

MAXIMIZING INFORMATION YIELD FROM PHEROMONE-
BAITED MONITORING TRAPS: ESTIMATING PLUME REACH,
TRAPPING RADIUS, AND ABSOLUTE DENSITY OF
RANDOMLY MOVING INSECTS

By

Christopher Glen Adams

A DISSERTATION

Submitted to
Michigan State University
in partial fulfillment of the requirements
for the degree of

Entomology - Doctor of Philosophy

2016

ABSTRACT

MAXIMIZING INFORMATION YIELD FROM PHEROMONE- BAITED MONITORING TRAPS: ESTIMATING PLUME REACH, TRAPPING RADIUS, AND ABSOLUTE DENSITY OF RANDOMLY MOVING INSECTS

By

Christopher Glen Adams

Monitoring traps perform very well for recording what insect pests are present in a crop and when they are active. However, translating catch numbers into actual pest numbers per area has, until now, rarely been accomplished. This dissertation reveals how novel methods of data analysis were used to interpret catch data from both single-trap, multiple-release and single-release to a 5 x 5 trapping grid experiments using two model organisms, codling moth (*Cydia pomonella*) and Japanese beetle (*Popillia japonica*) responding to standard pheromone baited and floral plus pheromone baited traps, respectively. The main objectives were to produce reliable measurements of: 1) the attractant plume reach from the trap, 2) the maximum dispersive distance of a population of responders, 3) the proportion of the population in the trapping area caught (T_{fer}), and 4) the patterns of movement (C.S.D.). Two release designs were employed for codling moth: 1) releases in the four cardinal directions, and 2) even releases across 16 ha orchard blocks using both high and low CM populations. For both release designs at high populations, the unadjusted mean proportion caught (T_{fer}) was 0.01 as compared to

0.02 for even releases of low populations. Mean maximum dispersive distance for released codling moth males was ca. 260 m. Plume reach for the standard CM trap was only ca. 2 m, total trapping area for a single trap was ca. 21 ha., and the measure of meander of 37° . These estimates were consistent across three growing seasons and are supported by extraordinarily high replication for this type of field experiment. For Japanese beetle a foraging meander of $9 \pm 3^\circ$ C.S.D. was revealed and the plume reach from the Trécé Catch Can trap baited with the dual baitpack lure was 10 m. Measures of T_{fer} and maximum dispersive distance of 0.06 and 120 m per day were measured. Knowing the trapping area and the T_{fer} values for these model insects permits catch numbers from single monitoring traps to be translated into absolute pest density using the equation: males per trapping area = catch per trapping area / T_{fer} . Finally, the mean of 5 traps spaced one tree apart produced considerably more precise measures of absolute codling moth density than did a single trap. This fundamental knowledge of how to space traps and interpret catch numbers will enable pest managers to make considerably more precise projections of damage and therefore more precise and reliable decisions on whether insecticide applications are justified. The principles and methods established here for estimating absolute insect density should be broadly applicable and thereby set a new standard for IPM decisions based on trapping.

Copyright by
CHRISTOPHER GLEN ADAMS
2016

I dedicate this work to my loving wife Lane.
Her unwavering faith in the Lord
and her belief in me is what made this
possible.

ACKNOWLEDGEMENTS

First and foremost, I would like to thank Piera Siegert for thinking of me when there was a need for a construction-minded bug-loving summer employee to build Jim's large field cages at Palmer Farm. What an amazing introduction to science! I would like to thank Jim Miller for taking a chance and giving me the opportunity to pursue my life-long dream of a PhD in entomology. This work is the crowning jewel in Jim's long and distinguished career and I am grateful to have had the opportunity to contribute to this important work. I thank the other members of my guidance committee: Jeffery Schenker, Matthew Grieshop, and Larry Gut for their outstanding advice, contributions, and insights throughout the planning, execution and interpretation of this research. I have enjoyed our collaborations and look forward to working together in the future.

I am especially grateful to Peter McGhee for finding orchards for my research, for showing me the ropes in the field, and for his close friendship. Peter's positive attitude and unwavering faith made the effort seem easy. I am fortunate to have had the opportunity to work with and become good friends with Keith Mason whose positive attitude and sense of humor helped make the heavy load lighter. My sincere thanks to the many undergraduate students who reared

insects, helped set up experiments, and collected data; your efforts made this work possible.

I acknowledge Jim Miller's aid and significant contribution to the writing and editing of this dissertation. This work would not have been possible without his generous assistance.

I would like to express my gratitude for a Department of Entomology Teaching Assistantship that supported me for two years during my research. I truly enjoyed my time in the classroom and my interactions with the great people within the Center for Integrative Studies-General Science, especially Amanda Lorenz and Gabe Ording.

This research was supported in part by MSU, Michigan Project GREEN, the Michigan Apple Committee, and by the National Science Foundation (#1411411) to J.H.S and J.R.M. Their support is gratefully acknowledged.

For their continuing love, support, and encouragement, I thank my parents: Karyn Flynn and Glenn and Beth Adams

TABLE OF CONTENTS

LIST OF TABLES	xii
LIST OF FIGURES	xiii
KEY TO ABBREVIATIONS.....	xviii
CHAPTER ONE Introduction	1
INTRODUCTION.....	2
Apple Agriculture around the World.....	2
Codling Moth (CM) Biology and Phenology.....	2
Management of Codling Moth	3
Mating Disruption (MD)	3
Monitoring and Economic Injury Levels	4
Path Leading to this Research	4
The Role and Current Limits of Trapping and Justification for this Research.....	6
Current Knowledge of Trapping	7
Future Applications	9
KEY CONCEPTS	11
Interception.....	11
Plume Reach	11
Patterns of movement	12
Ballistic Movers.....	13
Interception.....	14
Single-Trap Multiple-Release	16
MAG plot.....	17
Measures of Plume Reach for Non-Ballistic Movers.....	19
Change in C.S.D.	20
Random walk.....	21

Trapping Gain.....	23
MAIN OBJECTIVES.....	24
CHAPTER TWO Maximizing Information Yield from Pheromone-Baited	
Monitoring Traps: Estimating Plume Reach, Trapping	
Radius, and Absolute Density of Codling Moth (<i>Cydia</i>	
<i>pomonella</i>) in Michigan Apple.	25
ABSTRACT	26
INTRODUCTION.....	28
Knowledge Required to Translate Catch Numbers into Absolute Pest	
Density	29
Evidence for Random Walks by Flying Insects Not in Contact with	
Pheromone	31
Examples of Past Attempts to Estimate Absolute Pest Density from	
Capture Number in Attractive Traps	33
Study Objectives and Rationale for Using CM for Proof-of-Concept.....	36
Simulation Results Guiding the Current CM Study	37
MATERIALS AND METHODS	40
Source and Handling of Codling Moths.....	40
Estimates of Proportion of Purchased CM Capable of Participating	41
Orchards.....	41
Experiment 1 -- Cardinal-Direction Releases	42
Experiment 2 -- Even-Release with High Populations	45
Experiment 2.5 -- Even-Releases with Very High Populations	45
Experiment 3 -- Even-Release with Low Populations	45
Data Analysis.....	48
RESULTS.....	50
Estimates of Proportion of Purchased CM Capable of Participating	50
Experiment 1 -- Cardinal-Direction Releases.	50
Experiment 2-- Even-Releases with High Populations	53
Experiment 2.5 -- Even-Releases with Very High Populations	55
Experiment 3 -- Even-Release with Low Populations	57

DISCUSSION	Error! Bookmark not defined.
Estimates of Proportion of Purchased CM Capable of Participating	60
Pattern and Consistency of Trapping Outcomes Indicate CM is a Random Walker	60
Approximations of Male CM Flight Paths when Foraging for a Pheromone Plume	62
Plume Reach of the CM Trap in Apple is Small	64
Radius and Sampling Area of the CM Trap in Apple are Large	67
Converting CM Catch Number to Absolute Density	68
Reducing the Normal Variance in Catch Data	70
Should Females be Present During Trapping Calibrations?	70
Recommendations for Applying this Approach to Other Pests	71
 CHAPTER THREE Flight meander of male codling moths searching for plumes of female sex pheromone is optimized.....	75
ABSTRACT	76
INTRODUCTION.....	78
MATERIALS AND METHODS	84
RESULTS AND DISCUSSION	84
 CHAPTER FOUR Line-Trapping of Codling Moth, <i>Cydia pomonella</i> , (Lepidoptera: Tortricidae): a Novel Approach to Improving the Precision of Capture Numbers in Monitoring Traps	87
ABSTRACT	88
INTRODUCTION.....	90
MATERIALS AND METHODS	96
RESULTS.....	99
DISCUSSION	102
 CHAPTER FIVE Interactions of Japanese Beetles, <i>Popillia japonica</i> , (Coleoptera: Scarabaeidae) with Monitoring Traps: Estimation of Plume Reach and Foraging Meander	103

ABSTRACT	104
INTRODUCTION.....	105
MATERIALS AND METHODS	107
Source and Handling of Beetles	107
Experiment 1 – Single-Trap, Multiple-Release for Measuring Plume Reach	108
Experiment 2 – Estimating Path Meander of <i>P. japonica</i> Foraging for Plumes	109
RESULTS AND DISCUSSION	111
Experiment 1 – Single-Trap, Multiple-Release for Measuring Plume Reach	111
Experiment 2 – Estimating Path Meander of <i>P. japonica</i> Foraging for Plumes	113
SUMMARY AND CONCLUSIONS	118
APPENDIX	123
LITERATURE CITED	125

LIST OF TABLES

Table 1 Orchard planting details and GPS coordinates	42
Table 2 Color and number of codling moth per distance of Experiment 1, and the resulting moths per area.....	44
Table 3 Color and number of codling moth per distance for Experiment 2, and the resulting moths per area.....	46
Table 4 Color and number of codling moth per distance for Experiment 3, and the resulting moths per area.....	47
Table 5 Record of voucher specimens	124

LIST OF FIGURES

Figure 1. The tracks of 100 ballistic movers, starting from a common origin and dispersing in random directions independently from one another. Image generated with the Weston mover model.	14
Figure 2. Large circle shows the population (P) of ballistic movers as it intersects the trap and its plume (T) depicted as the smaller circle. Image generated with the Weston mover model.	15
Figure 3. The dashed circles show the positions of several populations of ballistic movers released from varying release distances (R) as they would appear at the point they intercept the trap and plume (T).	16
Figure 4. Mean proportion of 1,000 ballistic movers caught from varying release distances $\pm$ S.E.M. at a trap with a diameter of 10 units (n = 100).	17
Figure 5. Transformation of data from Figure 4 showing 1/ mean proportion of ballistic movers caught over varying release distances. The slope from the straight line relationship of the transformed data can be used to reveal the size of the plume.	18
Figure 6. Standard curve of plume reach against MAG plot slope generated with Weston Multi Mover.	19
Figure 7. Weston mover model image of 100 movers starting from a common origin and dispersing for 1,000 steps using a C.S.D. of 2° . Note the track of one mover that has come back near the origin.	21
Figure 8. Weston mover model image of 100 movers starting from a common origin and dispersing for 1,000 steps using a C.S.D. of 360° . Note that the net displacement from the origin is greatly reduced relative to the movers in Figure 7.	22

Figure 9. Change in pattern of capture profiles to be expected if not all of the codling moths released into the forthcoming experiments lived. Individuals not participating simulate those killed by an insecticide. SpT_{fer} indicates proportion of insects captured after release at a particular distance, for the purpose of this demonstration always calculated on the basis of 800 released. Annulus area indicates a ring out of the full trapping area (composite of all annuli) sampled by a single trap. In all cases the C.S.D. for headings for new steps was 30° and the total number of steps was 3,000. The trend lines were fit using a second order polynomial.	38
Figure 10. Release pattern of codling moth for cardinal direction release (Experiment 1).....	44
Figure 11. Release pattern for even-release of codling moth over 20 ha (Experiment 2).....	46
Figure 12. Release pattern for even-release low-population of codling moth (Experiment 3).....	47
Figure 13. Mean specific trap findability (spT_{fer}) for central-trap, cardinal-direction release, Experiment 1 ($n = 9 \pm S.E.M.$).....	51
Figure 14. MAG plot transformation of Figure 13 data.	52
Figure 15. Miller Plot of Figure 13 data and their mean (dashed line).	52
Figure 16. Mean specific trap findability (spT_{fer}) for the central-trap, even-release Experiment 2 ($n = 9 \pm S.E.M.$).....	54
Figure 17. MAG plot transformation of Figure 16 data.	54
Figure 18. Miller plot of Figure 16 data, and their mean (dashed line).....	55
Figure 19. Mean specific trap findability (spT_{fer}) for 3 replicates of even-release at very high codling moth populations Experiment 2.5 ($\pm S.E.M.$).	56

Figure 20. MAG plot of Figure 19 data.	56
Figure 21. Miller plot of Figure 19 data.	57
Figure 22. Mean spT_{fer} for low-population, even-release of codling moth Experiment 3. ($n = 15 \pm S.E.M.$).	58
Figure 23. MAG plot of Figure 22 data.	59
Figure 24. Miller plot of Figure 22 data.	59
Figure 25. Panels A – G illustrate the tracks of individual Weston MultiMover (Miller et al. 2015) random walkers operating with a circular standard deviation for headings for new steps of 30° and 3,000 total steps. When scaled to steps of 1 m, these records estimate typical foraging paths for male codling moths released near the center of a 9 ha orchard plot as viewed from above. Each plot would contain ca. 1,600 apple trees.	63
Figure 26. A. Capture of computer-simulated random walkers as a function of C.S.D. ($n= 35$). B. Mean catch for 5 runs at each C.S.D. when 400 simulated movers were released 10 units from a 5 x 5 grid of traps spaced 15 units apart. Other conditions were as for A. The optimal C.S.D. for search is ca. 50° when plumes are clustered near the movers' point of origin. The optimal compromise C.S.D. covering both conditions is expected to be ca. 35°	80
Figure 27. A. Layout for grid of twenty-five 2 x 2-unit traps (small rectangles) separated by 15 units and numbering system for edge traps whose catch numbers were used for graphing data to quantify a decay constant. R = release site for movers. B. to D. shows final destinations of movers after 5, 100, and 400 steps. The spatial scale for panels varies. Movers caught on traps in Panel D. enlarge trap appearance. E. shows plot of catch by trap number (as in A.) after 400 movers released at R of Panel A. took 400 steps of 1 unit with a C.S.D. of 30° . For traps designated T2- T5 the mean of two trap was plotted.	82

Figure 28. Standard curve inter-relating decay constant (e) with C.S.D. This graph was generated using the computer-simulation procedures of Figure 19 and a run time of 400 steps. Ten replicate runs generated 10 e values for each C.S.D. Values of S.E.M. around the mean e values were too small to be visible.....	83
Figure 29. Decay curve averaged across all 39 replicates of codling moth males penetrating a 5 x 5 grid of traps in the field. An exponent of - 0.99 equates to a C.S.D. of 37°.....	85
Figure 30. Frequency histogram of the 39 individual determinations of C.S.D. for codling moth foraging for pheromone plumes in a Michigan apple orchard.....	86
Figure 31. Probabilities of getting respective catches from 0 through 12 as ascertained from the binomial distribution formula.....	93
Figure 32. Schematic of the layout for one block of the experiment comparing capture in a single trap (represented by a triangle) relative to that in a line of 5 traps each separated by 4 m; the distance separating triangles in the line is not drawn to scale. At each release point (R), ca. 100 CM males were released at the start of each trial lasting 1 wk.	98
Figure 33. Capture of codling moth males as influenced by their position in the line of five traps. Means sharing a common letter are not significantly different at $p = 0.05$, $n=36$. Mean capture in a single trap was 6.2.....	100
Figure 34. Frequency histograms of field data showing the realized capture numbers of codling moth under the single-trap (unfilled bars) vs. $\mu 5$ configuration (filled bars) in Michigan apple orchards, $n=36$. The range of the $\mu 5$ catch was 1 to 12, while the range of the single trap was 0 to 32.	101
Figure 35. A. Untransformed plot of capture of <i>P. japonica</i> as a function of distance of release from a single trap. B. Inverse of proportion of released beetles captured by distance of release. Plume reach can be deduced from the slope of this MAG plot using the Figure 4.12 standard curve of Miller et al. (2015).....	112

Figure 36. Number of <i>P. japonica</i> captured as a function of trap position in a 5 x 5 rectangular grid of traps separated by 15 m. Beetles were released 10 m from trap number 1. The exponent of negative 0.53 was then used to deduce meander using the standard curve.	114
Figure 37. Standard curve for translating the decay constant in catch from Figure 36 into meander expressed as circular standard deviation (C.S.D.) of turn angles. These data were generated using the MultiMover software of Miller et al. (2015). 300 movers executing 400 steps were released 10 units from the corner of a 5 x 5 grid of 10 x 2 unit plumes whose sources were separated by 15 units. Ten replicates were run for each C.S.D.	115
Figure 38. Representative tracks for a mover like <i>P. japonica</i> executing meander of C.S.D. 10°. These tracks were generated by the MultiMover software. Movers initiating at s took 5,000 steps many of which occurred outside the frame of view of the computer screen.	116

KEY TO ABBREVIATIONS

π	the ratio of a circle's circumference to its diameter, approximated as 3.14159
ac	acre
C	catch in a trap
ca.	<i>circa</i> ; from Latin, meaning “approximately”
CM	codling moth
C.S.D.	circular standard deviation = one standard deviation (+/-) in a normal (Gaussian) distribution of possible turn angle headings where 0° is straight ahead; 68% of the time the randomly chosen new heading between steps will be within the indicated angle
e	in T_{fer} , the efficiency of the trap or the probability that a mover having arrived at a trap gets captured
e	in the trend-line formula $y = e^{-x}$, the irrational constant (Euler's number) approximated as 2.71828
EIL	economic injury level
f	in T_{fer} , the findability of the trap or the probability that a mover arrives at a trap from the within the trapping area
GPS	global positioning system
ha	hectare
IPM	integrated pest management
m	meter

MAG plot	Miller-Adams-McGhee plot; a graph of the distance movers originate from a trap on the x-axis vs. $1/\text{proportion captured}$ on the y-axis
MD	mating disruption
M_{den}	density of movers per unit area
Miller plot	a graph plotting the distance of release on the x-axis vs. $\text{sp}T_{\text{fer}} \times$ annulus area on the y-axis
R	release point
r	in T_{fer} , the retention of the trap or the probability that a mover having been caught in a trap is retained by the trap
R_1	outer radius of an annulus
R_2	inner radius of an annulus
T	trap and its plume
T_{fer}	the proportion of the population caught, comprised of the composite probability of the trap's findability, efficiency, and retention
S.E.M.	standard error of the mean
$\text{sp}T_{\text{fer}}$	the proportion of the population caught from a specific distance from the trap

CHAPTER ONE

Introduction

INTRODUCTION

This introductory chapter offers information that will be useful in understanding the four chapters to follow that comprise the main body of this dissertation and that were written as manuscripts for publication.

Apple Agriculture around the World

Worldwide apple production exceeds 76 million tons per year (WAPA). China leads the world in apple production (37 million tons), followed by the United States at ca. 4 million tons; Turkey, Poland, and India each produce ca. 2 million tons (WAPA). Michigan is the third largest producer of apples in the United States behind Washington and New York (usapple.com). Michigan's average annual harvest is around 20 million bushels or 828 million pounds of apples (michiganapples.com).

Codling Moth (CM) Biology and Phenology

The codling moth, *Cydia pomonella* (L.) (Lepidoptera: Tortricidae) (CM) is the key insect pest of apple (*Malus* spp.) in Michigan, the United States, and across the world. Originally from Asia Minor, this insect pest can now be found everywhere apples are grown. An adult CM is on average 12 mm long. Their wings are covered in gray or white bands tipped with bronze and held roof-like over its body. Females attract male conspecifics using a sex attractant pheromone (Wyatt 2003). After mating, the female forages for ovipositional sites such as

pome fruits and deposits up to 200 eggs. Eggs hatch and the newly emerged neonate larva borrows into developing fruit. Once development is complete, the mature larva exits the fruit and pupates in protected areas such as beneath the tree bark. In Michigan, CM realizes 2-3 generations per growing season. The late generation of CM overwinters as mature larvae inside cocoons spun under loose bark on the tree or in the litter around the base of the tree. Adults emerge beginning early May (depending on temperature) through June. The adults become active at dusk when temperatures are above 15° C (Howitt, 1993).

Management of Codling Moth

For over 50 years, CM had been controlled through broad spectrum organophosphates. Pesticide resistance and secondary pest outbreaks, such as spider mites, have reduced the effectiveness of these pesticides (Codling and Bearon 2010, Epstein et al. 2000, Mota-Sanchez 2008) and created a need for an integrated pest management system including mating disruption using pheromones (Gut and Brunner 1996, Gut et al. 2004).

Mating Disruption (MD)

Where implemented, mating disruption has helped to reduce codling moth numbers and minimize the need for insecticides (McGhee 2011, Witzgall et al. 2008). In Michigan mating disruption is most widely practiced using hand-applying dispensers of synthetic sex pheromones, (E, E)-8, 10-dodecadienol

(codlemone) (Gut et al. 2004), at ca. 400 dispensers per acre before adults emerge in the spring. These dispensers function as false females that compete for the male's attention, thereby reducing the number of successful matings (Miller and Gut, 2015). Dispensers are designed to release pheromone throughout the season thereby providing mating disruption across multiple generations.

Monitoring and Economic Injury Levels

Pheromone-baited traps are used to monitor the emerging populations and action thresholds have been established for the application of pesticide sprays (Beers et al. 1993, Riedl 1980, Riedl and Croft 1974, Riedl et al. 1976, Riedl et al. 1986). These action thresholds have been established indirectly by evaluating fruit injury at the end of the season and comparing it to records of catch in sex pheromone baited monitoring traps (Knight 2005). Economic injury levels (EILs) along with action thresholds have been established for the number of target insects found captured in monitoring traps (Robertson et al. 2005, Mumford and Norton 1984, Stern et al. 1959). This protocol has been widely adopted (Brunner et al. 2004, Thomson et al. 2001), but relies heavily upon historical trapping data with no estimates of population numbers.

Path Leading to this Research

I was fortunate to have the opportunity to work for several years on a research project conducted in large field cages that enclosed 12 apple trees in

which moths acted like substrates in large test tubes (Miller et al. 2010). This project led by Dr. James Miller produced novel insights into the mechanisms of mating disruption (Miller and Gut 2015). Moreover this research shed light on the mechanisms of trapping. One key finding was that the probability of a moth getting caught in a monitoring trap (T) can be calculated as the product of the probability of a moth finding the trap (f), multiplied by the probability of getting caught in the trap, (efficiency (e)), multiplied by the probability of the moth being retained in the trap (r). This product of probabilities was labeled T_{fer} (Miller et al. 2010). Once the T_{fer} of a monitoring trap is known additional information can be obtained from catch data in a monitoring trap. For example, if the population of moths (which can be thought of as the density of movers (M_{den}) is known, then catch (C) can be understood as the product of trap findability and mover density.

$$C = T_{fer} \times M_{den}.$$

More importantly, the population of movers in the cage can be computed from catch divided by the T_{fer} .

$$M_{den} = C / T_{fer}$$

The predictive power of this relationship holds great potential not only for measuring populations of insect pests in cropping systems but also for measuring populations of invasive species as well as endangered or threatened species.

Understanding how this formula, along with a number of other novel graphical interpretations of catch data, would work in an unbounded arena such as an orchard was the next logical step in this research that comprises the current dissertation.

The Role and Current Limits of Trapping and Justification for this Research

Pheromone-baited traps play an important role in monitoring insect pest populations in agricultural crops such as apple. Catch in traps can inform growers of the spatial and temporal distribution of insect pests such as codling moth. Catch data compared to fruit injury from codling moth larvae have been used to establish action thresholds. For example, it has been established that 3 weeks of sustained catch in monitoring traps represents the bio fix date which, along with degree day charts, can help growers predict population growth of codling moth and establish spray timing of life stage appropriate pesticides (Beers et al. 1993, Riedl 1980, Riedl and Croft 1974, Riedl et al. 1976, Riedl et al. 1986). While male moth catch has been used to predict fruit damage (Riedl and Croft 1974) the true population (absolute density) in the orchard remains unknown. Because these action threshold numbers are based on relative and not absolute abundance, it is likely that many of these sprays are insurance sprays that are potentially unnecessary. Knowledge of absolute pest abundance could be used to make more precise decisions about whether or not to spray. Withholding sprays could amount to substantial savings

for the growers. For example withholding one pesticide application, at a cost of \$30/acre, from Michigan's ca. 57,000 acres would save growers over a million dollars per year. Other benefits of withholding insecticide sprays include improved biocontrol from natural predators, reduced incidence of spider mite flare ups, and minimizing the potential for pesticide resistance.

Current Knowledge of Trapping

The ability to understand catch data in a monitoring trap is crucial to the success of any IPM program. Monitoring traps baited with synthetic sex-pheromone or other attractants are important tools for monitoring many pest insects including Lepidoptera. Meaningful interpretations of catch in a monitoring trap require detailed understanding of the component parts of trapping. Inroads of understanding have been made into this subject. As early 1976 it was recognized that trapping efficiency is determined by the active space (plume reach) of the pheromone and by the male's flying behavior (Nakamura and Kawasaki 1976). Wall and Perry (1987) identified the need for more suitable definitions for these components and offered the following definitions: 1) the range of sampling (R_S) is the maximum distance from which insects can be shown to reach the source in a given time period, 2) the range of attraction (R_A) is the maximum distance over which an insect can be shown to direct their movements towards the source (Wall and Perry 1987). Among Wall and Perry's stated goals was to stimulate

conversation and to that end I offer here clarification of the aforementioned terms.

I offer that R_A (Wall and Perry 1987) and active space (Nakamura and Kawasaki 1976) would be more accurately termed plume reach (Miller et al., 2015).

Additionally R_S (Wall and Perry 1987) will be referred to as trapping radius. Wall and Perry also correctly identified that trapping radius is comprised of two components; the net radial, non-directed movement towards the source and the attractive phase from when the odorant source is encountered (Wall and Perry 1987). In this research I will refer to the non-directed movement phase as the maximum dispersion of the insect. These distances will be unique for certain variables that will need to be specified such as: odorant source, insect species, environmental conditions, and duration of experiment. Another definition that appears in the literature is the effective attraction radius (R_{EA}) (Byers et al. 1989). The challenge with this EAR concept is that it does not take into account any specific behavior of the insect but is defined as the relative trapping power of baited vs. unbaited traps, expressed in terms of the “effective” trap size (radius) of the baited, active trap (Byers et al. 1989). R_{EA} was initially developed for open sticky traps which always give a nonzero catch even on passive traps, and therefore cannot be utilized in situations where catch in unbaited traps is almost always zero.

Other insights into trapping were developed by Turchin and Odendaal (1996a) working on southern pine beetle. These authors correctly deduced that

catch in a monitoring trap, when conducting the single trap multiple release experiment, could be broken into the probabilities of catch from these different release distances multiplied by the area of their respective annuli (Turchin and Odendaal 1996b). Unfortunately they rely on log transformations of their data, making intuitive interpretations difficult. To further complicate understanding, these authors report that the “effective sampling area” for their pheromone baited multi-funnel traps is only 0.1ha despite reporting marked re-capture from 1,000 m (Turchin and Odendaal 1996b). This example highlights the need for clarification of terminology around trapping and the need for straight forward methods of data analysis.

Future Applications

The application of these principles and terminology reaches far beyond entomology. Other authors have begun to explore these movement patterns in their own work. The concept of random walk has been seen as most appropriate for species for which their movement is relatively simple such as crickets (Brouwers and Newton 2009) and ants (Pearcd-Duvet et al. 2011). However, on a large enough scale, the random walk may be an appropriate model for the more complex behavior of larger animals such as wolves (Lewis 2001) and coyote (Moorcroft et al. 1999) and elephants (Dai et al. 2007). Researchers have begun to combined the emerging field of robotics with the behavioral capabilities of insects(Ando et al.

2013, Edwards et al. 2005). New technologies like remote sensing (Newton et al. 2009), underwater vehicles (Li 2006), and unmanned aircraft (Watts and Perry 2010) are being used to track animals, birds, hazardous chemicals, crop diseases, and insects. These devices will need to optimize their movement and search patterns just as searching insects do. Finally, while we currently operate within the paradigm of static traps there may come a time when technology allows for diffusing traps (Van Wijland 2002) and the need to partner with other disciplines will require a codification of trapping terminology that is currently lacking.

KEY CONCEPTS

Research led by Dr. Miller and participated in by the author of this dissertation has resulted in a book in SpringerBriefs in Ecology titled *Trapping of Small Animals Moving Randomly* (Miller et al. 2015). Some of the key concepts developed in the book are summarized here.

Interception

Understanding catch in a monitoring trap requires understanding of the interaction between the insect and the trap. The components of this interaction are the plume emanating from a trap and the path of the foraging insect. Due to the high efficiency of the male moth getting to the source of the plume once he has contacted it, we can consider the trap and its plume to be one unit. Both of these two components need to be quantified to better understand trapping mechanisms.

Plume Reach

Much early pheromone research focused on the size and structure of the plume (Elkinton et al. 1987). Measures of plume reach have been successfully made; for example the plume for a codling moth trap was estimated at less than 10 m (Grieshop et al. 2010). These measures have typically been in a single direction that favors success and under ideal conditions that maximize plume structure. Measures of plume reach under these conditions only tell part of the story because, in reality, insects can approach the plume from all directions, including directly

opposite of the downwind plume structure. In practice, the plume emanating from a trap must operate in variable wind and temperature conditions, over days and weeks. For these reasons the measure of plume reach must be made from the point of view of the entire population of moths as they approach the plume from all directions. It is this working plume length that needs to be measured in order to calculate the trapping area. In the following examples we will consider the plume to be a time-averaged circle with a trap at its center (T).

Patterns of movement

The foraging pattern of the male moth has evolved to find what is likely a small plume from the female codling moth. As a male moth pursuing a female moves through an orchard, it must: 1) displace away from its point of origin, and 2) search for the female's plume as it travels. The male moth must optimize these two tasks simultaneously. To do this, he moves by a set of preprogramed behaviors designed to optimize his search pattern and increase the chances of successfully intercepting the female's plume. The female and her plume are a relatively small target for the male moth searching within the large landscape of an apple orchard. Therefore, it is reasonable to postulate that the male moth must be well adapted at searching for pheromone. Much of the early literature depicts huge plumes that stretch across the orchard so as to find the male moth (Elkinton et al. 1987). This large-plume paradigm underestimates the dispersive capabilities of the

male moth. Flight mills have been effectively used to measure a moth's potential physiological capacity for linear displacement (Schumacher et al. 1997), However these measures do not tell us the net displacement of a moth, (or more importantly, a population of moths) as it navigates and searches along a meandering path through an orchard. Measures of net dispersive distance need to account for this meandering path.

Ballistic Movers

To begin to understand trapping as an interception problem, it helps to examine the simplest case first, ballistic movers. A ballistic mover would travel in a straight line away from its origin and follow that initial random heading for the duration of the experiment. This pattern of movement will only succeed in finding the target if that target lies in the path of the mover. This can be thought of as all displacement and no search. If this mover's displacement track were traced, we would see a straight line develop. If we released a population of ballistic movers from a common origin and each mover randomly selected headings independently of one another from the full 360 degrees possible, their tracks would form a pattern that would look similar to the spokes of a bike (Figure 1), with movers distributed in a random pattern.

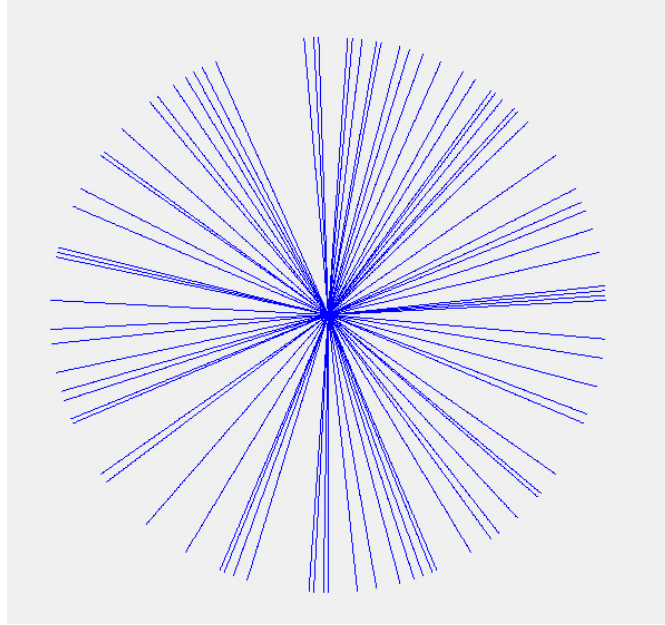


Figure 1. The tracks of 100 ballistic movers, starting from a common origin and dispersing in random directions independently from one another. Image generated with the Weston mover model.

Interception

If all the movers started at the same time and traveled at the same speed, then the population would form a circle with a circumference that increased with time. If a trap and its plume (considered together as a circle) were introduced into the experiment, it becomes obvious that some proportion of the movers would intercept and be caught by the trap (Figure 2). We want to know what proportion of the population was caught by this trap. For ballistic movers, this is simply the ratio of the trap diameter (T) to the circle formed by the population as it expands to the trap. The circumference of the circle formed by the population of movers can

be calculated by multiplying the release radius by 2π . With repeated sampling, this process gives an accurate ratio of the trap diameter (or length) to the population circumference from this release distance.

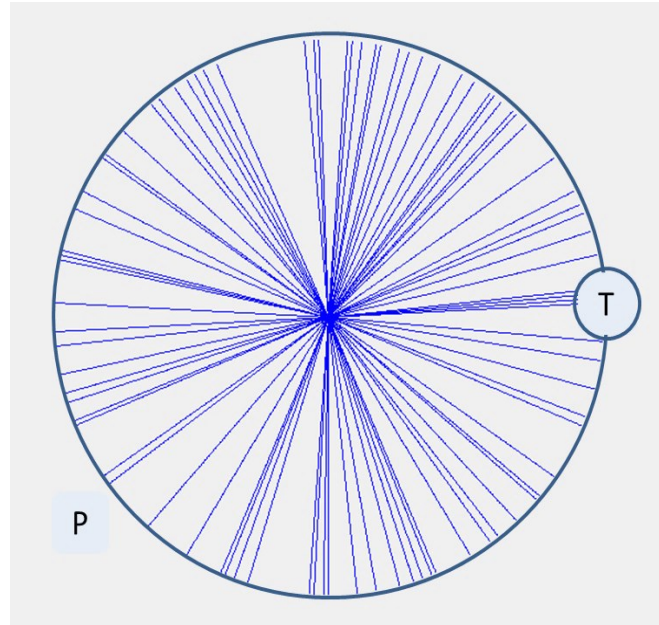


Figure 2. Large circle shows the population (P) of ballistic movers as it intersects the trap and its plume (T) depicted as the smaller circle. Image generated with the Weston mover model.

Single-Trap Multiple-Release

Let us consider how catch would be affected by changing release distances. If multiple populations of ballistic movers were released at increasing distances from the trap (Figure 3), the proportion caught of each population should decrease as distance increases.

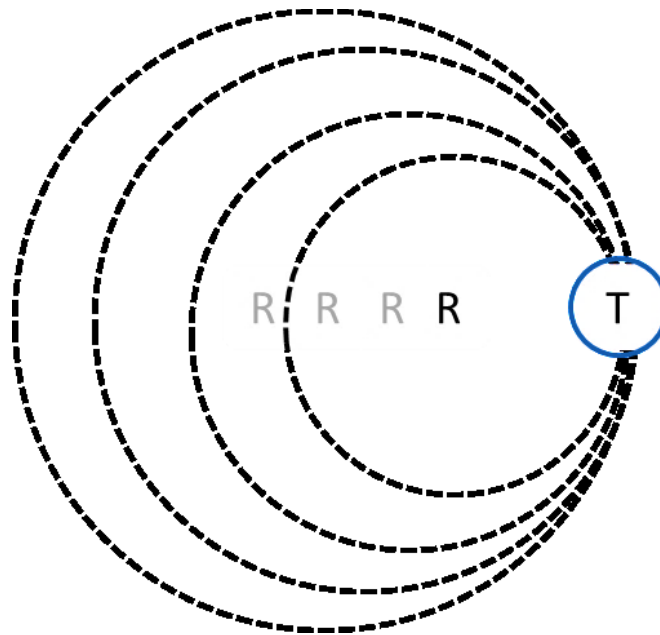


Figure 3. The dashed circles show the positions of several populations of ballistic movers released from varying release distances (R) as they would appear at the point they intercept the trap and plume (T).

MAG plot

Plotting the catch data from Figure 3 as proportion caught over distance (Figure 4) generates a concave curve with an asymptotic approach to the x axis as catch decreases with distance. If we then transform these data as the inverse of the proportion caught over distance, hence forth referred to as the MAG plot (Miller-Adams-McGhee) (Figure 5), a straight line is generated having a useful a slope.

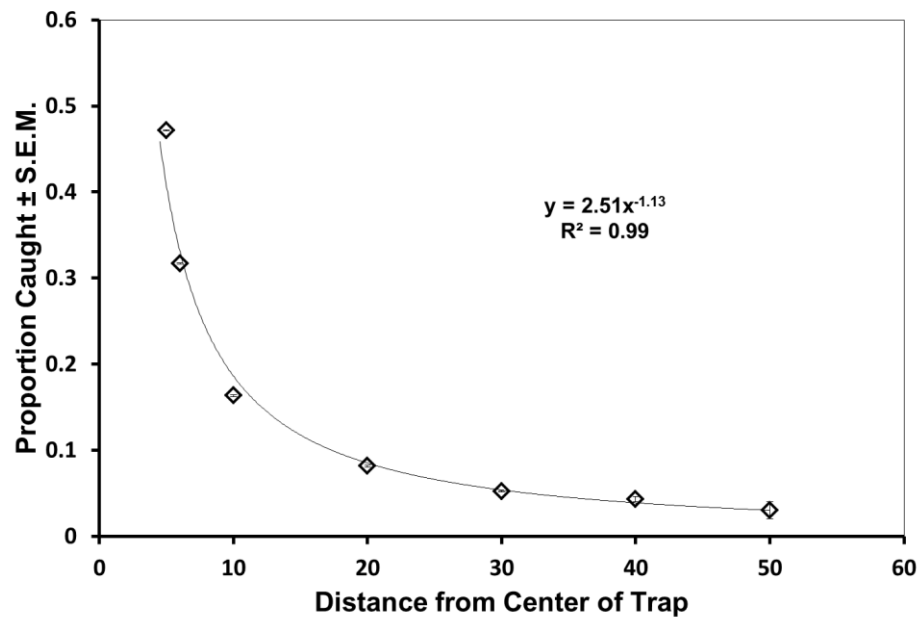


Figure 4. Mean proportion of 1,000 ballistic movers caught from varying release distances $\pm$ S.E.M. at a trap with a diameter of 10 units ($n = 100$).

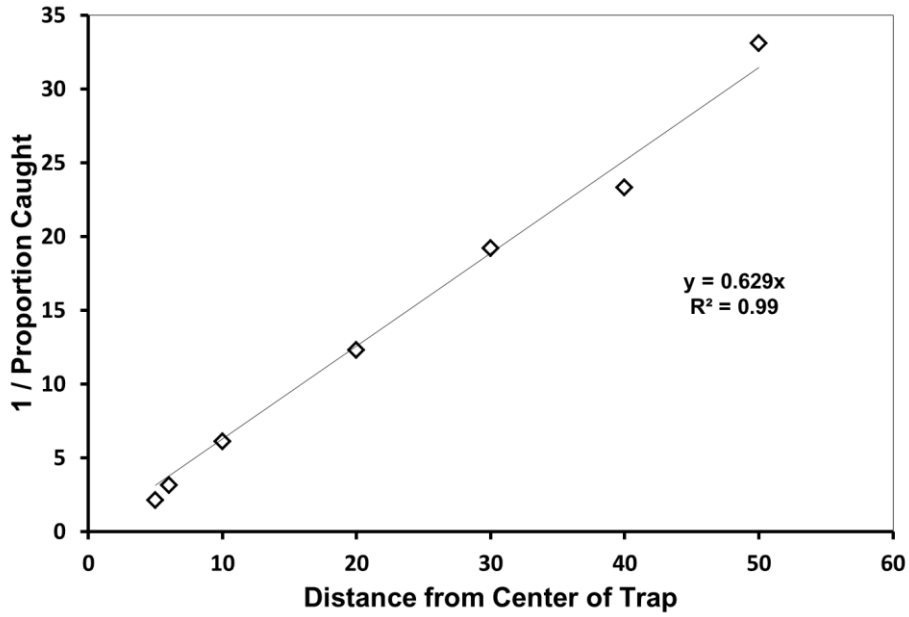


Figure 5. Transformation of data from Figure 4 showing 1/ mean proportion of ballistic movers caught over varying release distances. The slope from the straight line relationship of the transformed data can be used to reveal the size of the plume.

Finally, dividing 2π by the slope of the MAG plot ($2\pi / \text{Slope}_{\text{MAG}} = T$) returns the diameter of the plume and trap (T). Thus, using this simple experimental design of a single trap with multiple-releases and the MAG plot interpretation of the data, we have devised a way to measure the size of the plume emanating from a trap for ballistic movers.

Measures of Plume Reach for Non-Ballistic Movers

Using the Multi Mover simulator the single-trap multiple-release experiment described above was run with plumes of known sizes using various run times and C.S.D. to produce the standard curve (Figure 4.12 in Miller et al. 2015) of Figure 6. This standard curve allows the plume reach from field data to be looked up from the slope of a MAG plot graph.

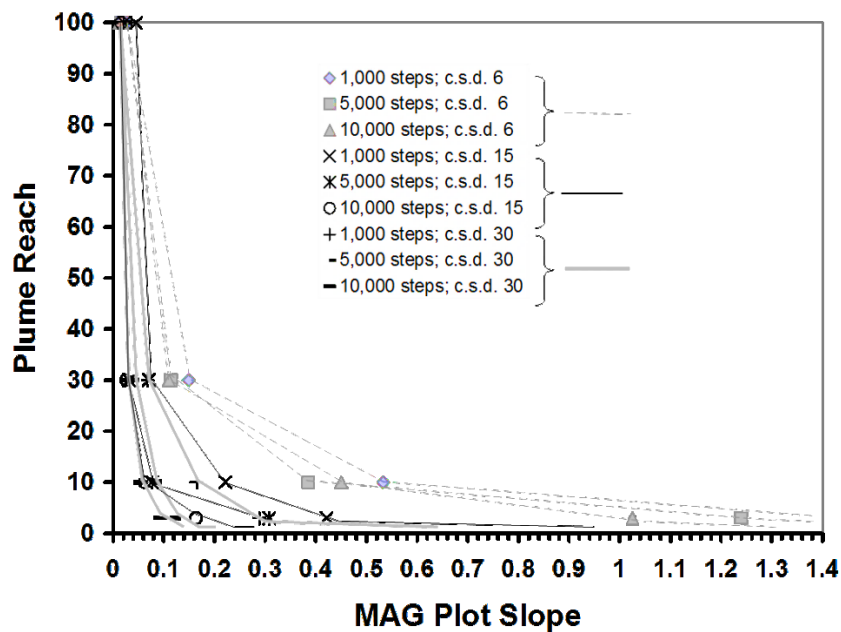


Figure 6. Standard curve of plume reach against MAG plot slope generated with Weston Multi Mover.

Change in C.S.D.

Since most organisms do not move in straight lines, we must consider more complex movements in our modeling and characterize these movements. To describe the distribution of turn angles a mover can make between steps the Weston mover model allows for the circular standard deviation (C.S.D.) to be adjusted. The C.S.D. is simply one standard deviation of a normal distribution of turn angles from the possible 360° of a circle. For example a C.S.D. of 2° results in a mover choosing a turn angle, from one step to another, within a range of 2° left and 2° right of its previous heading 68% of the time. Changing the C.S.D. of a mover affects its movement or foraging behavior. Figure 9 shows the tracks of 100 Weston movers for 1,000 steps using a C.S.D. of 2° . The C.S.D. of 2° generates tracks with a gentle side to side meander to them. The foraging success (number of interceptions) of these movers is only slightly better than ballistic movers. However not all of the movers maintain an outward direction, relative to their starting location. Changing the C.S.D. just this small amount allows for the possibility that some movers may return to the origin, as we can see in Figure 7, increasing the amount of local foraging.

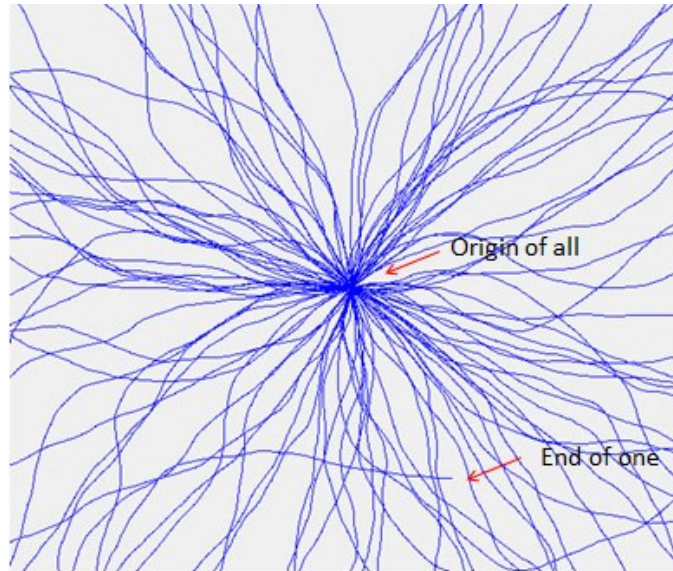


Figure 7. Weston mover model image of 100 movers starting from a common origin and dispersing for 1,000 steps using a C.S.D. of 2° . Note the track of one mover that has come back near the origin.

Random walk

Now consider the tracks made by the same number of movers (100) for the same number of steps (1,000) when we change the C.S.D. to 360° . When the C.S.D. is set to 360° the mover can choose any direction from one step to the next. Figure 8 shows the tracks of movers displacing from a common origin using a C.S.D. of 360° . This type of displacement is known as a classical random walk. Movers displacing in this way could be thought of as over-searching their environment because of the amount of backtracking performed by the movers.

High backtracking occurs when, from one step to another, all directions are equally probable.

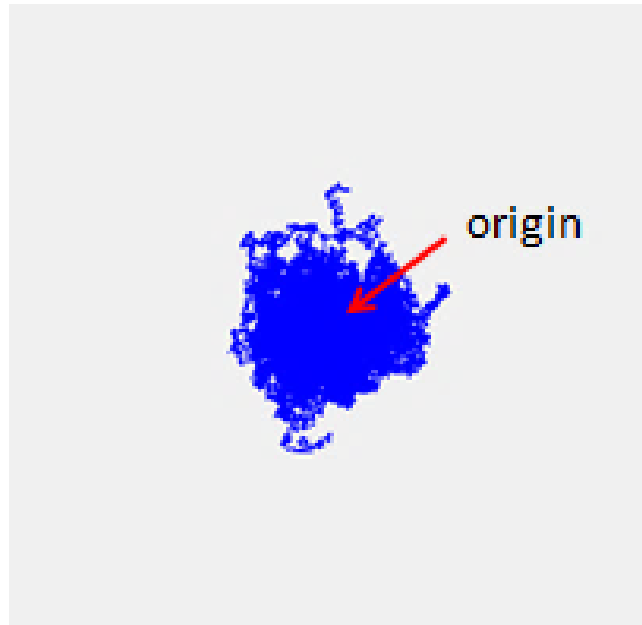


Figure 8. Weston mover model image of 100 movers starting from a common origin and dispersing for 1,000 steps using a C.S.D. of 360° . Note that the net displacement from the origin is greatly reduced relative to the movers in Figure 7.

When searching for resources, animals must find a balance between searching the local area very thoroughly and dispersing to searching new areas. Evolution should have pushed organisms toward an optimum C.S.D. that is a balance of local searching and dispersing when trying to locate finite resources/mates/rewards.

Trapping Gain

Recall that trap and plume (T) could be calculated from the central trap multiple release experiment with straight-line movers by dividing 2π by the slope of the MAG plot line. This calculation gives a good estimate of the size of the trap and its plume. When this experiment is conducted with C.S.D. values greater than 0, a value that is larger than the actual trap and plume is calculated. This extra catch is due to the turning behavior, or the amount of local search, of the mover which we refer to as Gain. Gain is therefore defined as the additional catch of a population of movers above what would have been caught if the movers had moved ballistically toward a trap of the same size.

$$\text{Gain} = (2\pi / \text{slope of MAG plot}) - T$$

Now well armed with some of the fundamental trapping concepts, an understanding of interception geometry, terminology, and the graphical tools and formulas for quantifying the constitute parts of the trapping area we are prepared to test these concepts and technics under the highly variable conditions of the field using biological movers.

MAIN OBJECTIVES

The main objective of the trapping research in our research group, of which this dissertation is a part, is to understand the mechanics of trapping sufficiently to deduce absolute pest density from catch number in a monitoring trap baited with sex pheromone. The two-part approach of this research program is to investigate these trapping mechanics with: 1) robust data sets of computer simulations to develop the theories and methods of data interpretation, and 2) through field experiments to test whether these emerging theories and methods maintain their predictive power for real animals in the field. Specifically, this research will 1) measure the change in proportion caught (T_{fer}) of a known population throughout the growing season, 2) measure the change in proportion caught of a known population with distance (spT_{fer}), 3) measure the plume reach emanating from a standard monitoring trap, 4) quantify dispersive distance of populations of the two target insects, 5) use these quantitative measures to calculate a total trapping area, 6) describe the search pattern, characterized as circular standard deviation of turn angles (C.S.D.) of these animals, and 7) demonstrate that these methods of data analysis can be used to accurately estimate absolute pest density within a given area, and finally 8) Demonstrate that these methods extend beyond these model insects (tortricid moths) to any organism that disperses by random walks.

CHAPTER TWO

Maximizing Information Yield from Pheromone-Baited Monitoring Traps:
Estimating Plume Reach, Trapping Radius, and Absolute Density of Codling Moth
(*Cydia pomonella*) in Michigan Apple.

ABSTRACT

Novel methods of data analysis were used to interpret codling moth (*Cydia pomonella*) (CM) catch data from central-trap, multiple-release experiments using a standard codlemone-baited monitoring trap in commercial apple orchards not under mating disruption. The main objectives of the study were to produce reliable and consistent measures of: 1) the plume reach, 2) the maximum dispersive distance of a population of responders, and 3) the proportion of the population caught, comprised of the composite probability of trap findability, efficiency, and retention (T_{fer}). Two release designs were employed: 1) releases at regular intervals in the four cardinal directions, and 2) even releases across entire ca. 16 ha orchard blocks using both high and low CM populations. For both release designs at high populations the unadjusted mean proportion caught (T_{fer}) was 0.01 as compared to 0.02 for even releases of low populations. Mean maximum dispersive distance for released codling moth males was ca. 260 m. Plume reach for the standard CM trap was only ca. 2 m, and total trapping area for a single trap was ca. 21 ha. These estimates were consistent across three growing seasons and are supported by extraordinarily high replication for this type of field experiment. Knowing the trapping area and T_{fer} , catch number per single monitoring trap can be translated into absolute pest density using the equation: males per trapping area = catch per trapping area / T_{fer} . Thus catches of 1, 3, 10 and 30 CM males per trap

translate to ca. 5, 14, 46, and 140 males / ha and reflect equal densities of females, since the CM sex ratio is 1:1. Combined with life-table data on CM fecundity and mortality, along with data on crop yield per trapping area, this fundamental knowledge of how to interpret catch numbers will enable pest managers to make considerably more precise projections of damage and therefore more precise and reliable decisions on whether insecticide applications are justified. A cumulative catch of 12 CM males per single monitoring trap not under mating disruption per season is suggested as the economic threshold for CM in Michigan. The principles and methods established here for estimating absolute CM density should be broadly applicable to pests generally and thereby set a new standard for IPM decisions based on trapping.

INTRODUCTION

Insect monitoring traps baited with species-specific sex pheromones are ideal tools for determining if particular pests are present in a crop and when they are active (Witzgall et al. 2010), thereby allowing control measures to be optimally timed (e.g., Judd and Gardiner 1997). Such traps and lures are relatively inexpensive and provide critical information quickly. Thus, it is not surprising that the number of lure-baited monitoring traps deployed annually around the globe can now be estimated by extrapolation from the data of Witzgall et al. (2010) at well over 15 million. Such traps have become a cornerstone of integrated pest management (IPM).

Nevertheless, lure-baited monitoring traps have fallen short of original hopes (Cardé 1976) that they might be used to quickly and inexpensively estimate the *absolute density* (abundance) of pests, which is the key variable for setting economic injury levels (Stern et al., 1959). In the absence of vetted procedures for translating capture numbers into absolute pest density estimates, pest managers resort to experience-based relative thresholds for action (e.g., Cuthbertson and Murchie 2005; Reddy and Manjunatha 2000; Schouest and Miller 1994; Riedl and Croft 1974) that are usually based upon years of correlating given catches with unacceptable levels of damage in particular locals. Currently, Michigan growers are advised to apply an insecticide when a cumulative catch of 5 to 7 moths during

the first generation or 3 to 5 moths during the second generation is recorded in a single codling moth (*Cydia pomonella*) (CM) trap baited with codlemone in an apple crop not under mating disruption (Gut and Wise 2016). Adoption of such indices for action has greatly improved pest management practices over regular calendar spraying. However, relative thresholds often lack sufficient precision and scientific grounding to guarantee that, rather than suffering a loss when withholding sprays, growers will raise profits (Pimentel et al. 1992) while reducing environmental damage (Miranowski 1980) as well as the incidence of insecticide resistance (Varela et al. 1993; Bouvier et al. 2001). Lack of understanding of the operative mechanisms governing pest-trap interactions has been a barrier to progressing beyond indices of pest abundance to the higher standard of using economic injury levels based on absolute pest abundance, as IPM theory professes (Stern et al. 1959).

Knowledge Required to Translate Catch Numbers into Absolute Pest Density

Accurate estimation of absolute pest density from a catch number recorded in a pheromone-baited trap requires knowledge of: 1) the pest's distribution in space and time, 2) the pest's movement patterns before and after encountering pheromone, 3) the farthest distance from which the trap can be reached, both unassisted and assisted by pheromone, and 4) the average probability of capture for all individuals in the trapping area (T_{fer}) of known size (Miller et al. 2015). As

pointed out by Riedl and Croft (1974), reliably linking catch number with absolute pest density across a growing season would be difficult or perhaps impossible if the pest's distribution were never reproducible and its movement patterns and activity levels over a lifetime varied substantially through time and with particular geography. On the other hand, estimating absolute pest density by trapping becomes feasible if insects foraging for pheromone plumes behave like molecules and execute "random walks" (Berg, 1993; Miller et al., 2015) that ironically cause the overall population to exhibit highly reproducible properties like those well quantified in physics and chemistry, e.g., diffusion, temperature, molecular flux, heat transfer. Any population of molecules or other random walkers (fliers) (Berg, 1993), including those simulated by computer (Weston 1986; Byers 1993; Miller et al. 2015), released from one point into a thin layer of static medium will always disperse equally in all directions so as to impart spatial regularity by forming a disk populated throughout, but with highest mean density at its center. Moreover, random walkers unevenly released as hot-spots will very quickly come to randomly populate their arena. Likewise, the trapping area for a trap collecting randomly distributed random walkers will itself be a regular disk (Miller et al. 2015) whose radius is comprised of the reach of the attractive plume from the trap plus the maximum dispersive distance for the local pest population (Hartstack et al. 1970; Wall and Perry 1987). Thus, the feasibility for translating capture numbers

into absolute pest density depends upon whether or not the pest behaves as a random-walker so as to impart regularity to all four points listed at the beginning of this paragraph.

Evidence for Random Walks by Flying Insects Not in Contact with Pheromone

The flight patterns of male insects responding to pheromone plumes have been very well characterized (Kennedy and Marsh, 1974; Baker et al., 1984; Cardé, 1984; Cardé and Willis 2008), often in wind tunnels (Miller and Roelofs 1978) and sometimes in the field (e.g., Baker and Roelofs, 1981; Elkinton et al., 1984; Baker and Haynes, 1996). Males so stimulated execute positive optomotor anemotaxis. This entails using visual cues from perceived ground flow over the visual system to steer upwind when flying in and along the edges of the pheromone plume while regularly zig-zagging to maintain plume contact. Doing so enables the responder to efficiently arrive at the pheromone source, be it a female or a trap.

On the other hand, flight patterns before pheromone contact have been rarely studied and are subject to considerable speculation. As summarized by Cardé et al. (2012 and references therein), the dominant expectation has been that insects foraging for pheromone plumes, but not yet encountering one, will raise their probability of contacting a presumed large plume by moving in straight lines and employing some non-random search strategy like flying up- or cross-wind.

Arguments have been offered for the superiority of certain of these wind-directed

tactics over others (e.g., Janzen 1984; Dusenbery 1990). However, despite their appeal to humans inclined toward sophistication rather than simplicity, very little evidence is available that insects actually employ wind- or geography-directed tactics when foraging for plumes. In fact, the extraordinary study by Cardé et al. (2012), using videography to directly measure the headings of day-flying bog moths, *Virbia lamae*, foraging for but not yet contacting pheromone plumes, elegantly demonstrated that no heading with respect to instantaneous wind direction or geographic feature was favored over any other. The flight headings of foraging bog moth males were random and consistent with random walking while employing modest path meander.

A related prediction that should hold for a population of insects foraging for pheromone plumes using random walks is that, when released from one point and not limited by crop edges, they should disperse so as to occupy a radially symmetric disk. The regularity of such dispersal can be measured by superimposing a dense grid of traps over the population after allowing it time to disperse from a single point. Symmetric dispersion from a single release point has been reported for CM (Worthley 1931), pine saw fly (*Neodiprion sertifer*) (Wedding et al. 1995, Östrand and Anderbrant 2003), oriental fruit moth (*Grapholita molesta*) (Ellis and Hull 2013), and painted apple moth (*Teia anartoides*) (Guichard et al. 2010). We are aware of no studies yielding notably

skewed dispersive disks that would falsify the random walk search model.

Such results and a growing body of evidence that local dispersion of small animals not yet guided by cues from resources frequently occurs by diffusion (Kareiva and Shigesada 1983; Rudd and Gandour 1985; Fagan 1997; Okubo and Levin 2001; Benhamou 2007; Patterson et al. 2008; Nathan and Giuggioli 2013) justify renewed attempts, like that of Miller et al. (2015), to derive estimates of absolute pest density from capture numbers in attractive traps based on a foundation of random walks.

Examples of Past Attempts to Estimate Absolute Pest Density from Capture Number in Attractive Traps

The first such attempt of which we are aware (Hartstack et al. 1970) occurred before pheromone-baited traps were available and used captures of marked moths released at defined distances from a single UV light-trap, an experimental approach pioneered by Wolf et al. (1971). Coming from an engineering background and familiar with molecular processes, Hartstack et al. (1970) were willing to assume that, over their lifetimes and despite the difference in size, dispersing insects would not behave differently in principle from molecular dispersal. They therefore assumed: 1) marked and released moths behaved no differently from wild moths; 2) moths dispersed randomly; 3) irrespective of exact initial origin, pests per area would quickly become homogeneous and directly

proportional to area alone; and 4), trapping area would be a regular disk whose radius was maximum dispersive distance for the local pest population plus attraction distance of the UV lamp. Hartstack et al. (1970), as clarified by Turchin and Odendaal (1996a), reasoned that the number of insects present in a narrow annulus of trapping area centered on the trap would equal annulus area x insects per area. Moreover, the probability of capture of insects from a given annulus could be and was measured experimentally after releasing known numbers of pest from defined distances. Maximum trapping radius was taken as the farthest distance yielding a capture. Then the number of insects caught would be given by the sum (for all annuli of the trapping area) of probability of capture for each annulus multiplied by its area. Finally, the absolute density of insects in some new trapping area with size equal to that found during calibration can be calculated algebraically by dividing the catch number per single trap by the weighted average probability of capture for all annuli (T_{fer} in the current vocabulary) (Hartstack et al. 1970, Turchin and Odendaal 1996a, Miller et al. 2015), or more directly by integrating using calculus. Although their methods varied slightly from the above, Hartstack et al. (1970) reported that, for the highly mobile tobacco hornworm, *Heliothis virescens*, the trapping radius for their single light trap was over 3,000 m, yielding a huge trapping area of ca. 2,800 ha. A capture of 100 moths per single light trap equated to 110 moths per ha. Östrand and Anderbrant (2003) extended

this approach when estimating absolute density of pine sawfly, *Neodiprion sertifer*, in Sweden using traps baited with female sex pheromone. The trapping radius for their single trap was ca. 1,000 m; therefore the trapping area was ca. 314 ha. Capture of 112 males over one adult generation translated into only 23 males per ha per generation.

Additional researchers (Byers et al. 1989; 2012; Mason et al., 1990; Dodds and Ross, 2002; and Sufyan et al., 2011) have used these and similar approaches to propose that absolute pest density could be quantified for various types of insect pests using capture number. But, curiously, there are yet no examples where such procedures are actually implemented in pest management for any crop. Reasons for this gap should be explored and remedied. Explanations for the failure to adopt practices that could elevate pest management decisions from an art best done by a select few experts with vast experience with the given cropping system into a science open to all might include: lack of confidence in the molecular assumptions upon which the translations from capture number to absolute density have been based; lack of confidence that the calibrations will hold for other times and places where crop and environmental conditions differ; and lack of awareness of this timely opportunity. The science of pest management stands in need of a well-replicated proof-of-concept study that examines the feasibility and reproducibility

of using capture numbers in traps for absolute pest density determinations over several growing seasons and at multiple locations.

Study Objectives and Rationale for Using CM for Proof-of-Concept

The current study aims to provide definitive proof-of-concept for procedures to translate capture number in monitoring traps into absolute pest density based upon field experiments using the single-trap, multiple-release design (Wolf et al., 1971; Turchin and Odenaal 1996a) measuring probability of male CM catch from specified distances of release (specific T_{fer} , designated spT_{fer} by Miller et al., 2015). Here we put into practice the techniques for interpreting catch and measuring maximum dispersive distance and pheromone plume reach recently detailed by Miller et al., (2015). Moreover, these experiments were conducted at both high and low CM densities, so as to represent conditions encountered by growers spraying often or little. The techniques and principles being validated here should be broadly applicable to diverse pests that forage using random walks.

Codling moth is a cosmopolitan pest of pome fruits having high economic importance whose management is based on relative capture numbers in pheromone traps (Witzgall et al. 2008). Pheromone chemistry for CM has been thoroughly studied and optimized (Witzgall et al. 2008) along with trap design. Estimates for maximum dispersive distance of male CM have ranged from 150 m (Worthley 1931) to 8 km along a river valley in a male only environment to as low as 55 m in

a short-term, mixed-sex experiment (Howell and Clift 1974) and as much as 10 km on flight mills (Schumacher et al. 1997). As summarized by Mani and Wildbotz (1977), however, the maximum dispersive distance achieved by ca. 95% of laboratory-reared and wild-collected populations is thought to be under 400 m. Very important for feasibility of the current study, large numbers of reproductively sterilized CM, shown to disperse similarly to wild moths (Mani and Wildbolz 1977), could be purchased for well replicated field studies.

Simulation Results Guiding the Current CM Study

Once we obtained preliminary data for pheromone plume reach and maximum dispersive distance for a given population of CM released into Michigan apple orchards, a preview (Figure 9) of likely catch patterns under differing survival regimes was generated using computer software (Weston MultiMover (Miller et al. 2015)) simulating trapping of correlated random walkers operating across varying run times.

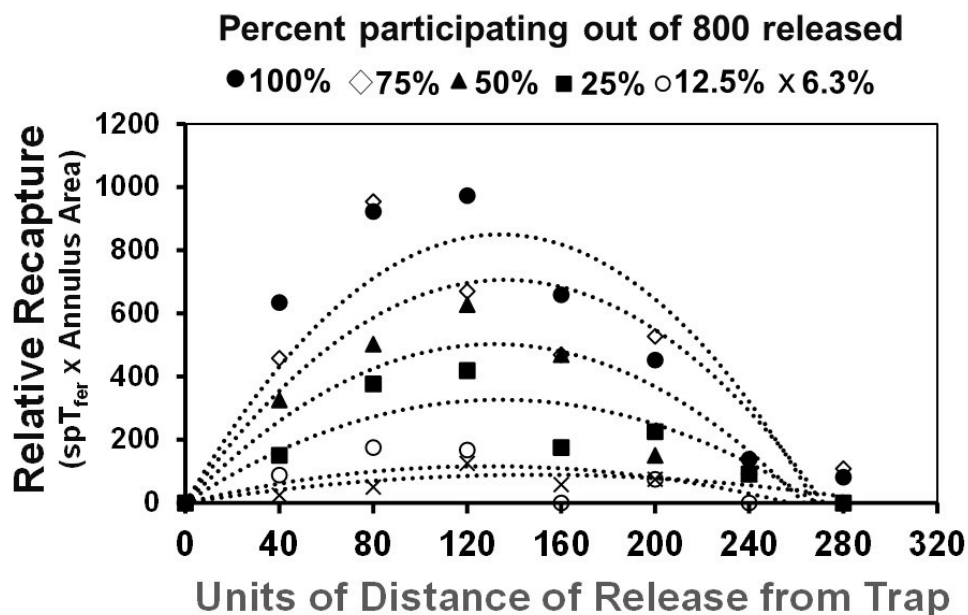


Figure 9. Change in pattern of capture profiles to be expected if not all of the codling moths released into the forthcoming experiments lived. Individuals not participating simulate those killed by an insecticide. SpT_{fer} indicates proportion of insects captured after release at a particular distance, for the purpose of this demonstration always calculated on the basis of 800 released. Annulus area indicates a ring out of the full trapping area (composite of all annuli) sampled by a single trap. In all cases the C.S.D. for headings for new steps was 30° and the total number of steps was 3,000. The trend lines were fit using a second order polynomial.

The results of Figure 9 are typical. Here the number of simulated insects released at each distance was 800 (similar to the number used below in Experiment 1 of the CM study) and the number of steps taken with a meander of circular

standard deviation 30° (Miller et al. 2015) was 3,000, so as to approximate the realized maximum dispersion distance for CM measured preliminarily. The distance of release from the trap appears on the x-axis of Figure 9, while the y-axis is a measure of the relative capture of released individuals from defined distances. As seen in Figure 9, the height of capture profiles is expected to diminish as participant numbers decrease and proportion caught from any given distance (spT_{fer}) continues to be calculated on the basis of 800 released. Such results could be obtained if the number of codling moth participants in some replicates of the current field experiments were reduced due to insecticide treatments while other replicates received no insecticide. The data of Figure 9 demonstrate that, even so, measures of maximum dispersion for the pest will not be greatly impacted by such mortality, even if its severity is unknown. Note that no profile reached the x-axis before a release distance of 280 distance units (nearly 80% of the maximum measurable under the highest level of participants). This knowledge helps legitimize the field experiments to follow conducted in commercial apple orchards under grower rather than experimenter management and where the actual number of surviving CM could not always be ascertained.

MATERIALS AND METHODS

Source and Handling of Codling Moths

Mixed-sex codling moths, internally dye-marked with Calco red vegetable dye, were purchased from the mass-rearing SIR facility in Osoyoos, British Columbia. Newly eclosed moths were sterilized at the rearing facility with 33 krad of gamma radiation from a Cobalt 60 source. A prescribed number of 9-cm diameter petri dishes filled with sterilized CM were shipped overnight to Michigan State University in coolers maintained at ca. 5° C with cold-packs; they always arrived ca. 10:30 am allowing moths to be released the same day. Due to the very high numbers of moths used (from 2,100 to 20,000 per run), and the need for rapid releases, separation by sex was not possible; therefore, all releases were males plus females. Arriving moths were immediately dispensed into 540 ml polypropylene cups (Fabri-Kal Corp., Kalamazoo, MI) in batches of ca. 100 and dusted with ca. 10 mg of fluorescent powder (DayGlo Color, Cleveland, OH). Periodically one cup was chosen at random and all moths were counted and sexed to confirm release numbers and sex ratio, which was confirmed to be 50:50 (± 0.08 S.E.M.) across all experiments. Moths for each release distance were uniquely colored. Capped cups containing moths and dye were repacked into coolers (Igloo Products Corp, Katy, Texas) at ca. 5° C for the 1 h transport to the orchard blocks. Moths were released the day of receipt at ca. 13:00 h. Chilled moths were allowed to

acclimate to ambient temperature before being lightly swirled to promote uniform coloration and ejected into the air at each pre-flagged release point. Ejected moths flew in all directions and alighted in the foliage of adjacent trees within a few seconds.

Estimates of Proportion of Purchased CM Capable of Participating

At semi-regular intervals during these experiments, ca. 400 males (and 400 females) were deployed into 3 x 3 x 2 m tents (Instant Up Canopy Quest[®] model: CEH00296 American Sports Licensing, Wilmington, DE 19805) with insect netting sidewalls enclosing two 2 m tall apple trees and four pheromone- baited delta traps suspended near the upper portion of the trees. Traps were checked and liners changed daily so as to ascertain what proportion of released males was eventually captured and in what time-frame.

Orchards

Experiments were conducted in various commercial apple orchards (details in Table 1) in and around Sparta, Michigan during the growing seasons of 2013 - 2015. These orchards were not and had not been under mating disruption the preceding 3 yr. Experiments were timed to avoid releases of moths during weeks when insecticide sprays occurred.

Table 1 Orchard planting details and GPS coordinates

Orchard Number	Tree Spacing	Row Spacing	Tree Height	Planting Style	Apple Varieties	GPS Coordinates	Insecticide Sprays
1	5.5 m	7.6 m	4.5 m	Large trees	I, R	43°02'04.36" N 85°42'06.72" W	no
2	5.5 m	7.6 m	3.5 m	Trellis trees	F, G, D, J	43°10'03.24" N 85°46'55.36" W	yes
3	1.5 m	4.8 m	3 m	Trellis trees	G, D, J, M	43°07'35.22" N 85°21'37.24" W	yes
4	2.5 m	5.0 m	3.5 m	Trellis trees	F, G, H, S	43°09'11.50" N 85°46'57.04" W	yes
5	2.5 m	5.0 m	3.5 m	Trellis trees	F, G, H, S	43°09'08.89" N 85°47'49.65" W	yes

Varieties: Fuji – F, Gala – G, Golden delicious –D, Honey Crisp – H, Ida Red – I, Jonagold – J, McIntosh – M, Rome – R, Sweet Tango – S.

Experiment 1 -- Cardinal-Direction Releases

These experiments were conducted June through August of 2013 in three 18 ha orchard blocks of mixed cultivars (Orchards: 1, 2, and 3 of Table 1). Release distances were flagged in the four cardinal directions from the central trap (Figure 10) at distances of 40, 80, 120, 160, 200 and 240 m. In five of the replicates, ca. 800 males (and ca. 800 females) were released per distance. One replicate was conducted with ca. 1,000 males (and females). Three additional replicates were conducted with populations of ca. 1,600 males (and equal numbers of females) per distance. A single delta trap (Pherocon VI; Trécé Inc., Adair, OK, U.S.A.), baited with a CML2 gray septum (Trécé, Inc.) containing (E,E)-8, 10-dodecadien-1-ol (codlemone) held aloft of the TanglefootTM-covered cardstock liner by a pin

through the trap roof, was placed in the top third of the canopy of a tree near the center of each block. Traps were checked daily; populated sticky liners were replaced and examined in the laboratory under a combination of UV illumination (22W fluorescent Circline BL #2851L, BioQuip products Rancho Dominguez, CA; 15 W fluorescent tube BL #2806, BioQuip products, Rancho Dominguez, CA; and 32 UV LED retrofit bulb, Battery Junction Old Saybrook CT, in an ML300L 3-cell D flashlight, Mag Instruments Ontario, CA) to determine coloration of powder on moths. Moths were always inspected for sex and internal red dye to distinguish them from the sparse wild population.

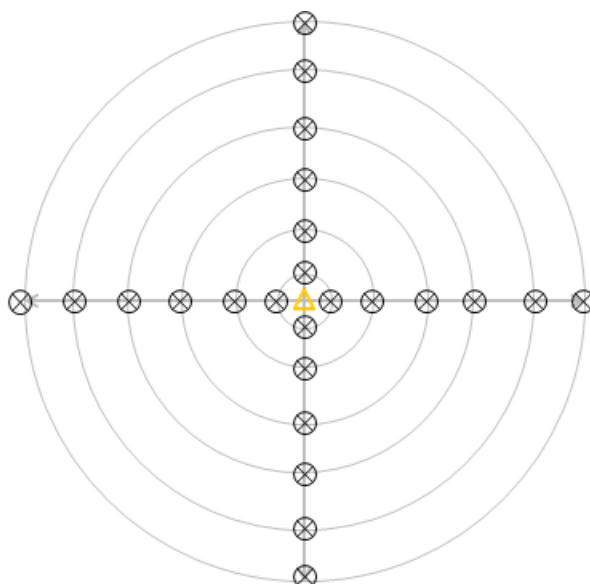


Figure 10. Release pattern of codling moth for cardinal direction release (Experiment 1).

Table 2 Color and number of codling moth per distance of Experiment 1, and the resulting moths per area.

Color	Release Distance (m)	Number of Releases	Moths /Annulus	Annulus Area m ²	Acres /Annulus	Hectares /Annulus	Moths /m ²	Moths /acre	Moths /ha
No	40	4	800	10048	2.48	1	0.08	323	800
Green	80	4	800	20096	4.97	2	0.04	161	400
Orange	120	4	800	30144	7.45	3	0.03	107	267
Blue	160	4	800	40192	9.93	4	0.02	80	200
Pink	200	4	800	50240	12.41	5	0.02	64	160
White	240	4	800	60288	14.9	6	0.01	54	133

Experiment 2 -- Even-Release with High Populations

These experiments were conducted from June through August of 2015. Here moths were released in ca. 30 min with the aid of a 4 x 4 motorcycle at 84 release sites (Figure 11) throughout orchards (1, 4, and 5 of Table 1) concurrently, so that ca. 400 male moths / ha were evenly distributed across the 18 ha for a total of ca. 8,400 males in each of the 9 replicates. Moths within each annulus were uniquely powered. Radii of annuli were 40, 80, 120, 160, 200, and 240 m.

Experiment 2.5 -- Even-Releases with Very High Populations

In July and August of 2014 a total of 20,000 moths was released evenly throughout the 16 ha of Orchard 1 (Table 1) at 100 flagged locations in a configuration similar to Figure 11, creating a population of ca. 1,250 male moths / ha. Distances of 40, 80, 120, 160, and 200 m were uniquely marked with Dayglow powder as described above. Three replicates were conducted through time.

Experiment 3 -- Even-Release with Low Populations

These experiments followed the protocols for Experiment 2. However, a low-population, even-release condition was achieved by releasing the same number of moths per release point, as Experiment 2, but with only one quarter of the release points (Figure 12) across the 18 ha experimental plots resulting in 100 moths per ha for a total of 2,100 male moths in each of the 15 replicates.

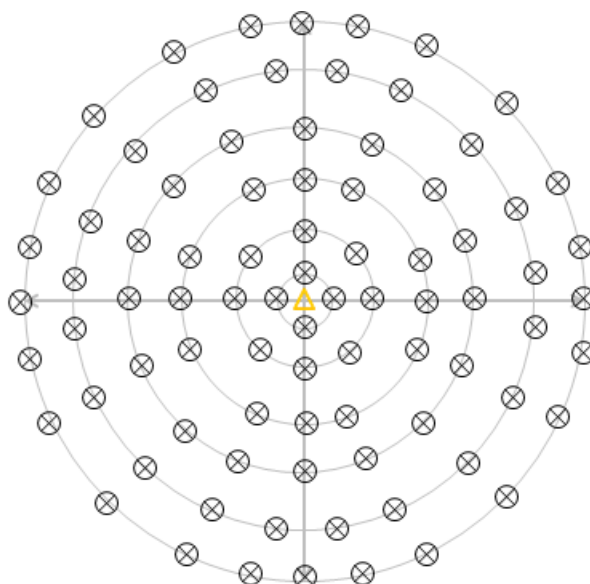


Figure 11. Release pattern for even-release of codling moth over 20 ha
(Experiment 2).

Table 3 Color and number of codling moth per distance for Experiment 2, and the
resulting moths per area.

Color	Release Distance (m)	Number of Releases	Moths /Annulus	Annulus Area m ²	Acres /Annulus	Hectares /Annulus	Moths /m ²	Moths /acre	Moths /ha
No	40	4	400	10048	2.48	1	0.04	161	400
Green	80	8	800	20096	4.97	2	0.04	161	400
Orange	120	12	1200	30144	7.45	3	0.04	161	400
Blue	160	16	1600	40192	9.93	4	0.04	161	400
Pink	200	20	2000	50240	12.41	5	0.04	161	400
White	240	24	2400	60288	14.9	6	0.04	161	400

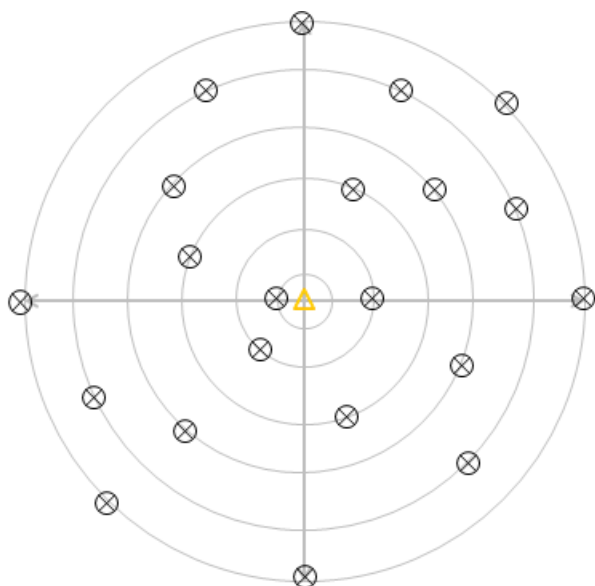


Figure 12. Release pattern for even-release low-population of codling moth (Experiment 3).

Table 4 Color and number of codling moth per distance for Experiment 3, and the resulting moths per area.

Color	Release Distance (m)	Number of Releases	Moths /Annulus	Annulus Area m ²	Acres /Annulus	Hectares /Annulus	Moths /m ²	Moths /acre	Moths /ha
No	40	1	100	10048	2.48	1	0.01	40	100
Green	80	2	200	20096	4.97	2	0.01	40	100
Orange	120	3	300	30144	7.45	3	0.01	40	100
Blue	160	4	400	40192	9.93	4	0.01	40	100
Pink	200	5	500	50240	12.41	5	0.01	40	100
White	240	6	600	60288	14.9	6	0.01	40	100

Data Analysis

Data were graphed according to the conventions of Miller et al. (2015) to yield: 1) an untransformed plot of proportion of released moths captured vs. distance of release; 2) $1 / \text{proportion of released moths captured}$ vs. distance of release (MAG plot); and 3) annulus area x proportion of released moths captured vs. distance of release (Miller plot).

Areas of annuli were calculated by; $\text{annulus area} = \pi (R_1^2 - R_2^2)$. Where R_1 is the outer radius and R_2 is the inner radius of each annulus centered on the release radius. When release distances are regularly spaced the inner and outer radii of each annulus would be the release radius minus $\frac{1}{2}$ the distance between release points and the release radius plus $\frac{1}{2}$ the distance between release points respectively. The untransformed plot provides confirmation that release distances were appropriately selected and that the responders were random-walkers when it produces a smooth concave line with an asymptotic approach to the x axis. The untransformed plot is also the only appropriate place to report the variance measured around mean captures that, upon transformations, becomes distorted and not useful. For random walkers, MAG plots will be initially linear (Miller et al. 2015), yielding a slope that can be used to deduce plume reach from the standard curve of Miller et al. (2015; Figure 4.12). Slopes of MAG plots and therefore determinations of plume reach are independent of and therefore insensitive to the

actual number of participants. Miller plot data can be fitted to a second-order polynomial whose x-intercept gives ca. 95% of the maximum dispersive distance. Height of the polynomial line on the Miller plot at points along the x-axis reveals the relative contribution of those distances to overall catch. Additional release distances would theoretically appear as an asymptotic tail that would represent the last 5% of possible catch. Catch from this outer edge becomes highly improbable in field experiments, thus a procedure to estimate 95% of the dispersive distance of the population is more useful to applied researchers.

For even-release experiments, where all annuli were populated with the same CM density, T_{fer} was calculated by dividing catch by total number of male CM released across the experiment. Calculation of T_{fer} for cardinal direction experiments, where number released did not increase with distance, were performed by dividing mean annulus area $\times$ spT_{fer} (proportion caught at a defined distance) by mean annulus area (see Table 5.1 example of Miller et al., 2015).

RESULTS

Estimates of Proportion of Purchased CM Capable of Participating

Based upon captures of CM males released into insecticide-free apple trees under field tents, we estimate ca. 80% (range 60-90%) of purchased SIR males were available to participate in these experiments. Subsequent to release, the data below will show that the proportion of participants in some orchards was further reduced, apparently by the chemical treatments they received. In all cases, catch was highest 1-3 d after release and tailed off to zero by one week. Powder-marked moths performed identically to unmarked moths.

Experiment 1 -- Cardinal-Direction Releases.

Of the 4,000 to 9,600 male moths released, less than 3% were recovered on average. The mean spT_{fer} for the closest release at 40 m was 0.056 ± 0.01 (mean $\pm$ S.E.M., $n = 9$). As is typical for random walkers (Miller et al., 2015), catch decreased smoothly with a regular increment of distance from the central trap to a mean spT_{fer} of 0.008 at the furthest distance of 200 m (Figure 13) and approached the x-axis asymptotically. The MAG plot (Figure 14) produced a straight line over the closest 4 data points with a slope of 0.43. Using the MAG plot standard curve (Figure 4.12 of Miller et al., 2015), plume reach was estimated at ca. a mere 2 m. The Miller plot of these data (Figure 15) bears a striking resemblance to the random-walker simulation data of Figure 9. Its projected x-intercept indicated the

maximum trapping radius was ca. 240 m. Thus, trapping radius, comprised by plume reach plus maximum dispersive distance, was overwhelmingly dominated by the latter. A trapping radius of 240 m equates to a trapping area of 20 ha. Mean T_{fer} was 0.01 ± 0.003 ($\pm$ S.E.M., $n=9$); values across the respective replicates ranged from 0.001 to 0.025.

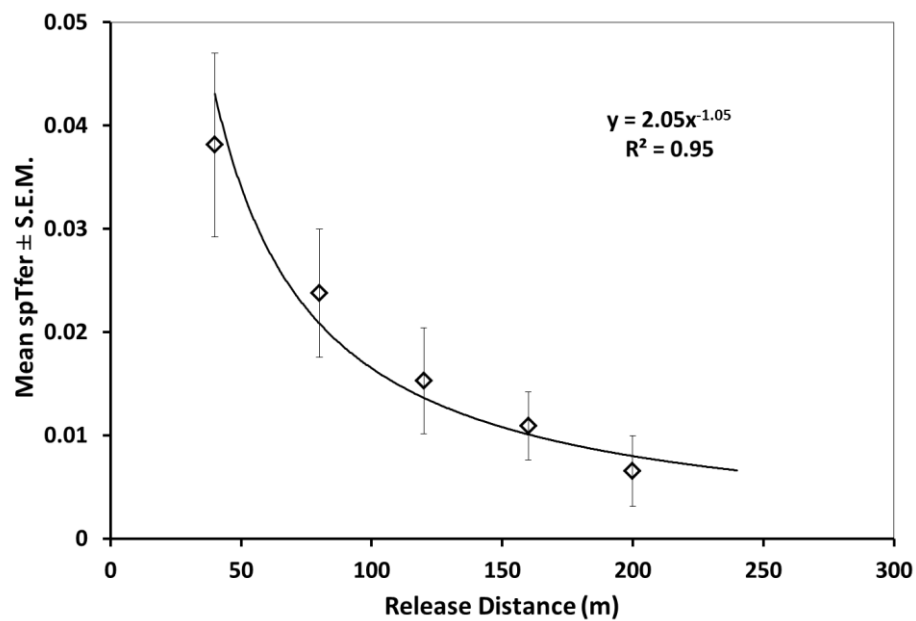


Figure 13. Mean specific trap findability (spT_{fer}) for central-trap, cardinal-direction release, Experiment 1 ($n = 9 \pm$ S.E.M.).

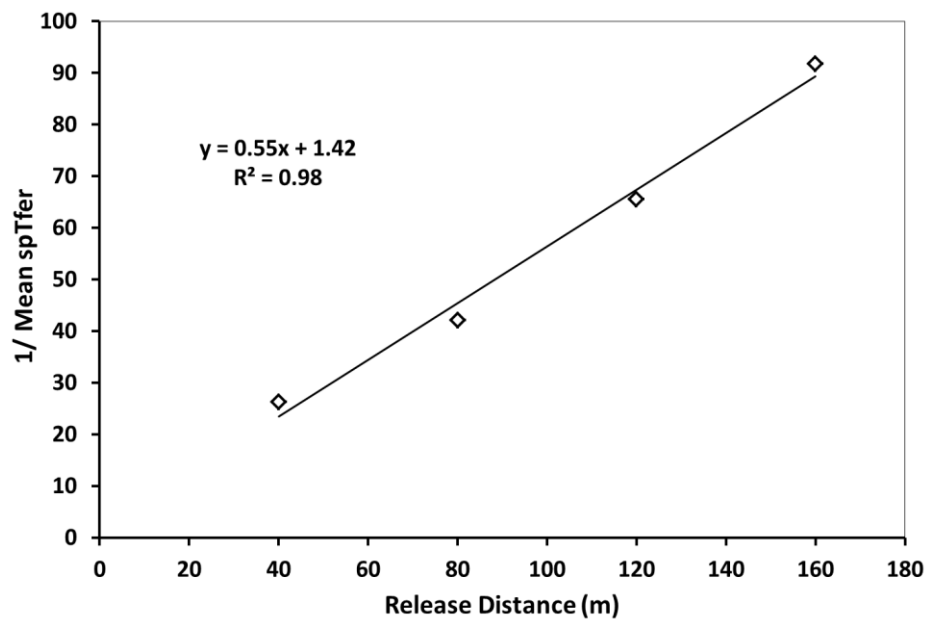


Figure 14. MAG plot transformation of Figure 13 data.

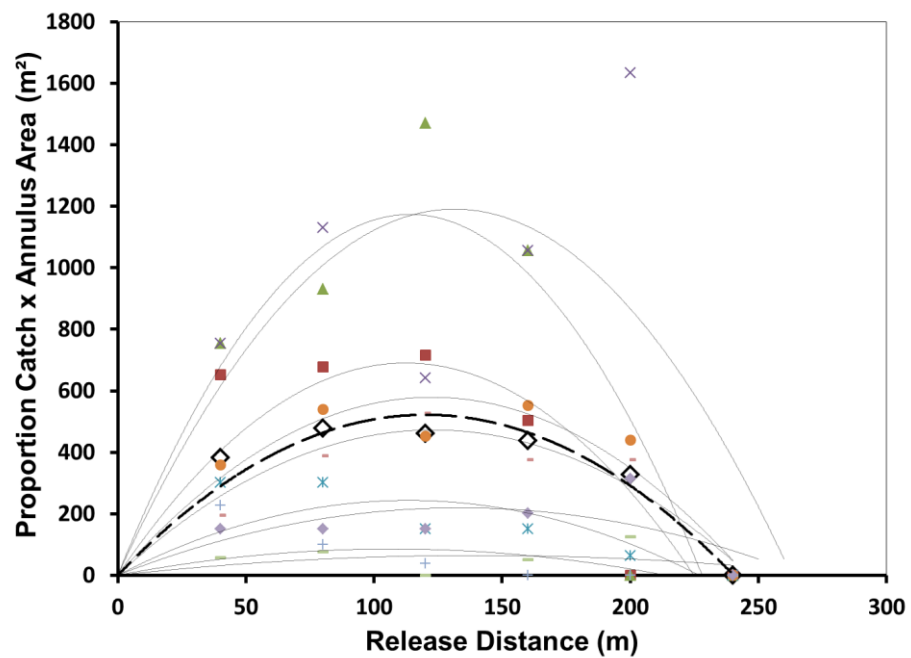


Figure 15. Miller Plot of Figure 13 data and their mean (dashed line).

Experiment 2-- Even-Releases with High Populations

As expected, results of Experiment 2 were strikingly similar to those of Experiment 1. For even releases at 8,000 moths per 18 ha, the average T_{fer} was 0.01 ± 0.005 (range 0.002 to 0.05). The untransformed data from the even release with high populations (Figure 16) show that the mean $\text{sp}T_{\text{fer}}$ for the closest distance of 40 m was 0.05 ± 0.02 (mean $\pm$ S.E.M., $n = 12$). Catch decreased with distance from the central trap to a mean $\text{sp}T_{\text{fer}}$ of 0.006 at the furthest distance of 240 m (Figure 16). The MAG plot (Figure 17) produced a straight line with a slope of 0.52. Again, this slope equates to a plume reach of only ca. 2 m. The Miller plot (Figure 18) supports a maximum dispersive distance for the population as ca. 275 m, resulting in a trapping area of ca. 23 ha.

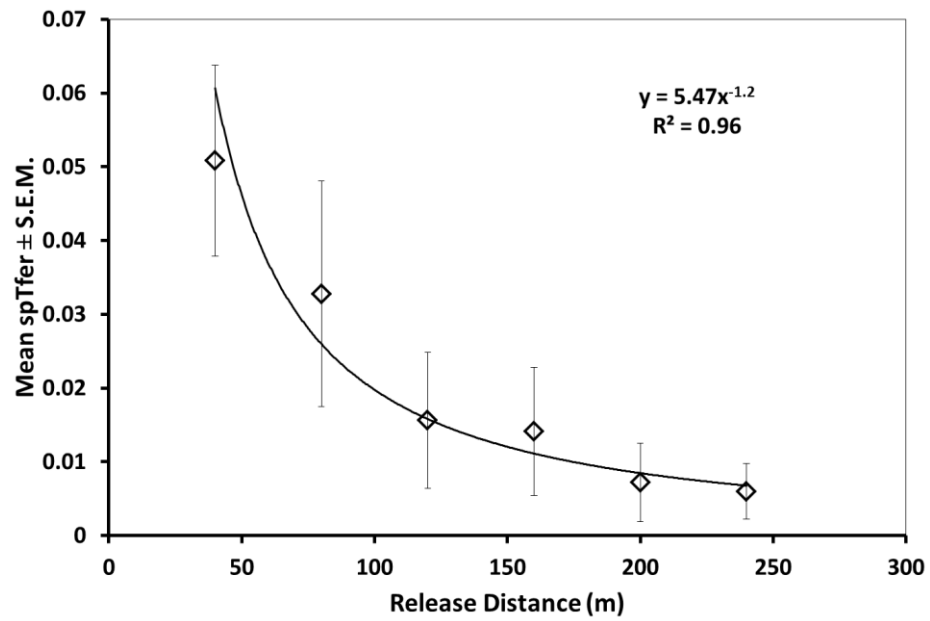


Figure 16. Mean specific trap findability (spT_{fer}) for the central-trap, even-release Experiment 2 ($n = 9 \pm S.E.M.$).

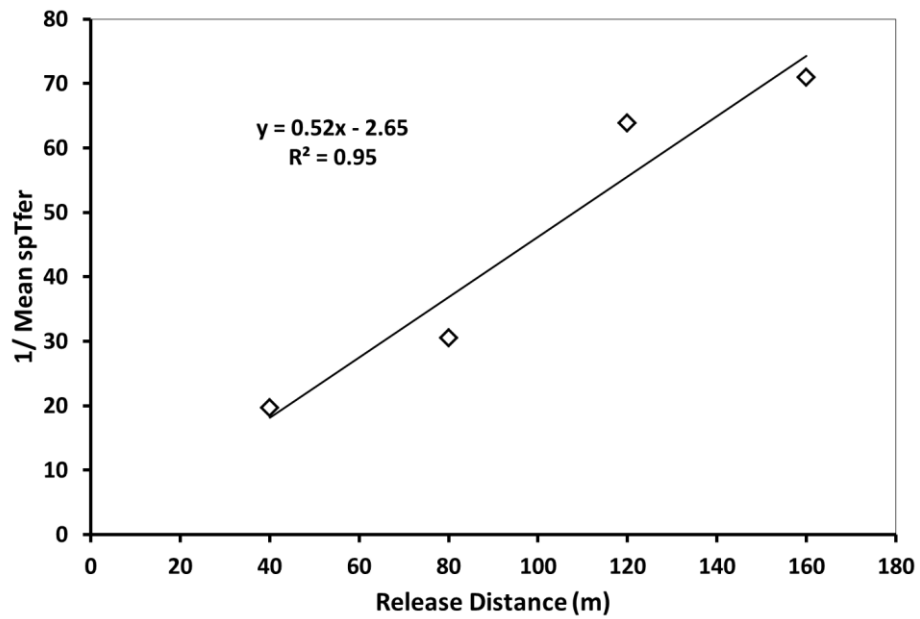


Figure 17. MAG plot transformation of Figure 16 data.

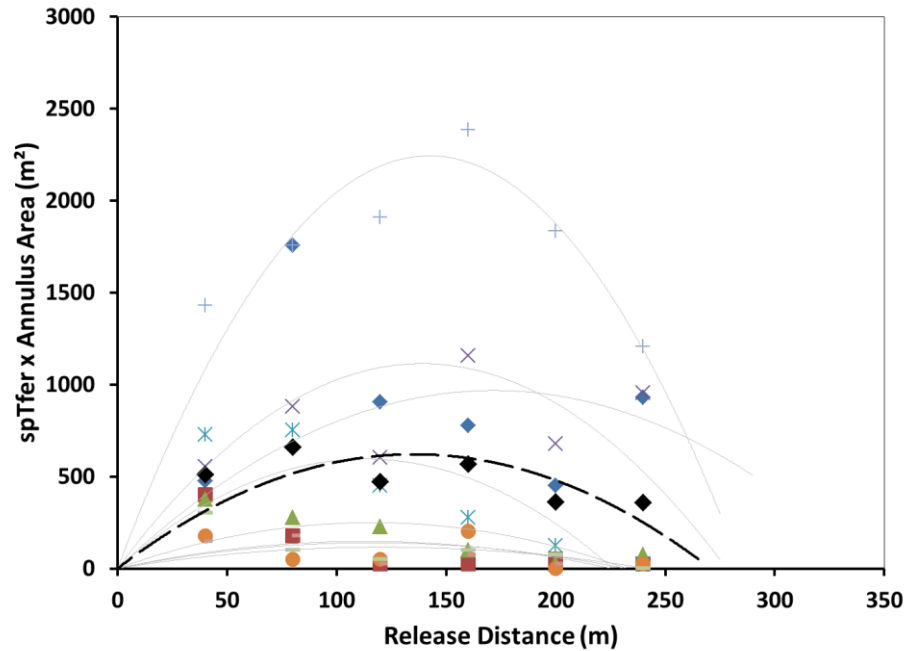


Figure 18. Miller plot of Figure 16 data, and their mean (dashed line).

Experiment 2.5 -- Even-Releases with Very High Populations

Results for Experiment 2.5 were very similar to those of Experiments 1 and 2. With released populations of 20,000 in 16 ha, T_{fer} averaged 0.02 ± 0.01 ($\pm$ S.E.M.). The highest T_{fer} measured was 0.05 and the lowest was 0.003. The expected concave line with asymptotic approach to the x axis can be seen in Figure 19. The slope of the line from Figure 20 predicts a plume reach of less than 2 m. Transformation of these data to the Miller plot, Figure 21, predicts that the dispersive distance of 95% of the population is ca. 250 m.

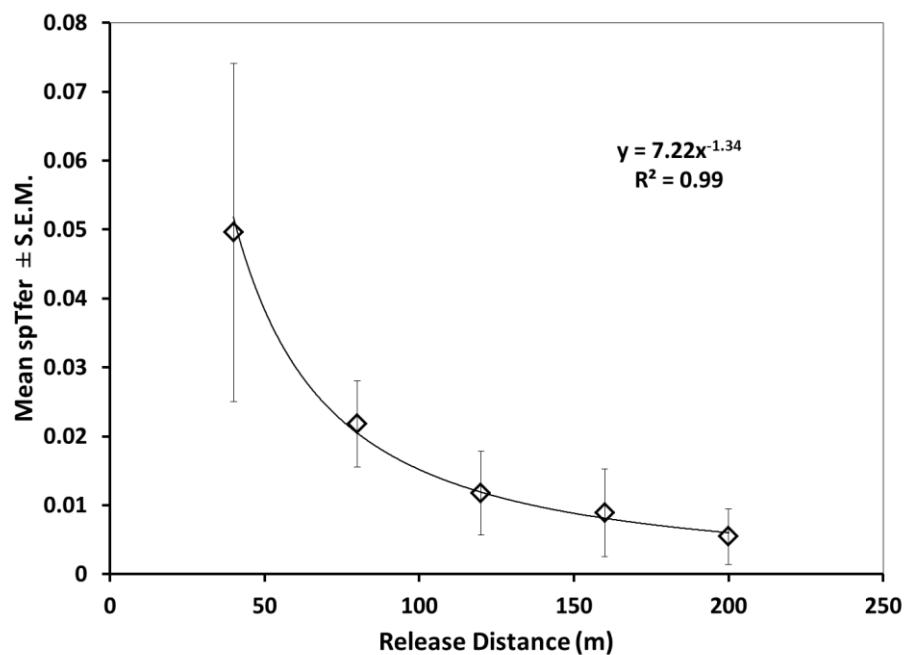


Figure 19. Mean specific trap findability (spT_{fer}) for 3 replicates of even-release at very high codling moth populations Experiment 2.5 ($\pm$ S.E.M.).

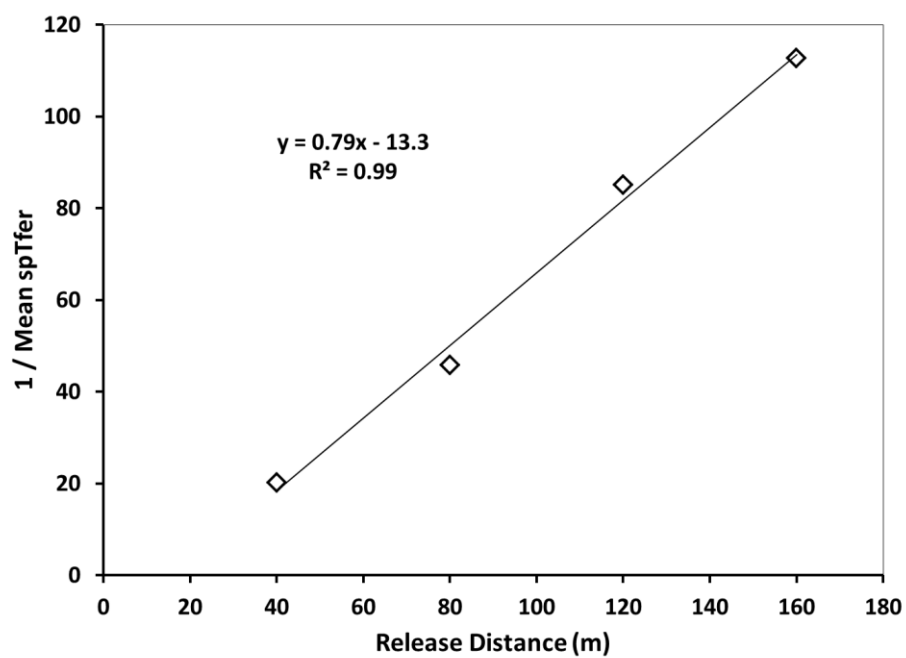


Figure 20. MAG plot of Figure 19 data.

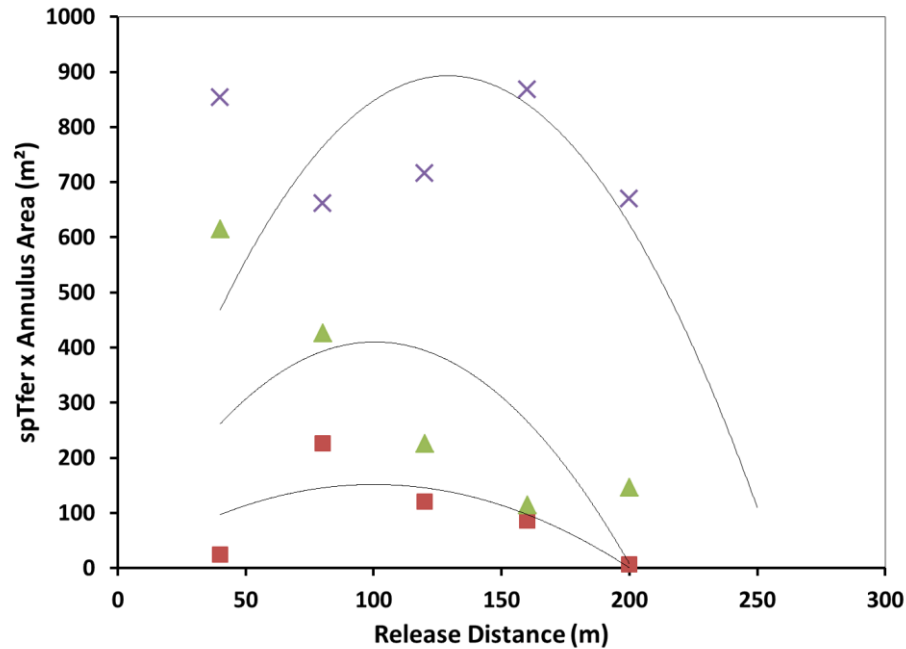


Figure 21. Miller plot of Figure 19 data.

Experiment 3 -- Even-Release with Low Populations

When populations were released at only ca. 2,000 per 18 ha, the mean proportion caught was ca. 0.02 ± 0.007 ($\pm$ S.E.M.) (range 0.001 to 0.08). Plotting the data as spT_{fer} over release distance produced a concave line with an asymptotic approach to the x axis (Figure 22). The mean spT_{fer} for the closest release distance of 40 m was $0.08 (\pm 0.02, n = 15)$. Catch decreased with distance away from the central trap to a mean spT_{fer} of $0.01 (\pm 0.006, n = 15)$ at the furthest distance of 240 m. Transformation of the data to the MAG plot produced a straight line (Figure 23) whose slope was 0.33, which equates to a plume reach of only 2 m.

Transformation of the data to the Miller plot (Figure 24) revealed a mean dispersive distance of the population of ca. 270 m.

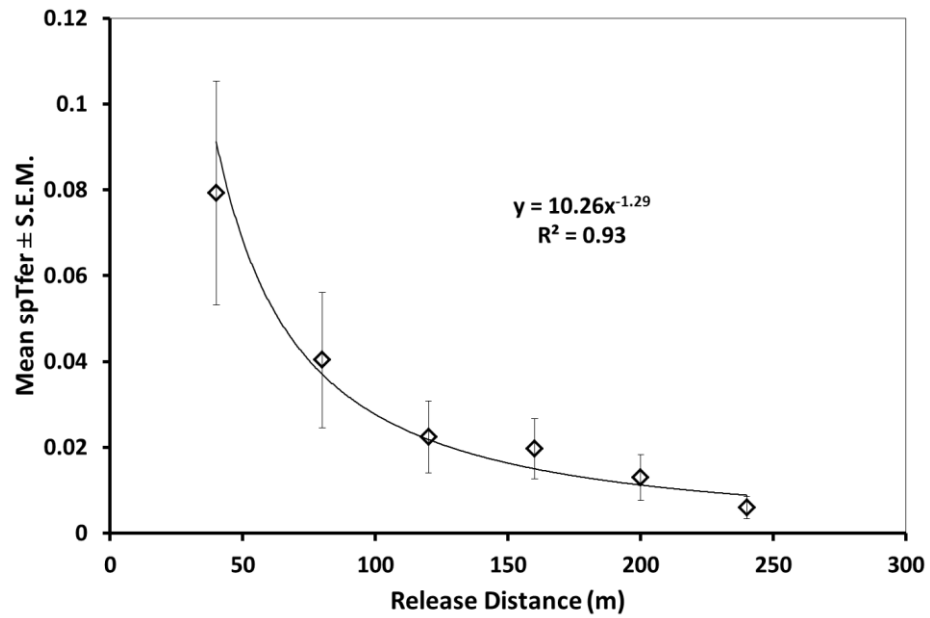


Figure 22. Mean spT_{fer} for low-population, even-release of codling moth Experiment 3. ($n = 15 \pm S.E.M.$).

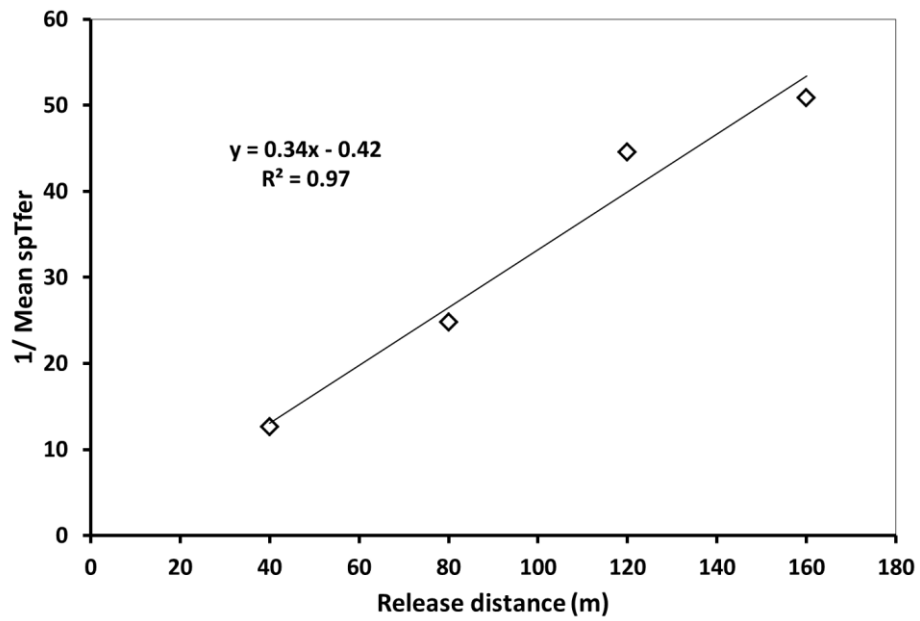


Figure 23. MAG plot of Figure 22 data.

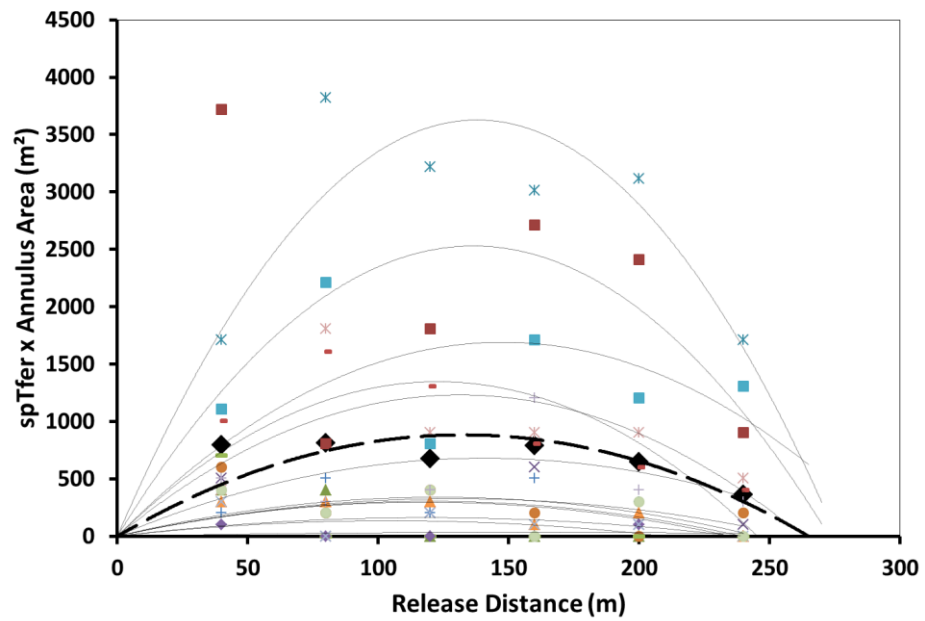


Figure 24. Miller plot of Figure 22 data.

DISCUSSION

Estimates of Proportion of Purchased CM Capable of Participating

Releasing known populations of CM within small field cages permitted males to fly and respond to standard monitoring traps, confirming that an overwhelming majority (ca. 80%) of these SIR moths was able to participate in these experiments. Similar levels of competency were recorded in the very large cage experiments of Miller et al. (2010). High competency is independently corroborated by the good match between the maximum y-values (ca. 1,000) for the Miller plots of Figure 9 and the three highest lines in Figure 15, which represent orchards not receiving insecticide. Although probably not surprising given the level of attentiveness this rearing facility pays to quality control, very high robustness of these CM is remarkable when considering the shipping and handling they experienced before release.

Pattern and Consistency of Trapping Outcomes Indicate CM is a Random Walker

A remarkable outcome of the present research is the consistency recorded for plume reach (2-3 m), trapping radius and trapping area (240-280 m and 18-25 ha, respectively), as well as T_{fer} (0.01-0.025) despite using cardinal direction releases and even releases and varying pest population densities across three growing seasons at eight different experimental sites in Western Michigan using laboratory-reared CM produced across three different years, airmailed cross-

country, powdered with fluorescent dye, and then immediately released into apple orchards to find sources of pheromone plumes across their short lifespans. Rather than showing that trapping results are too variable to provide a foundation for estimates of pest population density, current results strongly support the hypothesis that trap-insect interactions consistently and reliably express themselves despite variation in environmental factors like wind direction and local geography. The capture patterns emerging from these field data closely match those from a wide range of controlled manipulative experiments using computer-simulated random walkers (Miller et al., 2015), i.e., smoothly concave untransformed profiles of distance of release vs. spT_{fer} that asymptotically approach a catch of zero; initial linearity of plots of release distance vs. $1/spT_{fer}$ (MAG plots); rapidly rising, peaking, then tailing plots for distance of release vs. annulus area $\times spT_{fer}$ (Miller plots) that gradually rather than abruptly reach zero catch. These multiple lines of unique evidence compel us to conclude that CM is a biological random walker. As defined by Miller et al. (2015), this means that headings for new displacement steps are randomly picked from a normal distribution of possible headings centered on straight ahead and with a circular standard deviation of ca. $6-30^\circ$. When each individual CM male follows this simple rule of stochastic behavior, a population of such movers will exhibit spatially consistent properties that can reveal absolute pest density via capture number. Furthermore, we conclude that the principles

governing movement by small mobile animals like insects can be very similar to those governing molecular displacement. Therefore, the vast knowledge base from studies of atomic and molecular populations can and should be put to use on the problem of estimating pest density via trapping.

Approximations of Male CM Flight Paths when Foraging for a Pheromone Plume

Given the good match between the outcomes of the computer simulations that produced Figure 9 and the CM field results, representative flight paths for CM males could be produced using the Weston MultiMover software of Miller et al. (2015). Panels A-F of Figure 25 show simulated flight paths of six individual CM males as they would appear after 3,000 steps (3 km) to a viewer looking down upon a 9 ha rectangular orchard when a single male was released near its center. By overlaying such tracks on a Google Earth image of our orchard having 180 trees per ha it was possible to count that about 280 tree canopies would be visited in one moth lifetime. As seen in Figure 25, foraging with a meander of circular standard deviation of 30° balances intensive local search with intermittent forward excursions. However, the probability is very low that successive forward excursions will have a common heading; thus, a typical CM track folds back upon itself to produce a radius of net dispersion of little more than 100 m. But entirely due to chance alone, the forward excursions of an individual occasionally follow a common heading to produce a track with unusually long net displacement like that

of Figure 25 Panel G. Over 1,000 simulation records were examined to find this one atypical example of net displacement of 350 m.

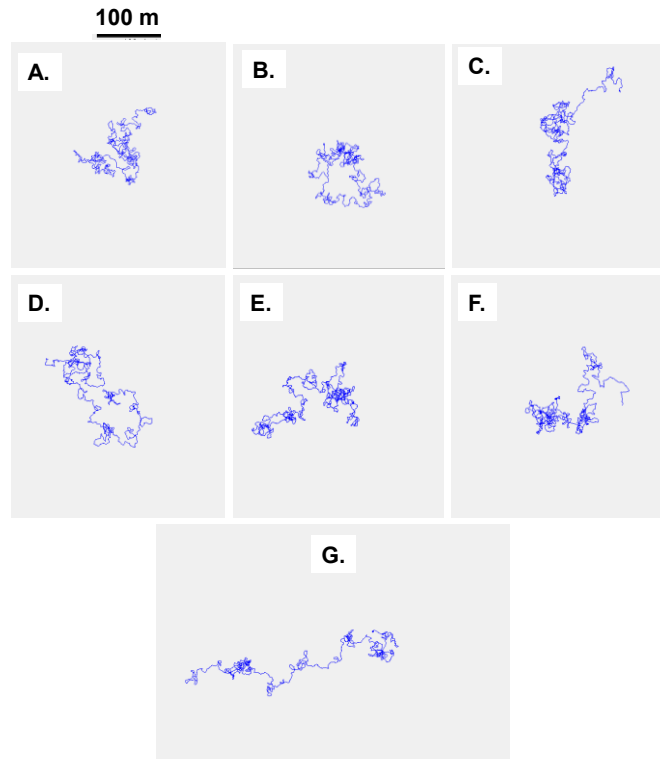


Figure 25. Panels A – G illustrate the tracks of individual Weston MultiMover (Miller et al. 2015) random walkers operating with a circular standard deviation for headings for new steps of 30° and 3,000 total steps. When scaled to steps of 1 m, these records estimate typical foraging paths for male codling moths released near the center of a 9 ha orchard plot as viewed from above. Each plot would contain ca. 1,600 apple trees.

Only after techniques are found to continuously track tiny animals like CM across great distances in the field will it become possible to directly test whether actual CM tracks match the deduced approximations of Figure 25. Nevertheless, we are quite confident of this bold prediction because it is congruent with the findings of other researchers using entirely different approaches. For example, Schumacher et al. (1997) used flight-mills to document that flight output by both CM males and females is a consistent 8-10 km of total displacement over a lifetime. However, because insects on a flight-mill only propel themselves forward and do not carry their own weight, we suggest that values for flight propensity on flight mills need to be cut at least in half for estimates of total displacement for free flight. Thus, our estimate of 3 km for total lifetime displacement by released CM becomes a good match to the Schumacher et al. (1997) data.

Plume Reach of the CM Trap in Apple is Small

Plumes as long as 60, 50, and 30 m have been revealed in the field by direct behavioral observations of e.g., responding gypsy moths (*Lymantria dispar*) (Elkinton et al. 1984), European pine sawflies (*Neodiprion sertifer*) (Östrand and Anderbrant 2003), and oriental fruit moths (*Grapholita molesta*) (Baker and Roelofs 1981), respectively. By that standard, our estimate of ca. a 2 m plume reach for the CM monitoring trap in apple is tiny. Nevertheless, we are confident in the accuracy of the current measures of CM plume reach based on the MAG-

plot slope method (Miller et al. 2015) because: 1) it has a strong basis in mathematical theory and simulation-modeling, 2) it returned an estimate of 30-50 m for European pine sawfly data previously measured by direct observation (Östrand and Anderbrant 2003) to be ca. 50 m, and 3) Grieshop et al. (2010) previously found that plume reach from the identical CM monitoring trap was likely < 10 m because traps never ensnared marked and released CM males quickly unless releases were directly down-wind and very close to the trap. Other short plumes have occasionally been reported. In a field study looking at large fruit-tree tortrix (*Archips podana*) Van der pers and Minks (1993) found that when measuring the plume from a pheromone source using the single sensillum recording technique, contact with the plume was only realized at distances of approximately 1 m from the source.

We postulate that the dense and complex architecture of an apple orchard caused atmospheric turbulence that dispersed the CM pheromone plume more quickly in our study than would happen in a simpler environment. The above and other reports of large plumes come mainly from experiments conducted in open understories of forests or in open fields of low-growing vegetation. Additionally, codlemone (12-carbon primary alcohol) is one of the smallest of lepidopteran pheromones and thus adsorbs less strongly (Gut et al. 2004) onto the antennae. Furthermore, the antennae of CM males are not notably specialized for collecting

pheromone from air as are the antennae of male gypsy moths and pine sawflies. Thus, the active space for CM sexual communication might be already small irrespective of crop architecture.

Measurements of plume reach for monitoring traps have been rare because of the difficulty in attaining such measures. Diverse methodologies have been brought to bear on this problem (Murlis et al. 1992, Murlis et al. 2000), including: direct observations of responding insects, release-recapture, field EAGs, and GLC analysis of air samples. Even when achieved, a given estimate for plume reach by these methods may apply only to a subset of all possible environmental conditions encountered by a trap operating in the field over many days. By contrast, the MAG-plot slope method has the advantages of being less labor-intensive and of always time-averaging. Thus, the novel approach of estimating plume reach via geometry solves a difficult and long-standing problem in chemical ecology and could make plume-reach determinations routine.

Knowing plume reach will be helpful in assessing the sampling power of a trapping system and judging its potential for direct pest control by e.g., mass trapping. Traps emitting long plumes will be better sampling devices than those emitting short plumes like that found here for CM. A long plume can increase trapping radius appreciably and it will actively collect many more pests than will a short plume when shifting wind directions cause such respective plumes to sweep

across the crop. The computer simulations of Miller et al. (2015, Chapter 8) suggest that effective mass trapping requires a trapping grid with a spacing no more than 1.5 times the plume reach. Thus, the number of traps required for control by mass trapping is entirely dependent upon plume reach, which must be ascertained before mass-trapping or attract-and-kill tactics are attempted.

Radius and Sampling Area of the CM Trap in Apple are Large

The plume reach for the CM trap was tiny, while the 95% trapping radius (TR_{95}) was ca. 260 m. Therefore, the maximum dispersive distance for 95% of members of a local CM population was nearly 260 m. The radii of trapping annuli contributing the most to overall catch are revealed by the x-values associated with the maximum y-values of a Miller plot. For CM, the annulus contributing the most to catch (see Figures 15, 18, 21 and 24) had a radius of ca. 125 m. It follows that travel of CM males from their points of origin to the plume contributes far more (ca. 98 % of the total) to the average overall journey into a trap than does travel of males in the plume. Nevertheless, guidance of the final leg by pheromone is critical to trapping success, as it is rare that any catch is registered when no pheromone is emitted from a CM trap.

The above information and the simple calculation of trapping area of ca. 21 ha given a trapping radius of ca. 260 m enables informed suggestions for optimal deployment of CM monitoring traps in orchards not under mating disruption.

Despite a surprisingly tiny plume reach, the trapping area for CM is considerably larger than once presumed. Suggestions can be found that optimal monitoring requires one CM trap for every hectare of orchard; one trap per 2-4 ha is acceptable in large, uniform blocks (Gut and Wise 2016). However, such a trap density is excessive in light of the current data. Deployment of multiple traps per 21 ha orchard is likely to provide a more reliable (less noisy) measure of pest abundance and this approach to enhancing precision will be explored in a subsequent report on line-trapping. But placing multiple traps with small plumes close to one another will have little influence on the distance from which CM is being sampled.

Counter-intuitively, the CM males found in a trap are more likely to have originated > 100 m away from the trap than near the trap. Although the probability of capturing a given individual is highest when the trap is nearby, the nearby area and therefore the total number of randomly distributed pests associated with a nearby annulus is considerably less than that for a distant annulus (Miller et al. 2015). It therefore would be unsafe to restrict insecticide applications targeting CM to apple blocks only immediately adjacent to a trap registering a catch number interpreted as above threshold.

Converting CM Catch Number to Absolute Density

Once trapping area and T_{fer} are known and shown to be highly reproducible, as in the current study, absolute pest density in a new location and given in units of

pests per trapping area is obtained simply by dividing capture number by T_{fer} (Miller et al., 2015). In the current case, T_{fer} uninfluenced by insecticide treatment was ca. 0.02. Therefore, a CM male catch of 1, 3, 10, 30, etc. equates to 50, 150, 500, and 1,500 males per 21 ha, or 2.4, 7, 24, and 71 males per ha, respectively. Given that the sex ratio of CM is 1:1, this estimate holds for females. The curtain can now be lifted on how many CM are present in a typical commercial apple orchard in Michigan where capture numbers are maintained at or under 5 males per trap per control period. The answer has surprised growers and pest managers alike; catch of 5 males per trap equates to only 250 females per 21 ha, or 12 females per ha (5 per ac). Thus, when spraying at this threshold and spending \$120 per ha for the insecticide, cost per killed female CM is a surprisingly high \$10 per female.

The above information provides a platform for computing an actual economic threshold for CM in Michigan apple. The threshold for rejection of an apple crop by a fresh-market packer is 0.5% infestation. According to Michigan Agricultural Statistics, the fruit load for Michigan averages ca. 360,000 apples per ha. A 0.5 % infestation translates into 1,800 infested apples per ha. If each female CM produced an average of 100 larvae, of which 40% successfully established singly in apples, the infestation rate per female would be 40 apples. The threshold of tolerance would then be crossed at 45 females per ha, which would be reflected

by a cumulative catch of 19 males per trap. We suggest a cumulative catch of 12 males per trap per season as a reasonable and conservative starting point for an economic threshold for CM. These calculations are in remarkable agreement with recommendations in the Michigan Fruit Management Guide (Gut and Wise 2016) that were established by years of practical field experience and research.

Extension personnel are now encouraged to investigate whether and how much this threshold might be raised, as it appears that the existing threshold has a considerable safety margin built into it that might be reduced without undue risk to growers.

Reducing the Normal Variance in Catch Data

While measures of mean catch over time showed remarkable consistency and reliability over the three years of experiments, normal variation in any single measure remains a concern. An efficient solution, demonstrated by Adams et al. (2016c Chapter 4) is to place multiple traps (e.g. 5) closely in a line, with their mean taken as a single T_{fer} measure. In this way the variance around a single measure can be reduced without substantially increasing the time needed to check traps.

Should Females be Present During Trapping Calibrations?

Any females that call during the flight window of the males would represent a competing point source and could therefore theoretically impact instantaneous

catch in the monitoring trap and the measures of the trapping performance reported here. However, including females more closely approximates conditions that growers and pest managers actually experience with a sex ratio of 1:1. Excluding females from experiments might produce larger estimates of the maximum dispersive distance of the males; but these would be artificial because CM females are always present at 1:1. However, we have seen very high consistency of these measures using mixed sex populations ranging from ca. 130 / ha to 1,250 / ha in the unbounded orchard conditions, suggesting that there is little female effect on eventual outcomes. One reason for modest female impact could be that the period of male sexual activity brackets that for females so as to provide males ample opportunity to interact with traps when females are silent. Another would be that mated females drop out of the competition, eventually leaving only the trap. Finally, the work of Miller et al. (2015) demonstrates that the distances over which attractive point sources compete appreciably is much more limited than previously guessed because of the probabilistic requirement that males would have actually have visited both alternatives.

Recommendations for Applying this Approach to Other Pests

The principles and methods used here to estimate absolute density via trapping should be broadly applicable to any pest or beneficial animal that forages locally by random walks. Measures of plume reach and trapping radius are critical

for understanding pest detectability and should be a research priority for any invasive species. We recommend the following set of steps in executing this approach to ascertaining absolute pest density from capture data in a monitoring trap:

- 1) Using a minimum of five distances, establish regularly spaced release points in the four cardinal directions around a central trap, with the furthest release point at a distance where probability of catch is non-zero. Individuals released at each distance should be uniquely and indelibly marked, and if possible each direction should be distinguishable from the others to measure bias in catch by direction. Probability of catch falls with distance, so released populations should be large and can be increased with distance to provide a stronger measure of catch probability where catch will be low.
- 2) Catch data should be collected regularly until catch from the middle distance tapers off or stops.
- 3) Proportion of released populations capable of participating should be determined separately in an enclosed arena that allows insects to orient normally to the baited trap. Appropriate adjustments should be made when calculating T_{fer} values if a substantial proportion ($> 30\%$) of populations fail to respond to the trap.
- 4) Catch data should be plotted and analyzed as follows:

- i. Untransformed plot – The proportion caught from each distance (spT_{fer}) over release distance. For random movers the line fit of these data should be a curvilinear concave line that approaches the x axis asymptotically. Data that cannot be fitted thusly indicates that release distances were not spaced properly. For example if the first three distances all had spT_{fer} values of 0.20, these distances may have been too close to one another.
- ii. MAG plot – Inverse of spT_{fer} over release distance. The first part of this graph will be linear and the slope from the fitted straight line can be used to look up the plume reach on the standard curve (Figure 4.12 of Miller et al. 2015). Properly spaced release distances that produce very small spT_{fer} values for the furthest distance (on the untransformed plot), will equate to extremely large values on the MAG plot and can be omitted.
- iii. Miller plot – $SpT_{fer} \times$ annulus area over release distance. Areas of each annulus are calculated per Formula 1 with annuli centered on release distances. Data should be fitted with a 2nd order polynomial forced through the point zero, zero. The x intercept estimates the dispersive distance of 95 % of the population.

- iv. T_{fer} – Calculation of T_{fer} , for cardinal direction experiments, is performed by dividing mean annulus area $\times spT_{fer}$ by mean annulus area (see Table 5.1 example of Miller et al., 2015).

Armed with these principles and methods investigators can now calculate absolute pest density of any pest by dividing number caught in a monitoring trap by its T_{fer} ; and the area to which this density applies can be calculated as π times the square of the sum of dispersal distance and plume reach. With this knowledge more accurate calculations of economic injury levels can be made with cost control measures calculated down to a per-insect level, and trap spacing can be optimized based on the area it samples.

CHAPTER THREE

Flight meander of male codling moths searching for plumes of female sex
pheromone is optimized

ABSTRACT

It is generally accepted that evolution optimizes animal traits (Pyke 1978, Stephens and Krebs 1986, Parker and Smith 1990) including search tactics (Katz 1974, Bell 1991, Viswanathan et al. 1999, Sakiyama and Gunji 2013). For example, animals like walking ants (Crist and MacMahon 1991, Pearce-Duvet et al. 2011) and flies (Bell 1991) foraging at some distance from food items are known to search via correlated random walks with path meander assumed to appropriately balance search thoroughness with distance and area (Bell 1991, Viswanathan et al. 1999, Sakiyama and Gunji 2013, Pearce-Duvet et al. 2011, Larralde et al. 1992, Bartumeus et al. 2002). On the other hand, moths foraging for plumes of sex pheromone are postulated to optimize their foraging by flying cross-wind (Cardé et al. 2012) so as to increase the probability of intersecting plumes moving down-wind. However, actual experimental proofs of search optimizations have been weak to non-existent. Here we used the novel approach (Miller et al. 2015) of measuring and interpreting the decay constant in capture number of codling moths moving through a 5 x 5 grid of sex pheromone-baited traps to show that males do not forage in straight lines for pheromone plumes. Rather, they use correlated random walks (flights) with a meander equivalent to that produced by computer-simulated movers randomly selecting headings for new steps from a normal distribution having a circular standard deviation (C.S.D.) of $37 \pm 2^\circ$

(S.E.M) relative to the previous heading. Efficiency of computer-simulated biological random walkers (Miller et al. 2015) foraging for plumes under codling moth-like conditions peaked at C.S.D. ca. 20° and 50° when plumes were sparse and distant from the origin of movers vs. close and dense, respectively. Thus, the 37° C.S.D. measured for codling moth in the field represents an expected compromise averaging these extremes and provides the first strong evidence for actual search optimization by an animal. Moreover, these methods for quantifying path meander and its significance should be applicable to many types of motile organisms.

INTRODUCTION

An unexpected benefit of recent advances in understanding mechanisms whereby sex pheromone-baited monitoring traps interact with their insect targets (Miller et al. 2015, Adams et al. 2016a Chapter 2) has been the ability to accurately estimate plume reach based upon the rate of fall in capture number with distance from which responders are released from the trap (Miller et al. 2015). This approach has been used to quantify the reach of attractive plumes from traps for various invertebrates (Miller et al. 2015). For example, the plume from an optimized pine sawfly (*Neodiprion sertifer*) trap was estimated at ca. 40 m (Miller et al. 2015), while that for codling moth (*Cydia pomonella*), whose larvae are the preverbal worm in the apple, was a mere 2 m (Adams et al. 2016a Chapter 2).

Quantified plume reaches then offer a unique opportunity for assessing optimization of foraging behaviors. Figure 26 A. reveals the rise and fall in catch of 5,000 computer-simulated random walkers seeded randomly into an unbounded 6,000 x 10,000 unit cyber space having a codling moth-like 2 x 2 unit trap plume at its center. Runs were terminated after 3,000 steps. Levels of meander were manipulated in increments of C.S.D. This situation mimics that when female density is sparse. Ballistic movers (C.S.D. 0°) performed poorly when the plume was tiny because they under-searched all spaces through which they traversed. Molecular-like random walkers (C.S.D. 100° and greater) performed equally

poorly because they over-searched their area of origin and rarely achieved sufficient distance to reach the target. Meander values maximizing search thoroughness with distance peaked at C.S.D. ca. 20° under a low density of plumes. When simulated movers originated near a cluster of traps that also could represent clustered females arising from a localized insect outbreak, catch as a function of C.S.D. peaked at ca. 50° (Figure 26 B.). If evolution has tuned their search for efficient finding of both sparse and distant as well as dense and nearby females, we postulate that codling moth males in the field should forage for pheromone plumes by executing search paths with a meander equivalent to ca. C.S.D. 35° , which is halfway between 20° and 50° .

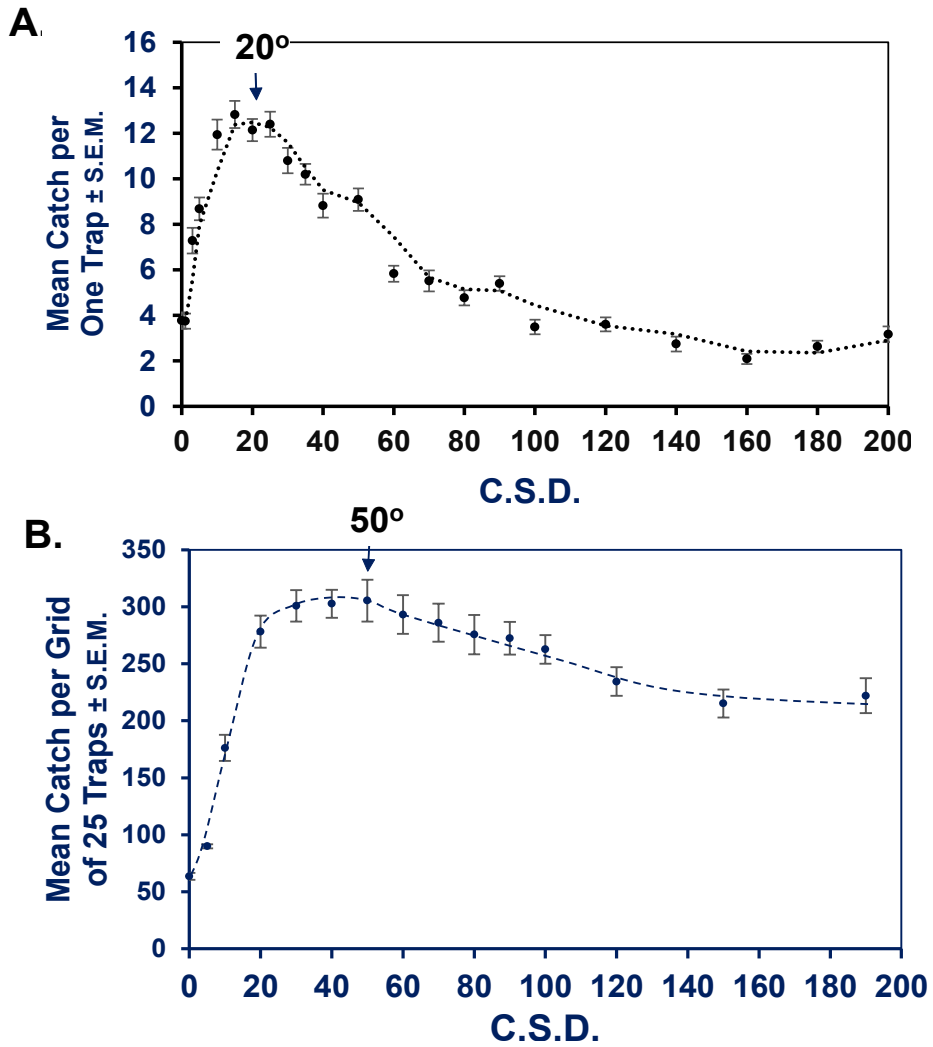


Figure 26. A. Capture of computer-simulated random walkers as a function of C.S.D. ($n=35$). B. Mean catch for 5 runs at each C.S.D. when 400 simulated movers were released 10 units from a 5 x 5 grid of traps spaced 15 units apart. Other conditions were as for A. The optimal C.S.D. for search is ca. 50° when plumes are clustered near the movers' point of origin. The optimal compromise C.S.D. covering both conditions is expected to be ca. 35° .

The most straight-forward method for measuring path meander for an animal like codling moth would be analysis of the distribution for turns between segments of foraging tracks recorded by, e.g., videography (Cardé et al. 2012). However, codling moths are tiny (< 1 cm), delicate fliers incapable of carrying transmitters. Moreover, they fly at night for an estimated 3 km (Adams et al. 2016a Chapter 2) over their lifespans of ca. 1 wk. Because these challenges have not yet been surmounted, we turned to an indirect method of quantifying foraging meander first proposed by Miller et al. (Chapter 7 of Miller et al. 2015) and tested only on computer-simulated movers. Level of meander strongly influences the extent to which movers, released on the corner of a small grid of traps, penetrate the array and are captured in individual traps (Figure 19). Computer simulations (Miller et al. 2015) using random walkers proved that decrease in catch with distance from the grid corner is exponential – a classical decay constant (Bauer and Westfall 2014) (see Figure 27 E. for example). Moreover, the relationship between C.S.D. and decay constant is conveniently linear so as to function well as a standard curve useful in interpreting results of parallel studies of codling moth in the field using a trapping grid (Figure 28).

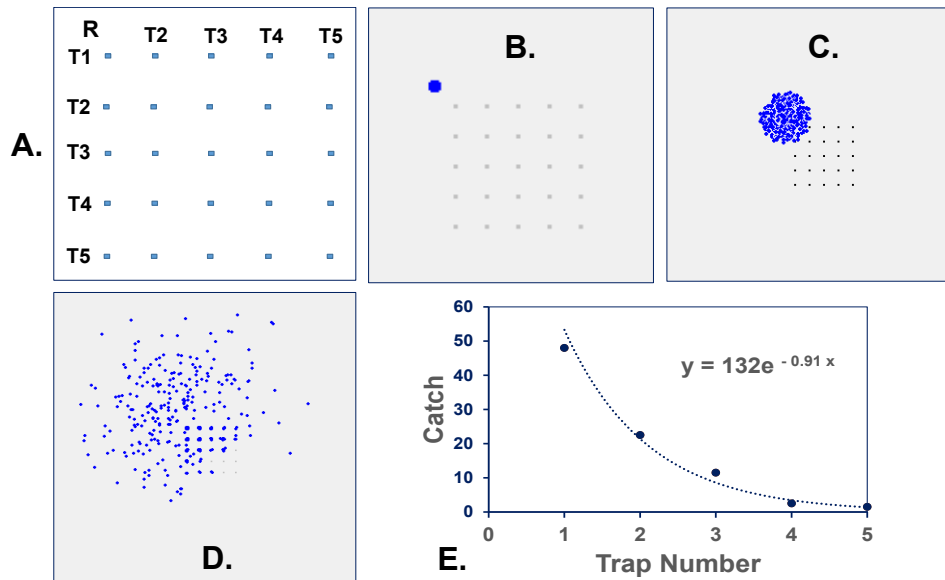


Figure 27. A. Layout for grid of twenty-five 2 x 2-unit traps (small rectangles) separated by 15 units and numbering system for edge traps whose catch numbers were used for graphing data to quantify a decay constant. R = release site for movers. B. to D. shows final destinations of movers after 5, 100, and 400 steps. The spatial scale for panels varies. Movers caught on traps in Panel D. enlarge trap appearance. E. shows plot of catch by trap number (as in A.) after 400 movers released at R of Panel A. took 400 steps of 1 unit with a C.S.D. of 30°. For traps designated T2-T5 the mean of two trap was plotted.

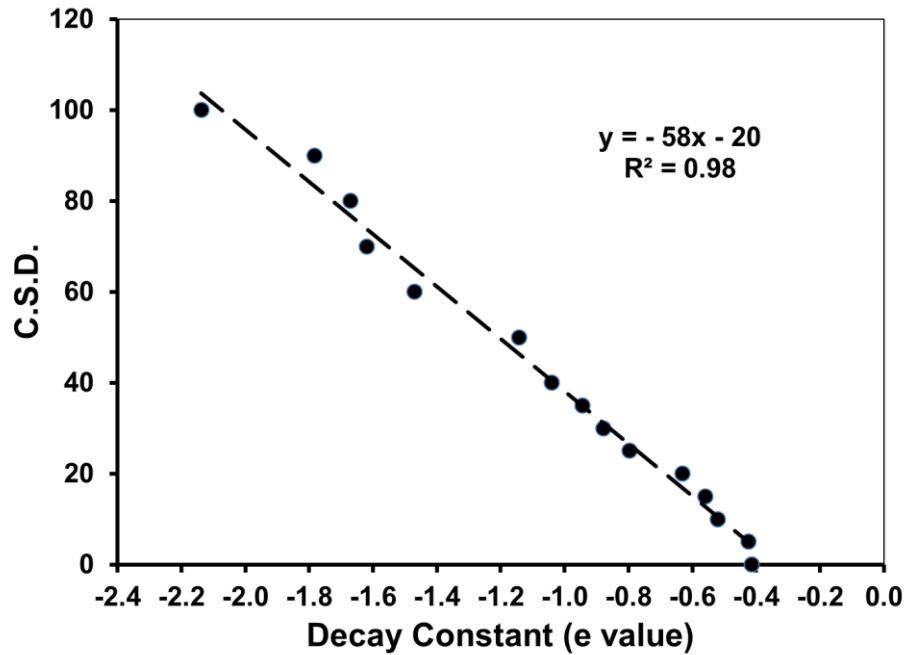


Figure 28. Standard curve inter-relating decay constant (e) with C.S.D. This graph was generated using the computer-simulation procedures of Figure 19 and a run time of 400 steps. Ten replicate runs generated 10 e values for each C.S.D. Values of S.E.M. around the mean e values were too small to be visible.

MATERIALS AND METHODS

To put this approach to measuring path meander to its first test with real animals, we released batches of ca. 800 codling moths (1:1 M:F) marked with fluorescent powders of unique color 10 m outside of the corner of a 5 x 5 grid of optimized traps separated by 15 m and using orchard, moth, marking, and trapping protocols detailed previously (Adams et al. 2016a Chapter 2). Thirty nine replicates of this experiment were accumulated between June 6 and July 9 of 2014 during which time coolness of the season kept flight activity appropriately low so that moths rarely moved into the trap grid from the side opposite of release. Captures in all traps were recorded daily and catch was plotted for each run as in Figure 27 and C.S.D. computed using Figure 28.

RESULTS AND DISCUSSION

Average fall in catch of male codling moths as a function of trap position in the grid is given in Figure 29. Placing the resultant decay constant of negative 0.99 into the line formula of the standard curve (Figure 28) estimates a C.S.D. of 36°. Alternatively the mean decay constant (e value) arising from the 39 individual determinations was negative 0.97 ± 0.02 (S.E.M.), with a mean C.S.D. of $37 \pm 1.7^\circ$. Both methods closely match our prediction of average meander equivalent to a C.S.D. of 35°.

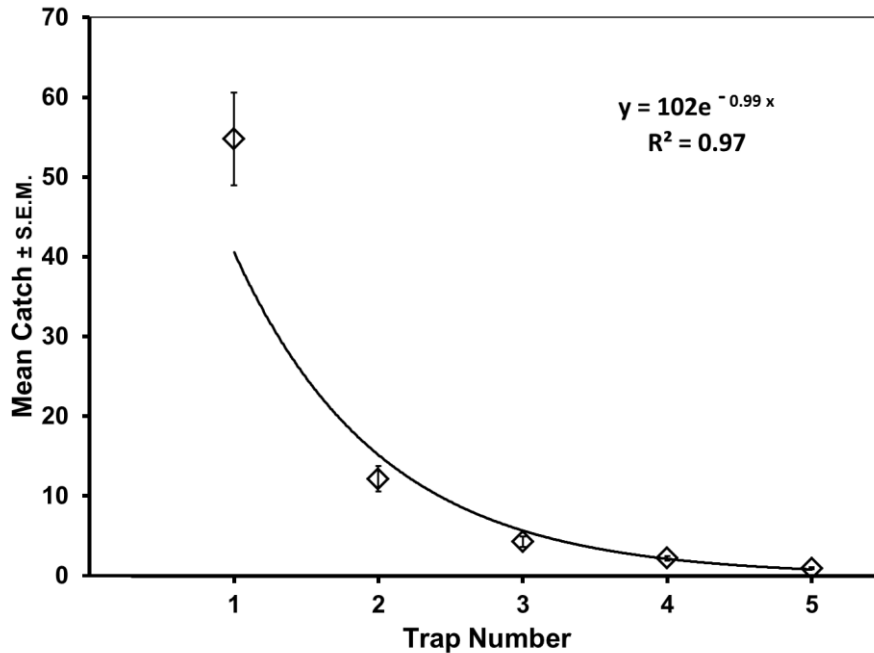


Figure 29. Decay curve averaged across all 39 replicates of codling moth males penetrating a 5 x 5 grid of traps in the field. An exponent of - 0.99 equates to a C.S.D. of 37°.

Moreover, the frequency histogram of meander values (Figure 30) appears bimodal by exhibiting what appears to be one peak near C.S.D. 25°, a valley at 35°, and another peak near 45°. Such a pattern might result if the females released with the males called during some of the runs but not others. The higher C.S.D. would have been appropriate under dense pheromone plumes and the somewhat lower C.S.D. would make sense when females were not calling. Insects are known to modulate their search meander in accordance with encounter rate of resources

(Bell 1991). But, such nuances notwithstanding, the overall hypothesis of this study is supported – evolution has indeed optimized flight meander of male codling moths foraging for plumes of female sex pheromone.

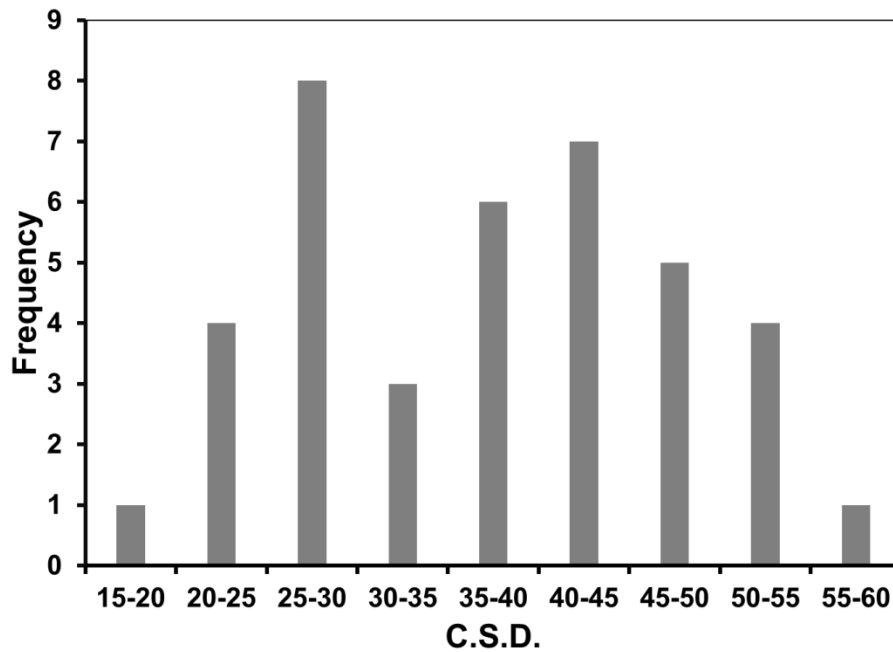


Figure 30. Frequency histogram of the 39 individual determinations of C.S.D. for codling moth foraging for pheromone plumes in a Michigan apple orchard.

CHAPTER FOUR

Line-Trapping of Codling Moth, *Cydia pomonella*, (Lepidoptera:Tortricidae): a
Novel Approach to Improving the Precision of Capture Numbers in Monitoring
Traps

ABSTRACT

This study of codling moth, *Cydia pomonella*, (CM) response to monitoring traps baited with codlemone demonstrates that precision of a given capture number is alarmingly poor. As documented using the binomial distribution formula and computer simulations, captures as low as 0 and as high as 12 CM males per single trap will sometimes occur under conditions where the expected modal catch for CM is 3. Here we demonstrate that the frequency of false negatives and overestimated positives for CM trapping can be substantially reduced by employing the tactic of *line-trapping*, where five traps were deployed 4 m apart along a row of apple trees. Under paired contrasts with treatments spaced 70 m apart and using marked SIR CM, mean catch in a single trap was 6.2 ± 1.2 (S.E.M.) males vs. 4.5 ± 0.53 from a line of five traps. This outcome and examination of catch within the trap line demonstrated that CM traps spaced closely compete only slightly. Therefore, deploying 5 traps closely in a line is a sampling technique nearly as good as deploying 5 traps widely. But line trapping offers a substantial savings in time and therefore cost when servicing aggregated vs. distributed traps. As the science of pest management matures by mastering the ability to translate capture numbers into estimates of absolute pest density, it will be important to employ a tactic like line-trapping so as to shrink the troublesome variability associated with capture numbers in single traps that thwarts accurate

decisions about if and when to spray. Line-trapping is likely to offer similar advantages for other pests beyond CM.

INTRODUCTION

Monitoring traps baited with sex pheromones have been playing a key role in pest management since the 1970s by identifying what insect pests are present in a crop and when they are active (Witzgall et al. 2010). Moreover, significant advances have recently been made in the development (Miller et al. 2015) and validation (Adams et al. 2016a Chapter 2) of methodologies for converting catch numbers in pheromone baited traps into estimates of absolute pest density, the key parameter required for generating economic thresholds intended to optimize control decisions. It is becoming increasingly evident that insects foraging for pheromone plumes displace via biological random walks (Miller et al. 2015; Adams et al. 2016a Chapter 2) and that captures result from stochastic intersections of movers travelling large distances with relatively small plumes from monitoring traps. For example, codling moth, *Cydia pomonella*, (CM) males typically displace an estimated ca. 3 total km while foraging for pheromone along a convoluted path that results in a net displacement rarely more than 300 m (Adams et al. 2016a Chapter 2). By contrast, plume reach for an optimized CM monitoring trap has proven to be a surprisingly short 2 m.

Given the stochastic nature of trapping, an important consideration when using catch numbers to guide control decisions will be precision – what is the level of agreement of a particular catch number with itself when repeated measures are

taken under identical conditions? Despite heavy use of catch numbers in insect pest management, precision of such numbers has provoked very little attention to date, save for a brief introduction to the topic by Miller et al. (2015).

The following specific example drawn from our recent CM trapping research (Adams et al. 2016a Chapter 2) illustrates the normal range of catch numbers possible from a single trap and the risks of using data from a single trap as the driver of pest management decisions. Adams et al. 2016a (Chapter 2) found that the trapping radius for a single CM monitoring trap not under mating disruption was ca. 260 m, yielding a sampling area of ca. 21 ha. The mean probability of capture (T_{fer}) of a standard monitoring trap baited for CM males was ca. 0.015 across the sampling area, and therefore the mean probability that the males in the sampling area *would not* be caught was 0.985. As documented by Miller et al. (2015), catch per sampling area equals $T_{fer} \times$ males per trapping area. Knowing the T_{fer} (probability of success) value, it is also possible to calculate the total distribution of catch probabilities, for a given population, using the binomial distribution formula:

$$b(x; n, P) = {}_nC_x * P^x * (1 - P)^{n-x}$$

where: b = binomial probability; x = total number of successes; n = number of trials (males per trapping area); P = probability of a success on an individual trial;

and ${}_nC_x$ = the combination of n entities taken x at a time. Figure 31 shows a probability distribution generated using a probability of success of 0.015 with $n = 220$ which equates to a credible CM population of ca. 10 CM per ha (4/ac). These values were selected to produce a catch mode of 3 so as to match the action threshold for spraying CM in Michigan. A virtually identical frequency histogram was produced using Weston MultiMover software (Weston 1986; Miller et al. 2015) and randomly seeding 220 movers into a discoid trapping area having radius 260 pixels and containing a central trap with a reach of 2×2 pixels. When the number of steps taken was 3,000 and the circular standard deviation of headings for new steps was 30° , T_{fer} was ca. 0.015. Thus, the probability distribution in this figure was arrived at both by theory and by simulations using movement parameters very similar to those of CM (Adams et al. 2016a Chapter 2).

In this example the most frequently observed catch value is 3 CM, the recommended threshold for action against second generation CM (Gut and Wise 2016). However, this catch of 3 is realized in only 22% of trials, while a lower catch of 0,1, or 2 is to be expected in ca. 36% of trials, and a higher catch of 4, 5 or 6 can be expected 36% of the time. While rare, catches as high as 12 can occur. However, the preponderance of catches will fall between 1 and 6.

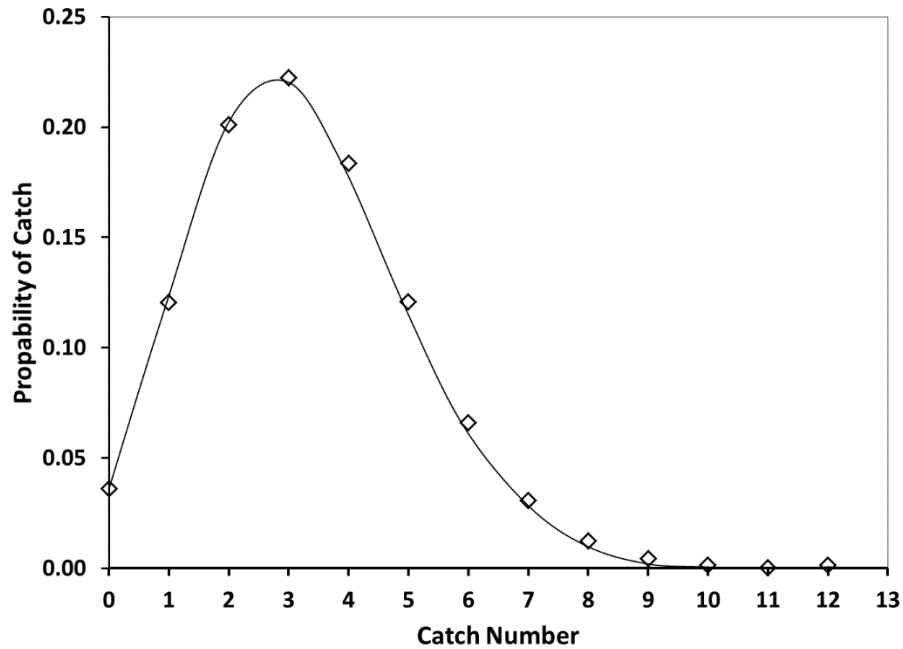


Figure 31. Probabilities of getting respective catches from 0 through 12 as ascertained from the binomial distribution formula.

This range in CM catch outcomes is sobering because it directly impacts accuracy of decisions on whether or not to spray for this key pest of Michigan apple production, where the action threshold is a cumulative catch of 5 to 7 moths per trap during the first generation or 3 to 5 moths during the second generation (Gut and Wise, 2016). In light of Figure 31, the current system of interpreting capture numbers from single CM monitoring traps could result in numerous occasions where sprays are applied to perceived hot-spots when they are not

needed. But worse, the probability of falsely capturing low numbers and not spraying is also substantial.

Such realizations led us to seek improvements in CM monitoring so as to tighten the correlation between catch number and the probability of a damaging pest density actually being present. One possibility might be to raise the codlemone release rate so as to enlarge the reach of the plume emanating from the CM trap, thus making it a larger target for intersections with foraging males. However, this approach is refuted by documentation (Kehat et al. 1994; Vacas et al. 2013) that, although it might attract more CM males to the vicinity of the trap, doing so diminishes rather than raises overall catch. Apparently, trap entry rate is inhibited by excessive pheromone near the source. Another tactic might be to deploy multiple traps per 21 ha rather than one. Indeed, more CM would then be captured because of a guaranteed increase in summed plume area. However, Michigan fruit growers and pest-management consultants are reticent to deploy multiple monitoring traps per orchard mainly because of the unacceptable time demands and thus costs required to travel to the various traps for servicing each one across the whole growing season.

In pondering this problem, we were struck by its parallels to the challenge fishermen face in needing to deploy multiple baited hooks across fish habitat while minimizing travel and service time per hook. A favored solution to this

optimization problem in fishing is *long-line fishing* (Yamaguchi 1989; Otto and von Brandt 2005), where multiple baited hooks descend from short snood lines at increments from a tow line. This configuration dramatically raises the probability of catch by summing the reaches of plumes emanating from all bait point-sources. Optimized spacing of snood lines from the main line essentially creates a continuous and potentially very long plume acting upon any fish intersecting the long line at any point. Competition between hooks is considered inconsequential when their spacing approximates the plume reach of a single baited hook. It turns out to be far more efficient to retrieve the main line while servicing all hooks than to travel to a unique location to tend each hook.

Here we test the idea that the line-trapping approach can successfully be applied to trapping of insect pests like CM, where the plume from each trap is small (ca. 2 m) (Adams et al. 2016a Chapter 2). In this case we deployed five traps, not literally from a line, but along a row of apple trees so as to be slightly more than one plume-reach apart. Thus, little more time and effort would be required for the pest manager to drive to and service this trap aggregation than is required for tending a single trap. The specific hypotheses tested here were: 1) average CM catch for five traps spaced 4 m apart in a line will be only moderately lower than that for a trap operating alone, and 2) the precision associated with a

mean catch of five traps in a line, hereafter referred to as μ_5 , will be substantially increased over the precision of a single trap.

MATERIALS AND METHODS

The above hypotheses were tested using a paired experimental design whose treatments were: 1) a single delta trap (Pherocon VI; Trécé Inc., Adair, OK, U.S.A.) baited with a CML2 gray septum (Trécé, Inc.) containing (E,E)-8, 10-dodecadien-1-ol (codlemone) and held aloft of the TanglefootTM-covered cardstock liner by a pin through the trap roof, and 2) a line of five such traps each separated by 4 m. Traps were hung in the upper third of tree canopies. The commercial apple orchards used were located near Sparta, MI and detailed by Adams et al. (2016a Chapter 2). The horticultural and management protocols were standard for this area and included some insecticide sprays. Because feral CM populations at these sites were so low as to yield barely 1 male per trap per wk, CM of mixed sex (1:1 M:F) were purchased from the SIR rearing facility in Osoyoos, British Columbia, marked by distinctive fluorescent powers, and released into the test blocks (see Adams et al. 2016a Chapter 2 for details on handling, marking, release, and color identifications). Each block of this experiment consisted of the above two treatments separated by 70 m as shown schematically in Figure 32. Pairs of treatments were separated by at least 150 m. To guarantee sufficient sampling power for strong tests of the above hypotheses, 100 males along with 100 females

were released at the four locations marked R in Figure 32 at the beginning of each trial lasting only 1 wk, which exceeded the life span of purchased CM males. Six blocks of this experiment were set up simultaneously on each of six dates collectively spanning the entire 2015 growing season so as to yield an exceptionally large n of 36 data pairs. The position of the single trap vs. the line-trap with respect to cardinal direction was balanced to guard against possible bias due to prevailing wind direction that, during the daytime, was from the west. The data were collected nearly daily for each week-long replicate of this season-long experiment. CM males harvested from each individual trap were tallied after identification as wild or released. Statistical comparisons were made via paired t-tests.

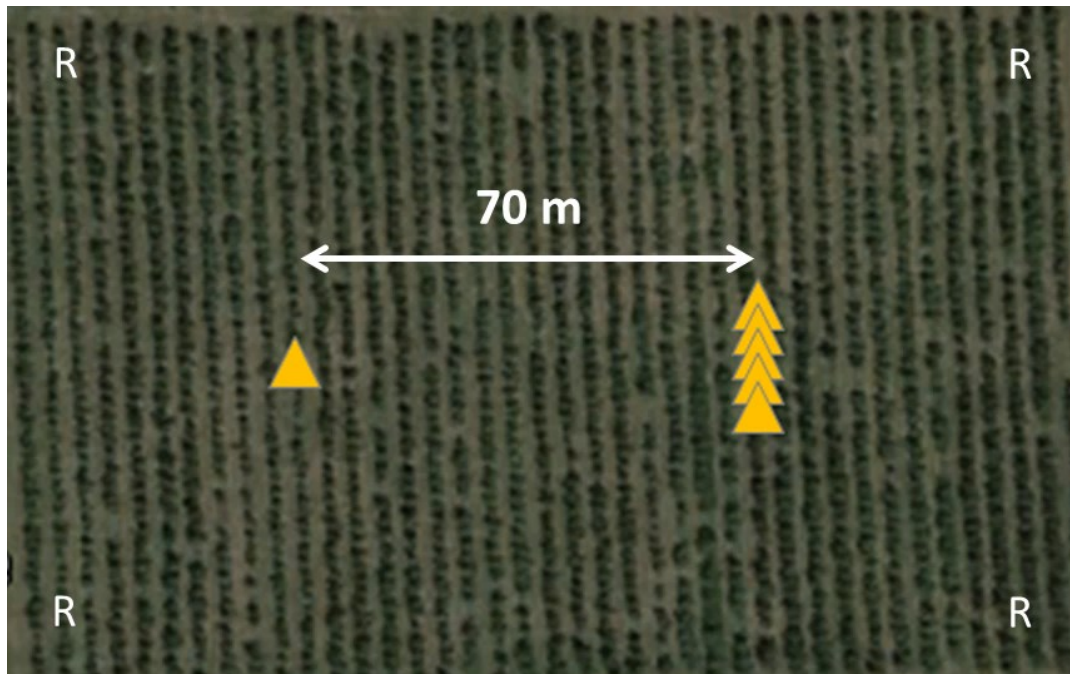


Figure 32. Schematic of the layout for one block of the experiment comparing capture in a single trap (represented by a triangle) relative to that in a line of 5 traps each separated by 4 m; the distance separating triangles in the line is not drawn to scale. At each release point (R), ca. 100 CM males were released at the start of each trial lasting 1 wk.

RESULTS

Feral males comprised less than 2% of all the CM captured, hereafter given as total catch. Mean capture in the single trap was 6.2 ± 1.2 (S.E.M.) males vs. 4.5 ± 0.53 for the $\mu 5$. This 35 % reduction was not significant at $p = 0.05$. This supports Hypothesis 1; the level of competition between traps spaced only 4 m apart is shown to be modest. As anticipated, the severity of competition increased significantly in the middle of the trap line compared to its ends (Figure 33). Pleasingly, traps in a common position along the trap line performed nearly identically. Collectively, these data validate the notion that, when reach of the plume from a trap is small as for CM, spacing multiple traps closely in a line can yield catches per trap almost as high as would isolated traps spaced widely.

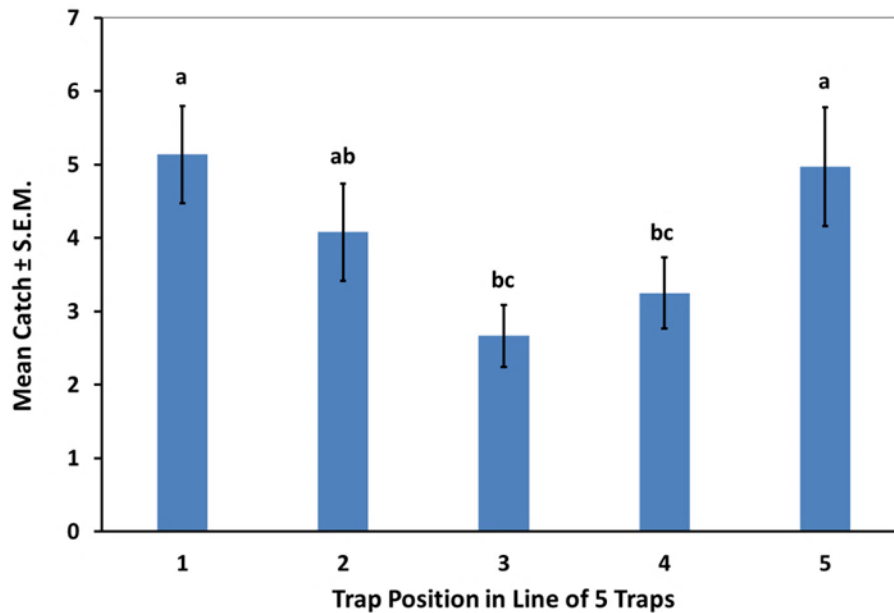


Figure 33. Capture of codling moth males as influenced by their position in the line of five traps. Means sharing a common letter are not significantly different at $p = 0.05$, $n=36$. Mean capture in a single trap was 6.2.

The data of Figure 34 support the claim (Hypothesis 2) that precision around μ_5 will be substantially higher than that around the capture number for a single trap. Or stated conversely, the variance for catch by a single trap is substantially greater than that for the mean of five traps in a line. While the single trap occasionally caught no CM males per week, such false negatives never occurred for the μ_5 . Furthermore, the single trap occasionally registered catches of > 15 males per week, while this never happened in the μ_5 . Startlingly, the single trap

returned a catch of 32 CM males per week on one occasion when the overall mean for the 36 replicates was only 6.2; proving single traps will sometimes return extremely variable catch. Catch data such as this would certainly lead to erroneous pesticide application of this perceived hot-spot in the orchard.

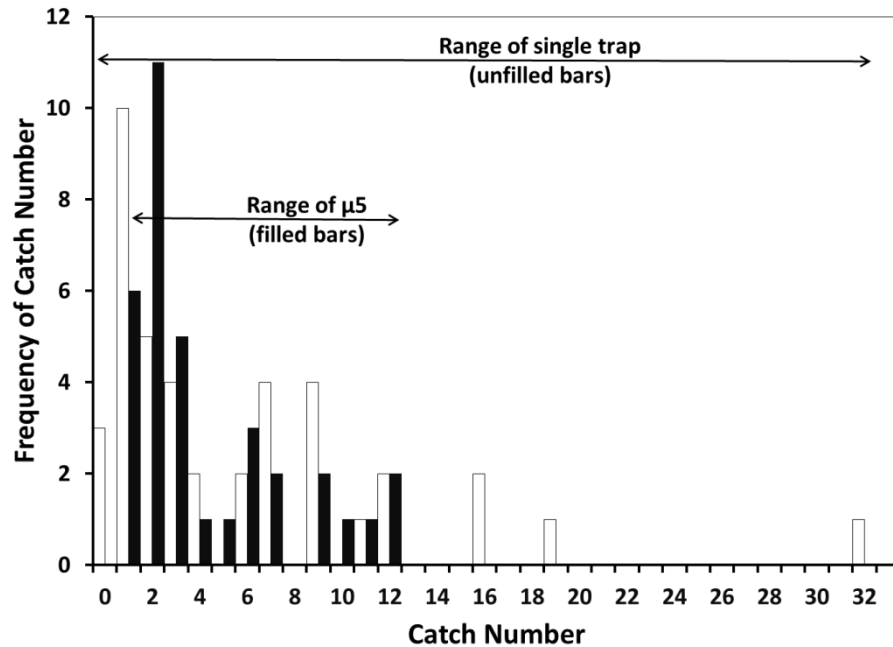


Figure 34. Frequency histograms of field data showing the realized capture numbers of codling moth under the single-trap (unfilled bars) vs. $\mu 5$ configuration (filled bars) in Michigan apple orchards, $n=36$. The range of the $\mu 5$ catch was 1 to 12, while the range of the single trap was 0 to 32.

DISCUSSION

The conclusion of this study is straightforward. Deploying a single trap to monitor CM density in the 21 ha sampling area does a poor job of proffering a reliable estimate of the density of this pest. It is far better to deploy five traps in a line with trap spacing of ca. 4 m. The cost of the additional traps (\$11 per trap plus lure) is dwarfed by: 1) the savings that could be realized by withholding sprays when they are indeed not justified ($> \$400$ per CM spray per trapping area of 20 ha), or 2) the losses that could be realized because the single monitoring trap presented false negatives. As the science of pest management matures by mastering the ability to translate capture numbers into estimates of absolute pest density (Miller et al. 2015; Adams et al. 2016a Chapter 2), it will be important to employ a tactic like line-trapping so as to shrink the troublesome variability associated with capture numbers in single traps. It is likely that the advantages of line-trapping will be substantial even when plume sizes are moderate to large. Thus, the value of this approach is likely to be far-reaching. For example, detectability of invasive species is critical and requires maximally powerful monitoring tools that reduce the possibility of false negatives. Thus it follows that the detection power of the line-trapping tactic will rise correspondingly with the length of the trapping line. In certain cases, long-line and very long-line trapping could be justified.

CHAPTER FIVE

Interactions of Japanese Beetles, *Popillia japonica*, (Coleoptera: Scarabaeidae)
with Monitoring Traps: Estimation of Plume Reach and Foraging Meander

ABSTRACT

The single-trap, multiple-release experimental design has proven very useful in providing basal data from which measures like reach of the attractive plume, trapping radius and area, and foraging meander can be deduced using recently developed analytical procedures for interpreting catch in traps. Past work has focused mainly on tortricid moths responding to sex pheromone-baited sticky traps. The current study confirmed that this approach could be successfully extended to the Japanese beetle (*Papilio japonica*), an economically important generalist pest for which traps and lures have been optimized. Patterns of *P. japonica* capture strongly suggested that this pest displaces by random walks (flights). The plume reach of Trécé Catch Can traps baited with the dual baitpack (floral and sex pheromone) lures deployed in large alfalfa fields was measured at ca. 10 m while foraging meander, expressed as circular standard deviation for turn angles, was $9 \pm 3^\circ$ (S.E.M.). The respective values for codling moth (*Cydia pomonella*) were 2 m and 37° . This outcome confirms predictions from theory and computer simulations that the amount of meander expressed by a forager will be inversely related to plume size. To be highly effective, attempts to manage this beetle by mass trapping should use a trap spacing no greater than 15 m.

INTRODUCTION

Recent elucidations of the interactions of randomly moving small animals with their monitoring traps (Miller et al. 2015; Adams et al. 2016a Chapter 2) have paved the way for translating data from single-trap, multiple-release experiments into estimates of: 1) plume reach of the trap, 2) trapping radius and area, and 3) overall capture probability across the trapping area. These data can then be used to estimate of absolute density of the responders from capture number. Moreover, methods have been developed to convert the decay constant in captures of animals moving into a trapping grid into an estimate of the amount of meander displayed by the searcher as it forages for attractant plumes (Miller et al. 2015; Adams et al. 2016a Chapter 2). Plume reach and foraging meander are parameters strongly influencing the effectiveness of a trap for monitoring a pest or the manner in which a group of traps should be deployed to manage a pest.

To date this research having high potential impact on pest management has been conducted mainly on moths responding to monitoring traps baited with sex pheromone (Miller et al. 2015, Adams et al. 2016a,b, Chapters 2 and 3). Here we extend this line of research to a beetle (Japanese beetle, *Papillio japonica*,) of high economic importance (Potter 1998) and for which much effort has gone into optimizing traps (Alm 1994) and lures (Ladd 1980). The hypotheses to be tested were: 1) mathematical patterns in data will demonstrate that *P. japonica* is a

random-walker (flier) amenable to the analytical approaches of behavioral analyses outlined by Miller et al. (2015) so as to produce initial measures of plume reach and foraging meander, and 2) based on our direct visual observations of these insects in the field, the foraging meander of *P. japonica* will be smaller than that we previously measured for codling moth, *Cydia pomonella*. Confirmation of these hypotheses should encourage additional research on this and other types of pests that will vastly increase the knowledge gain from trapping with the applied benefit of improved pest management.

MATERIALS AND METHODS

Source and Handling of Beetles

Experiments were conducted from August 20th to 30th, 2014. Prior to each experiment large numbers of *P. japonica* were collected from various locations around Michigan State University campus using Trece Catch Can traps (Trece, Salinas, CA) hung from 1.6 m galvanized rods and baited with the dual baitpack (floral and sex pheromone) lures (Trece, Salinas, CA). Beetles were washed and sorted in the laboratory to remove damaged or moribund individuals. For Experiment 1, ca. 7,000 beetles were collected for each replicate. Beetles were distinctively marked by assigned distance with DayGlo fluorescent powders (DayGlo Color, Cleveland, OH) by placing ca. 350 beetles into 540 ml polypropylene cups (Fabri-Kal Corp., Kalamazoo, MI) with ca. 400 mg of DayGlow powder. For Experiment 2, populations of beetles ranging from 230 to 350 were individually marked with a small brush dispensing ca. 5 mg of acrylic paint (Testors, Vernon Hills, IL). Extra effort in color assignment and determination was justified here because the numbers of beetles captured were designed to be low. Marked beetles were released at assigned locations by overturning captive beetles in 6 liter plastic boxes (Sterilite Co., Townsend, MA) and permitting them to escape of their own volition. Individuals unable to take flight after ten minutes were recaptured and removed from experiments.

Experiment 1 – Single-Trap, Multiple-Release for Measuring Plume Reach

Distinctively marked beetles were released in the four cardinal directions at defined distances from a single Trécé Catch Can trap baited with a dual baitpack (floral and sex pheromone) lure deployed at a height of ca. 1.6 m on a galvanized rod at the center of large alfalfa fields on the South Campus of Michigan State University, chosen because they presented a large uniform area devoid of strongly competing host plants. Catch was recorded for the next two days. A pilot test, replicated three times and using release distances of 20, 40, 80, 120, and 160 m and releases of ca. 1,000 beetles at each release site, yielded catch of marked beetles in all traps at 120 m and closer, but no catch in any trap at 160 m. Thus, the two-day maximum dispersion distance detected for these populations of *P. japonica* was ca. 140 m. Because we prioritized measurement of plume reach from this trapping system, this experiment was repeated (three more replicates) using release distances of closer spacing (10, 20, 30, 40, and 50 m) and ca. 7,000 beetles per replicate. It generated data that did not up-turn when release distance was plotted on the x-axis against 1/proportion of released beetles captured on the y-axis. A standard curve (Figure 4.12 of Miller et. al (2015)) could then be used to translate slope of this plot into length of the attractive plume.

Experiment 2 – Estimating Path Meander of *P. japonica* Foraging for Plumes

This experiment followed the methods of Miller et al. (2015 Chapter 7) and Adams et al. (2016a,b, Chapters 2 and 3) for translating decline in catch in a trapping grid of insects released just outside the grid corner into foraging meander. Doing so was accomplished using a graph of catch decay-constant vs. meander expressed as circular standard deviation (C.S.D.) (Miller et al. 2015, Adams et al. 2016b Chapter 3). This standard curve was generated by computer simulations (Miller et al. 2015; Adams et al. 2016a Chapter 2) using conditions matching those established here for *P. japonica*. This experiment was replicated five times using field sites like those for Experiment 1. Traps were arranged as a 5 x 5 rectangular grid where each trap was 15 m from a neighbor. Approximately 300 distinctively painted beetles were released 10 m from a given corner of the trapping grid. Releases across replicates used different grid corners with respect to North so that effects of prevailing wind might be detected if they came into play; but, they did not. Counts in every trap were recorded for only one day after beetle release because this experimental approach works best under short dispersion times for movers (Miller et al. 2015). The corner trap closest to the point of release was labeled Trap 1; the set of adjacent edge traps leading away from the corner (Miller et al. 2015, Chapter 7) were designated Traps 2 through 5 and their counts were averaged. The resulting catch in Traps 1 through 5 plotted on the x-axis vs. catch

on the y-axis was fit exponentially. Then C.S.D. was estimated from the above standard curve generated by computer simulations using the equivalent of a 10 m plume.

RESULTS AND DISCUSSION

Experiment 1 – Single-Trap, Multiple-Release for Measuring Plume Reach

As expected for random walkers, a graph of beetle capture vs. distance of release (Figure 35 A) fell smoothly and asymptotically toward zero catch and yielded a straight line with positive slope when release distance was plotted vs. the inverse of proportion catch (Figure 35 B). These properties strongly support Hypothesis 1 – *P. japonica* is a random-walker. The slope of the latter plot (0.36) was then used to estimate plume reach using the standard curve of Miller et al. (2015, Figure 4.12). Plume reach of this trap under the above conditions was estimated at a minimum of 5 and a maximum of 15 m, taken hereafter as ca. 10 m. These methods (Miller et al. 2015) have estimated plume reaches as large as 70 m and as small as <1 m; the mean of nine insect pest species so measured was 19 m. Thus the plume reach for the *P. japonica* trap has appreciable but modest reach.

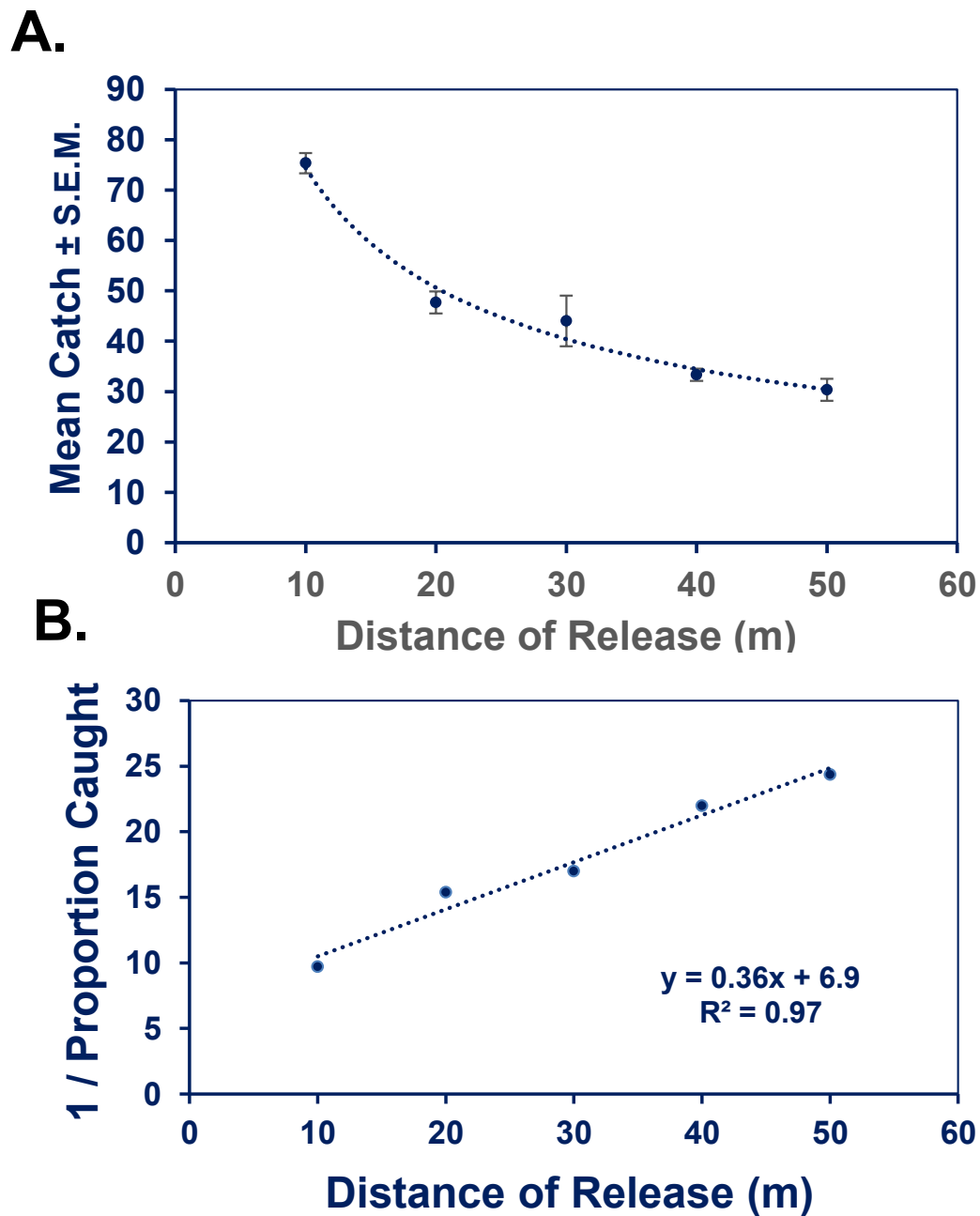


Figure 35. **A.** Untransformed plot of capture of *P. japonica* as a function of distance of release from a single trap. **B.** Inverse of proportion of released beetles captured by distance of release. Plume reach can be deduced from the slope of this MAG plot using the Figure 4.12 standard curve of Miller et al. (2015).

This knowledge has immediate practical ramifications. Deployment of traps to rid a landscape of this pest are not likely to be highly effective because each trap has limited power to draw pests from a great distance. Rather, it is far more likely that beetles disperse at random over long distances to reach the trap. As documented by Miller et al. (2015 Chapter 8), mass trapping of pests becomes effective only when traps are deployed with spacing no more than 1.5 times the plume reach. Thus for *P. japonica* traps to appreciably reduce populations of this pest, trap separation should be no greater than ca. 15 m, which translates to ca. 44 per ha. Whether or not this management tactic would be economical then depends of the value of the protected crop. Indeed, this recommended trap spacing agrees quite well with the ca. 20 m spacing employed by the several publications reporting useful levels of *P. japonica* populations reductions via mass trapping (Hamilton et al. 1971, Wawrzynski and Ascerno 1998)

Experiment 2 – Estimating Path Meander of *P. japonica* Foraging for Plumes

A plot of capture by trap position in the grid is shown in Figure 28 along with its fit to an exponential function. Its exponent of negative 0.53 can then be translated into an estimate of path meander using the standard curve of Figure 37.

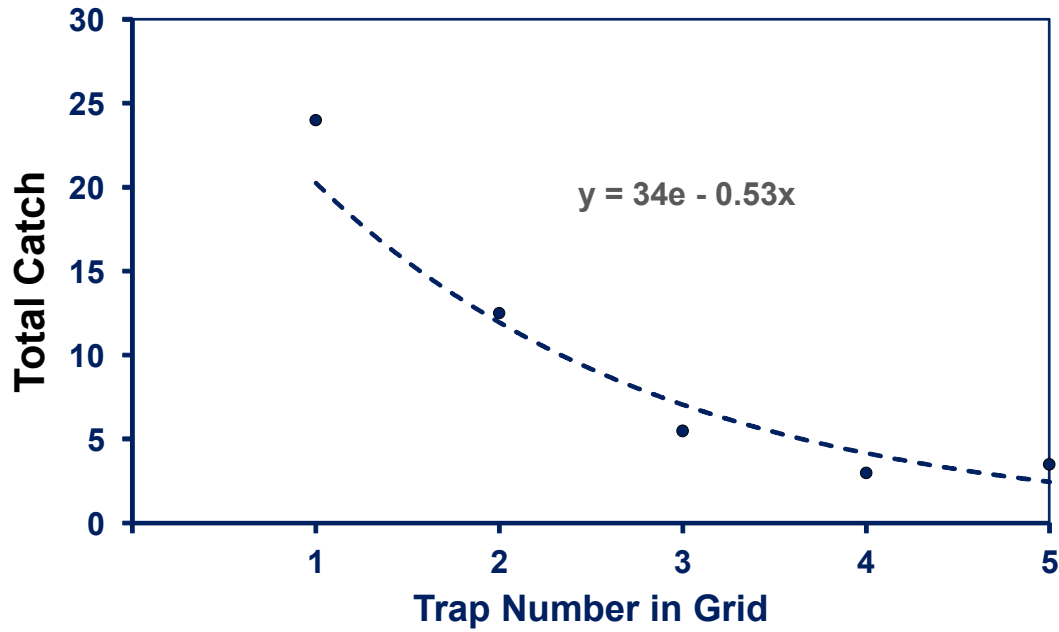


Figure 36. Number of *P. japonica* captured as a function of trap position in a 5 x 5 rectangular grid of traps separated by 15 m. Beetles were released 10 m from trap number 1. The exponent of negative 0.53 was then used to deduce meander using the standard curve.

The meander estimate returned from Figure 36 was C.S.D. 12° and that for the mean derived from the individual experimental replicates was $10 \pm 3^\circ$ (S.E.M.).

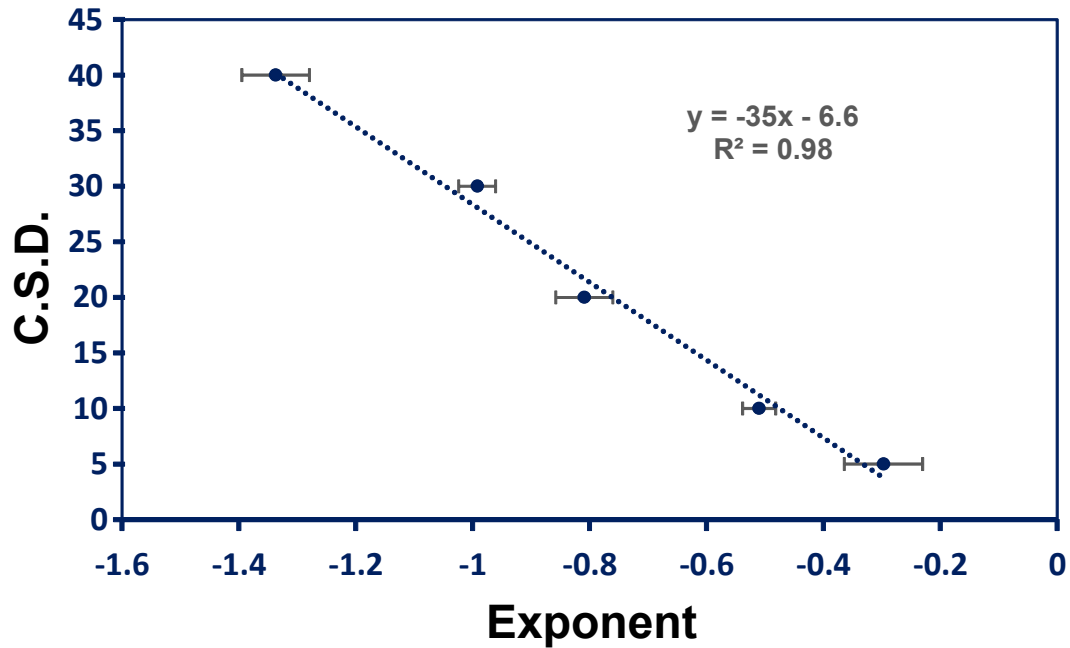


Figure 37. Standard curve for translating the decay constant in catch from Figure 36 into meander expressed as circular standard deviation (C.S.D.) of turn angles. These data were generated using the MultiMover software of Miller et al. (2015). 300 movers executing 400 steps were released 10 units from the corner of a 5 x 5 grid of 10 x 2 unit plumes whose sources were separated by 15 units. Ten replicates were run for each C.S.D.

Figure 38 offers four sample paths with a distance scale representative of a mover like *P. japonica* random-walking with a C.S.D. of 10°.

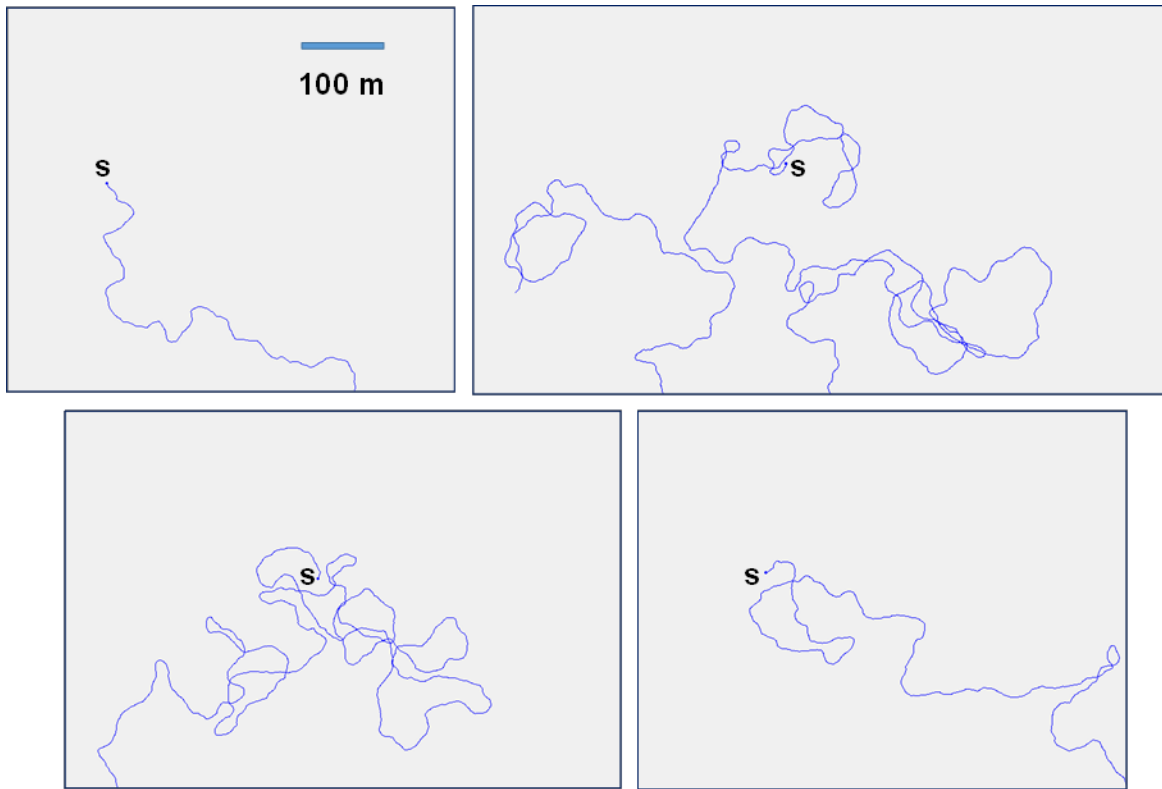


Figure 38. Representative tracks for a mover like *P. japonica* executing meander of C.S.D. 10° . These tracks were generated by the MultiMover software. Movers initiating at s took 5,000 steps many of which occurred outside the frame of view of the computer screen.

The C.S.D. for male codling moths foraging in apple was estimated at $37 \pm 2^\circ$ (S.E.M.); thus, Hypothesis 3 is confirmed – foraging meander for Japanese beetle is smaller than that for codling moth. This finding was expected because meander is inversely correlated with size of attractant plume (Miller et al. 2015). A larger C.S.D. results in more thorough search, which is necessary to find a small target. As plume size increases, search paths can become straighter allowing

sufficient intersection with a larger plume while allowing more territory to be searched.

Collectively, this opening study applying the methods of Miller et al. (2015) to a beetle demonstrates that this approach can be successfully applied to diverse organisms. We expect that these tools will add substantially to the field of movement ecology (Ran 2008, Holyoak 2008).

SUMMARY AND CONCLUSIONS

The main objective of this research was to validate the methods of data analysis laid out in the book *Trapping of Small Organisms Moving Randomly: Principles and Applications in Pest Management* on which I am a co-author. Extensively replicated field studies have shown that reliable and consistent measures of the dispersive distance of a population of insects as well as the T_{fer} and the plume reach of a trap can be made by proper analysis of the catch data from the single-trap multiple-release experiment. Absolute pest density can be successfully calculated under normal field conditions and across the season. Furthermore the meander of an insect's movement patterns, characterized as the circular standard deviation (C.S.D.) of turn angles, can also be measured from the analysis of catch data from a single release of a population of insects along the edge of a 5 x 5 grid of attractive traps. Finally this research demonstrates that the variance around catch data from a single trap can be mitigated by considering the mean of a line of 5 closely placed traps. These findings unlock the black box that trapping has been for the past century.

This work validates the approach for measuring the plume reach and the trapping radius by producing a consistent and reliable measure of these values throughout the 30 replicates spanning three seasons, with two different release configurations, and three different population levels. This work also falsifies the

paradigm that pheromone plumes are always large. The plume reach from a standard CM pheromone baited monitoring trap was shown to be surprisingly small at 2 m, while the 95% trapping radius was ca. 260 m. This finding illustrates that catch in a trap is the result of the extensive searching done by the male moths, not a giant plume of pheromone sweeping the orchard. Moreover the T_{fer} (proportion of the population sampled) of the monitoring trap, has been consistently shown to be only 0.015. This is the first time that the consistency in trapping has been tested and verified by known populations.

These results have notable benefits for pest managers of not only CM but for pests generally. This approach to understanding the constituent parts of the black box of trapping should be put into practice for the entire throng of insect pests impacting growers in Michigan and across the globe. The ability to make accurate estimations of the absolute pest density over a given area when considering economic threshold decisions will undoubtedly lead to sprays being withheld which will have positive impacts on worker health, environmental impacts, and food costs.

This research confirms that the C.S.D used by CM matches that shown to be optimal in computer simulations. Figure 26 illustrates simulation data showing that the optimal C.S.D. for insect like movers is estimated to be ca. 35° . The plot of the mean of 39 replicates of the 5 x 5 trapping grid experiment (Figure 29) gives

an exponent of -0.99 which equates to a C.S.D. of 37° for this insect. This close agreement to the predicted strongly supports the idea that these insects have indeed optimized their search behavior. Circular standard deviation of turns for a population of CM can indeed be measured in the field by plotting the catch along the leading edge of a 5 x 5 trapping grid. This experimental approach should prove useful to the new field of animal movement ecology (Ran 2008, Holyoak 2008).

The variance in catch from a single isolated monitoring trap poses a substantial challenge to pest managers. A solution to this problem is to increase the number of traps and take a mean of the catch data. However the time required to visit and service additional remotely placed traps makes this approach impractical. This research demonstrates that taking the mean of five traps, placed in a line spaced ca. 4 m apart, can substantially reduce the variance around mean catch values and increase the accuracy of estimates of pest populations without significantly increasing the time required to service them. The line-trapping technique is therefore highly recommended for many types of pests.

These techniques were applied to species other than CM. Japanese beetle is an introduced pest that impacts many valuable crops in the Eastern United States. These methods were used to quantify the plume reach of a standard trap and the C.S.D. of flying Japanese beetles. For Japanese beetle a foraging meander of $9 \pm 3^\circ$ C.S.D. was revealed and the plume reach from the Tréré Catch Can trap baited

with the dual baitpack (floral and sex pheromone) lure was measured at 10 m. A T_{fer} value of 0.06 and a maximum dispersive distance of 120 m should be considered preliminary until release distances can be extended to the edge of the total trapping area, but demonstrates the effectiveness of these methods. Pilot studies have also been conducted with oriental fruit moth (*Grapholita molesta*) that produced similar estimates of plume reach at 2 m, and a dispersive distance of 140 m.

While these techniques require large populations of the target insect there is substantial economic incentive to maximize information yield from pheromone-baited monitoring traps in high value crops. For example two species of moths, the naval orangeworm (*Amyelois transitella*) and peach twig borer (*Anarsia lineatella*) are important pests of almonds in California. These pests are managed through careful IPM programs that rely on detection of adults with pheromone baited monitoring traps. Securing a measure of the trapping area and T_{fer} for these pests, would allow pest managers to calculate absolute pest populations and potentially reduce sprays and increase profits on these valuable crops. Other insects with high economic and environmental impacts include invasive species such as gypsy moth (*Lymantria dispar*) and emerald ash borer (*Agrilus planipennis*). Translating monitoring trap catch data into accurate measures of population per area would greatly improve control program decisions.

The major limiting factor for monitoring traps is the time required to manually inspect distantly located traps. Automated pheromone baited traps offer a solution to this problem. Monitoring traps equipped with wireless sensors have the ability to deliver accurate and timely catch data to a central data collection and process point where growers can make real time decisions about pest control. As the future of trapping moves towards automation the need for a complete understanding of the trapping area as well as the dispersive capacity of the target pest will become more and more important. The principles laid out in the book *Trapping of Small Organisms Moving Randomly: Principles and Applications to Pest Monitoring and Management*, and validated by this research will be critical to companies wishing to optimize trap placement and maximize information yield.

It is my sincere hope that the principles validated here will be broadly adopted and practiced so as to increase accuracy of IPM decisions. Hopefully unnecessary pesticide applications will be reduced or eliminated so that the human food supply is safer, less expensive, and sustainable.

APPENDIX

RECORD OF DEPOSITION OF VOUCHER SPECIMENS

The specimens listed below have been deposited in the named museum as samples of those specimens or other taxa, which were used in this research. Voucher recognition labels bearing the voucher number have been attached or included in fluid preservation specimens.

Voucher Number: 2016 – 01

Author and Title of thesis:

Christopher Glen Adams

Maximizing Information Yield from Pheromone-Baited Monitoring Traps:
Estimating Plume Reach, Trapping Radius, and Absolute Density of Randomly
Moving Insects

Museum (s) where deposited:

Albert J. Cook Arthropod Research Collection, Michigan State University (MSU)

Table 5 Record of voucher specimens

Family	Genus-Species	Life Stage	Quantity	Preservation
Tortricidae	<i>Cydia pomonella</i> (L)	Adult	10 male	Pinned
Tortricidae	<i>Cydia pomonella</i> (L)	Adult	10 female	Pinned
Scarabaeidae	<i>Popillia japonica</i> (Newman)	Adult	10 male	Pinned
Scarabaeidae	<i>Popillia japonica</i> (Newman)	Adult	10 female	Pinned

LITERATURE CITED

LITERATURE CITED

- Adams, C. G., P. S. McGhee, J. H. Schenker, L. J. Gut, J. Brunner, and J. R. Miller. 2016a. Maximizing information yield from pheromone-baited monitoring traps: estimating plume reach, trapping radius, and absolute density of codling moth (*Cydia pomonella*) in Michigan apple. *Journal of Economic Entomology*. *submitted*. Chapter two this dissertation.
- Adams, C. G., P. A. Weston, J. H. Schenker, and J. R. Miller. 2016b. Flight meander of male codling moths searching for plumes of female sex pheromone is optimized. *Nature – Letters*. *submitted*. Chapter three this dissertation.
- Adams, C. G., P. S. McGhee, J. H. Schenker, L. J. Gut, and J. R. Miller. 2016c. Line-trapping of codling moth, *Cydia pomonella*, (Lepidoptera: Tortricidae): a novel approach to improving the precision of capture numbers in monitoring traps. *Pest Management Science*. *Submitted*. Chapter four this dissertation.
- Adams, C. G., P. A. Weston, J. H. Schenker, and J. R. Miller. 2016d. Interactions of Japanese Beetles, *Popillia japonica*, (Coleoptera: Scarabaeidae) with Monitoring Traps: Estimation of Plume Reach and Foraging Meander. *Environmental Entomology*. *Submitted*. Chapter five this dissertation.
- Alm, S. R., T. Yeh, M. L. Campo, C. G. Dawson. 1994. Modified trap design and heights for increased capture of japanese beetle adults (Coleoptera: Scarabaeidae). *Horticultural. Entomology*, 87: 775-780.
- Ando, N., S. Emoto, and R. Kanzaki. 2013. Odour-tracking capability of a silkmoth driving a mobile robot with turning bias and time delay. *Bioinspiration and Biomimetics*, 8(1).
- Bartumeus, F., J. Catalan, U.L. Fulco, M. L. Lyra, G. M. Viswanathan. 2002. Optimizing the encounter rate in biological interactions: lévy vs. brownian strategies. *Physical Review Letters*, 88: 097901.
- Baker, T. C. and K. F. Haynes. 1996. Pheromone-mediated optomotor anemotaxis and altitude control exhibited by male oriental fruit moths in the field. *Physiological Entomology*, 21: 20-32.
- Baker, T. C. and W. L. Roelofs. 1981. Initiation and termination of oriental fruit

- moth responses to pheromone concentrations in the field. *Environmental Entomology*, 10: 211-218.
- Baker, T. C., M. A. Willis and P. L. Phelan. 1984. Optomotor anemotaxis polarizes self-steered zigzagging in flying moths. *Physiological Entomology*, 9: 365-376.
- Bauer, W. and G. Westfall. 2014. *University Physics*. McGraw Hill Publishers.
- Beers. E. H., J. F. Brunner, M. Willett, and G. M. Warner. 1993. *Orchard pest management: a resource book for the Pacific Northwest*. Good Fruit Grower, Yakima, WA. 276 pp.
- Bell, W. J. 1991 *Searching behaviour: the behavioural ecology of finding resources*. Chapman & Hall
- Benhamou, S. 2007. How many animals really do the Lévy walk? *Ecology*, 88: 1962-1969.
- Berg, H. C. 1993. *Random walks in biology*. Princeton University Press, Princeton, N.J. 152 pp.
- Bouvier, J. C., R. Bues, T. Boivin, L. Boudinhon, D. Beslay, B. Sauphanor. 2001. Deltamethrin resistance in codling moth (Lepidoptera: Tortricidae): inheritance and number of genes involved. *Heredity*, 87: 456-462.
- Bovet, P. and S. Benhamou. 1991 Optimal sinuosity in central place foraging movements. *Animal Behaviour*, 42: 57-62.
- Brouwers, N. C., and C. Newton. 2009. Movement analyses of wood cricket (*Nemobius sylvestris*) (Orthoptera: Gryllidae). *Bulletin of Entomological Research*, 100(6), 623–34.
- Brunner, J., S. Welter, C. Calkins, R. Hilton, El. Beer, J. Dunley, P. Van Buskirk. 2002. Mating disruption of codling moth: a perspective from the Western United States. use of pheromones and other semiochemicals in integrated production IOBC WPRS Bulletin, 25, 1–11.
- Byers, J. A. 1993. Simulation and equation models of insect population control by pheromone-baited traps. *Journal of Chemical Ecology*, 19: 1939-1956.
- Byers, J. A., O. Anderbrant, J. Löfqvist. 1989. Effective attraction radius: a method for comparing species attractants and determining densities of flying insects. *Journal of Chemical Ecology*, 15: 749-765

- Byers, J. A. 2012. Modeling female mating success during mass trapping and natural competitive attraction of searching males and females. *Entomologia Experimentalis et Applicata*, 145: 228-237.
- Cardé, R. T. 1976. Utilization of pheromones in the population management of moth pests. *Environmental Health Perspectives*, 14: 133-144.
- Cardé, R. T. 1984. Chemo-orientation in flying insects. *In* W. Bell and R. Cardé (eds.) *Chemical ecology of insects*. Springer, New York, NY. pp. 111-124.
- Cardé, R. T. and M. A. Willis. 2008. Navigational strategies used by insects to find distant, wind-borne sources of odor. *Journal of Chemical Ecology*, 34: 854-866.
- Cardé, R. T., A. M. Cardé, R. D. Girling. 2012. Observations on the flight paths of the day-flying moth *Virbia lamae* during periods of mate location: do males have a strategy for contacting the pheromone plume? *Journal of Animal Ecology*, 81: 268-276. (and references therein)
- Codling, E. A., and R. N. Bearon. 2010. Diffusion about the mean drift location in a biased random walk. *Ecology*, 91(10), 3106–3113.
- Crist, T. O. and J. A. MacMahon. 1991. Individual foraging components of harvester ants: movement patterns and seed patch fidelity. *Insectes Sociaux*. 38: 379-396
- Cuthbertson, A., G. S. and A. K. Murchie. 2005. Economic spray thresholds in need of revision in Northern Irish Bramley orchards. *Biodiversity News*, 32: 19
- Dai, X., G. Shannon, R. Slotow, B. Page, and K. J. Duffy. 2007. Short duration daytime movements of a cow herd of African elephants. *Journal of Mammalogy*, 88(1), 151–157.
- Dodds, K. J. and D. W. Ross. 2002. Sampling range and range of attraction of *Dendroctonus pseudotsugae* pheromone-baited traps. *The Canadian Entomologist*, 134: 343-355
- Dusenbery, D. B. 1990. Upwind searching for an odor plume is sometimes optimal. *Journal of Chemical Ecology*, 16: 1971-1976.
- Edwards, S., A. J. Rutkowski, R. D. Quinn, and M. Willis. 2005. Moth-inspired plume tracking strategies in three-dimensions. *Proceedings – Institute of*

- Electrical and Electronics Engineers, International Conference on Robotics and Automation, 2, 1669–1674.
- Elkinton, J. S., R. T. Cardé and C. J. Mason. 1984. Evaluation of time-averaged dispersion models for estimating pheromone concentrations in a deciduous forest. *Journal of Chemical Ecology*, 10: 1081-1108.
- Ellis, N. H. and L. A. Hull. 2013. Factors influencing adult male *Grapholita molesta* dispersal in commercial *Malus* and *Prunus* host crops. *Entomologia Experimentalis et Applicata*, 146: 232-241.
- Epstein, A. D. L., R. S. Zack, J. F. Brunner, L. Gut, and J. J. Brown, 2000. Effects of broad-spectrum insecticides on epigeal arthropod biodiversity in Pacific Northwest apple orchards. *Environmental Entomology*, 29(2), 340–348.
- Fagan, W. F. 1997. Introducing a “boundary-flux” approach to quantifying insect diffusion rates. *Ecology*, 78: 579-587.
- Grieshop, M. J., J. F. Brunner, V. P. Jones, and N. M. Bello. 2010. Recapture of codling moth (Lepidoptera: Tortricidae) males: influence of lure type and pheromone background. *Journal of Economic Entomology*, 103(4), 1242–1249.
- Guichard, S., D. J. Kriticos, A. Leriche, S. P. Worner, J. M. Kena and D. M. Suckling. 2010. Evidence of active or passive downwind dispersal in mark-release-recapture of moths. *Entomologia Experimentalis et Applicata*, 134: 160-169.
- Gut, L.J., and J. F. Brunner. 1996. Implementing codling moth mating disruption in Washington pome fruit orchards. *Tree Fruit Research Extension Center Information Series*, No. 1. Washington State University, Wenatchee, Washington.
- Gut, L. J., L. Stelinski, D. Thompson and J. R. Miller. 2004. Behavior-modifying chemicals: prospects and constraints in IPM. pp. 73-121 *In* O. Koul, G. S. Dhaliwal and G. W. Cuperus (eds.) *Integrated Pest Management: Potential, Constraints, and Challenges*. CABI Press, Cambridge MA.
- Gut, L. and J. Wise. 2016. Codling moth integrated pest management. pp. 109-113 *In* J.C. Wise, L.J Gut, R. Isaacs, A.M. C. Schilder, G.W. Sundin, B. Zandstra, R. Beaudry and G. Lang (eds.) 2016 *Michigan Fruit Management Guide*. Michigan State University Extension Bulletin E154, East Lansing, MI.

- Hamilton, D. W., P. H. Schwartz, B. G. Townshend, C. W. Jester. 1971. Traps reduce an isolated infestation of Japanese beetle. *Journal of Economic Entomology*, 64 (1) 150-153.
- Hartstack, A. W., J. P. Hollingsworth, R. L. Ridgway, H. H. Hunt. 1970. Deterioration of trap spacings required to control an insect population. *Journal of Economic Entomology*, 64: 1090-1100.
- Holyoak, M., Casagrandi, R. and Nathan R. 2008. Trends and missing parts in the study of movement ecology. *Proceedings of the National Academy of Sciences*, 105.49: 19060-19065.
- Howell, J. F. and A. E. Clift. 1974. The dispersal of sterilized codling moths released in the Wenatchee valley, Washington. *Environmental Entomology*, 3: 75-81.
- Howitt, A.H. 1993. *Common Tree Fruit Pests*, Michigan State University Extension
- Janzen, J. H. 1984. Two ways to be a big tropical moth: Santa Rosa saturniids and sphingids. *Oxford Surveys in Evolutionary Biology*, 1: 85-140.
- Judd, G. and M. Gardiner. 1997. Forecasting phenology of *Orthosia hibisci guenée* (Lepidoptera: Noctuidae) in British Columbia using sex-attractant traps and degree-day models. *The Canadian Entomologist*, 129: 815-825.
- Kareiva, P. M. and N. Shigesada. 1983. Analyzing insect movement as a correlated random walk. *Oecologia*, 56: 234-238.
- Katz, P. L. 1974. A long-term approach to foraging optimization. *The American Naturalist*, 108: 758-782.
- Kehat, M., L. Anshelevich, E. Dunkelblum, P. Fraishtat and S. Greenberry. 1994. Sex pheromone traps for monitoring the codling moth: effect of dispenser, pheromone dose and type of trap on male capture. *Entomologia Experimentalis et Applicata*, 70: 55-62.
- Kennedy, J. S. and D. Marsh. 1974. Pheromone-regulated anemotaxis in flying moths. *Science*, 184, 999-1001.
- Knight, A. L. 2005. Developing action thresholds for codling moth (Lepidoptera: Tortricidae) with pear ester- and codlemone-baited traps in apple orchards treated with sex pheromone mating disruption. *The Canadian Entomologist*, 747(May), 739-747.

- Ladd, T. L. and McGovern, T. P. 1980. Japanese beetle: a superior attractant, phenethyl propionate + eugenol + geraniol, 3:7:3. *Journal of Economic Entomology*, 73: 689-691
- Larralde, H., P. Trunfio, S. Havlin, H. E. Stanley, G. H. Weiss. 1992. Territory covered by N diffusing particles. *Nature*, 355: 423-426.
- Lewis, M. 2001. ESS Analysis of mechanistic models for territoriality: the value of scent marks in spatial resource partitioning. *Journal of Theoretical Biology*, 210, 449–461.
- Li, W., J.A. Farrell, S. Pang, and R. M. Arrieta. 2006. Moth-inspired chemical plume tracing on an autonomous underwater vehicle. *Institute of Electrical and Electronics Engineers, Transactions on Robotics*, 22(2), 292–307.
- Mani, E. and T. Wildbotz. 1977. The dispersal of male codling moths (*Laspeyresia pomonella* L.) in the Upper Rhine Valley. *Journal of Applied Entomology*, 83: 161-168.
- Mason L. J., R. K. Jansson, R. R. Heath. 1990. Sampling range of male sweetpotato weevils (*Cylas formicarius elegantulus*) (Summers) (Coleoptera: Curculionidae) to pheromone traps: influence of pheromone dosage and lure age. *Journal of Chemical Ecology*, 16: 2493-2502.
- McGhee, P. 2011. Quantifying the benefits of areawide pheromone mating disruption programs that target codling moth. *American Entomologist*, 57(2), 94–100.
- Michigan Apple Committee. 2013. Accessed at <http://www.michiganapples.com>
- Miller J. R. and L. J. Gut. 2015. Mating disruption for the 21st century: matching technology with mechanism. *Environmental Entomology*, 427-453.
- Miller J. R. and W. L. Roelofs. 1978. Sustained-flight tunnel for measuring responses to wind-born sex pheromones. *Journal of Chemical Ecology*, 4: 187-198.
- Miller, J. R., P. S. McGhee, P. Y. Siegart, C. G. Adams, J. Hung, M. J. Grieshop and L. J. Gut. 2010. General principles of attraction and competitive-attraction as revealed by large cage studies of moths responding to sex pheromone. *Proceedings of the National Academy of Science*, 107: 22-27.

- Miller J. R., C. G. Adams, P. A. Weston, J. H. Schenker. 2015. Trapping of small organisms moving randomly: principles and applications to pest monitoring and management. Springer Briefs in Ecology, New York NY. 114 pp.
- Miranowski, J. A. 1980. Estimating the relationship between pest management and energy prices, and the implications for environmental damage. American Journal of Agricultural Economics, 65: 995-1000.
- Moorcroft, P. R., M. A. Lewis, and R. L. Crabtree 1999. Home range analysis using a mechanistic home range model. Ecology, 80(5), 1656–1665.
- Mota-Sanchez, D., J. Wise, R. Vander Poppen, L. Gut, and R. Hollongworth, 2008. Resistance of codling moth, *Cydia pomonella* (L.) (Lepidoptera: Tortricidae), larvae in Michigan to insecticides with different modes of action and the impact on field residual activity. Pest Management Science, 64, 881–890.
- Mumford, J. D., and G. A. Norton. 1984. Economics of decision making in pest management. Annual Review of Entomology, 29:157-74
- Murlis, J., J. S. Elkinton, R. T. Carde. 1992. Odor plumes and how insects use them. Annual Review of Entomology, 37: 505-532
- Murlis, J., M. A. Willis, and R. T Carde. 2000. Spatial and temporal structures of pheromone plumes in fields and forests. Physiological Entomology, 25: 211-222.
- Nakamura, K., and K. Kawasaki. 1976. The active space of the *Spodoptera litura* (F.) sex pheromone and the pheromone component determining this space. Applied Entomology and Zoology, 12(2), 162–177.
- Nathan, R. and L. Giuggioli. 2013. A milestone for movement ecology research. Movement Ecology, 1: 1-3.
- Newton, A. C., R. Hill, C. Echeverría, D. Golicher, J. M. R. Benayas, L. Cayuela, and S. Hinsley. 2009. Remote sensing and the future of landscape ecology. progress in physical geography, 33(4), 528–546.
- Okubo, S. A. and S. A. Levin. 2001. Diffusion and ecological problems: modern perspectives. Springer, Berlin.
- Östrand, F. and O. Anderbrant. 2003 From where are insects recruited? A new model to interpret catches of attractive traps. Agricultural and Forest Entomology, 5: 163-171.

- Otto, G. and A. von Brandt. 2005. Fish catching methods of the world. 4th edition. Blackwell Publishers. Oxford.
- Parker, G. A. and J. M. Smith. 1990. Optimality theory in evolutionary biology. *Nature*, 348: 27-33.
- Patterson, T. A., L. Thomas, C. Wilcox, O. Ovaskinen, and J. Matthopoulos. 2008. State-space models of individual animal movement. *Trends in Ecology and Evolution*, 23: 87-94.
- Pearce-Duvet, J. M. C., C. P. H. Elmans, D. H. Feener Jr. 2011. Walking the line: search behavior and foraging success in ant species. *Behavioral Ecology*, 22: 501-509.
- Pimentel, D., H. Acquay, M. Biltonen, P. Rice, M. Silva, J. Nelson, V. Lipner, S. Giordane, A. Horowitz and M. D'Amore. 1992. Environmental and economic costs of pesticide use. *American Institute of Biological Science*, 42: 750-760.
- Potter DA. 1998. Destructive insects: biology, diagnosis, and control. Chelsea MI: Ann Arbor Press, 344 pp
- Pyke, G. H. Are animals efficient harvesters? 1978. *Animal Behaviour*, 26: 241-250.
- Ran, N, 2008 An emerging movement ecology paradigm. *Proceedings of the National Academy of Sciences*, 105.49 19050-19051
- Reddy, G. V. P. and M. Manjunatha. 2000. Laboratory and field studies on the integrated pest management of *Helicoverpa armigera* (Hubner) in cotton, based on pheromone trap catch threshold level. *Journal of Applied Entomology*, 124: 213-221.
- Riedl, H. 1980. The importance of pheromone trap density and trap maintenance for the development of standardized monitoring procedures for the codling moth (Lepidoptera: Tortricidae). *The Canadian Entomologist*, 112: 529–544.
- Riedl, H. and B. A. Croft. 1974. A study of pheromone trap catches in relation to codling moth (Lepidoptera: Olethreutidae) damage. *The Canadian Entomologist*, 106: 525-537.
- Riedl, H., B. A. Croft, A. J. and Howitt. 1976. Forecasting codling moth phenology based on pheromone trap catches and physiological time models. *The Canadian Entomologist*, 108: 449– 460.

- Riedl, H., J. F. Howell, P. J. McNally, and P. H. Westigard. 1986. Codling moth management: use and standardization of pheromone trapping systems. Bulletin 1918, University of California Division of Agriculture and Natural Resources, Oakland, California.
- Robertson, A. S. P., L. A. Hull, and D. D. Calvin. 2005. Tufted apple bud moth (Lepidoptera : Tortricidae) management model for processing apples based on early season pheromone trap capture. *Journal of Economic Entomology*, 98(4), 1229–1235.
- Rudd, W. G. and R. W. Gandour. 1985. Diffusion model for insect dispersal. *Journal of Economic Entomology*, 72: 295-301.
- Sakiyama, T., and Y.-P. Gunji. 2013. Emergence of an optimal search strategy from a simple random walk. *Journal of the Royal Society Interface*, 10: 1-6.
- Schouest, J. L. P. and T. A. Miller. 1994. Automated pheromone traps show male pink bollworm (Lepidoptera: Gelechiidae) mating response is dependent on weather conditions. *Journal of Economic Entomology*, 87: 965-974.
- Schumacher, P., A. Weyeneth, D. C. Weber and S. Dorn. 1997. Long flights in *Cydia pomonella* L. (Lepidoptera: Tortricidae) measured by flight mill: influence of sex, mated status and age. *Physiological Entomology*, 22: 149-160.
- Stephens, D. W. and J. R. Krebs. 1986. Foraging theory. Princeton University Press
- Stern, V. M., R. F. Smith, R. V. d. Bosch and K. S. Hagen. 1959. The integration of chemical and biological control of spotted alfalfa aphid: the integrated control concept. *Hilgardia*, 29: 81-101.
- Sufyan, M., D. Neuhoﬀ, L. Furlan. 2011. Assessment of the range of attraction of pheromone traps to *Agriotes lineatus* and *Agriotes obscurus*. *Agricultural and Forest Entomology*, 13: 313-319.
- Thomson, D., J. Brunner, L. Gut, G. Judd, and A. Knight. 2001. Ten years implementing codling moth mating disruption in the orchards of Washington and British Columbia : starting right and managing for success ! Pheromones for insect control in orchards and vineyards IOBC Wprs Bulletin, 24(2), 23–30.

- Turchin, P., and F. Odendaal. 1996a. Measuring the effective sampling area of a pheromone trap for monitoring population density of southern pine beetle (Coleoptera: Scolytidae). *Population Ecology*, 25(3), 582–588.
- Turchin, P., and F. Odendaal. 1996b. Measuring the effective sampling area of a pheromone trap for monitoring population density of southern pine beetle (Coleoptera: Scolytidae). *Environmental Entomology*, 25(3), 582–588.
- United States Apple Association. 2013. Accessed at <http://www.usapple.org>
- Vacas, S., M. Miñarro, M. D. Bosch, J. Rrimo and V. Navarro-Llopis. 2013. Studies of the codling moth (Lepidoptera: Tortricidae) response to different codlemone release rates. *Environmental Entomology*, 42: 1383-1389.
- Van der Pers, J. N. C. and A. K. Minks. 1993. Pheromone monitoring in the field using single sensillum recording pp. 359-371 *In* R. T. Cardé and A. K. Minks (eds.) *Insect Pheromone Research: new directions*. Chapman and Hall, New York, NY.
- Van Wijland, F. 2002. Trapping of a random walk by diffusing taps. *Journal of Physics*, 35, 5391–5401.
- Varela, L. G., S. C. Welter, V. P. Jones, J. F. Brunner, and H. Riedl. 1993. Monitoring and characterization of insecticide resistance in codling moth (Lepidoptera: Tortricidae) in four western states. *Journal of Economic Entomology*, 86: 1-10
- Viswanathan, G. M., S. V. Buldyrev, S. Havlin, M. G. E. da Luz, E. P. Raposo, H. E. Stanley. 1999. Optimizing the success of random searches. *Nature*, 401: 911-914.
- Wall, C. and J. N. Perry. 1987. Range of action of moth sex-attractant sources. *Entomologia Experimentalis et Applicata*, 44: 5-14
- Wawrzynski R. P., M. E. Ascerno. 1998. Mass trapping for Japanese beetle (coleoptera: Scarabaeidae) suppression in isolated area. *Journal Arboric* 24:303-307
- Wedding, R., O. Anderbrant, and P. Jönsson. 1995. Influence of wind conditions and intertrap spacing on pheromone trap catches of male European pine sawfly, *Neodiprion sertifer*. *Entomologia Experimentalis et Applicata*, 77: 223-232

- Weston, P. A. 1986. Experimental and theoretical studies in insect chemical ecology: ovipositional biology of *Delia* flies and simulation modeling of insect movement. Ph. D. Dissertation, Michigan State University, E. Lansing, MI.
- Witzgall, P., L. Stelinski, L. Gut, D. Thomson. 2008. Codling moth management and chemical ecology. *Annual Review of Entomology*, 53: 503-522.
- Witzgall, P., P. Kirsch and A. Cork. 2010. Sex pheromones and their impact on pest management. *Journal of Chemical Ecology*, 36: 80-100.
- Wolf, W. W., A. N. Kishaba, and H. H. Toba. 1971. Proposed method for determining density of traps required to reduce an insect population. *Journal of Economic Entomology*, 64: 872-877.
- Worthley, H. N. 1931. Studies of codling moth flight. *Journal of Economic Entomology*, 25: 559-565.
- Yamaguchi, Y. 1989. Tuna long-line fishing I: historical aspects. *Marine Behaviour and Physiology*, 15: 1-11.