SAMPLING TECHNIQUES AND POPULATION ESTIMATION FOR *ATHERIGONA REVERSURA* VILLENUEVE (DIPTERA: MUSCIDAE) IN BERMUDAGRASS HAY FIELDS

by

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(Under the Direction of William G. Hudson)

ABSTRACT

Atherigona reversura Villenueve (Diptera: Muscidae) has the potential to become a substantial threat to bermudagrass (*Cynodon dactylon* Pers.) hay fields through much of the southeastern and south central USA. In order to determine a protocol for treatment, a better understanding of *A.reversura* biology and a method for population estimation is needed. Regression analysis of an absolute and relative sampling technique yielded an equation [Density = $2(e^{-0.723}$ x sweep net count$^{0.698}$), where Density = the number of flies/m² and sweep net count = the number of flies /10 sweep sample] to estimate the population of adult flies in a given hay field. The pupation period of *A. reversura* was determined to be 7-10 days on a 14:10 (L: D) photoperiod at two different temperature regimes (29.4°C/27.4°C and 27.0°C/25.0°C). The adult lifespan was 15-25 days when fed sugar water.

INDEX WORDS: bermudagrass, *Cynodon dactylon*, population estimation, biology, invasive, *Atherigona reversura*, sampling techniques

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CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

Bermudagrass Production. Bermudagrass (*Cynodon dactylon* (L). Pers.) is a warm-season perennial grass and is the most common forage used for livestock grazing and hay production in the Southeastern United States. Believed to have been introduced in the mid 1700's in Savannah, Georgia, bermudagrass dispersed naturally through much of the South by the 20th century. In 1928, the University of Georgia's Coastal Plain Experimental Station (CPES) in Tifton, GA, initiated a bermudagrass breeding program in order to develop the forage potential of the grass. Local cultivars were crossbred with varieties from all over the world including southern Africa and South America (Hanna et al. 2010). The ongoing program has developed many improved (i.e. increased digestibility, growth rate, hardiness) cultivars of bermudagrass such as 'Coastal', 'Tifton 85', and 'Tifton 44' (Hancock et al. 2010). In addition to improving the grass, the CPES also established techniques to vegetatively propagate improved cultivars, which ultimately led to its widespread cultivation throughout an estimated 10-15 million acres in the Southeastern United States today (Hanna et al. 2010; Taliaferro et al. 2004).

Information on *Atherigona.* In 2010, reports of extensive damage to bermudagrass hay fields in Georgia, Florida, South Carolina and Alabama subsequently revealed *Atherigona reversura* Villenueve (Diptera:Muscidae), commonly known as the bermudagrass stem maggot (BSM), as the causal agent (Hudson 2010). Additional reports from California and northern Mexico were confirmed in 2013 and it is thought that the BSM can now be found throughout much of

southeastern USA and south central North America (Grzywacz et al. 2013). Whether its presence is a result of multiple introductions or a single event is unknown. The BSM causes the death of the apical shoot of bermudagrass as it feeds just outward of the terminal node where it damages the vascular tissue and subsequently feeds on the decaying tissue. This feeding mechanism stunts the growth of the plant forcing any regrowth to initiate through new tillers from basal nodes or the apical node directly below the damage.

Overall, literature on *A. reversura* itself is largely limited to morphological descriptions for taxonomic purposes. First described by Villenueve in 1936, further detail of the adult stages were provided by Pont and Magpayo (1995) which also included geographical ranges and a list of plants from which *A. reversura* larvae were reared. The immature stages were recently described, with SEM documentation, by Grzywacz et al. in 2013.

A. reversura is a member of the muscid shoot fly genus *Atherigona* Rondani which includes over 200 described species widely distributed throughout tropical and subtropical regions of the Old World (Pont 1981; Pont and Magpayo 1995). The flies are distinguished from other muscids in that they are small (3-5 mm), gray or yellowish gray flies with a long face and large antennal flagellomeres (Pont 1981; Pont and Magpayo 1995). The genus is divided into two subgenera, *Acritochaeta* Grimshaw and *Atherigona* s. str., which are distinguished by the feeding habits of the larval instars (Pont and Magpayo 1995). The feeding habits of adult members of *Atherigona* remain undescribed. Larvae of the species of *Acritochaeta* Grimshaw are considered saprophages; feeding on dead plant material and in some cases animal carrion (Pont and Magpayo 1995). Although it is known that one species, *Atherigona orientalis* Schiner, may attack fresh produce, they are not considered to be pests of fruits or vegetables (Ogbalu 2005).

Members of the subgenus *Atherigona* s. str. are separated from those of *Acritochaeta* Grimshaw in that they attack living plant material. Known commonly as shoot-flies, they are considered as primary pests of several species of Poaceae often causing serious economic damage. Eggs are laid on the shoot of the host plant and upon hatching the larvae bore down the shoot, cut the growing point and subsequently feed their way upwards on the decomposing tissue (Nwanze 1998; Pont and Magpayo 1995). There is some uncertainty as to whether larval instars feed on living or dead plant material. *Atherigona soccata* Rondani larvae were believed, based solely on observational data, to feed and derive nourishment from the living tissue in the 1st and 2nd instars (Raina 1981). However, Kalaisekar et al. (2013) found that the 1st instar larvae of *A.soccata* molt subsequently after cutting of the growing point and the 2nd instar feeds solely on the decomposing tissue. The 3rd instar larvae are known to feed on decomposing tissue and are described to move up and down the shoot feeding on the decomposing tissue (Hardy 1985; Raina 1981). The presence of oral ridges in the mouthparts of larvae is a characteristic of cyclorrhaphan fly species that use the structures to direct fluid into the mouth and these are reduced or absent in phytophagus species (Courtney et al. 2000). Oral ridges were found to be reduced in 3rd instar larvae of both *A. soccata* and *A. reversura* (Deeming 1971; Grzywacz et al. 2013). These findings, as well as the presence of massive oral bars used for cutting tough plant material, suggest both phytophagous and saprophagous feeding behaviors (Grzywacz et al. 2013).

Species of *Atherigona* s. str. are known to be pests of sorghum, rice, wheat, corn, and barley in the Oriental region (Pont and Magpayo 1995). *A. soccata*, in particular, is considered to be the major pest of sorghum and causes serious economic damage (Nwanze 1998). *A. orientalis* and *A. reversura* are the only known species to be introduced into North America (Carvalho et al. 2005; Hudson 2010). While *A. orientalis* has been described as a minor pest of pepper plants

and melons, the only reports of *A. reversura* damage have been in bermudagrass hay fields and turfgrass (Ogbalu 2005; Hudson 2010). However, *A. reversura* has been reported to have been reared from the following: *Echinochloa colona* (L.) Link, *Eleusine coracana* (L.) Gaertn., *Eriochloa procera* (Retz.) C.E. Hubb., *Sehima nervosum* (Rottl.) Stapf., *Sorghum bicolor* (L.) Moench and *Zea mays* L. (Pont and Magpayo 1995). In addition, the ovipositional preference of *A. reversura* was shown to be for finer textured varieties of bermudagrass such as 'Coastal', 'Alicia' and common which provide denser canopies than coarse textured cultivars (Baxter 2014, Ikeda 1995). These findings coupled with field observations lead us to concentrate on fields with 'Alicia' and other fine textured varieties for the comparison of sampling techniques. Finally, a recent study of the reproductive potential of *A.reversura* found the total number of ovarioles per female ranged from 24 to 45 with a mean of 32.1 ± 3.4 and the total number of mature oocytes per female ranged from 11 to 40 with a mean of 27.6 ± 5.7 (Baxter 2014). Male and female *A.reversura* can be distinguished from each other in that the female has a longer, sharper abdomen in contrast to the male's which is short and round.

It is the purpose of this study to further explore the biology of *A. reversura* with particular emphasis on pupation period, because larvae are known to exit grass shoots upon cutting of hay fields. Moreover, a reliable method for estimating the population of *A. reversura* in a given hay field is needed in order to monitor population dynamics and conduct the ecological studies necessary to formulate coherent management plans.

Sampling insect populations. In order to implement effective Integrated Pest Management (IPM) strategies in agriculture, a sampling plan must first be established that can estimate with precision the number of pests per unit of measure (Norris 2003). This population estimation, through proven sampling methods, is essential to the calculation of thresholds involved in

management decisions (Norris 2003). There are several elements of a sampling plan that must be developed in order to correctly monitor insect pests. The variability of sampling methods, sample unit size, number of samples to be taken, sample technique used, as well as the time of day and temperature at which samples are taken are all aspects that need to be considered in order to provide precise population estimates in a given sampling protocol (Norris 2003, Dent 2000). Furthermore, a phenological knowledge of the life stages present and spatial distribution of the pest are also required to help direct these assignments (Dent 2000). When considering holometabolous insects, as is the case with the BSM, developmental periods of larvae and pupae as well as the life span of adults need to be considered in order to choose the appropriate sample unit and technique (Norris 2003).

A sample unit can be defined as a given area of habitat in which the pest can be found and collected (Morris 1955). Whereas, the sampling technique is the device and manner in which data are gathered from the sample unit, which in turn often directs the size and form of the latter (Buntin 1994). It is necessary when first initiating a sampling program to experiment with various sampling techniques and examine the variability of each through statistical analysis as well as comparing them to one another (Dent 2000). Estimates of population that are derived from sampling techniques can be classified as being either absolute or relative (Morris 1955). Absolute samples are derived from a census count of pests per unit of land area and are often tedious and costly because they require every pest in the defined area to be accounted for. For mobile insects, this often involves the use of a cage combined with a vacuum or fumigant. In general, relative estimates are quicker and less costly and therefore used more often. They are often not related to a unit of area and are instead based on the unit of effort of the sampling technique employed and must be calibrated through absolute estimates (Buntin 1994). An

example of a relative estimate would be that of a sweep net, where pests are represented per sweep or number of sweeps.

CHAPTER 2

MATERIALS AND METHODS

Sampling. In the summer of 2013, we attempted to initiate a sampling plan for the purpose of monitoring populations of the *A.reversura* in bermudagrass hay fields. In order to determine the most appropriate methods needed for such a plan, sampling techniques needed to be compared and analyzed in terms of efficiency and precision. Emergence traps were considered but ultimately proved to be too costly to build in order to provide the appropriate amount of data needed. Because bermudagrass is a relatively small, thin stemmed plant, the sweep net is an ideal sampling technique to employ for relative estimates of the adult fly population. It is inexpensive, time efficient, and relatively easy to use. Also, sweep nets have been used in studies involving rice and alfalfa, plants similar in size and structure to bermudagrass, with a standard of 10 sweeps per sample unit common in published studies (Espino 2008, Rashid 2006, Way 2006). Although results of the catch may vary somewhat depending on the user, when calibrated with absolute estimates the sweep net can provide multiple, reliable data points and thus was chosen to test as a possible sampling technique. Considering the aforementioned research involving the sweep net, 10 sweeps of the net while walking forward was accepted as an appropriate unit size for our study.

Through observations it became apparent that adult flies preferred the protection of the grass canopy and were never seen to be flying very far above the surface of the grass. Consequently, a Vortis Insect Suction Sampler (Burkard Mfg., Ltd) was first experimented with

to provide absolute estimates of adult populations. However, the height of the vacuum chamber proved to be too small to enclose 2-3 foot grass tillers and the surface area covered was impractical in comparison to the size of the field. Therefore, a cage was built to trap flies perching in the canopy. Constructed of a PVC frame with 0.5 m x 1 m footprint x 1 m height, mesh fabric fixed to 4 sides, with open bottom and clear acrylic sheeting on top in order to view the flies during capture, the cage was placed quickly in the field so as not to allow the flies to escape (Figure 2.1). The vacuum from the Vortis Sampler was fitted with a hose and then used to capture flies from within the cage by sucking them into mesh bags attached to the hose (Figure 2.1). At first we employed the vacuum throughout the space inside the cage including the topsoil. However, it quickly became evident that the flies would immediately fly to the top of the cage when disturbed. We then compared samples collected with the vacuum to a simple visual count of flies clinging to the ceiling using a matched paired t-test (Figure 2.2) and found the differences to be negligible when fly numbers in a given sample were less than 15. Any sample with over 15 flies was collected with the vacuum. This allowed more data points to be taken on a given day in the field.

For every cage sample, a sweep net sample (10 sweeps of net/sample) was taken in the immediate area and a sample of grass from within the cage footprint was collected. Sweep net samples were placed in collapsible cages and taken back to the lab where they were placed in the freezer overnight. The flies were then counted the next day and recorded. Cage samples were treated in the same manner unless the number of flies was under 15 in which case their number was recorded in the field.

A grass sample consisted of all the grass in a circle with a 17.8 cm diameter, clipped 1-2 inches above the ground. The samples were then bagged and later processed in the lab, recording

grass height and the number of damaged shoots per sample. Reliable data on the presence of larvae in bermudagrass hay fields proved difficult to obtain. The larval stages of the BSM are found in the growing point of the apical shoot of the plant and are difficult to extract; often they are not found in the damaged tillers or pulverized in the extraction process. Therefore, grass samples were scored on the basis of damage levels only. Shoots were considered damaged if they were discolored and the uppermost 2-3 leaves were easily removed from the plant (Figure 2.3). After the numbers of damaged shoots were recorded, the larvae were allowed to emerge from the clippings and pupate.

All techniques were employed in sample units of 10 m x 10 m plots in 6 different locations in the state of Georgia in the summers of 2013 and 2014. At first, 3 plots were sampled in a field moving from the border to the interior at different times of day with varying conditions from the May-October. Overtime, as techniques improved, 10-20 plots were sampled in a given field and a zig-zag pattern of data points was adopted as this allowed for a better representation of the field as a whole. The total number of observations was 207. Data was used to determine the potential number of samples needed for each technique, based on the variance to mean relationship, for precise estimates. Thus, the optimum number of samples (n) required to attain a precision (D) level of 0.25, expressed as a proportion of the mean, was calculated using the general formula, $n = s^2/D^2*\bar{\bar{x}}^2$ (Karandinous 1976). Data from absolute and relative sampling techniques were related to each other using a Poisson regression analysis with JMP-pro 11 statistical software.

Population dynamics. Mowing practices in bermudagrass hay fields appeared to affect the dynamics of fly populations. The grass is cut regularly during the growing season (every 3-6 weeks depending on growing conditions) and often the perimeter is left longer than the rest of

the field. During preliminary sampling it was typical to find flies and damage only in the uncut perimeter of recently harvested fields. Once the grass is cut, there are no growing shoots to lay eggs on and flies are not found in observable levels until the grass begins to regrow. Considering this, it is important to know the reinvasion and damage patterns of the fly throughout the growing cycle for the purposes of future sampling plans and management decisions.

In 2013, five hay fields were sampled with a total of 67 observations from July to October at irregular intervals. These fields were mostly unmanaged and were thus subjected to a number of variables which could affect the growth of grass, and consequently populations of flies, such as rainfall, uneven growth, and weeds. In 2014, however, two hay fields (cultivar 'Alicia') outside of Valdosta, GA were sampled with a total of 140 observations from May to September. These fields belonged to a commercial grower who fertilized, irrigated, and controlled for weeds on a regular basis which allowed the grass to grow faster and more evenly. Samples were taken at weekly intervals beginning shortly after mowing (7-10 days, as weather allowed) and continuing until the next mowing to investigate the pattern of fly population development throughout the hay growth cycle. Data from absolute (cage) samples were compared to grass damage, grass height, and date using generalized linear mixed models (Type III test of fixed effects), with JMP-pro 11 statistical software.

Developmental biology *A.reversura* larvae emerging from cuttings were allowed to pupate, placed in petri dishes (Figure 2.4) and then placed in temperature cabinets at the University of Georgia, Athens, GA, Forest Entomology Lab for pupation period trials (n=6) from 8 August to 13 September 2014. The pupae were maintained on a 14:10 (L:D) photoperiod with 65% RH at three different temperature regimes (32.2°C/30.2°C, 29.4°C/27.4°C and 27.0°C/25.0°C). Emerged adults were kept in cages and provided sugar water to determine the potential lifespan

of adults. In addition to these trials, flies were sexed under a dissecting microscope and the male to female ratio was recorded from samples on 13 different dates from May to October.

Figure 2.1. Cage constructed of a pvc frame, mesh fabric fixed to 4 sides, with open bottom and clear acrylic sheeting on top. If flies were too numerous to be counted they were captured with vacuum.

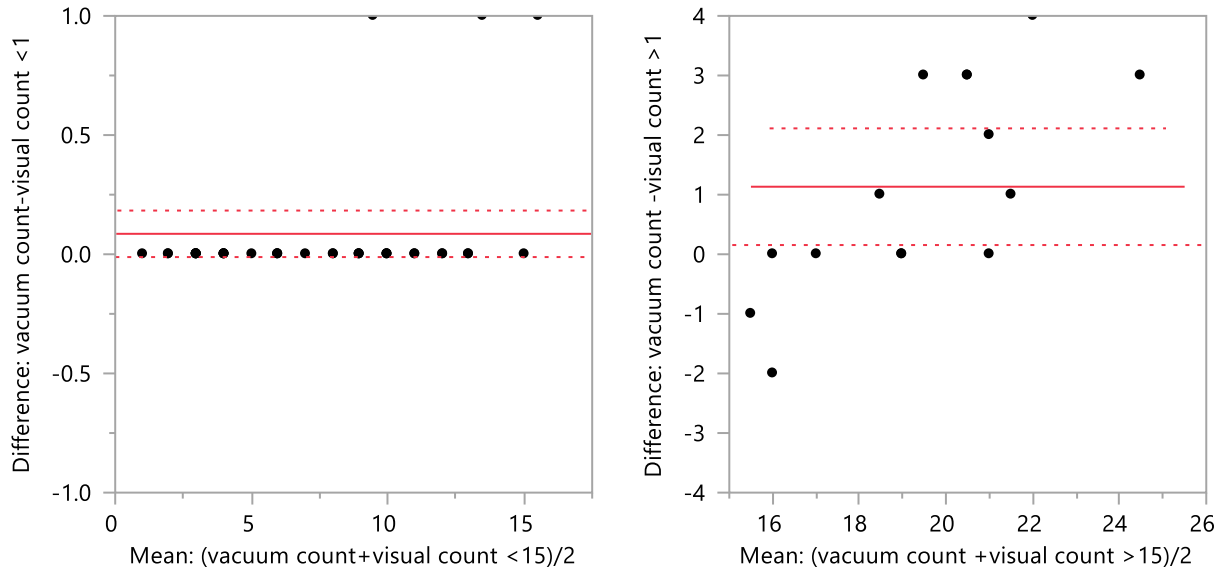


Fig 2.2. Matched pair t-tests for vacuum counts and visual counts. Visual count < 15: Mean difference = 0.086, Std Error = 0.048, Upper 95% = 0.183, Lower 95% = -0.012, Correlation = 0.998, t-Ratio = 1.785, Prob > |t| = 0.083, Prob > t = 0.042, Prob < t = 0.958. Visual count > 15: Mean Difference = 1.133, Std Error = 0.456, Upper 95% = 2.112, Lower Upper 95% = 0.154, Correlation = 0.886, t-Ratio = 2.483, Prob > |t| = 0.026, Prob > t = 0.013, Prob < t = 0.987.

Figure 2.3. Shoots were considered damaged if they were discolored and easily removed from the plant.

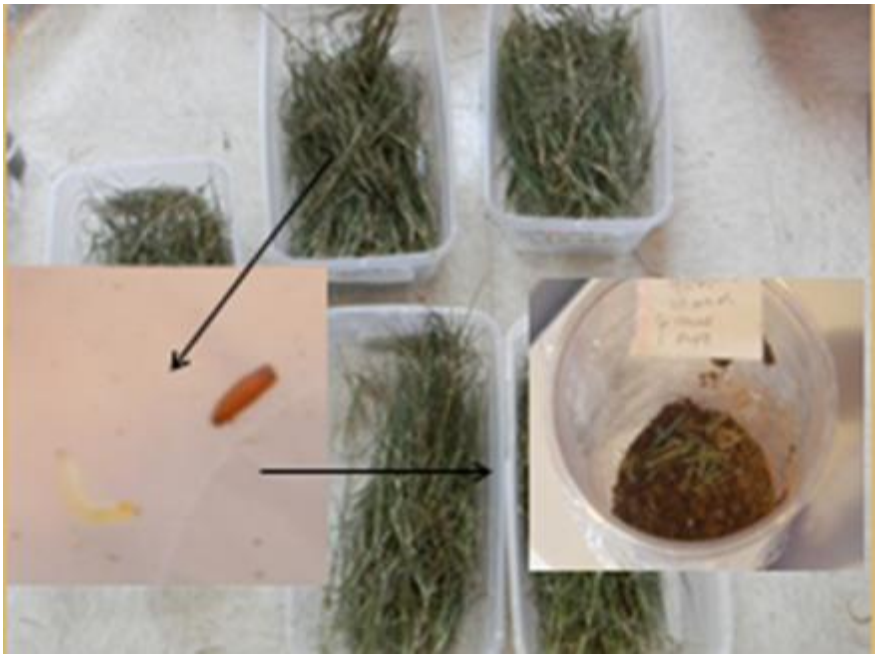


Figure 2.4. Once larvae emerged from grass and pupated they were then placed in petri dishes and maintained on a 14:10 (L:D) photoperiod with 65% RH at three different day and night temperature regimes (32.2°C/30.2°C, 29.4°C/27.4°C and 27.0°C/25.0°C respectively).

CHAPTER 3

RESULTS AND DISCUSSION

Sampling. The count of flies by the cage technique ranged from 0 to 18. Its mean, median and standard deviation were 2.70, 2 and 3.25, respectively. Similarly, the count of flies by the relative method ranged from 0 to 146 per ten sweep sample. Its mean, median and standard deviation were 14.45, 7 and 20.88, respectively. Through regression analysis the dependent variable (count of flies by the cage technique) was related to the predictor (count of flies by the sweep netting technique). A simple regression model was not appropriate for this study because the dependent variable is a count, which can only be positive. In addition, the distribution of data from both techniques was not normal being skewed to the right with a few particularly large numbers (Figure 3.1). Therefore, a Poisson regression, which models the log of the expected count as a linear function of the predictor variables, was utilized for fitting the model.

The estimations of the regression parameters are -0.7234 and 0.6978. Therefore, the regression equation can be written as follows: expected cage count = ($e^{-0.723}$ x sweep net count$^{0.698}$). Thus, when the count by sweep net doubles, the count by cage is $2^{0.698}$ = 1.62 times its original value. In addition, since the estimate of the parameter is positive, the response and the predictor have a positive relationship. Figure 3.2 shows that the higher the relative count, the higher the absolute count. Considering that the cage had a .5 m^2 footprint, we can formulate an equation which, based on sweep net counts, can estimate the density of flies for a unit of land

area. The equation for this estimation is: Density = $2(e^{-0.723} \times \text{sweep net count}^{0.698})$, where Density = the number of flies/m^2 and sweep net count = the number of flies /10 sweep sample.

Optimum sample size (n) for sweep net samples ranged from 1.4 to 10.8 (Figure 3.3). There was no clear indication of a relationship between population density and the optimum sample size. Conversely, the sample size curve for cage samples, with a range of 2.3 to 34.6, indicated that as population density increases, optimum sample size decreases dramatically (Figure 3.3).

From this investigation we discerned that a cage with a ~ .5 m^2 footprint is the appropriate size for absolute sampling as anything larger would prove unwieldy. A clear acrylic top allows for the subsequent count of disturbed flies. However if fly numbers exceed ~15 a vacuum is needed for accurate counts. We also provide an equation which can be used to estimate the number of flies per m^2 with a 10 sweep sample unit.

Population dynamics. Data from 2013 and 2014 were analyzed using general linear mixed models, with Type III tests of fixed effects, in order to relate the absolute count to the grass damage %, square of damage %, grass height and date. The results revealed that date and grass height were not significant ($p = 0.575$ and $p = 0.159$ respectively). When date was dropped from the model, there was still no significant correlation for height ($p = 0.863$). This could be due to variability in location such as management practices and the degree of infestation at the time samples were taken. Also, the range of grass heights was limited and may not have been adequate enough to suggest a relationship between either absolute counts or even damage percentages. A linear regression of the damage % and grass height was only able to account for 14 % of the variation (Rsquare = 0.145, RMSE = 18.022, F Ratio = 34.6291, Prob > F = <.0001) (Figure 3.4). However, Table 3.1 suggests that damage levels could be shown to increase with

height if the range for grass height were to be expanded and the number of samples large enough to account for the variability. When grass height and date were dropped and the model refitted, it was revealed that absolute count is associated with grass damage and square of damage ($p < 0.0001$ and $= 0.0030$, respectively). The regression equation is: expected count = $e^{(0.2278 + 0.04810*\text{damage} - 0.00032*\text{damage}^2)}$. Figure 3.5 shows the function relating damage and damage squared to the counts. The number of flies in a particular spot initially increases as grass damage percentage increases, but then starts to decrease when grass damage percentage reaches around 75%. This indicates that as resources are exhausted, flies move to new or less damaged areas.

Weekly data from two fields (Herron 1, Herron 2) near Valdosta, GA in 2014 further revealed the correlation between fly counts and damage percentages. Figure 3.6 illustrates a six week growth cycle from May 12 - June 19th. Counts and damage are shown to increase, gradually at first, over the growth cycle with a sharper increase in damage towards the end. During the next cycle, on July 5th, the pyrethroid insecticide cypermethrin was used in both fields to control fly numbers (Figures 3.7 and 3.8). This caused an initial decrease of flies but populations were seen to rebound towards the end of the cycle. Interestingly, damage percentages decreased as well. This is most likely due to the fact that shoot density increases throughout the growth cycle. In fact, a t-test of shoot density per sample from the week before treatment and the week after revealed that mean density increased from 78.35 shoots/sample to 92.85 shoots/sample with a mean difference of 14.5 shoots/sample (N = 20, Std Error = 3.97, t-Ratio = 3.67, Prob > |t| = 0.0017, Prob > t = 0.0008, Prob < t = 0.9992).

These patterns suggest that management decisions may not be appropriate based on damage levels alone. This is especially true in unmanaged fields where the growth may be slower, more clumped, and less dense allowing damage to accumulate over a longer time. When

these factors are considered, one can see the difficulty in correlating fly numbers sampled at a particular time and place to damage. Moreover, the slow buildup of fly populations after harvest indicates that certain developmental processes (i.e. pupation period) may contribute to the accelerated population increase later in the cycle. Thus, control practices also may not be appropriate shortly after hay cuttings.

Developmental biology. In order for management decisions to be effective, the determination of the developmental periods for *A.reversura* is crucial. Targeting of the adults is more feasible due to the fact that a systemic insecticide would need to be used for the larvae. Therefore, the duration of the pupal stage is key to determining when to employ control measures especially when considering that once hay is cut mature larvae emerge and pupate. If the pupation period is known then an appropriate response can be timed to target the subsequent adult emergence in the early growth stage of the grass before damage occurs.

Larvae, emerged from grass samples, pupated within 24 – 72 hours, but all pupae that emerged as adults did so on the same day. All pupae maintained at 32.2°C/30.2°C failed to emerge as adults. Pupae maintained at 29.4°C/27.4°C emerged as adults 7-10 days after pupation with limited success rate: trial 1= 1/7, trial 2= 2/5, trial 3= 0/8, trial 4= 3/16, trial 5= 1/5, and trial 6= 2/4 (Table 3.2). Similarly, pupae maintained at 27.0°C/25.0°C emerged as adults 7-10 days after pupation with limited success, however did not undergo as many trials: trial 1= 1/5, trial 2= 2/7, and trial 3= 2/5 (Table 3.2). The average female to male ratio of field collected flies was 4.6:1 (Table 3.3). Emerged adults lived 15-25 days when raised on sugar water (Figure 3.9).

The data from pupation period trials reaffirms that the use of control measures may prove ineffective if employed too soon after cuttings. This coupled with the data of delayed reinvasion after cutting suggests control measures would be appropriate no less than 10 days after hay has

been harvested. A female biased population ratio combined with a potential robust fecundity and 15-25 day adult lifespan, indicate that the BSM can have a substantial impact on the growth cycle of bermudagrass hay fields.

Table 3.1. Average grass height and damage percentages.

Height in cm	7.5	15	25	45	75
No. of obs	30	64	29	43	41
Mean damage%	0	13.7	2.8	18.5	26.2

Table 3.2. Pupae were maintained on a 14:10 (L:D) photoperiod with 65% RH at three different temperature regimes (32.2°C/30.2°C, 29.4°C/27.4°C and 27.0°C/25.0°C). Larvae pupated 24-72 hours after emerging from grass samples, but all emerged as adults on the same day.

Date	Temperature(°C)	# of pupae	# of emerged adults	Pupation period in days
8/08/2014	32.2°C/30.2°C	6	0	-
8/14/2014	32.2°C/30.2°C	8	0	-
8/23/2014	32.2°C/30.2°C	11	0	-
8/08/2014	29.4°C/27.4°C	7	1	9
8/14/2014	29.4°C/27.4°C	5	2	8-10
8/23/2014	29.4°C/27.4°C	8	0	-
9/02/2014	29.4°C/27.4°C	16	3	7-10
9/09/2014	29.4°C/27.4°C	5	1	7
9/13/2014	29.4°C/27.4°C	4	2	7-9
9/02/2014	27.0°C/25.0°C	5	1	9
9/09/2014	27.0°C/25.0°C	7	2	8-10
9/13/2014	27.0°C/25.0°C	5	2	8-10

Table 3.3. Female to male ratio of flies over 13 dates from May to October. The average female to male ratio of field collected flies was 4.6:1. However, the date (5/05/2014) with the highest female to male ratio also had the lowest count of total flies.

Date	Location in GA	Female to Male Ratio	# of flies
7/19/2013	Dublin	3.2:1	25
7/30/2013	Eatonton	5.1:1	258
8/06/2013	Eatonton	5.2:1	235
8/13/2013	Lexington	3.1:1	37
8/24/2013	Madison	2.1:1	482
8/25/2013	Lexington	4.6:1	117
9/06/2013	Madison	2.5:1	276
9/10/2013	Eatonton	5.3:1	64
9/14/2013	Tifton	5.1:1	86
9/17/2013	Eatonton	5.1:1	320
10/04/2013	Madison	6:1	130
5/05/2014	Valdosta	9.5:1	19
5/12/2014	Valdosta	2.9:1	25

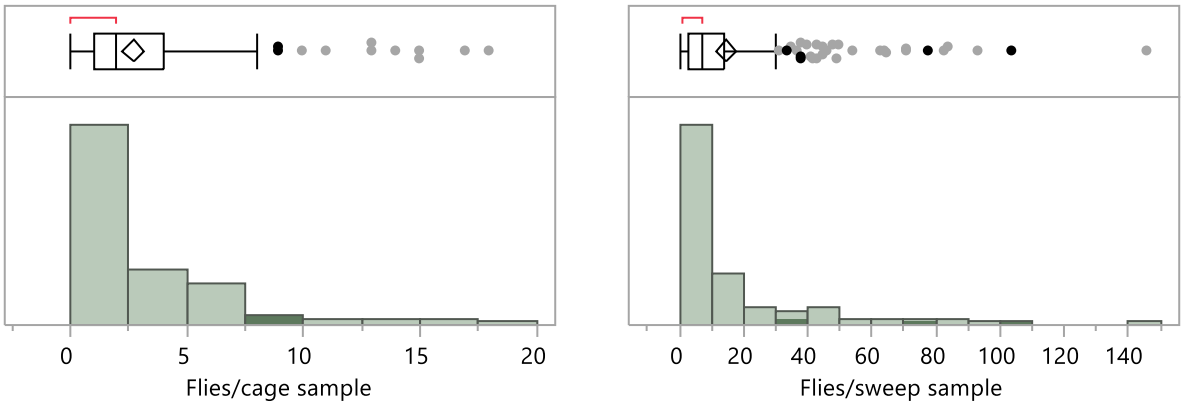


Figure3.1. Distribution of counts from cage and sweep net samples (N = 207). Cage: Mean = 2.696, Std Dev = 3.248, Std Err Mean = 0.226, Upper 95% Mean = 3.141, Lower 95% Mean = 2.251. Sweep net: Mean = 14.459, Std Dev = 20.889, Std Err Mean = 1.452, Upper 95% Mean = 17.321, Lower 95% = 11.596.

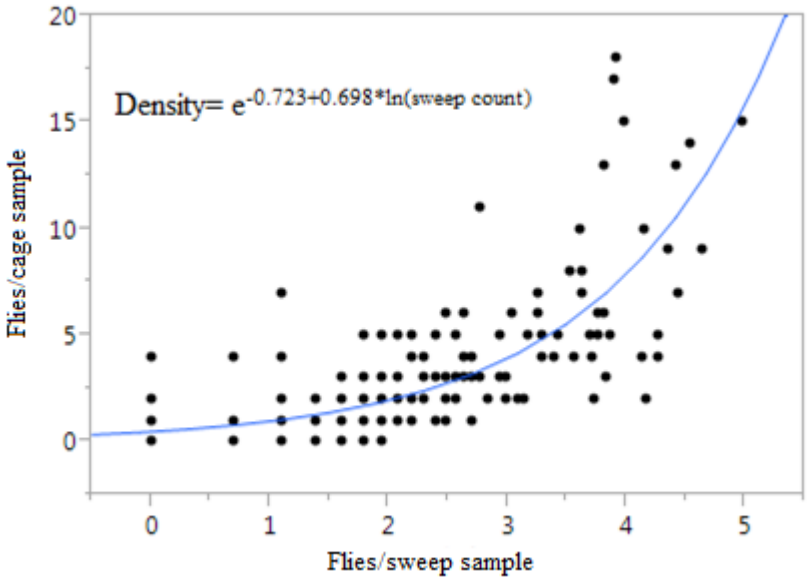
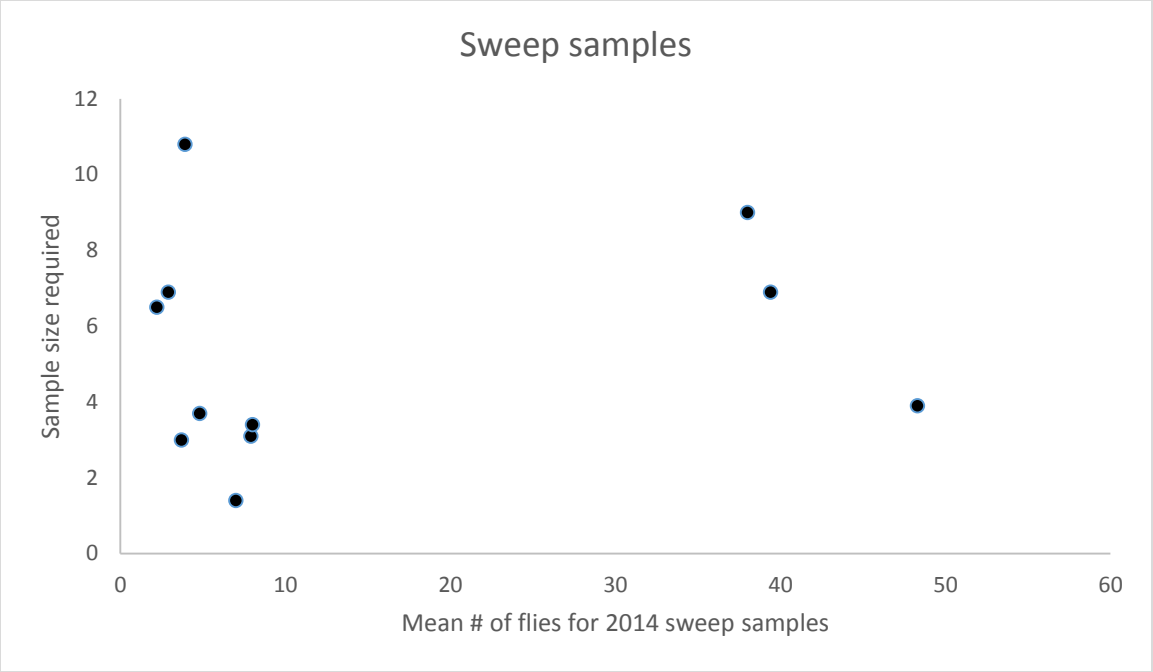


Figure 3.2. Poisson regression model of absoulte(cage) samples vs the logrelative (sweep net) samples. Intercept: Estimate= -0.7234, Std Error= 0.1453, Prob>ChiSq= <.0001*, Lower CL= -1.0140, Upper CL= -0.4443. Logrelative: Estimate= 0.6978, Std Error= 0.0454, Prob>ChiSq= <.0001*, Lower CL= 0.6093, Upper CL= 0.7872.



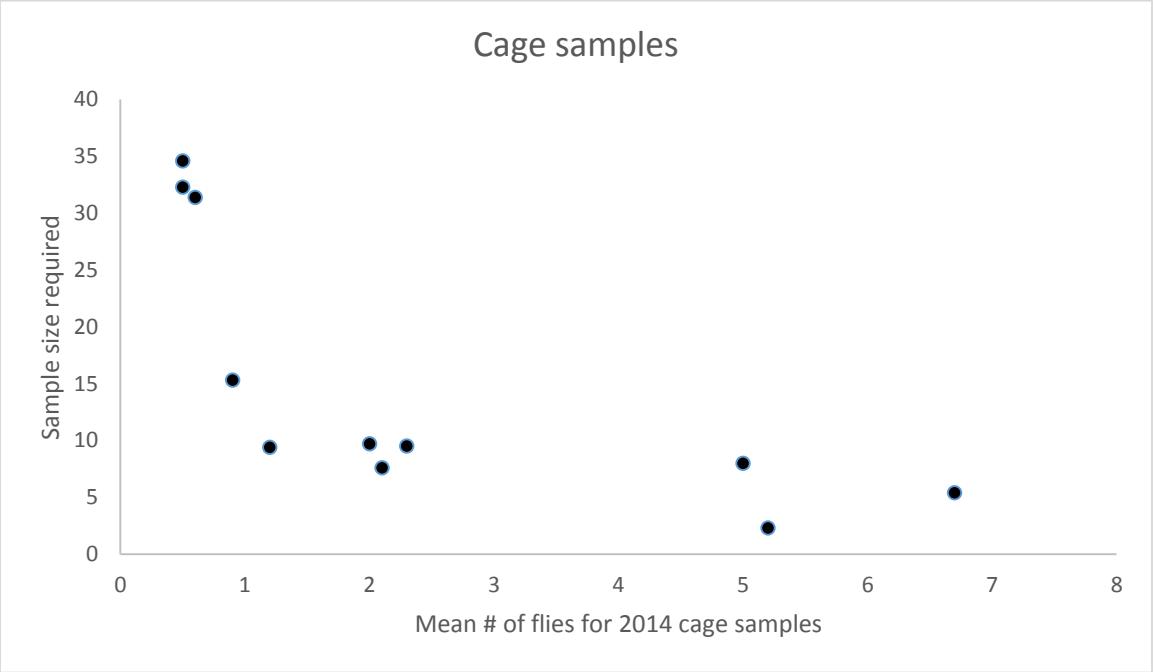


Figure 3.3 The optimum sample size for both techniques at different fly densities for a 0.25 precision level with the general formula.

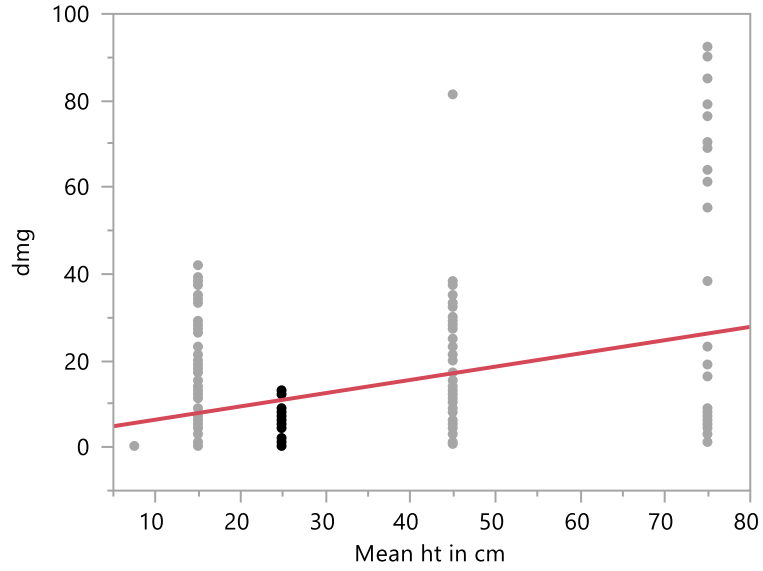


Figure 3.4. Linear regression of damage % to mean ht in cm of grass samples. Rsquare = 0.145, RMSE = 18.022, F Ratio = 34.6291, Prob > F = < 0001.

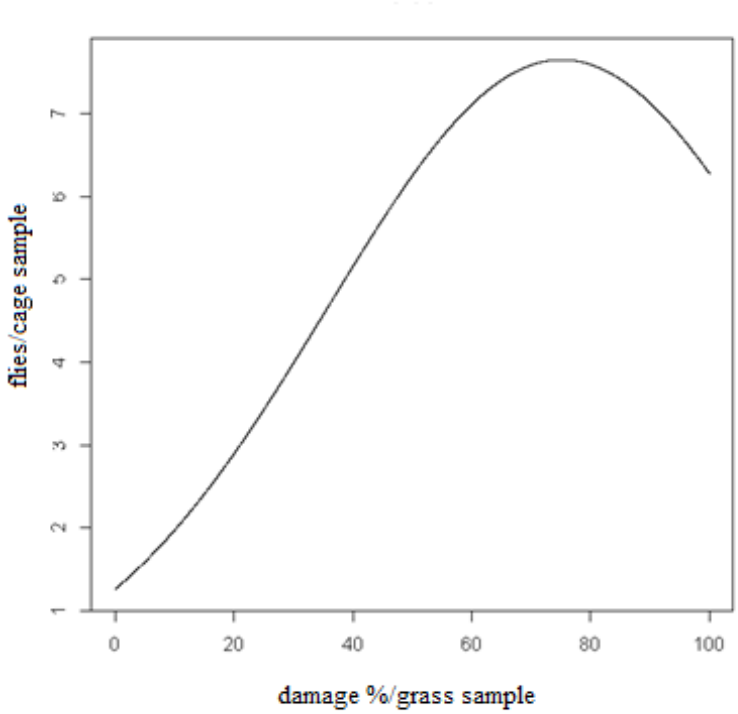


Figure 3.5. Relationship of absolute count to damage. The p-values for damage and the square of damage are < 0.0001 and 0.0030, respectively. Fit Statistics: -2 Res Log Pseudo-Likelihood = 558.79, Generalized Chi-Square = 217.57, Gener. Chi-Square / DF = 1.07.

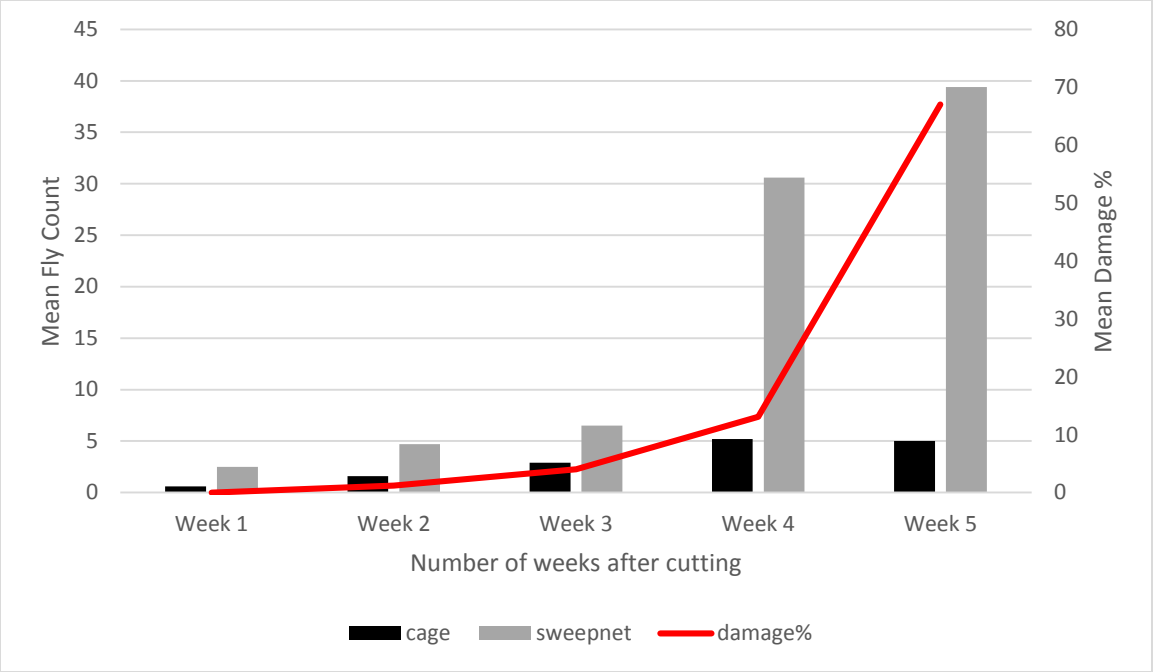


Figure 3.6. Mean fly count and mean damage % over six week growth cycle from May 12 - June 19 (Herron1: Valdosta, GA).

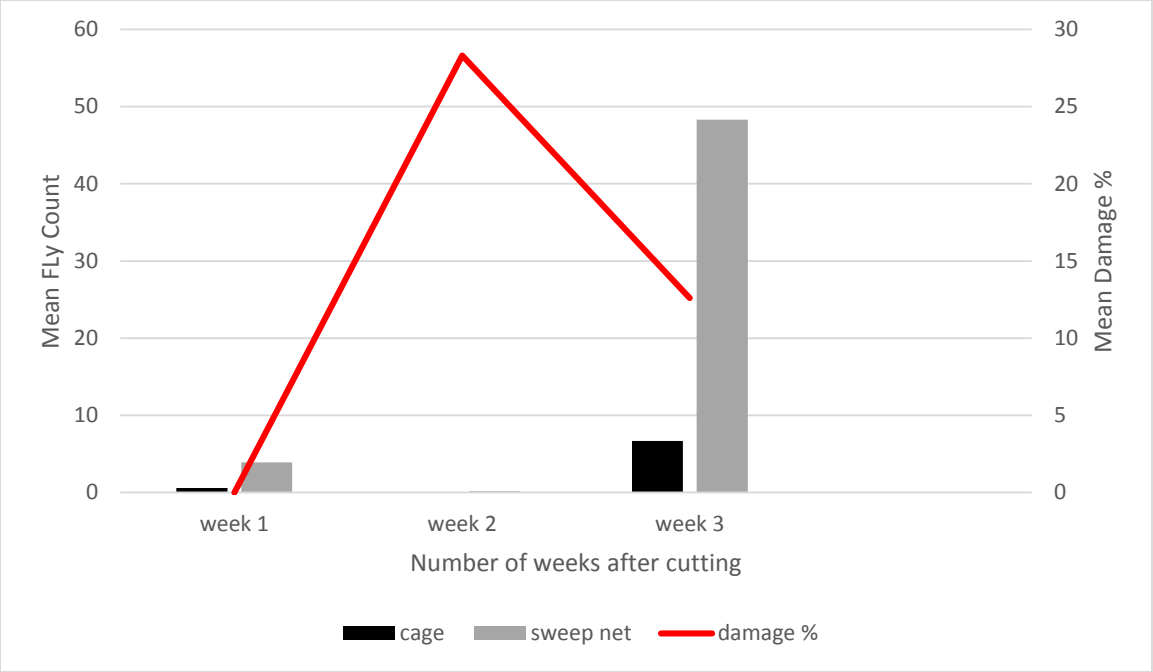


Figure 3.7. Mean fly count and mean damage % over 4 week growth cycle from June 27-July 13 (Herron1: Valdosta, GA). The field was treated on July 5th (week 2) and sampling was conducted after treatment.

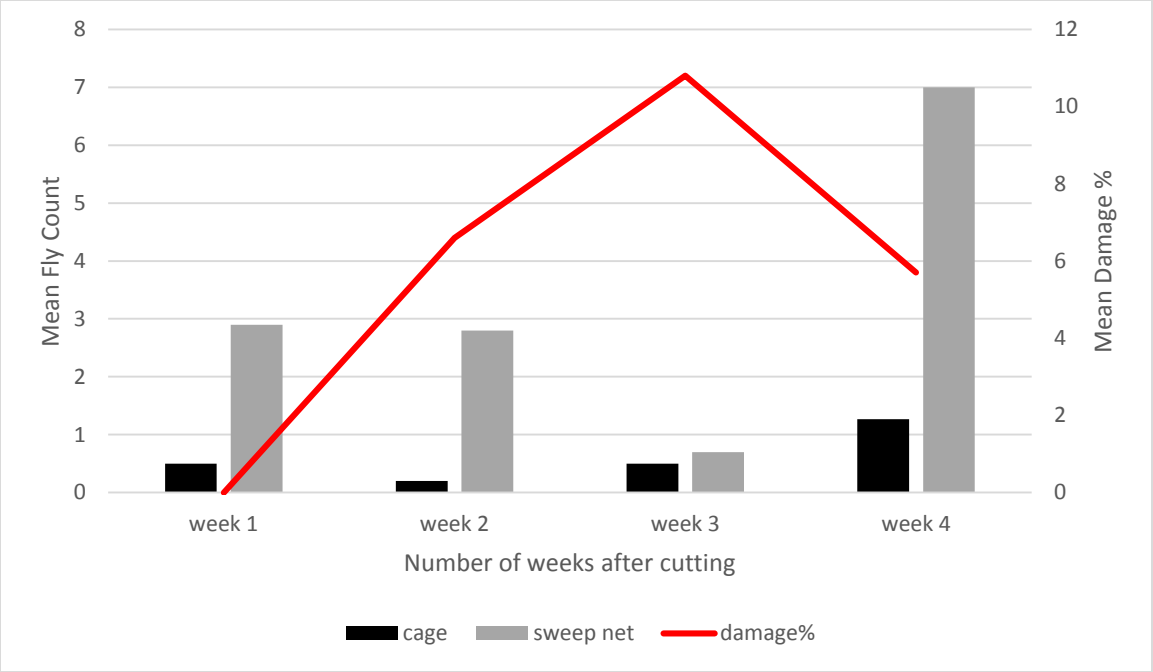


Figure 3.8. Mean fly count and mean damage % over 5 week growth cycle from June 27-July 20 (Herron2: Valdosta, GA). The field was treated on July 5th (week 2), however, sampling was conducted before treatment.

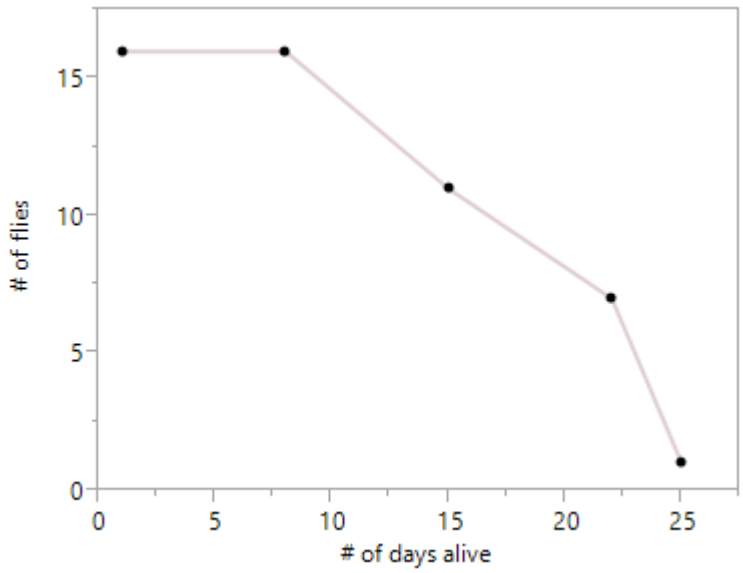


Figure 3.9. Lifespan of emerged adults kept in cage and fed sugar water.

CHAPTER 4

CONCLUSION

A significant association between absolute and relative sampling techniques provided an equation which can be used to estimate populations of *A.reversura* adults per m^2 in a given field from a 10 sweep sample unit. A cage with a footprint of ~0.5 m^2 and a clear top is an effective means to obtain an absolute sample unless fly numbers exceed ~15 at which point a vacuum will be needed.

If control measures are to be taken, consideration of the fly counts are necessary in order for control to be effective. We believe fly numbers are unlikely to warrant control measures for at least 1 - 2 weeks after harvest or before the grass reaches 15 - 30 cm (6 inches to 1 foot) due to the slow initial buildup of adult populations, although it must be said that weekly data came only from 3 fields over two different growth cycles. Similarly, when damage levels near 75% measures may prove to be too late as fly numbers were shown to decline for percentages above that threshold. Also, damage may accumulate in unmanaged fields with slower growth. Based on this assessment, management decisions may be more appropriate based on fly count numbers alone.

Our findings suggest the pupation period of *A.reversura* to be 7-10 days with an adult life span of 15-25 days in duration. This considered with the potentially robust fecundity and an average 4.6:1 female to male ratio, indicates the BSM has the potential to cause yield loss,

delayed harvest times, and reduce quality in the 10-15 million acres of bermudagrass hay fields throughout the Southeastern United States.

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