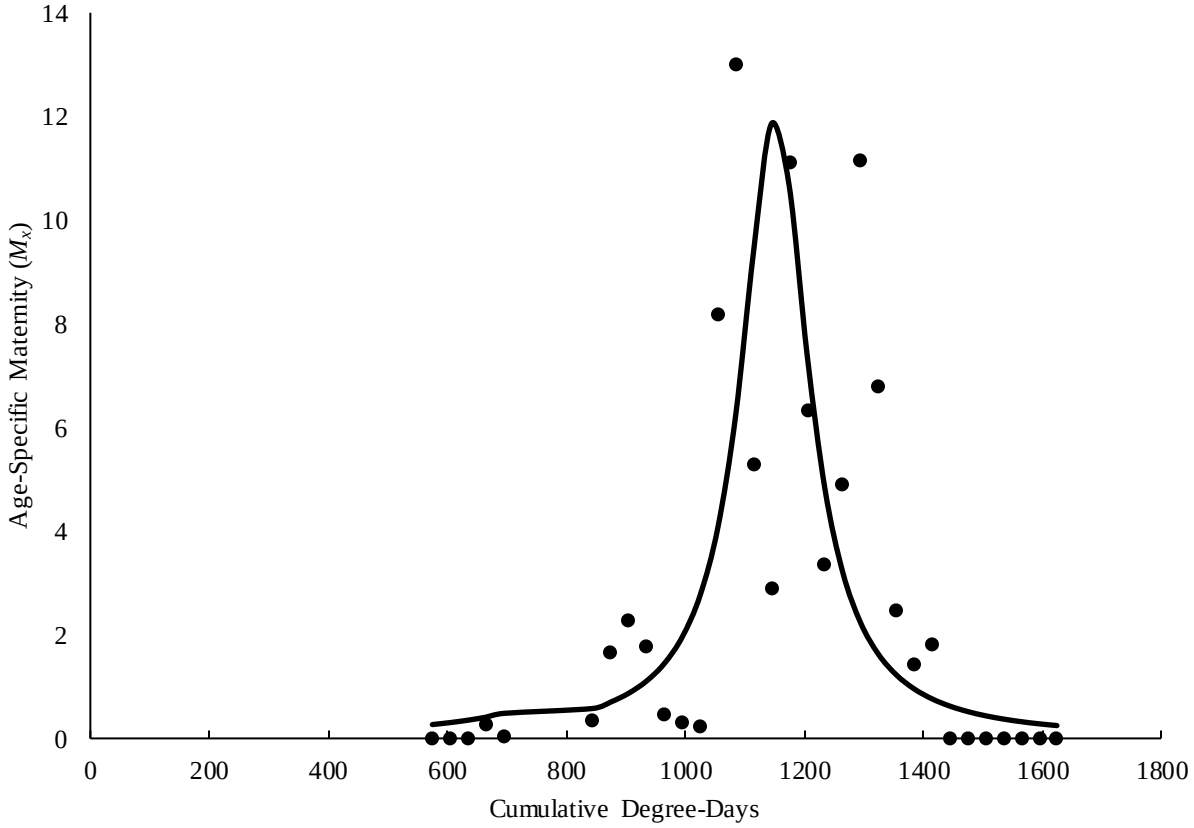

Figure 2.5. Age-specific maternity (M_x) of *Halyomorpha halys* over physiological time (degree-days).

Table 2.1. *Halyomorpha halys* adult female survival and reproductive parameters (±SE) at eleven constant temperatures (ºC) (DD = degree-days; EM = egg mass; OD = ovipositional days; EFD = mean egg laying per female per day; EF = mean eggs per female).

Temperature (ºC)	Egg to Adult (Days)[1]	Adult to Mortality (Days)	Day First EM	Day Last EM	Total OD	EFD	EF	N
15	-	58.8±9.5a	-	-	-	-	-	24
18	-	76.1±8.6a	23	63	40	0.2±0.0c	69.7	44
22	-	44.8±5.1b	8	121	113	1.0±0.1b	78.4	42
25	-	29.3±2.5c	10	74	64	1.4±0.2a	141.1	50
27	-	22.2±3.1c	14	71	57	1.1±0.2ab	76.3	44
30	-	14.7±1.5c	7	46	39	1.4±0.2a	65.7	50
32	-	16.7±1.9c	12	18	6	0.3±0.2c	5.1	44
17[1]	121.5±0.5	-	-	-	-	-	-	100
20[1]	81.2±0.8	-	-	-	-	-	-	100
25[1]	44.9±0.8	47.97	-	-	-	-	212.3[3]	100
25[2]	-	-	-	-	12	-	-	6
27[1]	35.8±0.5	-	-	-	-	-	-	80
30[1]	33.4±0.5	-	-	-	-	-	-	100
33[1]	37.8±0.9	-	-	-	-	-	-	95
35[1]	-	-	-	-	-	-	-	-

[1] Nielsen et al., 2008
[2] Haye et al., 2014
[3] N = 28

Table 2.2. Estimated life table parameters in relation to temperature (°C) of *Halyomorpha halys* (R_0 = net reproductive rate; T = mean generation times in days; and r_m = intrinsic rate of population increase).

Temperature (°C)	R_o	T	r_m
18	0.12	1361	0.000
22	6.01	123	0.014
25	3.98	91	0.015
27	8.25	78	0.027
30	4.20	53	0.027
32	0.33	53	0.000
25[1]	60.02	59.59	0.07

[1] Nielsen et al., 2008

CHAPTER 3

Halyomorpha halys (Hemiptera: Pentatomidae) Feeding Pressure by Temperature and Life Stage Using Wine Grapes as a Model Host

Erika A. Maslen

Introduction

Halyomorpha halys (Stål), brown marmorated stink bug (BMSB, Hemiptera: Pentatomidae) are serious pests of many different agricultural and ornamental crops in the world. An extremely wide range of host plants can be damaged by feeding on susceptible tissues of agronomic crops, vegetables, tree nuts and fruits such as apples (*Malus domestica* Mill), blueberries (*Vaccinium corymbosum* L.), peaches (*Prunus persica* (L.) Batsch), pears (*Pyrus* spp. L.) and wine grapes (*Vitis vinifera* L.) (Lee et al., 2013; USAA, 2010; Wiman et al., 2015a). BMSB has been reported in wine grape vineyards in Maryland, New York, New Jersey, Oregon and Virginia (Ingels, 2014; Loeb, 2012; Pfeiffer, 2011; Smith et al., 2014). Commercially processed wine and wine grapes grown in Oregon and California are not currently economically affected by *H. halys* (Leskey et al., 2012a), but is a nuisance in Oregon wineries. BMSB is a rapidly spreading and reproducing insect that could potentially threaten the wine grape industry. In 2014, Oregon produced 58,000 tons of wine grapes valued at $118 million, while California produced 3.89 million tons, valued at $5.24 billion (CDFA, 2014; ODA, 2015).

Halymoprha halys has been detected or established in counties that produce or surround wine grape vineyards in Oregon and California (CDFA, 2015; Leskey, 2015; NIPMC, 2014). The species is capable of dispersing long distances on its own (Ingels, 2014; Wiman et al., 2014b) and is also spread by tourist activities and agricultural commodities (Poplin et al., 2014). BMSB is bivoltine in western parts of the United States and its multigenerational life history generates considerable risk and potential damage to crops (Wiman et al., 2015).

All life stages of BMSB, excluding eggs and first instar nymphs, are thought to cause berry damage by piercing the fruit skin during feeding (Fiola, 2011a), though the feeding activity and

related damage associated with each life stage is unknown. Feeding on wine grapes and other crops can cause wounds, discoloration, raisening and weight loss on individual berries (Smith et al., 2014; Walton, 2012). Wounds can become infected by *Botrytis* and other rots, causing further damage (Fiola, 2011b). Feeding damage also causes berry abortion and possible economic losses due to reduction in yield (Ingels, 2014; Pfeiffer, 2011).

Halyomorpha halys feeding damage can differ depending on the crop. Damage on apples occurs as depressed and discolored areas with corky flesh. The fruit surface is irregular and may contain puncture sites (Brown, 2003; Leskey et al., 2009). BMSB can feed on corn (*Zea mays mays* L.) and soybean (*Glycine max max* (L.) Merr.) kernels through the husk and pod (Tooker, 2012). Corn ear length and number of kernels per ear decreases significantly when feeding occurs during the late growing season (Cissel et al., 2015a). Kernels and seeds become discolored and sunken in both corn and soybeans (Cissel et al., 2015a & 2015b; Tooker, 2012). Late-season feeding in soybeans can delay plant development or cause seed abortion (Cissel et al., 2015b). Feeding damage in hazelnuts (*Corylus avellana* L.) depends on feeding timing. Blank nuts occur when feeding happens in the early season. Kernels are deformed during mid-season, and late-season feeding causes corky tissues in kernels (Hedstrom et al., 2014).

Halyomorpha halys also can produce defensive secretions that can contaminate and persist in wines. These secretions may result in undesirable flavors in wine if the insects are unintentionally incorporated into fermentations, leaving a taint that is detectable and undesired by consumers (Walton et al., 2013; Wiman et al., 2015). BMSB can become lodged in grape clusters during the harvest period, making removal by hand, chemical sprays or mechanical shaking difficult (Fiola, 2011c). Removal efforts disturb *H. halys*, making them more likely to

release defensive secretions, although the majority of wine taint comes from pressing of grapes and *H. halys* (Wiman et al., 2015). Sensory tests of processed juice showed that very low rates of BMSB pheromone and insect presence is perceivable (Fiola, 2011d; Ingels & Fiola, 2014; Wiman et al., 2015). Trans-2-decenal is excreted by stressed BMSB and has a strong cilantro smell. Consumers can taste its pungent characteristics as soon as it is present (Tomasino et al., 2013).

Halyomorpha halys is a nuisance pest in wine tasting rooms (Ingels & Fiola, 2014; Loeb, 2012; Pfeiffer, 2011). BMSB can take refuge in non-crop habitat along vineyard edges, invading tasting rooms or infesting grapes (Ingels & Fiola, 2014). As ectotherms, *H. halys* rely on an external heat source to fuel physiological functions. Vineyard temperatures can vary depending on location and bordering plants, greatly influencing BMSB feeding activity and damage levels.

The term insect days is the expression of intensity and duration of pest infestation in a crop. The area under the expression curve is the combination of the number of insects and time of infestation (Ruppel, 1983). Cumulative insect days can be used as an index in crop protection and is similar to degree-days (DD), which combines heat units over time. Degree-days establish the rate of insect development at temperatures between their upper and lower limits (Murray, 2008). Both are useful tools that can be used by field scouts to reduce feeding damage.

Research activities are an uncommon pathway for invasive species spread and introduction, but spread can happen when species escape, are discarded, released or spread on scientific equipment (Ruiz & Carlton, 2003). Hazard Analysis and Critical Control Points (HACCP) plans are preventative measures constructed when working with hazards to create a safe end project. They can be used when working with invasive species to reduce the unintentional spread of

nonnative and non-target species. Because this research worked with an invasive insect, creating a HACCP plan prior to the experimental period would have been recommended to reduce the spread of BMSB and other invasive species in the vineyard. A HACCP plan was created after the experiment to determine what measures should have been taken and will be used in subsequent seasons of field research (Appendix).

The goal of this study was to estimate the impact of temperature on feeding activity of the different BMSB life stages. This study will be used to estimate *H. halys* feeding and determine risk by creating a feeding model to use for other crops .

Materials and Methods

Collection and Rearing

Halyomorpha halys stock of all life stages was collected in the Willamette Valley, OR in June and July 2015 using a beat sheet (Model DP1000; BioQuip, Rancho Dominguez, CA). Colonies were reared in 30 x 30 x 30 cm bug dorms (#2840R; BugDorm, Taichung, Taiwan) at 22 ± 1ºC and a photoperiod of 16:8 light:dark (L:D). Each cage contained a 473-mL plastic container (460938; Arrow Plastic Stor, Elk Grove, IL) filled with water and a modified mesh-topped lid to provide a water source and humidity. A diet of organic Spanish peanut (*Arachis hypogaea hypogaea* L.), soybean, pumpkin seed (*Cucurbita* spp.) and sunflower (*Helianthus annuus* L.) was provided in an open petri dish on the cage floor. Jalapeño pepper (*Capsicum annuum* L.), empress tree (*Paulownia tomentosa* (Thunb.) Steud) and green bean (*Phaseolus vulgaris* L.) plants grown in an Oregon State University (OSU) greenhouse and English ivy (*Ilex aquifolium* L.) branch clippings with berries from Corvallis, OR were provided for additional nutrition, habitat and egg-laying substrate. Plants were grown in 800-mL plastic pots filled with

Professional Growing Mix Custom Blend soil (4v; Pro Grow, Portland, OR) and were approximately 0.5 meters tall.

Field Trials

Field trials were conducted at the OSU Botany and Plant Pathology Farm in Corvallis, OR (44º 33'56.952" N, 123º 14'28.4748" W) on Pinot noir grapes. The grape clusters were thinned before trial commencement, leaving approximately 30 clusters per block (1.8 x 10.4 x 1.2 m). Mesh, 48.0 x 39.5 cm feeding inclusion sleeves (60597; Premier Paint Roller, Richmond Hill, NY) were placed over grape clusters for four weeks at the start of post-véraison, from August 21 to September 21, 2015.

Within each block, four life stage treatments with 10 replicates were randomly applied to individual clusters: 1) a partial egg mass of ten hatching *H. halys* eggs (eggs), 2) two second-instar and one third-instar *H. halys* nymphs (nymphs), 3) one female and two male *H. halys* adults (adults), and 4) no *H. halys* (controls). The backsides of the partial egg masses were moistened with water and attached to a strip of paper that was wedged in between the grapes in the cluster and secured with adhesive tape, allowing nymphs to emerge onto the grape clusters. Treatments were divided into two temperature regimes per life stage. These two temperature regimes were a shady east-facing (shady) and a sunny west-facing (sunny) regime. Forty sleeves (ten per life stage treatment) were placed in each of the two temperature regimes (80 total). Sleeves in the shady regimes had a paper cover placed at the top of the cluster to provide additional shade.

Two temperature and light recording HOBO data loggers (UA-002-08; Onset, Bourne, MA) were placed in each of the five vineyard blocks in the control life stage treatments, one in

the shady and one in the sunny regime (10 total). Supplemental water was provided using 163-ml plastic cups (P550N; Solo, Urbana, IL), with a hole in the top of the lid (PL4N; Solo, Urbana, IL) to hold a piece of sponge. These containers were placed in sleeves containing egg and nymph treatments.

Data were collected each Monday and Friday to determine mortality. Dead insects were replaced with *H. halys* of the same life stage from the lab-reared colonies. Weekly °Brix measurements were taken using a refractometer (HR-032; AFAB Enterprises, Eustis, FL) from a random grape cluster from each of the five vineyard blocks during the experimental period (four measurements per block). Average °Brix for each grape cluster was calculated as the average °Brix of five berries collected from the top, middle and lower portions of each cluster.

All contents of the sleeves were removed from the vineyard for lab analysis of the fruit quality at the end of the four-week experimental period. A random sample was taken for °Brix from each block, as described above. Feeding activity was documented by counting the number of stylet sheaths per berry under a microscope. The average berry weight was measured by weighing the cluster and dividing by the number of berries for each cluster. The average berry diameter was measured by averaging the diameter of the above six randomly selected berries. Cluster °Brix was measured by using the mixed juices of the six randomly sampled berries.

A BMSB feeding model was created based on BMSB life stage and temperature (Ruppel, 1983). For life stages, a factor of 1 was attributed to controls, 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 in each life stage treatment by the number of stylet sheaths in the control treatments (0.009). The effect of temperature was determined by estimating the number of DD for each temperature

regime. The DD were estimated using the lower and upper thresholds of 14 and 34°C (Nielsen et al., 2008), respectively. From these values, the accumulated DD in shady regimes was 198 and 281 in sunny regimes. Based on the relative number of DD in each regime, a factor of 1 was attributed to shady regimes and a factor of 1.4 was attributed to sunny regimes. The life stage factor was multiplied by the temperature factor to create a feeding index value (Table 3.3).

A protocol was needed to reduce spread of non-target species during set-up and weekly checks after observing BMSB escaping during the experimental period. A HACCP plan (Appendix) was constructed, setting critical control points and monitoring requirements to reduce threats. Action points were established to control threats when they happened.

Statistical Analysis

The impact of temperature on insect feeding and life stage was analyzed using ANOVA with temperature as the independent factor and feeding factor as the dependent factor. Differences in the means were separated using Tukey's HSD. All analyses were conducted using Statistica (Statsoft 7.1; Tulsa, OK). The feeding model was estimated by fitting multiple regression using values obtained at each experimental temperature. The mortality rates over the four-week period of the feeding trial were calculated to determine whether temperature regimes effected insect mortality during the experimental period.

Results

There were significantly higher temperatures recorded in sunny regimes (18.7°C) than shady regimes (17.7°C) ($F_{1, 4074} = 45.1$, $p < 0.001$; Fig. 3.1). Mean temperatures ranged from 12.3 to 23.8°C during the experimental period. Temperature differences between sunny and

shady regimes were as wide as 4.7ºC on days with full sun exposure, to virtually none on cloudy days.

The mean berry weight was 1.3 ± 0.03 and 1.1 ± 0.03 grams, while mean cluster weight was 90.0 ± 4.9 and 56.7 ± 4.9 grams for the shady and sunny regimes, respectively. Mean berry diameter was 12.1 ± 0.1 and 11.6 ± 0.1 mm for the shady and sunny regimes, respectively. Mean number of berries per cluster was 72.4 ± 3.9 and 50.2 ± 3.9 berries for the shady and sunny regimes, respectively. Berry and cluster weight, berry diameter and number of berries per cluster were significantly different between the sunny and shady regimes ($p = 0.001$; $p < 0.001$; $p = 0.002$; $p < 0.001$; Table 3.1). The mean number of stylet sheaths per berry was 9.8 stylet sheaths. The mean °Brix was 21.8°. There were no significant differences between regimes in mean number of stylet sheaths and mean °Brix ($p = 1.61$; $p = 0.280$). There were no significant differences in cumulative mortality rates between temperature regimes (Fig. 3.2).

The mean berry weight was 1.15 grams for the life stage treatments, with a mean cluster weight of 73.38 grams and a mean berry diameter of 11.85 grams. Mean berries per cluster was 61.25 berries. Berry and cluster weight, berry diameter and berries per cluster were not significantly different between the life stage treatments ($p = 0.999$; $p = 0.273$; $p = 0.999$; $p = 0.429$). The mean number of stylet sheaths per berry differed depending on the life stage treatment and temperature regime. There were significantly higher numbers of stylet sheaths per berry in adult treatments compared to all other life stage treatments ($F_{9, 170.51} = 6.9$, $p < 0.001$; Table 3.1).

There were significantly higher numbers of stylet sheaths per berry in shady (0.335 ± 0.092) and sunny adult treatments (1.226 ± 0.282) than all other combined temperature regimes

and life stage treatments ($p < 0.001$; Fig. 3.3 and Table 3.2). There were significantly higher numbers of stylet sheaths per berry in sunny adult than shady adult treatments ($F_{9, 170.51} = 3.7$, $p < 0.001$; Fig. 3.3 and Table 3.2).

For the BMSB feeding model, 0.005 stylet sheaths with a feeding factor of 0 was estimated. For 0.04 stylet sheaths, the feeding factor was 1 (sunny regime) and increased to 1.4 (shady regime) for 0.07 stylet sheaths. At 0.3 stylet sheaths, the feeding was 49 and increased to 68.6 for 0.9 stylet sheaths. The multiple regression of stylet sheaths on the feeding index value resulted in a significant fit using the function $y = 0.013377x-0.01975$ ($R^2 = 0.92$; $F = 94.73$; df = 1, 7; $p < 0.001$; Fig. 3.4).

Discussion

Halyomorpha halys was not given the choice of food source and feed on wine grapes in the experiment. *H. halys* may not prefer wine grapes as a host plant, but this study attempted to provided data on relative feeding activity based on temperature and life stage. All life stage and temperature treatments showed some evidence of feeding damage, suggesting that wine grapes are susceptible to economic losses if *H. halys* is present and feeding during the season.

The least damaging life stages were first instar nymphs because first instars primarily feed on the egg mass instead of the host plant (Rice et al., 2014). Although the mean accumulation of stylet sheaths was greater in sunny regimes, there were stylet sheaths observed in each of the life stage treatments, including the controls. Stylet sheaths may have been deposited by other pentatomids or BMSB before the experiment, but could not have occurred during the experimental period. Although some stylet sheaths were observed on the shady regimes, mean accumulation in all life stage treatments was low.

There were significantly higher levels of stylet sheaths per berry in sunny compared to shady regimes. Adult sunny regimes had significantly higher mean stylet sheath accumulation than the shady regimes. Feeding activity was affected by temperature, increasing damage levels in warmer conditions. Vineyards and rows in warmer localities or microclimates are more likely to experience greater *H. halys* feeding damage than vineyards or rows at lower temperatures. As temperature increases and nears upper developmental thresholds, the rate of development to adult increases, though would decrease at temperatures above the upper threshold (Nielsen et al., 2008). The environmental data measured may not have accurately measured the temperature experienced by *H. halys* in this study. These data provide a relative representation of temperatures likely occurring in the temperature regimes.

The data from the feeding index suggest that both life stage and DD accumulation contribute to feeding intensity and probable fruit feeding damage. These results indicate that greater DDs result in increased feeding activity. Younger life stages resulted in relatively less feeding activity as compared to adults. Adult BMSB cause more damage in wine grapes than all other life stages. BMSB spends the majority of its life as an adult, increasing the amount of damage a crop can accumulate. The potential for feeding damage caused by *H. halys* on wine grapes is high because adults cause the most damage, are the most mobile and have the highest survival rates relative to other life stages (Haye et al., 2014; Wiman et al., 2014b). The BMSB feeding model can be used to estimate feeding activity levels of other crops.

Other factors that may play a role and not investigated in this study include *H. halys* aggregation pheromone. The aggregation pheromone of *H. halys* is more attractive to adults than other life stages and could potentially cause more adults to aggregate in vineyards later in the

season (Harris et al., 2015). Grapes are harvested late in the season and can potentially accumulate more damage from adult BMSB during the harvest period.

This work presents the first BMSB feeding model and the importance of life stage and temperature to feeding activity. This study is intended to use wine grapes as a host model of *H. halys* feeding pressure on other agricultural crops. More work on environmental factors, such as humidity, a longer time range and insect density contributing to feeding pressure is needed to establish more accurate damage predictions.

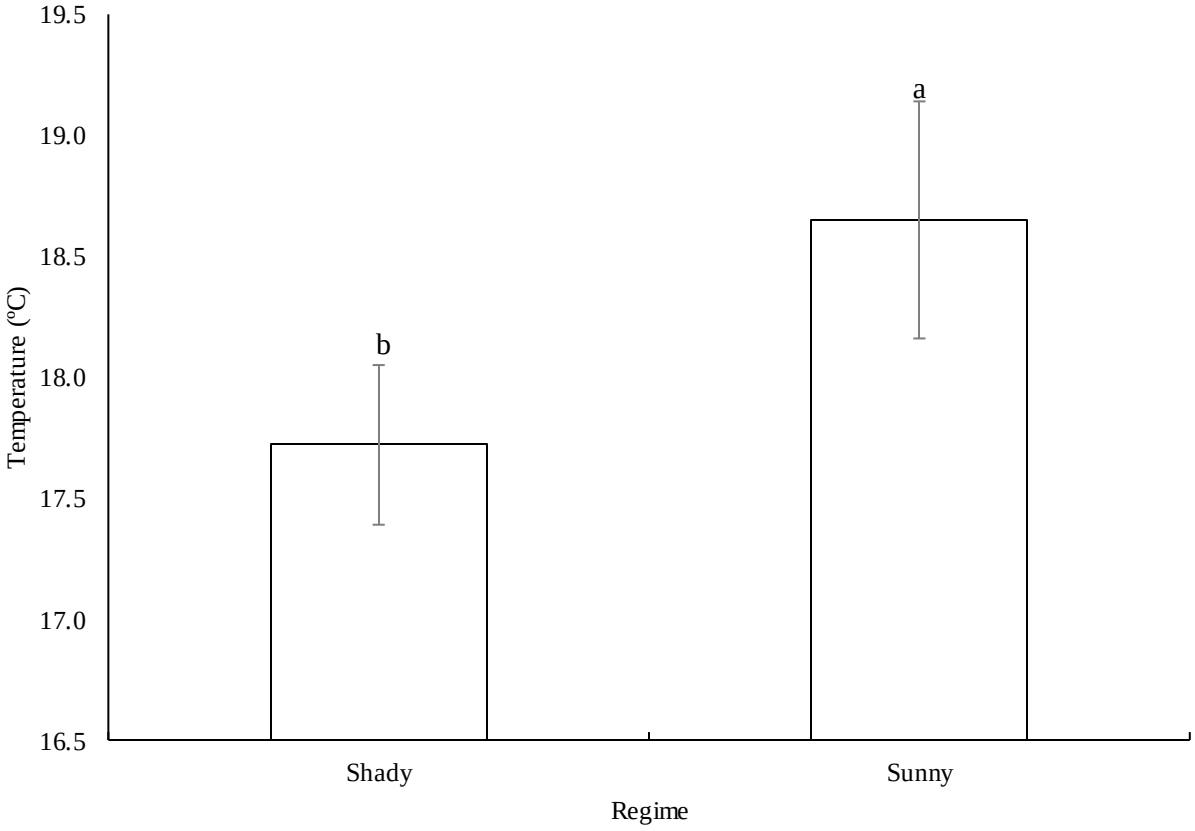

Figure 3.1. Mean temperatures (ºC) of shady and sunny temperature regimes recorded of Pinot noir grapes grown in Corvallis, OR.

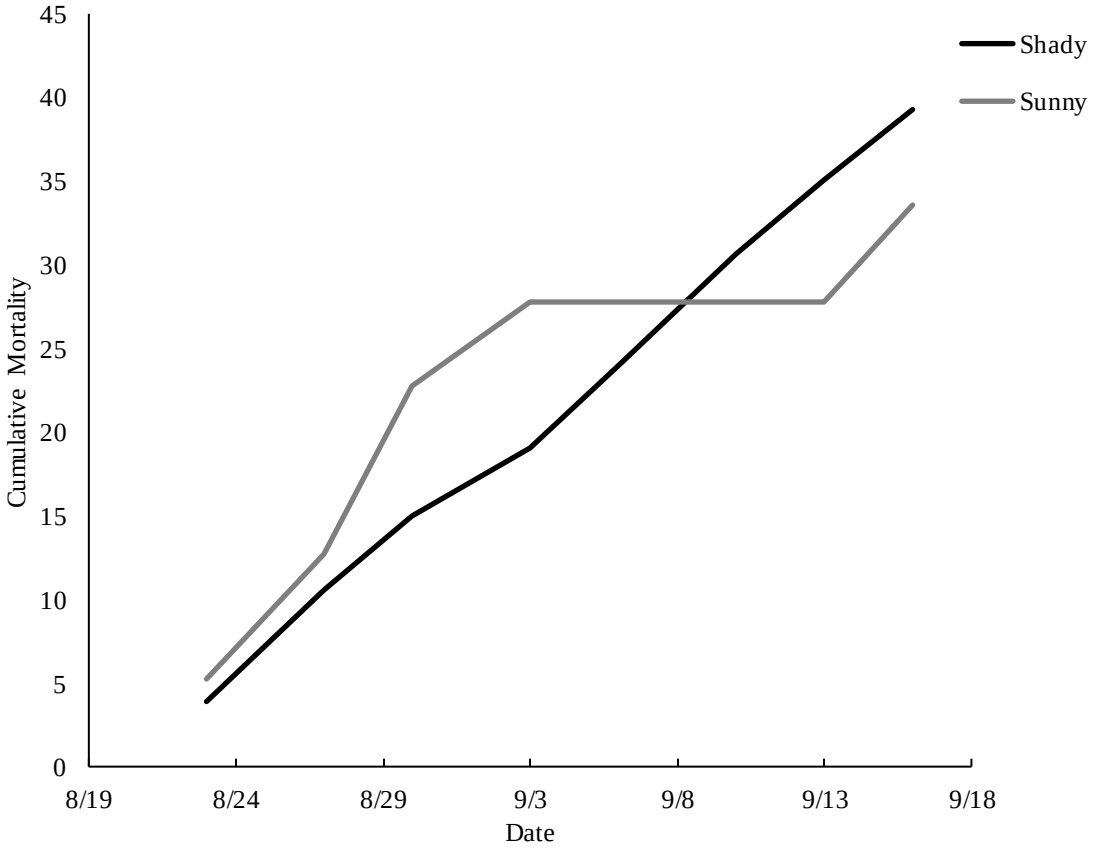

Figure 3.2. Cumulative mortality of *Halyomorpha halys* on Pinot noir wine grapes in shady and sunny temperature regimes.

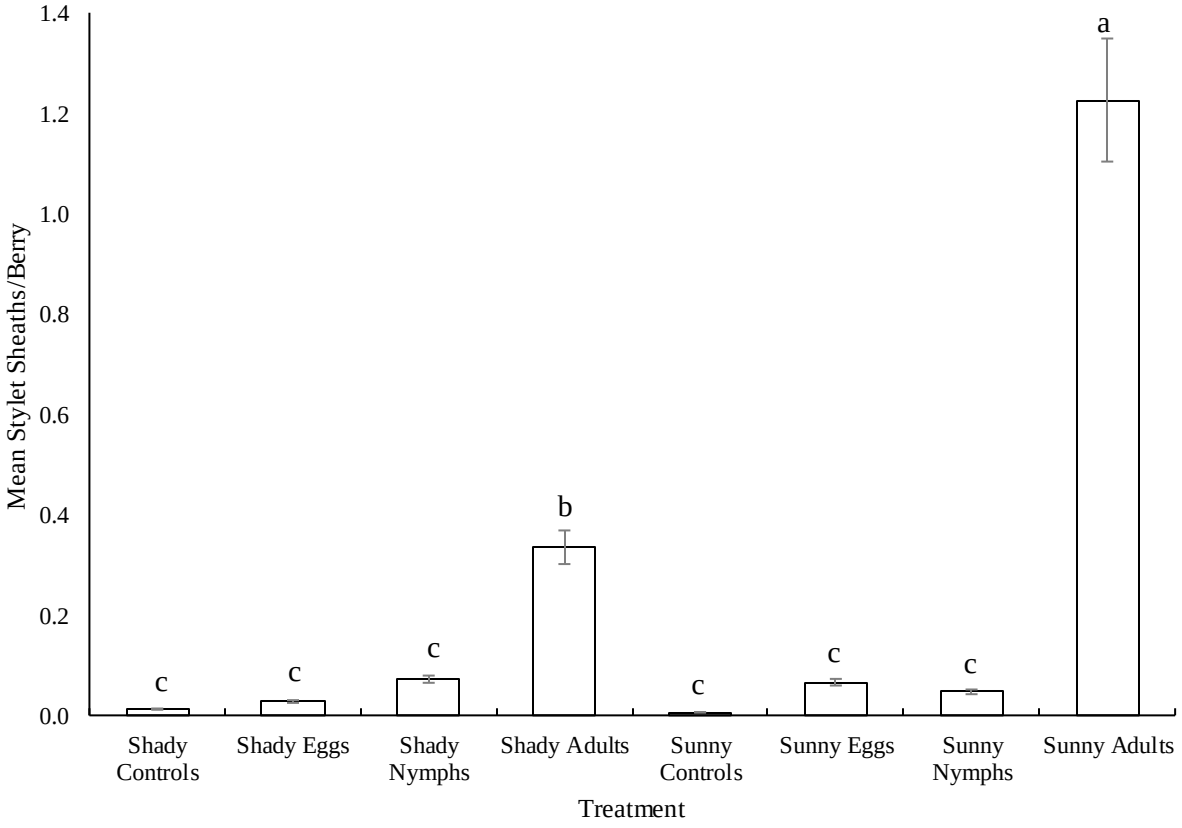

Figure 3.3. Mean number of *Halyomorpha halys* stylet sheaths per berry on temperature regimes and life stage treatment.

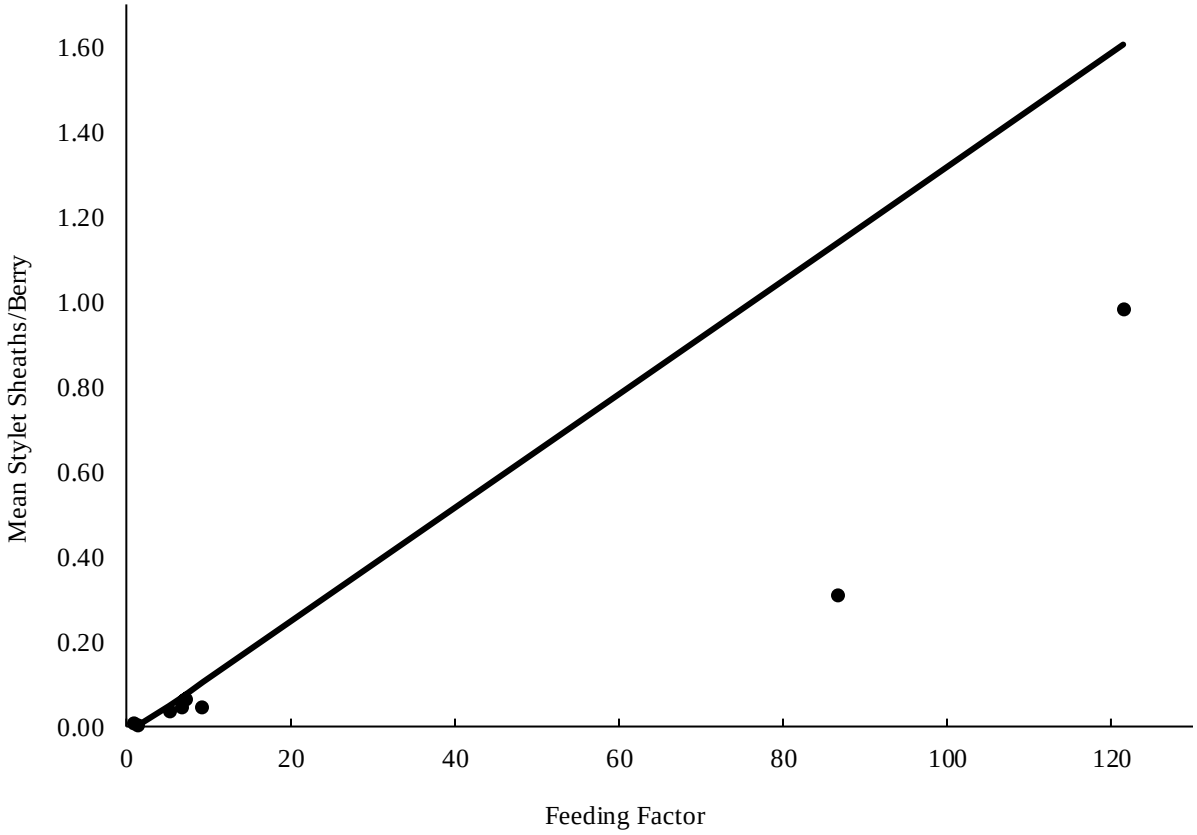

Figure 3.4. Mean number of *Halyomorpha halys* stylet sheaths per berry on the feeding factor from life stage and temperature factors.

Table 3.1. Mean berry characteristics of Pinot noir grapes and *Halyomorpha halys* feeding activity (±SE) for temperature regimes (N = 40) and life stage treatments (N = 20).

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±3.0a	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
Controls	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 3.2. Mean berry characteristics of Pinot noir grapes and *Halyomorpha halys* feeding activity (±SE) for temperature regimes and life stage treatments (N = 10).

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

Table 3.3. BMSB feeding model life stage, temperature and feeding factors of *Halyomorpha halys* feeding activity on Pinot noir.

Life Stage Treatment	Temperature Regime	Life Stage Factor	Temperature Factor	Feeding Factor
Controls	Shady	1	1	1
Controls	Sunny	1	1.4	1.4
Eggs	Shady	5.22	1	5.22
Eggs	Sunny	5.22	1.4	7.31
Nymphs	Shady	6.67	1	6.67
Nymphs	Sunny	6.67	1.4	9.34
Adults	Shady	86.78	1	86.78
Adults	Sunny	86.78	1.4	121.49

CHAPTER 4

General Conclusions

Erika A. Maslen

Halyomorpha halys has grown to become a more serious agricultural concern for more industries in Oregon and California since originally invading the United States in 1996. BMSB can be a potential economic threat to many crops in both native and invaded ranges.

BMSB developmental and reproductive thresholds have not been intensively researched, leaving gaps in the body of knowledge. Adults were exposed to constant temperatures ranging from 15 to 32ºC. Life table analyses help predict reproduction and survival of invasive insects such as *Halyomorpha halys*. Mortality, survival and reproduction were tracked and found to be significantly affected by temperature. Results from the life table analysis indicate that BMSB adult survival and egg-laying temperatures range from 18 to 32ºC. The ideal temperature for *H. halys* reproduction and survival was found to be 27ºC. Future work should continue to develop monitoring and control techniques that will establish thresholds for economically important crops. Other research should focus on the missing temperatures from past life table analyses, integrating different humidity and photoperiod regimes. Development and survival of all life stages of *H. halys* at fluctuating temperatures could further refine prediction models and risk assessments in different geographic regions.

Data on BMSB crop damage due to temperature and life stages are also lacking. Wine grapes were exposed to all life stages of BMSB during the growing season in sunny and shady temperature regimes. Factors of mortality, temperature, berry dimensions and feeding activity were determined. Temperature and life stage significantly affected feeding activity, with berries in sunny regimes that were exposed to adults experiencing the most feeding activity. At higher degree-day accumulation and later life stages, feeding activity increased. Only one wine grape variety was tested, but this study determined the relative role of life stage and environmental

conditions on feeding activity and can be used as a model for other host plants. There are no reasons that suggest that other cultivars, vineyards or crops in other regions would be less susceptible to feeding damage *by H. halys* compared to the Pinot noir used in this study.

Results from the research presented here, along with evidence from previous and ongoing studies, confirm that *H. halys* is a potential pest to wine grapes and other crops and will continue to disperse and reproduce through the United States. Many regions where *H. halys* is not currently detected have ideal environmental conditions for survival, including the Pacific Northwest (Wiman et al., 2012). Although knowledge of feeding damage in vineyards is limited, and wine grapes are not believed to be a current preferred host (Ingels, 2015; Fiola, 2011d; Pfeiffer, 2011), these data can be used as a benchmark of comparison to other cropping systems. BMSB feeding pressure is higher during warmer temperatures and older life stages. The constructed models show the biggest proportion of a BMSB population are adults and cause the most feeding damage of the life stages. This research is one of the first steps involved in developing management and prevention strategies for BMSB in agricultural crops.

Halyomorpha halys is a serious potential threat to the wine industries of Oregon and California, along with other agricultural commodities in the Pacific Northwest. Life table analyses and feeding indices can be incorporated into projection models to estimate population growth and damage levels under different temperature ranges.

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Appendix

Hazard Analysis and Critical Control Points Plan
HACCP Step 1 – Activity Description

Management Objective & Contact Information	
HACCP Plan Title: Brown Marmorated Stink Bug (BMSB) Spread Prevention Plan	
Management Objective: BMSB Wine Grape Damage Trials	Contact Person: Erika Maslen
	Phone: 541-490-2761
	Email: maslene@oregonstate.edu

Activity Description i.e. Who; What; Where; When; How; Why
Who: BMSB Graduate Student (Erika Maslen) What: Placement and data collection of insects and grape damage Where: Oregon State University Botany and Plant Pathology Test Site When: August to September 2015 How: Gather required insects for the day, place in bags on grapes and collect previous data Why: Determine BMSB wine grape damage and collect data for the California Department of Food and Agriculture

HACCP Step 2 – Activity Flow Chart
Outline Sequential Tasks of Activity

Task 1	Title: Gather insects and travel to site
	Description: Count out required insects for day into cages, load insects, travel to site

Task 2	Title: Unload gear and set up tools
	Description: Take insects and tools out of car; set up data sheets, tools, and insects to prepare for placement

Task 3	Title: Place insects
	Description: Place required insects for day in bags on grapes

Task 4	Title: Data collection
	Description: Enter mortality data from previous days, collect sample grapes

Task 5	Title: Load car and return to lab
	Description: Place tools and insect in car and travel back to lab

Task 6	Title: Analyze grapes in lab
	Description: Search and collect grape data about damage and characteristics, discard recorded contents in garbage

HACCP Step 3 – Identify Potential Non-Targets

Non-Targets That May Potentially Be Moved/Introduced
Vertebrates: None
Invertebrates: Earwigs (*Forficula auricular*) Spiders (Araneae) Brown marmorated stink bug (*Halyomorpha halys*)
Plants: False brome (*Brachypodium sylvaticum*)
Other Organisms (pathogens, parasites etc.): Powdery mildew (*Erysiphe nectator*) Anthracnose (*Elsinore ampelina*) Downy mildew (*Plasmopara viticola*)

HACCP Step 4 – Non-Target Analysis Worksheet

1 **Tasks** From Step 2	2 **Potential Non-targets** From Step 3	3 **Risk Assessment** Are any non-targets significant? Yes or No	4 **Justification** Justify your answer in Column 3	5 **Control** What control measures can be applied during this task to reduce the risk of non-targets?	6 **CCP?** Is this task a CCP? Yes or No	7 **Justification** Justify your answer in Column 6
Task # 1 Title: Gather insects and travel to site	Vertebrates None	No	No organisms	N/A	No	No targets
	Invertebrates Earwigs Spiders BMSB	No	Clean from previous days	N/A	No	No targets
	Plants False brome	No	Clean from previous days	N/A	No	No targets
	Others Powdery mildew Anthracnose Downy mildew	No	Clean from previous days	N/A	No	No targets

HACCP Step 4 – Non-Target Analysis Worksheet (Continued)

Task # 2 Title: Unload gear and set up tools	Vertebrates None	No	No organisms	N/A	No	No targets
	Invertebrates Earwigs Spiders BMSB	No	Clean from previous days	N/A	No	No targets
	Plants False brome	No	Clean from previous days	N/A	No	No targets
	Others Powdery mildew Anthracnose Downy mildew	No	Clean from previous days	N/A	No	No targets
Task # 3 Title: Place insects	Vertebrates None	No	No organisms	N/A	No	No targets
	Invertebrates Earwigs Spiders BMSB	Yes	BMSB can escape	Place bugs in cool area and place individually	Yes	Can introduce BMSB to site
	Plants False brome	No	Clean from previous days	N/A	No	No targets
	Others Powdery mildew Anthracnose Downy mildew	No	Removed insects not in contact with other plants	N/A	No	No targets

HACCP Step 4 – Non-Target Analysis Worksheet (Continued)

Task # 4 Title: Data collection	Vertebrates None	No	No organisms	N/A	No	No targets
	Invertebrates Earwigs Spiders BMSB	No	Clean from previous days	N/A	No	No targets
	Plants False brome	No	Clean from previous days	N/A	No	No targets
	Others Powdery mildew Anthracnose Downy mildew	No	Clean from previous days	N/A	No	No targets

Task # 5 Title: Data collection	Vertebrates None	No	No organisms	N/A	No	No targets
	Invertebrates Earwigs Spiders BMSB	Yes	High risk that invertebrates could be on grapes	Remove all invertebrates and bag grapes	Yes	BMSB could spread
	Plants False brome	Yes	High risk that seeds could be on clothing	Remove all seeds	Yes	Plants could spread
	Others Powdery mildew Anthracnose Downy mildew	Yes	High risk that others could be on tools, hands and grapes	Wipe off all tools and hands with alcohol solution, bag sample grapes	Yes	Others spread next day from tools or to new areas from hands

HACCP Step 4 – Non-Target Analysis Worksheet (Continued)

Task # 6 Title: Analyze grapes in lab	Vertebrates None	No	No organisms	N/A	No	No targets
	Invertebrates Earwigs Spiders BMSB	Yes	High risk that invertebrates could be on collected grapes	Discard or kill all invertebrates	Yes	BMSB could spread from discarded grapes
	Plants False brome	No	Clean from previous days	N/A	No	No targets
	Others Powdery mildew Anthracnose Downy mildew	Yes	High risk that others could be on tools, hands and grapes	Wipe off all tools and hands with alcohol solution, autoclave	Yes	Others could spread

HACCP Step 5 – Non-Target Risk Action Plan (NTRAP)

<table>
<tr><td colspan="5">(Use this form for any "Yes" from Column 6 of HACCP Step 4 - Non-Target Analysis Worksheet)
One page for each Critical Control Point</td></tr>
<tr><td colspan="2">Mangement Objective From Step 1</td><td colspan="3">BMSB Wine Grape Damage Trials</td></tr>
<tr><td>Critical Control Point: Task #</td><td>3</td><td>Title:</td><td colspan="2">Place Insects</td></tr>
<tr><td colspan="2">Significant Non-Target(s) (Step 4, Column 3)</td><td colspan="3">Invertebrates</td></tr>
<tr><td colspan="2">Control Measure(s) (Step 4, Column 5)</td><td colspan="3">Place insects in cold room before travelling to site, place insects in mesh bag or cage individually</td></tr>
<tr><td colspan="2">Precribed ranges, limits, or citeria for control measure(s): (PRLC)</td><td colspan="3">Place insects in 5ºC cold room for 1 hour before travelling to site, transport in cooler to site to keep cool and reduce activity, place and remove insects one at a time to reduce escape</td></tr>
<tr><td rowspan="4">Monitoring the Control Measure(s)</td><td>Who?</td><td colspan="3">Graduate Student</td></tr>
<tr><td>How?</td><td colspan="3">Check cold room time with timer</td></tr>
<tr><td>Where?</td><td colspan="3">At field site and in lab</td></tr>
<tr><td>How often?</td><td colspan="3">Everytime insects are placed</td></tr>
<tr><td colspan="2">Corrective Action(s) if Control Measures Fail (or PRLC cannot be met)</td><td colspan="3">Search for to catch or kill escaped insects</td></tr>
<tr><td colspan="5">Supporting Documents
(For example, Management Plan, Checklist, Decontamination Techniques, SOPs, Scientific Journal Articles, etc.)</td></tr>
<tr><td colspan="2">Development Team Members</td><td colspan="3">Erika Maslen</td></tr>
<tr><td>Date Developed:</td><td>11/11/15</td><td colspan="2">Date(s) Reviewed:</td><td>5/5/16</td></tr>
</table>

HACCP Step 5 – Non-Target Risk Action Plan (NTRAP) (Continued)

(Use this form for any "Yes" from Column 6 of HACCP Step 4 - Non-Target Analysis Worksheet) One page for each Critical Control Point			
Mangement Objective From Step 1		BMSB Wine Grape Damage Trials	
Critical Control Point: Task #	**5**	**Title:**	Load car and return to lab
Significant Non-Target(s) (Step 4, Column 3)		Invertebrates, Plants and Others	
Control Measure(s) (Step 4, Column 5)		Wipe off all tools and hands with alcohol solution, bag sample grapes, remove all seeds, remove all invertebrates and bag grapes	
Precribed ranges, limits, or citeria for control measure(s): (PRLC)		Disinfect tools and hands with 80 to 95% ethanol, tools must sit for at least 1 minute, bag sample grapes in ziplock bags and place sealed bags in cooler, remove seeds from clothing, remove invertebrates from grapes and bag	
Monitoring the Control Measure(s)	**Who?**	Graduate Student	
	How?	Check immersion time with timer and recheck seals, visual checks	
	Where?	At field site	
	How often?	Everytime tools and grapes are packed back into car at end of day	
Corrective Action(s) if Control Measures Fail (or PRLC cannot be met)		Use higher percent ethanol and repeat disinfection, recheck for seeds during immersion, recheck seals and for invertebrates by shaking bags upside down, visual car inspection	
Supporting Documents (*For example, Management Plan, Checklist, Decontamination Techniques, SOPs, Scientific Journal Articles, etc.*)			
Development Team Members		Erika Maslen	
Date Developed:	11/11/15	**Date(s) Reviewed:**	5/5/16

HACCP Step 5 – Non-Target Risk Action Plan (NTRAP) (Continued)

(Use this form for any "Yes" from Column 6 of HACCP Step 4 – Non-Target Analysis Worksheet) One page for each Critical Control Point			
Mangement Objective From Step 1		BMSB Wine Grape Damage Trials	
Critical Control Point: Task #	**6**	**Title:**	Analyze grapes in lab
Significant Non-Target(s) (Step 4, Column 3)		Invertebrates and Others	
Control Measure(s) (Step 4, Column 5)		Wipe off all tools and hands with alcohol solution, remove and kill invertebrates, bag sample grapes to discard	
Precribed ranges, limits, or citeria for control measure(s): (PRLC)		Disinfect tools and hands with 80 to 95% ethanol, tools must sit for at least 1 minute, remove seeds from clothing, remove invertebrates from grapes, bag sample grapes in ziplock bags before discarding	
Monitoring the Control Measure(s)	**Who?**	Graduate Student	
	How?	Check immersion time with timer and recheck seals, visual checks	
	Where?	In lab	
	How often?	Everytime grapes are discarded and at end of day	
Corrective Action(s) if Control Measures Fail (or PRLC cannot be met)		Use higher percent ethanol and repeat disinfection, recheck seals and for invertebrates by shaking bags upside down	
Supporting Documents (***For example, Management Plan, Checklist, Decontamination Techniques, SOPs, Scientific Journal Articles, etc.***)			
Development Team Members		Erika Maslen	
Date Developed:	11/11/15	**Date(s) Reviewed:**	5/5/16

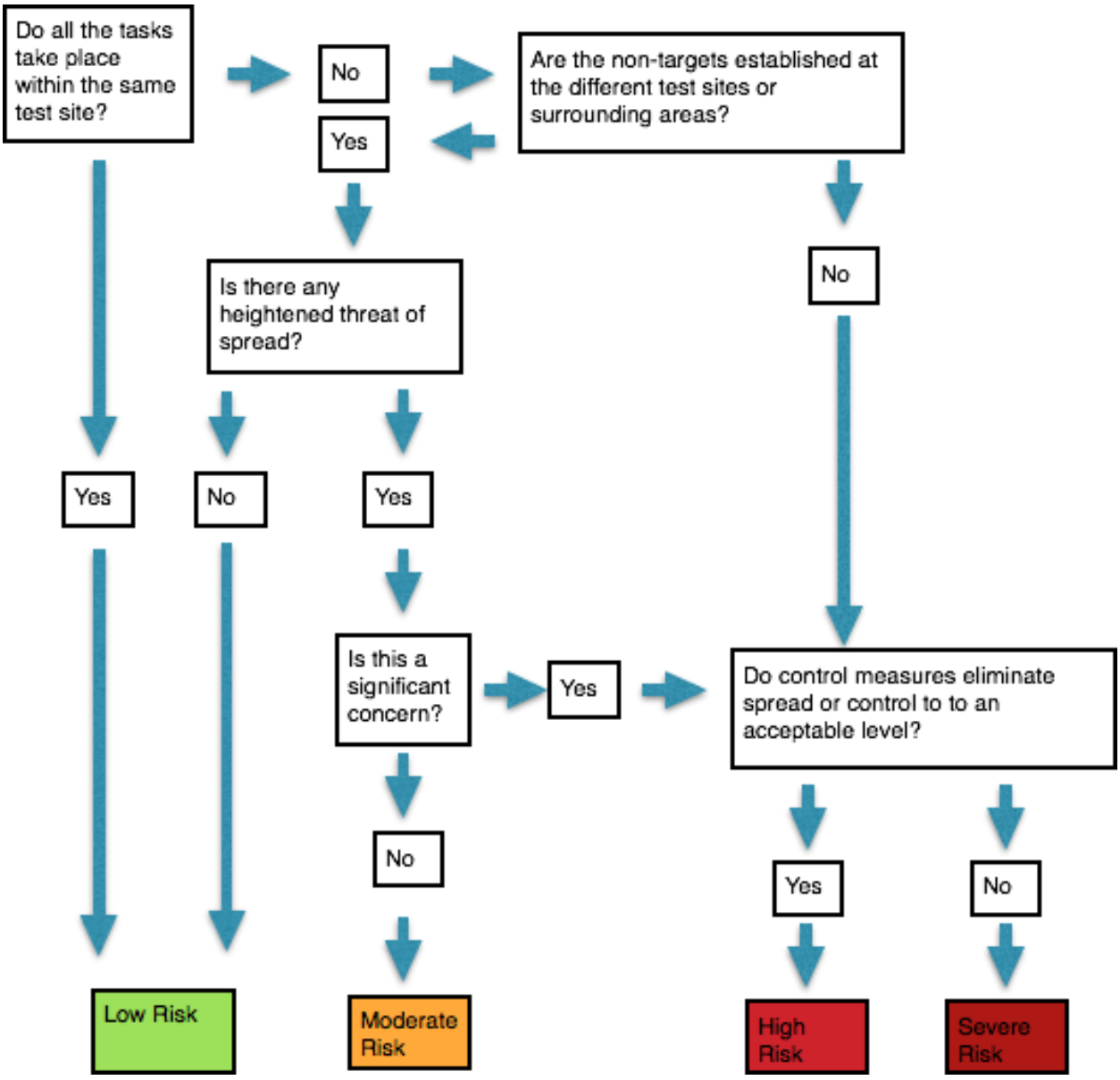

Figure A.1. Nontarget species risk diagram.