Improving Stink Bug Management in The Midwest: Understanding Dispersal Capacity and Developing Sampling Plans

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Dedication

To my mom, Marta
Thank you for teaching me the values of life and to dream big. Your unconditional love and support guide me through the challenges of life.

To my dad, Celso
Thank you for encouraging me to pursue my dreams, and to teach me that knowledge is something that nobody can ever take it from you.

Abstract

Stink bugs are an emerging threat for horticultural and field crops in the North Central Region of the United States. This threat can be attributed to the increase in abundance of Pentatomidae species native to North America over the years, and to the spread of invasive stink bugs throughout this region, in particular, the brown marmorated stink bug, (*Halyomorpha halys*). There is a lack of decision-making frameworks that provide more efficient sampling for stink bugs in soybean for this region, and there is a need to understand the dispersal capacities of laboratory-reared *H. halys*. Therefore, my research developed more efficient sampling for herbivorous stink bugs in this region and assessed the dispersal capacity of *H. halys* under laboratory conditions.

First, I developed a binomial or decision-making sequential sampling plan for herbivorous stink bugs by using data from 11 states of the North Central Region. Results showed that using a tally threshold of 3 stink bugs per 25 sweeps for action thresholds corresponding to the current action thresholds of 5 (soybean grown for seeds) and 10 (soybean grown for grain) stink bugs per 25 sweeps, provided the best sampling outcomes in terms of efficiency and accuracy. In addition, we showed that 18 and 12 sample units, for action thresholds of 5 and 10 stink bugs per 25 sweeps respectively, would be needed for reliable decision-making sampling. The development of a binomial sequential sampling plan for stink bugs in soybean should provide increased efficiency with regards to the number of sample units required to reach a management decision.

Secondly, I assessed the effects of starvation, age, mating status, and pre-flight weight on flight parameters of laboratory-reared *H. halys* using flight mills. Although some statistical differences were observed across the experiments, these differences represented minimum ecological significance. These results could reflect the resilience of

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H. halys as a successive invasive species to North America and could help predict the dispersal potential of this species in Minnesota.

The results of my thesis will provide valuable information to fine-tune integrated pest management strategies for stink bugs in the North Central Region, enabling more efficient scouting for growers and consultants as well as better forecasting the dispersal capacity of an important invasive species to the region.

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Introduction

Soybean, *Glycine max* (L.) Merrill, is the most valuable bean in the world, constituting a major source of protein for humans and high-quality animal feed (Wilson 2008, Pagano and Miransari 2016). The United States (U.S.) is the world's leading soybean producer, followed by Brazil and Argentina, combining for a total of 82% of the world's soybean production (Meade et al. 2016, ASA 2018). In 2020, approximately 34 million hectares of soybean were planted in the U.S., with the North Central Region responsible for over 80% of the national soybean acreage (USDA-NASS 2019, USDA-ERS 2020). Stink bugs (Hemiptera: Pentatomidae) are among the most significant pests of soybean (Kogan 1976, McPherson and McPherson 2000). Within the North Central Region of the U.S., the stink bug fauna is relatively diverse, with approximately 45 – 57 species encountered per state (McPherson 1982, Koch et al. 2017). In soybean, approximately 23 herbivorous stink bug species can be found in this region (Panizzi et al. 2000, Koch et al. 2017, Pezzini et al. 2019b).

Stink bugs are generally polyphagous pests that can be best described by their oval (often shield-shaped) bodies, a well-developed triangle-shaped scutellum, and five-segmented antennae (Panizzi et al. 2000, Koch et al. 2017). In temperate regions, stink bugs generally prefer to overwinter as adults in protected areas such as under crop residues in the field and leaf litter in woodlands, or inside man-made structures (Saulich and Musolin 2012, Rice et al. 2014). The overwintering is passed in a physiological state called diapause, which is a necessary state for increasing cold tolerance and successful overwintering where sexual development is suppressed and associated with an increase in body fat and reduced oxygen consumption (Saulich and Musolin 2012, Koch et al. 2017). Insects resume their developmental activity early in the season, when there is a

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significant increase in day length, temperature, and host availability, potentially leading to their movement throughout the landscape where feeding and reproduction occurs (Panizzi 1997, Saulich and Musolin 2012).

Stink bugs can cause injury to plants by inserting their piercing-sucking mouthparts into the plant tissues to inject digestive enzymes that will suck up nutrients from the plants, causing abnormal plant growth, deformation, discoloration and abortion of seeds and fruits (McPherson and Mcpherson 2000, Panizzi et al. 2000). In soybeans, stink bugs prefer to feed on reproductive parts, such as the developing seeds and pods (Todd and Herzog 1980). The degree of injury caused by stink bug feeding depends on a variety of factors, such as the insect density, duration of infestation and on the soybean growth stage (Owens 2012, Koch et al. 2017). Among these, soybean growth stage is the critical factor, with beginning pod set (i.e., R3) to beginning seed set (i.e., R5) (Fehr and Caviness, 1977) being the most sensitive growth stages, resulting in more severe damage due to pod loss and seed abortion (McPherson and McPherson 2000, Owens 2012, Koch et al. 2017). In addition, feeding of stink bugs can result in deformation and discoloration of seeds, reduced germination and emergence of seeds, and delayed plant maturity (i.e., "stay green" syndrome), significantly impacting seed quality and yield (Boethel et al. 2000, McPherson and McPherson 2000, Musser et al. 2011, Vyavhare et al. 2015, Koch et al. 2017).

In the North Central Region stink bugs have historically not been considered an economic issue to soybean or other agronomic crops, unlike in southern states where stink bug populations are a more persistent economic problem (McPherson and McPherson 2000, Reay-Jones 2010, 2014, Babu and Reisig 2018a). However, there is an

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increasing threat posed by stink bugs in the North Central Region, and this can be attributed to several factors (Koch et al. 2017). First, there has been an increase in abundance of native stink bugs over the recent years in field crops in the North Central Region (Hunt et al. 2011, 2014; Michel et al. 2015, Koch et al. 2017). Particularly, the green, *Chinavia hilaris* (Say), brown, *Euschistus servus* (Say), onespotted, *E. variolarius* (Palisot de Beauvois), and redshouldered, *Thyanta custator acerra* McAtee stink bugs have been increasing in abundance and frequency in field crops over the recent years (Koch et al. 2017). The explanation is uncertain, but some researchers believe that this could be due to increasing winter temperatures or greater use of reduce-tillage systems (Panizzi 2015, Kistner 2017, McPherson 2018).

Management tactics to control stink bug infestations in field crops rely on chemical control tactics such as the application of broad-spectrum insecticides (Willrich et al. 2003, Kamminga et al. 2009, Leskey et al. 2012). The current treatment thresholds for stink bugs in soybean in the North Central Region, are 5 stink bugs per 25 sweeps for soybean grown for seed production and 10 stink bugs per 25 sweeps for soybean grown for grain (Kogan 1976, Koch et al. 2017). To determine if populations are at the treatment threshold, scouting for pests in the field is necessary, as it is a fundamental aspect of integrated pest management (IPM) programs (Moon and Wilson 2009, Pedigo and Rice 2009). The currently available sampling plan for stink bugs in the North Central Region is for density estimation and more relevant to research purposes (Pezzini et al. 2019a). There is a need for a decision-making framework where more judicious and targeted use of insecticides could be achieved to avoid any unnecessary losses in soybean production.

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Therefore, there is a need to develop a binomial or decision-making sampling plan for stink bugs in soybean in the North Central Region.

The second reason for the increasing threat posed by stink bugs in the North Central Region, is due to the recent spread of an invasive species, the brown marmorated stink bug, *Halyomorpha halys* (Stål), throughout this region. *Halyomorpha halys* was first detected in North America in Pennsylvania in 1996 (Hoebeke and Carter 2003). It is now established in 4 Canadian provinces and 46 U.S. states, including 11 of 12 states in the North Central Region, where its status ranges from detected or nuisance pest only (i.e., 5 states) to agricultural and nuisance pest of moderate to severe status (i.e., 6 states) (NIPMC 2020). This insect is highly polyphagous, known to feed on over 300 host species including many of horticultural and agricultural significance (Rice et al. 2014, Bergmann et al. 2016). Most of the economically significant infestations of *H. halys* have been limited primarily to the Mid-Atlantic Region , where a 50% yield reduction in soybeans were associated to this pest including a $37 million loss in apples reported in this region in 2010 (Leskey et al. 2012, Leskey and Nielsen 2018). As *H. halys* impacts are spreading westward, this pest is causing a growing concern for growers in states like Minnesota, where *H. halys* was first detected in 2010 (Koch 2014), and currently, breeding populations have been detected in apple orchards and soybean fields (Koch and Aita 2019, Pezzini et al. 2019b, MDA 2020).

Halyomorpha halys is a highly mobile pest, capable of flying > 70 km per day and frequently move throughout the landscape in search for crops and wild host plants after overwintering emergence in the spring (Lee et al. 2014, Wiman et al. 2014, Lee and Leskey 2015, Leskey and Nielsen 2018). The current projected climate warming

scenarios for Minnesota and the Midwest U.S., could potentially enhance the biological fitness of *H. halys* at its current northern limit and advance the continued expansion into new areas, likely contributing to significant infestations of this pest in agricultural crops in this region (Kriticos et al. 2017, Govindan and Hutchison 2020). Given the potential to emerge as a serious crop pest in Minnesota, understanding the dispersal capacity of *H. halys* will inform the development of monitoring and management tactics, as well as to developing dispersal and forecasting models to aid in the management of *H. halys* (Lustig et al. 2017, Naranjo 2019). Insect dispersal by flight is a challenging ecological process to study in the field (Naranjo 2019), therefore, researchers have moved to the laboratory to investigate how different biotic or abiotic factors could impact insect dispersal, more specifically, using laboratory flight mills as a tool (Minter et al. 2018, Naranjo 2019). For *H. halys*, there is a limited number of studies that investigated the flight capabilities of this pest using flight mills (Wiman et al. 2014, Lee and Leskey 2015), however, neither of these investigated how variables manipulated under laboratory conditions could impact the flight capacity of *H. halys*. Therefore, there is a need to understand how several ecologically relevant variables affect the dispersal capacity of laboratory-reared *H. halys*, which could enhance risk assessment analyses for future expansion of this pest in Minnesota.

The focus of this thesis is to develop more efficient sampling for decision-making for herbivorous stink bugs in the North Central Region, and to understand the dispersal capacity of *H. halys* under controlled laboratory conditions. In chapter I, I developed a binomial or decision-making sequential sampling plan for herbivorous stink bugs in soybean in the North Central Region. This sampling plan will provide a more efficient,

time-saving option for growers to sample herbivorous stink bugs in this region. In chapter II, I investigated the flight capacity of laboratory-reared *H. halys* under different controlled experimental conditions. This work will help to better predict the dispersal potential of this pest in Minnesota. In general, results obtained from this thesis will provide information to fine-tune IPM strategies for stink bugs in the North Central Region, providing more efficient scouting efforts for growers as well as to better forecast the dispersal capacity of an important invasive species to the region.

Chapter I: Development of Binomial Sequential Sampling Plans for Herbivorous

Stink Bugs in Soybean in the North Central Region of the United States

Summary

Stink bugs are an emerging threat soybean production in the North Central Region of the United States. The current available sampling plan for stink bugs in the North Central Region of the U.S. is tailored for density estimation and more relevant to research purposes. The development of a decision-making framework for more efficient sampling for herbivorous stink bugs in this region is needed. Therefore, the aim of this study was to develop and validate a binomial sequential sampling plan for herbivorous stink bugs in the North Central Region. In 2016, 2017, and 2018, 146 soybean fields were sampled across eleven states using sweep nets. The binomial sequential sampling plans were developed using combinations of five tally thresholds at two action thresholds to identify those that provided the best sampling outcomes. Final analysis of the operating characteristic curves for each plan indicated that the tally threshold of 3 stink bugs per 25 sweeps, and action thresholds corresponding to the proposed action thresholds of 5 and 10 stink bugs per 25 sweeps (0.75 and 0.95, respectively), provided the optimal balance between highest probability of correct decisions ($\geq$ 99%) and lowest probability of incorrect decisions ($\leq$ 1%). In addition, the average sample size for both plans (18 and 12, respectively) were lower than the other proposed plans. Results of this research showed that the binomial sequential sampling plan can improve efficiency with regards to the number of sample units required to reach a management decision for stink bugs in soybean in this region.

Introduction

Soybean, *Glycine max* (L.) Merr. (Fabales: Fabacea), is one of the most valuable crops globally, representing one of the largest sources of vegetable oil and animal protein feed (Pagano and Miransari 2016). The United States is the world's leading soybean producer and second-leading exporter, with soybean comprising about 90% of the country's total oilseed production (USDA-ERS, 2020). The North Central Region of the United States is responsible for 82% of the national soybean acreage (USDA-NASS 2019). Worldwide, Pentatomidae (Hemiptera: Heteroptera), commonly referred to as stink bugs, are significant pests of soybean (Kogan 1976). In the North Central Region, stink bugs were historically insignificant pests (McPherson and McPherson 2000). However, in recent years the threat posed to soybean production by stink bugs in the region is increasing (Koch et al. 2017).

The increasing risk posed by stink bugs can be attributed to several factors (Koch et al. 2017, Pezzini et al. 2019b). First, the abundance of native Pentatomidae species is increasing in field crops in the North Central Region (Hunt et al. 2011, 2014; Michel et al. 2015, Koch et al. 2017), perhaps due to warmer winter temperatures or the increased use of reduce-tillage systems (Panizzi 2015, Kistner 2017, McPherson 2018). Second, invasive stink bugs are spreading into this region. An example is the brown marmorated stink bug, *Halyomorpha halys* (Stål), first detected in North America in Pennsylvania in 1996 (Hoebeke and Carter 2003). Since its introduction, it has spread to 46 U.S. states, including 11 of 12 states in the North Central Region (NIPMC 2020), and is responsible for causing up to 50% yield reduction in soybean in the Mid-Atlantic Region (Leskey et al. 2012).

In soybean, stink bugs feed on seeds and pods by inserting their piercing-sucking mouthparts into these tissues, causing irreversible damage to developing seeds which can impact seed quality, yield, and germination rates (McPherson and McPherson 2000, Koch et al. 2017). This potential damage to soybean necessitates the development of practical, cost-effective sampling plans for making integrated pest management (IPM)-based control decisions (Binns et al. 2000). The time required to sample pest insects is a key bottleneck to effective monitoring in the field. Sampling for stink bugs in soybean can be done with a sweep net or drop cloth (Koch et al. 2017). Both methods are efficient, but sweeping is reported to be more convenient and cost-effective for several stink bug species (Todd and Herzog 1980, Sane et al. 1999, Koch et al. 2017).

Sampling plans can have different purposes, such as detection, estimation, and decision-making (Moon and Wilson 2009, Pezzini et al. 2019a). Sequential sampling plans can be more efficient than fixed sample size sampling plans, allowing for more flexibility in sample size while reducing sampling effort, and retaining high levels of precision (Binns et al. 2000). Density estimation, or enumerative sequential sampling, is used to estimate pest density in the field based on a pre-established precision level, and can be used for decision-making (Hodgson et al. 2004); however, this approach is often used for research purposes due to high sample size requirements (Hutchison 1994, Galvan et al. 2007, Pezzini et al. 2019a). For decision making in IPM programs, binomial sequential sampling plans are more commonly used because the tally threshold (i.e., number of insects required to consider a sample unit infested) requires that only the presence or absence of the pest above a specified level is needed to make a decision, resulting in a more efficient, time-saving option (Jones 1994, Burkness and Hutchison

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2009, Paula-Moraes et al. 2011, Araújo et al. 2020). In addition, when pest densities are low or high, a decision can be made quickly, potentially reducing sample size by 50% compared to fixed sample size sampling plans (Jones 1994, Burkness and Hutchison 1997, 2009, Hodgson et al. 2004)

There have been no binomial sequential sampling plans developed for stink bugs in the North Central Region. Given the advantages of practical and efficient binomial sequential sampling plans, and the need to aid growers with decision making for improved IPM of these pests, the objective of this study was to develop and validate the performance of several binomial sequential sampling plans. The sampling plans we evaluated included the composite action thresholds (all major stink bug species combined) currently used in the North Central Region (i.e., 5 stink bugs per 25 sweeps for soybean grown for seed and 10 stink bugs per 25 sweeps for soybean grown for grain (Kogan 1976, Koch et al. 2017). We also proposed several different tally thresholds that can be used in the field for characterizing a sample unit as infested. Finally, the sampling plan combinations were developed and validated using the resampling software Resampling for Validation of Sampling Plans (RVSP) (Naranjo and Hutchison 1997), which included estimates of the probability of making correct treat or no-treat decisions.

Materials and Methods

Sampling Procedure

Stink bug nymphs and adults were sampled from 146 soybean fields distributed across 11 states in the North Central Region during 2016, 2017, and 2018 (Table 1.1). For the data collected in 2016 and 2017, sampling protocols (i.e., field sites and data

collection) are described in Pezzini et al. (2019a). Briefly, 63 fields were sampled in eight

states (i.e., Indiana, Kansas, Minnesota, Missouri, Nebraska, North Dakota, Ohio, and

South Dakota) in 2016 and 62 fields were sampled from the same eight states plus

Michigan in 2017 (Table 1.1). In each state, one to four sites were selected with 13 – 368

km between sites, and at each site, one to four fields were sampled. In 2018, 21 fields

were sampled in eight states (i.e., Illinois, Indiana, Michigan, Minnesota, Nebraska,

North Dakota, Ohio, and Wisconsin) (Table 1.1). In each state, two separate fields were

sampled, except for Michigan which had seven fields. Within each state, fields were 0.92

– 116 km apart. In general, fields were sampled on a weekly basis from R1 (i.e.,

beginning-bloom) through R8 (i.e., full maturity) soybean growth stages (Fehr and

Caviness 1977).

Sampling was done using sweep nets (39-cm net diameter). A sample unit was a

set of 25 sweeps (i.e., sample unit size of 25 sweeps). Sweeps were performed using

vertical pendulum-style swings of the net through the soybean canopy as the sampler

walked between adjacent soybean rows, resulting in the sweep net passing only once

through the same plants in the field; each pass of the net counted as one sweep (Kogan

and Pitre 1980). In 2016 and 2017, sample units were collected from randomly selected

locations at the field edge (i.e., < 10 m into the field) and in the field interior (i.e., > 10 m

into the field). Generally, a total of 12 sample units were taken per field on a given date;

however, additional sample units were collected from selected fields depending on other

research projects (Pezzini et al. 2019a, b). In 2018, 15 sample units were collected from

the interior of each field (i.e., >10 m into the field) on a given date. For all years, sample

units were spaced at least 10 m apart in each field. Immediately after each set of 25

sweeps was collected, the contents of the net were transferred into a 20.3×25.4-cm plastic

zipper-locking bag, labeled accordingly, placed in a cooler, and transported to the

laboratory where they were maintained in freezers at approximately −20 °C. Samples

were sorted to retain only stink bug nymphs and adults and sent to the University of

Minnesota for identification. Nymphs were identified to genus or species using

DeCoursey and Esselbaugh (1962) and Evans (1985); adults were identified to species

and subspecies using keys in McPherson and McPherson (2000), Rider (2012), and

Paiero et al. (2013). For the purpose of this research, only the herbivorous stink bugs

were quantified.

Development and validation of the binomial sequential sampling plans

A total of 885 data sets (i.e., samples) were collected over all years. However,

samples with a mean density of zero (i.e., without any stink bugs; 178 samples) were

excluded from the analysis. The remaining 707 data sets were used to validate the

binomial sequential sampling plans using the RVSP software v. 2.0 (Excel add-in)

developed by Naranjo and Hutchison (1997). Steps to develop the binomial sequential

sampling plans followed methodologies used by Hodgson et al. (2004) and Galvan et al.

(2007). Stop lines for the Wald's sequential probability ratio test (Wald 1947) require

several parameters including: a tally threshold (TT), which is the number of insects

required to consider a sample unit infested; an action threshold (PI_{AT}), which is based on

proportion of infested sample units (PI); upper (θ_1) and lower (θ_2) boundaries around the

PI_{AT}; and α (type I) and β (type II) error rates associated with the decision boundaries

(Jones 1994). The type I error is defined as the probability of making a decision to treat

when PI is below the AT and type II error is defined as the probability of making a

decision to not treat when PI is above the AT. All parameters were held constant for all tally threshold and action threshold combinations.

The lower and upper boundaries (θ_1 and θ_2, respectively) were set at 0.03 (above or below the proportion infested action threshold (PI_{AT}), and type I and II (α and β, respectively) errors were set at 0.10 (Binns 1994, Burkness and Hutchison 2009, Paula-Moraes et al. 2011, Araújo et al. 2020). The stop lines for each action threshold and tally threshold combination were calculated using Wald's plan and are defined as follows:

$$T_{n(t)} \geq Rx + Q \text{ and } T_{n(t)} \leq Rx - S,$$

where $T_{n(t)}$ is the cumulative number of samples infested with at least t insects, and Q, R, and S are functions of α and β (Wald 1947). The 707 data sets were iteratively resampled (500 resampling bouts) until a treat or no-treat decision was made. The software simulations provided the average proportion of infested sample units, the operating characteristic (OC) function (i.e., the probability of not taking action against an insect population relative to the observed proportion of infested sample units) (Onsager 1976), and the average sample number (ASN) which represents the required sample size over all sampling iterations (i.e., 500 resampling bouts).

We evaluated multiple combinations of TT and AT to identify those that provided the best sampling outcomes. Two mean densities were used to develop the PI_{AT} (i.e., ATs in terms of proportion of infested sample units). These densities, which are the recommended action thresholds for stink bugs in soybean in the North Central Region, are 5 stink bugs per 25 sweeps for soybean grown for seed and 10 stink bugs per 25 sweeps for soybean grown for grain (Kogan 1976, Koch et al. 2017). In addition, we independently evaluated five TTs (i.e., 1, 2, 3, 4, and 5 stink bugs per 25 sweeps),

meaning that for a sample unit (i.e., set of 25 sweeps) to be considered infested, at least 1, 2, 3, 4, or 5 stink bugs must be present, respectively. For each TT, the relationship between the mean density and proportion of infested sample units based on that TT was used to calculate PI_{AT} for the densities of 5 and 10 stink bugs per 25 sweeps (Figure 1.1).

The applicability of a sampling plan for decision making should be based on precision and efficiency of the plan (Galvan et al. 2007). The OC function determines precision and ASN function determines efficiency (Binns and Nyrop 1992). Therefore, to validate the sampling plans' precision, OC functions calculated by RVSP software were plotted against the observed proportion of infested sample units for each TT and PI_{AT} combination. In addition, to validate the efficiency of the sampling plans, the ASN functions calculated by RVSP software were plotted against the observed proportion of infested sample units for each TT and PI_{AT} combination. Models for the relationships between mean density and proportion of infested sample units, OC, and ASN were fitted using the automated curve-fitting software, TableCurve 2D (Systat Software, Inc. 2002). The predicted lines for the relationship between mean density and proportion of infested sample units and OC functions were fitted using a logistic dose response function:

$$[\, y = a/(1 \,+\, (x/b)^c)],$$

where, a, b, and c are parameters to be estimated, and x is the population level (i.e., mean density of stink bugs). These parameter estimates are presented in Tables 1.3 and 1.4. The ASN functions were fitted using a Pearson VII function:

$$[\, y = a \,+\, b/(1 \,+\, 4 \times ((x \,-\, c)/d)^2 (2^{(1/e)^{-1}}))^e\,],$$

where, a, b, c, d, and e are parameters to be estimated, and x is the population level. These parameter estimates are presented in Table 1.5.

As an extension of the validation process, a four-cell probability matrix was used to evaluate the accuracy of the sampling plans (Calvin et al. 1986, Hodgson et al. 2004, Galvan et al. 2007, Burkness and Hutchison 2009, Paula-Moraes et al. 2011). The results obtained from the OC curves, jointly with the observed and average PI, were used in the four-cell probability matrix to calculate the probability of making a correct decision (i.e., treating when population exceeds the threshold or not treating when the population is below the threshold). The decision to treat or not treat was determined by comparing the observed PI (i.e., average proportion of infested sample units calculated from actual field data) to the average PI (i.e., average proportion of infested sample units generated from the 500 iterations in the software simulation) at each AT. Therefore, the four-cell probability matrix included cells A, correct decision to treat, when the observed and average PI both exceeded the PI_{AT}; D, correct decision to not treat, when the observed and average PI both did not exceeded the PI_{AT}; B, incorrect decision to not treat, when the observed PI exceeded the PI_{AT} and the average PI did not exceed the PI_{AT}; and C, incorrect decision to treat, when the observed PI did not exceed the PI_{AT} and the average PI exceeded the PI_{AT} (Burkness et al. 1999, Hodgson et al. 2004, Galvan et al. 2007, Burkness and Hutchison 2009). For each data set evaluated, an appropriate decision is fixed by the magnitude of density and must be correct or incorrect in the matrix, where A + B = 1 or C + D = 1 (Burkness et al. 1999, Hodgson et al. 2004, Galvan et al. 2007). Therefore, when the infestation is large enough to require treatment, the probability of A = 1 – OC and the probability of B = OC. When the density is too low to require treatment, the probability of C = 1 – OC and the probability of D = OC. The probability

of making a correct decision (i.e., treat or not treat) was summarized for all data sets at

each TT and PI_{AT} combination with

$$1 = \sum p_i (A_i + D_i) + \sum p_i (B_i + C_i),$$

where, p_i is the proportion of *n* data sets represented by data set i, and A, D, B, and C

were described above.

The correct and incorrect decisions were totaled for each TT and PI_{AT}

combination, and the best sampling plan was selected based on the greatest probability of

making correct decisions while minimizing the probability of incorrect decisions, and on

the balance among OC, ASN, and actual α and β (Table 1.2; Figures 1.2 and 1.3).

Results

The mean number of stink bugs per 25 sweeps ranged from 0.02 to 15.58 for the

707 data sets collected and used in the RVSP software. The relationship between mean

density and proportion of infested sample units for each TT was used to calculate PI_{AT} for

the densities of 5 and 10 stink bugs per 25 sweeps (Figure 1.1). For the mean density of 5

stink bugs per 25 sweeps and TTs of 1, 2, 3, 4, and 5 stink bugs per 25 sweeps, the

calculated PI_{AT} values were 0.95, 0.86, 0.75, 0.62, and 0.49, respectively. For the mean

density of 10 stink bugs per 25 sweeps and TTs of 3, 4, and 5 stink bugs per 25 sweeps,

the calculated PI_{AT} values were 0.95, 0.94, and 0.88, respectively. The OC and ASN

function curves for all TT and PI_{AT} combinations were plotted (Figure 1.2A – J; Figure

1.3A – F). PI_{AT} values that correspond to the mean density of 10 stink bugs per 25

sweeps for the TTs of 1 and 2 stink bugs per 25 sweeps were not included in the OC and

ASN function calculation, because the values were above 1 (i.e., more than 100% of

proportion infested sample units) based on extrapolating the values from the relationship

between the proportion of infested sample units and mean density of stink bugs functions

(Figures 1.1A and 1.1B).

The OC function curves for each PI_{AT} associated with the respective TTs showed

varying levels of precision among sampling plans. For a mean density threshold of 5

stink bugs per 25 sweeps, the OC for TTs of 1, 2, 3, 4, and 5 stink bugs per 25 sweeps

were 0.36, 0.36, 0.51, 0.46, and 0.42 respectively (Figures 1.2A, 1.2C, 1.2E, 1.2G, and

1.2I). Ideally, when $\alpha = \beta$ the OC should be equal to 0.5 at the PI_{AT} (i.e., 50% chance of

treating or not treating) (Naranjo and Hutchison 1997, Binns et al. 2000). For all the

sampling plans, except for TT of 3 stink bugs per 25 sweeps, the OC values at the PI_{AT}

were less than 0.5, suggesting the sampling plans were conservative (i.e., treatment was

more likely to occur than not treating at the PI_{AT}). For the TT of 3 stink bugs per 25

sweeps, the sampling plan was slightly liberal (OC = 0.51) that is, not treating at the PI_{AT}

was more likely to occur. For the mean density threshold of 10 stink bugs per 25 sweeps,

the OC for TTs of 3, 4, and 5 stink bugs per 25 sweeps were 0.39, 0.32, and 0.36

respectively (Figures 1.3A, 1.3C, and 1.3E). Because the OC values at the PI_{AT} were less

than 0.5, these sampling plans can be considered conservative, meaning that treatment

was more likely to occur than not treating at the PI_{AT}.

Resampling analysis resulted in eight summary outputs that were used to create

the treatment decision probability matrix to determine the optimal TT and PI_{AT}

combination (Table 1.2). All simulations had high probabilities (i.e., 0.994 to 0.999) of

correctly deciding to treat or not treat (A + D) and low probabilities (i.e., 0 to 0.005) of

incorrectly deciding to treat or not treat (B + C) (Table 1.2). The ASN required to reach

either a treat or no-treat decision ranged from 12 to 25 sample units across all simulations. For the TT and PI_{AT} combinations that corresponded to a mean density of 5 stink bugs per 25 sweeps, the ASN ranged from 15 to 25 sample units. For the TT and PI_{AT} combinations that corresponded to a mean density of 10 stink bugs per 25 sweeps, the ASN ranged from 12 to 13 sample units (Table 1.2). The maximum ASN required to reach a decision is near or at the AT and it ranged from 67 to 357 sample units required for TT and PI_{AT} combinations that corresponded to a mean density of 5 stink bugs per 25 sweeps (Figures 1.2B, 1.2D, 1.2F, 1.2H, and 1.2J), and from 49 to 88 sample units required for TT and PI_{AT} combinations that corresponded to a mean density of 10 stink bugs per 25 sweeps (Figures 1.3B, 1.3D, and 1.3F).

Based on *a priori* criteria to select the optimum TT and PI_{AT} combination (i.e., A + D > B + C and C > B; balance between actual α and β values, and ASN) a TT of 3 stink bugs per 25 sweeps was selected as the optimal TT to be used for field implementation of the binomial sequential sampling plans that represented the proposed action thresholds of 5 and 10 stink bugs per 25 sweeps. The use of a single TT for all field situations facilitates the development and execution of binomial sampling plans for herbivorous stink bugs. This TT represents the best suitable balance among the highest probability of correct (i.e., A + D ≥ 99%) and lowest probability of incorrect (i.e., B + C ≤ 1%) decisions, while minimizing actual α (i.e., for PI_{AT} of 0.75 and 0.95, α = 0.07) and β (i.e., for PI_{AT} of 0.75 and 0.95, β = 0.09 and 0.07, respectively) error rates and ASN (i.e., for PI_{AT} of 0.75 and 0.95, ASN = 18 and 12, respectively) compared to the other TTs examined.

Based on the above results, decision stop-lines graphs for the TT of 3 stink bugs per 25 sweeps and PI_{AT} of 0.75 and 0.95 were developed (Figures 1.4A and 1.4B). The proportion of infested sample units for the specific PI_{AT} (i.e., 0.75 and 0.95) is calculated from the field-examined sample units and compared with the stop line graphs (Figures 1.4A and 1.4B). Subsequently, a management decision can be made if the proportion is in the "treat" or "do not treat" area. If the value is in the "continue sampling" area, additional samples will be required before a treatment decision is made. For each sampling plan, upper and lower stop-lines were generated for classifying the number of infested sample units as treatable or not. The minimum number of sample units to reach a decision to treat or not treat is determined when the lower stop line intercepts the x-axis. When the PI_{AT} is 0.75 (i.e., 75% of infested sample units) at least 9 sample units must be sampled to make a decision (Figure 1.4A). When the PI_{AT} is 0.95 (i.e., 95% of infested sample units) at least 2 sample units must be sampled to make a decision (Figure 1.4B).

Discussion

In this study we developed and validated eight binomial sequential sampling plans based on five TTs and two ATs, using resampling software based on Wald's binomial sequential sampling plan (Naranjo and Hutchison 1997) for herbivorous stink bugs in the North Central Region. The goal of this study was to identify the sampling plan combination that provided the most efficient decision-making outcome. Unlike enumerative sequential sampling plans, which are typically evaluated based on the precision level (Southwood 1978), binomial sequential sampling plans are evaluated using the OC and ASN functions based on Monte Carlo simulations (e.g., theoretical

distributions of insects) or by bootstrap simulations (Jones 1994, Naranjo and Hutchison 1997). In the present study, binomial sequential sampling plans were evaluated using bootstrap simulations from independent field data sets and resampling simulations of the sequential sampling process. This approach is often more robust and more predictive than the Monte Carlo method (Fowler and Lynch 1987, Hutchison et al. 1988, Nyrop and Binns 1990), because actual field data are used to develop OC functions rather than theoretical distributions (Hutchison 1994, Naranjo and Hutchison 1997). The OC obtained using bootstrap simulations from the resampling software were used with a decision probability matrix to select the optimal TT and AT combination (Burkness et al. 1999, Hodgson et al. 2004, Galvan et al. 2007).

All sampling plans delivered highly accurate classifications (i.e., the probability of making a correct treat or no-treat decision was always 99% or greater). For TTs ≤ 3 stink bugs per 25 sweeps and ATs corresponding to a mean density of 5 stink bugs per 25 sweeps, the ASN was ≤ 18 sample units. As the TT increased (i.e., 4 and 5 stink bugs per 25 sweeps), the ASN increased by approximately 28%. For TTs of 3, 4, and 5 stink bugs per 25 sweeps and ATs corresponding to a mean density of 10 stink bugs per 25 sweeps, the ASN was near the minimum sample size of 12 sample units. A TT of 3 stink bugs per 25 sweeps was selected as the optimal TT to be used in the field, because this TT presented the proper balance among OC, ASN, actual α and β, and high probability of correct decisions compared to other proposed TTs. Although all sampling plans delivered highly accurate decisions, a TT of 3 stink bugs also provided the smallest actual α and β error values over all the sampling plans. The α and β error values are variable as a function of threshold, variation in the data, and the resampling process (Naranjo and

Hutchison 1997, Subramanyam et al. 1997, Paula-Moraes et al. 2011). The actual α and β

values were lower than the preset value of 0.10 and represent a reduced probability of

committing a type I (implementing control measures when they are not necessary) and

type II (not implementing control measures when they are necessary) errors. This is

essential in today's soybean production where profit margins are tight and making such

errors would result in either unnecessary insecticide applications, which increase

financial costs and environmental contamination, or missing necessary insecticide

applications, which could result in significant yield loses that reduce financial gains. Of

these, the latter error is potentially more serious for producers, and any sampling method

with a high type II error rate will be unacceptable.

Stink bugs exhibit an aggregated spatial pattern in cropping systems such as

soybean (Fernandes et al. 2019, Pezzini et al. 2019a), cotton, *Gossypium hirsutum* L.

(Malvales: Malvaceae) (Reay-Jones et al. 2009), rice, *Oryza sativa* L. (Poales: Poaceae)

(Espino et al. 2008), and corn, *Zea mays* L. (Poales: Poaceae) (Babu and Reisig 2018b).

With highly aggregated species, generally higher sample sizes are needed with

enumerative sequential sampling plans designed to estimate population density (Jones

1994, Reay-Jones et al. 2009). Because binomial sequential sampling plans only classify

the pest as either present or absent based on a tally threshold rather than a full count, this

approach can greatly reduce sampling costs for highly aggregated pests (Mcgraw and

Koppenhöfer 2009). Overall, the ASN of our proposed sampling plans with a TT = 3

stink bugs per 25 sweeps was 18 and 12 sample units for PI_{AT} of 0.75 and 0.95,

respectively, which are reasonable numbers for use in field-based decision-making. In

addition, these sampling plans corroborate other proposed binomial sequential sampling

plans for stink bugs in soybean (Souza et al. 2014, Fernandes et al. 2019). Based on the

ASN estimates for our proposed binomial sequential sampling plans, a soybean field

could be sampled in approximately 22 and 15 min for ASN of 18 and 12 sample units,

respectively, based on our records of time required to sample two fields on two different

dates in Minnesota (unpublished data). A reasonable next step in this effort is

implementing this approach across the region and determining whether these time

estimates are representative.

Pezzini et al. (2019a) proposed an enumerative sequential sampling plan for

herbivorous stink bugs in the North Central Region and found that an ASN of 40-42 25-

sweep sample units were necessary to achieve a desired precision of 0.25. Our findings

represent a significant reduction in sample size requirements when compared to an

enumerative sequential sampling plan while retaining high levels of accuracy, efficiency,

and improved practicality. Similarly, Hodgson et al. (2004) found that a binomial

sequential sampling plan developed for the soybean aphid, *Aphis glycines* Matsumura

(Hemiptera: Aphididae), improved efficiency in IPM decision-making with an ASN of 11

plants compared to an ASN of 38 plants for a desired precision level of 0.25 for an

enumerative sequential sampling plan.

As with most binomial sequential sampling plans, the greatest ASN values

required to make a treatment decision occur near the ATs (Jones 1994, Naranjo and

Hutchison 1997, Paula-Moraes et al. 2011). In a binomial sequential sampling plan, the

number of sample units collected will be closely related to the density of the pest

population (Paula-Moraes et al. 2011). When stink bug densities are low or high, the

binomial sequential sampling plans proposed here will provide the greatest savings in sampling since a no-treat or treat decision will be reached quickly.

For binomial sequential sampling plans the intercept of the lower stop line on the x-axis indicates the minimum number of sample units that must be sampled to reach a decision to treat or not treat (Subramanyam et al. 1997, Burkness and Hutchison 2009); however, in practice, we recommend that at least 5 sample units be collected prior to making a decision. Furthermore, because this is a sequential sampling plan, the proportion of infested sample units can potentially indicate that it is necessary to "continue sampling" indefinitely. One of the main advantages of a binomial sequential sampling plan is that it is practical (i.e., a decision could be made after each sample unit), minimizing the effort and time of the scout. Therefore, based on our analysis, we recommend a limit of 20 sample units to arrive at a decision. If 20 sample units have been taken, and no decision has been reached, the field should be resampled in 7 days. The period for resampling the soybean field is based on the soybean reproductive growth stage, time of harvest, and the geographic distribution of stink bugs across the region (Shepard 1980, Pezzini et al. 2019a).

To our knowledge, this is the first proposed binomial sequential sampling plan for herbivorous stink bugs in soybean in the North Central Region. In Brazil and the U.S., which are worldwide leaders in soybean production, IPM can be challenging, where the grower is confronted with the need for a quicker and easier pest sampling methodology that may reduce prophylactic insecticide applications that occur when scouting is too tedious and time intensive. (Bueno et al. 2020). Results of this research showed that the binomial sequential sampling plan can improve efficiency with regards to the number of

sample units required to reach a management decision for the region. In addition, the

sampling plan parameters used in this study can easily be adjusted to accommodate other

action thresholds and allowable error rates suitable for a specific geographic region.

However, continued efforts should be made to evaluate the cost-effectiveness of the

proposed binomial sequential sampling plans for different states of the North Central

Region.

Table 1.1: Number of soybean fields sampled for herbivorous stink bugs across the cooperating states in the North Central Region of the United States for 2016, 2017, and 2018. Adapted from Pezzini et al. (2019a)

State	2016	2017	2018
Illinois	0	0	2
Indiana	8	8	2
Kansas	8	7	0
Michigan	0	8	7
Minnesota	12	8	2
Missouri	8	4	0
Nebraska	7	8	2
North Dakota	8	8	2
Ohio	8	8	2
South Dakota	4	3	0
Wisconsin	0	0	2
Total	63	62	21

Table 1.2: Probability of correct and incorrect decisions as influenced by various tally thresholds and action thresholds combinations for a binomial sequential sampling plan for herbivorous stink bugs in the North Central Region of the United States, based on the observed and average PI and OC results from the resampling validation; independent data sets from 11 states from 2016 – 2018

AT[a]	PI_{AT}[b]	α[c]	β[c]	Correct decisions			Incorrect decisions			ASN
				A[d]	D[e]	A + D[f]	B[g]	C[h]	B + C[i]	
Tally threshold = 1										
5	0.95	0.083	0.078	0.048	0.948	0.996	0.000	0.003	0.003	14.55
Tally threshold = 2										
5	0.86	0.098	0.097	0.036	0.960	0.997	0.001	0.001	0.002	16.47
Tally threshold = 3										
5	0.75	0.077	0.095	0.029	0.965	0.994	0.004	0.000	0.005	18.47
10	0.95	0.077	0.075	0.005	0.993	0.998	0.000	0.001	0.001	12.40
Tally threshold = 4										
5	0.62	0.096	0.082	0.028	0.971	0.999	0.000	0.000	0.000	23.87
10	0.94	0.083	0.032	0.004	0.995	0.999	0.000	0.000	0.000	12.34
Tally threshold = 5										
5	0.49	0.093	0.105	0.028	0.970	0.998	0.001	0.000	0.001	24.52
10	0.88	0.144	0.057	0.004	0.995	0.999	0.000	0.000	0.000	12.59

[a] Mean stink bug density per 25 sweeps that are the proposed action thresholds (AT) for stink bugs (i.e., soybean grown for seed = 5 stink bugs per 25 sweeps; soybean grown for grain = 10 stink bugs per 25 sweeps).
[b] Proportion infested action threshold (PI_{AT}), based on the proportion of infested sample units and mean relationship curves (Figure 1.1).
[c] Type I error (α) is defined as the probability of making a treatment decision when PI is below the AT. Type II error (β) is defined as the probability of making a no-treat decision when PI is above the AT. Actual error values were estimated from the fitted curves in Figures 1.2 and 1.3
[d] A, probability of both the observed and average PI exceeding the AT, resulting in a correct treat decision.
[e] D, probability of both the observed and average PI not exceeding the AT, resulting in a correct no-treat decision.
[f] A + D, probability of making a correct treat or no-treat decision.
[g] B, probability of the observed PI exceeding the AT and the average PI not exceeding the AT, resulting in an incorrect no-treat decision.
[h] C, probability of the observed PI not exceeding the AT and the average PI exceeding the AT, resulting in an incorrect treat decision.
[i] B + C, probability of making an incorrect treat or no-treat decision.

Table 1.3: Parameters from equations used to fit the relationship between the proportion of infested sample units as a function of the mean density of stink bugs per 25 sweeps.

TT[a]	Parameter estimates $\pm$ SEM[b]			R^2	$F_{2,\,704}$	P
	a	b	c			
1	1.12 ± 0.02	0.99 ± 0.05	-1.07 ± 0.02	0.93	5103.06	< 0.001
2	1.06 ± 0.02	1.99 ± 0.07	-1.60 ± 0.04	0.94	5564.02	< 0.001
3	1.05 ± 0.03	3.10 ± 0.11	-1.92 ± 0.05	0.93	4909.61	< 0.001
4	1.11 ± 0.03	4.43 ± 0.15	-2.12 ± 0.06	0.94	5565.71	< 0.001
5	1.08 ± 0.03	5.37 ± 0.17	-2.42 ± 0.07	0.93	5054.42	< 0.001

[a]This relationship for the data sets at a given tally threshold (TT) was used to transform the proposed mean density action thresholds to proportion infested action thresholds.
[b]The equation type is logistic dose response of the form: $y = a/(1 + (x/b)^c)$.

Table 1.4: Parameters from equations used to fit the operating characteristic (OC) curves as a function of the observed proportion of infested sample units obtained from resampling software (RVSP) using field data for each tally threshold (TT) and proportion infested action threshold (PI$_{AT}$) combination.

PI$_{AT}$	TT	Parameter estimates ± SEM[a]			R^2	F$_{2, 704}$	P
		a	b	c			
0.95[b]	1	0.99 ± 0.00	0.94 ± 0.00	77.11 ± 0.72	0.99	381832.70	< 0.001
0.86[b]	2	0.99 ± 0.00	0.85 ± 0.00	63.82 ± 0.19	0.99	3693079.00	< 0.001
0.75[b]	3	0.99 ± 0.00	0.75 ± 0.00	59.18 ± 0.43	0.99	2011823.00	< 0.001
0.95[c]	3	0.99 ± 0.00	0.94 ± 0.00	67.36 ± 0.74	0.99	226607.00	< 0.001
0.62[b]	4	1.00 ± 0.00	0.62 ± 0.00	58.20 ± 0.14	0.99	32948592.00	< 0.001
0.94[c]	4	0.99 ± 0.00	0.93 ± 0.00	91.17 ± 3.17	0.99	1507724.00	< 0.001
0.49[b]	5	0.99 ± 0.00	0.48 ± 0.00	35.99 ± 0.38	0.99	3685510.00	< 0.001
0.88[c]	5	0.99 ± 0.00	0.87 ± 0.00	67.26 ± 0.13	0.99	10948715.00	< 0.001

[a]The equation type is logistic dose response of the form: $y = a/(1 + (x/b)^c)$.
[b]This PI$_{AT}$ corresponds to the action threshold (AT) of 5 stink bugs per 25 sweeps.
[c]This PI$_{AT}$ corresponds to the action threshold (AT) of 10 stink bugs per 25 sweeps.

Table 1.5: Parameters from equation used to fit the average sample number (ASN) curves as a function of the observed proportion of infested sample units obtained from resampling software (RVSP) using field data for each tally threshold (TT) and proportion infested action threshold (PI_{AT}) combination.

| PI_{AT} | TT | Parameter estimates $\pm$ SEM[a] | | | | | R^2 | $F_{4, 702}$ | P |
		a	b	c	d	e			
0.95^b	1	11.50 ± 0.03	55.91 ± 0.38	0.95 ± 0.00	0.08 ± 0.00	0.80 ± 0.01	0.99	48038.66	< 0.001
0.86^b	2	9.93 ± 0.07	164.84 ± 0.49	0.86 ± 0.00	0.09 ± 0.00	0.72 ± 0.00	0.99	80739.14	< 0.001
0.75^b	3	5.95 ± 0.24	258.74 ± 0.63	0.74 ± 0.00	0.09 ± 0.00	0.61 ± 0.00	0.99	71954.19	< 0.001
0.95^c	3	11.93 ± 0.00	37.26 ± 0.24	0.94 ± 0.00	0.09 ± 0.00	1.22 ± 0.02	0.99	37155.50	< 0.001
0.62^b	4	4.88 ± 0.16	411.61 ± 2.62	0.62 ± 0.00	0.09 ± 0.00	0.59 ± 0.00	0.99	295217.50	< 0.001
0.94^c	4	11.91 ± 0.00	57.66 ± 0.23	0.93 ± 0.00	0.09 ± 0.00	1.20 ± 0.01	0.99	131581.80	< 0.001
0.49^b	5	2.72 ± 0.31	357.05 ± 0.95	0.49 ± 0.00	0.09 ± 0.00	0.55 ± 0.00	0.99	163170.50	< 0.001
0.88^c	5	11.32 ± 0.03	116.60 ± 1.30	0.88 ± 0.00	0.11 ± 0.00	0.93 ± 0.01	0.99	53160.65	< 0.001

[a]The equation type is Pearson VII of the form: $y = a + b/(1 + 4 \times ((x - c)/d)^2(2^{(1/e)^{-1}}))^e$.
[b]This PI_{AT} corresponds to the action threshold (AT) of 5 stink bugs per 25 sweeps.
[c]This PI_{AT} corresponds to the action threshold (AT) of 10 stink bugs per 25 sweeps.
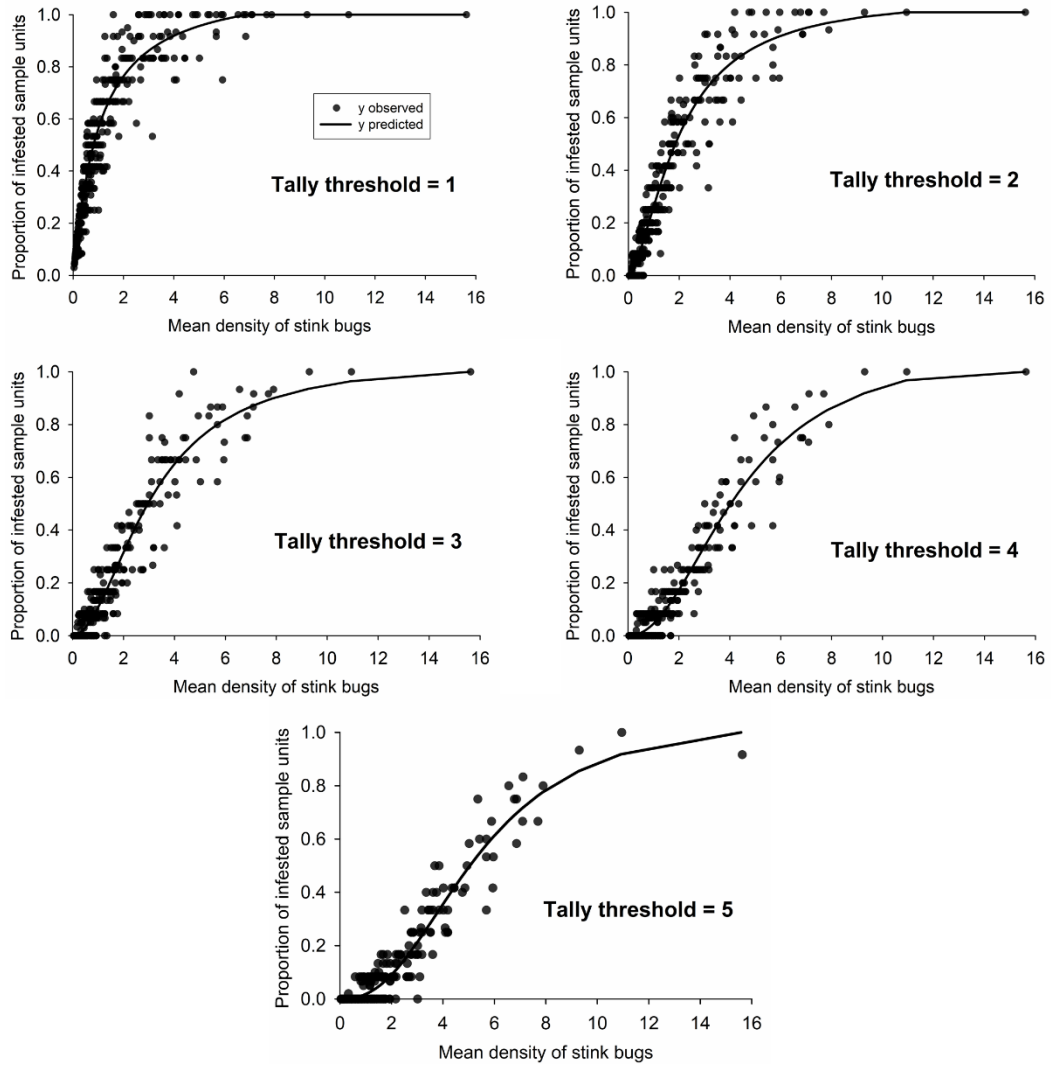
Figure 1.1: Nonlinear relationship between the proportion of infested sample units (i.e., sets of 25 sweeps) and mean density of herbivorous stink bugs (i.e., bugs per 25 sweeps) for tally thresholds ≥ than 1, 2, 3, 4, or 5 stink bugs per sample unit.
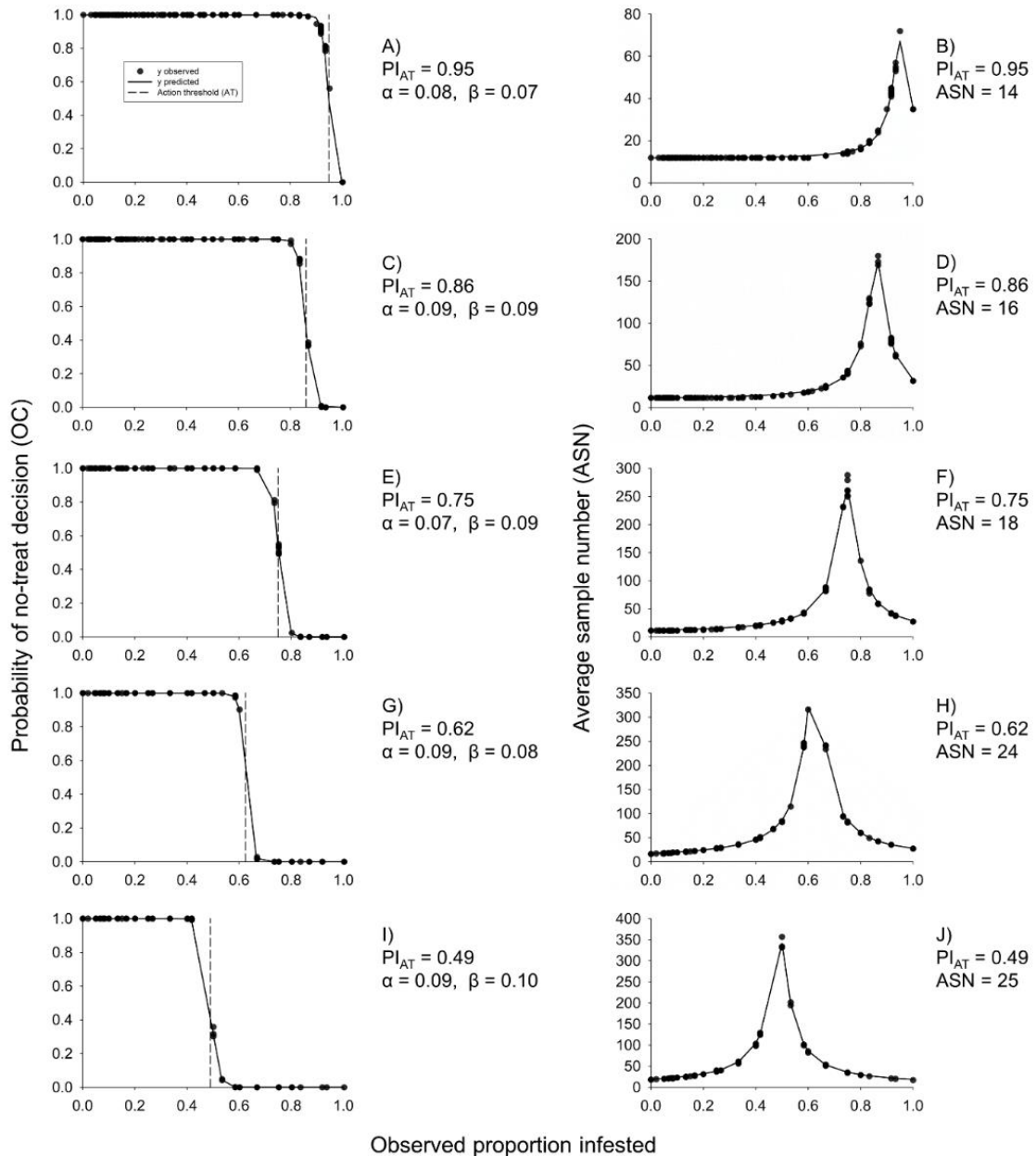
Figure 1.2: Operating characteristic (OC) and average sample number (ASN) functions for binomial sequential sampling plans for action thresholds (AT) based on the proportion of infested sample units that correspond to a mean density of 5 stink bugs per 25 sweeps. The OC (panels A, C, E, F, G and I) and ASN (panels B, D, F, H and J) functions were plotted against the observed proportion infested obtained from resampling software. Panels A and B correspond to tally threshold of 1; C and D correspond to tally threshold of 2; E and F correspond to tally threshold of 3; G and H correspond to tally threshold of 4; I and J correspond to tally threshold of 5. ATs and actual α and β values are shown, based on preset values of $\alpha = \beta = 0.10$ (see Table 1.2).
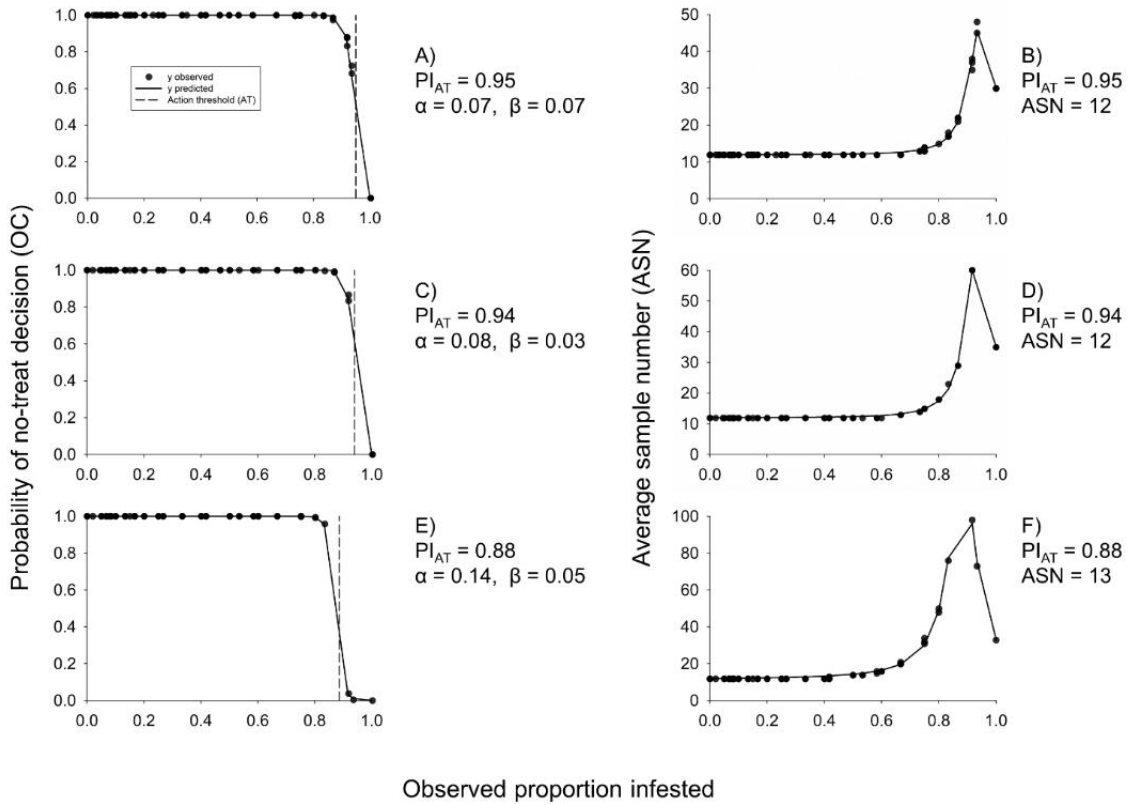
Figure 1.3: Operating characteristic (OC) and average sample number (ASN) functions for binomial sequential sampling plans for action thresholds (AT) based on the proportion of infested sample units that correspond to a mean density of 10 stink bugs per 25 sweeps. The OC (panels A, C, and E) and ASN (panels B, D, and F) functions were plotted against the observed proportion infested obtained from resampling software. Panels A and B correspond to tally threshold of 3; C and D correspond to tally threshold of 4; E and F correspond to tally threshold of 5. ATs and actual α and β values are shown, based on preset values of α = β = 0.10 (see Table 1.2).

Figure 1.4: Decision stop lines for the binomial sequential sampling plans for herbivorous stink bugs in the North Central Region of the United States based on resampling analysis, where sample unit size = 25 sweeps, $\alpha = \beta = 0.10$, and tally threshold = 3 ($\geq$ 3 stink bugs per sample unit to be considered infested). Panel A represents the action threshold of 75% of infested sample units, which corresponds to a mean density action threshold of 5 stink bugs per 25 sweeps (upper line is $y = 6.84 + 0.75x$ and lower line is $y = -6.84 + 0.75x$). Panel B represents the action threshold of 95% of infested sample units, which corresponds to a mean density action threshold of 10 stink bugs per 25 sweeps (upper line is $y = 1.39 + 0.95x$ and lower line is $y = -1.39 + 0.95x$)

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Chapter II: Effects of Starvation, Age, and Mating Status on Flight Capacity of Laboratory-Reared Brown Marmorated Stink Bug (Hemiptera: Pentatomidae)

Summary

The brown marmorated stink bug, *Halyomorpha halys* (Stål) (Hemiptera: Pentatomidae), is an invasive species to North America and has spread throughout most of the territory. Understanding flight in *H. halys* is crucial to understanding the dispersal capacity and developing forecasting models for this pest. The purpose of this research was to assess the effects of starvation, age, mating status, sex, and pre-flight weight on flight parameters of laboratory-reared *H. halys* using computer-monitored flight mills. The mean flight distance observed over a 24-h period was 266 m and the maximum distance was 7.3 km. Overall, the flight capacity of males and females was similar, even though females weighed more than males. The proportion of *H. halys* that initiated flight was not affected by starvation, age, or mating status. The number of bouts of individual flights and velocity significantly increased with longer durations of starvation. The number of bouts significantly decreased with increasing age. Total distance flown and total flight time were not affected by starvation, age, or mating status. Although some statistical differences were seen across the experiments, these differences likely represent minimal ecological significance. Therefore, these results suggest that *H. halys* are remarkably resilient, which may contribute to their success as an invasive species. Findings of this study could help better predict the dispersal potential of *H. halys* in Minnesota.

Introduction

Halyomorpha halys (Stål) (Hemiptera: Pentatomidae), commonly known as the Brown Marmorated Stink Bug, is an invasive species native to east Asia first introduced into the United States in the mid-1990s (Hoebeke and Carter 2003). This insect is polyphagous, with > 300 reported host species, including many of agricultural importance (e.g., tree fruits, vegetables, and row crops) (Kuhar et al. 2012, Rice et al. 2014, Bergmann et al. 2016). *Halyomorpha halys* is a highly mobile pest, able to fly >70 km per day and easily move throughout the landscape in pursuit of preferred host plants (Lee et al. 2014, Wiman et al. 2014, Lee and Leskey 2015). Based on human-mediated transport and innate dispersal capacity, *H. halys* has rapidly spread throughout the globe, including much of North America (e.g., 46 U.S. states and four Canadian provinces), western and eastern Europe, and South America (e.g., Chile) (Haye et al. 2015, Faúndez and Rider 2017, Kriticos et al. 2017, Leskey and Nielsen 2018, Acebes-Doria et al. 2020, NIPMC 2020).

Since its introduction in the United States, feeding from *H. halys* has caused significant agricultural losses in the mid-Atlantic region. A widespread outbreak within this region in 2010 resulted in a ~US$37 million loss in apples and caused severe damage in many other specialty and row crops (Leskey et al. 2012, Leskey and Nielsen 2018). Additionally, feeding damage and economic losses are being reported in the Pacific Northwest and Midwestern United States (NIPMC 2020). The high dispersal capacity, polyphagy, and inter-species competition of *H. halys* is causing growing concern throughout the Midwestern United States, where its status ranges from recently detected or nuisance pest only (i.e., North Dakota, Nebraska, Kansas, Iowa, and Missouri) to an

agricultural and nuisance pest of moderate (i.e., Ohio, Indiana, Illinois, Wisconsin, and Minnesota) to severe (i.e., Michigan) status (NIPMC 2020).

In Minnesota, *H. halys* was first detected in 2010 (Koch 2014). Within the last decade, it has been primarily a nuisance invader of homes and buildings (MDA 2020). Since 2015, mature and immature stages of *H. halys* detected in apple orchards and soybean fields have suggested the existence of breeding populations (Pezzini et al. 2019b, Koch and Aita 2019). Given the diversity of agronomic and horticultural crops at risk, *H. halys* is an emerging threat to Minnesota; if populations continue to increase and spread as the insect has in other states, significant agricultural losses are likely to occur in the future. This pest is able to fly > 75 km (e.g., Wiman et al. 2014, Lee and Leskey 2015) and benefits from human-mediated dispersal through hitch-hiking, facilitating range expansion. Therefore, it is pivotal to understand the dispersal capacity of *H. halys* in the state of Minnesota to mitigate future economic losses.

Dispersal is a critical component for understanding the dynamics and ecology of pest populations (Stinner et al. 1983, Naranjo 2019). Understanding dispersal behavior is crucial for the development of monitoring and management tactics (Zhang et al. 2009, Lee et al. 2014, Lee and Leskey 2015, Grettenberger and Joseph 2019) as well as developing dispersal and forecasting models (Hudgins et al. 2017, Lustig et al. 2017). Various techniques have been used to study the dispersal and movement of *H. halys* in field settings, such as harmonic radar (Lee et al. 2014, Morrison et al. 2016, Kirkpatrick et al. 2019), mark-release-recapture using fluorescent dusts (Rice et al. 2015); protein markers (Blaauw et al. 2016), and the use of black light traps (Nielsen et al. 2013). Tracking insect movement in the field would be the ideal method for examining dispersal

behavior; however, dispersal remains one of the most challenging and intractable processes to accurately characterize in the field (Naranjo 2019). Low recapture rates and limited distance and duration of tracking devices make it impractical to assess long-distance dispersal of insects using some of the methods described above (Yamamura et al. 2003, Franzén and Nilsson 2007, Lopez et al. 2017).

Laboratory tethered flight mills are relatively simple and inexpensive tools for measuring flight characteristics that are difficult to capture in the insect's natural habitat (Minter et al. 2018, Naranjo 2019). Although flight mills have limitations and do not truly represent natural flight conditions (Minter et al. 2018, Naranjo 2019), they are effective tools in determining how multiple experimental treatments can potentially affect the relative flight capacity of individuals (Taylor et al. 2010, Lopez et al. 2014). Flight mills have been used to determine how variable traits such as age (Sarvary et al. 2008, Zhang et al. 2008), feeding status (Fahrner et al. 2014, Wong et al. 2018), sex (Hughes and Dorn 2002, Kees et al. 2017), weight (Lopez et al. 2014, Lee and Leskey 2015), and reproductive status (Colvin and Gatehouse 1993, Schumacher et al. 1997) affect the flight capacities of insects.

To date, there have been a limited number of studies that have examined the flight capacities of pentatomid pests using flight mills. A recent study by Babu et al. (2020) assessed the baseline flight potential of the brown stink bug, *Euschistus servus* (Say), across multiple hosts and found that the longest distance flown by an individual was approximately 16 km and that higher mean dispersal occurs soon after overwintering emergence. In previous work with *H. halys*, the baseline flight potential of tethered field-collected adults showed that approximately 85% of individuals flew up to 5 km in 24 h,

and the longest distance flown by an individual was 117 km (Wiman et al. 2014, Lee and Leskey 2015). To our knowledge, few studies have examined how variables manipulated under controlled laboratory conditions affect the flight capacity of *H. halys* on flight mills. Therefore, the objectives of this study were to assess the effects of starvation, age, mating status, sex, and pre-flight weight on flight parameters of laboratory-reared *H. halys*, including distance flown, total flight time, flight velocity, and bouts of flight (i.e., number of flight events of an individual). Findings from this study could help better predict the dispersal capacity of *H. halys*, enhancing risk assessment analyses for future range expansion of this pest.

Materials and Methods

Insects

Halyomorpha halys adults were reared in a laboratory colony at the University of Minnesota. The colony was first established in 2012 with individuals collected from a residence in Wyoming, MN (Govindan and Hutchison 2020). In the fall of each year, additional individuals were collected from this location subjected to diapause conditions in the laboratory and added to the colony to preserve genetic diversity (Govindan and Hutchison 2020). Mixed-sex groups of 40-50 *H. halys* were maintained in 34×34×61-cm mesh rearing cages (BioQuip Products, Rancho Domingues, CA, USA) at ~25°C, ~70% RH, and a 16:8 (L:D) h photoperiod in walk-in laboratory rearing rooms. Insects were provisioned with an *ad libitum* supply of dried raw organic sunflower (*Helianthus annuus* L.) (Asterales: Asteraceae) and soybean (*Glycine max* (L.) Merr.) (Fabales: Fabaceae) seeds, carrots (*Daucus carota* subsp. *sativus* (Hoffm.) Schübl. & G. Martens) (Apeales: Apeaceae), and pods of green beans (*Phaseolus vulgaris* L.) (Fabales: Fabaceae)

(Dingha and Jackai 2017). To maintain humidity, cages were misted with deionized water every weekday. In addition, each cage was provided with flats of vegetative-stage green bean plants (V2-V3 stage) to serve as an ovipositional substrate (Shanovich et al. 2020). Egg masses were collected on a daily basis from the underside of the green bean leaves and placed in 55×15-mm Petri dishes (Fisher Scientific, Pittsburgh, PA, USA) with a half piece of moistened 5.5-cm-diameter filter paper (Fisher Scientific, Pittsburgh, PA, USA). Petri dishes were held in a growth chamber (Percival Scientific, Inc., Perry, IA, USA) under similar environmental conditions as the walk-in laboratory rearing rooms and eggs were reared to second instar nymphs. The second instars were then transferred to mesh cages (31×31×31-cm) and reared under the same conditions as described for the adults. Upon eclosion, newly emerged adults (i.e., ≤ 24-h old) were maintained according to the specifications of each experimental protocol described below.

Flight mills

Twenty-two computer-monitored flight mills were used to investigate the flight capacity of *H. halys*. Detailed information for flight mill construction (i.e., design, data acquisition and output) can be found in Fahrner et al. (2014) and Kees et al. (2017). Insects were attached to the tip (bent 90°) of an 8-cm tether arm (solid 33-gauge [American] copper wire) using a droplet of glue (Surebonder®, Wauconda, IL, USA) from a low-temperature glue gun (Stanley®, Jackson, TN, USA), gently pressed against the center of *H. halys* pronotum. This attachment method was similar to that used for field-collected *H. halys* by Wiman et al. (2014) and Lee and Leskey (2015). After attachment, but prior to placement on the flight mills, the hemelytra of *H. halys* were carefully examined to ensure that wing movement was not noticeably inhibited by the

tether arm or glue. To ensure proper balance of the tether arm on the flight mill, a counterbalance was added to the opposite end. Once the insects were successfully attached, the tether arm was placed into the flight mill to begin recording data.

All movements of the tether arm, including actual flight activity and pseudo-movements due to air current or accidental bumps during trial initiation, were recorded by an infrared (IR) sensor. The pseudo-movements can be identified and omitted to avoid misleading results (Fahrner et al. 2014, Kees et al. 2017). To exclude potential spurious recordings, thresholds for flight speed and bouts of flight were implemented. Following the initial flight, a limit for maximum speed was set at 2 m/s (7.2 km/h) and a minimum of twelve revolutions were set as criteria for counting flights. These thresholds were set following personal observations of *H. halys* during a tethered flight.

Experiments

All flight experiments took place at room temperature of ~24 ºC, ~55% RH, and were of 24-h duration in constant light (2700K fluorescent; ~1955 lux). Individual insects were not re-used for other experiments after a 24-h flight trial. For all experiments, insects were weighed to the nearest 0.001g using an analytical balance (Sartorius Entris® 224, Sartorius AG, Göttingen, Germany). In addition, the sex of *H. halys* was recorded for all experiments after the methods of Vétek et al. (2014). A maximum of 22 insects could be flown on a given day, so each experiment was carried out over multiple days (i.e., temporal blocks). Three separate experiments were conducted to test the effects of starvation (i.e., number of days without food), age (i.e., number of days after emergence of adults), and mating status (i.e., unmated vs. mated) on the flight capacity of *H. halys*.

Experiment 1 – Effects of starvation: To determine the effects of starvation on

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flight capacity of *H. halys*, groups of mixed-sex adults were selected from the laboratory colony. Insects were of uniform age, collected approximately 7d after emergence of adults. These adults were transferred into circular 18.5×8-cm plastic dishes (Pioneer Plastics, Inc., North Dixon, KY, USA) with a circular 9.0-cm-diameter filter paper (Fisher Scientific, Pittsburgh, PA, USA) and provided water *ad libitum* through moistened cotton wicks. Each dish with insects was randomly assigned to one of four durations of starvation: 0 (i.e., no starvation), 1, 3, and 7 days. Dishes were held in a growth chamber (Percival Scientific, Inc., Perry, IA, USA) under the same environmental conditions as the laboratory colony for the desired durations of starvation. To have individuals from each starvation duration ready to fly on the same day, food was provided to the individuals from the time of set up of the dishes for the entire 7 days (i.e., 0-d starvation), for the first 6 days (i.e., 1-day starvation), for the first 4 days (i.e., 3-day starvation), or no food provided over the entire 7 days (i.e., 7-day starvation). A total of 319 *H. halys* were placed on the flight mills for this experiment.

   Experiment 2 – Effects of age: To determine the effects of age postemergence on flight capacity of *H. halys*, newly emerged second instars were transferred to individual mesh cages and held in a growth chamber under the same environmental conditions and food provisioning as the laboratory colony described earlier. Each individual cage contained a cohort of mixed-sex *H. halys* adults of the same age. Age groups ranged between 3 and 47 d post emergence of adults and insects from multiple age groups were randomly assigned across the 22 positions on the flight mill on a given day. A total of 106 *H. halys* were placed on the flight mill.

   Experiment 3 – Effects of mating status: To determine the effects of mating

status on flight capacity of *H. halys*, individuals were assigned upon emergence as adults to two groups: unmated (i.e., males and females were kept isolated from one another for 15 days in preparation for the experiment) or mated (i.e., male-female pairs were kept together for 15 days in preparation the experiment and visually checked daily to ensure that mating occurred). Both groups were placed inside 100×20-mm Petri dishes (Fisher Scientific, Pittsburgh, PA, USA) with ~25 0.5-mm diameter holes in the lid for ventilation. A 9.0-cm-diameter piece of filter paper (Fisher Scientific, Pittsburgh, PA, USA) was placed in the dish and moistened as needed, and food was provided by adding fresh organic green beans, carrots, and dry soybean seeds every two to three days. Immediately following a 24-h flight trial and weighing of individuals, adults were placed into the freezer at approximately -20 °C for later dissection. Females were dissected in Dulbecco's Phosphate Buffered Saline 1X (DPBS) with calcium and magnesium solution (Meditech Inc., Manassas, Virginia, USA) to record the mating status. To determine mating status, spermatheca condition was examined following methodologies from Cullen and Zalom (2006) and Nielsen et al. (2017), which described virgin females having a slender and translucent spermatheca and mated females having an enlarged and opaque spermatheca. A total of 141 *H. halys* were placed on the flight mill, with between 28 to 40 individuals for each mating status of each sex.

Data extraction and statistical analyses

The recording of the flight parameters such as the number of rotations per flight and flight duration was performed using customized LabVIEW software (National Instruments Corporation, Austin, TX, USA). The extraction of flight metrics from the raw phase-change data were conducted using R Development Core Team software

(version 3.6.3, 2020) following methodologies described by Fahrner et al. (2014) and Kees et al. (2017). Flight variables calculated to characterize the flight capacity of *H. halys* were total flight distance, time, velocity and bouts. The use of more than one response variable is appropriate for flight mill experiments, because using a single variable might fail to reveal flight differences or can lead to misleading results (Dingle 1985, Luo et al. 2002).

All data were analyzed using R version 4.0.0 (R Core Team 2020). Linear mixed-effect models were used to analyze the data for all experiments. The effects of starvation and the covariates of sex, pre-flight weight and their 2- and 3-way interactions on distance flown, total flight time, flight velocity, and bouts of flight were analyzed using separate simple linear regressions for each response variable using the function '*lmer*' from package 'lme4' (Bates et al. 2015). The effect of age on the flight-related response variables was analyzed the same as for the effect of starvation, with the same covariates. The effects of mating status (i.e., categorical variable) and the covariates of sex, pre-flight weight and their 2- and 3-way interactions on distance flown, total flight time, flight velocity, and bouts of flight were analyzed using separate analysis of variance (ANOVA type II) with chi-square tests for significance of each response variable using the function '*Anova*' from package 'car'. Means were compared by obtaining least-square means using the function '*lsmeans*' from package 'lsmeans' (Lenth 2016) adjusted for Tukey's HSD test. The effects of starvation, age and mating status on pre-flight weight were tested separately, with each analysis including sex as a covariate and the 2-way interaction using analysis of variance (ANOVA type II), similarly as described above. For all models, graphical examinations of residual plots were used to check assumptions

of normality and homoscedasticity of the errors. To meet model assumptions, some

response variables (e.g., distance flown, total flight time, flight velocity, and bouts of

flight) were subjected to variance-stabilizing logarithmic transformations. In addition,

generalized linear mixed-effect models were used to analyze the probability of insects

flying as a function of starvation, age, and mating status including the covariates of sex,

pre-flight weight and their 2- and 3- way interactions for each analysis. The function

'*glmer*' from the package 'lme4' with a binomial distribution and logit as the link-

function was used. For all described above models, terms for electronic flight channel

and day of the trial were included as random effects. The best fitting models were

selected from excluding non-significant factors and interaction terms through stepwise

backward process. Pre-flight weight showed high correlation coefficients (≥ 0.7) with the

response variables for all models and therefore was removed as a predictor from the

analysis. Logarithmic model predictions and 95% CIs were back-transformed using

exponential function and are reported in the text.

Results

General flight performance

The flight parameters of *H. halys* across all three experiments are summarized in

Table 2.1. Overall, 566 adults were flown during the three experiments (283 females, 283

males). From this total, 80% ($n = 453$) of the insects initiated flight; only 1% ($n = 6$) of

the individuals had died at the end of a 24-h flight trial and were not included in the

analysis. Among the individuals that initiated flight, the frequency distribution for total

flight distance for both sexes was highly right-skewed with 93.1% ($n = 422$) of

individuals flying <1 km in 24 h. Approximately 2% ($n = 9$) of individuals flew >2 km.

Mean flight distance was 266.51 ± 31.44 SEM m with a maximum distance flown of 7.3 km during a 24-h flight mill trial (Table 2.1). Total flight time ranged from 11.4 s to 3.6 h (6.57 ± 0.85 SEM min), and mean flight velocity was 0.64 ± 0.01 SEM m/s (Table 2.1). Number of flight bouts ranged from 1 to 25 (3.57 ± 0.16 SEM) (Table 2.1).

Effects of starvation, age, and mating status on pre-flight weight of *H. halys*

Females were significantly heavier (0.193 ± 0.005 g) than males (0.119 ± 0.002 g) ($F = 103.07$; df = 1, 62; $P < 0.001$) for the starvation experiment. Preflight weight decreased with increasing duration of starvation ($F = 100.28$; df = 1, 122; $P < 0.001$), and the rate of weight loss varied between sexes ($F = 30.28$; df = 1, 118; $P < 0.001$) (Figure 2.1a). Females lost weight at a rate of 10 ± 1 mg/d, while males only lost 3 ± 1mg of weight per day when food was withheld (Figure 2.1a).

Females were significantly heavier (0.238 ± 0.005 g) than males (0.130 ± 0.001 g; $F = 361.2$; df = $_1$, 103; $P < 0.001$) for the age experiment. Individuals gained weight as they grew older (F = 12.00; df = 1, 78; $P < 0.001$). Females gained weight at a rate of 1.5 ± 0.3 mg/day and males gained weight at a rate of 0.2 ± 0.1 mg/day (F= 6.30; df = 1, 98; P = 0.013) (Figure 2.1b).

Females were significantly heavier (0.219 ± 0.003 g) than males (0.119 ± 0.001 g; $F = 233.06$; df = 1, 8; $P < 0.001$) for the mating status experiment. Preflight weight of individuals was significantly affected by mating status ($\chi^2 = 14.54$, df = 1, $P < 0.001$), and by the interaction of mating status and sex of individuals ($\chi^2 = 12.76$, df = 1, $P < 0.001$) (Figure 2.1c). Mated females were heavier (0.230 ± 0.005 g) than unmated females (0.207 ± 0.004 g), while for males the preflight weight of mated (0.1189 ± 0.002) and unmated (0.1193 ± 0.002) were not statistically different ($P > 0.05$) (Figure 2.1c).

Effects of starvation on flight capacity of *H. halys*

Of the 319 *H. halys* placed on flight mills for this experiment, 245 individuals

(76.8%) initiated flight (128 females, 117 males). The proportion of *H. halys* that

initiated flight was not affected by duration of starvation, sex of individuals or the

interaction of duration of starvation and sex ($P > 0.05$). The number of bouts of flight

during a 24-h period was significantly affected by duration of starvation ($F = 5.60$; df =

1, 191; $P = 0.018$), with number of bouts increasing with longer durations of starvation

(1.04 bouts/d; 95% CI [1.00, 1.09]) (Figure 2.2c). However, number of bouts was not

affected by sex or the interaction between duration of starvation and sex ($P > 0.05$).

Although flight velocity was affected by duration of starvation ($F = 6.16$; df = 1, 220; $P =$

0.013) and sex ($F = 14.54$; df = 1, 98; $P < 0.001$), the interaction was not significant (P >

0.05) (Figure 2.2d). Across durations of starvation, males flew 0.088 m/s faster (95% CI

[0.083, 0.093]) than females and for both sexes a 1-d increase in duration of starvation

was associated with 1.02 m/s increase (95% CI [1.00, 1.03]) in flight velocity (Figure

2.2d). Total distance flown (Figure 2.2a) and total flight time (Figure 2.2b) were not

affected by duration of starvation, sex, or the interaction ($P > 0.05$).

Effects of age on flight capacity of *H. halys*

Of the 106 *H. halys* placed on flight mills for this experiment, 94 individuals

(88.7%) initiated flight (44 females, 50 males). The proportion of *H. halys* that initiated

flight was not affected by age, sex of individuals or the interaction of age and sex ($P >$

0.05). Number of bouts was not affected by sex or the interaction of sex and age ($P >$

0.05). However, number of bouts was significantly affected by age ($F = 8.14$; df = 1, 88;

$P = 0.0053$), with the number of bouts decreasing by 0.98 bouts per day with age (95%

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CI [0.97, 0.99]) (Figure 3.3c). Total distance flown (Figure 3.3a), total flight time (Figure 3.3b) and flight velocity (Figure 3.3d) were not affected by the age of individuals, sex, or the interaction ($P > 0.05$).

Effects of mating status on flight capacity of *H. halys*

Of the 141 *H. halys* placed on flight mills for this experiment, 114 individuals (80.8%) initiated flight (63 females, 51 males). The proportion of *H. halys* that initiated flight was not affected by mating status, sex or the interaction of mating status and sex of individuals ($P > 0.05$). Mating status did not affect the response variables in this study (i.e., total distance flown, number of bouts, total flight time, and flight velocity) ($P > 0.05$).

Discussion

Halyomorpha halys is a significant nuisance and agricultural pest that is highly mobile and can rapidly spread throughout the landscape. Although some statistical differences were seen across our experiments, especially for number of flight bouts and velocity, these differences likely represent minimal ecological significance. These results show that *H. halys* are remarkably resilient and demonstrate little to no increase or decrease in flight capacity with food deprivation, advancing age, or mating status, which likely contributes to their emergence as a highly successful invasive species.

In contrast to our results, some studies with other insects have shown increasing movement over an initial period of starvation followed by a decrease in movement due to mortality, lack of energy reserves, or other chronic stresses as starvation continues (Elsey 1974, Fadamiro and Wyatt 1995, Perez-Mendoza et al. 2011, Scharf 2016). One study investigated the aspects of flight metabolism on two stink bug species, *Encosternum*

delegorguei (Hemiptera: Tessaratomidae) and *Nezara viridula* (Hemiptera:

Pentatomidae), and found that during intensive flight activity lipid oxidation in the flight

muscles is the main source of energy production. If durations of food deprivation greater

than one week were used in the present study, *H. halys* would likely have shown an

eventual decrease in flight capacity. The increasing number of bouts with increasing

duration of starvation indicates the insects were stopping and restarting flight more

frequently as their hunger increased, which likely increases their success in finding food

across a landscape.

Individuals used in the present experiment ranged from 3 to 47 d old, but

laboratory-reared *H. halys* males and females can survive for approximately 60 and 85

days at 27 ℃, respectively (Govindan and Hutchison 2020). If individuals older than

those flown in the present study had been used, the results may have shown an eventual

decrease in flight capacity, as has been shown with other insects. For example, Maharjan

and Jung (2009) compared flight parameters of the bean bug, *Riptortus clavatus*

(Thunberg) (Hemiptera: Alydidae) in relation to its age and found that flight activities

were higher in 25-30 d old individuals compared to 5 and 45 d old individuals. Similarly,

for three species of *Adelphocoris* (Hemiptera: Miridae), which are pests of cotton in

China, the flight capacity of individuals was low for 1-d old adults, gradually increased

with age until 10- to 13-d old, and then gradually decreased until 25-d old individuals (Lu

et al. 2009). The number of bouts decreased with increasing age, which may suggest that

older *H. halys* are more prone to preserve energy resources that would otherwise be used

for more frequent flights.

Impacts of mating status on flight capacity seem to be species-specific. In the present study, mating status of females and males had no significant effect on flight capacity of *H. halys*. Similarly, mating status of males and females had little to no effect on flight performance of three *Adelphocoris* spp. (Heteroptera: Miridae) (Lu et al. 2009). In addition, (Lu et al. 2007) found no significant effect of mating status on flight capacity of *Lygus lucorum* (Meyer-Dür) (Heteroptera: Miridae).

Flight performance of insects generally tends to increase as the percentage of body weight comprised of flight muscle increases (Lopez et al. 2017). However, the size of flight muscles may fluctuate based on varying biotic and abiotic factors and therefore might not always be correlated with body size (Marden 2000, Lopez et al. 2017). The experimental manipulations (e.g., starvation, age and mating status) performed in this study affected *H. halys* body mass and therefore were expected to impact flight capacity of this insect. In addition, female Pentatomidae are generally larger than their male counterparts (Panizzi et al. 2000), which corroborates the higher pre-flight body weights found for female *H. halys* in the present experiments (Figure 2.1). However, the relatively larger females in our experiments did not demonstrate higher flight capacity than the males, perhaps due to the fact that body weight also includes lipid content, water content and non-flight musculature (Shelton et al. 2006, Lopez et al. 2014). Lu et al. (2019) actually found that males of the sycamore lace bug, *Corythucha ciliata* (Hemiptera: Tingidae) had significantly greater flight speed than females over a 24-h flight mill study.

While laboratory flight mill studies provide useful insights regarding several variables that are difficult to quantify in the field, appropriate caution must be exercised

when interpreting flight mill data (Riley et al. 1997, Taylor et al. 2010, Naranjo 2019). Limiting factors such as handling and tethering of insects, lack of natural stimuli, increased weight load, and reduced energy expenditure of tethered insects can impact results; therefore, it is important to design experiments that use a comparative approach (Wong et al. 2018). We investigated the flight capacity of *H. halys* through a comparative process focusing on the relative changes in behavior due to specific biotic factors that we manipulated, while controlling other aspects of rearing and the flight mill process. Distances flown for the laboratory reared *H. halys* in this experiment were generally lower than those observed for wild-collected *H. halys* (Wiman et al. 2014, Lee and Leskey 2015) and other wild-collected Pentatomidae (Babu et al. 2020) assessed on flight mills. Such differences between laboratory-reared and wild-caught individuals have been observed for other insects (Baker et al. 1980, Nakamori and Simizu 1983, McKibben et al. 1988). Despite this, we chose to use laboratory-reared individuals, so we could carefully control the rearing conditions to allow manipulation and comparison of the effect of certain factors. Flight mill studies should be performed in a comparative process for laboratory-reared individuals (Naranjo 2019).

Individuals used in this study, which originated from individuals collected in Minnesota, represent a distinct population possibly with different traits from those used in previous *H. halys* flight studies. For example, the Minnesota population developed faster and survived at higher rates compared to eastern populations (Govindan and Hutchison 2020). Understanding the biological attributes of local *H. halys* dispersal is key to the improvement of integrated pest management strategies to successfully manage this pest in Minnesota, especially exploiting strategies that depend on insect movement

such as the attract-and-kill strategy (El-Sayed et al. 2009, Morrison et al. 2019). Future

research will use data from this study to parametrize dispersal models for *H. halys* to help

predict its spread throughout the state.

Table 2.1: Summary of flight parameters calculated for all three experiments of *H. halys* tested on flight mills. Only insects that flew were included in the analysis. Insects were placed on the flight mill for 24 hours and were not re-used.

$n = 453$	Distance (m)	Total flight time (min)	Velocity (m/s)	Bouts
Minimum	6	0.19	0.23	1.0
Median	48	1.30	0.55	2.0
Mean	266	6.57	0.64	3.6
Maximum	7269	216.11	1.45	25.0
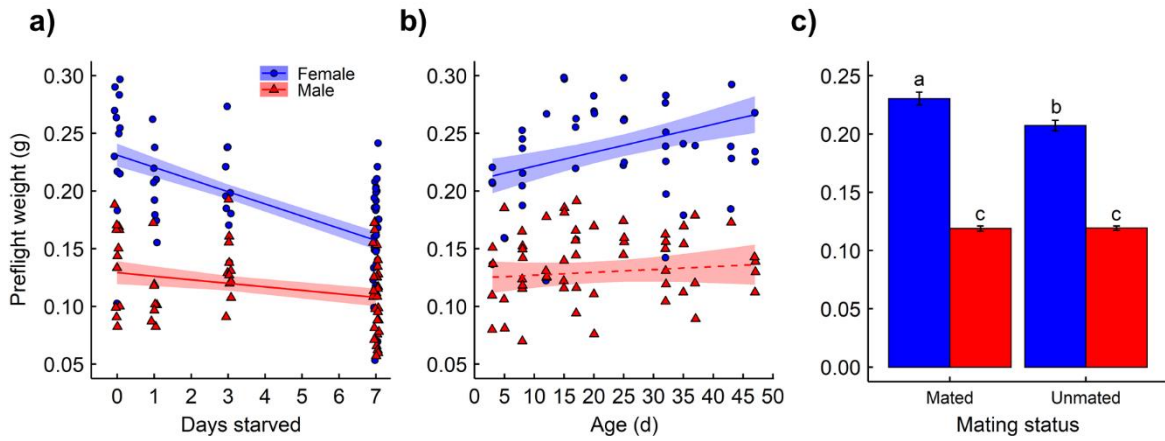
Figure 2.1: Effects of duration of starvation (a), age post emergence (b), and mating status of adult *H. halys* on preflight weight of males and females. For (a) and (b), solid lines represent statistically significant trend lines and shaded regions represent 95% confidence interval for model predictions. For (c), treatment means with the same letter are not significantly different (Tukey HSD, $P > 0.05$).
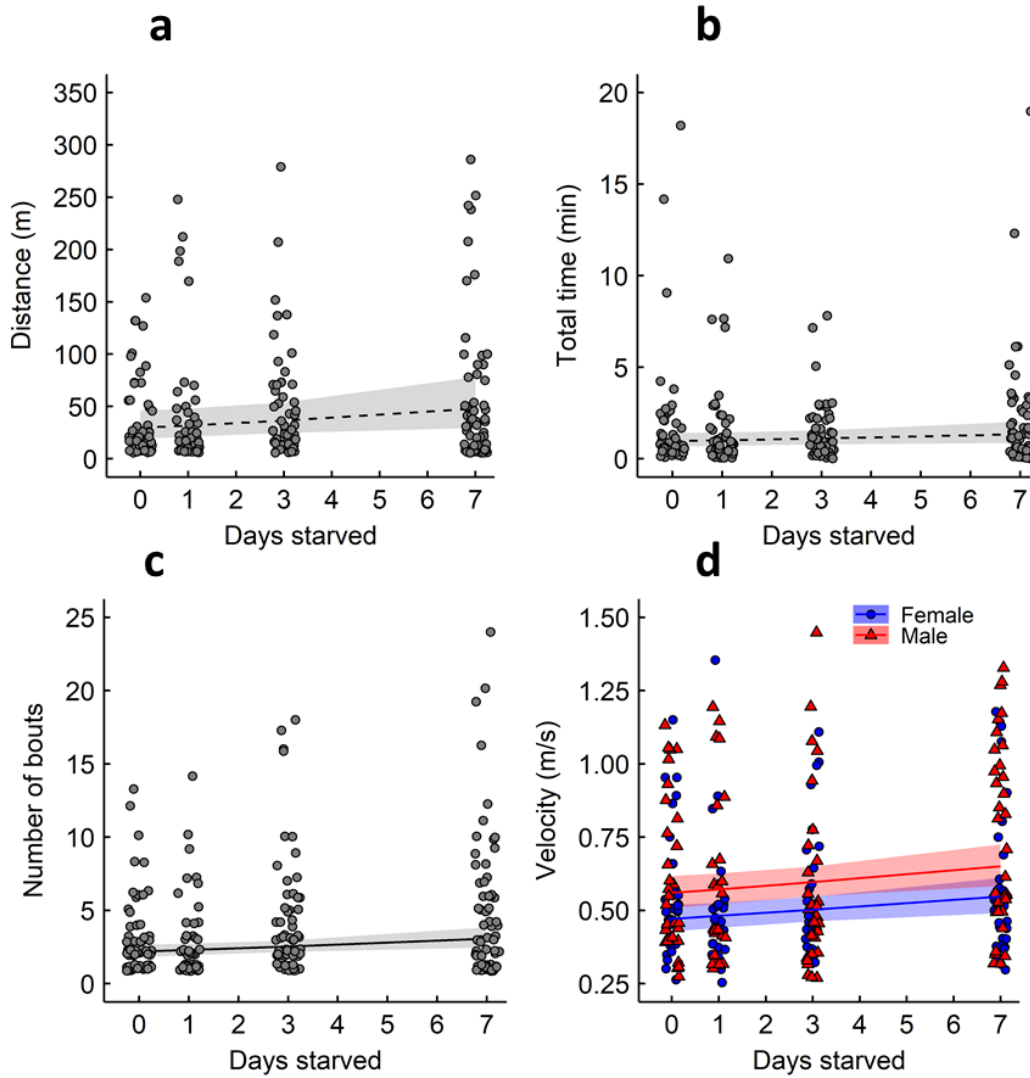
Figure 2.2: Effect of duration of starvation of *H. halys* adults on (a) total distance flown, (b) total flight time, (c) number of bouts, and (d) velocity over 24-h flight trials. Solid lines represent statistically significant trend lines. Shaded regions represent 95% confidence interval for model predictions. Only individuals that flew were included in these analyses (*n* = 245).
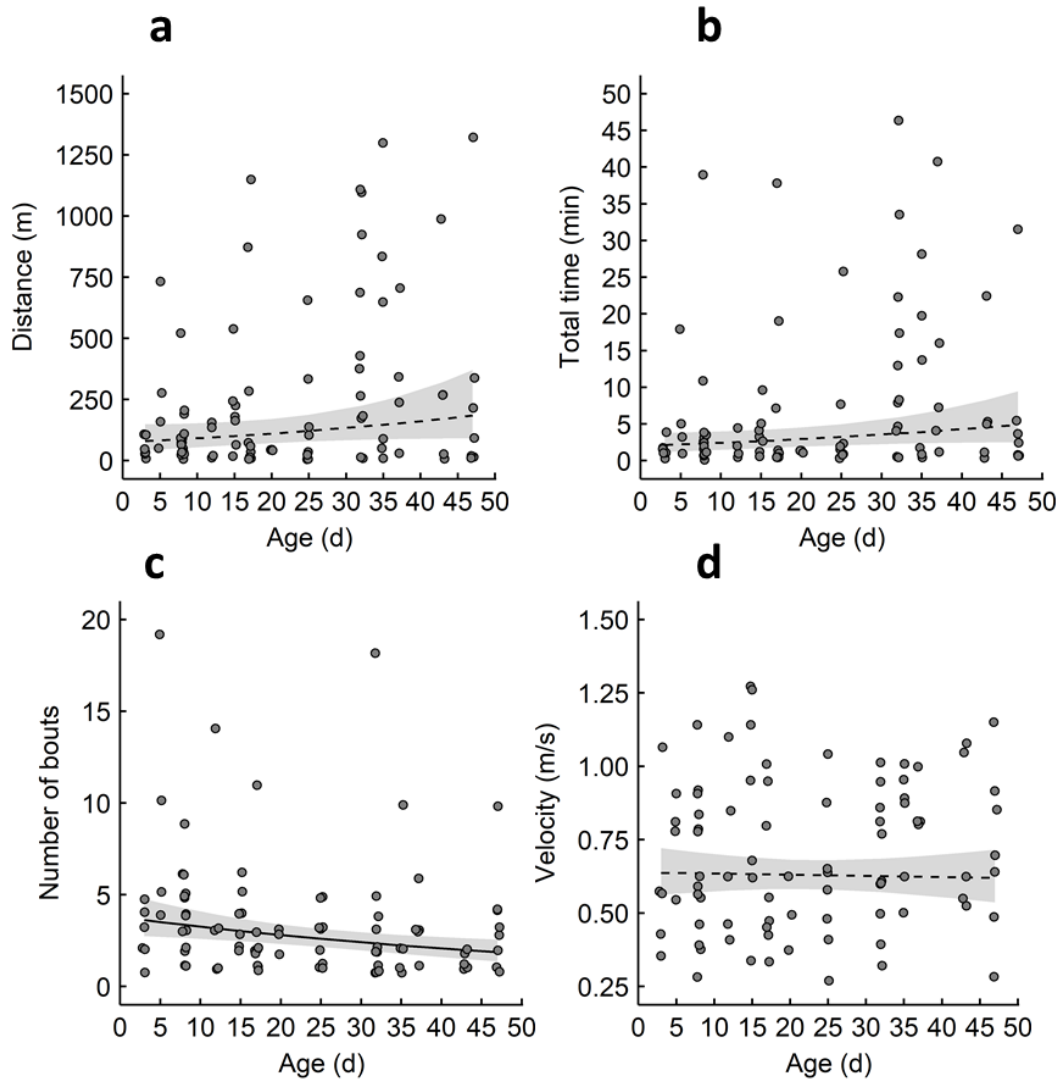
Figure 2.3: Effect of age postemergence of *H. halys* adults on (a) total distance flown, (b) total flight time, (c) number of bouts, and (d) velocity over 24-h flight trials. Solid lines represent statistically significant trend lines. Shaded bands represent 95% confidence interval for model predictions. Only individuals that flew were included in these analyses (*n* = 94).

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