Pesticide exposure patterns and avoidance behaviour in non-target arthropods

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Abstract

For a pesticide to be registered for use, the risk to non-target arthropods must first be assessed. Standardised laboratory tests are conducted to evaluate lethal and sublethal effects on these species, with sublethal assessments focusing on reproductive effects. One other sublethal effect reported in the scientific literature is behavioural changes – such as hyperactivity or arrested movement – in non-target arthropods exposed to pesticide residues. Avoidance behaviour is also of interest in this context, where individuals display signs of irritation or repellence, thus showing a preference for untreated surfaces. Behavioural changes can be a precursor to more deleterious effects of pesticides, while avoidance behaviour is seen as both positive and negative, depending on the context. Regulatory studies expose individuals to homogeneous residues, even though pesticide spray within crop systems is often heterogeneous. Regulatory studies thus consider the worst case scenario yet this leaves no consideration of effects arising from realistic exposure in the environment, in particular effects of avoidance behaviour. Additionally, little is known of pesticide exposure patterns at spatial scales relevant to non-target arthropods. This thesis documents studies of movement behaviour and avoidance behaviour in a predatory mite (*Typhlodromus pyri*) when exposed to three insecticides. Irritation, reduced activity and avoidance behaviour were observed in mites exposed to residues in arenas with residues covering half and the whole surface. The thesis also documents the quantification of pesticide residues and spray patterns at small spatial scales, and these results were combined with movement data to investigate how populations are impacted by heterogeneous residues and pesticide avoidance behaviour through individual-based modelling. The simple model showed that both heterogeneous pesticide residues and avoidance behaviour lead to increased longevity and reproduction in individuals. Additionally, novel methods for quantifying spray residues, spray patterns, and behavioural bioassays are presented.

Table of contents

Abstract 3

Table of contents 4

List of tables 10

List of figures 16

Acknowledgements 20

Author's declaration 21

Chapter 1 – Introduction 22

1.1 Background 22

1.2 Knowledge gaps 25

1.3 Project aims 26

1.3.1 *Chrysoperla carnea* 27

1.4.2 *Typhlodromus pyri* 29

1.5 Thesis outline 31

Chapter 2 – Quantifying pesticide deposits and spray patterns at micro-scales on apple (*Malus domesticus*) leaves with a view to arthropod exposure 34

2.1 Introduction 34

2.2 Materials and methods 37

2.2.1 Orchard sampling 37

2.2.2 Residue sample collection 39

2.2.3 Leaf sample extraction and clean-up 40

2.2.4 Gas chromatography-mass spectrometry (GC-MS) 41

2.2.5 Analytical method development 42

2.2.6 Spray pattern analysis 43

2.2.7 Testing DepositScan 43

2.2.8 Statistical analysis 47

2.3 Results 47
2.3.1 Spray pattern analysis 47
2.3.2 Leaf residues 50
2.3.3 Comparing residue analysis methods 50
2.3.4 Testing DepositScan 53
2.4 Discussion 54
2.4.1 Comparison of trends in leaf residue and water sensitive paper data 54
2.4.2 Comparing residue analysis methods 55
2.5 Conclusions 58
2.6 Using spray data to contextualise laboratory studies 60
Chapter 3 – Pesticide induced changes in movement behaviour in the predatory mite *Typhlodromus pyri* 62
3.1 Introduction 62
3.1.1 Sublethal effects of pesticides 62
3.1.2 Behavioural effects of pesticides 63
3.1.3 Avoidance Behaviour 64
3.1.4 Study aim, species and pesticide selection 66
3.1.5 Hypotheses 67
3.2 Materials and methods 68
3.2.1 Insects 68
3.2.2 Insecticides 68
3.2.3 Behavioural bioassays 72
3.2.4 Pesticide residue analysis 78
3.2.5 Statistical analysis 80
3.3 Results 89
3.3.1 Environmental conditions 91

3.3.2 Mite movement behaviour 93
3.4 Discussion 103
3.4.1 Pesticide residue analysis 103
3.4.2 Control mite movement 104
3.4.3 Mite activity/inactivity 104
3.4.4 Movement behaviour 105
3.4.5 Avoidance behaviour 108
3.4.6 Ecological relevance of findings 108
3.4.7 Scope for further study 112
Chapter 4 – Can the predatory mite *Typhlodromus pyri* avoid pesticides? A study of movement behaviour and avoidance in choice arenas 116
4.1 Introduction 116
4.1.1 Why choice arenas? 116
4.1.2 Studying avoidance behaviour in choice arenas 117
4.1.3 Study aim 121
4.1.4 Hypotheses 122
4.2 Materials and methods 122
4.2.1 Insects 122
4.2.2 Insecticides 122
4.2.3 Behavioural bioassays 124
4.2.4 Pesticide residue analysis 128
4.2.5 Analytical method development 128
4.2.5 Statistical analysis 129
4.3 Results 137
4.3.1 Environmental conditions 139
4.3.2 Mite activity/inactivity 141

4.3.3 Zone preference study 143
4.3.4 Mite movement behaviour 145
4.4 Discussion 153
4.4.1 Pesticide residue analysis 154
4.4.2 Control mite movement and environmental conditions 154
4.4.3 Mite activity/inactivity 156
4.4.4 Zone preference study 157
4.4.5 Mite movement behaviour 158
4.4.6 Avoidance behaviour 163
4.4.7 Study findings compared to hypotheses 163
4.4.8 Ecological relevance of findings 164
4.4.9 Summary of findings and comparison to full coverage study 167
4.4.10 Risk assessment implications 168
4.4.11 Further study 169
Chapter 5 – From an individual to a population: modelling the effects of pesticide residues on *Chrysoperla carnea* and *Typhlodromus pyri* in the PANTA model 172
5.1 Introduction 172
5.1.1 Models in ecotoxicology 172
5.1.2 Population consequences of avoidance behaviour 174
5.1.3 Aim 176
5.1.4 Research questions 176
5.2 The model 177
5.2.1 Biological context 177
5.2.2 Model Overview 183
5.3 Model testing 202
5.3.1 Control development and reproduction 202

5.3.2 Survival after 48 hours (lacewings) 208
5.3.3 Mortality after 7 days (mites) 211
5.3.4 Movement parameters 215
5.4 Results 218
5.4.1 Movement patterns 218
5.4.2 Simulation analysis methods 219
5.4.3 *Chrysoperla carnea* survival 219
5.4.4 *Chrysoperla carnea* reproduction 224
5.4.5 *Typhlodromus pyri* survival 226
5.4.6 *Typhlodromus pyri* reproduction 230
5.5 Discussion 232
5.5.1 Model output verification and current status 232
5.5.2 Model simulation results 232
5.5.3 Model limitations 237
5.5.4 Research needs 239
Chapter 6 – Overall discussion 242
6.1 Key findings 242
6.2 Novel method contributions 244
6.3 Future research needs 246
6.3.1 Potential for pesticides to attract 246
6.3.2 Interactive effects of environment and pesticide 247
6.3.3 Studies to increase ecological relevance 247
6.4 Implications for pesticide manufacturers 250
6.5 Implications for regulators 251
Appendices 254
Appendix A – Measures of environmental conditions 254

Appendix B – PANTA lacewing model code (NetLogo) 255

Appendix C – PANTA mite model code (NetLogo) 267

References..... 280

List of tables

Table 2.1 – Variable spread factor values determined on water and used as an alternative to DepositScan's built-in spread factor calculation (Syngenta, 2002; Cunha, Carvalho and Marcal, 2012) 45

Table 2.2 – Pesticide spray coverage – expressed as proportion of target covered by spray – and spray density – expressed as the number of droplets in an area – determined from water sensitive paper cards set within apple trees. 49

Table 2.3 – Penconazole residues in apple leaves from Patch A, expressed as mean residue with 95% confidence intervals, split by tree and tree zone. 50

Table 2.4 – The effect of two different spread factors on 6 stain diameter sizes. The spread factors were determined on water droplets. 57

Table 3.1 – Insecticidal active substances used in the present study with the lethal rate at which 50% of a *Typhlodromus pyri* population displays mortality after 7 days (7d-LR_{50}) expressed as $g_{a.s.}$ Ha^{-1} and $mg_{a.s.}$ L^{-1}; both of these values are based on an application rate of 200 L Ha^{-1} (Blümel et al., 2000a). 71

Table 3.2 – The number of active and inactive mites for each studied insecticide split by treatment. Active mites comprised the final sample populations for statistical analysis of movement behaviours. 81

Table 3.3 – Influence of air temperature and relative humidity (covariates) on *T. pyri* movement behaviours, derived from correlation. 83

Table 3.4 – Summary of comparison of movement behaviours between male and female *T. pyri* adults observed in control test arenas. 84

Table 3.5 – Results of Box's M Test for equality of covariance for the three studied insecticides. 86

Table 3.6 – Results of the Brown-Forsythe test for equality of variance, assessed for each movement behaviour included in the multivariate model. 87

Table 3.7 – Results of the slope comparison test investigating variance in covariate response in the dimethoate dataset. Each movement behaviour was assessed against each environmental covariate. 89

Table 3.8 – Mean insecticide residue values, 95% confidence intervals, coefficient of variance (CV) based on residue, mean recovery compared to expected activity levels and associated 95% confidence intervals for each insecticide treatment level. 90
Table 3.9 – Summary of air temperature and relative humidity measured during mite movement observations in each treatment. 92
Table 3.10 – Summary of mean difference ± 95% confidence intervals and Games-Howell multiple comparisons test of differences in relative humidity measurements taken during mite movement observations. 92
Table 3.11 – Summary of Games-Howell post-hoc analysis of the differences in measures of angular velocity as measured in *T. pyri* adults observed in control or treated arenas containing acetamiprid residues. 99
Table 3.12 – Summary of Games-Howell post-hoc analysis of the differences in measures of distance moved, angular velocity, meander and time active as measured in *T. pyri* adults observed in control or treated arenas containing deltamethrin residues. 100
Table 3.13 – Summary of Games-Howell post-hoc analysis of the differences in measures of angular velocity as measured in *T. pyri* adults observed in control or treated arenas containing dimethoate residues. 102
Table 3.14 – Insecticide treatment concentrations used in the behaviour studies expressed as their equivalent field rate if applied at a rate of 200 L Ha^{-1} and as measured residues from the laboratory studies. 110
Table 3.15 – Field application rates for acetamiprid and deltamethrin expressed as estimations of residues landing on apple leaves in one hectare of orchard and per unit of leaf area. 111
Table 4.1 – Insecticidal active substances used in the present study with the lethal rate at which 50% of a *Typhlodromus pyri* population displays mortality after 7 days (7d-LR_{50}) 123
Table 4.2 – Detection limits (L_d, expressed as counts) and minimum counts (as counts per minute, CPM)) for the two counting methods used to analyse insecticide residues extracted from half treated test arenas. 129
Table 4.3 – Influence of air temperature and relative humidity on *T. pyri* movement behaviours in half treated arenas, derived from correlation. 131

Table 4.4 – Results of Box's M Test for equality of covariance for the three insecticides. 135
Table 4.5 – Results of the Brown-Forsythe test for equality of variance, assessed for each movement behaviour in each arena zone.. 136
Table 4.6 – Mean measured insecticide residue values, 95% confidence intervals and coefficient of variance (CV) of measurements, mean recovery compared to expected radioactivity levels and associated 95% confidence intervals, and mean counting efficiency with 95% confidence intervals for each insecticide treatment level. 138
Table 4.7 – Summary of air temperature and relative humidity measured during mite movement observations in each treatment. 140
Table 4.8 – Summary of mean difference ± 95% confidence intervals and Tukey's multiple comparisons test of differences in air temperature measurements taken during mite movement observations. 140
Table 4.9 – Summary of mean difference ± 95% confidence intervals and Tukey's multiple comparisons test of differences in relative humidity measurements taken during mite movement observations. 141
Table 4.10 – Summary of Tukey's post-hoc analysis of the differences in time spent in treated arena halves, comparing time spent by mites on the control treated surface to the time spent on surfaces treated with dimethoate residues. 145
Table 4.11 – Summary of paired t tests applied as post hoc analysis of the effect of acetamiprid residues on the velocity of *T. pyri*. 148
Table 4.12 – Summary of paired t tests applied as post hoc analysis of the effect of acetamiprid residues on the time *T. pyri* individuals spend active. 151
Table 4.13 – Summary of Tukey's multiple comparisons test, applied as post hoc analysis of the effect of deltamethrin residues on the overall distance walked by *T. pyri* in test arenas half treated with deltamethrin. 152
Table 4.14 – Summary of paired t tests applied as post hoc analysis of the effect of dimethoate residues on the distance walked by *T. pyri* individuals. 153
Table 4.15 – Summary of mean difference ± 95% confidence intervals and paired t tests of differences in the proportion of time spent on untreated and treated surfaces in deltamethrin treated test arenas. 161

Table 4.16 – Insecticide treatment concentrations used in the behaviour studies expressed as their equivalent field rate if applied at a rate of 200 L Ha^{-1} and as measured residues from the laboratory studies. 165
Table 4.17 – Field application rates for acetamiprid and deltamethrin expressed as estimations of residues landing on apple leaves in one hectare of orchard and per unit of leaf area.. 166
Table 5.1 – Life stages and developmental times for the green lacewing *Chrysoperla carnea* presented in terms of mean number of days and survival (%) ± standard deviation (SD) for each developmental stage at 25°C, plus survival to adulthood. 179
Table 5.2 – Life stages and developmental times for female predatory mites (*Typhlodromus pyri*) presented in terms of mean numbers of days ± standard deviation (SD) and survival for each developmental stage at 25°C. 180
Table 5.3 – Comparison of life table parameters for female *Chrysoperla agilis* (member of *C. carnea* cryptic group) and *Typhlodromus pyri*, derived from development studies conducted at 25°C. 180
Table 5.4 – Movement data used to parameterise individual movement behaviour for the two modelled species *Chryosperla carnea* and *Typhlodromus pyri*. 183
Table 5.5 – Descriptions of state variables implemented in the PANTA model for *Chrysoperla carnea* and *Typhlodromus pyri*. 185
Table 5.6 – 48 hour mortality of *Chrysoperla carnea* larvae exposed to homogeneous acetamiprid residues at three concentrations (Nasreen, Mustafa and Ashfaq, 2005), and p-mortality values derived from mortality rates to assign “toxicity” to pesticide contaminated patches within the model. 187
Table 5.7 – Seven day mortality (± standard deviation) of *Typhlodromus pyri* larvae exposed to homogeneous acetamiprid residues at five concentrations (EFSA, 2016; Volume 3 B.9 p. 182), and p-mortality values derived from mortality rates to assign “toxicity” to pesticide contaminated patches within the model. 188
Table 5.8 – Parameters and values implemented in the PANTA *Chrysoperla carnea* model. 193
Table 5.9 – Parameters and values implemented in the PANTA *Typhlodromus pyri* model. 195

Table 5.10 – Model output verification of *Chrysoperla carnea* development and reproduction in a control exposure landscape (0% coverage). 204
Table 5.11 – Comparisons of *Chrysoperla carnea* mean development time to adulthood, adult longevity and total oviposition between simulated control populations exhibiting avoidance behaviour and no avoidance behaviour, and empirical data used to parameterise the model (Pappas et al., 2013). 205
Table 5.12 – Model output verification of *Typhlodromus pyri* development and reproduction in a control exposure landscape (0% coverage). 207
Table 5.13 – Comparisons of *Typhlodromus pyri* mean development time to adulthood, total longevity and total oviposition between modelled control populations exhibiting avoidance behaviour and no avoidance behaviour, and published data (Gadino and Walton, 2012). 208
Table 5.14 – Model output verification of *Chrysoperla carnea* survival over 48 hours in a 100% coverage exposure landscape. 210
Table 5.15 – Comparisons of survival after 48 hours of exposure on a 100% coverage surface between simulated populations exhibiting avoidance behaviour and no avoidance behaviour and published data used to parameterise the model (Nasreen, Mustafa and Ashfaq, 2005). 211
Table 5.16 – Model output verification of *Typhlodromus pyri* mortality over 7 days in a 100% coverage exposure landscape. 213
Table 5.17 – Comparisons of *Typhlodromus pyri* mortality after 7 days of exposure on a 100% coverage surface between simulated populations exhibiting avoidance behaviour and no avoidance behaviour, and published data used to parameterise the model (EFSA, 2016). 214
Table 5.18 – Model output verification of movement behaviour in *Chrysoperla carnea* and *Typhlodromus pyri* in a control exposure landscape. 216
Table 5.19 – Comparisons of movement behaviour in *Chrysoperla carnea* and *Typhlodromus pyri* in a control exposure landscape. 217
Table 5.20 – Mean differences in survival to adulthood in *Chrysoperla carnea* individuals avoiding or not avoiding acetamiprid residues at three surface coverage levels. 221

Table 5.21 – Mean differences in longevity in *Chrysoperla carnea* individuals avoiding or not avoiding acetamiprid residues at three surface coverage levels. 223
Table 5.22 – Mean differences in oviposition per female in *Chrysoperla carnea* individuals avoiding or not avoiding acetamiprid residues at three surface coverage levels..... 225
Table 5.23 – Mean differences in survival over seven days in Typhlodromus pyri individuals avoiding or not avoiding acetamiprid residues at three surface coverage levels..... 227
Table 5.24 – Mean differences in longevity in *Typhlodromus pyri* individuals avoiding or not avoiding acetamiprid residues at three surface coverage levels. 229
Table 5.25 – Mean differences in total oviposition in *Typhlodromus pyri* individuals avoiding or not avoiding acetamiprid residues at three surface coverage levels. .. 231
Table 5.26 – Acetamiprid application rates utilised in the PANTA model expressed as the laboratory application rate and their equivalent liquid concentration if applied at a rate of 200 L ha^{-1}. 236

List of figures

Figure 2.1 - Schematic of the nested orchard sampling design showing the four spatial scales used for apple leaf residue and water sensitive paper coverage analysis. 39

Figure 2.2 – Illustration of the overestimation of droplet size by DepositScan when presented with overlapping droplets. 46

Figure 2.3 – Penconazole residues derived from GC-MS based on leaf mass (horizontal axis) and whole leaf upper surface area (vertical axis). 51

Figure 2.4 – Comparison of penconazole residue values based on surface area derived from leaf residue samples analysed via GC-MS and water sensitive paper (WSP) samples analysed via DepositScan image analysis with no correction factor (a) and with a correction factor applied to individual data points (b). 52

Figure 2.5 – (a) Deposition values calculated from the same artificial stain using the default DepositScan spread factor and an alternative spread factor that varies based on stain diameter. *n* = 33 (b) Deposition values calculated by DepositScan based on whether two artificial circular stains of the same size touch or not. *n* = 33 53

Figure 2.6 – Distribution of droplet diameters as a function of spray proportion on water sensitive papers from patch A. 57

Figure 3.1 – Screenshot of a sample video of the preliminary movement test arena with a bean leaf disc. 73

Figure 3.2 – Screenshot of a sample video from the captan experiment as analysed in EthoVision XT 13, with a detected mite highlighted in yellow in the right hand arena and further highlighted by the red arrow. 76

Figure 3.3 – Illustrative movement tracks from individual mites observed in the control (blue tracks) and insecticide treated test arenas (red tracks). 93

Figure 3.4 – Proportions of *T. pyri* individuals displaying activity (grey) or inactivity (white) during the 10 min observation period in control and insecticide treated arenas. 95

Figure 3.5 – Effect of acetamiprid (left column), deltamethrin (middle column) and dimethoate (right column), each at three concentrations, on the distance walked, velocity, angular velocity, meander and time spent active as observed in *Typhlodromus pyri* individuals over 10 min. 97

Figure 3.6 – Time taken for each trapped mite to become trapped when exposed to deltamethrin. Line and whiskers represent mean ± 95% confidence intervals. 103
Figure 4.1 – Representative movement tracks of individual lacewing larvae of the two species *Chrysoperla externa* and *Ceraeochrysa cubana* over 10 minutes in 9 cm test arenas half-treated with dried insecticide residues (upper sector of each arena). 119
Figure 4.2 – Representative movement tracks of individual psocids (top row = *Liposcelis entomophila*; bottom row = *L. bostrychophila*) over 10 minutes in 2.5 cm test arenas half treated with dried insecticide formulations (right hand sector of each arena). 120
Figure 4.3 – Twelve freshly treated arenas for the half coverage behaviour bioassay. 125
Figure 4.4 – Screenshot of a sample arena from the dimethoate half coverage experiment with the two test arena zones identified in EthoVision XT 13. 127
Figure 4.5 – Proportions of *T. pyri* individuals displaying activity (dark grey) or inactivity (light grey) during the 10 min observation period in control arenas and arenas half treated with insecticides. 142
Figure 4.6 – Effect of a) acetamiprid, b) deltamethrin, and c) dimethoate residues, each at three concentrations, on time spent by *Typhlodromus pyri* in the treated (right hand) arena zone over 10 min.. 144
Figure 4.7 – Representative movement tracks from individual mites observed in the control (blue tracks) and insecticide treated test arenas (red tracks). 146
Figure 4.8 – Effect of acetamiprid (left column), deltamethrin (middle column) and dimethoate (right column), each at three doses, on the distance walked, velocity, angular velocity, meander and time spent active in the untreated (left hand; blue) and treated (right hand; pink) sections of the test arena, as observed in *Typhlodromus pyri* individuals over 10 min. 150
Figure 4.9 – Correlation between the distances walked by control mites in both the full and half coverage experiments, and air temperature (a), and relative humidity (b).. 155
Figure 4.10 – Effect of deltamethrin residues at three concentrations, on the proportion of time spent by *Typhlodromus pyri* on the untreated (blue; left) and treated (pink; right) surfaces over 10 min.. 161

Figure 5.1 – An example exposure landscape within the PANTA model, with pesticide residues shown as yellow patches, and unexposed surfaces shown as green patches. 186
Figure 5.2 – Movement tracks of individual larvae after 2.5 hours of simulation in a heterogeneous pesticide exposure landscape. 218
Figure 5.3 – Proportion of *Chrysoperla carnea* larvae surviving to adulthood when a) not avoiding acetamiprid residues (left) and b) avoiding acetamiprid residues (right). 220
Figure 5.4 – Differences in survival to adulthood in *Chrysoperla carnea* individuals that do not avoid acetamiprid residues (grey dots, left), and individuals that do avoid acetamiprid residues (pink dots, right). 221
Figure 5.5 – Mean longevity of *Chrysoperla carnea* individuals when a) not avoiding acetamiprid residues (grey dots, left) and b) avoiding acetamiprid residues (pink dots, right). 222
Figure 5.6 – Differences in longevity in *Chrysoperla carnea* individuals that do not avoid acetamiprid residues (grey dots, left), and individuals that do avoid acetamiprid residues (pink dots, right). 223
Figure 5.7 – Total oviposition per adult female *Chrysoperla carnea* when a) not avoiding acetamiprid residues (grey dots) and b) avoiding acetamiprid residues (pink dots)..... 224
Figure 5.8 – Differences in total oviposition per female in *Chrysoperla carnea* individuals that do not avoid acetamiprid residues (grey dots, left), and individuals that do avoid acetamiprid residues (pink dots, right)..... 225
Figure 5.9 – Proportion of *Typhlodromus pyri* individuals surviving seven days of exposure when a) not avoiding acetamiprid residues (left) and b) avoiding acetamiprid residues (right). 226
Figure 5.10 – Differences in survival over seven days in *Typhlodromus pyri* individuals that do not avoid acetamiprid residues (grey dots, left), and individuals that do avoid acetamiprid residues (pink dots, right). 227
Figure 5.11 – Mean longevity of *Typhlodromus pyri* individuals when a) not avoiding acetamiprid residues (grey dots, left) and b) avoiding acetamiprid residues (pink dots, right). 228

Figure 5.12 – Differences in longevity in *Typhlodromus pyri* individuals that do not avoid acetamiprid residues (grey dots, left), and individuals that do avoid acetamiprid residues (pink dots, right). Lines represent mean ± 95% confidence intervals. 229
Figure 5.13 – Total oviposition per adult female *Typhlodromus pyri* when a) not avoiding acetamiprid residues (grey dots) and b) avoiding acetamiprid residues (pink dots). 230
Figure 5.14 – Differences in total oviposition per female in *Typhlodromus pyri* individuals that do not avoid acetamiprid residues (grey dots, left), and individuals that do avoid acetamiprid residues (pink dots, right). 231

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This thesis is dedicated to my parents and to the memory of Matthew Pyke.

Author's declaration

I declare that this thesis is a presentation of original work and that I am the sole author. In chapters presenting experiments and data, I have chosen to use the personal pronoun "we" as these chapters were written either for publication, or in preparation for publication, and so acknowledge the involvement of my supervisors and others. The work within this thesis has not previously been presented for an award at this, or any other, University. All sources are acknowledged in the References section. The table below reports the papers arising from this thesis.

Title	**Thesis chapter**	**Status**	**Journal**
Quantifying pesticide deposits and spray patterns at micro-scales on apple (*Malus domesticus*) leaves with a view to arthropod exposure	2	Published online July 2018	Pest Management Science
Move it! Assessing effects of pesticide exposure on movement behaviour in the predatory mite *Typhlodromus pyri*	3/4	In preparation	

Chapter 1 – Introduction

1.1 Background

Pesticides are widely used in agriculture to manage pests effectively within the crop environment, and in 2016 over 16 thousand tonnes were applied to crops in Great Britain, of which 315 tonnes were insecticides (Fera, 2018). Although insecticides are effective in controlling populations of target organisms that cause damage to crops, they also affect beneficial species. Such species, including insects and arachnids, exist within agricultural ecosystems and can provide ecosystem services such as pollination and biocontrol of crop pests (Costanza et al., 1997; Fisher et al., 2009). However, insecticide use has been linked to reduced pollination services, vital for food production (Goulson, 2013).

Insecticide use is known to be potentially lethal to beneficial insects – from this point on defined as non-target arthropods (NTAs) – through both direct and residual exposure (Medina et al., 2004; Rezaei et al., 2007). However, sublethal effects have also been reported, such as shortened lifespan, reduced fecundity, changes in gender ratio, and changes in reproductive and feeding behaviour (Stark and Banks, 2003; Tison et al., 2016). Such effects are certain to have wider implications on populations, especially life table parameters such as development time and fecundity (Stark et al., 2004), and are deemed to be especially important for NTAs due to their longer term exposure to sublethal insecticide concentrations (Cordeiro et al., 2010; Joao Zotti et al., 2013). In addition, behavioural effects such as avoiding applied pesticides and reduced movement have been reported in a range of beneficial species, including pirate bugs, mites, and lacewings (Beers and Schmidt-Jeffris, 2015; Cordeiro et al., 2010; Pereira et al., 2014), though the amount of research focusing upon behavioural effects is currently very limited, in spite of behavioural ecotoxicology being seen as an early warning for worse effects (Hellou, 2011).

For a pesticide to be registered for use in the European Union, the submission is required to include details regarding the toxicity of an ingredient or formulation to non-target organisms (EU, 2009a). In addition, during assessment consideration must be made towards the relevance of the product in the integrated pest management

context – defined by the EU (2009b) as a philosophy aimed at discouraging the growth of pest populations while keeping the use of pesticides at a minimum in favour of alternative forms of intervention, such as natural pest control. Ecological risk assessment is a process that allows for such evaluation by investigating the likelihood, and estimating the scale of adverse ecological effects arising from exposure to an environmental stressor (Munns, 2006). Although it is a tool developed for a wide range of stressors, it is most frequently applied to chemicals in the regulatory context (Galic et al., 2012).

Laboratory experiments have traditionally been the source of toxicological data that assist in decision making in ecological risk assessment. Numerous methods for laboratory-based toxicity tests involving non-target arthropods have been defined (Candolfi et al., 2000), though these typically study lethal effects, and sublethal effects such as fecundity, with no consideration for behavioural effects. Additionally, population-level consequences are rarely considered, as ecological risk assessment concentrates upon effects at the individual level (Amelie Schmolke et al., 2010).

Ecological models offer the ability to study the effect of chemicals at a larger spatial and temporal scale than laboratory studies would usually allow (EFSA Panel on Plant Protection Products and their Residues, 2018; Galic et al., 2012). Individual-based modelling, one such ecological modelling approach, involves simulation models that handle individuals within a population as unique entities, with properties such as age and weight that change through the life cycle of the individual giving rise to modelled population dynamics (Grimm and Railsback, 2005). Such models are a valuable tool for risk assessment with an ecological relevance, as they allow for laboratory-based risk assessment findings to be extrapolated to gain understanding of population-level consequences (Bartell et al., 2003; Grimm and Thorbek, 2014). Additional advantages of integrating modelling into risk assessment include the ability to reduce the financial and time costs (Galic et al., 2012). It is also relatively easy to develop a model for one species and the effects of one substance, but then adjust the model to work with another substance (Hommen et al., 2010). An ethical advantage to model-based risk assessment is the reduced need for *in vivo* laboratory studies, an advantage raised by experts within industry and regulatory bodies (EFSA Panel on Plant

Protection Products and their Residues, 2018; Hunka et al., 2013). When study methods can involve as many as 50 individuals per treatment concentration studied (Candolfi et al., 2000), this would be a significant benefit.

While the argument for the integration of models into risk assessment is strong, there are many issues that lead to only a small number of population models being used in risk assessment frameworks (Galic et al., 2010). One issue highlighted by policymakers is the concern relating to models being overly complex, meaning such models are difficult to parameterise and any results produced by the model are difficult to understand (Amelie Schmolke et al., 2010). A lack of successful model validation is another regulatory concern (EFSA Panel on Plant Protection Products and their Residues, 2018). Another issue, instead arising from an academic perspective, is the need to consider heterogeneity of exposure when modelling population-level effects (Munns, 2006). Finally, issues relating to models not being transparent, and poor documentation and justification for specific decisions can lead to a model supporting a wrong decision, though this can be countered by the use of good modelling practice and clear and transparent documentation (Grimm et al., 2010; e.g. A Schmolke et al., 2010).

Few ecological models are currently being used to inform ecological risk assessment; however, the European Food Safety Authority (EFSA) recently reviewed toxicokinetic-toxicodynamic modelling for aquatic environmental risk assessment, and concluded that one type of model, General Unified Threshold models of Survival (GUTS), is now ready to be used to predict effects (EFSA Panel on Plant Protection Products and their Residues, 2018). However, model applications in the terrestrial context are not yet as well accepted, though EFSA highlighted the value of using models to investigate landscape level pesticide effects on NTAs by saying such models would allow the ability to study source-sink dynamics with the hope of better understanding the resilience and recovery of NTA populations beyond a field scale (EFSA, 2015).

The compatibility of pesticides and beneficial insects is a topic that is receiving attention within ecotoxicology, due to the increasing adoption of integrated pest management regimes. However, research has only recently started to consider the sublethal effects of pesticide exposure on NTAs that reside within the on- and off-

crop environments (e.g. Beers and Schmidt-Jeffris, 2015; Joao Zotti et al., 2013), and behavioural effects such as pesticide avoidance are poorly understood with just a small number of published studies (e.g. Cordeiro et al., 2013; Porcel et al., 2011). Results from these studies suggest that more research into avoidance behaviour is vital for understanding the compatibility of pesticides and NTAs. We also need to better understand other behavioural changes that may arise from pesticide exposure, so that we can understand what population consequences may arise. This would assist in better understanding landscape-scale effects.

1.2 Knowledge gaps

Limited scientific evidence exists of avoidance behaviour in NTAs. Examples do exist within scientific literature: the lacewing *Chrysoperla carnea* exhibiting avoidance behaviour when exposed to kaolin film (Porcel et al., 2011), and *C. externa* showing avoidance when exposed to azadirachtin (Cordeiro et al., 2013); while predatory mites have also been observed avoiding certain compounds (Beers and Schmidt-Jeffris, 2015). However, the knowledge base is limited, and authors frequently emphasise the need for further study into behavioural effects.

Most reported examples of avoidance behaviour exist for below-ground arthropods, with springtails being one well studied group. Avoidance behaviour has been observed in *Folsomia candida* when exposed to substrates contaminated with the polycyclic aromatic hydrocarbon naphthalene (Boitaud et al., 2006), copper (Boiteau et al., 2011), and the pyrethroid insecticide cypermethrin (Zortéa et al., 2015). Avoidance bioassays have also been cited as a potential rapid screening tool for soil-based ecological risk assessment, gauging the potential toxicity of contaminants in collembolan, earthworms and isopods (da Luz et al., 2004; Loureiro et al., 2005), and such work led to a standardised test being created that studied avoidance behaviour in choice situations in *F. candida* (Filser et al., 2014; ISO, 2011).

Regarding pesticide application to crops, little is known about the distribution of residues at the micro-scale, such as across a single leaf. A recent study looked to map the distribution of a fungicide across a wheat leaf surface (Annangudi et al., 2015);

however, the published method had a number of limitations (Dong et al., 2015) and this showed the method required more work before further application. Little work has otherwise been undertaken to investigate spatial dimensions of residues, with researchers instead investigating patterns qualitatively using materials such as water sensitive paper (Jaeken et al., 2000).

There is also a lack of research into long term, population-scale consequences of pesticide exposure to NTAs, and the effect any behavioural changes might have on populations. Ecological models have been developed for a small number of species, with only one investigating the interaction of avoidance behaviour and population dynamics (Meli et al., 2013). This is a distinct gap in the investigation of pesticide effects.

1.3 Project aims

The overall aim of this project was to understand pesticide avoidance behaviour in NTAs and the spatial heterogeneity of pesticide exposure, and to consider population consequences of exposure for NTA species. Additionally, this project aimed to improve the realism of regulatory risk assessment processes. The study aims can be broken down into four objectives, which are as follows; these will form the basis of completing the project:

1. Develop ecological models for the two test species, *Chrysoperla carnea* and *Typhlodromus pyri*;
2. Quantify the heterogeneity of pesticide spray exposure in an apple orchard, a crop environment relevant to both test species;
3. Assess the effect of pesticides on pesticide avoidance behaviour in NTAs through the use of simple and novel choice arenas established within the laboratory;
4. Combine behaviour study results and spatial heterogeneity of pesticide exposure with the species' ecological models to produce an integrated individual-based population model.

When planning the project, it was decided that two NTA species would be investigated in the laboratory, and then modelled. The species needed to be native to the UK and Europe, be from different phyla to investigate differing sensitivities to pesticides, and be relevant to ecological risk assessment by being species that are regularly used in testing. Most importantly, they needed to be easy to handle and rear under laboratory conditions. To this end, the two species selected were the green lacewing *Chrysoperla carnea* (order *Neuroptera*), and the predatory mite *Typhlodromus pyri* (order *Acarina*). *C. carnea* is widely used within ecological risk assessments and is highlighted as a "universal indicator" in the context of pesticide ecotoxicological tests (Andow and Hilbeck, 2004). Similarly, *T. pyri* are regularly used in pesticide registration tests (Jepson, 1997). While both species are used widely, the guidance concerning species selection for registration tests emphasises testing on *T. pyri*. Though the intention was to study avoidance behaviour in both species in the laboratory, project time constraints meant that it was only possible to study *T. pyri*.

1.3.1 *Chrysoperla carnea*

Rather than being a single morphological species, the common green lacewing (*Chrysoperla carnea*) is a complex of eighteen "sibling" species, three of which are known to be present within the UK (Chapman et al., 2006; Henry et al., 2001; Charles S Henry et al., 2014). While they are both ecologically and morphologically similar, there are distinct differences in the mating song, suggested by Henry et al. (2014) to be a result of parallel speciation. DNA sequencing is a frequently applied method for differentiating species within the complex; it has been used to identify specimens (Price et al., 2015), and determine new sibling species (Henry et al., 2014) For the purpose of this project, the complex was treated as a single species as there was no scope to investigate differences in responses to pesticides between species within the complex.

C. carnea are found in a range of agricultural and forest ecosystems, and are often found in arable fields, fruit orchards, natural and semi-natural grassland, and woody and hedgerow margins surrounding arable fields (Szentkirályi, 2007; Trouve et al., 2002). Adults of some *Chrysopid* species, including *C. carnea*, are attracted to specific

locations by the presence of two chemical signal types: synomones and kairomones. Synomones are “habitat” signals, chemicals released by plants and that are attractive to lacewings (Hagen, 1986), and these identify food and oviposition sites for adult *C. carnea* (Zhu et al., 2005). Kairomones are attractants associated with food, and in combination with synomones, these encourage adult *C. carnea* to land as they signal the location of food and ideal oviposition sites (Hagen, 1986).

The lacewing life cycle consists of four main life stages: egg, larva, pupa and adult. The larval stage has three sub-stages, based on larval moults. In the first three main stages of the life history of *C. carnea*, an individual will likely remain within the area of one, or a few plants (Szentkirályi, 2007), in part because *Chrysopid* larvae tend to prefer plants to soil (Clark and Messina, 1998). However, adults are highly mobile: upon emergence from the cocoon, an adult *C. carnea* in its migratory (or pre-ovipositional) phase can travel up to 40 km in a 4 hour period, though a small number of individuals take advantage of high altitude winds and migrate up to 150 km in a 4 hour period (Chapman et al., 2006). The different dispersal ranges exhibited by different life stages requires major consideration in the context of integrating population models with pesticide exposure, and is considered in more depth in Section 5.2.1.2.

C. carnea are more active at night, and are most active in the 2 hours following sunset, with dispersal and migratory flights occurring during this time – this enables lacewings to avoid activity from predators, such as birds and dragonflies (Duelli, 1984a). Rather than overwintering at the larval or pupal stage, which is the method of winter survival practised by many lacewing species, *C. carnea* adults go into diapause in the autumn – this is initiated by shortening day light cycles, and falling ambient temperatures (Honek, 1973). Diapause termination occurs in January or February; however the adults remain dormant until increasing day light cycles and temperature allow mating and oviposition to occur (Tauber et al., 1970). In Central Europe, *C. carnea* produce up to 2 generations per year (Honek, 1977), though this increases in warmer climates (Duelli, 1984a).

As larvae, *C. carnea* provides an ecosystem service through being a natural predator to many agricultural crop pests. While adults feed on honeydew created by aphids,

nectar and pollen (Hagen, 1986), the larvae have been found to feed upon aphids, whiteflies, fruit flies, thrips and butterfly and moth eggs and caterpillars in field conditions (Canard, 2007). The larval life stages are those that are of interest for ecological risk assessment.

Due to time constraints, it was not feasible to undertake laboratory studies with *C. carnea* and the focus was placed on refining methods with *T. pyri*. However, an individual-based model was still developed for the species, and this is reported in Chapter 5.

1.4.2 *Typhlodromus pyri*

Typhlodromus pyri is a generalist predatory mite found in fruit trees that feeds on a wide range of foods, both prey and non-prey (Zemek and Prenerova, 1997). Prey species are wide-ranging, and include thrips (McMurtry and Croft, 1997), mites such as the red mite *Panonychus ulmi* (Lorenzon et al., 2012) and the apple rust mite *Aculus schlechtendali* (McMurtry, 1992). *T. pyri* also feed on honeydew and plant juices (Duso et al., 2003). A key property of *T. pyri* is its ability to survive and reproduce while local prey populations are small by consuming plant products such as juices and pollen (Duso et al., 2003), honeydew, and even the fungus powdery mildew (Zemek and Prenerova, 1997). This is an advantage for farmers as it means the predator can be retained within a crop system while pest populations are low.

While as a species *T. pyri* is tolerant of a number of pesticides, it is sensitive to sulphur-based compounds, and especially fungicides used to treat powdery mildew (Gadino et al., 2011). However, there are examples of local populations, or strains, being resistant or susceptible to certain pesticides (Bonafos et al., 2008). Metabolic changes can lead to resistance and tolerance and has been observed in earthworms exposed to metals (Otomo et al., 2016; Spurgeon and Hopkin, 2000). Changes in metabolism, such as increased activity of detoxifying enzymes, have also been identified in the polyphagous mite *Tetranychus urticae* in Dutch rose crops, where wild strains even displayed resistance to novel modes of action not yet used in the field (Khajehali et al., 2011). *T. urticae* also benefits from accelerated resistance

development arising from high fecundity and short life cycle; as a result, field resistance to new acaricides occurs within just a few years (Dermauw et al., 2012). *T. pyri* in central Europe averages three generations per year (Khan and Fent, 2005); therefore, it would be expected that resistance development is similarly accelerated in this species.

T. pyri also react to kairomones from prey species. Female individuals were found to react to three prey mite species: *P. ulmi*, *Tetranychus urticae*, and *A. schlechtendali*, with responses to the kairomones altered depending on the food on which they were reared, and also their level of hunger (M Dicke, 1988).

The life cycle of *T. pyri* involves four developmental stages: six-legged larva; and two eight-legged stages, the protonymph and deutonymph (Sabelis, 1985). To advance through the juvenile stages (i.e. from egg to end of protonymph), the developmental rate ranges from 8 – 13 days at 20 – 25˚C (Genini et al., 1991). The deutonymph, or adult stage can be further split into pre-oviposition, oviposition, and post-oviposition stages; while there is no morphological difference, Hayes (1988) suggests differentiating between the three stages is useful for modelling purposes, as his experiment suggests a prey consumption threshold exists for completing the pre-oviposition development phase. *T. pyri* prefer humid conditions created by mature orchards, and can survive without prey food sources (Helle and Sabelis, 1985).

In its lifetime, *T. pyri* covers a far smaller geographical range than lacewings. Spider mites such as *T. pyri* have been observed to move from the “parent” colony on one leaf, to found a new colony on a nearby leaf – this then creates so called “spider mite patches”, where a high density of individuals and colonies is found in a small area (Sabelis and Dicke, 1985).

Longevity and fecundity are strongly related to both air temperature and prey availability (Sabelis, 1985). In a study with variable temperature and prey availability, Hayes (1988) found that adult longevity ranged between 12.6 – 99 days, with oviposition lasting 0 – 67 days when temperature ranged between 15 and 26.5˚C and prey availability ranged between 1 – 12 larvae per day. An ideal combination seemed to be 25˚C and 12 larvae per day, giving an oviposition period of 40.5 days and total

fecundity of 29.3 eggs per female (Hayes, 1988). However, this contrasts with the suggestion that oviposition lasts just 10 days when *T. pyri* are reared at 25˚C (Blümel et al., 2000a). Another study suggested that development rate was altered by two correlated factors: species strain and geographical location, with a Canadian strain favouring temperatures below 20˚C when compared to European strains that favoured above 20˚C (Hardman and Rogers, 1991).

1.5 Thesis outline

The thesis is split into six main sections, including the current chapter, and is structured as follows. In addition to this broader introduction to the overall project topic, Chapters 2-5 include focused reviews of the state of the science for each topic, and identification of specific knowledge gaps.

Chapter 2 details the field study of pesticide spray exposure in an apple orchard. We developed methods to allow both the quantification of leaf residues and spray patterns within the crop. We then compared the methods, and used the spray exposure data derived from the field study to contextualise our discussions in later chapters.

Chapter 3 reports methods we developed for investigating movement behaviour in *T. pyri*. Results are discussed and placed into context. An extended review of current knowledge on pesticide effects on movement behaviour is also included.

Chapter 4 extends the previous study by discussing pesticide avoidance behaviour, and reports the results of a study investigating avoidance behaviour in *T. pyri* in novel choice arenas.

Chapter 5 introduces the two ecological models for our test species, and documents the development and testing undertaken. Results of survival and longevity studies are reported for both species in the context of exposure to heterogeneous pesticide spray patterns.

Chapter 6 provides an overall discussion and reflection for the project, offering thoughts for future studies and directions for the science of movement behaviour

and population modelling of pesticide effects. We also offer thoughts on implications for pesticide regulators.

Within the following chapters a number of pesticides were studied, either in formulation or as active substances. We discuss the compounds in further detail in the respective chapters, but we have briefly introduced them in turn below with the rationale behind the decisions.

In Chapter 2 we studied the fungicide penconazole, formulated as Topenco 100 EC (100 g L^{-1}, Globachem NV, Belgium). This was selected for study as we wanted to study a real pesticide application within a commercial apple orchard. While the preferred choice was to study an insecticide application, the logistics involved with our research schedule combined with the apple grower's obligation to observe pre-harvest intervals led to the decision to sample a fungicide spray application.

In Chapters 3 and 4, we studied three insecticidal active substances: the neonicotinoid acetamiprid, the pyrethroid deltamethrin, and the organophosphate dimethoate. We chose these to cover a range of insecticidal classes and modes of action; we also selected them based on relevance to the test species, the apple crop context, and known sublethal responses to the substances. We were also limited in choice by the range of available radiolabelled substances.

Note: *The following chapter has been accepted for publication in Pest Management Science and is available online at doi:10.1002/ps.5136. We have presented the paper in the same format in which it was accepted for publication, but edited for presentation in this thesis.*

Chapter 2 – Quantifying pesticide deposits and spray patterns at micro-scales on apple (*Malus domesticus*) leaves with a view to arthropod exposure

2.1 Introduction

Pesticides are a globally important tool in the control of pests and diseases in commercial crop systems (Cooper and Dobson, 2007; Rugno et al., 2016; Santos et al., 2015); however, their use can adversely affect non-target populations of arthropods, with both lethal and sublethal effects reported for a wide range of species (Bowie et al., 2001; Garzón et al., 2015; Giolo et al., 2009; Kalajahi et al., 2014; Kim et al., 2006). In recent years the focus has turned more towards the sublethal effects on non-target species (Desneux, Decourtye and Delpuech, 2007; Stark, J D, Banks, 2003); this is partly due to the potentially prolonged exposure of both target and non-target species to sublethal concentrations in real conditions (Cordeiro et al., 2010).

Sublethal effects such as reproduction changes are studied in non-target arthropods as part of regulatory pesticide testing (Candolfi et al., 2000), and many studies have reported negative effects at recommended field rates (Owojori, Waszak and Roembke, 2014; Bowie et al., 2001; Cordeiro et al., 2010). Regulatory studies typically apply pesticide products using total coverage of a test arena as they must expose test organisms in a heterogeneous environment to reduce variability. Uniform residues can be considered to be worst-case exposure scenarios where individuals may avoid residues. However, this causes a lack of realism in terms of the test exposure environment not matching the exposure realistically achieved within crop systems where exposure is patchy and varies spatially; heterogeneous coverage may also lead to greater residues where spray lands Therefore, to bring realism to pesticide risk

assessment real exposure patterns must be considered at spatial scales relevant to the test species, either by modified toxicity and behaviour assays within laboratory settings or by computational modelling.

Various methods exist for assessing pesticide residue and exposure patterns, from the conventional method of taking samples from a field and extracting the residues in the laboratory (Hall et al., 2004), to the use of tracers such as fluorescent dyes (Cilgi and Jepson, 1992) and artificial collectors such as water sensitive paper (de Moor, Langenakens and Vereecke, 2000) to investigate spray patterns such as deposition and coverage. Conventional residue testing – involving extraction, clean-up and analysis steps – is usually conducted in the context of human exposure, for instance to measure concentrations in food crops for dietary risk assessment (Angioni et al., 2003; Ferrer et al., 2005; Hall et al., 2004). Some studies have also investigated residues on foliage (Li et al., 2015; Rueegg and Siegfried, 1996), a more relevant substrate in the context of non-target arthropods (e.g. parasitoids and predators) in crop systems. These foliage methods typically work with large samples of several leaves with a mass of 10 – 20 g, but residues averaged across many leaves are not very relevant for predatory insects who are so small that heterogeneous exposure within one leaf is what matters. One study worked with individual leaves weighing approximately 4.5 g (Sántis et al., 2012), though apple leaves are typically smaller. A recently developed method is able to map pesticide residues on individual wheat leaves using MALDI-MS (Annangudi et al., 2015), and this method shows promise but has limitations such as inaccuracies when working with dense spray coverage (Dong, Zheng and Zhao, 2015), access to such instruments and operational costs. Therefore, to investigate pesticide residues at spatial scales relevant to predatory arthropods, conventional residue testing methods need to be adapted to the scale of individual leaves.

One method that has been widely applied to the study of pesticide spray patterns is the use of water sensitive paper (Kunimoto and Inoue, 1997; Martin et al., 2000; Nansen et al., 2011). Water-sensitive paper is yellow card that is coated on one surface with bromophenol blue, a compound that turns from yellow to blue on contact with water and retains this colour once dry, thus creating a stain (Turner and

Huntington, 1970). As such, these cards can be used to evaluate spray patterns as long as the pesticide spray mixture contains water (Cunha, Carvalho and Marcal, 2012). Water sensitive paper has received a lot of attention from researchers who have developed methods for assessing spray quality manually using either a microscope, or by scanning and magnifying the image to evaluate cards by eye, though these are time consuming (Chaim et al., 2002; Hill and Inaba, 1989; Salyani and Fox, 1994). More recent technology has allowed for the development of automated, computer-based analysis of cards, with a number of programs available (Nansen et al., 2015; Panneton, 2002; Wolf, 2003; Zhu et al., 2011). Studies comparing the efficacy of computer-based programs against manual analysis of cards have found that a number of programs show strong correlations with manual analysis when comparing droplet diameters, volumes and counts computed from the same water sensitive paper cards (Cunha, Farnese and Olivet, 2013). However, the authors found that there were differences of up to 10.4 times in droplet density values reported from different software packages, and thus suggest choosing one image analysis program and using it exclusively. Another comparison study found inconsistencies in relative droplet diameter values reported by three different programs, but also found that factors relating to 10^{th}, 50^{th} and 90^{th} percentile diameters ($D_{V0.1}$, $D_{V0.5}$, $D_{V0.9}$) were consistent (Hoffmann and Hewitt, 2005).

While correlations between manual and digital assessment of water sensitive papers were strong enough to prove that digital analysis successfully emulated manual analysis, this only showed that it was effective in assessing spatial patterns in spray such as droplet density and droplet size (Hoffmann and Hewitt, 2005; Cunha, Farnese and Olivet, 2013). A further question is whether water sensitive paper can be used to determine pesticide residues. Through extraction and analysis of pesticide residues from water sensitive paper, a previous study demonstrated that droplet density and total mass of deltamethrin residues correlate well (Hill and Inaba, 1989). One study suggested that pesticide deposition could be estimated through droplet analysis using a microscope having studied the efficacy of the method with chemical tracers (Chaim, Maia and Pessoa, 1999). Additionally, liquid volumes derived through digital image analysis were consistent with microscopic droplet analysis (Chaim et al., 2002).

In summary, we know that water sensitive paper can be used to study spray distribution, and that digital image analysis of these paper samples can be quick, repeatable and consistent with manual analysis of samples (Zhu, Salyani and Fox, 2011; Cunha, Farnese and Olivet, 2013). We also know that pesticide residue analysis on an apple leaf substrate can be both accurate and precise (Hall et al., 1997; Rueegg and Siegfried, 1996; Xu et al., 2008). However, we do not know whether it is possible to accurately estimate pesticide residues using data derived from the analysis of pesticide exposed water sensitive paper, which would provide a low cost analysis method that allows for greater understanding of residues at micro spatial scales, such as within a single apple leaf.

In this study we aimed to assess the accuracy of methods used to analyse spatially distributed pesticide residues. The first objective was to assess pesticide residues and spray patterns in an apple orchard at spatial scales relevant to orchard-dwelling non-target arthropods (e.g. leaf, tree, orchard scale). The second objective was to evaluate the potential for digital image analysis of water sensitive paper to be an effective alternative to conventional pesticide residue analysis for non-target arthropod exposure estimation. To achieve this, patterns in data from both methods were compared.

2.2 Materials and methods

2.2.1 Orchard sampling

Field sampling was conducted in a commercial apple orchard in Cambridgeshire, UK in August 2015. The orchard had an area of 1.25 Ha and contained five-year-old dessert apple trees (*Malus domestica cv.* Braeburn) growing in rows 3.5 m apart and running from south-west to north-east in aspect. Trees were grown with a trellis support system with tree spacing within rows at 0.8 m. Trees were approximately 3 m tall and in growth stage 8-9 according to the BBCH scale for pome fruit. (Meier et al., 1994) Samples were collected following a routine spray application of the fungicide penconazole formulated as Topenco 100 EC (100 g L^{-1}, Globachem NV, Belgium), an emulsifiable concentrate diluted in tap water for spray application at a

rate of 450 mL diluted in 250 L water per hectare (final penconazole concentration = 0.18 g L^{-1}). Penconazole has a photolytic degradation half-life (λ) of 1.32 – 1.99 days; however, it is stable in darkness and also hydrolytically stable at air temperatures of 50˚C for seven days (ECHA, 2012). Spray application was undertaken using a tower sprayer (Kirkland tower triprop sprayer, Kirkland UK), fitted with six each of Albuz ATR 80 yellow and green hollow cone nozzles (Solcera, France) working at a spray pressure of 6 bar. Crop spraying commenced at 09:40 in overcast conditions at an air temperature of 19.8˚C with wind speeds of 0.54 m s^{-1} (60 s average) and 1.97 m s^{-1} (60 s maximum). Samples were collected once pesticide residues were dry, after approximately 1 hour.

An experimental design with nested spatial scales was established for the orchard sampling, similar to one previously outlined (Xu et al., 2006). Three rows were selected within the orchard, with one patch (A, B and C) per row. Each patch contained three consecutive trees and was located away from row ends to ensure pesticide spray was representative. Each tree was then split into three zones: top, middle, and outer, with the top portion starting approximately 2 m above ground (Figure 2.1).

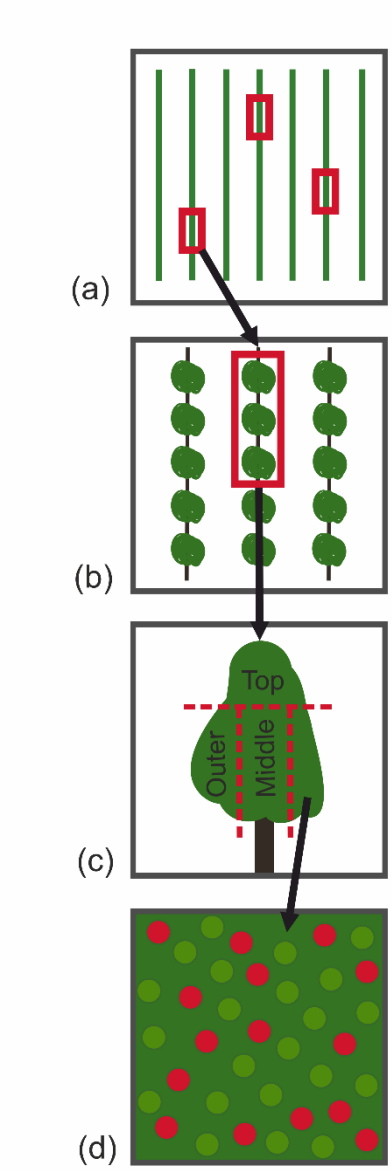


Figure 2.1 - Schematic of the nested orchard sampling design showing the four spatial scales used for apple leaf residue and water sensitive paper coverage analysis. Each spatial scale is represented: (a) patch locations; (b) one patch comprising three trees; (c) one tree comprising three zones; (d) several samples within one zone.

2.2.2 Residue sample collection

Prior to the scheduled pesticide application, twelve water sensitive paper cards measuring 26 × 76 mm (Syngenta, Basel, Switzerland) were placed in the middle and outer zones in each tree across the three patches; these were attached to the upper

leaf surface using a small bulldog clip at the stem to minimise the impact of the additional weight. We were unable to access the top tree zone for placement of water sensitive paper cards due to health and safety constraints. After allowing 60 minutes for the pesticide spray to dry, the water sensitive paper cards were collected and stored at ambient air temperature in sealed plastic bags within an opaque box until analysis.

Five apple leaves were collected from trees prior to pesticide application to act as blank samples for residue analysis. Following pesticide application and a drying period of approximately 60 minutes, 45 apple leaves were sampled from each tree in Patch A using telescopic secateurs, with 15 leaves from each of the three tree zones in each tree. Leaf samples were stored individually in centrifuge tubes in the dark at 10˚C in the field before being transferred after 24 hours to a -20˚C freezer until analysis.

2.2.3 Leaf sample extraction and clean-up

Our method was adapted from one for extracting pesticide residues from bulk samples of leafy vegetables (10 g; González-Rodríguez et al., 2008), to the extraction of individual leaves by adjusting extractant volume to be of a similar ratio to the original method. The sampled leaves had a mean mass of 0.57 g (95% CIs [0.53 g, 0.62 g]). Leaf samples were removed from the freezer and allowed to defrost at ambient temperature. Weight and upper leaf area were determined for each leaf before they were extracted, with leaf area determined by scanning and image analysis in ImageJ (version 1.38x, NIH, USA). Each leaf was cut into smaller pieces and returned to the centrifuge tube for extraction with 10 mL acetonitrile. Samples were homogenised in an ultrasonic bath for 10 min. An 8.5 mL aliquot of the extraction solution was then transferred into a glass test tube and concentrated down to 1-2 mL under a gentle stream of nitrogen gas using a heated sample concentrator (Techne Dri-Block DB-3; 40˚C, N_2 flow rate of 8 L min^{-1}).

For sample clean-up, a solid-phase extraction (SPE) cartridge (Supelclean ENVI-Carb-II/PSA 500 mg/500 mg, 6 mL size) was conditioned with 3 mL acetonitrile:toluene (3:1

v/v). The acetonitrile sample extract was then loaded onto the cartridge and the retained pesticide was eluted slowly with 3 mL acetonitrile:toluene (3:1 v/v). The final eluate was then evaporated to dryness and reconstituted in 1 mL acetonitrile. The sample was mixed using a vortex mixer (25,000 rpm, 5 s) before it was transferred to an amber autosampler vial for analysis via gas chromatography-mass spectrometry (GC-MS).

To compare leaf residue data from analytical chemistry with residues estimated from image analysis, penconazole residue data was converted so that residues were based on leaf upper surface area (Equation 2.1). It was not within the scope of this study to determine what proportion of pesticide spray lands on the upper and lower leaf surfaces, therefore the leaf residue values assume all residue was present on the upper leaf surface. Previous studies have investigated differences in upper and lower leaf surface residues (Hall et al., 1997).

$$R_{area} = \frac{R_{leaf}}{A} \qquad \textbf{Equation 2.1}$$

R_{area} denotes residue based on leaf surface area in µg cm^{-2}, R_{leaf} denotes residue detected on a leaf sample in µg, and A denotes leaf area in cm^2.

2.2.4 Gas chromatography-mass spectrometry (GC-MS)

Penconazole residues were determined using a Clarus 680/600C GC-MS (PerkinElmer, UK) fitted with an Elite-5MS fused silica capillary column (L 30 m × 0.25 mm i.d. × 0.25 µm film thickness; PerkinElmer, UK). Samples were prepared in acetonitrile for analysis and 1 µL injected via a split-splitless injection port operated in splitless mode (splitless time 1 min; injector temperature 250°C). The oven was programmed from an initial temperature of 50˚C (hold 1 min) to 300˚C at a rate of 20˚C min^{-1} where it was held for 3 min. Helium (99.999% purity) was used as the carrier gas at a flow rate of 1 mL min^{-1}. The MS was operated in electron ionisation (EI) mode with an ionisation energy of 70 eV, source temperature of 180°C and inlet line temperature of 240°C. Data was acquired in selected ion monitoring (SIM) mode

at *m/z* 159 and 248 (dwell time 100 ms), used for quantification of penconazole (González-Rodríguez et al., 2008). The solvent delay was set to 4 min and the total run time was 16.5 min. Penconazole eluted with a retention time of 11 min. Instrument control, data acquisition and processing was by Turbomass software v5.4.1617 (PerkinElmer, UK).

2.2.5 Analytical method development

To determine the accuracy of the GC-MS method, apple leaves washed in deionised water were spiked with known quantities of penconazole analytical standard (Sigma Aldrich, Dorset, UK) in acetonitrile at five concentrations ranging from 0.1 – 4 µg per leaf, a range that covered all field residues determined in this study. These leaves were subject to extraction and SPE using the method previously outlined in Section 8.3. Penconazole recovery was calculated along with the co-efficient of variation (CV). Method precision was determined by extracting five apple leaves spiked with 2 µg penconazole, and was expressed as CV. In a further step to validate the experimental method, a storage stability study was conducted where ten washed apple leaves were spiked with 2 µg penconazole each. One set of five leaves were extracted and analysed once residues were dry, and one set of five leaves were stored at -20°C after drying for a total of 37 weeks before being defrosted, extracted and analysed. The penconazole recovery rate was determined along with CV.

Recoveries were in the range of 74 – 119% (mean 90%; n = 21), with CV of 5.9 – 24.9% (mean 11.9%) for all standard concentrations. Precision, calculated from five 2 µg mL^{-1} samples, was 7.9% and within the acceptable CV limit of 20% (EU, 1996). Our storage stability study showed that recovery was in the range 83 – 110% (mean 99%) after 37 weeks.

Analytical limit of detection (LOD) and limit of quantitation (LOQ) were calculated using signal to noise ratios of 3:1 and 10:1 respectively (Vial and Jardy, 1999), determined for spiked apple leaves. LOD and LOQ for penconazole were 0.08 mg kg^{-1} and 0.26 mg kg^{-1} respectively. No field samples had detected residues that were below the LOQ, including the controls collected before the spray event.

2.2.6 Spray pattern analysis

Following a review of the various image analysis packages available, DepositScan, a freely available droplet analysis program was selected for use in this study (Zhu, Salyani and Fox, 2011). Individual water sensitive paper cards (WSP) were scanned on a flatbed scanner (Canon CanoScan 9000F) at a resolution of 600 dpi as greyscale bitmap images and cropped and converted to GIF file type using Irfanview v4.4. Several parameters were determined by DepositScan including spray coverage (percentage of target covered), spray density (droplets cm^{-2}), and liquid deposition (µL cm^{-2}). The smallest droplet diameter that can be detected by DepositScan is 17 µm (Zhu, Salyani and Fox, 2011); however, in the present study the smallest droplet diameter was 52.8 µm. These were analysed at the various spatial scales sampled (within tree, between tree, within orchard). Volume median diameter (VMD) is a common measure when describing droplet sizes; however this is easily skewed by factors such as a few large droplets amongst a mostly small droplet pattern (Cunha, Carvalho and Marcal, 2012); thus this metric was omitted.

It is possible to calculate estimated pesticide deposits on apple leaves using data from DepositScan, if the concentration of pesticide in the spray tank mixture is known (Equation 2.2).

$$R = C \times D$$ **Equation 2.2**

R denotes active ingredient residue in µg cm^{-2}; C denotes concentration of active ingredient in spray tank mixture in µg μL^{-1}; D denotes the liquid deposition value calculated by DepositScan in µL cm^{-2}.

2.2.7 Testing DepositScan

To test the way in which DepositScan calculates factors such as deposition, a number of tests were designed involving computer generated images of “stains”, with all images generated using Microsoft Paint. Stains on water sensitive paper are larger

than the area covered by the initial droplet due to the solution spreading (Cunha, Carvalho and Marcal, 2012). As water sensitive paper absorbs and expands the aqueous portion of a pesticide spray, it has been suggested that the measurement of water sensitive paper stains can overestimate the liquid deposition (Garcera, Molto and Chueca, 2014), as the stains created appear larger than the initial droplet. However, through using a spread factor, this error can be accounted for.

To calculate the initial droplet diameter from which droplet volume can be derived, a spread factor was applied – DepositScan uses a formula where a single factor is applied to the stain area (Equation 2.3; Zhu, Salyani and Fox, 2011). An alternative spread factor calculation exists based upon stain diameter, with the spread factor varying based upon stain diameter. Documentation providing further information about water sensitive paper reports a range of spread factors, with spread factor values increasing as stain size increases (Table 2.1). The factors were determined using water sprayed at 20˚C and 40% relative humidity, though the authors state that pH and relative humidity have no effect (Syngenta, 2002).

$$d = SF \times A^{0.455}$$ **Equation 2.3**

d denotes droplet diameter in µm; SF denotes the spread factor (the DepositScan default used here is 1.06); A denotes the spot area calculated by DepositScan in µm^2.

Table 2.1 – Variable spread factor values determined on water and used as an alternative to DepositScan's built-in spread factor calculation (Syngenta, 2002; Cunha, Carvalho and Marcal, 2012)

Stain diameter of droplet (µm)	Spread factor	Droplet diameter (µm)
100	1.7	58.8
200	1.8	111.1
300	1.9	157.9
400	2.0	200.0
500	2.1	238.1
600	2.1	285.7

To apply this variable spread factor, the stain diameter was calculated from the stain area derived from DepositScan (Equation 2.4), and from this the droplet diameter was calculated using Equation 2.5 and applying the relevant spread factor from Table 2.1 – for example, the spread factor for a droplet with a stain diameter in the range of 301-400 µm would be 2.0 (Syngenta, 2002).

$$d_s = \left(\sqrt{A/\pi}\right) \times 2 \qquad \textbf{Equation 2.4}$$

d_s denotes stain diameter in µm and A denotes spot area calculated by DepositScan in µm^2.

$$d = \frac{d_s}{SF} \qquad \textbf{Equation 2.5}$$

d denotes droplet diameter in µm; d_s denotes stain diameter in µm and SF denotes the spread factor value from Table 2.1 that applies to d_s.

To assess whether calculated deposition changed when different spread factors were applied to stain size data, we generated 33 artificial circular "stains" with diameters

in the range 95.5 – 1438 µm. These were analysed using DepositScan, and calculated deposition values derived from DepositScan's own calculation and from the application of the varied spread factor were compared. To determine deposited volume, each droplet diameter was converted to volume.

We also quantified the effect of droplets of equal size touching each other. The software creators state that DepositScan cannot differentiate between droplets that overlap, and so the software makes the assumption that two touching droplet stains are one single deposit (Zhu, Salyani and Fox, 2011). To assess whether this affected the estimation of deposition, the same artificial stains from the spread factor tests were used, with two images at each stain diameter analysed: one containing two stains of equal size that did not touch, and one containing two stains that touched at one side, but did not overlap. This meant that an image with two droplets each of 96 µm diameter had a horizontal diameter of 192 µm, but still measured 96 µm at the longest vertical point, or vice versa (Figure 2.2).

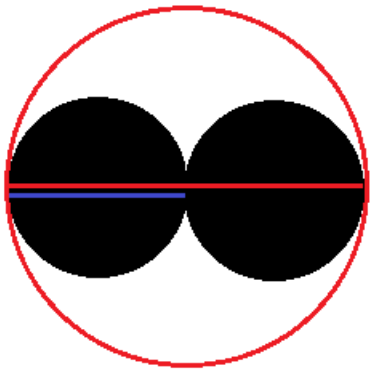


Figure 2.2 – Illustration of the overestimation of droplet size by DepositScan when presented with overlapping droplets. In this example, both black stains had diameters of 96 µm (blue horizontal line). However, because the two droplet stains overlapped slightly, in this example, DepositScan would count the two droplets as one single stain measuring 192 µm in diameter (red horizontal line), giving a deposition value based on a much larger assumed stain, illustrated by the red circle.

To calculate the droplet volume for each droplet, DepositScan uses Equation 2.5, and the sum of each volume is reported as deposition expressed as µL cm^{-2} (Zhu, Salyani and Fox, 2011). In this test, we compared the deposition value in µL per image for each image pair.

2.2.8 Statistical analysis

Analyses were undertaken using GraphPad Prism (Version 7.01, GraphPad Software Inc., California, US). All residue and water sensitive paper data were initially tested for normality using the D'Agostino & Pearson normality test (D'Agostino, 1986); the Brown-Forsythe test for equality of variances was also used to determine the best test for data sets (Brown and Forsythe, 1974). Data were analysed using one-way ANOVA with Tukey HSD test for multiple comparisons. When comparing exposure between trees, there was no grouping so all nine trees were compared with each other, with Trees 1-3 from Patch A, Trees 4-6 from Patch B, and Trees 7-9 from Patch C. Datasets comparing exposure between two tree zones were tested for normality using D'Agostino & Pearson, and then analysed using the unequal variances t-test (also known as Welch's t test), chosen for its ability to handle unequal population variances (Ruxton, 2006).

Data relating to testing DepositScan were tested for normality as above, but due to non-normal distributions in both tests, data were analysed using the Wilcoxon matched pairs ranked test. Regression analysis was performed to investigate whether leaf residues based on leaf area were comparable to residues based on leaf mass.

2.3 Results

2.3.1 Spray pattern analysis

Mean pesticide coverage of the water sensitive paper surface was 16.3% [95% CIs: 15.1%; 17.5%] across all samples (Table 2.2; *n* = 215). Mean surface coverage in Patch A was 20.1%, 14.9% in Patch B (mean difference A vs B 5.2%; 95% CIs [8.5%; 1.8%]; P = 0.0009), and 14% in Patch C (mean difference A vs C 6.1%; 95% CIs [9.4%; 2.7%]; P < 0.0001).

When analysing variance between all nine trees, the mean difference in pesticide coverage was 3.4% (P = 0.002). Tree 1 received the greatest pesticide coverage (Table 2.2) and significantly greater coverage than Tree 4 (mean difference 7.7%; 95% CIs [15.3%; 0.03%]; P = 0.048), Tree 6 (mean difference 8.7%; 95% CIs [1.1%; 16.3%]; P = 0.012), Tree 7 (mean difference 8.2%; 95% CIs [0.53%; 15.8%]; P = 0.026), Tree 8 (mean difference 9.3%; 95% CIs [1.66%; 16.95%]; P = 0.026) and Tree 9 (mean difference 8.9%; 95% CIs [1.23%; 16.52%]; P = 0.026). Mean coverage in the middle zone was 15.3% and 17.2% in the outer zone (P = 0.12).

Spray density averaged 120 droplets cm^{-2} [95% CIs: 114 droplets cm^{-2}; 126 droplets cm^{-2}] across all samples (Table 2.2; *n* = 215). On average, density varied significantly by 4.45 droplets cm^{-2} between patches (P < 0.0001), though in contrast to the trend shown in coverage, spray density was 31.4 droplets cm^{-2} higher in Patch A than Patch B (95% CIs: 47.5 droplets cm^{-2}; 15.3 droplets cm^{-2}; P < 0.0001), and 24.7 droplets cm^{-2} higher in Patch C than Patch B (95% CIs: 8.7 droplets cm^{-2}; 40.7 droplets cm^{-2}; P = 0.001). Spray density did not significantly vary within patches (Table 2.2). There was also no significant difference in spray density between tree zones, which is consistent with coverage trends.

Table 2.2 – Pesticide spray coverage – expressed as proportion of target covered by spray – and spray density – expressed as the number of droplets in an area – determined from water sensitive paper cards set within apple trees. Data are expressed as mean coverage with 95% confidence intervals, showing differences within the sampled orchard, within patches, and within trees. Co-efficient of variance (CV) is also presented. P values for within patch variance are for each group of 3 trees that made up each patch, e.g. Patch A is comprised of Trees 1 – 3.

			Target Covered (%)						Spray density (droplets cm^{-2})				
					95% CIs					95% CIs			
			n	Mean	Lower	Upper	CV (%)	P	Mean	Lower	Upper	CV (%)	P
Within Orchard	**Patch**	**A**	70	20.1	18	22.1	43.1		132.8	124.6	141	25.9	
		B	73	14.9	12.8	17	60.6		101.4	91.8	111	40.5	
		C	72	14	12.2	15.8	53.7	< 0.0001	126.1	115.3	136.9	36.3	< 0.0001
Within Patch	**Tree**	**1**	24	22.8	18.6	27	43.9		133.2	120.2	146.2	23.1	
		2	23	19.4	15.6	23.3	46.2		130.9	113	148.8	31.6	
		3	23	17.8	15.2	20.4	33.7	0.13	134.2	120.5	147.9	23.6	0.95
		4	24	15.1	10.9	19.3	65.9		101	85.34	116.6	36.7	
		5	24	15.6	12.2	18.9	50.4		104.5	88.7	120.4	36.7	
		6	25	14.1	10.2	18	67.2	0.85	98.6	78.1	119.1	49.2	0.88
		7	24	14.6	11.9	17.3	43.3		121.9	105.5	138.3	31.8	
		8	24	13.5	10	16.9	60.4		128.7	106.5	150.9	40.1	
		9	24	13.9	10.4	17.4	59.2	0.87	127.8	108	147.5	36.6	0.86
Within Tree	**Zone**	**Middle**	106	15.3	13.7	17	55.8		115.3	106.8	123.8	38.2	
		Outer	109	17.2	15.5	18.9	52.2	0.12	124.4	116.6	132.2	33.1	0.12

2.3.2 Leaf residues

Penconazole residues found on apple leaves in Patch A were between 0.35 – 6.56 mg kg^{-1} (Table 2.3). The mean difference in residues between trees was 0.22 mg kg^{-1} (P = 0.18). In contrast, residues varied within trees by 0.63 mg kg^{-1} on average (P < 0.0001), with residues in the top tree zone 0.95 mg kg^{-1} higher than in the middle zone (95% CIs [0.54 mg kg^{-1}; 1.36 mg kg^{-1}]; P < 0.0001) and 0.63 mg kg^{-1} higher in the outer zone than in the middle zone (95% CIs [0.22 mg kg^{-1}; 1.04 mg kg^{-1}]; P = 0.0005). The mean difference between top and outer zones of the trees was 0.32 mg kg^{-1} (95% CIs [-0.08 mg kg^{-1}; 0.74 mg kg^{-1}]; P = 0.657).

Table 2.3 – Penconazole residues in apple leaves from Patch A, expressed as mean residue with 95% confidence intervals, split by tree and tree zone. Co-efficient of variance (CV) is also reported.

			Penconazole residue in apple leaf (mg kg^{-1}) 95% CIs				
		n	**Mean**	**Lower**	**Upper**	**CV (%)**	**P**
Patch	**A**	135	2.28	2.09	2.47	48.31	-
Tree	**1**	45	2.03	1.70	2.36	63.4	
	2	45	2.44	2.08	2.79	40.8	0.18
	3	45	2.37	2.08	2.67	47.5	
Zone	**Top**	45	2.72	2.42	3.01	43.2	
	Middle	45	1.76	1.55	1.98	65.1	< 0.0001
	Outer	45	2.39	2.17	2.61	41.8	

2.3.3 Comparing residue analysis methods

To compare leaf derived penconazole residue data to penconazole deposits estimated from water sensitive papers by DepositScan, residue data had to be converted to a comparable unit, so the following data are expressed as µg cm^{-2}. Due

to leaf residue measurements only being undertaken on leaves from Patch A, water sensitive paper data from patches B and C were omitted to ensure data were comparable. Similarly, leaf residue data from the top tree zone were also omitted. Any residue estimates derived from water sensitive paper samples with coverage greater than 30% were also omitted, as volume estimates become inaccurate above this point (Zhu, Salyani and Fox, 2011). Regression analysis suggests that area-based leaf residue values correlate well with the mass-based leaf residue values (R^2 = 0.65; P < 0.0001; Figure 2.3). Two main outliers that deviate far from the 95% CI boundary represent samples with lower leaf mass, or smaller leaf areas than average.

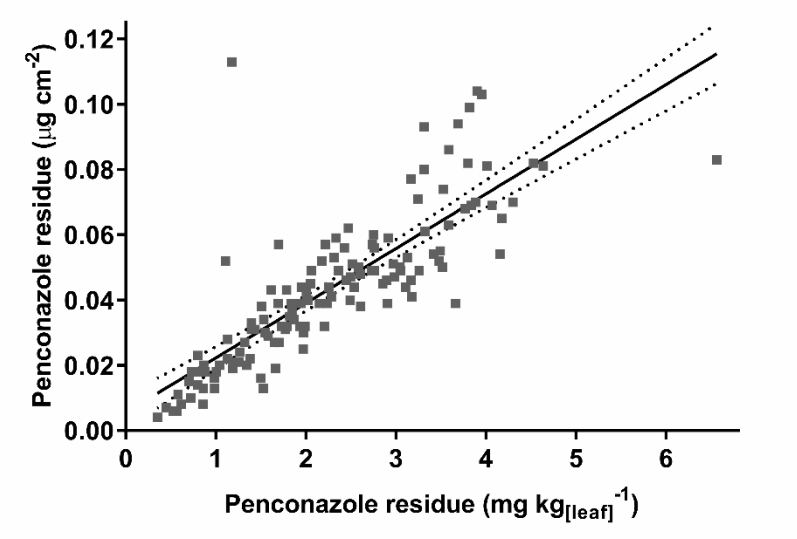


Figure 2.3 – Penconazole residues derived from GC-MS based on leaf mass (horizontal axis) and whole leaf upper surface area (vertical axis). Solid trend line shows regression with 95% confidence bands (dotted lines). Regression: Y = 0.01675 × X + 0.005528. R^2 = 0.65; P < 0.0001; n = 135

Penconazole residues based on leaf surface area averaged 0.039 µg cm^{-2} [95% CIs: 0.035 µg cm^{-2}; 0.044 µg cm^{-2}]; the water sensitive paper derived values averaged 0.24 µg cm^{-2} (95% CIs [0.21 µg cm^{-2}; 0.27 µg cm^{-2}]; P < 0.001; Figure 2.4a). The mean difference between the leaf residue and water sensitive paper estimate was 0.198 µg cm^{-2} (95% CIs [0.17 µg cm^{-2}; 0.23 µg cm^{-2}]; P < 0.001). To adjust the water sensitive

paper mean penconazole deposit to that of the apple leaf residues, an empirical correction factor of 0.1625 was applied to the water sensitive paper data (Equation 2.6), and this successfully adjusted the mean penconazole deposit to 0.039 µg cm^{-2} (95% CIs [0.035 µg cm^{-2}; 0.044 µg cm^{-2}]; Figure 2.4b). The 0.00013 µg cm^{-2} mean difference between mean corrected water sensitive paper residue values and leaf residue values was not significant (95% CIs [-0.006 µg cm^{-2}; 0.006 µg cm^{-2}]; P = 0.97).

$$R_{corrected} = R \times CF \qquad \textbf{Equation 2.6}$$

$R_{corrected}$ denotes corrected penconazole residue in µL cm^{-2}; R denotes penconazole residue on water sensitive paper in µL cm^{-2}; CF denotes the correction factor of 0.1625.

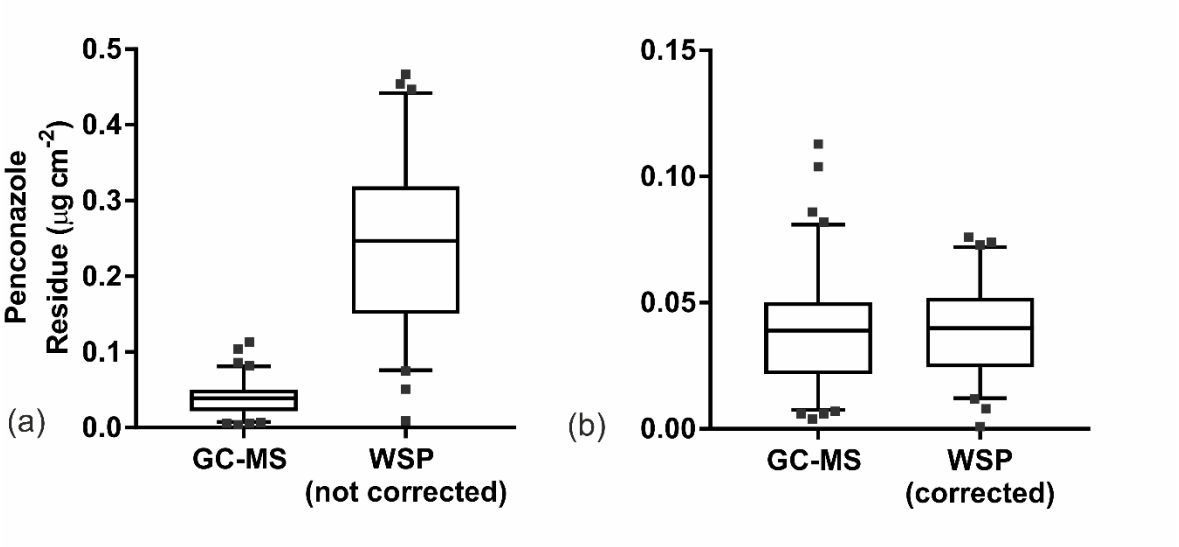


Figure 2.4 – Comparison of penconazole residue values based on surface area derived from leaf residue samples analysed via GC-MS and water sensitive paper (WSP) samples analysed via DepositScan image analysis with no correction factor (a) and with a correction factor applied to individual data points (b). The horizontal middle, lower and upper lines within each box indicate mean, 25th and 75th percentiles; caps at the top of the vertical lines indicate the 5th and 95th percentiles; dots depict extreme data points (i.e. values less than 25th Percentile - 1.5 × inter-quartile distance, or greater than 75th Percentile + 1.5 × inter-quartile distance). *n* = 90; 61

2.3.4 Testing DepositScan

When comparing different spread factor calculations, mean droplet volume for the DepositScan spread factor was 0.055 µL per image [95% CIs: 0.035 µL; 0.075 µL], 19.6% higher than the average droplet volume from the varied spread factor of 0.046 µL (95% CIs [0.028 µL; 0.064 µL]; $P < 0.0001$; ratio paired t test). Deposition ranged from 0.00009 – 0.1882 µL when calculated using the DepositScan spread factor, and 0.00009 – 0.1681 µL when calculated with the varied spread factor (Figure 2.5a). When assessing whether deposition is overestimated when 2 droplets touch compared to when there is no contact, the respective mean deposition volumes were 0.14 µL [95% CIs: 0.09 µL; 0.19 µL] and 0.11 µL [CIs: 0.07 µL; 0.15 µL], thereby showing deposition was overestimated on average by 22.3% when the droplets were touching ($P < 0.0001$; Figure 2.5b).

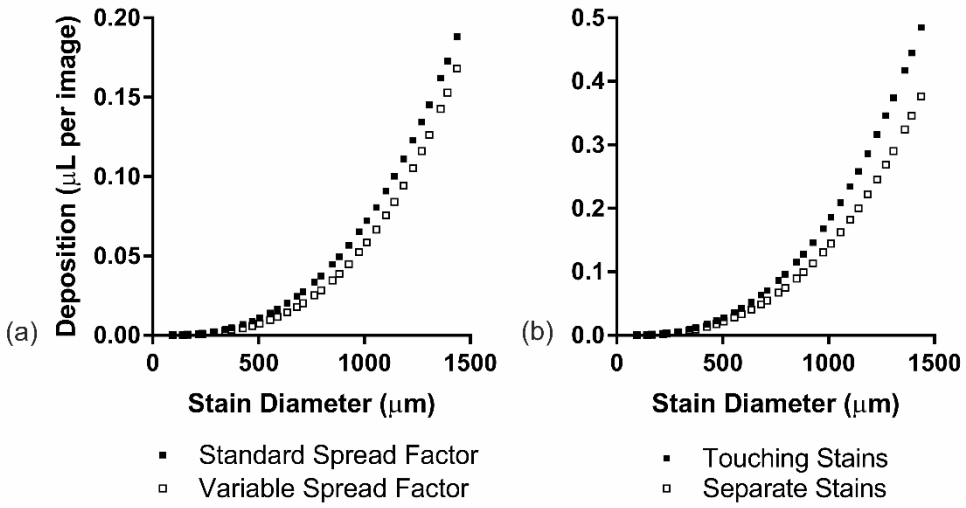


Figure 2.5 – (a) Deposition values calculated from the same artificial stain using the default DepositScan spread factor and an alternative spread factor that varies based on stain diameter. *n* = 33 (b) Deposition values calculated by DepositScan based on whether two artificial circular stains of the same size touch or not. *n* = 33.

2.4 Discussion

2.4.1 Comparison of trends in leaf residue and water sensitive paper data

Measurements derived from water sensitive paper analysis indicated there was a significant average difference of 28% in spray coverage and 20% difference in spray density between patches within the same orchard (Table 2.2). This is in contrast to findings from a study where a zinc tracer was sprayed in an apple orchard where no significant differences between plots within the same orchard were observed (Xu et al., 2006). These contrasting findings could be due to differences in weather conditions during the study, spray systems or settings, the methodology to identify spray coverage and density, sample numbers, tree type, tree spacing etc. and highlight the problems with inter-study comparisons. However, the nested sampling design used in our study enables detailed analysis of sources of variation within a single orchard which can be compared to findings in other studies. When studying trends within orchard patches, we found no significant differences in spray coverage or density; this was also the case within Patch A for apple leaf residues. When focusing upon Patch A, Tree 1 displayed the highest spray coverage, the second highest spray density, and the lowest leaf residue. This suggests that trends differ depending on the measurement analysed. Though the trees chosen for this study were uniform in height, growth habit and management, such differences in spray patterns between trees have previously been justified by tree architecture, in particular variability in leaf position (Xu et al., 2006). This seems to be consistent with our findings.

In considering variance within trees, spray cover, density, and leaf residues were all higher in the outer zone than in the middle zone; leaf residues were higher in the top tree zone than in the middle (Table 2.3). The trend of outer tree sections receiving more residue than the centre is consistent with trends previously reported in a similarly designed apple orchard study using EDTA chelates of various metals as tracers (Cross et al., 2001) and also in the zinc EDTA chelate tracer study (Xu et al., 2006), suggesting that our study method was a successful model of real pesticide exposure when compared to other studies. Between tree variation for measures based on water sensitive paper such as spray coverage and spray density showed

consistent but different trends to the actual residue analysis, and all were not significant, suggesting natural variation was responsible for observed differences. However, within a single patch of trees, spray pattern measurements showed no significant trends between trees. With observed trends differing at different spatial scales, we suggest an unmeasured factor may have an impact, such as variable proximity of the crop sprayer to trees.

When considering within tree differences, leaf residue trends were consistent with spray coverage and density trends, with values in the outer zone higher than in the middle zone, though effect size varied: leaf residues were 36% higher in the outer zone, a significant difference, while spray coverage and density showed non-significant differences where coverage was 12% higher and density was 8% higher in the outer zone. This suggests that, while water sensitive paper analysis accurately estimates overall trends in within tree differences, effect size would be underestimated.

2.4.2 Comparing residue analysis methods

Overall, penconazole deposits estimated through image analysis of water sensitive paper were over five times higher than residues determined through GC-MS analysis of exposed apple leaves (Figure 2.4b). However, there was also a significant difference in variances, with overall CV higher for the leaf residues measured with GC-MS (52%) than it was for the water sensitive paper deposits (44%; F test; $P < 0.001$). This suggests trends derived from water sensitive paper analysis are less affected by random variation. Our analytical method validation demonstrated that the penconazole extraction method was within validation guidelines (EU, 1996); additionally, the storage stability study demonstrated minimal penconazole losses of 1% on average over time. Therefore, our findings suggest that DepositScan consistently overestimated penconazole residues when compared to the leaf residues.

The source of overestimation could come from the farmer's dilution of pesticide product when preparing spray tank mixtures, as varying precision in the preparation

could provide a source of error. The overestimation could also come from the spray tank mixture behaving differently on the water sensitive paper surface in comparison to water, which is used by the paper manufacturer in the preparation of varied spread factors (Syngenta, 2002). Additionally, many factors relating to DepositScan's estimation of residues could lead to overestimation. Firstly, the software assumes droplet stains are circular, and is not capable of identifying droplets that are overlapping (Zhu, Salyani and Fox, 2011), and this can cause an overestimation of spray deposition. Additionally, when coverage is over 30%, spray density and deposition are potentially inaccurate (Zhu, Salyani and Fox, 2011), as droplets are more likely to be touching when there is a high coverage value. In the present study, 12% of all water sensitive paper cards displayed coverage greater than 30%.

A comparison of spread factors found that the factor used by DepositScan produced deposition values that were on average 11.5% higher than the deposition calculated from the varied spread factor. Table 2.4 shows how the two different spread factors would calculate droplet diameter from six hypothetical stain sizes, and displays a range of differences, from 5.8% at 200 µm diameter, to 12.3% at 500 µm diameter. While no studies have investigated whether image analysis programs overestimate deposition, one study showed that, when compared to manual analysis of water sensitive paper, DepositScan overestimated droplet density by 89% when dealing with fine droplets (Cunha, Farnese and Olivet, 2013). From further analysis of water sensitive paper samples from Patch A, on average 33% of all droplets were determined to be 100 µm or lower in diameter, with over 70% of all droplets measuring below 200 µm in diameter (Figure 2.6). This strong skew suggests that DepositScan's overestimation of droplet density associated with fine droplets is a contributing factor in this study, and could also affect other calculated factors.

Table 2.4 – The effect of two different spread factors on 6 stain diameter sizes. The spread factors were determined on water droplets.

	Droplet Diameter (µm)		
Stain Diameter (µm)	**DepositScan Spread Factor**	**Varied Spread Factor**	**Difference**
100	62.74	58.82	6.25%
200	117.9	111.1	5.76%
300	170.5	157.9	7.40%
400	221.5	200.0	9.72%
500	271.4	238.1	12.3%
600	320.4	285.7	10.8%

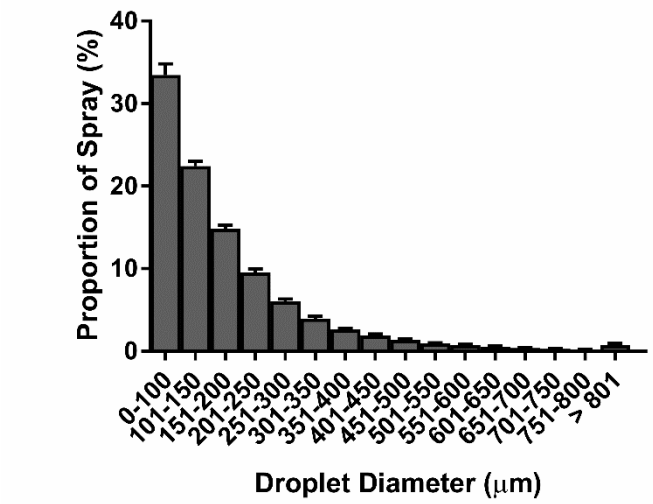


Figure 2.6 – Distribution of droplet diameters as a function of spray proportion on water sensitive papers from patch A. Bars show 95% confidence intervals (*n* = 70)

One other reason for the overestimation could be that wetting agents within the pesticide formulation could cause the droplet to spread further on impact with a surface due to an altered surface tension, therefore creating a larger stain than that created by water alone. The aforementioned variable spread factors were developed based on droplets of water, as opposed to pesticide mixtures (Syngenta, 2002), which would in theory produce smaller stains for a droplet of the same volume. Finally,

when a pesticide is sprayed onto a plant some of the active ingredient might translocate into the plant, thus leading to lower residue values on the plant surface. This may be another source of the overestimation seen when analysing water sensitive paper.

Despite the overestimation of residue from water sensitive paper, it is possible to use water sensitive paper and DepositScan to estimate pesticide residues with a correction factor. However, this correction factor might only apply to the penconazole formulation and application regime in this study, and more work is necessary to determine whether a single correction factor would work for all spray situations and pesticide formulations, or whether correction factors would be specific to formulations (e.g. due to different wetting agents, application concentrations).

Digital image analysis of water sensitive paper to estimate pesticide deposits provides a time- and cost-effective high throughput method of studying pesticide residues in agricultural systems. At the time of writing, water sensitive paper cost under £40 (GBP) for 50 pieces, and digital analysis of each sample can be completed in a matter of minutes. By comparison, conventional residue analysis methods involve pesticide extractions that can take over a day to complete, with chromatographic analysis time requirements varying based on method and retention times for the chosen study compounds. The analysis cost can be prohibitive due to the use of solid phase extraction techniques and the operation of analytical equipment. Therefore, the time and cost savings of using water sensitive paper are attractive as they allow for large sample numbers in study designs; water sensitive paper also allows for examination of pesticide spray patterns at a fine spatial scale, something that is not currently widely undertaken by conventional residue analysis.

2.5 Conclusions

Pesticide spray patterns can differ within an orchard, between and within trees during a single pesticide spray event. Trends showing differences in coverage, spray density and residue within trees are important for understanding the exposure patterns that tree-dwelling arthropods are subjected to in commercial apple

orchards. Together with the micro-scale exposure patterns derived from the water sensitive papers, these data will inform future experiments looking at effects of pesticide exposure on their behaviour.

Through the study of pesticide spray patterns, we have been able to demonstrate that water sensitive paper could act as a replacement for conventional residue testing by offering a fast method of estimation, but the sources of uncertainty need to be fully understood. Water sensitive paper is a highly effective tool for analysing differences in spray patterns and allows for large sample numbers and rapid estimation of pesticide deposits on a surface. It also provides data on spray density and coverage that residue analysis cannot; however, if residue values are an important factor in the study of an untested compound then conventional residue analysis techniques should still be considered. Once the correlation between water sensitive paper data and conventional residue analysis is established for a pesticide product, with an adjustment factor if necessary, large numbers of water sensitive paper samples can be deployed for high throughput exposure analysis. Further work should explore whether pesticide residues derived from water sensitive paper correlate well with other pesticide formulations, and whether different correction factors are necessary for different pesticide formulations or situations.

2.6 Using spray data to contextualise laboratory studies

Note: *this section does not appear in the published article and is an addendum to aid in discussion of concepts in the following chapters*

In the following chapters, we have conducted laboratory studies and want to consider whether observed effects would occur in an apple orchard in a real spray event. As a result, we needed to know what proportion of a pesticide spray application landed on apple leaf surfaces, and data from this chapter made this possible. Data from water sensitive paper showed that 1.066 µL cm^{-2} was deposited in the apple tree canopy. However, from our comparison of penconazole residues derived from water sensitive paper and analysis of apple leaves, we found that water sensitive paper overestimated deposition (Section 2.4.3). After adjusting the aforementioned value using the correction factor of 0.1625, we determined penconazole spray deposition on apple leaves was 0.173 µL cm^{-2}. This equated to 1.73 mL m^{-2}, or 17.3 L Ha^{-1}. However, this did not account for the fact that one hectare of apple orchard did not equate to one hectare of apple leaf surface.

To determine exactly what proportion of spray landed on apple leaves, we utilised the leaf area index (LAI) concept. LAI is the ratio of one-sided leaf area per unit of ground area and is used for modelling atmosphere-vegetation processes (Jonckheere et al., 2004). A value greater than one indicates there is more leaf surface area than ground area (e.g. in broadleaved trees), while a value below one indicates there is more ground area than leaf surface area (e.g. in coniferous trees). LAI has been used in considering pesticide efficacy in grapevines (Siegfried et al., 2007). In apple orchards, LAI varies through the growing season and is also affected by the production system used for growing the apples (Wunsche et al., 1996). As we made no estimation of total leaf number or area in the studied apple orchard, we used information available to us to identify which literature LAI value was most appropriate. Based on tree growth stage, cultivar, tree height, row and tree spacing and the knowledge that trees we sampled were growing based on the slender spindle cultivation style, we identified the most appropriate LAI value to be 2.46, derived

from a study of dessert apples in Chile (Poblete-Echeverría et al., 2015). This value indicates that leaf area is 24,600 m^2 per hectare of ground surface.

Based on our measurements that showed penconazole spray deposition of 17.3 L Ha^{-1} on apple leaves, by multiplying this value by the LAI, we conclude that 42.6 L, or 17%, of penconazole spray landed on apple leaves. This value will vary based upon many factors such as climatic conditions and spray equipment performance; however, this value allows us to consider the environmental relevance of effects observed in laboratory studies in the following chapters.

Chapter 3 – Pesticide induced changes in movement behaviour in the predatory mite *Typhlodromus pyri*

3.1 Introduction

3.1.1 Sublethal effects of pesticides

The registration of pesticide active substances requires consideration of lethal effects (mortality) and sublethal effects (fecundity) in non-target arthropods as an absolute minimum. Parallel to this, most laboratory bioassays in the past have studied acute mortality of pesticide active substances and formulations, and only recently has a focus developed in the study of sublethal effects (Desneux et al., 2007). Sublethal effect studies in the regulatory context only typically cover changes in reproductive capacity (Beers and Schmidt-Jeffris, 2015); however, there is a greater scope to study sublethal effects. In a review of sublethal effects of pesticides on beneficial arthropods, Desneux et al. (2007) stated that sublethal effects cover physiological and behavioural effects and categorised such effects arising from intoxication into two classes: direct intoxication, which involves responses such as knock down, uncoordinated movement and excessive cleaning; and secondary intoxication which includes disruption in responses to kairomones and pheromones, repellence and irritation.

Sublethal effects have been seen across a range of species. A study observing the carabid beetle *Nebria brevicollis* showed that individuals that ate prey contaminated with the pyrethroid deltamethrin went on to eat fewer uncontaminated prey, suggesting a physiological effect on feeding behaviour (Wiles and Jepson, 1993). A study by Beers and Schmidt (2014) also observed reduced prey consumption in the predatory mite *Galendromus occidentalis* when exposed to field rate doses of the neonicotinoids acetamiprid and imidacloprid, and the micro-organism derived insecticide spinosad. The organophosphate dimethoate also reduced feeding – and

therefore predatory capacity – in the ladybird *Coccinella septempunctata* (Singh et al., 2004).

3.1.2 Behavioural effects of pesticides

The need for greater understanding of ongoing behavioural effects arising from pesticide exposure, and any population- or ecosystem-level consequences arising from such effects on non-target arthropods has recently been identified as a priority by the European Food Standards Agency (EFSA, 2015). Changes in behaviour can have a deleterious effect on non-target arthropods, such as increasing their vulnerability to predation (Kunkel et al., 2001), decreased energy efficiency as a result of hyperactivity (Desneux et al., 2006), and reduced predatory efficiency (Singh et al., 2004). Reproduction can also be impacted via changing sex pheromone communication and mating behaviours (Wang et al., 2018). Irritation, a term also used in the study of avoidance behaviour, has been defined to include behaviour changes such as increased walking rate, reduced resting time, and increased grooming (Wiles and Jepson, 1994). Effects are often seen at sublethal concentrations. Reproduction behaviours were disrupted in the parasitoid wasp *Trichogramma brassicae* when exposed to the insecticide chlorpyrifos at a dose with no apparent mortality (LD0.1, Delpuech et al., 1998). Effects can also be rapid: a study of movement behaviour in the plant bug *Deraeocoris brevis* found that at the maximum field rate of acetamiprid, 89% of individuals displayed immobility after just two hours with the remaining samples showing impaired mobility; at 0.1× maximum field rate 22% showed immobility and the rest showed impaired mobility (Kim et al., 2006).

Several studies have looked at how movement behaviour can be affected. Adult female mites (*G. occidentalis*) sprayed directly with acetamiprid and exposed to residues were observed to be lethargic after 48 hours of exposure to 0.1× maximum field rate (Beers and Schmidt, 2014). Orientation behaviour was affected in the parasitic wasp *Aphidius ervi* when exposed for 24 hours to dry residues of the pyrethroid lamba-cyhalothrin at a sublethal dose ($LD_{0.1}$), though negative responses had disappeared 24 hours after exposure ended, suggesting capacity for recovery

(Desneux et al., 2004). Another study demonstrated ability to recover from pesticide intoxication in *A. rhopalosiphi* exposed to deltamethrin within 12 hours of being removed from the insecticide source (Longley and Jepson, 1996). Effects on movement can also change over time: exposure to the neonicotinoid imidacloprid initially affected movement, particularly coordination and hyperactivity in the bee *Apis mellifera*; however, the symptoms gradually disappeared but eventually led to hypoactivity (Suchail et al., 2001). Singh *et al.* (2004) noted that behavioural responses, especially pesticide avoidance, could reduce exposure and therefore cancel out deleterious effects. However, a very recent study has shown that adaptive behaviours may not always arise, by showing that worker bees (*Bombus terrestris*) developed a preference for feeding from neonicotinoid treated flowers (Arce et al., 2018).

3.1.3 Avoidance Behaviour

Avoidance behaviour is an umbrella term covering behavioural responses to pesticide residues, and is seen as a rapid response to contaminants that protects individuals from their effects (Hellou, 2011). Repellence and irritation are two key defined avoidance behaviours, each with differing definitions in the scientific literature. Cordeiro *et al.* (2010) defined the behaviours in relation to studies where individuals have a choice on whether to reside on a contaminated surface or not, with repellence as spending < 1 sec on a treated surface, while irritability was defined as spending < 50% of an observation period on a treated surface. A similar definition was proposed by Beers and Schmidt-Jeffris (2015), with repellence defined as avoidance of treated surfaces by resting on residue-free surfaces; however, their definition of irritation varied, with it being defined as the tendency to run off a test arena to escape residues entirely. Like Cordeiro and colleagues, the definition of repellence reported by Beers and Schmidt-Jeffris assumes the test individuals have a choice of resting on residues or not, though the latter authors accept that most bioassay test arenas represent a worst case exposure scenario where harmfulness may be overstated as test species would have no option of a surface with no residue. Guedes *et al.* (2016) offers an alternative demarcation of the two behaviours, defining repellence as a behavioural

response following extensive contact with residues, while irritation arises from little to no pesticide contact.

There are both positive and negative implications of repellence: on the individual level, contact with toxicants is reduced which reduces negative effects; however, repellence can also disperse non-target arthropod populations, therefore decreasing predator populations within crop systems where their biocontrol service is desired (Gerson et al., 2003; Hislop et al., 1981). Resurgences of spider mite pest populations have been observed in apple orchards, with the effect attributed to the use of pyrethroids that repel predatory mites (Gerson and Cohen, 1989); resurgences were also associated with deltamethrin and acetamiprid spray in aubergine fields (Barbar, 2017). Therefore, repellence is an important side effect to consider at the population level and in the biocontrol context.

The examples discussed above cover a range of spatial scales. Laboratory studies investigate avoidance at small, highly controlled spatial scales often at 10 cm or below to quantify avoidance behaviours in individuals in terms of time spent in contact with residues. Field studies on the other hand consider much larger spatial scales and investigate population-scale avoidance and repellence, with experimental areas of one or more field systems covering many hectares and even larger areas of leaf surface area. Consequently, avoidance behaviour also needs to be considered in the context of the spatial scales in which certain behaviours would be observed.

There is also an ecological context to spatial scales, with the natural range of an organism being an important point to consider when investigating avoidance behaviour. Morphological differences mean that different species can cover vastly different ranges, meaning different spatial scales would be of relevance to different species and even within species where large morphological changes occur during development. For example, a 10 cm test arena may be appropriate for some predatory mites, or ladybirds, or for a lacewing larva that is capable of covering over 100 cm in ten minutes (Porcel, Cotes and Campos, 2011). However, this would be inappropriate for a lacewing adult that can easily cover several kilometres (Duelli, 1984), or for predatory mites or collembolan that would be better suited to smaller test arenas of 5 cm or below (e.g. Zortéa et al., 2015).

Avoidance behaviour has been reported in many species. Avoidance (reported as escaping test arenas) has been observed in the predatory mite *Typhlodromus pyri* when exposed to residues of the carbamate fungicides mancozeb and metiram (Blümel et al., 2000b). A study of *C. septempunctata* feeding behaviour observed avoidance of dimethoate-treated surfaces and treated prey, showing avoidance behaviour extends to avoidance of contaminated food sources (Singh et al., 2004). Another study investigating changes in foraging behaviour showed that the parasitoid *A. rhopalosiphi* spent less time in areas treated with both honeydew (a food cue for the parasitoid) and deltamethrin compared to areas of just honeydew (Longley and Jepson, 1996).

3.1.4 Study aim, species and pesticide selection

With the present study we aimed to investigate whether pesticide residues affected movement behaviour in the non-target arthropod *Typhlodromus pyri*. *T. pyri* is a generalist phytoseiid species that consumes spider mite prey, but also subsists on honeydew, pollen and fungi (McMurtry and Rodriguez, 1987), and it lives in many on-crop habitats such as grapevines (Pozzebon et al., 2015), and apples (Duso and Pasini, 2003) throughout humid climates in Europe, North America, Asia and New Zealand where it is a major biocontrol agent against the pest European red mite, *Panonychus ulmi* (Bowie et al., 1999; Helle and Sabelis, 1985; Nauen et al., 2001). It is also one of the most important predatory mites in Europe and North America due to a combination of its abundance and beneficial capacity (Kostiainen and Hoy, 1996). The species also has major relevance to pesticide regulation as it is one of two mandatory study species in the risk assessment of plant protection products and their effects on non-target arthropods in the EU (EFSA, 2015).

We chose four pesticide active substances – active substances were studied so that any effects observed were due to the active substance and not any co-formulants contained within a product. To cover several scenarios, we selected study substances based on current literature, known toxicity to *T. pyri* as a non-target arthropod and relevance to both environmental exposure scenarios and regulatory testing. Based on these criteria, the following substances were chosen – three insecticides and one fungicide – each from different chemical classes: acetamiprid, a neonicotinoid

acetylcholine receptor agonist; deltamethrin, a pyrethroid sodium channel modulator; dimethoate, an organophosphate acetylcholinesterase inhibitor; and the fungicide captan, a phthalimide with protective and curative actions against fungal pests. All three insecticides have both contact and stomach action (Lewis et al., 2016).

Acetamiprid and deltamethrin are registered within the EU for use in pome fruit e.g. apples, hence their inclusion in this study (EFSA, 2016; European Commission, 2002). Acetamiprid is of interest due to a near 2000% increase in usage in UK orchards between 2014 and 2016, apportioned to the withdrawal of chlorpyrifos (Garthwaite et al., 2017); therefore it is of environmental relevance in real world exposure considerations. Additionally, a recent study observed reductions in prey consumption when another predatory mite, *G. occidentalis* was exposed to acetamiprid doses of 10% of the usage rate (Beers and Schmidt, 2014). Deltamethrin was included due to observations of repellence and avoidance behaviour in predatory mites exposed to pyrethroids in both laboratory and field studies (e.g. Penman and Chapman, 1981; Riedl and Hoying, 1983). Dimethoate is not registered for use in pome fruit crops; however, it is used as the toxic reference chemical in laboratory- and field-based registration studies (Blümel et al., 2000a), hence its inclusion in this study. Captan, the only fungicide in the study, was selected based on it being the most extensively used fungicide within UK fruit orchards in 2016 (Garthwaite et al., 2017). Though the substance is classed as having low toxicity to *T. pyri*, with 7% mortality observed at a rate of 1.7 $kg_{a.s.}$ Ha^{-1} when the maximum rate in pome fruit is 2.4 $kg_{a.s.}$ Ha^{-1} (EFSA, 2008), it was included to investigate whether a low toxicity substance could elicit behavioural changes and also whether this fungicide would elicit avoidance behaviours like those observed in carbamate fungicides (Blümel et al., 2000b).

3.1.5 Hypotheses

Based on the information on the test substances above, it was appropriate to develop individual hypotheses for each substance. We hypothesised that acetamiprid, deltamethrin and dimethoate residues would lead to changes in movement behaviour based on previously reported changes in feeding behaviour in a phytoseiid (Beers and Schmidt-Jeffris, 2015), repellency observed in phytoseiids exposed to

pyrethroids (e.g. Riedl and Hoying, 1983) and high toxicity to the test species (Blümel et al., 2000a) respectively. Though we intended to analyse movement behaviour in mites exposed to captan residues, analysis of the experiment was not possible due to poor analytical recoveries and crystallisation of the active substance on the test arena surface. Consequently, we have included the methodology used for captan but have not explored a hypothesis for this substance.

3.2 Materials and methods

3.2.1 Insects

We sourced synchronised *Typhlodromus pyri* eggs from a pesticide sensitive culture at Bias Labs Ltd. (Kirkcaldy, Fife, UK), and these were grown in culturing arenas comprising acrylic sheet placed above a deionised water reservoir contained within a plastic box with a loosely placed lid. The culturing arena was developed based on guidance for regulatory testing and training in *T. pyri* culturing at a contract research laboratory (Blümel et al., 2000a). The culturing arena surface included filter paper providing constant water to the mites, small plastic shelters created from the plastic substrate on which eggs were delivered, and insect barrier glue (Agralan Ltd, Wiltshire, UK) to prevent mite escape. The culture was kept in a growth chamber (Sanyo MLR-351H, Sanyo Electric Co., Osaka, Japan) at a target temperature of 25˚C (mean 25.1˚C; 95% Confidence Intervals (CIs) [25.05; 25.19]) and a target relative humidity of 80% (mean 78.2%; 95% CIs [77.2; 79.2]) in a 24-hour constant light cycle providing approximate illuminance of 15 000 lx. Eggs hatched approximately 24 hours after placement in the growth chamber and protonymphs were reared on apple pollen to adulthood, which took seven days. All test individuals were 7 – 9 days old when analysed.

3.2.2 Insecticides

To allow for rapid, high throughput quantification of pesticide residues in this experiment, we decided to use radiolabelled active substances. ^{14}C radiolabelled active substances were sourced from two providers: ^{14}C-acetamiprid (methylene-

labelled; 98.3% purity; 1188 MBq $mmol^{-1}$) was sourced from Triskelion B.V. (Zeist, Netherlands); ^{14}C-captan (carboximide-labeled; 96.7% purity; 2173 MBq $mmol^{-1}$), ^{14}C-deltamethrin (benzyl-7-labeled; 98.7% purity; 1432 MBq $mmol^{-1}$) and ^{14}C-dimethoate (carbonyl-labeled; 96.7% purity; 1681 MBq $mmol^{-1}$) were sourced from the Institute of Isotopes (Budapest, Hungary). To create the stock solutions, we separately mixed ^{14}C-acetamiprid, ^{14}C-captan and ^{14}C-dimethoate in methanol with analytical grade standards of the respective substance (Sigma Aldrich, Dorset, UK) to achieve the desired concentrations. Due to the low concentrations studied, the deltamethrin stock solution comprised ^{14}C-deltamethrin in methanol only. Stock solutions were created at the following active substance concentrations: 0.18 mg mL^{-1} for acetamiprid; 0.48 mg mL^{-1} for captan; 1×10^{-4} mg mL^{-1} for deltamethrin and 0.115 mg mL^{-1} for dimethoate.

We prepared three dosing stocks from each solution; for acetamiprid, deltamethrin and dimethoate these were based on published lethal rates at which 50% of a *T. pyri* population was affected after seven days of exposure (7d-LR_{50}s) for the active substances with treatment levels based on 0.2, 1 and 2× LR_{50} (Table 3.1). For captan the dosing stocks were based on 15%, 7.5% and 1.5% of the maximum field concentration (3.2 $g_{a.s.}$ L^{-1} based on a product containing 80% captan). For acetamiprid and dimethoate we drew the seven day LR_{50} values from European Union substance registration documents; for deltamethrin, at the time of experimentation no published LR_{50} was publicly available in registration documents and therefore we derived the dosing stock concentrations from effect data derived from a three day exposure study in the scientific literature (Bonafos et al., 2007).

Pyrethroid resistance had to be considered when deciding on deltamethrin dosing stocks. The study published by Bonafos *et al.* (2007) studied two wild strains of *T. pyri* collected in France: one population was collected from an unsprayed and uncultivated area of bramble (*Rubus spp*); the other population suspected to be resistant to synthetic pyrethroids was collected from vineyards where synthetic pyrethroids and organophosphate insecticides were regularly sprayed. The two strains displayed very different LC_{50}s after three days: the first population had a 3d-LC_{50} of 0.05 mg L^{-1} (95% CIs [0.03, 0.08]), while the second population was much less

susceptible with a 3d-LC_{50} of 79 mg L^{-1} (95% CIs [46; 150]). The managing director of the company that supplied the *T. pyri* for study confirmed that the strain used in our studies had never been exposed to synthetic pyrethroids (M Wainwright, 2018, personal communication, 17 August); therefore, we based the dosing stocks on the lower 3d-LC_{50} of 0.05 mg L^{-1}. Since the experiments were completed a 7d-LR_{50} was published in a draft EU renewal report from a higher tier *T. pyri* study conducted on leaf discs; this is quoted in Table 3.1 and equated to 0.0002 mg L^{-1} based on the application being carried in 200 L water (European Commission, 2017).

Table 3.1 – Insecticidal active substances used in the present study with the lethal rate at which 50% of a *Typhlodromus pyri* population displays mortality after 7 days (7d-LR_{50}) expressed as $g_{a.s.}$ Ha^{-1} and $mg_{a.s.}$ L^{-1}; both of these values are based on an application rate of 200 L Ha^{-1} (Blümel et al., 2000a). Also shown are the permitted application rates for formulated products in pome fruit orchards; and the three treatment levels for the present study expressed as a liquid concentration and active substance mass per unit area of test arena.

Active substance	**7d-LR_{50} (g Ha^{-1})**	**7d-LR_{50} (mg L^{-1})**	**Field application Rate ($g_{a.s.}$ Ha^{-1})**	**Dose concentrations ($mg_{a.s.}$ mL^{-1})**			**Target residue ($\mu g_{a.s.}$ cm^{-2})**		
				1	**2**	**3**	**1**	**2**	**3**
Acetamiprid	18 [a]	90	75	0.018	0.09	0.18	0.95	4.77	9.54
Captan	1490 [b]	7450	2000	0.049 [f]	0.24 [f]	0.49 [f]	25.44	12.72	2.54
Deltamethrin	0.000043 [c]	0.00022	7.5	0.00001 [g]	0.00005 [g]	0.0001 [g]	0.00053	0.0027	0.0053
Dimethoate	2.24 [d]	11.2	- [e]	0.0045	0.022	0.045	0.24	1.19	2.38

n.b. *1 µg cm^{-2} equates to 100 g Ha^{-1}*. Field application rates derived from pesticide formulation product labels.

[a] – European Food Safety Authority (EFSA, 2016)
[b] – LR_{50} was not identified in studies; 7.2% mortality observed at this rate (EFSA, 2008)
[c] – European Commission (2017)
[d] – European Food Safety Authority (2013)
[e] – Dimethoate use in apple orchards is not permitted
[f] – Concentrations based on 1.5%, 7.5% and 15% of field application rate due to solubility issues
[g] – Based on an LC_{50} of 0.05 mg L^{-1} (Bonafos et al., 2007)

3.2.3 Behavioural bioassays

3.2.3.1 Method development

Initially, we aimed for the behaviour study experimental set up to be as environmentally relevant and realistic as possible, while also creating a consistent and controlled setting. *T. pyri* adults are known to reside on most parts of an apple tree throughout the year (Breth et al., 1998), but the most practical substrate for a behaviour bioassay was the apple leaf where female adults spend most time and prefer to oviposit (Sengonca et al., 2004). Mites are observed to occur most frequently on leaf undersides (Gerson et al., 2003), and higher tier registration studies are conducted on leaf undersides, with bean (*Vicia faba*) and bell pepper leaves commonly used (Blümel et al., 2000a; European Commission, 2017). We cultivated beans in the laboratory for leaves to harvest; however, the leaf underside had a significant ridge where leaf veins were, and mites would disappear in the shadow of the ridge, becoming undetectable for a period. Therefore, we transferred individual *T. pyri* adults onto the upper surface of a leaf disc placed with the underside on damp paper towel, used to prevent leaf desiccation and to maintain humidity.

Mites were recorded using a Dino-Lite USB digital microscope mounted on a microscope pole stand (10 frames per second; Dino-Lite AM7013MT fitted with polarising filter; AnMo Electronics, Taiwan) and the videos were initially analysed in the free software package Ctrax (Branson et al., 2009). However, animal tracking in such software requires contrast between the test animal and test substrate, and the green leaf offered insufficient contrast against the cream mite (Figure 3.1). We therefore discounted natural substrates as test arenas and instead decided that the two priorities were a flat surface for accurate movement tracking and homogeneous pesticide coverage, and a substrate that allowed for a dark background to optimise contrast between the mite and the surface. We explored filter papers as they had been used previously in studies (Cordeiro et al., 2010; e.g. Pekár and Haddad, 2005), and would provide a consistent pesticide exposure environment. Black filter papers (Thomas Scientific, Swedesboro, New Jersey, United States) were tested; however, we found the surface was not consistent and small white fibres within black filter paper affected mite detection, so these were also discounted. We also considered

the ease of recovering pesticide residues following behaviour observation, and as a result, we decided upon a glass surface placed on black cardboard as the final test arena design. This decision had the added advantage of homogenising the surface texture, therefore removing the influence of leaf morphology (Penman and Chapman, 1981). A previous study highlighted the difficulty of tracking movement in *T. pyri* due to their translucent body and poor camera resolution, and used a 16 mm glass microscope slide coverslip as the test arena (Bowie et al., 1999). With improved resolution in contemporary cameras, we were able to use a 24 mm diameter circular glass coverslip as the test arena while also retaining clear focus with the USB microscope recording the mite.

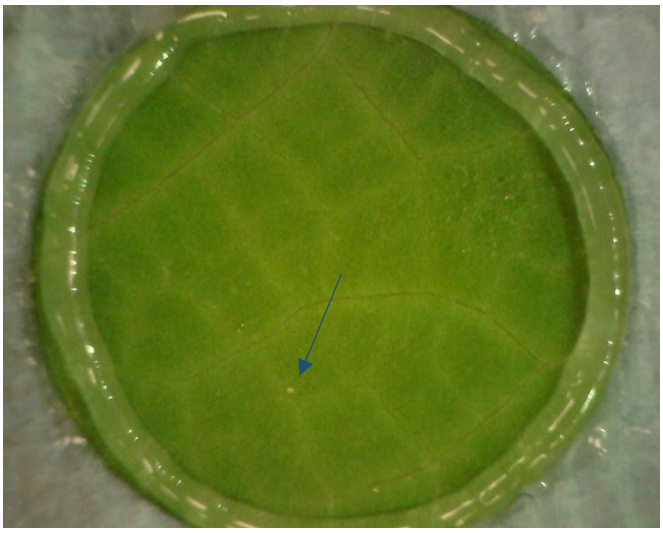


Figure 3.1 – Screenshot of a sample video of the preliminary movement test arena with a bean leaf disc. The *T. pyri* individual can be seen towards the centre bottom of the test arena and is highlighted by the blue arrow.

We also decided to change movement behaviour software and, as a result, the final experiments were analysed in Noldus EthoVision XT 13 (Noldus Information Technology, Wageningen, Netherlands). We chose the software package for the more efficient working process, greater ability to cancel out spurious detections (e.g. those caused by uneven lighting and reflections), and the ability to manually draw arenas and zones, ensuring only the area available to mites was considered in

analysis. It is also the main software used in contemporary studies of digital movement behaviour analysis of non-target arthropods (Azandeme-Hounmalon et al., 2016; Nansen et al., 2016; Porcel et al., 2011; e.g. Prasifka et al., 2008).

3.2.3.2 Experimental set up

We created test arenas by placing circular glass microscope slide coverslips (24 mm diameter, Scientific Laboratory Supplies Ltd., Nottingham, UK) in duplicate on black cardboard, giving two replicates per card. Insect barrier glue (Agralan, Wiltshire, UK) was applied using a syringe around the coverslip edges to act as an arena barrier and adhesive to hold the arenas in place. Mean arena surface area was approximately 2.83 cm^2. 0.15 mL of dosing stock was applied to each arena surface using a pipette, ensuring the solution fully covered the surface. Control arenas were treated with 0.15 mL methanol. Test arenas were left to dry for 75 min in a fume cupboard before the behavioural bioassay commenced. We used a randomised block design for the experiment, with random allocation of insecticide concentrations to test arenas to minimise any effects relating to time of day. To eliminate the risk of cross-contamination of insecticides, one active substance was studied at a time.

A single sexed *T. pyri* adult was removed from the culture and placed in the centre of a test arena using a fine (size 0) nylon paintbrush and was given 10 min to acclimatise to the new environment; however, we reduced the acclimatisation time to 30 sec for deltamethrin residues due to immediate responses to residues. Twenty four arenas were used for each treatment, and each mite was used once (12 female; 12 male), giving 24 independent replicates per treatment. Control contained 30 observed mites (15 female; 15 male) as the initial experimental design included 30 replicates per treatment; however, this was unfeasible in the time scale, so replicate numbers were reduced. Replicates were handled in duplicate, with two test arenas established at a time for simultaneous recording. Test arenas were placed on a thermostatically controlled heated propagator base set to 26°C (aiming to achieve 24°C) in preparation for measurement. Test arenas were lit using a targeted small white LED light. Humidity was increased in the heated propagator through the addition of blue roll saturated with deionised water. However, humidity was otherwise ambient and

heavily influenced by the prevailing weather conditions and intermittent issues with the air circulation systems in the laboratory.

We recorded the movement of each mite for a 10 min observation period using a Dino-Lite USB digital microscope mounted on a microscope pole stand (10 frames per second; Dino-Lite AM7013MT fitted with polarising filter; AnMo Electronics, Taiwan); however, equipment failure during the study necessitated a change of recording equipment to a Canon DSLR camera (25 frames per second; EOS 1300D, Canon Inc., Tokyo, Japan) equipped with an 18-55 mm lens mounted on a metal hood (Syngene DigiGenius, Synoptics Ltd., Cambridge, UK). At the end of each recording, we lightly brushed mites that had ceased moving with the paintbrush to see whether mortality had occurred. Air temperature and relative humidity were recorded once during each observation period, approximately 1 min into the observation. The videos were then converted to mpeg format using the open source video transcoder software HandBrake (version 1.1.1) for movement behaviour analysis.

3.2.3.3 Movement analysis

We analysed mite movement behaviours using the video tracking software Noldus EthoVision XT 13. Videos were analysed at a rate of 5 frames per second to allow consistency in analysis between recordings from the different cameras. There were problems with successfully detecting and tracking mites in arenas treated with captan as the active substance left a white crystalline residue on the surface that meant the mite was often undetectable (Figure 3.2); this was thought to be due to the low substance solubility and subsequent precipitation as the methanol evaporated from the arena surface. As such it was not possible to successfully analyse movement behaviour and we did not analyse captan samples further.

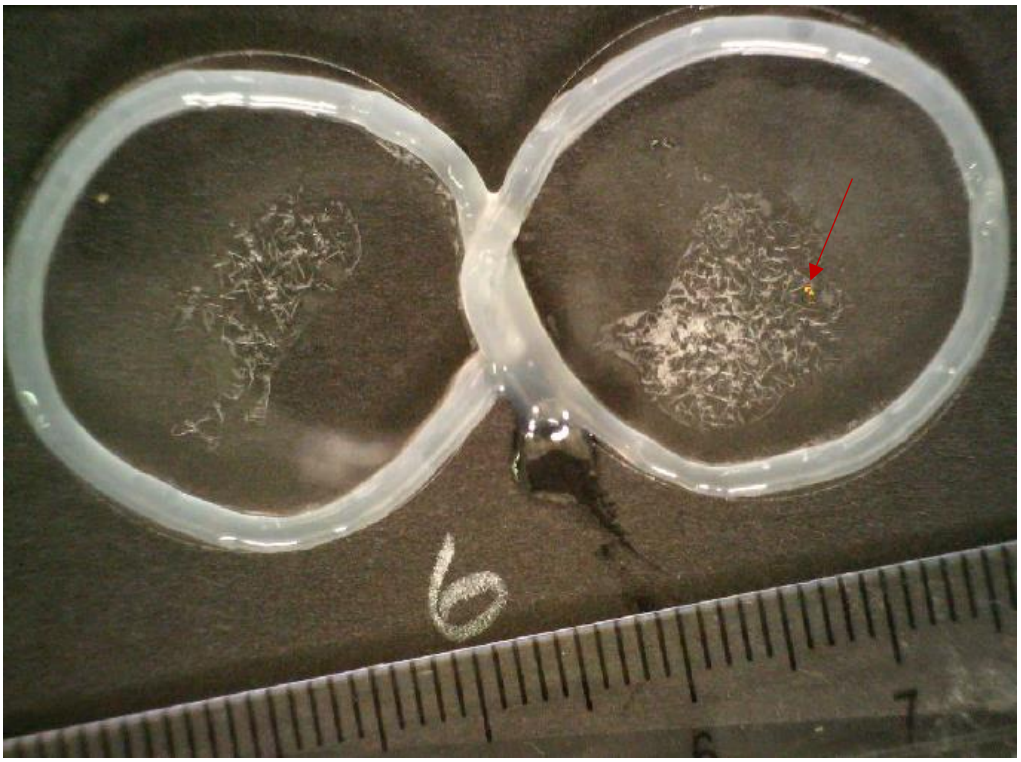


Figure 3.2 – Screenshot of a sample video from the captan experiment as analysed in EthoVision XT 13, with a detected mite highlighted in yellow in the right hand arena and further highlighted by the red arrow. Note the white crystalline residues in the two test arenas – the left hand arena was treated with the second dosing level, and the right arena treated with the third dosing level. The crystalline structures created spurious detections in the software, thus rendering it impossible to successfully analyse *T. pyri* movement behaviour.

We approximated arena surface area using the arena width readings generated by the software (Equation 3.1).

$$Arena\ area\ (cm^2) = \pi \left(\frac{\bar{x}_d}{2}\right)^2 \qquad \textbf{Equation 3.1}$$

$\bar{x}_d$ denotes mean arena diameter.

Five movement behaviours were automatically analysed by the software. The definitions for each behaviour are as follows, adapted from Kaur *et al.* (2015); mean measurements were averaged over measurements taken every sampled frame, with 3000 measurements taken per 10 min observation period:

1. Distance walked (total; cm): the total distance covered by a mite over the 10 min observation period. This estimated general mite activity;
2. Velocity (mean; cm s^{-1}): the walking speed of the mite within the arena over 10 min. This also estimated general mite activity;
3. Angular velocity (mean; deg s^{-1}): the mean change in mite moving direction over time. This was calculated as the ratio between turn angle and sample interval;
4. Meander (mean; deg cm^{-1}): the change in mite moving direction relative to distance covered. This measure quantifies the level of tortuosity of the mite's movement track;
5. Time active (total; s): the time spent moving and not moving was determined by the software through use of thresholds in the distance moved per frame. The application of these thresholds (0.1 mm s^{-1} for moving; 0.09 mm s^{-1} for not moving averaged over 3 samples) minimised the influence of small wobbling movements created as an artefact of video resolution and frame rate. Only the time spent moving is reported.

Though the sampling rate and thresholds applied for movement analysis helped to reduce noise, artefacts and noise arising from the analysis of videos is unavoidable and measured behaviours are highly sensitive to this noise (Hen et al., 2004). We applied two smoothing methods to each sample: locally weighted scatter plot smoothing (LOWESS; Cleveland, 1979); and minimal distance moved (MDM). The LOWESS process is iterative and involves fitting a curve to the data in sample windows – in this case each window is 10 samples. Hen *et al.* (2004) explain the process in detail regarding its application in EthoVision. It is useful for reducing the noise created by body wobble or the video resolution. The second method, MDM, filters out small movements caused by random noise, especially when the individual is inactive, by setting a threshold (Noldus, 2018). We used the "direct" method, which

is calculated based on the shortest distance between samples, and the threshold was 0.03 mm.

We manually analysed a sixth movement behaviour, time to trap, by watching each video and recording the point at which a mite became trapped in the arena barrier with a keystroke. Where this occurred, it was necessary to correct velocity and angular velocity as these were calculated based on the overall observation duration (10 min); therefore, the EthoVision readings for these behaviours were skewed by the time spent trapped. We corrected these values to ensure the measure only accounted for the time in which the mite remained in the arena (Equation 3.2).

$$v_{corr}\ or\ \omega_{corr} = \frac{v_{est}\ or\ \omega_{est}}{\left(\frac{T_t}{T_e}\right)} \qquad \textbf{Equation 3.2}$$

v_{corr} denotes velocity corrected for a mite becoming trapped. ω_{corr} denotes angular velocity corrected for a mite becoming trapped. v_{est} denotes velocity as estimated by EthoVision. ω_{est} denotes angular velocity as estimated by EthoVision. T_t denotes time at which a mite became trapped in seconds. T_e denotes experimental test duration in seconds (600 s).

3.2.4 Pesticide residue analysis

We quantified actual pesticide residues by measuring the radioactivity present following extraction of the test arena. Following the observation period, each test arena was transferred into a tall 50 mL glass beaker and 10 mL methanol was added. Beakers were covered to reduce methanol evaporation and left for 10 min. Following extraction an 8 mL aliquot of the sample was transferred into a 20 mL HDPE screw cap scintillation vial containing 10 mL Ecoscint A (National Diagnostics, Nottingham, UK) and the activity quantified via liquid scintillation counting (Hidex 300 SL, Hidex Oy, Turku, Finland). Analysis commenced after a time delay of 2.5 hours for each sample run; each vial was counted three times for 60 sec. We corrected samples for background activity by subtracting readings derived from blank (control) vials containing 10 mL Ecoscint A + 8 mL methanol. Activity related to insecticide

concentration in dosing stocks was calculated (Equation 3.3 (1)), and from this, we calculated insecticide residue on the test arena surface (Equation 3.3 (2)). Finally, we adjusted the measured residue in each arena to express residues based on approximate arena area.

(1) $$M_{\beta} = \frac{\rho_{a.s.}}{A_{a.s.}}$$ **Equation 3.3**

(2) $$Residue\ in\ arena\ (mg) = \frac{M_{\beta}}{A_s}$$

M_{β} denotes active substance mass per unit activity, expressed as mg KBq^{-1}. $\rho_{a.s.}$ denotes mass concentration of the active substance expressed as mg mL^{-1}. $A_{a.s.}$ denotes measured activity of dosing stock, expressed as kBq mL^{-1}. A_s denotes activity measured in each sample, expressed as kBq.

3.2.4.1 Analytical method development

To quantify the counting method detection limits, we calculated three metrics: the critical level (L_c), the detection limit (L_d), and the lower limits of detection (LLD), which meant we could derive a minimum counts value to use as our methodological detection limit. L_c is the value used to decide whether a signal is a "true" signal (Currie, 1968), whereas the L_d is the true "net" signal that can be reliably detected, and the LLD is the minimal detectable activity, i.e. the lowest concentration of material that produces a signal above the background with a 95% probability (Passo and Cook, 1994). The formulae used to determine these are below and were selected as our background counts were below 70 counts (Equation 3.4; Prichard et al., 1992).

(1) $L_c\ (counts) = 0.1B + 2.33B^{0.5}$

(2) $L_d\ (counts) = 1.1(2.71 + 4.65B^{0.5}) + 0.1B$ **Equation 3.4**

(3) $LLD\ (Bq\ L^{-1}) = \frac{L_d}{60EVTX}$

$\boldsymbol{B}$ denotes mean background reading in counts. $\boldsymbol{E}$ denotes counting efficiency. $\boldsymbol{V}$ denotes sample volume in L. $\boldsymbol{T}$denotes counting time in min.

Finally, to determine how many counts must be measured to achieve the calculated LLD, we used Equation 3.5 (Passo and Cook, 1994):

$Minimum\ counts = L_c + B$ **Equation 3.5**

Using this method, we determined that for this experiment, L_c = 15.3 counts; L_d = 31.4 counts; and LLD = 3.45 Bq L^{-1}. To achieve this threshold, the minimum counts was 58.2.

3.2.5 Statistical analysis

In all subsequent analyses, we excluded mites that were not active during the observation period from further analysis of movement behaviour as the intention was to study the behavioural changes in mites that had moved. Final sample populations are presented in Table 3.2. All statistical analyses were undertaken in GraphPad Prism 7 (GraphPad Software Inc., La Jolla, USA) or SPSS 25 (IBM Inc., Armonk, USA), with software selection for each statistical test specified below.

Table 3.2 – The number of active and inactive mites for each studied insecticide split by treatment. Active mites comprised the final sample populations for statistical analysis of movement behaviours.

Insecticide	Concentration (µg cm^{-2})	Active mites	Inactive mites
Control	0	24	6
Acetamiprid	0.95	21	3
	4.77	18	6
	9.54	16	8
Deltamethrin	0.00053	23[a]	0
	0.0027	23[b]	0
	0.0053	25	0
Dimethoate	0.24	21	3
	1.19	19	5
	2.38	20	4

[a] – One replicate at this concentration comprised residues measuring in line with the highest concentration. Therefore, the sample was re-assigned to 0.0053 µg cm^{-2}

[b] – One replicate at this concentration was removed from final analysis after having been identified as a multivariate outlier. The process of identification is explained in section 3.2.5.2.

3.2.5.1 Environmental conditions

As each pesticide treatment was investigated at different time points, we wanted to investigate whether the environmental conditions were consistent from one pesticide to the next (i.e. from one week to the next) as this could help to inform conclusions. We compared air temperature and humidity measurements grouped by insecticide using one-way ANOVA in SPSS. The Brown-Forsythe test for equality of variances was used to determine what post-hoc test to apply: if equal variances could be assumed, Tukey's post hoc test was applied; if equal variances could not be assumed then the Games-Howell test was applied as the test is more robust where equal variances are not observed (Games et al., 1979). P values were automatically adjusted for multiple comparisons ($\alpha = 0.05$).

3.2.5.2 Environment effects on mite movement behaviour

Prior to investigating whether mite movement was affected by insecticide residues, we investigated whether air temperature and relative humidity affected mite movement behaviours to determine the final statistical test for each insecticide dataset. Linear regressions were undertaken in GraphPad Prism for each insecticide, correlating the five observed movement behaviours with air temperature and relative humidity. The outcome informed whether the environmental measures should be included in statistical analysis as covariates: where a slope was significantly not zero ($\alpha = 0.05$), and with a fit greater than $R^2 = 0.2$, then we included the relevant covariate. If these criteria were not met, then covariates were not included in final analysis. The fit criteria were selected to ensure that a weak trend arising between a behaviour and either temperature or humidity would be included in the final analysis for further exploration.

All results are summarised in Table 3.3. Three movement behaviours displayed significant slopes with weak correlations ($R^2 < 0.2$) to relative humidity when mites were exposed to acetamiprid; therefore, no covariates were included in the final acetamiprid test. No slopes generated between covariates and movement behaviours were significant when mites were exposed to deltamethrin and as such no covariates were included. When exposed to dimethoate, mite angular velocity correlated strongly with air temperature ($R^2 = 0.65$; $P < 0.0001$) and relative humidity ($R^2 = 0.57$; $P = 0.0005$); there were also significant but weak correlations ($R^2 < 0.2$) between both behaviours and relative humidity. Therefore, we included both environmental factors as covariates in the final dimethoate statistical test.

Table 3.3 – Influence of air temperature and relative humidity (covariates) on *T. pyri* movement behaviours, derived from correlation. Goodness of fit (R^2) is shown with *F* and *P* values indicating whether a slope is significant. Significant correlations (i.e. slope ≠ 0; α = 0.05) are in italic. *n* = 24 (control); 56 (acetamiprid); 71 (deltamethrin); 59 (dimethoate).

		Air temperature			**Relative humidity**		
Treatment	**Behaviour**	**R^2**	***F***	***P***	**R^2**	***F***	***P***
Control	Distance walked	0.106	2.6	0.121	0.065	1.54	0.228
	Velocity	0.028	0.62	0.439	<0.0001	0.0006	0.981
	Angular velocity	0.018	0.39	0.536	*0.171*	*4.52*	*0.045*
	Meander	0.073	1.74	0.2	0.119	2.97	0.099
	Time moving	<0.0001	0.0005	0.983	0.0003	0.006	0.94
Acetamiprid	Distance walked	0.012	0.68	0.413	*0.079*	*4.61*	*0.036*
	Velocity	0.013	0.73	0.395	*0.087*	*5.11*	*0.028*
	Angular velocity	0.013	0.68	0.412	0.01	0.53	0.47
	Meander	0.011	0.59	0.448	0.004	0.24	0.625
	Time moving	0.003	0.19	0.668	*0.09*	*5.37*	*0.024*
Deltamethrin	Distance walked	0.021	1.54	0.22	0.01	0.73	0.395
	Velocity	0.046	3.41	0.069	0.04	2.94	0.091
	Angular velocity	0.035	2.52	0.117	0.038	2.74	0.103
	Meander	0.004	0.31	0.581	0.005	0.37	0.55
	Time moving	0.03	2.18	0.144	0.009	0.63	0.431
Dimethoate	Distance walked	0.001	0.07	0.792	<0.0001	0.0003	0.99
	Velocity	0.0003	0.02	0.893	0.0001	0.007	0.93
	Angular velocity	*0.65*	*106*	*<0.0001*	*0.566*	*74.5*	*<0.0001*
	Meander	*0.202*	*14.4*	*0.0004*	*0.194*	*13.7*	*0.0005*
	Time moving	0.001	0.06	0.81	<0.0001	0.001	0.97

3.2.5.3 Mite movement behaviour

Prior to any analysis of movement behaviours, it was necessary to determine whether behaviour differed between mite sex as this would need to be included in the final statistical design if there were significant differences in response. Previous studies of avoidance and behaviour have focused on females only (Beers and Schmidt-Jeffris, 2015; Bowie et al., 2001, e.g. 1999), so we studied both sexes to see whether there were sex-specific responses.

We conducted t tests, one per movement behaviour, comparing male and female average measurements in the control mites. As the Student's t test is not robust when examining populations with unequal variances, we chose to use the unequal variance t test (also known as Welch's test) due to its more robust nature, in particular in handling behavioural data (Ruxton, 2006). Table 3.4 summarises the results of comparison; as there were no significant differences in movement behaviour we did not include mite sex as a factor in subsequent analyses.

Table 3.4 – Summary of comparison of movement behaviours between male and female *T. pyri* adults observed in control test arenas. Mean differences (comparing male to female), 95% confidence intervals of the difference, and Welch's test results are shown. n = 11 (female); 13 (male). $\alpha = 0.05$.

Movement Behaviour	Mean Difference	95% Confidence Intervals	*t*	*P*
Distance walked (cm)	-17.81	-44.51 – 8.9	1.39	0.18
Velocity (cm s^{-1})	-0.017	-0.054 – 0.019	0.97	0.34
Angular velocity (deg s^{-1})	-2.08	-6.43 – 2.27	0.33	0.99
Meander (deg cm^{-1})	-6.27	-117.4 – 104.8	0.12	0.91
Time active (s)	10.87	-179 – 201	0.12	0.91

Mite activity/inactivity

To investigate whether the number of inactive (resting) mites was changed by insecticide residues, we conducted the binomial observed versus expected test in GraphPad Prism, comparing each insecticide treatment (observed) to the control (expected). As the test required equal sample populations, we adjusted the control sample population total from 30 to 24 by multiplying the active and inactive counts by 0.8. Due to the multiple tests the original significance value threshold of $\alpha = 0.05$ was adjusted using Bonferroni's Correction, leading to a new level of $\alpha = 0.0167$.

Insecticide effects on movement behaviour

To test whether insecticide residues affected mite movement behaviour, we subjected the movement behaviour data related to acetamiprid and deltamethrin residues to general linear models in SPSS, with the two options being multivariate analysis of variance (MANOVA) or multivariate analysis of covariance (MANCOVA), with all five measured behaviours included in the statistical model and one analysis per insecticide. Based on the outcome of the linear regressions plotting movement behaviour and environmental factors, we decided that acetamiprid and deltamethrin would be subjected to MANOVA, and dimethoate subjected to MANCOVA, as this allowed for statistical elimination of the influence of temperature and humidity (D'Alonzo, 2004).

Prior to analysis, each dataset was tested for assumption violations. Multivariate outliers, defined as replicates with unusual combinations of values across the measured variables, are of importance as these can lead to skewed results (McCune and Grace, 2002). We conducted Mahalanobi's Distance test to identify such outliers in each insecticide dataset as measurement error could have been responsible (e.g. through inaccurate tracking in EthoVision). The test was based on a critical chi value of 20.52 ($\alpha = 0.001$; df = 5); and one replicate – from the 2.65×10^{-3} µg cm^{-2} deltamethrin treatment – was identified. On scrutiny of the data and video recording it appeared the mite may have been injured during transfer into the arena as the walking behaviour was atypically convoluted; therefore, the replicate was excluded from further analysis.

Box's M and Brown-Forsythe tests were conducted to assess equality of covariance and variance and inform selection of test statistic (Tables 3.5 – 3.6). For each test, the mean and mean difference ± 95% CIs are presented with interpretation of the Pillai's Trace results. Based on the significant Box's Test results, all three tests were interpreted based on the Pillai's Trace value as this statistic is more robust to violations of the equality of covariance assumption (Pillai and Hsu, 1979). We used the Brown-Forsythe test to decide which post-hoc test was appropriate to apply to any significant results. As before, where equal variances were observed, Tukey was applied; where variances were unequal, the Games-Howell test was applied instead. For acetamiprid, Tukey was applied as the post-hoc test; for deltamethrin and dimethoate Games-Howell was applied. P values were automatically adjusted for multiple comparisons ($\alpha = 0.05$).

Table 3.5 – Results of Box's M Test for equality of covariance for the three studied insecticides. Each insecticide included four treatment levels: three insecticide treatments and one control. $\alpha = 0.05$. Significant values suggesting assumption violation are highlighted in bold.

Insecticide	**Box's M**	***F***	***P***
Acetamiprid	344.6	**6.7**	**<0.0001**
Deltamethrin	457.5	**9.2**	**<0.0001**
Dimethoate	420.7	**8.3**	**<0.0001**

Table 3.6 – Results of the Brown-Forsythe test for equality of variance, assessed for each movement behaviour included in the multivariate model. Each insecticide included four treatment levels: three insecticide treatments and one control. $\alpha = 0.05$; significant values that suggest assumption violation are highlighted in bold.

	Distance walked		**Velocity**		**Angular velocity**		**Meander**		**Time active**	
Insecticide	***F***	***P***	***F***	***P***	***F***	***P***	***F***	***P***	***F***	***P***
Acetamiprid	1.02	0.39	0.08	0.97	0.39	0.76	1.67	0.18	1.56	0.21
Deltamethrin	**24.5**	**<0.0001**	2.64	0.054	**4.55**	**0.005**	**2.72**	**0.049**	**2.98**	**0.036**
Dimethoate	**3.08**	**0.03**	0.85	0.47	2.43	0.07	1.14	0.34	**7.99**	**<0.0001**

n.b. *Dimethoate values are derived from the final model design*

For the dimethoate experiment, one additional assumption required testing: homogeneity of regression slopes in relation to the air temperature and relative humidity covariates. We tested this assumption by comparing the slopes of each movement behaviour, split by insecticide treatment level, to examine whether the responses differed between treatments. Responses to both air temperature and humidity differed between treatments when measuring angular velocity (Table 3.7). Where the assumption of slope homogeneity is violated, it is recommended that the Johnson-Neyman procedure is applied to determine regions of insignificance in the covariates (D'Alonzo, 2004); however, due to complexity we did not undertake this. As such we interpret results regarding angular velocity with caution. To confirm the final MANCOVA model design, the model was run to ascertain whether treatment*covariate interactions were significant ($\alpha = 0.05$); if so, the interaction was retained in the model; if not, it was removed from the final model design. In the practice model run treatment*temperature was a significant interaction ($P < 0.0001$); however, treatment*humidity was not ($P = 0.31$). Therefore, we included dimethoate treatment; air temperature and relative humidity as covariates; and treatment*temperature as an interaction in the final MANCOVA model.

Table 3.7 – Results of the slope comparison test investigating variance in covariate response in the dimethoate dataset. Each movement behaviour was assessed against each environmental covariate. α = 0.05. A P value below 0.05 indicates that slopes generated for each dimethoate treatment are different, suggesting different responses to the covariate at different treatment levels. Significant outcomes are highlighted in bold.

Movement Behaviour	Covariate	*F*	*P*
Distance walked	Air Temperature	1.51	0.22
	Relative Humidity	1.08	0.36
Velocity	Air Temperature	0.27	0.85
	Relative Humidity	0.43	0.73
Angular velocity	**Air Temperature**	**4.07**	**0.01**
	Relative Humidity	**9.35**	**<0.0001**
Meander	Air Temperature	1.84	0.15
	Relative Humidity	2.57	0.06
Time active	Air Temperature	0.06	0.98
	Relative Humidity	0.64	0.59

3.3 Results

We observed no mite mortality during the observation period. Table 3.8 summarises the measured residue values for each insecticide and treatment level. Activity-based recoveries averaged 96% (95% CIs [91.4; 99.5]) for acetamiprid, 42% (95% CIs [39; 44.7%]) for captan, 79% (95% CIs [77.5; 81.2]) for deltamethrin and 95% (95% CIs [90.4; 99.5]) for dimethoate compared to calculated expected activity. We found no samples measured below the minimum counts threshold, therefore all sample measurements were deemed valid.

Table 3.8 – Mean insecticide residue values, 95% confidence intervals, coefficient of variance (CV) based on residue, mean recovery compared to expected activity levels and associated 95% confidence intervals for each insecticide treatment level. *n* is based on the final sample populations arising from discounting inactive mites.

Insecticide	Nominal dose (µg cm^{-2})	*n*	Mean residue (µg cm^{-2})	95% Confidence Intervals (µg cm^{-2})	CV (%)	Mean Recovery (%)	95% Confidence Intervals (%)
Acetamiprid	0.95	21	0.98	0.89 – 1.08	21	98.1	89.6 – 106.6
	4.77	19	4.49	4.01 – 4.98	22	95.1	85.3 – 105
	9.54	16	9.46	8.39 – 10.54	21	98.4	88.8 – 108.1
Captan	2.54	24	Not calculated due to poor recoveries			35.2	31.1 – 39.2
	12.72	24				48.0	42.0 – 54.0
	25.44	24				42.4	40.0 – 44.7
Deltamethrin	5.3×10^{-4}	23[a]	4.2×10^{-4}	$3.9 \times 10^{-4} – 4.4 \times 10^{-4}$	13	79.8	75.8 – 83.8
	2.65×10^{-3}	23	2×10^{-3}	$1.9 \times 10^{-3} – 2.1 \times 10^{-3}$	11	78	74.4 – 81.5
	5.3×10^{-3}	25[a]	4.2×10^{-3}	$4 \times 10^{-3} – 4.4 \times 10^{-3}$	11	80.7	78.4 – 83
Dimethoate	0.24	21	0.22	0.2 – 0.25	26	75.4	67.4 – 83.3
	1.19	19	1.19	1.07 – 1.3	20	98.3	88 – 108.5
	2.38	19	2.46	2.26 – 2.66	17	105.5	99.4 – 111.5

[a] *One sample replicate was erroneously dosed with the highest dose when the lowest dose was intended. The samples were reassigned based on their quantified residues and retained in the final analysis.*

3.3.1 Environmental conditions

Table 3.9 summarises the environmental conditions recorded throughout the movement behaviour observations. We found no significant difference in temperature between the treatments (ANOVA; $F = 0.82$; $P = 0.48$); however, there was a significant difference in relative humidity between the treatments (ANOVA; $F = 56$; $P < 0.0001$), with humidity lowest in the deltamethrin samples, followed by control, acetamiprid and with highest humidity in dimethoate. Table 3.10 summarises the post hoc analysis using the Games-Howell test. Graphs showing the spread of air temperature and humidity measurements for each treatment can be found in Appendix A.

Table 3.9 – Summary of air temperature and relative humidity measured during mite movement observations in each treatment.

		Air Temperature (°C)				Relative Humidity (%)			
Treatment	***n***	**Mean**	**95% Confidence Intervals**	**Minimum**	**Maximum**	**Mean**	**95% Confidence Intervals**	**Minimum**	**Maximum**
Control	24	23.7	23.4 – 23.93	22.8	24.8	48.8	45.96 – 51.62	43	67
Acetamiprid	56	23.8	23.44 – 24.07	22.6	26.8	53	52.11 – 53.93	46	66
Deltamethrin	71	23.5	23.25 – 23.64	22.2	25.9	44	43.39 – 44.66	39	50
Dimethoate	59	23.7	23.25 – 24.19	21.5	26.6	57	54.56 – 59.47	46	75

Table 3.10 – Summary of mean difference ± 95% confidence intervals and Games-Howell multiple comparisons test of differences in relative humidity measurements taken during mite movement observations. $\alpha = 0.05$; significant pairings are highlighted in bold.

Treatment Pairing	**Mean Difference (%)**	**95% Confidence Intervals**	***P***
Control vs Acetamiprid	-4.23	-8.16 – -0.29	**0.032**
Control vs Deltamethrin	4.76	0.91 – 8.62	**0.011**
Control vs Dimethoate	-8.23	-13.08 – -3.37	**< 0.0001**
Acetamiprid vs Deltamethrin	8.99	7.54 – 10.44	**< 0.0001**
Acetamiprid vs Dimethoate	-4	-7.44 – -0.56	**0.016**
Deltamethrin vs Dimethoate	-12.99	-16.33 – -9.65	**< 0.0001**

3.3.2 Mite movement behaviour

Tracks illustrative of typical mite walking behaviour on the control and insecticide treated arenas at each treatment level are shown in Figure 3.3. Comparing treated arenas to control, the largest visual difference in movement track comes between deltamethrin and control, where mites appear to cover less distance. The tendency to track around the outer arena barrier appeared absent in mites exposed to deltamethrin.

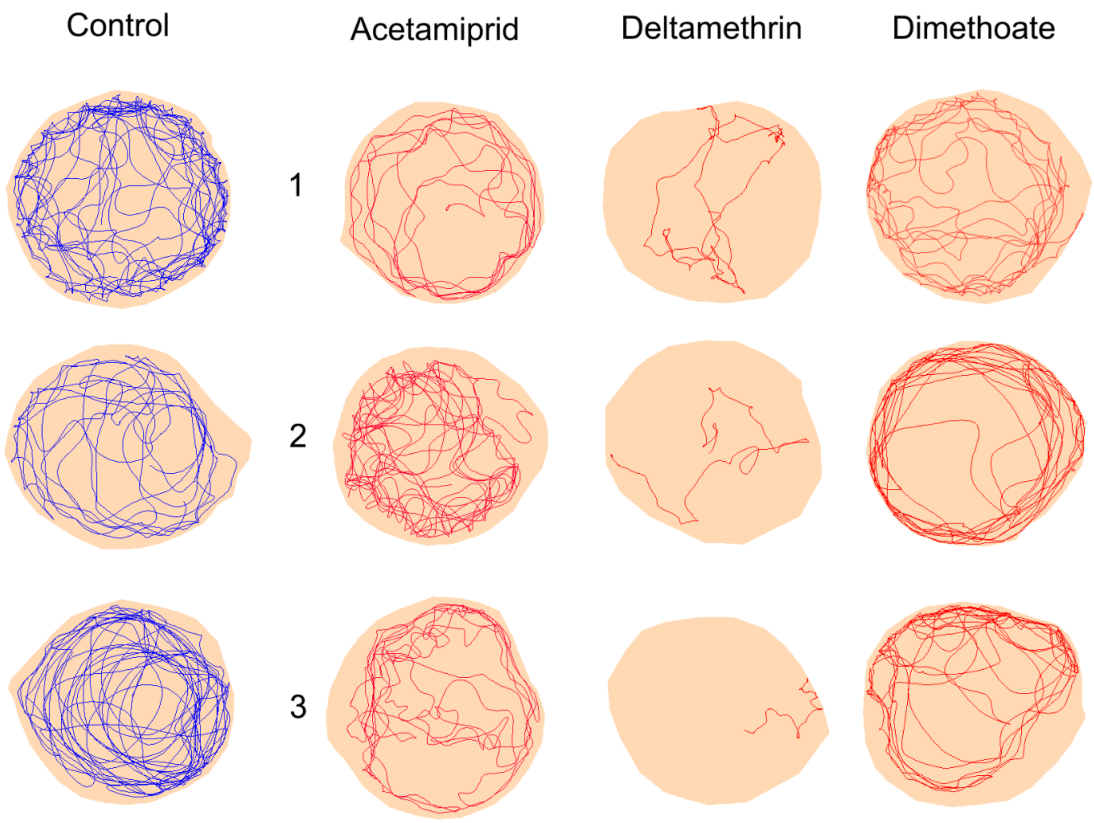


Figure 3.3 – Illustrative movement tracks from individual mites observed in the control (blue tracks) and insecticide treated test arenas (red tracks). Mean arena area was 2.83 cm^2. Each number corresponds to increasing insecticide treatment concentration – Acetamiprid 1 = 0.95 µg cm^{-2}; 2 = 4.77 µg cm^{-2}; 3 = 9.54 µg cm^{-2}. Deltamethrin 1 = 5.3 × 10^{-4} µg cm^{-2}; 2 = 2.65 × 10^{-3} µg cm^{-2}; 3 = 5.3 × 10^{-3} µg cm^{-2}. Dimethoate 1 = 0.24 µg cm^{-2}; 2 = 1.19 µg cm^{-2}; 3 = 2.38 µg cm^{-2}.

3.3.2.1 Mite activity/inactivity

Figure 3.4 shows the proportion of observed mites that were inactive or active in each insecticide treatment. When comparing these proportions at each treatment level (observed) against the control proportions (expected), we found no effect of acetamiprid residues at any concentration on the number of inactive mites (control = 20.8% inactive; 0.95 µg cm^{-2} = 12.5% inactive (95% CIs [4.3 – 31%]; P = 0.45); 4.77 µg cm^{-2} = 25% inactive (95% CIs [12 – 45%]; P = 0.62); 9.54 µg cm^{-2} = 33.3% inactive (95% CIs [18 – 53%]; P = 0.14)). Deltamethrin residues on the test arena led to no mites (0%) being inactive in any of the three treatments (95% CIs of observed [0; 13.8%]; P = 0.005 for all 3 treatments). Dimethoate residues did not change the proportion of inactive mites through the observation period (0.24 µg cm^{-2} = 12.5% inactive (95% CIs [4 – 31%]; P = 0.45); 1.19 µg cm^{-2} = 20.8% inactive (95% CIs [9 – 40%]; P > 0.999); 2.38 µg cm^{-2} = 16.7% inactive (95% CIs [7 – 36%]; P = 0.8).

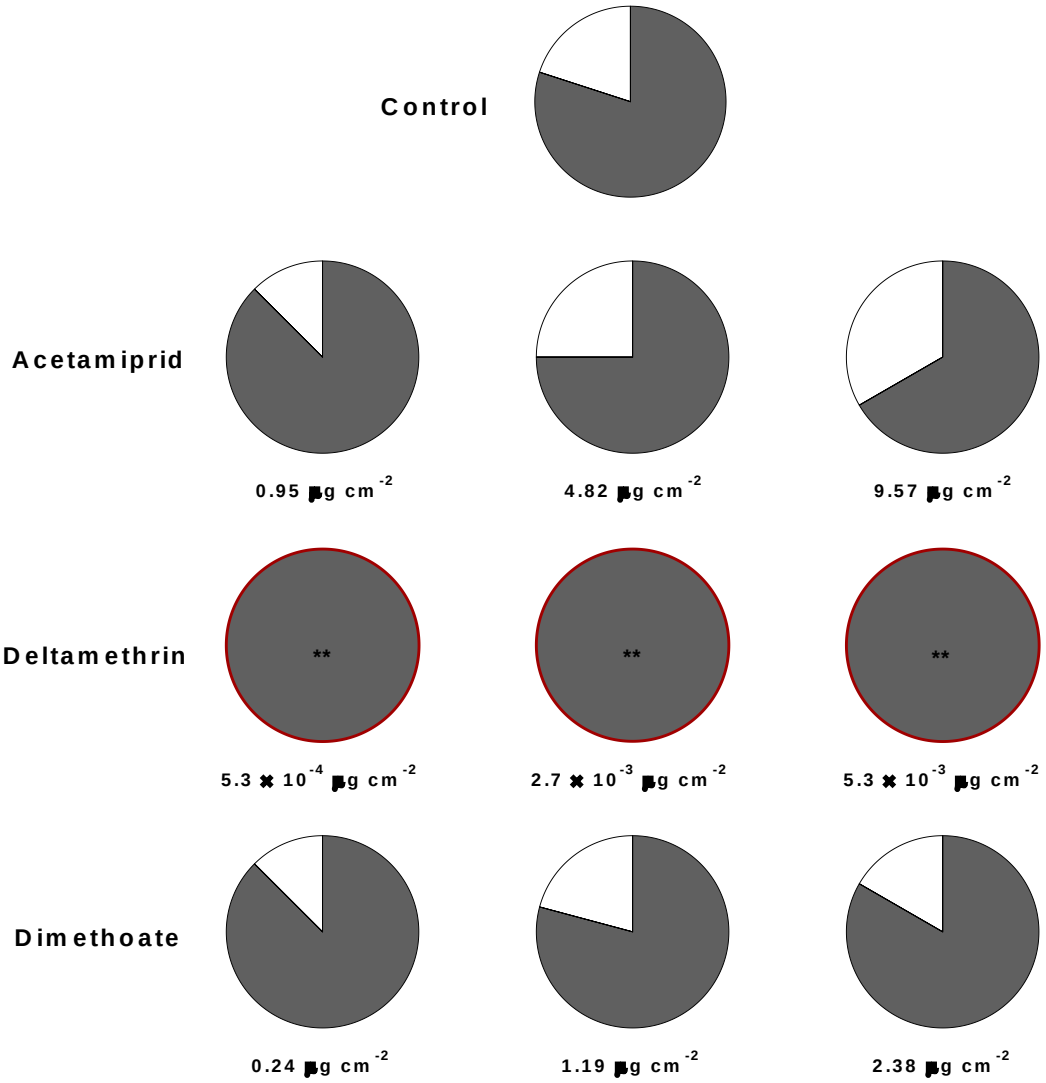


Figure 3.4 – Proportions of *T. pyri* individuals displaying activity (grey) or inactivity (white) during the 10 min observation period in control and insecticide treated arenas. Charts bordered in red signify a significant difference in the proportions when compared to control proportions ($\alpha = 0.016$; all significant pairings $P < 0.01$).

3.3.2.2 Insecticide effects on movement behaviours

Figure 3.5 summarises the effect of acetamiprid, deltamethrin and dimethoate on five movement behaviours (distance walked, velocity, angular velocity, meander and time active). For each insecticide we will discuss the effect of insecticide on movement behaviour considered as a whole; followed by a breakdown of effects on individual movement behaviours and finally effect of treatment levels. For dimethoate we will also discuss effect of environmental covariates. Effect sizes are discussed from interpretation of the partial eta squared (η^2) value.

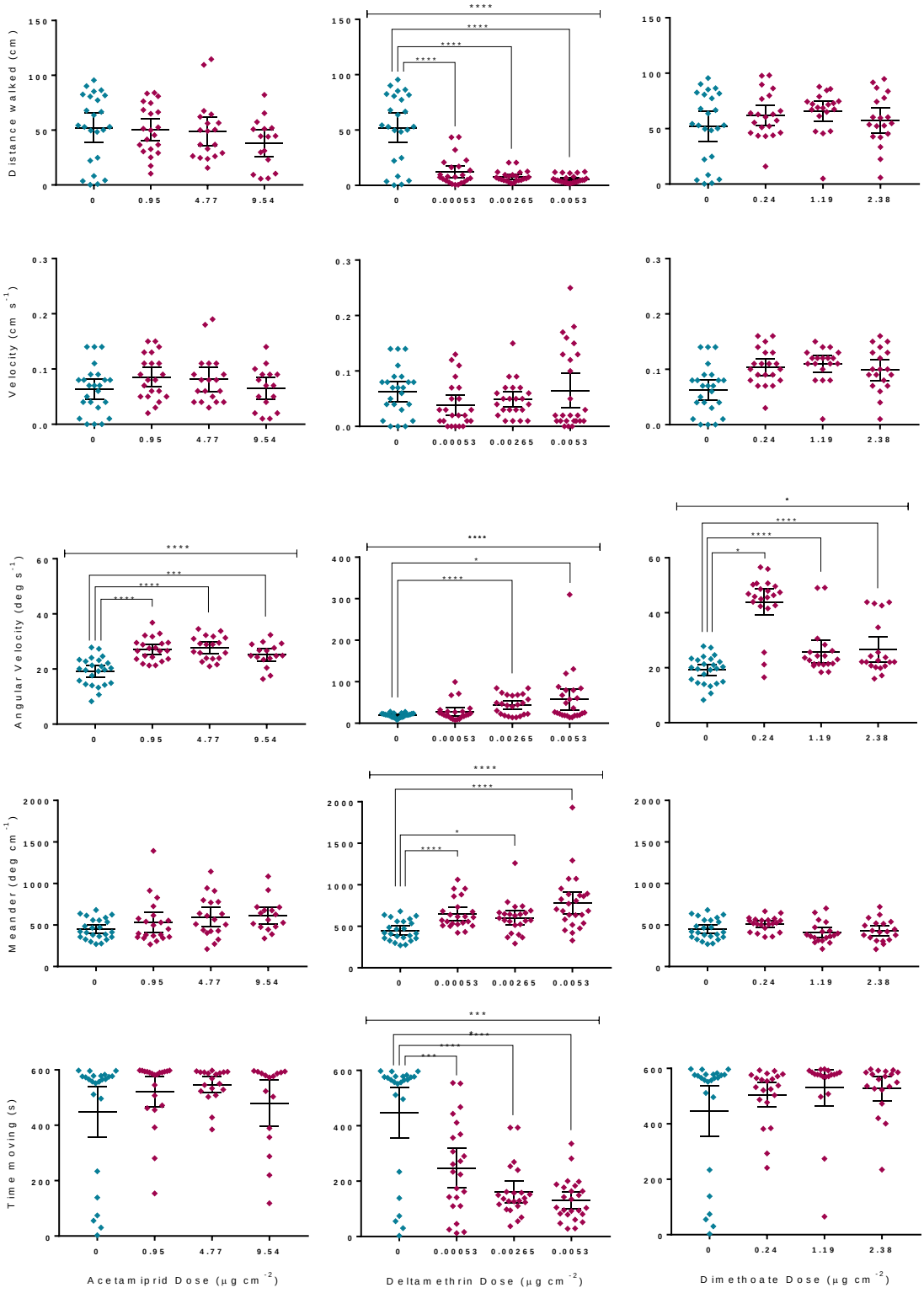


Figure 3.5 – Effect of acetamiprid (left column), deltamethrin (middle column) and dimethoate (right column), each at three concentrations, on the distance walked, velocity, angular velocity, meander and time spent active as observed in *Typhlodromus pyri* individuals over 10 min. Line and whiskers for each column shows

mean ± 95% confidence intervals at each treatment level. Individuals deemed inactive throughout the observation period were excluded from analysis. Note the different Y axis for deltamethrin versus angular velocity. Significant effects of insecticide are shown on each graph, with brackets between control and a treatment level displaying significance of effect between that treatment and control; brackets to the top of each graph display significant effects of the pesticide overall versus control (P values adjusted for multiple comparisons; * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$; **** = $P < 0.0001$). Control *n* = 24; acetamiprid *n* = 21; 18; 16; deltamethrin *n* = 23; 23; 25; dimethoate *n* = 21; 19; 20.

Acetamiprid

Exposure to acetamiprid residues affected the overall movement behaviour of *T. pyri* adults, though only one fifth of the variance seen is apportioned to acetamiprid (MANOVA; $F = 0.392$; $P < 0.0001$; $\eta^2 = 0.209$). When analysing each individual movement behaviour, angular velocity was found to be significantly higher in mites exposed to acetamiprid residues than those in control ($F = 16.99$; $P < 0.0001$; $\eta^2 = 0.4$). Angular velocity increased by 7.95 deg s^{-1} between 0.95 µg cm^{-2} and control, 8.5 deg s^{-1} between 4.77 µg cm^{-2} and control, and 6 deg s^{-1} between 9.54 µg cm^{-2} and control; results of the post-hoc analysis are summarised in Table 3.11. The other four movement behaviours displayed no significant effect of acetamiprid residue with negligible influence on the behaviours based on partial eta squared values of between 0.04 – 0.09.

Table 3.11 – Summary of Games-Howell post-hoc analysis of the differences in measures of angular velocity as measured in *T. pyri* adults observed in control or treated arenas containing acetamiprid residues. P values are corrected for multiple comparisons (α = 0.05). Significant differences between an acetamiprid concentration and control are highlighted in bold.

Movement Behaviour	Treatment Comparison	Mean Difference	95% Confidence Intervals		*P*
Angular velocity	0 vs 0.95 µg cm^{-2}	-7.95 deg s^{-1}	-11.48	-4.43	**<0.0001**
	0 vs 4.77 µg cm^{-2}	-8.53 deg s^{-1}	-12.15	-4.91	**<0.0001**
	0 vs 9.54 µg cm^{-2}	-5.98 deg s^{-1}	-9.78	-2.17	**0.0001**

Deltamethrin

When exposed to deltamethrin residues, overall mite movement behaviour was significantly affected (MANOVA; F = 6.42; P < 0.0001; η^2 = 0.27); the effect of deltamethrin was greater than that caused by acetamiprid. When investigating the effects of deltamethrin on each movement behaviour, four behaviours were significantly affected: distance walked by the mites was decreased by deltamethrin residues (F = 38.4; P < 0.0001; η^2 = 0.56); angular velocity increased (F = 5.4; P = 0.002; η^2 = 0.15); meander increased (F = 9.27; P < 0.0001; η^2 = 0.23); and time active decreased (F = 22.3; P < 0.0001; η^2 = 0.42). Velocity was not significantly affected by deltamethrin residues and treatment had a very small influence (η^2 = 0.05). The strongest effect of deltamethrin was observed in changes in distance walked and time spent active; deltamethrin had a relatively small influence on angular velocity and meander. Table 3.12 summarises the post-hoc Games-Howell analysis for the effect of each deltamethrin dose against control for each of the four significant movement behaviours. There were stepwise reductions in distance moved with increasing deltamethrin residue, increasing angular velocity with increasing residue levels, increasing meander with increasing residue level (though the mean meander observed in the second treatment was slightly lower than that observed in the first

treatment), and finally decreasing time spent active as deltamethrin residues increased.

Table 3.12 – Summary of Games-Howell post-hoc analysis of the differences in measures of distance moved, angular velocity, meander and time active as measured in *T. pyri* adults observed in control or treated arenas containing deltamethrin residues. P values are corrected for multiple comparisons ($\alpha = 0.05$). Significant differences between a deltamethrin concentration and control are highlighted in bold.

Movement Behaviour	Treatment Comparison	Mean Difference	95% Confidence Intervals		P
Distance walked	0 vs 5.3×10^{-4} µg cm^{-2}	39.8 cm	20.8	58.7	**<0.0001**
	0 vs 2.65×10^{-3} µg cm^{-2}	44.3 cm	26.2	62.3	**<0.0001**
	0 vs 5.3×10^{-3} µg cm^{-2}	46.5 cm	28.6	64.5	**<0.0001**
Angular velocity	0 vs 5.3×10^{-4} µg cm^{-2}	-8.6 deg s^{-1}	-21.8	4.6	0.3
	0 vs 2.65×10^{-3} µg cm^{-2}	-25.2 deg s^{-1}	-39.2	-11.3	**<0.0001**
	0 vs 5.3×10^{-3} µg cm^{-2}	-37.9 deg s^{-1}	-72.6	-3.3	**0.028**
Meander	0 vs 5.3×10^{-4} µg cm^{-2}	-199.3 deg cm^{-2}	-319.3	-79.2	**<0.0001**
	0 vs 2.65×10^{-3} µg cm^{-2}	-152.1 deg cm^{-2}	-280.5	-23.7	**0.015**
	0 vs 5.3×10^{-3} µg cm^{-2}	-331.5 deg cm^{-2}	-523.3	-139.7	**<0.0001**
Time active	0 vs 5.3×10^{-4} µg cm^{-2}	200.3 s	50.4	350.2	**0.005**
	0 vs 2.65×10^{-3} µg cm^{-2}	287 s	156.4	417.6	**<0.0001**
	0 vs 5.3×10^{-3} µg cm^{-2}	316.7 s	189.4	443.9	**<0.0001**

Dimethoate

Overall, once accounting for the environmental covariates, *T. pyri* movement behaviour was significantly affected by dimethoate residues, though the residues only had a small influence (MANCOVA; $F = 3.85$; $P < 0.0001$; $\eta^2 = 0.21$). Air temperature also significantly affected movement behaviour, and the influence was greater than the influence of dimethoate residues ($F = 7.84$; $P < 0.0001$; $\eta^2 = 0.36$). Although preliminary investigations of environmental measures showed relative

humidity affected angular velocity, the final model did not find a significant effect ($F = 1$; $P = 0.42$; $\eta^2 = 0.07$). There was a significant interaction between air temperature and dimethoate treatment, with the influence of this interaction as great as the effect of dimethoate ($F = 3.79$; $P < 0.0001$; $\eta^2 = 0.21$). When analysing movement behaviours individually, only angular velocity showed a significant response to any factor, though all effects were relatively small: dimethoate treatment increased the rate ($F = 3.6$; $P = 0.017$; $\eta^2 = 0.128$); air temperature ($F = 10.8$; $P = 0.002$; $\eta^2 = 0.127$), and the interaction of temperature and treatment ($F = 4.1$; $P = 0.01$; $\eta^2 = 0.141$) also significantly affected angular velocity. The interaction means that, while dimethoate residues did increase angular velocity, the effect varied depending on air temperature, and this effect was greater than individual effects of dimethoate or temperature. Post-hoc comparisons of estimated means – values corrected for the influence of air temperature (set at 23.7°C) and humidity (set at 54.6%) – showed that angular velocity increased when mites were exposed to dimethoate residues (Table 3.13) with an increase of 20.59 deg s^{-1} at 0.24 μg cm^{-2} (95% CIs [0.09; 41.2 deg s^{-1}]; $P = 0.048$), 15.4 deg s^{-1} at 1.19 μg cm^{-2} (95% CIs [7.1; 23.6 deg s^{-1}]; $P < 0.0001$), and 17.3 deg s^{-1} at 2.28 μg cm^{-2} (95% CIs [7.2; 27.3 deg s^{-1}]; $P < 0.0001$) compared to control mite angular velocity. Although these differences in response between dimethoate residues and control are significant, caution must be taken in the interpretation due to the relatively small effects and response to treatment being affected by temperature.

Table 3.13 – Summary of Games-Howell post-hoc analysis of the differences in measures of angular velocity as measured in *T. pyri* adults observed in control or treated arenas containing dimethoate residues. Comparisons are based on estimated means arising from multivariate analysis of covariance (MANCOVA) and are corrected for the influence of air temperature and relative humidity. P values are corrected for multiple comparisons (α = 0.05). Significant differences between a dimethoate concentration and control are highlighted in bold.

Movement Behaviour	Treatment Comparison	Mean Difference	95% Confidence Intervals		*P*
Angular velocity	0 vs 0.24 µg cm^{-2}	20.59 deg s^{-1}	0.09	41.2	**0.048**
	0 vs 1.19 µg cm^{-2}	15.4 deg s^{-1}	7.1	23.6	**<0.0001**
	0 vs 2.28µg cm^{-2}	17.3 deg s^{-1}	7.2	27.3	**<0.0001**

3.3.2.3 Avoidance behaviour

No mites became trapped in the arena barrier in the control, acetamiprid or dimethoate treated arenas. However, several mites became trapped at each dose level of deltamethrin, with 8, 16 and 14 mites becoming trapped during the observation period at 5.3×10^{-4}, 2.65×10^{-3}, and 5.3×10^{-3} µg cm^{-2} respectively. Figure 3.6 shows the time taken for each mite to become trapped. When comparing the average time to trap, there was a 122 sec reduction in the time to become trapped between the first and second treatments (95% CIs [-4.1; 248 s]); a 99 sec reduction between the first and third treatments (95% CIs [-29.6; 227.8 s]); and a 23 sec increase between the second and third treatments (95% CIs [-129; 84 s]).

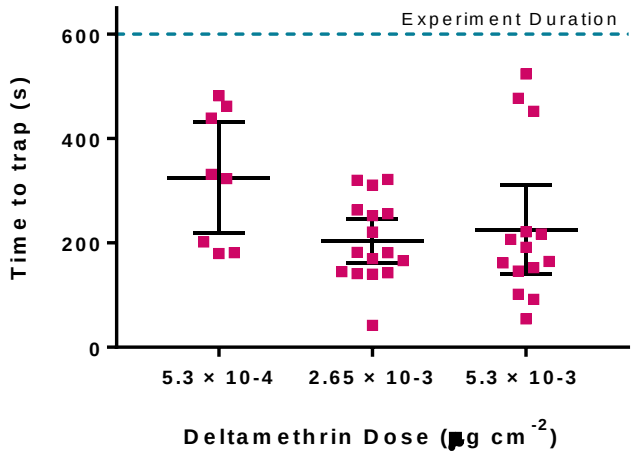


Figure 3.6 – Time taken for each trapped mite to become trapped when exposed to deltamethrin. Line and whiskers represent mean ± 95% confidence intervals.

3.4 Discussion

3.4.1 Pesticide residue analysis

Our novel method developed during this study produced high activity recovery rates and relatively low variance for acetamiprid, deltamethrin and dimethoate (Table 3.8). It is likely that recovery was below 80%, and variances above 20% for deltamethrin due to the low levels of activity being measured in each sample, and in any future studies methods should be adapted to increase the radioactivity within each sample, or to increase counting time to improve the counting. Radioactivity recoveries for captan were very low at 42%, and likely a result of the substance's low solubility in methanol (4 g kg^{-1}) and instability in non-acidic solutions (EFSA, 2008). We conclude that this analytical method provided a rapid, accurate and precise method for quantifying residues of acetamiprid, deltamethrin and dimethoate, but was not successful in quantifying captan, which has a reputation for being a challenging compound to analyse by conventional means due to rapid degradation in solution, including in solvents (EURL, 2017).

3.4.2 Control mite movement

We found that the movement of *T. pyri* observed in the control test arenas showed a tendency for individuals to track around the outer edge of the arena (Figure 3.3, Control 1), though there is a large degree of natural variation in movement behaviours as illustrated by the three control tracks. This behaviour has been noted previously in a comparison of movement with and without prey eggs within a test arena, where *T. pyri* individuals tracked the outside of the arena when no prey eggs were available, but displayed numerous visits to the prey egg site when these were within the arena (Varley et al., 1994). The mite movement observed in control test arenas therefore depicts typical food searching behaviour in individuals.

3.4.3 Mite activity/inactivity

Of the three insecticides, we found that deltamethrin residues caused a change in the activity of *T. pyri*, with no mites resting for the full ten minutes (Figure 3.4). In a study manually recording activity in *T. pyri* larvae, Croft and Zhang (1994) noted that mites on untreated surfaces tend to be most active in the first few hours of the study, though their data showed that 80% of individuals were noted to be resting at the first observation point four hours into the study, a much higher proportion of resting mites than observed in the control cohort of our study. This could be an artefact of natural variation amongst individuals; however, it could also suggest that in such studies, mites are less likely to rest in the hour after transfer into an arena. The significant response in activity related to deltamethrin residues is not unexpected as several studies have related pyrethroid exposure to hyperactivity in many species. Hyperactivity was observed in the assassin bug (*Triatoma infestans*) when they were exposed to topical applications of deltamethrin (Alzogaray et al., 1997), and in the two spotted spider mite (*Tetranychus urticae*) when exposed to fenvalerate and permethrin spray (Iftner and Hall, 1983). However, one study showed high concentrations of pyrethroids can arrest movement in two booklice species (Liposcelis bostrychophila and L. entomophila; Guedes et al., 2008), suggesting there is a threshold where low concentrations elicit hyperactivity, and high concentrations elicit arrest. In the present study it appears the residue levels mites were exposed to

were at levels that induce hyperactivity. We observed no significant differences in the number of resting mites in mites exposed to acetamiprid or dimethoate residues, suggesting that the residue levels were not high enough to induce changes in resting behaviour.

3.4.4 Movement behaviour

Prior to the experiments commencing, we hypothesised that residues of acetamiprid, deltamethrin and dimethoate would all lead to changes in movement behaviour. We came to this hypothesis for acetamiprid due to previously reported effects of acetamiprid on the feeding behaviour of a phytoseiid mite (Beers and Schmidt-Jeffris, 2015). For deltamethrin, our hypothesis was based on observed repellency in phytoseiids exposed to pyrethroids (Riedl and Hoying, 1983). For dimethoate, we based the hypothesis on the high toxicity of the active substance to *T. pyri* (Blümel et al., 2000a). Our broad hypotheses were proven to be correct for all three insecticides to varying degrees, with acetamiprid and dimethoate only affecting angular velocity, and deltamethrin affecting many of the measured movement behaviours.

Deltamethrin was the only pesticide to elicit a reduction in the distance walked by *T. pyri* when exposed to the residues, with reductions of 76%, 85% and 89% from the lowest to highest residue level compared to control (Figure 3.5). Additionally, the only significant response in time spent active was observed in mites exposed to deltamethrin, with 45%, 64% and 71% reductions respectively; the step-wise reduction in activity is consistent with the reduced distances walked. The measurements for deltamethrin were corrected for the mites that had escaped the arena (avoidance behaviour is discussed in section 3.4.5), meaning decreased activity is a result of increased resting behaviour rather than trapped individuals. Pyrethroids are known to arrest movement in spiders and psocids (Guedes et al., 2008; Shaw et al., 2006). A study of movement behaviour on leaves half treated with the pyrethroid esfenvalerate found that there were changes in walking behaviour in the red mite *P. ulmi* (Bowie et al., 1999), though the analysis to detect the differences was complex. In the present study, we checked each individual for mortality at the end of each observation period and no death was observed as each mite would move if provoked.

Therefore, we conclude the residues in the arena were likely at a threshold to cause reduced mobility via ataxia within the first few minutes of exposure; however, the exposure duration (or residue level) was not enough to turn the ataxia into paralysis of the mite. This contrasts with our finding in the previous section, suggesting the residues caused hyperactivity; therefore, we suggest the residues were at levels able to cause enough irritation to ensure each individual moved, but was also having an overall arresting effect.

Angular velocity, also described as turning speed, was significantly increased in mites exposed to residues of all three pesticides (Figure 3.5). In acetamiprid all three residue levels elicited increases in turning; in deltamethrin the second and third treatment levels led to increased turning speeds; and dimethoate residues increased angular velocity in all residue levels compared to control. The meander (turning relative to distance moved) also increased with increasing concentrations of deltamethrin residues and in relation to control, a behaviour that is strongly related to angular velocity as both are calculated from the turning angle metric calculated by EthoVision.

The increase in turning speed and path meander suggests either hyperactivity or agitation induced by the residues, especially when combined with the observed non-significant increases in time spent active, and would be classed as irritation under the definition of Wiles and Jepson (1994). It could also be interpreted as a decrease in directed movement through loss of muscle coordination, especially with the slight downward trend in distance moved and slight increase in meander where acetamiprid exposure occurred. Turning behaviour has been identified as a key component of search orientation patterns (Bayley, 1995), therefore the observed increases in angular velocity could signal a change in food searching behaviour in field settings. This interpretation is consistent with reports of more indirect movement in ground beetles exposed to lambda-cyhalothrin (Prasifka et al., 2008), and ataxia in the spider *Pardosa amentata* (Shaw et al., 2006). Shaw *et al.* also observed reductions in prey consumption arising from the ataxia, validating the idea that food searching could be affected.

Dimethoate was the only active substance where movement behaviours were found to be affected by environmental factors, with air temperature and relative humidity both correlating with the mite's angular velocity. The model analysed via MANCOVA showed that the initial correlation between angular velocity and humidity was not significant; however, air temperature significantly influenced the behaviour, and we found a significant interaction between dimethoate treatment and air temperature that explained more variance than each factor individually ($\eta^2 = 0.14$ compared to $\eta^2 = 0.13$ for treatment and temperature).

When comparing temperatures measured during the dimethoate experiment to temperatures measured during the other insecticide experiments, we found that the greatest temperature range was observed in the dimethoate samples (Table 3.9), but there was no significant difference when compared to the other treatments. Therefore, we may be observing a physiological reaction where dimethoate residues increase sensitivity to fluctuations in air temperature or humidity, especially since there were no strong correlations between control behaviour and environment (Table 3.3). There are no published findings to support this idea so we can only hypothesise at this point. However, there are a small number of studies that demonstrate insecticide toxicity changes with changes in air temperature. One study of insecticide toxicity to a field population of the lacewing *Chrysoperla carnea* found that 3d-LC_{50}s reduced with increasing temperature for acetamiprid and chlorpyrifos, but increased for lambda-cyhalothrin and spinosad between 20 and 40°C (Mansoor et al., 2015). Another study highlighted that organophosphate toxicity increased with increased temperature (Glunt et al., 2013). We found that angular velocity and meander increased with increased air temperature in mites exposed to dimethoate residues, but not in control mites (Table 3.3); therefore, our alternative conclusion is that the higher air temperatures increased the toxicity of dimethoate to *T. pyri*, and that the effects were shown as more agitated movement, or irritation, just ten minutes after exposure. Further experiments would be necessary to test our conclusions to determine what exactly is happening.

Ultimately, the analysis of variance showed a significant response in angular velocity once environmental effects were removed; however, due to the significant

interaction this conclusion must be interpreted with caution and further complex analysis would be necessary to see at what air temperatures the mite angular velocity is affected.

3.4.5 Avoidance behaviour

Avoidance behaviour was only observed in mites exposed to deltamethrin residues, with several mites attempting to escape the arena at all deltamethrin concentrations and thus becoming trapped in the arena barrier. We saw no attempts to escape arenas that led to trapping in any other mites in other treatments, and as such we conclude this response to deltamethrin is confirmed avoidance behaviour through running away. Based on the definitions given in the introduction, this avoidance behaviour is classed as repellence by Guedes et al. (2016), though it would class as irritancy under the definition of Beers and Schmidt-Jeffris (2015), who classed a tendency to escape as irritancy. Between the first and second concentration levels there was a 37% reduction in the time taken for mites to becoming trapped, and a 30% reduction in the trap time between the first and third concentration levels (Figure 3.6). This suggests that the residue level that triggers a more rapid repellence is between 0.0005 and 0.0027 µg cm^{-2}, giving a trigger below the maximum field rate.

Avoidance behaviour has previously been reported in female *T. pyri* protonymphs exposed to fungicidal formulations containing mancozeb and metiram (Blümel et al., 2000b). *G. occidentalis* mites displayed avoidance when exposed to acetamiprid and lambda-cyhalothrin residues in a choice arena (Beers and Schmidt-Jeffris, 2015). Avoidance by predatory insects of pesticide residues reduces the ability for such species to control pests (Umoru et al., 1996), which can lead to reduced efficacy as biocontrol agents (Hislop et al., 1981).

3.4.6 Ecological relevance of findings

Both changes in movement behaviours, such as ataxia and convoluted walking paths, and pesticide residue avoidance led to reduced prey consumption in spiders and parasitoids (Shaw et al., 2006; Umoru et al., 1996). Reduced prey consumption has

been linked to longer larval development periods in *T. pyri* (Hayes and McArdle, 1987); as a side effect of pesticide-induced behaviour changes, this would therefore have population-level consequences. Avoidance of pyrethroid residues on leaves led to reduced oviposition rates in *T. pyri*; however, the study authors noted that this may not be deleterious; as prey also preferred unsprayed surfaces, this would not necessarily lead to predators hatching away from their food source (Bowie et al., 2001). However, pesticide avoidance behaviour can ultimately lead to the dispersal of predatory mites like *T. pyri* from pyrethroid-treated crops, resulting in the loss of the predator from the ecosystem and a shift in species dynamics and pest stresses (Cordeiro et al., 2013; Gerson and Cohen, 1989).

Our study investigated behavioural effects at insecticide active substance concentrations no more than double the published 7d-LR_{50} for *T. pyri*, and found that movement behaviour in mites was affected within 10 minutes when exposed to residues equivalent to one fifth of the LR_{50} for all three insecticides. However, we want to consider whether these effects would occur in an apple orchard. In Chapter 2 we concluded that 42.6 L, or 17% of the sampled penconazole spray was deposited on the upper surface of apple leaves when 250 L was applied (Section 2.8). Our treatment concentrations for acetamiprid were based on regulatory studies, where active substances are applied at a rate to achieve no more than 200 L Ha^{-1} (Blümel et al., 2000a; EFSA, 2016); if 17% of spray landed on the leaf surfaces then this would equate to 34 L. Based on this, we have converted our nominal treatment concentrations for acetamiprid and deltamethrin to their equivalent application rate and the residue levels we would observe on apple leaves (Table 3.14). We also used the same basis to estimate how much active substance would land on apple leaves at the recommended application rate (i.e. label rate) for orchards (Table 3.15). This information has been used to inform our conclusions regarding these two active substances. We have not treated dimethoate in the same way as it is not permitted for use in apple orchards.

Table 3.14 – Insecticide treatment concentrations used in the behaviour studies expressed as their equivalent field rate if applied at a rate of 200 L ha^{-1} and as measured residues from the laboratory studies. Test concentrations are then expressed as estimations of residues landing on apple leaves in one hectare of orchard and per unit of leaf area. Presented values are based on the assumption that 17% of a 200 L ha^{-1} pesticide spray application lands on apple leaves in a 1 ha orchard.

	Test concentrations in laboratory studies			**Estimated residues on apple leaves in field**	
Active ingredient	**Nominal treatment concentration ($mg_{a.s.}$ mL^{-1})**	**Equivalent field application rate ($g_{a.s.}$ ha^{-1})**	**Measured residues on test arena surface ($\mu g_{a.s.}$ cm^{-2})**	**Apple leaf residues in orchard (g ha^{-1}_{ground})**	**Apple leaf residues ($\mu g_{a.s.}$ cm^{-2}_{leaf})**
Acetamiprid	0.018	3.6	0.983	0.61	0.0025
	0.09	18	4.493	3.06	0.0125
	0.18	36	9.464	6.13	0.0249
Deltamethrin	1×10^{-5}	0.002	0.00042	0.0003	1.4×10^{-6}
	5×10^{-5}	0.01	0.00201	0.002	6.9×10^{-6}
	1×10^{-4}	0.02	0.00423	0.003	1.4×10^{-5}

Table 3.15 – Field application rates for acetamiprid and deltamethrin expressed as estimations of residues landing on apple leaves in one hectare of orchard and per unit of leaf area. Presented values are based on the assumption that 17% of a 200 L Ha^{-1} pesticide spray application lands on apple leaves in a 1 Ha orchard.

Active ingredient	Field application Rate ($g_{a.s.}$ ha^{-1})	Apple leaf residues in orchard (g ha^{-1}_{ground})	Apple leaf residues ($\mu g_{a.s.}$ cm^{-2}_{leaf})
Acetamiprid	75	12.77	0.052
Deltamethrin	8.75	1.49	0.006

For acetamiprid, if acetamiprid is applied at the label rate of 75 $g_{a.s.}$ Ha^{-1}, then we estimate that 12.77 g of the applied active substance would land on the apple leaves based on assumptions outlined in Section 2.8. The acetamiprid residue levels measured in our laboratory study were 19 – 182 times higher than the expected leaf residues arising from a field rate application (Table 3.14); therefore, the behavioural effects we observed would not be of concern in the crop environment unless they occur at much lower residue levels. This difference is a result of our laboratory study method applying 150 µL of treatment to the test arena, giving 53 µL cm^{-2} when deposition in the field amounted to 0.173 µL cm^{-2}. We chose the application volume to ensure total coverage of residues on the test arena surface, though in future studies this method will require refinement for substances such as acetamiprid.

For deltamethrin, a label rate application of 8.75 $g_{a.s.}$ Ha^{-1} would lead to 1.5 g landing on apple leaves in one hectare of orchard, giving 0.006 $\mu g_{a.s.}$ cm^{-2}_{leaf} (Table 3.15). Equivalent residues measured in our laboratory studies were 1.4 – 14 times lower than this; therefore, the range of behavioural changes we observed along with avoidance behaviour would be expected to occur in the crop environment. Moreover, with effects being observed at rates several times lower than what could be expected as leaf residues, we could conclude that the behavioural effects and residue avoidance could be long lasting while deltamethrin persists. However, one study has shown that agrobiont spiders avoided fresh permethrin residues, but did not avoid 24 hour old residues that continued to be detrimental to the test species (Pekár and Haddad, 2005), so we cannot draw conclusions regarding avoidance of

degrading residues without further study. Ultimately, the behavioural effects observed in mites exposed to deltamethrin are likely to be less severe than what we observed due to pyrethroid resistance in agricultural mite populations (Bonafos et al., 2008). As dimethoate is no longer used in crop systems where *T. pyri* reside, we cannot comment on the implications for this insecticide. Further study would be required to investigate the severity of implications linked to these changes in field populations.

3.4.7 Scope for further study

Firstly, we have identified an opportunity for an in depth study involving dimethoate residues. The interaction between dimethoate effects and temperature effects led to our two hypotheses that *T. pyri* are either more sensitive to environmental fluctuations when exposed to dimethoate, or that dimethoate toxicity is increased with increasing air temperature. However, it was not possible for these to be explored further. As such, a study involving a range of controlled temperatures and humidity and dimethoate residues could be undertaken to further investigate this potential interaction between a pesticide and environmental conditions. The study could also be extended to investigate the other compounds, as scientific literature has shown toxicity of organophosphates, pyrethroids and neonicotinoids are changed with changing air temperature (Mansoor et al., 2015).

One major option for further laboratory-based study is for behavioural assays to be undertaken on pesticide formulations and the individual co-formulants contained within. The study could be extended to investigate any mixture effects, such as the combined effect of the active substance and an adjuvant combined on behaviour. Another option would be for numerous other non-target arthropods of different families to be studied. *Chrysoperla carnea* larvae are regularly included in higher tier laboratory and/or semi-field studies (EFSA, 2015). Our results also highlight the importance of considering environmental conditions to the response of mites to pesticides. A study of locomotory activity in *G. occidentalis* found that activity peaked at 24°C and reduced at temperatures above 25°C (Penman and Chapman, 1981); therefore, a more in depth study of responses at a range of air temperatures would

be useful, especially for risk assessment and the future modelling of predatory mite responses to pesticides in a range of climate zones or conditions.

There are also many opportunities for further study through investigations of data at higher resolutions. EthoVision could be used to analyse whether movement behaviour changes through the observation period, for example comparing the first and fifth minutes of an observation in a repeated measures design. This, combined with longer observation periods, would allow for a precise investigation into when mite movement behaviours recover from pesticide residues, as suggested by Desneux *et al.* (2004). Studies could also be designed to investigate whether mites spend more or less time in different parts of a test arena, such as quantifying the proportion of time spent tracking around the arena edge. Studies could also investigate whether the residues change the behaviour over 24 hours or more, as such time scales would be relevant to better understanding of the implications for field populations.

On a different perspective, there are many questions raised by our findings in relation to the risk to *T. pyri* populations as a result of pesticide induced behaviour changes. There are three pertinent questions to ask in this context: do behaviours change in the crop environment like we observed in the laboratory? Do any changes affect the biocontrol potential of *T. pyri*? Finally, do behavioural changes affect the overall population structure? These questions would require both field and laboratory studies: field investigations of mite behaviour in sprayed orchards; assessments of feeding and pest control in both the laboratory and the field; and laboratory studies of changes in reproductive behaviour would all inform the discussion of the risk to non-target arthropods arising from pesticide exposure. For example, the avoidance of pyrethroid residues (such as what we observed in the deltamethrin study) could reduce biocontrol potential, but could reduce mortality in field populations due to the ability to avoid contamination. This combination of effects would then give an arising question: what are the long term population consequences? Such studies could then be used to develop a population model to investigate the long term consequences of any behavioural changes; this would combine well with the previous

suggestion of studying effects at a range of temperatures to allow for the modelling of such consequences over large spatial scales.

Because the present study arena design offered no choice to the mites in being exposed to insecticide residues, the next logical step for further study was to offer the mites a choice by creating a partly exposed test arena to see whether the presumed avoidance behaviour observed in this study is true avoidance behaviour when mites have a choice. In the following chapter we explore this idea in full through a series of experiments similar to those discussed in the present chapter.

Chapter 4 – Can the predatory mite *Typhlodromus pyri* avoid pesticides? A study of movement behaviour and avoidance in choice arenas

4.1 Introduction

4.1.1 Why choice arenas?

In Chapter 3 we explored the necessity for the study of sublethal effects induced by pesticides, notably behavioural changes, and how the natural progression of the original study was to investigate behaviours in arenas offering free choice to individuals, where part of the surface is treated with pesticide. Avoidance behaviour was highlighted in a review by Hellou (2011) as a factor warranting further testing. Avoidance can be good or bad depending upon the chosen test subject and context, with avoidance of residues being beneficial to non-target species, or detrimental to the control of pests. For example, Pekár and Haddad (2005) devised choice arena experiments to investigate whether agrobiont spiders could recognise and avoid contaminated surfaces, therefore reducing their contact with harmful substances. Such avoidance has been highlighted as a protective rapid response for non-target species (Hellou, 2011). Conversely, a study of pest responses to stored product pesticides found that repellence reduced the efficacy of pyrethroid insecticides in controlling psocid species (Guedes et al., 2008). It would not have been possible to identify these behavioural responses from bioassays using full coverage test arenas, demonstrating that the bioassays used for EU regulatory testing methods should be taken as worst case scenarios (Beers and Schmidt-Jeffris, 2015), and that studies using free choice arenas should be implemented more frequently to augment understanding of responses.

The premise of studying partially treated arenas and the need for such experiments is also strengthened by literature regarding real pesticide spray patterns. Our study of fungicide spray exposure in an apple orchard demonstrated that spray covered on

average 16% of the upper surface of an apple leaf; therefore, pesticide exposure at the micro scale is heterogeneous (Chapter 2; Witton et al., 2018). Studies have also investigated differences between apple leaf surfaces. Spray retention on upper and lower apple leaf surfaces varied when sprayed with the same volume of pesticide, with upper leaf surfaces retaining 76% less pesticide than lower surfaces; this was related to more leaf hairs on the lower surface (Hall et al., 1997). Another study found that EDTA metal chelate deposits were at least twice as high on the lower leaf surface in apple trees sprayed by commercial sprayers (Cross et al., 2003). The heterogeneity and partial coverage of surfaces in the environment reiterate the previous point that experimental designs using full surface coverage demonstrate a worst case scenario, but also do not match reality.

4.1.2 Studying avoidance behaviour in choice arenas

In Chapter 3 we discussed the definitions of avoidance behaviour, reviewed literature that had already studied these behaviours, and also investigated avoidance in *T. pyri*. However, as highlighted at the end of our study, to better understand how movement behaviours are affected in a more realistic setting, studies need to investigate how behaviours change when individuals are offered a choice in whether to move or reside on exposed or unexposed surfaces. The use of such free choice experiments enables greater understanding of behaviour and its consequences in the spatially heterogeneous exposure landscapes found in agricultural systems. Additionally, the study of avoidance could fill gaps between the behaviour of natural enemies (such as *T. pyri*) in the field and the results of small scale, no-choice, worst-case laboratory experiments (Beers and Schmidt-Jeffris, 2015).

A number of studies have investigated behaviours of various pest species in choice arenas. Cordeiro et al. (2013) studied the behaviour of the pest Southern Red Mite (*Oligonychus ilicis*) in choice arenas where half of the surface was treated with a sublethal concentration of deltamethrin; the authors found no difference in the amount of time spent in the untreated and treated sectors, suggesting no avoidance behaviour occurred. Additionally, they observed no difference in the distance walked, the walking velocity, or the resting time, reinforcing the authors' conclusion that

there were no deltamethrin induced behavioural changes. An earlier study by the same authors defined repellence as spending less than 1 second on the treated surface, and irritability as spending less than 50% of the observation period on the treated surface (Cordeiro et al., 2010), and these definitions have been used widely in recent work. The grain weevils *Sitophilus granarius* and *S. zeamais* showed irritability when exposed to formulations containing the pyrethroid deltamethrin and the micro-organism derived spinosad, though showed no notable avoidance of residues (Vélez et al., 2017). A study of the phytophagous mite *Steneotarsonemus concavuscutum* found individuals would spend on average 20% of their time on surfaces treated with acaricide formulations containing abamectin, azadirachtin and fenpiroximate (França et al., 2018). One study investigated avoidance behaviour in the tomato leaf miner (*Tuta absoluta*) when exposed to azadirachtin and found that, while there was no significant residue avoidance on filter paper substrates, there was significant avoidance of the insecticide at concentrations relating to LD_{50} and LD_{90} on tomato leaves, suggesting test substrate can impact on behaviour studies (Tome et al., 2013). The authors suggested that azadirachtin itself may not be repellent, but may instead mask compounds emitted by tomato leaves that are attractive to the species.

A number of studies have also investigated avoidance behaviours in beneficial species in choice arenas. A study of repellence in three species (the ladybird *Cycloneda sanguinea*, the pirate bug *Orius insidiosus*, and the soldier beetle *Chauliognathus flavipes*) exposed to seven insecticide formulations in choice arenas half treated with insecticides found that bifenthrin, imidacloprid and acephate repelled all three species, and deltamethrin, a pyrethroid we studied in Chapter 3, also induced repellence in all three species with 75% repellence in *C. sanguinea*, and 65% repellence in *O. insidious* and *C. flavipes* (Fernandes et al., 2016). However, the methodology of this study was basic and involved counting how many individuals out of twenty were on the treated surface 15 minutes after their placement in the arena. Other studies used more sophisticated methods to determine repellence by using movement analysis software to calculate how much time was spent in each arena section. One such study involved two lacewing species, and showed that *Chrysoperla*

externa larvae were repelled by the plant derived insecticide azadirachtin in formulation, and *Ceraeochrysa cubana* larvae were completely repelled by the pyrethroid permethrin in formulation in half treated arenas (Cordeiro et al., 2010). Figure 4.1 illustrates this repellence when viewed as walking tracks derived from movement analysis, with no tracks falling within the treated sector. In contrast, Guedes et al. (2008) identified "weak repellence" caused by pyrethrins in a pest psocid species, with their statement describing a lower proportion of time spent on the treated surface versus the untreated surface; this subtler response is illustrated in Figure 4.2.

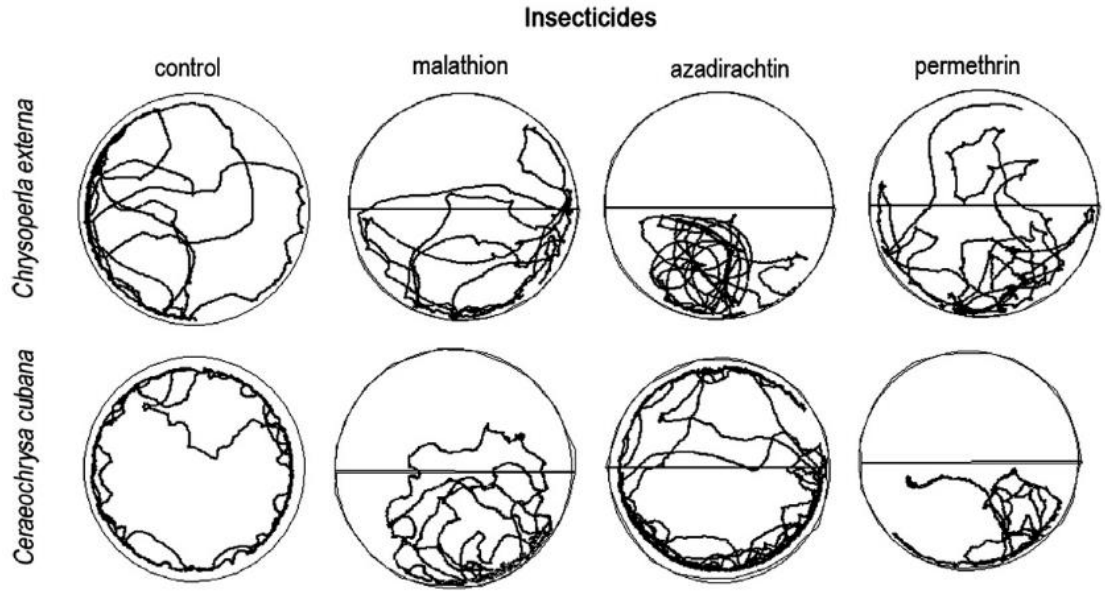


Figure 4.1 – Representative movement tracks of individual lacewing larvae of the two species *Chrysoperla externa* and *Ceraeochrysa cubana* over 10 minutes in 9 cm test arenas half-treated with dried insecticide residues (upper sector of each arena). Repellence is observed in *C. externa* exposed to azadirachtin, and *C. cubana* exposed to permethrin (Cordeiro et al., 2010).

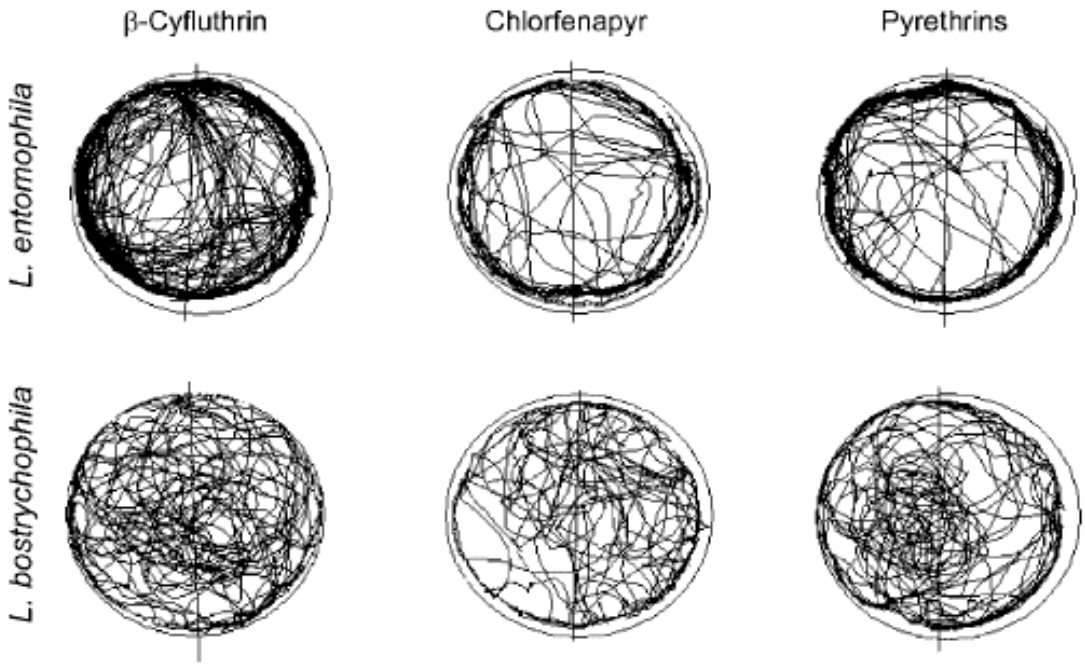


Figure 4.2 – Representative movement tracks of individual psocids (top row = *Liposcelis entomophila*; bottom row = *L. bostrychophila*) over 10 minutes in 2.5 cm test arenas half treated with dried insecticide formulations (right hand sector of each arena). Weak repellence is observed in *L. bostrychophila* when exposed to pyrethrin residues (bottom right), where individuals spent on average 42% of the observation time on the treated surface (Guedes et al., 2008)

A study of avoidance behaviour in *Galendromus occidentalis* on bean leaves found that many formulations did not induce avoidance behaviours, but acetamiprid and lambda-cyhalothrin induced "run off", where individuals escape the test arena (defined as irritancy in this study), and spirotetramat, flubendiamide and cyantriniliprole induced "repellence", the term given for avoiding the treated surface by remaining on the untreated surface (Beers and Schmidt-Jeffris, 2015). Another study used a more complex, four choice arena: the authors investigated avoidance and movement in four agrobiont spider species exposed to commercial formulations containing the organophosphate phosalone; permethrin; the micro-organism derived biopesticide *Bacillus thuringiensis*; and a water control, and found that three of the four species were repelled by fresh phosalone and permethrin residues, but

not by one day old residues, raising concerns that the beneficial spiders are not repelled by compounds that continue to be toxic (Pekár and Haddad, 2005).

In 2005 the lack of data on pesticide repellency to natural enemies was highlighted (Pekár and Haddad, 2005), and though many studies have been conducted, information continues to be limited for some species and compounds. There continues to be a lack of knowledge about pesticide induced repellency in phytoseiids (Beers and Schmidt-Jeffris, 2015).

One factor that the majority of current choice arena studies has in common is that they study the effects of pesticides in formulation. While this is more realistic when considering real world exposure, it raises the idea that co-formulants may be inducing avoidance or other behavioural changes. Adjuvants and solvents included in commercial formulations of the biopesticide neem can influence toxicity (Isman, 2006; Liang, Chen and Liu, 2003), and additives in deltamethrin formulations were attributed to honey bee repellency (Bos and Masson, 1983). Physical characteristics of spray (e.g. wetting agents) can also impact (Desneux, Decourtye and Delpuech, 2007). In summary, the discussed studies all investigated formulation pesticides and as such can only report on the repellence and behaviour changes induced by pesticide formulations, and not by active ingredients.

4.1.3 Study aim

With this study we aimed to investigate whether the movement behaviour of the predatory mite *Typhlodromus pyri* was altered by pesticide residues in a free choice arena that comprised treated and non-treated surfaces. To achieve this aim, we looked to answer two main questions: do mites show a preference for treated or non-treated surfaces; and do mite movement behaviours differ between the treated and non-treated surfaces?

We previously studied movement behaviour changes in *T. pyri* when exposed to pesticide residues in a non-choice (i.e. fully exposed) arena; please refer to Section 3.1 for the rationale for choosing this species for these studies. To eliminate the effect of co-formulants, we again focused on studying active substances, and investigated

three insecticidal active substances that were used in the no-choice experiment: acetamiprid, deltamethrin and dimethoate. The rationale for these choices can also be found in Chapter 3.

4.1.4 Hypotheses

Consistent with the previous study reported in Chapter 3, we developed individual hypotheses for each substance and based them on both the scientific literature and our findings in the previous study. We hypothesised that acetamiprid and deltamethrin would induce avoidance behaviour based on published observations for acetamiprid in the phytoseiid *G. occidentalis* (e.g. Beers and Schmidt-Jeffris, 2015), and on both published observations in three non-target arthropods and our own observations in *T. pyri* for deltamethrin (Fernandes et al., 2016). We hypothesised that dimethoate would not induce avoidance behaviour as there are no reports of repellency, though the active substance is highly toxic to *T. pyri* (Blümel et al., 2000a).

4.2 Materials and methods

4.2.1 Insects

In Chapter 3 we provided details on the source and culturing of *Typhlodromus pyri* from eggs to adulthood. Cultures were again kept in growth chambers at a target temperature of 25°C (mean 25.3°C; 95% Confidence Intervals (CIs) [25.21; 25.35]) and target relative humidity of 80% (mean 77.2%; 95% CIs [76.06; 78.37]) in a 24-hour constant light cycle providing approximate illuminance of 15 000 lx. Consistent with the previous study, all observations were undertaken on 7 – 9 day adults.

4.2.2 Insecticides

As outlined previously, we used ^{14}C-radiolabelled active substances to enable rapid quantification of residues. Three insecticidal substances were studied: acetamiprid, deltamethrin and dimethoate, with details on sourcing previously outlined in Chapter

3. Due to the inability to accurately quantify residues or measure behaviour on the residues, we did not include the fungicide captan in this experiment.

Dosing stocks remained at the same levels as those studied in the previous behaviour study: 0.18 mg mL^{-1} for acetamiprid; 1×10^{-4} mg mL^{-1} for deltamethrin and 0.115 mg mL^{-1} for dimethoate. However, with this study only partly covering the test arena, overall activity from residues would be lower than those measured in the full coverage study and were at risk of being close to limits of detection. While activity levels in the acetamiprid dosing stocks were acceptable, deltamethrin and dimethoate samples were at risk of being close to background. It was not possible to increase activity in the deltamethrin dosing stock due to the low concentrations being studied; however, we increased the activity in the dimethoate dosing stock while maintaining the same concentration by increasing the proportion of ^{14}C-dimethoate. Table 4.1 summarises the treatment levels expressed as residue per unit of treated arena area.

Table 4.1 – Insecticidal active substances used in the present study with the lethal rate at which 50% of a *Typhlodromus pyri* population displays mortality after 7 days (7d-LR_{50}); the application rate for formulated products in pome fruit orchards; and the three treatment levels for the present study expressed as active substance mass per unit area of treated test arena surface (i.e. half of the test arena area).

Active Substance	**7d-LR_{50} (g ha^{-1})**	**Application Rate ($g_{a.s.}$ ha^{-1})**	**Target Residue ($\mu g_{a.s.}$ cm^{-2})**		
			1	**2**	**3**
Acetamiprid	18[a]	75	0.95	4.82	9.57
Deltamethrin	0.000043[b]	7.5	0.00053[c]	0.0027[c]	0.0053[c]
Dimethoate	2.24 [d]	-[e]	0.24	1.19	2.38

n.b. *1 µg cm^{-2} equates to 100 g ha^{-1}*

[a] – European Food Safety Authority (EFSA, 2016)
[b] – European Commission (2017)
[c] – Based on an LC_{50} of 0.05 mg L^{-1} (Bonafos et al., 2007)
[d] – European Food Safety Authority (2013)
[e] – Dimethoate use in apple orchards is not permitted

4.2.3 Behavioural bioassays

4.2.3.1 Method development

We used the full coverage behaviour study as the basis for the design of this experiment. As a result, the test arena construction is as previously described in Chapter 3. There were two matters that required further method development, however: how to create half covered arenas, and the measurement of residues. The latter is discussed in the next section.

A literature review was undertaken to explore methods for creating half exposed arenas. Two studies used an artist's airbrush to target pesticide spray on surfaces (Guedes et al., 2008; Porcel, Cotes and Campos, 2011); another adapted this idea and sprayed filter paper with water, and then placed a half filter paper sprayed with insecticide on top to provide the test arena (Cordeiro et al., 2010). Another pipetted dosing solution onto quarters of filter paper (Pekár and Haddad, 2005), while another idea was to dip the test arena surface, as done with bean leaves by Beers and Schmidt-Jeffries (2015) and by Fernandes et al. (2016) with half-dipping filter paper. However, each of these methods created problems for the present study. Firstly, although the premise of spraying with an airbrush worked well, it was inappropriate in safety terms to spray radiolabelled pesticides in the laboratories, so this method was discounted. Additionally, the layering of several filter papers to create the choice arena generated the risk of mites being lost between the layers due to their small size. We also had issues with the surface not being homogeneous in colour and causing issues with mite detection. As such, filter papers were also dismissed. The glass coverslips decided upon for the full coverage experiment were therefore optimal for the half coverage experiment, but dipping glass coverslips for half coverage was a poor method for treating the surface due to uneven coverage and inconsistent residues on the surface.

These considerations led to the idea of pipetting on part of the surface, and one idea that was explored was to use a glass scoring pen to score the centre of the arena to encourage the pesticide solution to remain in one section; this would allow a perfect 50/50 exposed/unexposed surface. While it successfully prevented the spread of the pesticide solution, the glass scoring caused a groove deep enough to affect mite

movement. Our final method involved lightly dragging the tip of a loaded pipette in a line across the middle of the glass coverslip, and then steadily pipetting the remaining volume to the right of the liquid line. Experience in the laboratory showed that the surface tension of the initial line would prevent the liquid from spreading beyond the initial line if the concentration volume was ejected slowly from the pipette. Figure 4.3 shows a series of freshly exposed test arenas that were treated using this method.

Figure 4.3 – Twelve freshly treated arenas for the half coverage behaviour bioassay. The dosing volume was applied through careful use of a pipette and the volume can be seen on the right hand side of each test arena, or to the top half at this viewing angle.

4.2.3.2 Experimental set up

Test arenas were created as outlined for the full coverage arenas in Chapter 3; however, rather than applying 0.15 mL of dosing solution onto the entire arena surface, we applied 0.075 mL to the right half of the arena surface with a pipette. This volume ensured residues on the treated surface were consistent with the area-based residues in the full coverage study. Control arenas were treated with 0.075 mL methanol in the same way. Test arenas were left to dry for 75 min in a fume cupboard before the behavioural bioassay commenced. Arena area was determined as outlined

previously (Chapter 3, Equation 1); in this experiment, mean arena surface area was 2.81 cm^2 (95% CIs [2.78 – 2.84]).

As with the full coverage arena experiments, we placed single sexed *T. pyri* mites in the centre of the test arena and, in the case of methanol, acetamiprid and dimethoate exposures, these were given 10 min to acclimatise to the new environment; mites placed in deltamethrin treated arenas were given approximately 30 s to adjust due to previously observed immediate behavioural responses. There were 24 replicates per treatment and control (12 female, 12 male). Due to the USB microscope failing during the full coverage experiment (Chapter 3), there was a deviation in the physical set up of the testing chamber for this experiment. Test arenas were placed on a platform raised 15 cm above the heated propagator base, with the base thermostat set to 26°C (aiming to achieve 24°C). Damp blue roll was placed at the bottom of the platform to elevate humidity, and the test arenas were again lit using a targeted small white LED light.

All bioassays in this experiment were recorded for 10 min using a Canon DSLR camera (EOS 1300D, Canon Inc, Tokyo, Japan) equipped with an 18-55 mm lens recording at 25 frames per second and mounted on a metal hood (Syngene DigiGenius, Synoptics Ltd., Cambridge, UK). As the hood was opaque, temperature and humidity were noted at the end of each observation period, and the thermometer was placed to measure the conditions on the same level as the test arena. Video files were converted to mpeg format using HandBrake (Version 1.1.1) for movement behaviour analysis.

4.2.3.3 Movement analysis

As with the full coverage experiments, videos were analysed using Noldus EthoVision XT 13 (Noldus Information Technology, Wageningen, Netherlands) at a rate of 5 frames per second to ensure consistency with the first experiment. The arena was identified and split into two zones for the movement behaviour analysis – Figure 4.4 shows how this looked in EthoVision. The five movement behaviours previously described in Chapter 3 were automatically analysed again (distance walked, velocity,

angular velocity, meander, and time spent active); however, measurements were taken for each zone, giving two measurements per mite per movement behaviour. Additionally, a sixth metric was calculated: time in zone for each identified zone. The zone exit threshold – used to determine when a mite had fully transitioned from one zone to the next – was set to 0.03 mm, which is approximately 10% of the mite body length. Time to trap was also manually determined as previously described, though this was considered across the whole arena. Minimal distance moved and LOWESS smoothing were applied as previously outlined.

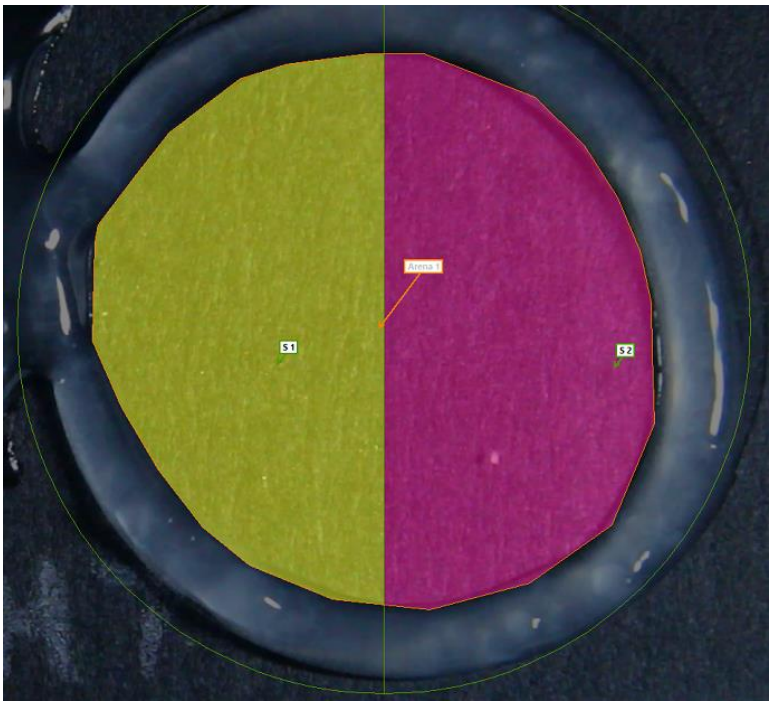


Figure 4.4 – Screenshot of a sample arena from the dimethoate half coverage experiment with the two test arena zones identified in EthoVision XT 13. The yellow zone represents the untreated zone; the pink zone represents the treated zone. White rectangular labels identify the overall arena (orange rectangle to the centre); green rectangles identify each arena zone (S1 to the left; S2 to the right). With this set up EthoVision calculates movement behaviours for each zone as well as the test arena considered as a whole.

4.2.4 Pesticide residue analysis

Pesticide residues were extracted from the arena surface as outlined previously in Chapter 3; however, we refined the liquid scintillation analysis due to lower expected activity arising from fewer residues.

Following extraction in 10 mL methanol an 8 mL aliquot of the sample was transferred to a 20 mL glass screw cap scintillation vial containing 10 mL Ecoscint A (National Diagnostics, Nottingham, UK). Samples were wrapped in foil and refrigerated at 4.5°C for a maximum of 18 hours (typically no more than 6 hours), until enough samples had been generated for a full sample run on the liquid scintillation counter (Hidex 300 SL, Hidex Oy, Turku, Finland). Analysis commenced after a time delay of 2.5 hours for each sample run to allow samples to settle. For acetamiprid and dimethoate, each vial was counted three times for 5 min, but due to low activity levels it was necessary to count deltamethrin vials three times for 20 min to sufficiently separate measured ^{14}C-deltamethrin activity and background activity. We measured background activity using blank (control) vials containing 10 mL Ecoscint A + 8 mL methanol; activity from these vials was averaged and subtracted from all samples to correct for background activity. Finally, we used the calculations previously reported to determine the activity related to insecticide concentration in each dosing stock, and the residues in each arena (Chapter 3, Equation 3.3). Residues were expressed as the mass of active substance in half of the arena area. Based on the mean arena surface area, the mean treated area was 1.41 cm^2 (95% CIs [1.39 – 1.42]). It was not possible to determine the precise treated arena area for each replicate as residues did not always leave a visible trace; therefore, we based this on half of the arena area.

4.2.5 Analytical method development

As before, we calculated detection limits for the counting methods so that it was possible to determine whether sample counts were valid. As the counting windows were increased for the half coverage samples, background counts were greater than 70; as such a different equation was used to determine the detection limit (L_d) and minimum counts (Equation 4.1; L'Annunziata and Kessler, 2012). Table 4.2 summarises the detection limits for the two methods.

(1) $L_d(counts) = 4.65\sqrt{B}$ **Equation 4.1**

(2) $Minimum\ counts = L_d + B$

$\boldsymbol{B}$ denotes mean background reading in counts

Table 4.2 – Detection limits (L_d, expressed as counts) and minimum counts (as counts per minute, CPM)) for the two counting methods used to analyse insecticide residues extracted from half treated test arenas.

Counting window	Samples analysed	L_d (counts)	Minimum counts
5 min	Acetamiprid, dimethoate	83.9	409 (82 CPM)
20 min	Deltamethrin	164.3	1413 (70.7 CPM)

4.2.5 Statistical analysis

4.2.5.1 Environmental conditions

As in the first behavioural study, one insecticide was studied per week and as a result we wanted to investigate whether the environmental conditions were consistent from one pesticide to the next to help inform conclusions. Air temperature and humidity readings were grouped by pesticide and compared via one-way ANOVA; please refer to the methods outlined in Chapter 3 for how this was undertaken.

4.2.5.2 Environment effects on mite movement behaviour

Consistent with the statistical approach in the full arena coverage study, we investigated the effect of air temperature and relative humidity on mite movement through a series of correlations and these determined whether environmental factors needed to be included in the final analytical model or not (Table 4.3). As before, where a slope was significantly not zero ($\alpha = 0.05$) and where it had a fit greater than $R^2 = 0.2$, there the relevant covariate was included. If these criteria were

not met, then covariates were not included in final analysis. There were four significant control slopes, with angular velocity and meander both correlating moderately with air temperature and humidity. In the control population, both temperature and relative humidity correlated with angular velocity and meander. There were also two significant slopes in the pesticide treatments (angular velocity vs temperature in the deltamethrin population; angular velocity vs relative humidity in the dimethoate population), though neither slope had a fit greater than 0.2, and as such, no analyses included environmental factors as covariates.

Table 4.3 – Influence of air temperature and relative humidity on *T. pyri* movement behaviours in half treated arenas, derived from correlation. Goodness of fit (R^2) is shown with *F* and *P* values indicating whether a slope is significant. Significant correlations (i.e. slope ≠ 0; α = 0.05) are in italic. *n* = 22 (control); 59 (acetamiprid); 50 (deltamethrin); 54 (dimethoate).

		Air temperature			**Relative humidity**		
Treatment	**Behaviour**	**R^2**	***F***	***P***	**R^2**	***F***	***P***
Control	Distance walked	0.0002	0.004	0.95	0.006	0.13	0.73
	Velocity	0.003	0.06	0.81	0.002	0.04	0.84
	Angular velocity	*0.43*	*15.06*	*0.001*	*0.43*	*15.22*	*0.001*
	Meander	*0.32*	*9.45*	*0.006*	*0.36*	*11.37*	*0.003*
	Time moving	0.03	0.69	0.42	0.002	0.03	0.86
Acetamiprid	Distance walked	0.03	1.75	0.19	0.006	0.33	0.57
	Velocity	0.022	1.3	0.26	0.004	0.25	0.62
	Angular velocity	0.023	1.32	0.26	0.002	0.12	0.73
	Meander	0.016	0.89	0.35	0.002	0.11	0.74
	Time moving	0.018	1.03	0.32	0.001	0.05	0.83
Deltamethrin	Distance walked	0.022	1.09	0.3	0.007	0.32	0.57
	Velocity	0.041	2.03	0.16	0.003	0.14	0.71
	Angular velocity	*0.136*	*7.55*	*0.008*	<0.0001	0.001	0.98
	Meander	0.061	3.05	0.09	0.001	0.05	0.82
	Time moving	0.0002	0.01	0.92	0.0002	0.01	0.92
Dimethoate	Distance walked	0.015	0.77	0.39	0.015	0.82	0.37
	Velocity	0.012	0.62	0.43	0.016	0.84	0.36
	Angular velocity	0.031	1.66	0.20	*0.083*	*4.72*	*0.03*
	Meander	0.002	0.09	0.76	0.0004	0.02	0.88
	Time moving	0.003	0.14	0.71	0.003	0.17	0.69

4.2.5.3 Zone preference study

Previous studies have shown that, when offered the choice of surfaces with and without pesticide residues, a mite's preference to reside on untreated surfaces indicates repellency (Beers and Schmidt-Jeffris, 2015); an alternative definition from Cordeiro et al. (2013) splits this into two behaviours, with repellency including no contact with residues, and irritancy arising from spending less time on the treated surface compared to the untreated one. Repellency was therefore of interest in this study, so to investigate whether mites preferred the treated (right) or untreated (left) surfaces, we conducted two statistical tests. Firstly, we conducted a paired samples t test to investigate whether residues left by the methanol control (or other unquantified effects) influenced whether mites spent more time on the treated or untreated arena half. The second test investigated whether there was a difference in time spent on the treated surface at each concentration level for each active substance. Though the mite was placed in the arena centre on the border of the treated and untreated areas, individuals were not in the arena centre at the start of the movement recording. The data were subjected to one-way ANOVA, with one test per pesticide, comparing the time spent in the treated arena half at each concentration level against the time spent on the treated half of the methanol control. Where mites spent less than 50% of the observation time in the treated zone, this indicated a preference for untreated surfaces and therefore repellency.

Prior to ANOVA and t tests, data were tested for normality using the D'Agostino & Pearson test (D'Agostino, 1986), and for ANOVA the equality of group variances was tested using the Brown-Forsythe test. No data transformations were necessary. Post hoc analysis for ANOVA compared responses at each concentration level to control and the test was chosen based on the criteria outlined for analysing differences in environmental conditions. $\alpha = 0.05$ for both tests.

4.2.5.4 Mite movement behaviour

In Chapter 3 we started by investigating whether movement behaviours differed between male and female mites. Since there were no significant differences in behaviours in the first experiment, we did not test for any effects in this experiment.

Mite activity/inactivity

Consistent with the full coverage behaviour study reported in Chapter 3, we investigated whether the number of inactive mites was affected by insecticide residues through the use of the binomial observed vs expected test in GraphPad Prism, where we compared each insecticide treatment (observed) to the control (expected). Three tests were undertaken per insecticide (one per treatment level), and as such Bonferroni's Correction was applied to adjust the significance value to $\alpha = 0.0167$.

Insecticide effects on movement behaviour in half exposed arenas

We also wanted to investigate movement behaviour changes between the untreated and treated surfaces and at different insecticide treatment levels. To investigate this, we conducted 3 × 2 mixed model analysis of variance (ANOVA), to analyse the effect of arena surface (two levels), insecticide treatment (three levels), and their interaction on the movement behaviours of mites. It was not appropriate to include the control test arenas in this statistical design; therefore, the three treatment levels are all insecticide concentrations. These tests were conducted separately for each of the three insecticides, giving a total of 15 ANOVAs. Each test analysed whether a movement behaviour differed between the untreated and treated zones, and whether insecticide concentration level interacted significantly with zonal differences (i.e. within-subject effects). If there was a significant interaction ($\alpha = 0.05$), paired t tests were applied, one test per concentration level, to investigate the significance further. As each post hoc test included multiple t tests, Bonferroni's Correction was applied to give an adjusted α of 0.0167. If there was a significant difference in response between zones, but no interaction, then post-hoc analysis was not necessary as the result showed that, while there was a difference in the behaviour in treated and untreated zones, there was no difference in effect at different treatment levels. In this case we reported mean difference ± 95% confidence intervals. Finally, the test would also assess whether there was a significant difference in response between treatment levels when considering test arenas as a whole (i.e. between-

subject effects). Where a significant effect was found, Tukey's multiple comparisons test was applied as post hoc analysis ($\alpha = 0.05$) to see where significant differences were between the insecticide treated test arenas.

As with the analysis of movement behaviour in Chapter 3, each dataset was tested for assumption violations prior to final analysis. Equality of variances and covariances were assessed using the Brown-Forsythe Test and Box's M test respectively, and the outcomes were used to inform selection of test statistic (Tables 4.4 – 4.5). Due to over half of the analyses displaying unequal covariances, we used Pillai's Trace for all test interpretation due to its robustness against violations of covariance equality (Pillai and Hsu, 1979). As we were applying mixed ANOVAs to each dataset, we would also normally inspect sphericity of data (i.e. equality of variances between all possible within-subject pairings) using Mauchly's test of sphericity (Mauchly, 1940); however, with only two within-subject levels, this test was not necessary.

Table 4.4 – Results of Box's M Test for equality of covariance for the three insecticides. Each insecticide included four treatment levels: three insecticide treatments and one control. α = 0.05. Significant values suggesting assumption violation are highlighted in bold.

Insecticide	Behaviour	Box's M	*F*	*P*
Acetamiprid	Distance walked	2.06	0.32	0.29
	Velocity	14.92	2.35	**0.03**
	Angular velocity	10.27	1.62	0.14
	Meander	8.7	1.37	0.22
	Time active	54	8.51	**<0.0001**
Deltamethrin	Distance walked	8.77	1.37	0.22
	Velocity	14.03	2.19	**0.04**
	Angular velocity	18.8	2.94	**0.007**
	Meander	40.95	6.39	**<0.0001**
	Time active	1.49	0.23	0.97
Dimethoate	Distance walked	33.75	5.29	**<0.0001**
	Velocity	10.88	1.71	0.12
	Angular velocity	33.57	5.26	**<0.0001**
	Meander	34.31	5.38	**<0.0001**
	Time active	21.81	3.42	**0.002**

Table 4.5 – Results of the Brown-Forsythe test for equality of variance, assessed for each movement behaviour in each arena zone. Each insecticide included three treatment levels. α = 0.1; significant values that suggest assumption violation are highlighted in bold.

		Untreated zone		**Treated zone**	
Insecticide	**Behaviour**	***F***	***P***	***F***	***P***
Acetamiprid	Distance walked	0.38	0.68	0.38	0.69
	Velocity	0.3	0.74	1.59	0.21
	Angular velocity	1.26	0.29	0.49	0.62
	Meander	3.54	**0.036**	1.15	0.32
	Time active	0.26	0.77	2.8	**0.07**
Deltamethrin	Distance walked	0.56	0.57	1.64	0.21
	Velocity	0.88	0.42	1.33	0.27
	Angular velocity	0.13	0.88	3.11	**0.054**
	Meander	0.2	0.82	3.94	**0.03**
	Time active	1.35	0.27	0.38	0.69
Dimethoate	Distance walked	6.25	**0.004**	2.59	**0.085**
	Velocity	2.65	**0.08**	3.89	**0.027**
	Angular velocity	4.91	**0.01**	1.37	0.26
	Meander	1.66	0.2	1.95	0.15
	Time active	5.57	0.007	1.04	0.36

4.3 Results

No mite mortality was observed during the observation periods. Table 4.6 summarises the expected and measured residue values for each insecticide and treatment level. Activity-based recoveries averaged 92% for acetamiprid (95% Confidence Intervals (CIs) [88 – 96%]); 56% for deltamethrin (95% CIs [53 – 60%]); and 90% for dimethoate (95% CIs [86 – 93%]). The poor recovery levels observed in deltamethrin samples are likely explained by the relatively low counting efficiency, probably caused by low activity in samples. In spite of the inconsistent recoveries, we found no samples measured below the minimum counts threshold, therefore all sample measurements were accepted as correct.

Table 4.6 – Mean measured insecticide residue values, 95% confidence intervals and coefficient of variance (CV) of measurements, mean recovery compared to expected radioactivity levels and associated 95% confidence intervals, and mean counting efficiency with 95% confidence intervals for each insecticide treatment level. Residues are calculated based on mass of active substance per unit of treated arena area, with half of the arena area treated. *n* is based on the final sample populations arising from discounting inactive mites.

	Nominal		**Surface residues (µg cm^{-2})**			**Residue recovery (%)**		**Counting efficiency (%)**	
Insecticide	**concentration (µg cm^{-2})**	***n***	**Mean**	**95% Confidence Intervals**	**CV (%)**	**Mean**	**95% Confidence Intervals**	**Mean**	**95% Confidence Intervals**
	0.95	20	0.93	0.87 – 0.98	13	96	90.4 – 101.5	80.8	80.5 – 81.1
Acetamiprid	4.77	18	4.6	4.22 – 4.97	16	91.7	82.9 – 100.6	86.1	86 – 86.3
	9.54	20	8.62	7.67 – 9.57	23	88.4	79.2 – 97.5	86.8	86.7 – 87
	5.3×10^{-4}	16	2.5×10^{-4}	$2.3 \times 10^{-4} – 2.8 \times 10^{-4}$	21	56.2	49.6 – 62.8	64	63.3 – 64.7
Deltamethrin	2.65×10^{-3}	16	1.7×10^{-3}	$1.6 \times 10^{-3} – 1.8 \times 10^{-3}$	11	54.4	48 – 60.9	80.3	79.9 – 80.7
	5.3×10^{-3}	18	3.5×10^{-3}	$3.3 \times 10^{-3} – 3.7 \times 10^{-3}$	13	58.1	52.4 – 63.8	83.5	83.2 – 83.8
	0.24	17	0.2	0.19 – 0.22	13	84.5	80.4 – 88.6	75	74.3 – 75.7
Dimethoate	1.19	20	1.07	0.98 – 1.15	17	90.4	83.7 – 97.1	84.9	84.6 – 85.1
	2.38	17	2.11	1.92 – 2.3	18	93.8	85.7 – 101.8	86.3	86 – 86.6

4.3.1 Environmental conditions

Table 4.7 summarises the environmental conditions recorded throughout behavioural bioassays. We found temperature varied significantly between insecticide treatments (ANOVA; $F = 88.7$; $P < 0.0001$), with mean temperature recorded during the deltamethrin experiment almost 3°C lower than that measured in any other experiment, and temperatures recorded during the dimethoate experiment on average 1.7°C higher than any of the other experiments. Table 4.8 summarises the mean differences and post hoc analysis conducted using Tukey's multiple comparisons test.

Relative humidity also varied significantly between treatments (ANOVA; $F = 9.03$; $P < 0.0001$), with mean humidity on average 5% higher in deltamethrin than in any other treatment. Table 4.9 summarises the mean differences and Tukey's multiple comparisons test.

Table 4.7 – Summary of air temperature and relative humidity measured during mite movement observations in each treatment.

		Air Temperature (°C)				Relative Humidity (%)			
Treatment	***n***	**Mean**	**95% Confidence Intervals**	**Minimum**	**Maximum**	**Mean**	**95% Confidence Intervals**	**Minimum**	**Maximum**
Control	22	26.3	25.9 – 26.8	24.4	27.5	66.4	62.3 – 70.4	49	77
Acetamiprid	59	26.8	26.6 – 27.1	24.4	28.3	64.5	62.5 – 66.5	49	76
Deltamethrin	50	24.1	23.7 – 27.2	20.7	26.6	69.6	68 – 71.3	57	77
Dimethoate	54	27.5	27.2 – 27.7	24.8	28.9	62.3	60.3 – 64.3	50	76

Table 4.8 – Summary of mean difference ± 95% confidence intervals and Tukey's multiple comparisons test of differences in air temperature measurements taken during mite movement observations. α = 0.05; significant pairings are highlighted in bold.

Treatment Pairing	**Mean Difference (°C)**	**95% Confidence Intervals**	***P***
Control vs Acetamiprid	-0.48	-1.2 – 0.24	0.32
Control vs Deltamethrin	2.24	1.5 – 2.97	**<0.0001**
Control vs Dimethoate	-1.12	-1.85 – -0.39	**0.0006**
Acetamiprid vs Deltamethrin	2.71	2.16 – 3.27	**<0.0001**
Acetamiprid vs Dimethoate	-0.64	-1.19 – -0.1	**0.013**
Deltamethrin vs Dimethoate	-3.36	-3.92 – -2.79	**<0.0001**

Table 4.9 – Summary of mean difference ± 95% confidence intervals and Tukey's multiple comparisons test of differences in relative humidity measurements taken during mite movement observations. α = 0.05; significant pairings are highlighted in bold.

Treatment Pairing	Mean Difference (%)	95% Confidence Intervals	*P*
Control vs Acetamiprid	1.9	-2.9 – 6.7	0.74
Control vs Deltamethrin	-3.3	-8.2 – 1.7	0.32
Control vs Dimethoate	4.1	-0.8 – 8.9	0.13
Acetamiprid vs Deltamethrin	-5.1	-8.8 – -1.5	**0.002**
Acetamiprid vs Dimethoate	2.2	-1.4 – 5.8	0.4
Deltamethrin vs Dimethoate	7.3	3.6 – 11.1	**<0.0001**

4.3.2 Mite activity/inactivity

Figure 4.5 shows the proportion of observed mites that were inactive (resting) or active in each insecticide treatment. As in the full coverage experiment, we compared these proportions at each treatment level (observed) against the control proportions (expected), and we found no effect of acetamiprid residues on half of the arena on the number of inactive mites (Control = 8.3% inactive; 0.95 μg cm^{-2} = 16.7% inactive (95% CIs [7 – 36%]; P = 0.13); 4.77 μg cm^{-2} = 20.8% inactive (95% CIs [9 – 40%]; P = 0.045); 9.54 μg cm^{-2} = 16.7% inactive (95% CIs [7 – 36%]; P = 0.13)). Deltamethrin residues on half of the arena caused more mites to rest throughout the 10 min observation at all three concentrations than in control, with more mites inactive in the first (0.2× LC_{50}) and second concentrations (1× LC_{50}) than in the third concentration (2× LC_{50}; 5.3 × 10^{-4} μg cm^{-2} = 33.3% inactive (95% CIs [18 – 53%]; P = 0.0005); 2.7 × 10^{-3} μg cm^{-2} = 33.3% inactive (95% CIs [18 – 53%]; P = 0.0005; 5.3 × 10^{-3} μg cm^{-2} = 25% inactive (95% CIs [12 – 45%]; P = 0.012)). Finally, more mites were inactive in the first and third dimethoate concentrations than in control, but not in the second concentration level (0.24 μg cm^{-2} = 29.2% inactive (95% CIs [15 – 49%]; P

= 0.003); 1.19 µg cm^{-2} = 16.7% inactive (95% CIs [7 – 36%]; P = 0.13); 2.38 µg cm^{-2} = 29.2% inactive (95% CIs [15 – 49%]; P = 0.003).

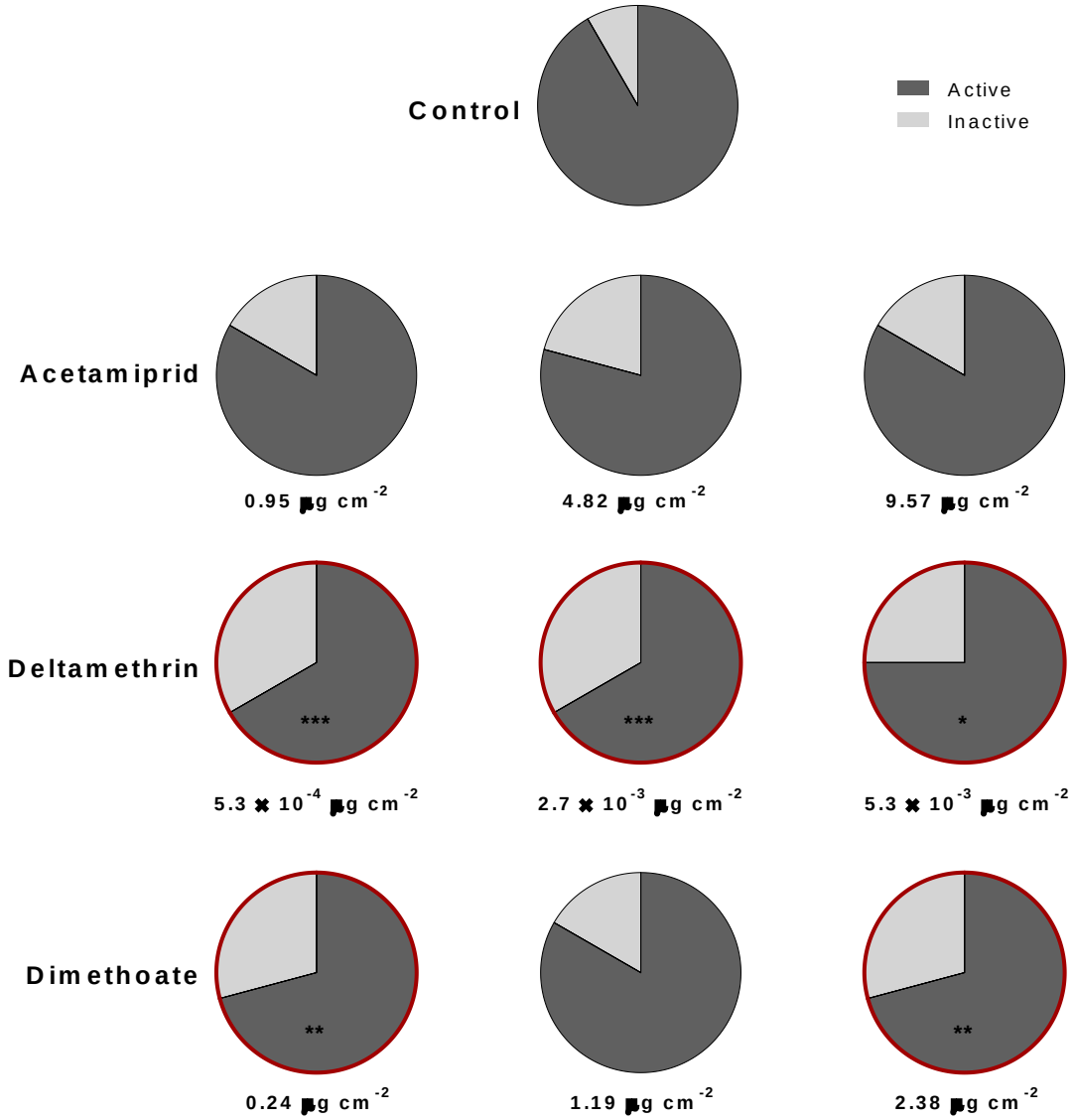


Figure 4.5 – Proportions of *T. pyri* individuals displaying activity (dark grey) or inactivity (light grey) during the 10 min observation period in control arenas and arenas half treated with insecticides. Charts bordered in red signify a significant difference in the proportions when compared to control proportions (α = 0.016; * = P < 0.016; ** = P < 0.01; *** = P < 0.001).

4.3.3 Zone preference study

There was no significant difference in the time spent in the treated and untreated arena zones when investigating mites in the control test arenas (paired t test; t = -0.88; P = 0.39). This suggests that visible methanol residues do not influence mite behaviour, nor do any other (unquantified) factors arising from the observation set up.

When comparing the amount of time spent in the treated arena half at each treatment level, we found no significant effects of acetamiprid (ANOVA; F = 1.8; P = 0.16) or deltamethrin (F = 1.1; P = 0.37) residues on the time spent in the treated arena half (Figure 4.6). However, there was a significant effect of dimethoate residues on the time spent in the treated zone (F = 3.8; P = 0.015). When comparing dimethoate concentration levels to control, there was no significant difference between the 0.24 µg cm^{-2} concentration and control, but there was a significant decrease in time spent on dimethoate residues at the 1.19 µg cm^{-2} and 2.38 µg cm^{-2} concentrations (Table 4.10).

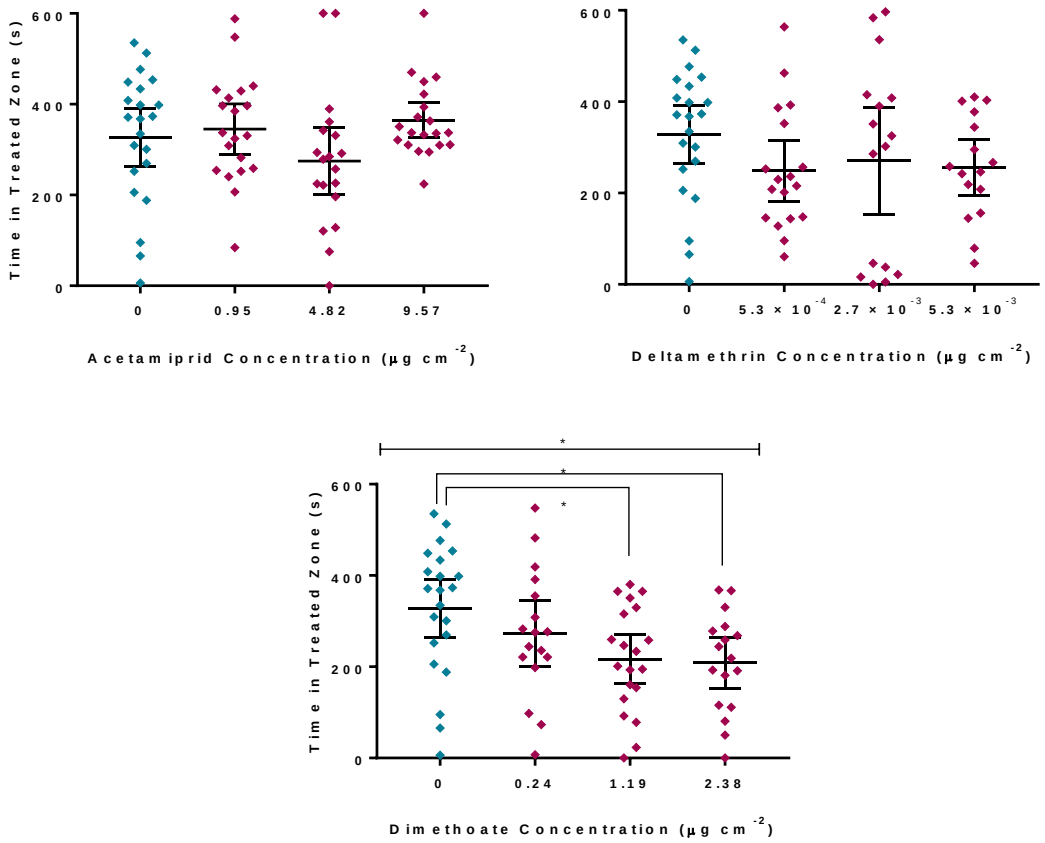


Figure 4.6 – Effect of a) acetamiprid, b) deltamethrin, and c) dimethoate residues, each at three concentrations, on time spent by *Typhlodromus pyri* in the treated (right hand) arena zone over 10 min. Line and whiskers for each column shows mean ± 95% confidence intervals at each treatment level. Individuals deemed inactive throughout the observation period were excluded from analysis. Significant effects of insecticide residues are shown on each graph, with brackets between control and a treatment level displaying significance of effect between that treatment and control; brackets to the top of each graph display significant effects of the pesticide overall versus control (P values adjusted for multiple comparisons; * = $P < 0.05$). Control $n = 22$; acetamiprid $n = 20; 19; 20$; deltamethrin $n = 18; 16; 16$; dimethoate $n = 17; 20; 17$.

Table 4.10 – Summary of Tukey's post-hoc analysis of the differences in time spent in treated arena halves, comparing time spent by mites on the control treated surface to the time spent on surfaces treated with dimethoate residues. P values are corrected for multiple comparisons (α = 0.05). Significant differences between a dimethoate concentration and control are highlighted in bold.

Treatment Comparison	Mean Difference (s)	95% Confidence Intervals	*P*
0 vs 0.24 μg cm^{-2}	54.87	-54.3 – 164.1	0.552
0 vs 1.19 μg cm^{-2}	110.9	6.4 – 215.4	**0.033**
0 vs 2.38 μg cm^{-2}	119	9.9 – 228.2	**0.027**

4.3.4 Mite movement behaviour

Figure 4.7 shows representative walking tracks of mites in test arenas half treated with methanol (control), and insecticides at each treatment level. Comparing insecticide treated arenas to control arenas, the largest visual difference in movement track comes between deltamethrin and control, where mites appear to cover less distance. There also appears to be a tendency to walk more on the untreated surface in the dimethoate treated arenas.

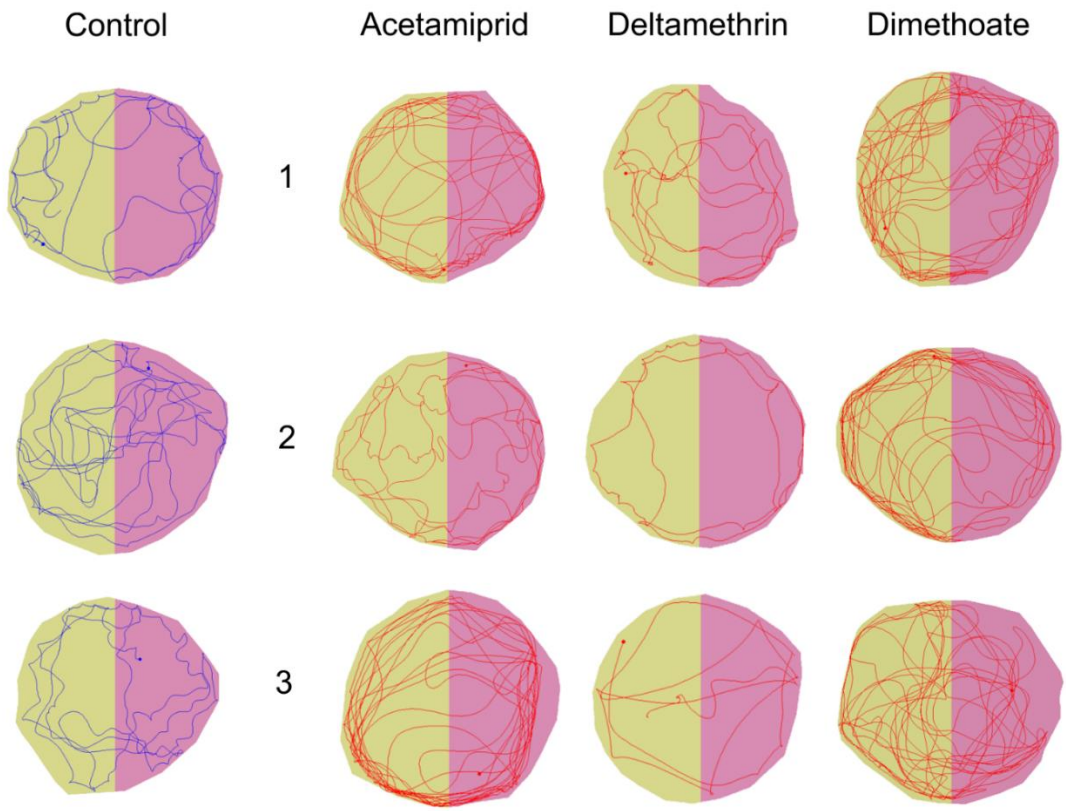


Figure 4.7 – Representative movement tracks from individual mites observed in the control (blue tracks) and insecticide treated test arenas (red tracks). Untreated arena zones are depicted in yellow and treated zones are depicted in pink. Mean arena area was 2.81 cm^2. Each number corresponds to increasing insecticide treatment concentration – Acetamiprid 1 = 0.95 µg cm^{-2}; 2 = 4.77 µg cm^{-2}; 3 = 9.54 µg cm^{-2}. Deltamethrin 1 = 5.3×10^{-4} µg cm^{-2}; 2 = 2.65×10^{-3} µg cm^{-2}; 3 = 5.3×10^{-3} µg cm^{-2}. Dimethoate 1 = 0.24 µg cm^{-2}; 2 = 1.19 µg cm^{-2}; 3 = 2.38 µg cm^{-2}.

4.3.4.1 Insecticide effects on movement behaviour in half exposed arenas

Figure 4.8 (page 148-149) summarises the effect of acetamiprid, deltamethrin and dimethoate on five movement behaviours (distance walked, velocity, angular velocity, meander and time active), with each behaviour quantified in the untreated and treated arena zones. For each insecticide we will discuss the effect of insecticide on each movement behaviour in the context of the results of the mixed ANOVAs, with three points discussed: the difference between responses in untreated and treated zones without accounting for insecticide concentration level and then any interaction

of insecticide concentration (i.e. within-subject effects); and the effect of insecticide concentration levels when comparing responses for arenas as a whole (i.e. between-subject effects). Effect sizes are discussed from interpretation of the partial eta squared (η^2) value.

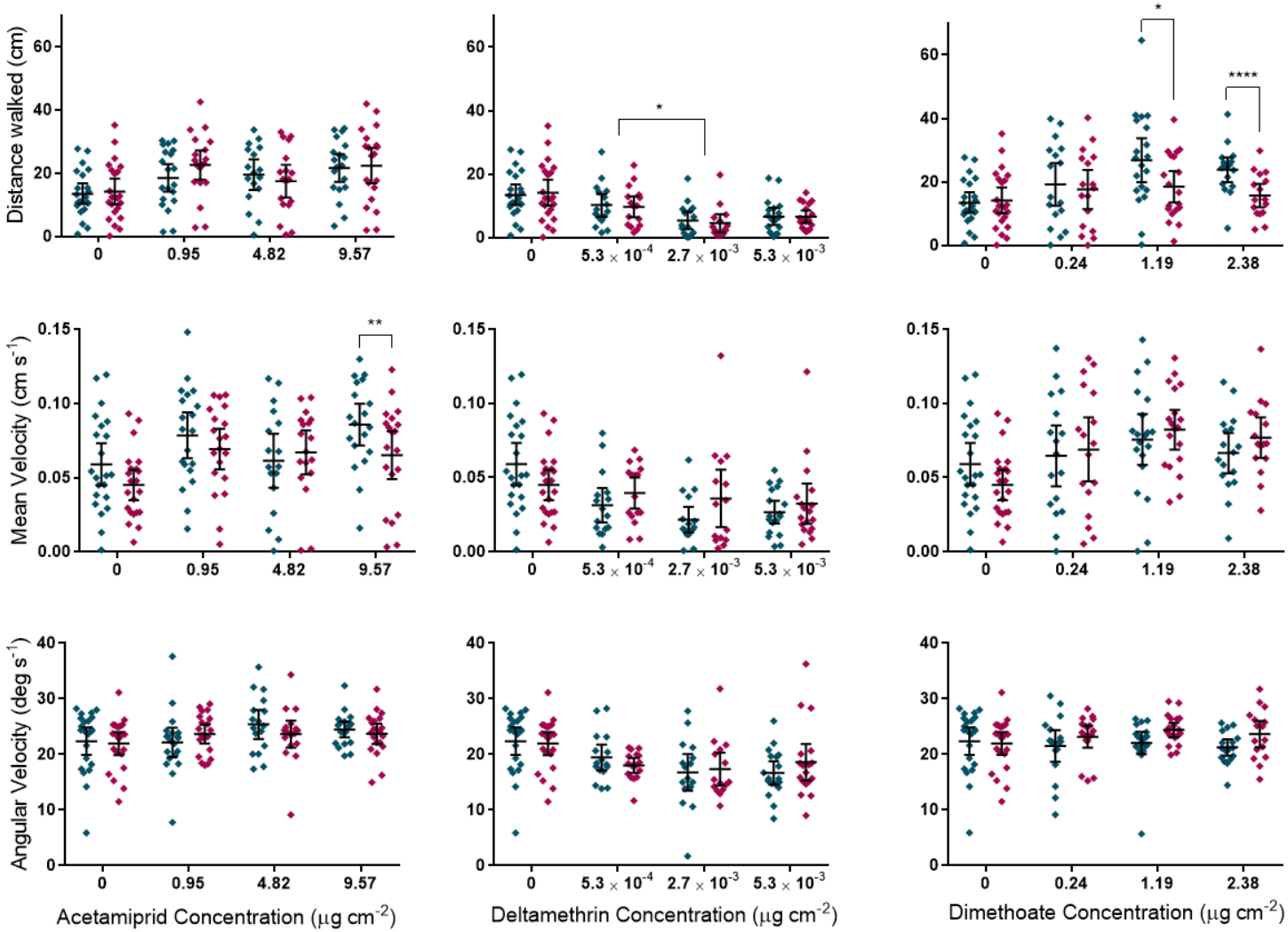
Acetamiprid

When observing mite velocity, we saw a significant interaction of mite velocity in untreated and treated arena zones and acetamiprid treatment ($F = 3.75$; $P = 0.03$; $\eta^2 = 0.13$), though velocity was not significantly different on the treated or untreated surface. We conducted three paired t tests to investigate further and found that mite velocity was 0.017 cm s^{-1} slower in the treated zone at the 9.57 µg cm^{-2} acetamiprid concentration, though there was no significant effect at any other concentration (Table 4.11). We also found that a mite's path was more tortuous when walking on acetamiprid residues, as meander increased by 38 deg cm^{-1} on the treated surfaces when comparing treated and untreated surfaces irrespective of treatment level (95% CIs [8.3 – 68.1 deg cm^{-1}]; $F = 6.6$; $P = 0.01$; $\eta^2 = 0.11$). However, there was no significant effect of concentration on meander ($F = 2.9$; $P = 0.06$; $\eta^2 = 0.1$). We also found time spent active was significantly affected by acetamiprid residues, with mites spending more time on treated than untreated surfaces ($F = 24$; $P < 0.0001$; $\eta^2 = 0.3$).

Table 4.11 – Summary of paired t tests applied as post hoc analysis of the effect of acetamiprid residues on the velocity of *T. pyri*. α = 0.0167 and is based on Bonferroni's correction for multiple comparisons. Mean differences are comparing measurements for the untreated surface to the treated surface. Significant differences are highlighted in bold.

Acetamiprid concentration (μg cm^{-2})	**Mean difference (cm s^{-1})**	**95% Confidence Intervals**	**t**	***P***
0.95	0.009	-0.009 – 0.027	1.06	0.3
4.82	-0.01	-0.025 – 0.005	-1.43	0.17
9.57	0.017	0.008 – 0.027	3.99	**0.001**

There was also a significant interaction with treatment, suggesting the effect of acetamiprid residues varied with concentration ($F = 5$; $P = 0.01$; $\eta^2 = 0.16$). We found that mites spent 73 seconds longer in the treated surface than the untreated surface at the 0.95 μg cm^{-2} concentration (95% CIs [22.4 – 123.8]; $t = 3.02$; $P = 0.007$), though the effect was not significant at any other concentration (Table 4.12). We found no effect of acetamiprid on the distance walked or angular velocity.


Distance walked (cm)
Mean Velocity (cm s-1)
Angular Velocity (deg s-1)
0
0.95
4.82
9.57
5.3 × 10-4
2.7 × 10-3
5.3 × 10-3
0.24
1.19
2.38
*
**
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Acetamiprid Concentration (μg cm-2)
Deltamethrin Concentration (μg cm-2)
Dimethoate Concentration (μg cm-2)

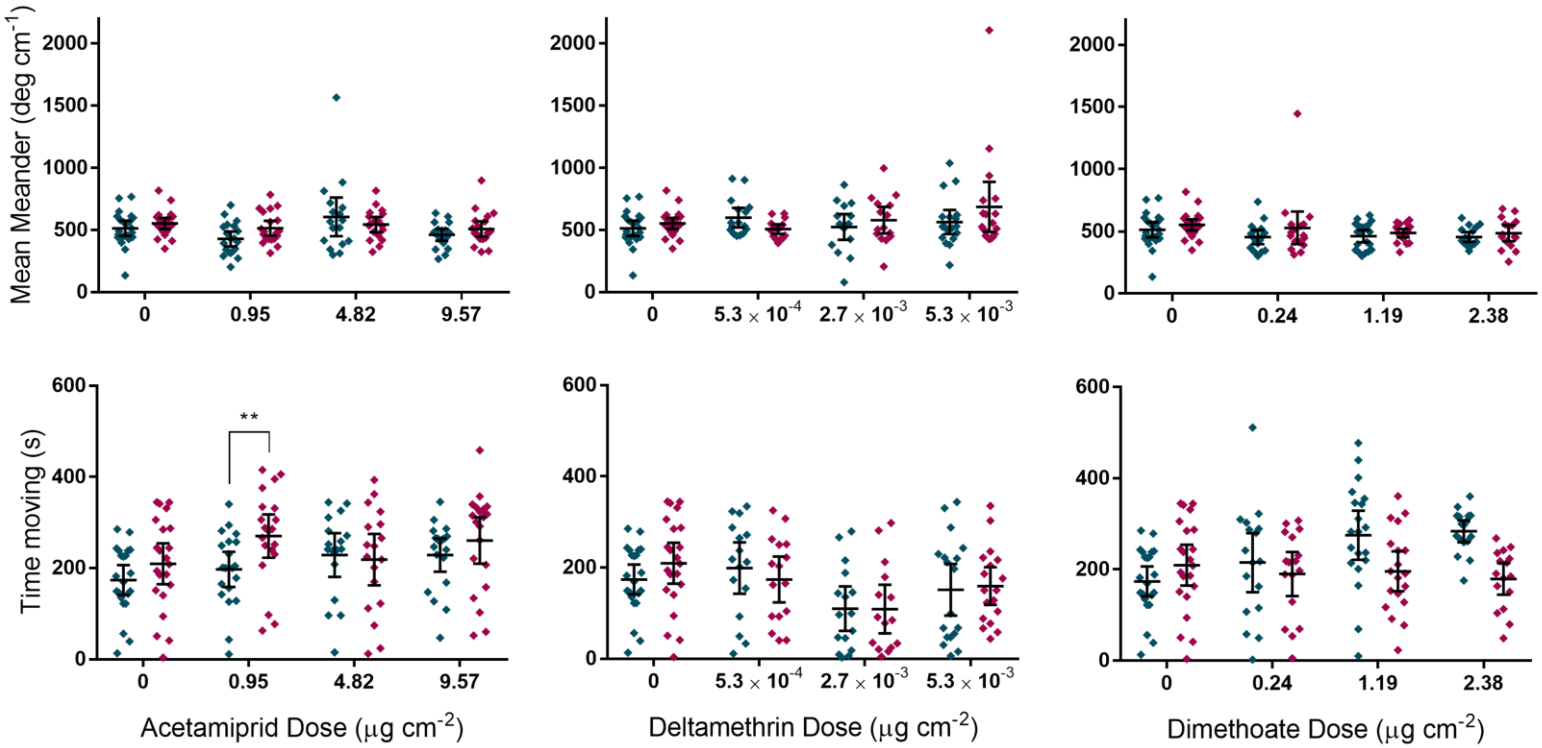

Figure 4.8 – Effect of acetamiprid (left column), deltamethrin (middle column) and dimethoate (right column), each at three doses, on the distance walked, velocity, angular velocity, meander and time spent active in the untreated (left hand; blue) and treated (right hand; pink) sections of the test arena, as observed in *Typhlodromus pyri* individuals over 10 min. Line and whiskers for each column shows mean ± 95% confidence intervals at each treatment level. Individuals deemed inactive throughout the observation period were excluded from analysis. Significant effects of insecticide are shown on each graph, with brackets between untreated and treated arena sections displaying significant differences in those measurements at that treatment level; brackets between two treatments display significant differences in overall differences between two treatment levels (P values adjusted for multiple comparisons; * = $P < 0.05$; ** = $P < 0.01$; *** = $P < 0.001$; **** = $P < 0.0001$). Control $n = 22$; acetamiprid $n = 20$; 19; 20; deltamethrin $n = 18$; 16; 16; dimethoate $n = 17$; 20; 17.

Table 4.12 – Summary of paired t tests applied as post hoc analysis of the effect of acetamiprid residues on the time *T. pyri* individuals spend active. α = 0.0167 and is based on Bonferroni's correction for multiple comparisons. Mean differences are comparing measurements for the untreated surface to the treated surface. Significant differences are highlighted in bold.

Acetamiprid concentration (μg cm^{-2})	**Mean difference (s)**	**95% Confidence Intervals**	**t**	***P***
0.95	73.2	22.4 – 123.8	3.02	**0.007**
4.82	1.28	-46.1 – 43.6	0.06	0.952
9.57	42.5	-85.1 – 0.17	2.09	0.051

Deltamethrin

Distances walked by mites did not differ between treated or untreated surfaces, nor was there an effect of concentration on this; however, we found that when arenas were analysed overall, distances walked in the overall arena were significantly affected by deltamethrin residues ($F = 3.7$; $P = 0.03$; $\eta^2 = 0.14$). We conducted Tukey's multiple comparisons test to identify the significant differences and found that distance walked by mites reduced by 4.8 cm between the lowest (5.3×10^{-4} μg cm^{-2}) and middle (2.7×10^{-3} μg cm^{-2}) deltamethrin concentrations (95% CIs [0.27 – 9.3]; adjusted $P = 0.034$), though no other comparison proved to be significant (Table 4.13). Mite velocity differed between the untreated and the deltamethrin treated surfaces, with mites moving on average 0.01 cm s^{-1} faster on the deltamethrin residues irrespective of concentration (95% CIs [0; 0.018]; $F = 4.2$; $P = 0.045$; $\eta^2 = 0.08$). There was no effect of deltamethrin concentration upon this difference. We found no effect of deltamethrin residues on angular velocity, meander, or time spent active in the half treated arena.

Table 4.13 – Summary of Tukey's multiple comparisons test, applied as post hoc analysis of the effect of deltamethrin residues on the overall distance walked by *T. pyri* in test arenas half treated with deltamethrin. P values are adjusted for multiple comparisons (α = 0.05). Mean differences are comparing measurements for the untreated surface to the treated surface. Significant differences are shown in bold.

Deltamethrin concentration (µg cm^{-2})	**Mean difference (cm)**	**95% Confidence Intervals**	***P***
5.3×10^{-4}	4.8	0.27 – 9.33	0.034
2.7×10^{-3}	3.3	-1 – 7.65	0.19
5.3×10^{-3}	-1.48	-5.9 – 2.92	> 0.999

Dimethoate

We found that mites covered less distance on the treated surfaces than untreated surfaces ($F = 22$; $P < 0.0001$; $\eta^2 = 0.31$), and that dimethoate concentration significantly interacted with this ($F = 3.3$; $P = 0.046$; $\eta^2 = 0.12$). Post-hoc analysis showed that mites covered 9.8 cm less distance on the treated surface at 1.19 µg cm^{-2} (95% CIs [2.2 – 17.4]; $t = 2.7$; $P = 0.014$), and 9.3 cm less at 2.38 µg cm^{-2} (95% CIs [6.3 – 12.3]; $t = 6.6$; $P < 0.0001$; Table 4.14). Mite paths were more convoluted on dimethoate treated surfaces, with meander measuring 1.7 deg cm^{-1} higher than on untreated surfaces (95% CIs [0.74 – 2.68]; $F = 5.7$; $P = 0.02$; $\eta^2 = 0.1$), though this was not affected by concentration. Time active was also significantly different, with mites spending 76 fewer seconds active on the dimethoate residues (95% CIs [41.6 – 111.2]; $F = 19.5$; $P < 0.0001$; $\eta^2 = 0.29$). Again, there was no effect of concentration. We found no significant effect of dimethoate residue on velocity or angular velocity.

Table 4.14 – Summary of paired t tests applied as post hoc analysis of the effect of dimethoate residues on the distance walked by *T. pyri* individuals. α = 0.0167 and is based on Bonferroni's correction for multiple comparisons. Mean differences are comparing measurements for the untreated surface to the treated surface. Significant differences are highlighted in bold.

Dimethoate concentration (μg cm^{-2})	**Mean difference (cm)**	**95% Confidence Intervals**	**t**	***P***
0.24	1.62	-1.2 – 4.4	1.2	0.24
1.19	9.77	2.2 – 17.4	2.7	**0.014**
2.38	9.28	6.3 – 12.3	6.6	**<0.0001**

4.3.4.2 Avoidance behaviour

No mites became trapped in the arena barrier in the control, acetamiprid or dimethoate treated arenas. In the deltamethrin experiment, just four mites became trapped in the half coverage experiment, with all four being trapped in the highest concentration (5.3×10^{-3} μg cm^{-2}). As mites only became trapped at the highest concentration, we decided a statistical test was unnecessary. On average mites became trapped in 297 seconds (95% CIs [144; 452]).

4.4 Discussion

In the following section we will first discuss the results of each analysis from the present experiment, and will then discuss these results in comparison to the full coverage experiment reported in Chapter 3.

4.4.1 Pesticide residue analysis

Our method, initially developed in Chapter 3 and refined for lower activity in this experiment, produced high recovery rates for acetamiprid and dimethoate, with recoveries across concentrations ranging 85 – 96% and co-efficients of variance within an acceptable range of 13 – 23% (Table 4.6). However, we experienced a reduction in quality of residue analysis for deltamethrin compared to the first experiment, with relatively poor recoveries of 54 – 58%. Variance was in the range 11 – 21% so was within range of the other two insecticides, and when considering all metrics for the residue analysis, we feel that the low recoveries are simply down to very low levels of activity within samples, arising from the very low deltamethrin concentrations we worked with in this study. In future it would be worth spiking deltamethrin samples with additional activity through the addition of a benign radiolabelled compound to the dosing stock. We conclude that this analytical method, refined for lower activity levels, continues to be a rapid, accurate and precise method for both acetamiprid and dimethoate at low quantities, though requires further refinement for very low activity levels.

4.4.2 Control mite movement and environmental conditions

Mite movement in control arenas tended towards mites tracking outer edges of the arena (Figure 4.7, Control 1 and 3); however, mites show a large degree of natural variation in their movement. The tendency to track outer edges had previously been reported in mites observed in arenas with no food available (Varley et al., 1994); therefore, like in the full coverage experiment, we feel the behaviour observed depicts typical food searching behaviour.

We observed that movement tracks appeared to be shorter in the control mites in this experiment, so compared the distance walked by control mites on full coverage arenas to distances walked by control mites in the present experiment and found that mites on average covered 21 cm less distance in the half coverage arenas (95% CIs [4.6 – 37.8]; Welch's t test; t = 2.6; P = 0.015). Examination of the air temperature and relative humidity ranges for the respective experiments showed that there were significant differences in the average conditions mites were subjected to during the

behaviour bioassays: temperature was 2.6°C higher in the half coverage experiment (95% CIs [2.13 – 3.17]; Welch's t test; t = 10.3; P < 0.0001); relative humidity was 17% higher in the half coverage experiment (95% CIs [12 – 22]; Welch's t test; t = 6.8; P < 0.0001). From this analysis we can infer that mite movement reduced between the first and second experiment due to the higher temperature and humidity levels within the measurement chamber, and when plotting a correlation of the control data from both experiments on the same chart, we see that mite movement reduced with increasing temperature (Figure 4.9a); and also reduced with increasing humidity (Figure 4.9b). Although these correlations were significant, they were not very strong (air temperature R^2 = 0.17; P = 0.007; relative humidity R^2 = 0.12; P = 0.025). In this instance the difference is most likely attributed to the change in experiment set up necessitated by the USB microscope failure during the full coverage experiment; however, we cannot dismiss natural, random variation in individual movement tendencies.

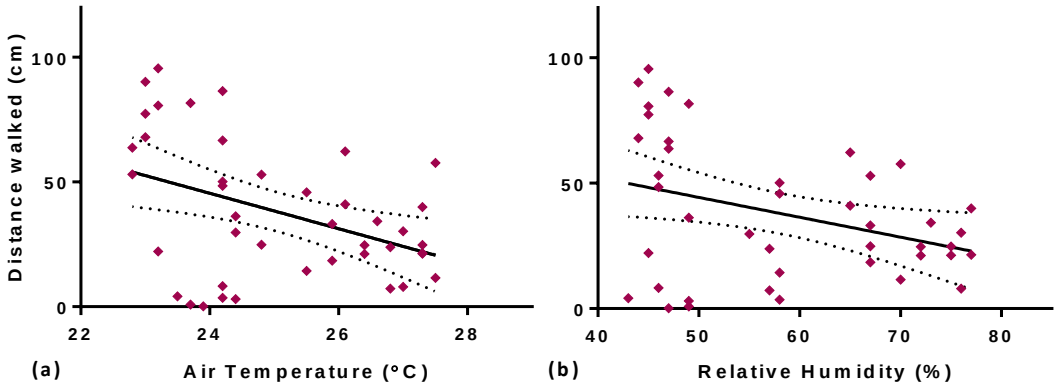

Figure 4.9 – Correlation between the distances walked by control mites in both the full and half coverage experiments, and air temperature (a), and relative humidity (b). Dotted lines depict 95% confidence intervals of plotted line. *n* = 42.

Research into the effect of temperature and humidity on movement in *T. pyri* is limited, although Dunley and Croft (1990) hypothesised low dispersal in orchards

could be due to high temperature. Unfortunately, most studies fail to report measured environmental conditions during their experiments, and instead report the expected condition within, for example, a controlled temperature room. Studies often take place in optimal environmental conditions to maximise the performance of control populations (Holmstrup et al., 2010); however, natural environments are rarely optimal, and as our study has shown, optimal conditions can be difficult to maintain in reality in laboratory conditions.

4.4.3 Mite activity/inactivity

We found that two of the three insecticides induced changes in mite resting behaviours in the half coverage study: deltamethrin residues caused higher rates of mites resting for the full observation period, with eight mites resting in the lowest two treatments compared to two in control, and six mites resting throughout in the highest treatment (Figure 4.5). Additionally, more mites were observed to be inactive throughout the bioassay in the lowest and highest dimethoate treatments, with seven mites observed to be inactive throughout in both treatments. These results were not expected as, based upon the results of the same analysis in the first experiment, there was an increase in mite activity in the deltamethrin treated arenas, and no significant differences in activity rates in dimethoate treated arenas (Figure 3.4).

Though many studies have observed hyperactivity in species exposed to pyrethroids (e.g. Alzogaray, Fontán and Zerba, 1997; Iftner and Hall, 1983), one study demonstrated that high concentrations arrest movement (Guedes et al., 2008). When we place our results from the full and half coverage experiments into the context of previous studies, we have two conclusions to draw: either the increase in the number of resting mites in the arenas half treated with deltamethrin shows that residues within the overall test arena were low enough to not cause hyperactivity in *T. pyri*; conversely, we may have demonstrated that, at very low concentrations, deltamethrin also arrests movement, though there are no literature data or reports to confirm this idea. This result also suggests that there is a threshold between the total residues measured in the full coverage and half coverage arenas where mites

become agitated by the residues, though it was not our intention with this experiment to identify this threshold.

The increase in the number of resting mites observed in those exposed to dimethoate residues was not expected and is in contrast to the limited information available regarding effects of the compound on insects. One study by Singh et al. (2001) demonstrated that ladybirds (*Coccinella septempunctata*) spent less time resting when exposed to dimethoate residues. Another study investigated movement behaviour in the soil dwelling collembolan (*Folsomia candida*) and found no significant effect of dimethoate residues on the time spent active at rates of 0.35 µg cm^{-2} and 0.7 µg cm^{-2} (Sørensen, Bayley and Baatrup, 1995). There are also reports of dimethoate residues increasing time spent active in woodlice (Bayley, 1995). Therefore, it seems that this could be the first report of dimethoate residues increasing the rate of resting behaviour in a predatory mite; alternatively, it could be a result of the large temperature difference between dimethoate and control experiments, with temperatures averaging 27.5°C and 26.3°C respectively. According to Figure 4.9b, 27.5°C would reduce distances covered in control mites.

Consistent with our previous, full coverage experiment, we observed no significant influence of acetamiprid residues on the number of resting mites (Section 3.3.2); following that experiment we suggested the residue levels were not high enough to induce changes in resting behaviour and our findings in the half coverage experiment reinforce this conclusion.

4.4.4 Zone preference study

We found that *T. pyri* were not affected by methanol residues on the test arena surface, as there was no significant difference in the time spent on the treated or untreated surfaces (Section 4.3.3). This also suggested there were no other unmeasured factors impacting upon a mite's preference for either side of the arena, such as light intensity.

We found no significant effect of acetamiprid or deltamethrin residues on the proportion of observation time spent on the treated arena surface (Figure 4.6); there

appeared to be a non-significant reduction in the time spent on deltamethrin residues and no trend relating to acetamiprid residues. We did however observe a significant reduction in the proportion of time spent on dimethoate surfaces, with mites spending approximately 33% and 36% less time on the treated surface at 1.19 μg cm^{-2} and 2.38 μg cm^{-2} respectively when compared to control. Our findings suggest a threshold exists between 0.24 μg cm^{-2} and 1.19 μg cm^{-2} where mites respond to the residues in such a way that causes them to spend time on untreated surfaces instead. This response equates to residue avoidance, likely through repellency based on the definition of Beers and Schmidt-Jeffries (2015); however, when applying the definition of Cordeiro et al. (2010) wherein irritation is observed in populations that spend < 50% of the observation period on treated surfaces, we can conclude that all three dimethoate concentrations lead to irrititation in *T. pyri*. By this definition we can also say that all three deltamethrin treatments also cause irritation, though mites exposed to deltamethrin residues display large variances in response and so we draw this conclusion with caution.

Our findings regarding dimethoate are consistent with reports of avoidance behaviour in *C. septumpunctata* on leaves (Singh, Walters and Port, 2001), though reports of dimethoate avoidance in predatory mites are limited. In a field study, dimethoate residues were observed to be repellent to bees (EFSA, 2006). Regarding acetamiprid, there is a report of extreme avoidance behaviour in *G. occidentalis* exposed to acetamiprid residues in a choice arena, where mites escaped the test arena completely (Beers and Schmidt-Jeffris, 2015). The authors studied a concentration similar to our highest treatment rate (178 mg L^{-1} compared to 180 mg L^{-1} in our study). This leads us to conclude *T. pyri* are less sensitive to acetamiprid residues than *G. occidentalis*, though more work would be necessary to confirm this.

4.4.5 Mite movement behaviour

Acetamiprid residues led to a 20% reduction in *T. pyri* velocity at the highest concentration studied (9.57 μg cm^{-2}), an 8% increase in meander on acetamiprid residues across the studied range, and also a 37% increase in the time spent active on acetamiprid residues at the lowest concentration (0.95 μg cm^{-2}; Figure 4.8). This

overall picture of responses to acetamiprid is convoluted, but suggests that there is a degree of toxic stress, identified by the slower movement rate and increased meandering behaviour. A previous study has identified velocity and angular velocity as the behaviours most sensitive to toxic stress in collembolan (Sørensen, Bayley and Baatrup, 1995), and suggests that changes in such behaviours would indicate a reduced fitness of the animals under field conditions. Interestingly there were no significant effects on these behaviours in the full coverage study, though angular velocity was increased by acetamiprid exposure (Section 3.2.2; Figure 3.5). We are not sure why a response would be observed in lower residue concentrations, but not in higher concentrations, though the high rates of variance in individual behaviours may be masking effects in full coverage studies, or producing a false positive effect here. One theory is that physiological controls are induced at the lower concentrations, leading to observed behavioural effects, and as concentrations increase, more toxic effects that do not show in behaviour – or mortality within 10 minutes – are occurring, overriding such physiological controls. Studies have shown that thiomethoxam, another neonicotinoid, affected thermoregulation, one such physiological control, in bees (Potts et al., 2018; Tosi et al., 2016). We would need to conduct further studies to determine whether physiological controls are the cause in our study.

We observed reductions in distances walked when mites were exposed to deltamethrin residues in choice arenas (Figure 4.8). This was a curious effect with no difference between treated and untreated zones; however, there was a near 50% reduction in the distance covered by mites when counting distance across the whole arena, and not in the two individual zones. This suggests that the toxic stress effect of deltamethrin residues at these levels arrests movement, as previously suggested in Section 4.4.3. It also suggests one of two things: either that the effect of deltamethrin is not just through contact, and that mites sense the residues remotely; or that the effect of contact with deltamethrin is instant and lingers after an individual has moved to an untreated surface. Due to the time scales we studied here, we conclude this effect is most likely the latter suggestion, with toxicokinetic effects continuing once individuals have moved away from treated areas. Mites also

displayed higher velocity on deltamethrin treated surfaces, though concentration was not relevant: this correlates with previous studies suggesting hyperactivity induced by pyrethroids (e.g. Alzogaray, Fontán and Zerba, 1997; Iftner and Hall, 1983), and when coupled with the reduced distances walked, it suggests that individuals may be irritated or stressed by contact with deltamethrin residues and subsequently move rapidly to escape the residues by moving to untreated surfaces. This combination of results would suggest that mites spent less time on the treated half.

To investigate this further, we examined the proportion of time spent on the treated and untreated surface by mites in the deltamethrin treated arenas (Figure 4.10), and conducted multiple paired t tests, one per treatment, to see whether there was a difference in time spent by mites on treated and untreated surfaces. Table 4.15 summarises the results and shows that, although less time was spent on the treated surface, there was no significant difference, likely due to the large variance in individual responses as illustrated in Figure 4.10.

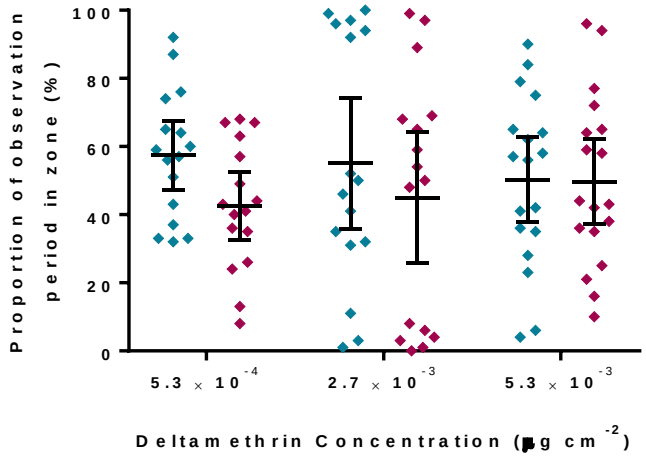

Figure 4.10 – Effect of deltamethrin residues at three concentrations, on the proportion of time spent by *Typhlodromus pyri* on the untreated (blue; left) and treated (pink; right) surfaces over 10 min. Line and whiskers for each column shows mean ± 95% confidence intervals at each treatment level. Individuals deemed inactive throughout the observation period were excluded from analysis. *n* = 18; 16; 16.

Table 4.15 – Summary of mean difference ± 95% confidence intervals and paired t tests of differences in the proportion of time spent on untreated and treated surfaces in deltamethrin treated test arenas. α = 0.0167; *n* = 16; 16; 18.

Deltamethrin concentration (µg cm^{-2})	**Mean difference (treated vs untreated surface; %)**	**95% Confidence Intervals (%)**	**t**	***P***
5.3×10^{-4}	-14.61	-34.73 – 5.52	1.55	0.14
2.7×10^{-3}	-9.98	-48.74 – 28.78	0.55	0.59
5.3×10^{-3}	-0.41	-25.5 – 24.69	0.03	0.97

Based on the full coverage study (Chapter 3), we would have expected to see reductions in distances walked, increased angular velocity and meander, and reduced time spent active as a result of deltamethrin residues (Figure 3.5). While the effects on distance walked have carried over into the choice experiment, there was no significant effect on the other three measures, suggesting that either the reduced

overall surface residues in the choice test arenas were not large enough to induce effects, or that natural variation amongst individual responses was too great to see a trend. Though there was a significant, yet very weak trend of angular velocity increasing with increasing temperature in mites within the deltamethrin arenas (Table 4.3), we conclude that natural variation in behaviour is the most likely explanation in this case.

Mites covered less distance in test arenas containing dimethoate residues, with a 34% reduction in distance walked on residues at the 1.19 μg cm^{-2} concentration, and 37% reduction at the 2.38 μg cm^{-2} concentration when compared to distances covered on untreated surfaces (Figure 4.8). Mite paths were increasingly convoluted on dimethoate residues, though this effect did not differ with concentration. Finally, mites spent less time active on the dimethoate residues than on the untreated surface, and again this was not affected by the different concentration levels, suggesting meander and time spent active are influenced by the presence of dimethoate residues at any level within the studied range. These findings are consistent with the findings that mites spend less time overall (i.e. whether active or resting) on dimethoate treated surfaces, and also display avoidance behaviour through a clear preference for untreated surfaces when given the choice (Section 4.4.4). These findings are corroborated by responses seen in the ladybird *C. septumpunctata* (Singh, Walters and Port, 2001), though as previously mentioned, we have found reports of avoidance relating to dimethoate to be limited. The reduction in time spent active on dimethoate residues also correlates with our findings that mites are less likely to move at all when exposed to dimethoate residues (Figure 4.5), and also spend less time on residues (Figure 4.6), further supporting our suggestions that dimethoate not only reduces activity, but also leads to residue avoidance.

A surprising difference between the full coverage and half coverage experiments was the lack of significant differences in angular velocity as a result of contact with pesticide residues. Our full coverage experiments found that angular velocity was affected by all three studied insecticides (Figure 3.5), and we were surprised to find no effects in this half coverage study. This suggests that angular velocity is a sensitive

measure of movement behaviour – an idea reinforced by Sorensen et al. (1995) – and that the behaviour was not affected by pesticides when placed in arenas with half of the overall residue level of that seen in the full coverage experiments.

4.4.6 Avoidance behaviour

Our study of avoidance behaviour following the same method as that applied in the full coverage study found that only four mites attempted to escape test arenas completely, and all four were at the highest deltamethrin treatment (Section 4.3.4). As there were no other examples of mites becoming trapped, we could not statistically investigate the behaviour within the half coverage study. When considering the response to residues in relation to responses seen in the full coverage experiment, 16 mites escaped the test arena when exposed to residues of 2.7×10^{-3} µg cm^{-2}; if residues in the half covered arena were considered across the whole arena, then the highest concentration in the half coverage arena would equate to the second concentration in the full coverage arena. When considering that only four mites escaped the test arena when residues were presented on half of the surface, we conclude that the rapid repellent effect of deltamethrin residues seen in the full coverage study is mitigated by mites having unexposed surfaces to rest or move on. This reinforces the suggestion that full coverage experimental designs overstate effects on species (Beers and Schmidt-Jeffris, 2015; Blümel, Pertl and Bakker, 2000). It also reinforces our conclusion from Chapter 3 that deltamethrin induces a rapid repellent effect.

4.4.7 Study findings compared to hypotheses

Prior to the experimental phase, we formulated working hypotheses for the three active substances. We hypothesised that acetamiprid and deltamethrin would induce avoidance behaviour, with the hypothesis for acetamiprid based on published observations involving *G. occidentalis* (Beers and Schmidt-Jeffris, 2015), and on our own observations in *T. pyri* for deltamethrin. We hypothesised that dimethoate would not induce avoidance behaviour as we observed no avoidance behaviour in our earlier studies; in addition there are no published reports of repellency in

predatory mites exposed to dimethoate, despite the substance being highly toxic (Blümel et al., 2000a).

We were interested to find that our hypotheses were proved wrong in all three cases, especially as our full coverage experiments informed the hypotheses. No avoidance behaviour was observed in individuals exposed to acetamiprid residues, in contrast to findings reports in the scientific literature. The only avoidance behaviour we observed in mites exposed to deltamethrin was identified with small numbers of individuals becoming trapped in the test arena glue barrier, though this effect was not as clear as in the full coverage study. We were surprised to observe a clear preference in mites exposed to dimethoate in choice arenas, where mites spent over 30% less time on treated surfaces than untreated surfaces.

4.4.8 Ecological relevance of findings

Our choice arena study investigated behavioural effects in mites exposed to residues amounting to no more than double the published 7d-LR_{50} for *T. pyri*, with residues on only half of the arena. In Chapter 2, we concluded that 17% of the sampled penconazole spray (250 L Ha^{-1}) was deposited on the upper apple leaf surfaces (Section 2.8). In the previous chapter, we inferred that a 17% deposition of a 200 L Ha^{-1} application rate – consistent with application rates used in toxicity studies used for determining treatment levels in this study – would amount to 34 L being deposited on apple leaves (Section 3.4.6). Based on this, and consistent with the process in Section 3.4.6, we converted nominal treatment concentrations for acetamiprid and deltamethrin to the equivalent field application rate; we then estimated field residues based on the treatment concentrations (Table 4.16). We applied the same basis to estimate how much active substance would land on apple leaves at the recommended application rate (i.e. label rate) for orchards (Table 4.17). We then informed our conclusions regarding acetamiprid and deltamethrin using this information. As before, we have not considered dimethoate in the same way as it is not permitted for use in apple orchards.

Table 4.16 – Insecticide treatment concentrations used in the behaviour studies expressed as their equivalent field rate if applied at a rate of 200 L ha^{-1} and as measured residues from the laboratory studies. Measured residues are based on the residues covering the whole test arena surface. Test concentrations are then expressed as estimations of residues landing on apple leaves in one hectare of orchard and per unit of leaf area. Presented values are based on the assumption that 17% of a 200 L ha^{-1} pesticide spray application lands on apple leaves in a 1 ha orchard.

	Test concentrations in laboratory studies			**Estimated residues on apple leaves in field**	
Active ingredient	**Nominal treatment concentration ($mg_{a.s.}$ mL^{-1})**	**Equivalent field application rate ($g_{a.s.}$ Ha^{-1})**	**Measured residues on test arena surface ($\mu g_{a.s.}$ cm^{-2})**	**Apple leaf residues in orchard (g Ha^{-1}_{ground})**	**Apple leaf residues ($\mu g_{a.s.}$ cm^{-2}_{leaf})**
	0.018	3.6	0.46	0.61	0.0025
Acetamiprid	0.09	18	2.29	3.06	0.0125
	0.18	36	4.31	6.13	0.0249
	1×10^{-5}	0.002	0.0001	0.0003	1.4×10^{-6}
Deltamethrin	5×10^{-5}	0.01	0.0009	0.002	6.9×10^{-6}
	1×10^{-4}	0.02	0.0018	0.003	1.4×10^{-5}

Table 4.17 – Field application rates for acetamiprid and deltamethrin expressed as estimations of residues landing on apple leaves in one hectare of orchard and per unit of leaf area. Presented values are based on the assumption that 17% of a 200 L Ha^{-1} pesticide spray application lands on apple leaves in a 1 Ha orchard.

Active ingredient	**Field application Rate ($g_{a.s.}$ ha^{-1})**	**Apple leaf residues in orchard (g ha^{-1}_{ground})**	**Apple leaf residues ($\mu g_{a.s.}$ cm^{-2}_{leaf})**
Acetamiprid	75	12.77	0.052
Deltamethrin	8.75	1.49	0.006

As before, we estimated that 12.77 g of a 75 $g_{a.s.}$ ha^{-1} application of acetamiprid would land on apple leaves, which equates to leaf residues of 0.052 $\mu g_{a.s.}$ cm^{-2}_{leaf} (Table 4.17). If we assume the residues applied to the choice test arenas were homogeneous across the test arena surface, then the acetamiprid levels we exposed *T. pyri* adults to were 9 – 83 times higher than the expected leaf residues, arising from field application of our test concentrations (Table 4.16). Consistent with our conclusion in Chapter 3, the effects of acetamiprid on velocity and time spent active would not be of concern in the crop environment, unless these effects also occur at much lower residue levels.

For deltamethrin, a label rate field application of 8.75 $g_{a.s.}$ ha^{-1} would lead to 1.5 g landing on apple leaves in one orchard hectare – this equates to leaf residues of 0.006 $\mu g_{a.s.}$ cm^{-2}_{leaf} (Table 4.17). When assuming deltamethrin residues were homogeneous across the test arena surface, the residue levels we assessed were 3.5 – 47 times lower than the residue levels we would anticipate based on the label rate field application (Table 4.16). We observed increased inactivity in mites exposed to deltamethrin residues at all three treatments when in choice arenas; therefore, we would anticipate this response to occur in the orchard environment. We also observed reduced distances covered by mites when comparing the first and second treatment levels and when considering mite movement across the whole arena – we would also anticipate these effects to occur in the crop environment when deltamethrin is sprayed at the field rate. Moreover, we would expect these

behaviours to be more likely as the effects were observed in heterogeneous exposure landscapes, meaning spray-free refugia are not enough to avoid negative effects of residues.

As dimethoate is no longer used in crop systems where *T. pyri* reside (e.g. grapevines, fruit orchards), we cannot comment on the implications for this insecticide.

4.4.9 Summary of findings and comparison to full coverage study

In summary, we found that acetamiprid residues in choice arenas did not increase or decrease the number of active mites, nor did the residues affect the time spent on the treated surface compared to control. In comparing movement behaviours on treated and untreated surfaces, acetamiprid did not affect distances walked, angular velocity or meander. However, mite velocity was 20% slower on surfaces treated at the highest concentration (9.57 µg cm^{-2}) than on untreated surfaces, and mites spent 37% more time active on surfaces treated at the lowest concentration (0.95 µg cm^{-2}) than on the untreated surface. In Chapter 3 we found that angular velocity was affected in full coverage arenas, with observed increases in angular velocity at all three concentrations. In considering all effects as a whole, we conclude that acetamiprid causes irritation to *T. pyri* at these concentrations, an effect especially highlighted by responses in the choice arenas.

Deltamethrin residues in choice arenas reduced the number of active mites in comparison to control at all three concentrations, but did not significantly affect the time spent on the treated surface, either compared to control mites, or when comparing paired data from individuals. Distances covered by mites across whole arenas were reduced by 24% between arenas treated with the lowest (5.3×10^{-4} µg cm^{-2}) and middle (2.7×10^{-3} µg cm^{-2}) concentrations, but deltamethrin residues did not affect any other movement behaviour. Avoidance behaviour was again observed, but only in four mites at the highest concentration, a much lower number than those observed in the full coverage experiments. By comparison, mite movement behaviour in arenas fully covered with deltamethrin residues was significantly altered, with reduced distances covered and time spent active, and increased angular

velocity and meander. In Chapter 3 we concluded that deltamethrin residues at these low levels both irritated and arrested mite movement, and also caused a high number of mites to escape residues completely. In considering all of these effects, we conclude that mite movement behaviour is significantly affected by deltamethrin residues, but only where they have no choice between avoiding or being exposed to residues.

We observed avoidance behaviour in *T. pyri* adults exposed to dimethoate residues of 1.19 and 2.38 µg cm^{-2} in choice arenas when comparing the time spent on the treated surface to the time mites spent on the control treated surface. Dimethoate residues also increased the number of inactive mites, but only at the lowest (0.24 µg cm^{-2}) and highest (2.38 µg cm^{-2}) residue levels. We also observed lower distances walked in the second and third concentrations, and an overall reduction in time spent active on all dimethoate treated surfaces. Considering these responses as a whole with responses in the full coverage arenas, there is a clear effect of dimethoate on *T. pyri* adults, with mites choosing to spend less time on residues if given the choice, and also to move less on these residues. When combined with mites displaying a more tortuous path when moving on dimethoate residues, we conclude that *T. pyri* adults detect the residues, are affected by the residues, and are avoiding residues as a result of irritancy.

The subtleties of behavioural responses to deltamethrin and dimethoate were completely missed by the analysis of behaviour in full coverage arenas. If we took the conclusions from the full coverage studies, we would not have known that *T. pyri* adults avoid dimethoate residues given the choice; likewise, the strong behaviour changes elicited by deltamethrin residues were diminished when individuals had a choice of treated and untreated surfaces. As a result, our conclusions regarding both insecticides were altered.

4.4.10 Risk assessment implications

Differences in results between the choice arenas and full coverage arenas reinforces the point that the full coverage arenas used in pesticide risk assessment are worst

case scenarios and may overstate effects (Beers and Schmidt-Jeffris, 2015), though as we have previously noted, heterogeneous coverage may also lead to greater residues where spray lands, therefore their assertion is not certain. However, our work suggests the overstatement comes as a result of an overly simple test design, and suggests caution when interpreting results elicited in test environments with small spatial and temporal scales, as these may differ in the crop environment. As previously discussed in Section 3.4, avoidance behaviour is a double edged sword: it could reduce the toxic effects of residues; however, it could also reduce the biocontrol capabilities of non-target arthropods such as *T. pyri*. Similar arguments have been made in other ecotoxicology contexts: Meli et al. (2013) highlighted the importance of knowing how homogeneous (or heterogeneous) chemical concentrations are in soil, as toxicity may be overstated if homogeneity is erroneously assumed. Additionally, a study comparing responses in *T. pyri* to fungicides in laboratory and field trials found disparities between responses, with laboratory studies overestimating deleterious effects (Blümel, Pertl and Bakker, 2000).

Based on our findings across the full- and partial coverage test arenas, we feel that regulators should consider the inclusion of choice arenas in the risk assessment of plant protection products. The inclusion of laboratory-based choice arena studies may help to bridge the gap between effects seen in the laboratory and in field studies, therefore improving the understanding of pesticide effects and subsequent ecosystem- and population-level consequences.

4.4.11 Further study

In Chapter 3 we considered several ideas for additional laboratory-based studies to develop further knowledge of behavioural changes (Section 3.4.7). These are also relevant to the choice arena study design we have reported here so will not be repeated. Instead, we will explore further study options in different directions.

There are many questions raised by our findings in relation to the risk to *T. pyri* populations as a result of pesticide induced behaviour changes. There are four pertinent questions to ask in this context: what mechanisms are behind avoidance behaviour? Do behaviours change in the crop environment in the same way as we

observed in the laboratory? Do any changes affect the biocontrol potential of *T. pyri*? Finally, do behavioural changes affect the overall population structure? These questions would require both field and laboratory studies: field investigations of mite behaviour in sprayed orchards; assessments of feeding and pest control in both the laboratory and the field; and laboratory studies of changes in reproductive behaviour would all inform the discussion of the risk to non-target arthropods arising from pesticide exposure. For example, the avoidance of pyrethroid (such as that we observed for deltamethrin, though to a greater extent in the full coverage study) and dimethoate residues could reduce biocontrol potential, but could reduce mortality in field populations due to the ability to avoid contamination. This combination of effects would then give an arising question: what are the long term population consequences? Such studies could then be used to develop a population model to investigate the long term consequences of any behavioural changes; this would combine well with the previous suggestion in Chapter 3 of studying effects at a range of temperatures to allow for the modelling of such consequences over large spatial scales.

In the case of our present work, the movement behaviour changes we quantified in this and the previous chapter will be used to parameterise and develop an individual based model to begin to answer the question of long term population consequences. The movement behaviour data will be integrated with existing life table parameters from the scientific literature to try to understand whether avoidance behaviour and movement behaviour changes would have long term consequences.

Chapter 5 – From an individual to a population: modelling the effects of pesticide residues on *Chrysoperla carnea* and *Typhlodromus pyri* in the PANTA model

5.1 Introduction

5.1.1 Models in ecotoxicology

Once lethal and sublethal effects of pesticides have been quantified in a species, it can be difficult to consider how this might affect populations in the short term, long term, and in the natural environment. More importantly, it is difficult to conclude whether any of the observed effects are important, such as in the context of population consequences. Though laboratory studies lack the ecological realism that would allow determination of population effects, field studies can be prohibitively expensive, complex and difficult to interpret (Jänsch et al., 2006). The European Food Safety Authority (EFSA) concluded in a scientific opinion report that field studies and modelling can be used to assess recovery following exposure, but also that modelling can go further than field studies by allowing extrapolation from small to larger scales, as well as the study of pesticide effects on species in different landscape scenarios and climatic conditions (EFSA, 2015).

Ecological modelling is increasingly being used in support of environmental decision making (A Schmolke et al., 2010). Models offer welfare advantages by reducing the need for animal testing; modelling also allows extrapolation of effects from the individual scale to the population (EFSA Panel on Plant Protection Products and their Residues, 2018; Amelie Schmolke et al., 2010). Models can also take into account various spatial and temporal scales that might prove impossible to study in experiments (Augusiak, Van den Brink and Grimm, 2014). Though models have in the past courted criticism and scepticism due to poor documentation and

communication of model processes, some modelling methods have recently been deemed ready for regulatory use for aquatic organisms (EFSA Panel on Plant Protection Products and their Residues, 2018; Grimm et al., 2010).

One popular approach for population modelling is individual-based modelling (IBM), also known as agent-based modelling (ABM); this is an approach that allows simulation of populations via creation of discrete individuals (Grimm and Railsback, 2005). Individuals, or agents, are governed by simple rules that define their behaviour, and these can be altered by interactions with other agents or the environment in which they are modelled (Macal and North, 2010). As opposed to top-down population parameters driving models as in differential equation modelling (e.g. Hanson and Stark, 2011), IBMs work from the bottom-up, allowing population-level behaviours to emerge from interactions between the discrete individuals and their environment (DeAngelis and Grimm, 2014). Recent IBM applications include studying soil warming and tillage effects on earthworm populations (Johnston, Sibly and Thorbek, 2018), metal contamination effects on springtail populations (Meli et al., 2013), and the effects of stressors, impaired foraging, and landscape changes on honeybee colonies (Becher et al., 2014).

In the study of contaminants affecting organisms, toxicokinetic-toxicodynamic (TKTD) modelling has emerged as the main method. Toxicokinetics cover what an organism does with a toxic chemical (e.g. the absorption, distribution, biotransformation and elimination), while toxicodynamics cover what the chemical does to that organism (Ashauer et al., 2011). Two main theories exist within this. The first, dynamic energy budget theory (DEB), works on the basis that individuals consume resources and convert them into energy for completing their entire life cycle (Kooijman, 2010), and this is tied to food availability and temperature (Lika et al., 2011). DEB in toxicology (DEBtox) is an extension of this, with models considering the mechanisms of action and subsequent effect of a contaminant on life history over time and different exposure concentrations (Billoir, Péry and Charles, 2007). Though the theory has led to the development of many models, including for earthworms (e.g. Baveco and Roos, 1996), there are still many gaps in the application due to a lack of knowledge of physiological modes of action (Ashauer and Jager, 2018); as such, DEBtox is not yet

routinely used for regulatory purposes (EFSA Panel on Plant Protection Products and their Residues, 2018).

The second main TKTD application is the general unified threshold model of survival (GUTS; Jager et al., 2011), which is used to model lethal effects through connecting external contaminant concentrations with damage dynamics. GUTS combines two assumptions regularly applied in ecotoxicology: individual tolerance, where each individual has their own tolerance of a contaminant and tolerance exceedance leads to mortality; and stochastic death, where all individuals share a common threshold, and once this is exceeded mortality occurs stochastically (Ashauer et al., 2015; Jager et al., 2011). The use of GUTS in ecological risk assessment has recently been recommended by EFSA, who suggest such models are now ready for regulatory use in studying effects on aquatic organisms (EFSA Panel on Plant Protection Products and their Residues, 2018); this contrasts with their opinion on DEBtox models which EFSA believes have the potential to be used in regulatory frameworks, but require further validation.

Although GUTS models have been recommended for aquatic applications, there is currently no recommendation for their application in terrestrial organisms. In 2015 EFSA highlighted the lack of modelling involving terrestrial species, in particular non-target arthropods (EFSA, 2015), and we have been unsuccessful in finding TKTD models relating to *Typhlodromus pyri*, the species we studied in Chapters 3 and 4, even though this is widely used in regulatory studies. At the time of writing, known physiological modes of action – necessary for TKTD model development – are limited to mainly soil dwelling arthropods and worms in the terrestrial compartment (Ashauer and Jager, 2018). Therefore, any attempts to consider the population level consequences of pesticides on non-target arthropods will be much simpler in application.

5.1.2 Population consequences of avoidance behaviour

In Chapters 3 and 4, we studied the effects of three insecticides on the movement behaviour of *T. pyri*, and as part of these studies we observed and quantified

avoidance behaviour when individuals were exposed to deltamethrin (Chapter 3) and dimethoate residues (Chapter 4). Having identified these behaviours in individuals in the laboratory, it is difficult to then understand how avoidance might affect populations in the natural environment. Previous studies in parasitoids and spiders have observed reduced prey consumption in individuals displaying more convoluted walking trajectories, arrested movement, and avoidance (Umoru, Powell and Clark, 1996; Shaw, Waddicor and Langan, 2006). Reduced prey consumption has been linked to longer development times in *T. pyri* larvae (Hayes and McArdle, 1987), which would in turn have population-level consequences. It has also been suggested that pesticide avoidance behaviour in crop systems would lead to the dispersal and loss of predatory mites from crops, leading to changes in species dynamics and pest stresses (Cordeiro et al., 2013; Gerson and Cohen, 1989).

Individual-based models can be utilised to gain greater understanding of the population consequences of avoidance behaviour. In an IBM developed to study metal contamination avoidance by springtails, Meli et al. (2013) demonstrated that the ability to detect and avoid toxicants influenced feeding behaviours and that a heterogeneous exposure landscape allowed population equilibrium to be achieved. However, applications of IBMs in the study of avoidance behaviour and population consequences are otherwise sparse.

Although we have studied movement behaviour in *T. pyri* at the individual level, we do not know how these changes, including avoidance behaviour, interact with a heterogeneous pesticide exposure landscape such as those reported in Chapter 2. We also do not know what population-level consequences could arise from changes in behaviour. To this end we developed an IBM to better understand these topics. Though our ideal model concept would include TKTD model principles, it was important to develop a simple model with as little uncertainty as possible. Therefore, we made the decision not to pursue the addition of TKTD principles in this initial model.

5.1.3 Aim

Our individual-based model, Pesticide Avoidance in Non-Target Arthropods (PANTA), was developed with the aim of investigating the population-level consequences of pesticide exposure and avoidance behaviour in non-target arthropods through the study of survival. We have parameterised the model for two species, the green lacewing *Chrysoperla carnea*, and the predatory mite *Typhlodromus pyri*.

5.1.4 Research questions

This study is intended as a proof of concept, to demonstrate that individual-based modelling can act as the bridge to understand how pesticide-induced behaviour changes, avoidance behaviour, and spatially heterogeneous pesticide exposure interact and affect non-target arthropod populations.

We produced a series of hypothetical questions for the model to answer for both modelled species. These were tested through the simulation of lacewings and mites in heterogeneous exposure landscapes to study the effect of avoidance behaviour at a range of pesticide coverage levels. The chosen insecticide for this study was acetamiprid as it was one of the substances studied in Chapters 3 and 4, is relevant to both species, and some data on mortality is available for both species. Evidence exists of avoidance behaviour in non-target arthropods when placed in choice arenas with neonicotinoids (e.g. Beers and Schmidt-Jeffris, 2015; Fernandes et al., 2016); however, our own laboratory studies failed to detect a significant response in *T. pyri* (Chapter 4).

Three questions were tested in relation to both *C. carnea* and *T. pyri*:

1. How much longer do individuals survive when they avoid contact with pesticide residues?
2. How many more individuals survive when they avoid contact with pesticide residues?
3. How many more eggs are laid by adults when they avoid contact with pesticide residues?

We hypothesise that the ability to avoid pesticide residues increases survival in both species, and through that, also the total fecundity of females as longer longevity would allow more reproduction. These questions were answered by running the PANTA individual-based model with 24 real life pesticide exposure patterns derived from water sensitive papers collected in the study reported in Chapter 2 (24 from one apple tree). We also included two "control" exposure patterns: 0% and 100% coverage.

The research questions allow us to consider the population consequences through changes in female oviposition rates. Standardised regulatory tests involve studying *C. carnea* survival to adulthood, which can take four weeks but do not have a specified temporal end point (Vogt et al., 2000). Such tests involving *T. pyri* study juvenile mortality after seven days of exposure (Blümel et al., 2000a), a time point where most individuals have developed into adults from our experience of rearing mites in the laboratory. Therefore, the first two questions were answered for *C. carnea* by studying survival to adulthood, and for *T. pyri* by studying survival after seven days. Female oviposition rates were studied through the life time of adult individuals, and data arising from these simulations also informed the investigation of pesticide effects on longevity.

5.2 The model

5.2.1 Biological context

As previously mentioned, we developed the model for two non-target arthropod species with differing lifestyles and developmental periods. We have previously introduced and described the two study species (Chapter 1), and will go into further detail below with details relevant to the modelling of the two species.

5.2.1.1 Development and life table parameters

Both *Chrysoperla carnea* and *Typhlodromus pyri* share apple orchards as habitats; however, they have very different developmental times and longevities. For example,

in laboratory studies at 25°C, *C. carnea* take on average 27 days to develop from egg to adult and live for 81 days (Pappas et al., 2013), but *T. pyri* take just 8 days to reach adulthood, and live for 45 days at the same temperature (Gadino and Walton, 2012). Detailed developmental studies have published life table parameters for both species at a range of air temperatures and displayed different developmental rates at different temperatures, and after scrutiny of data we chose two studies that had published data in a way that was compatible with parameterising individual-based models: for *C. carnea* we cited data from Pappas *et al.* (2013), as their data were derived from a study of *C. agilis*, a member of the *C. carnea* group; for *T. pyri* we cited data from Gadino and Walton (2012).

We intended to tie the development rate to the air temperature at which movement behaviours were measured for two reasons: firstly, the work published by Pappas and colleagues, and Gadino and Walton demonstrated that air temperature impacts significantly on development, survival and reproduction; secondly, our study of movement behaviours in *T. pyri* showed that air temperature can affect movement rates (Chapters 3-4). However, this proved difficult. Very little data are available pertaining to movement behaviour in lacewings, with one study from 1980 tracking larval movement by hand for five to eight minutes and providing little information of use (Bond, 1980), and one contemporary study investigating avoidance behaviour in *C. externa* through video analysis (Cordeiro et al., 2010). Neither study reported the air temperature at which behaviour was monitored, so it could be inferred to be one of two approximate temperatures: ambient air temperature, which could be assumed to be close to 20°C, or the temperature at which culturing and mortality studies take place, which is around 25°C. Since we undertook our own studies of movement behaviour in *T. pyri*, we have the air temperature range at which movement behaviour was recorded, which ranged 22 – 27°C (Chapters 3-4). Based on the data available to us, we decided to use published developmental times based on 25°C. Tables 5.1 and 5.2 summarise developmental time data for *C. carnea* and *T. pyri* respectively. Table 5.3 summarises reproduction data for the two species, highlighting the large differences in oviposition rates between lacewings and mites. A more complete model would have had development rates for each species

calculated as a function of temperature response, allowing movement behaviour and development to be modelled at different temperatures.

Table 5.1 – Life stages and developmental times for the green lacewing *Chrysoperla carnea* presented in terms of mean number of days and survival (%) ± standard deviation (SD) for each developmental stage at 25°C, plus survival to adulthood. Adult data relates to females only while other life stages comprise aggregated data for both sexes. Data derived from Pappas *et al.* (2013) and adapted for inclusion in the PANTA-lacewing model.

	Life stage	**Days**	**SD**	**Survival to adult (%)**
	Egg	4	2.24	
Larval	Instar 1	3.9	3.6	
	Instar 2	2.9	2.24	
	Instar 3	3.9	2.24	
Pupal	Prepupa	3.8	3.1	
	Pupa	8.3	4.9	74
Adult	Pre-ovipositional	6.9	0.89	
	Adult longevity	54.1	11.2	

Table 5.2 – Life stages and developmental times for female predatory mites (*Typhlodromus pyri*) presented in terms of mean numbers of days ± standard deviation (SD) and survival for each developmental stage at 25°C. Data derived from Gadino and Walton (2012) and adapted for inclusion in the PANTA-mite model.

	Life stage	Days	SD	Survival to adult (%)
Pre-imaginal	Egg	2.8	0.49	89.6
	Larvae	1	0.49	
	Protonymph	2.1	0.49	
	Deutonymph	2.1	0.49	
Adult	Pre-ovipositional	2.2	2.4	
	Ovipositional	31.3	13.9	
	Post-ovipositional	6.6	13.4	
	Total longevity	45	21.6	

Table 5.3 – Comparison of life table parameters for female *Chrysoperla agilis* (member of *C. carnea* cryptic group) and *Typhlodromus pyri*, derived from development studies conducted at 25°C. Means +/- standard deviation (SD) are presented. Data for *C. carnea* derived from Pappas *et al.* (2013); data for *T. pyri* derived from Gadino and Walton (2012).

	C. carnea		***T. pyri***	
	Mean	**SD**	**Mean**	**SD**
Total oviposition (eggs per female)	697.4	139.1	43.7	14.4
Egg hatchability (%)	85	5.8	98.1	-
Generation time (days)	51.4	-	23.8	-

5.2.1.2 Movement behaviour

Chrysoperla carnea is a complex species group to study and implement into a model due to vast differences in their locomotory behaviour at different life stages. Larvae

typically remain within one tree; however, upon hatching as an adult, lacewings will fly up to 40 km on the first night following emergence in what was described as an "obligatory migration flight" (Duelli, 1984b). They then also take flight each night, and as a result, the authors highlight that next to no eggs will be laid by a female in the habitat they emerged in. This migration and reproduction strategy complicated the implementation of reproduction in the model, and as a result, some decisions were made that oversimplify the life cycle and population dynamics for the function of the model. Rather than having adults emerge, take flight and leave the exposure landscape, or have previously unexposed adults enter the landscape for oviposition, we decided to keep the adults within the exposure landscape in which they developed. By doing this, we were able to study the effect of a certain level of pesticide coverage on an individual throughout its life.

As previously mentioned, data are scarce on lacewing larval movement behaviour, with few studies having investigated metrics such as distances covered, velocity and turning behaviour. We used control data from one choice arena study that investigated the effect of kaolin particles on *C. carnea* larval movement behaviour to parameterise the lacewing movement within the model (Porcel, Cotes and Campos, 2011). However, we could only find information on activity over 15 minutes, with no detailed information on how active larvae are over a 24 hour period. We were able to infer that larvae are active for 46% of the observation time in the Porcel et al. study; however, better quality data regarding activity over larger temporal scales would be ideal.

Parameterisation of short term *T. pyri* movement behaviour was more simple due to our own experiments (Chapters 3 and 4). To parameterise movement, we utilised the control mite movement data from the 100% coverage experiments. We considered including different behaviours based upon our findings in Chapters 3 and 4; however, there were few significant differences between mites exposed to acetamiprid and control mites. In Chapter 3 we only observed increases in mean velocity at the highest treatment (9.57 µg cm^{-2}, or 957 g ha^{-1}), a concentration that was not studied in the mortality tests we used to parameterise the model (Section 5.2.2). When mites were placed in choice arenas, we observed that mites were active for 45 seconds longer

on acetamiprid residues at the 0.97 µg cm^{-2} concentration, compared to the untreated surface (Chapter 4, Figure 4.6). However, when we looked at mite activity relative to how long individuals spent on untreated and treated surfaces, we found that the difference in activity was marginal, with mites being active for 79% of their time on the untreated surface, and 80.6% of their time on the treated surface (mean difference 1.6%; 95% confidence intervals [-14.2 – 17.4%]). Therefore, we did not include differences in movement behaviour on untreated and treated surfaces for acetamiprid, though based on our laboratory results, future implementation of deltamethrin and dimethoate would benefit from such implementation.

We have summarised the movement measures used to parameterise movement in Table 5.4. Mean velocity and time spent active were used to parameterise the model. As with life cycles, there were some similarities in movement behaviour, but also large contrasts: both species move in random food searching patterns until they sense food, at which point their movement is directed towards the source (e.g. Bond, 1980; Nyrop, 1988); however, the distances covered and proportions of time spent active are different. Though conclusions cannot be drawn from any comparisons due to the many differences in test set up and conditions, the data illustrate strong differences in both movement behaviour and development between the two species, justifying the need for different models for the two species.

Table 5.4 – Movement data used to parameterise individual movement behaviour for the two modelled species *Chryosperla carnea* and *Typhlodromus pyri*. Data for *C. carnea* are derived from Porcel, Cotes and Campos (2011); *T. pyri* data are derived from our own movement behaviour studies and are based on control mites (Chapter 3). Data for distance covered, velocity and time spent active are presented as mean and standard deviation (SD).

Movement behaviour	***C. carnea*[a]**	***T. pyri*[b]**
Distance walked (cm)	131.1	52.1
SD	44.9	31.7
Velocity (mm s^{-1})	1.51	0.63
SD	0.52	0.43
Time active (%)	46.0	74.6
SD	9.7	19.9

[a] *– Study duration = 15 min*

[b] *– Study duration = 10 min*

5.2.2 Model Overview

We have described the PANTA individual-based model following the ODD (Overview, Design concepts, Details) protocol widely used for describing ecological IBMs (Grimm et al., 2010, 2006). The model was implemented in NetLogo version 6.0.4 (Wilensky, 1999), a free-to-use modelling platform for developing and implementing individual-based models that is widely used in answering ecological and ecotoxicological questions (e.g. Becher et al., 2018; Johnston et al., 2014; Meli et al., 2013).

5.2.2.1 Purpose

The overall purpose of the PANTA model is to simulate the plant-dwelling non-target arthropod species *Chrysoperla carnea* and *Typhlodromus pyri* and to quantify the improvements in survival and longevity that arise from avoidance of pesticide contaminated surfaces. In its current state, the model can be used to investigate survival to adulthood and female longevity in both species when placed in

environments with spatially heterogeneous pesticide residues. Female fecundity can also be investigated by the model; however, at this point, this is only as a function of female longevity. The model does not currently take into consideration pesticide effects on reproduction through the application of e.g. GUTS or DEB theory, though it is hoped that future versions will implement this.

5.2.2.2 Entities, state variables, and scales

One time step equals one minute, and the duration of each model simulation depended upon the research question being answered by the simulation. Please refer to Section 5.3 for model simulation durations.

Individuals

The model includes several different entities for individuals, and these depend on whether the model is simulating *C. carnea* or *T. pyri*. For *C. carnea*, there are six entities: eggs, and female lacewings (larval instars 1-3, pupa, and adult). The pupa entity covers both the pre-pupal and pupal stages in Table 5.1. For *T. pyri*, there are five entities: eggs, and female mites (larva, protonymph, deutonymph, and adult). For each species, each larval life stage was modelled separately to maximise stochasticity amongst individuals. Lacewings and mites are represented as individuals moving across an exposure landscape and have numerous state variables, listed in Table 5.5. One state variable that requires highlighting is tolerance to pesticide: although we were not able to fully implement GUTS or DEB theory in the model as presented, we have included the premise of individual tolerance thresholds in the model (Ashauer et al., 2015). Tolerance was set as a random value from 1 – 100, and once the tolerance level was exceeded by cumulative pesticide exposure, the individual died. We note that this is simplistic as individuals with low tolerances would suffer near instant death within the model when we have no data to suggest this happened in laboratory experiments, and as such the tolerance variable would benefit from refinement.

Table 5.5 – Descriptions of state variables implemented in the PANTA model for *Chrysoperla carnea* and *Typhlodromus pyri*. Variables are grouped by those that are currently used for analysis, and those that currently exist and can be used for future analysis scenarios with model refinement.

State variable	Units	Description
Age (age)	Minutes	The age of the individual
Time as adult (adult-age)	Minutes	Duration of life spent as an adult
Duration of exposure (exposure-duration)	Minutes	Duration of exposure to pesticide residues
Cumulative effect of pesticide exposure (tox)		A value that accumulates with every minute spent on pesticide residues. Once this value is above the individual's tolerance, they die.
Tolerance to pesticide (tolerance)	Random 1-100	A randomly generated number that determines how sensitive an individual is to pesticide exposure. Used to inform mortality arising from exposure (tox). Based on the theory of individual tolerance to toxic compounds (Ashauer et al., 2015).
Oviposition of female (eggs-laid)		The number of eggs laid by an adult female.
Development time from egg hatch to adult (time-to-adult)	Minutes	The time an individual takes to develop into an adult.
Position within environment	X/Y co-ordinates	
Heading	Degrees	Direction of facing for the individual.
Fertility of individual (fertile)	True/false	A variable that determines whether the individual is fertile or not. Based on a randomly generated number (infertility-chance); 0-90 means the individual is fertile; 91 – 100 is infertile.
Cumulative time spent active (time-active)	Minutes	The amount of time spent moving as individuals do not move every model step.
Cumulative distance walked (distance-traveled)	mm	The distance covered by individuals.

Environment

The model world is a heterogeneous exposure landscape, a two-dimensional surface measuring 260 × 260 patches, with each patch measuring 1 mm^2. Landscapes are imported from cropped binary images of water sensitive paper (dimensions 26 mm × 26 mm) collected from the pesticide spray sampling conducted for the study reported in Chapter 2, with a representative landscape illustrated in Figure 5.1. 24 samples, all collected from one apple tree, were imported into the model. The global environment has no variability in time of day or season.

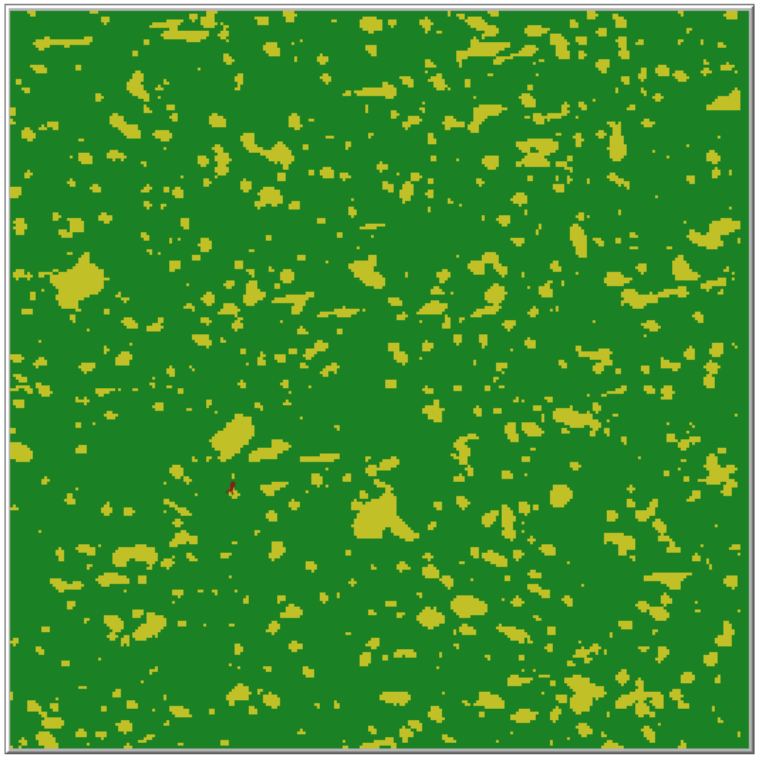

Figure 5.1 – An example exposure landscape within the PANTA model, with pesticide residues shown as yellow patches, and unexposed surfaces shown as green patches. The heterogeneous landscape shown was derived from pesticide spray patterns measured using water sensitive paper in an apple orchard (Witton et al., 2018), and depicts 13% surface coverage.

All patches were assigned two variables: whether a patch contained pesticide or not (true/false), and the scent of the patch (a value of 100 was assigned to unexposed patches, with 50% of this scent being diffused to neighbouring patches to create

gradients of scent). Patches containing pesticide residue were assigned two further variables: active substance, and the effect of the residue (p-mortality), based upon the concentration of the spray that caused the residues. Residue effects for *C. carnea* were drawn from toxicity data resulting from a 48-hour mortality study investigating effects of acetamiprid at three concentrations on larvae (Nasreen, Mustafa and Ashfaq, 2005), whereas residue effects for *T. pyri* were drawn from 7-day mortality studies at five concentrations (EFSA, 2016). To account for the time discrepancy, we adapted the method published by Meli et al. (2013) and converted the proportion of lacewing mortality over 48 hours to 1 minute (i.e. by dividing by 2880 min), and converted mite mortality over 7 days to 1 minute by dividing by 10080. Table 5.6 summarises the residue effect (p-mortality) values for the three acetamiprid levels included within the lacewing model, which then accumulate for individual lacewings and mites in the "tox" variable. Table 5.7 summarises the p-mortality values and mortality for the five acetamiprid levels included within the mite model.

Table 5.6 – 48 hour mortality of *Chrysoperla carnea* larvae exposed to homogeneous acetamiprid residues at three concentrations (Nasreen, Mustafa and Ashfaq, 2005), and p-mortality values derived from mortality rates to assign "toxicity" to pesticide contaminated patches within the model.

Acetamiprid concentration (%)	48 hour mortality (%)	p-mortality value
0.05	91.6	0.0318
0.1	100	0.0347
0.15	98.3	0.0342

Table 5.7 – Seven day mortality (± standard deviation) of *Typhlodromus pyri* larvae exposed to homogeneous acetamiprid residues at five concentrations (EFSA, 2016; Volume 3 B.9 p. 182), and p-mortality values derived from mortality rates to assign "toxicity" to pesticide contaminated patches within the model.

Acetamiprid concentration ($g_{a.s.}$ Ha^{-1})	**7 day mortality (%)**	**p-mortality value**
10.66	31.7 ± 25.7	0.00315
18.66	51.7 ± 14.4	0.00513
32.65	43.3 ± 2.9	0.00430
57.14	85.0 ± 15.0	0.00843
100	95.0 ± 5.0	0.00943

5.2.2.3 Process overview and scheduling

Every minute, each of the following processes are run in the order below. For each process, where necessary, we indicate what entities are affected by each process, and where these vary between modelled species. The submodels representing these processes are described in further detail in Section 5.2.2.7.

Ageing (all individuals)

Each minute, all individuals' ages increase by 1. When an egg hatches, its age is reset to 0.

Move (Lacewings: larvae I1, larvae I2, larvae I3, adults; mites: larvae, protonymphs, deutonymphs, adults)

Individuals move in a semi-random pattern across the landscape, following the scent of uncontaminated patches if the individuals are set to avoid pesticides.

Expose-pesticide (all individuals)

If individuals come into contact with acetamiprid residues, the cumulative exposure time and toxicity are increased accordingly, with no scope for decrease or recovery.

Reproduce (adults)

Lacewings and mites may reproduce once they have reached reproductive maturity and if they are fertile individuals. The procedure runs once every 24 hours, and the number of eggs laid is determined by an individual's reproduction rate parameter.

Lacewing development:

grow-adult (pupae); ***grow-pupa*** (larvae I3); ***grow-l3*** (larvae I2); ***grow-l2*** (larvae I1)

Mite development:

grow-adult (deutonymphs); ***grow-deuto*** (protonymphs); ***grow-proto*** (larvae)

Each of these processes run to allow for development from one life stage to the next, with the time at which an individual develops to the next stage determined by parameters set for each individual.

For lacewings and mites, one process also includes a mortality factor: to reflect lacewing survival to adulthood as published in literature, grow-adult determines whether pupae survive to adulthood based on a pre-defined value (pup_surv). For mites, this occurs also during the grow-adult process; however, it is based on the value of deuto-surv.

Hatch-egg (eggs)

Eggs hatch to first instar larvae based upon their viability and age at which they are ready to hatch. If an egg is laid on acetamiprid residues, then the individual may also die due to contamination before hatching.

Death (all individuals)

Mortality is handled in egg hatch and juvenile development based on the viability of individuals to make it to the next life stage. In addition to this, the death submodel introduces natural death in adults, and pesticide-related mortality in all individuals.

5.2.2.4 Design concepts

Basic principles

The key underlying hypothesis of the PANTA model is that, if individuals avoid pesticide residues, longevity will increase. This will then impact on populations as increased longevity is likely to increase the fecundity of adult females.

Stochasticity

Parameters used within the PANTA model are summarised in Tables 5.8 and 5.9 (pages 194-197). Almost all of these parameters are drawn from normal probability distributions, allowing the model to reflect heterogeneity in individuals in terms of development rates and life table parameters. Stochasticity is also used in the starting position of each individual at the model initialisation; it is also used in the determination of movement behaviour through the use of normal probability distributions.

Due to extreme values being returned by the use of the random-normal function (which draws from a mean and standard deviation), we would sometimes observe negative development periods, or the end of adult life occurring before an individual reached adulthood. To refine the values being returned by the random-normal function, we utilised a check-parameters submodel, which is described in full in Section 5.2.2.7.

Emergence

Emerging from the study of whether longevity increases with pesticide avoidance behaviour is the trend of total oviposition per female. Oviposition is based on life table parameters in the scientific literature. The PANTA model does not take into account effects of pesticide exposure on reproduction, so this emerging result is basic and only based upon changes in individual longevity.

Adaptation

Individuals within the model adapt to the exposure landscape by seeking out cells with the strongest “leaf” scent. Individuals then follow the strongest scent, and adjust movement trajectories. This behaviour therefore reduces the incidence of individuals

coming into contact with pesticide residues, thus reducing their individual exposure and increasing longevity.

Learning and prediction

No learning or prediction takes place within this model as a result of the adaptation or any other factor. If research identifies such learning within the two modelled species, it would be imperative to include this within future versions of the model.

Sensing

Individuals sense a scent given off by leaf surfaces not contaminated with pesticide residues. In doing so, they make decisions on where to move based on where the scent is strongest, which then minimises contact with pesticide residues. This sensing concept was developed based upon a study in the parasitoid wasp *Diaeretiella rapae*, where foraging behaviour was altered by pesticide residues, possibly due to the pesticide masking plant volatiles detected by the wasp (Umoru, Powell and Clark, 1996). Earlier studies had found that pesticides made plants less attractive to foraging pests (Jiu and Waage, 1990); furthermore, non-host plant volatiles are effective at masking plant odours, altering pest behaviour in tomatoes (Tosh and Brogan, 2015), Although such behavioural changes have not been specifically reported in lacewings or predatory mites, we have assumed that they would also exhibit such a behaviour as the two species also forage based upon host plant volatiles (Marcel Dicke, 1988; e.g. Duelli, 1984b).

Interaction

There is no interaction between individuals coded into the PANTA model currently; however, it would be important to study and include this in future versions of the model that include food.

Table 5.8 – Parameters and values implemented in the PANTA *Chrysoperla carnea* model. Means and standard deviations (SD) are presented as these are used to include stochasticity amongst individuals. Parameter names in the model are in parentheses beneath each parameter name.

Parameter	Description	Units	Distribution	Value Mean	SD	Reference
Lacewing development						
Time to hatch to larval instar 1 (grow-to-l1)	Time an egg takes to hatch into a larva	Minutes	Normal	5760	3225.6	(Pappas et al., 2013)
Time to moult to larval instar 2 (grow-to-l2)	The time spent as a first instar larva	Minutes	Normal	5616	5184	(Pappas et al., 2013)
Time to moult to larval instar 3 (grow-to-l3)	The time spent as a second instar larva	Minutes	Normal	4176	3225.6	(Pappas et al., 2013)
Time to pupate (grow-to-pup)	The time spent as a third instar larva	Minutes	Normal	5616	3225.6	(Pappas et al., 2013)
Time to hatch to adult (grow-to-adult)	The time spent as a pupa	Minutes	Normal	17424	11520	(Pappas et al., 2013)
Pupal survival (pup_surv)	A value set to determine whether the individual will hatch into an adult	Number	Normal	74	0	(Pappas et al., 2013)
Time to oviposition (be-mature)	An individual's pre-oviposition period	Minutes	Normal	9936	1281.6	(Pappas et al., 2013)
Adult longevity (natural-end)	An individual's longevity as an adult, assuming no pesticide exposure	Minutes	Normal	77904	16128	(Pappas et al., 2013)

Lacewing reproduction						
Infertility chance (infertility-chance)	A value drawn from random number generation to determine whether the individual is fertile or not. If this is < 90, the individual is fertile	Number	Uniform	Randomly generated (0 – 100)		
Reproduction rate (repro-rate)	The number of eggs laid per day	Number	Normal	14.78	2.95	(Pappas et al., 2013)
Lacewing fitness						
Tolerance (tol)	A value to introduce stochasticity in how much exposure causes mortality in individuals	Number	Uniform	Randomly generated (0 – 100)		Assumed
Lacewing movement						
Chance of being active (active-or-not)	A value that is reset every repetition of the movement process to determine whether the individual moves or not.	%	Normal	46	9.73	(Porcel, Cotes and Campos, 2011)
Distance moved (d)	A value that is reset every repetition of the movement process to determine how far the individual walks.	mm	Normal	1.46	0.499	(Porcel, Cotes and Campos, 2011)

Table 5.9 – Parameters and values implemented in the PANTA *Typhlodromus pyri* model. Means and standard deviations (SD) are presented as these are used to include stochasticity amongst individuals. Parameter names in the model are in parentheses beneath each parameter name.

Parameter	Description	Units	Distribution	Value Mean	SD	Reference
Mite development						
Time to hatch to larva (hatch-time)	Time an egg takes to hatch into a larva	Minutes	Normal	4032	705.6	Gadino and Walton (2012)
Time to develop into protonymph (grow-proto)	The time spent as a larva	Minutes	Normal	1440	705.6	Gadino and Walton (2012)
Time to develop into deutonymph (grow-to-deuto)	The time spent as a protonymph	Minutes	Normal	3024	705.6	Gadino and Walton (2012)
Time to hatch to adult (grow-to-adult)	The time spent as a deutonymph	Minutes	Normal	3024	705.6	Gadino and Walton (2012)
Survival to adulthood (deuto-surv)	A value set to determine whether the individual will develop into an adult	Number	Normal	89.6	0	Gadino and Walton (2012)
Time to oviposition (be-mature)	An individual's pre-oviposition period	Minutes	Normal	3168	3456	Gadino and Walton (2012)
Adult longevity (natural-end)	An individual's longevity as an adult, assuming no pesticide exposure	Minutes	Normal	64800	31104	Gadino and Walton (2012)

Mite reproduction						
Reproduction rate (repro-rate)	The number of eggs laid per day	Number	Normal	1.4	0.48	Gadino and Walton (2012)
Mite fitness						
Tolerance (tol)	A value to introduce stochasticity in how much exposure causes mortality in individuals	Number	Uniform	Randomly generated (0 – 100)		Assumed
Mite movement						
Chance of being active (active-or-not)	A value that is reset every repetition of the movement process to determine whether the individual moves or not.	%	Normal	74.6	36.3	Chapter 3
Distance moved (d)	A value that is reset every repetition of the movement process to determine how far the individual walks.	mm	Normal	1.166	0.71	Chapter 3

5.2.2.5 Initialisation

The initial state of the model (i.e. at time = 0) depends upon what model scenario is being initialised (and therefore what question is being answered). All model simulations begin with individuals as if they had just emerged: first instar larvae for lacewings, and larvae for mites. Individuals are randomly distributed across the landscape. Values for each individual's state variables are drawn from the distributions outlined in Table 5.8. If a model simulation is being started to investigate lacewing survival to adulthood, or mite survival over seven days, then the simulation begins with 20 individuals. If a simulation is being run to investigate reproduction and longevity, then simulations begin with just one individual.

5.2.2.6 Input data

The model has one input source: the model landscape. These are chosen by the user via a drop down box on the model interface, and each option calls a binary image of a pesticide spray pattern derived from water sensitive paper collected from a pesticide spray event within an apple orchard (Chapter 2; Witton et al., 2018). Upon calling the binary images, the model recolours these so that pesticide spray is represented by yellow patches, and unexposed surfaces are represented by green patches.

5.2.2.7 Submodels

All state variables and parameters mentioned in the following section are described in Tables 5.5, 5.7 and 5.8.

Move

This submodel comprises three steps: a decision on whether to move this time step; pesticide avoidance; and then the actual movement.

Every time step, each individual that can move is assigned a value that determines whether they will move or not (active-or-not). The following steps then occur 60 times within one time step, to simulate movement each second. If a randomly generated number (from 0 – 100) falls below "active-or-not", the individual initially turns according to a combination of random turning and then the submodel "repel". Turning is determined by individuals turning left and right based on random number generation each time they move: for lacewings, this is between 0 – 30.6 degrees; for mites this is a random normal distribution based on 19.2 ± 5.1 degrees. These numbers allowed individuals to walk in a generally forward direction while still accounting for turning behaviour (Table 5.4).

Repel is only applied if avoidance behaviour is being applied (via a switch on the user interface). Repel is a submodel that instructs individuals that are moving to determine the patch scent (p-scent) in adjacent patches within a 90° line of sight in front of the individual. The individual then determines which patch has the strongest scent, and then turns to face in that direction.

Following the repel submodel, the distance that the individual will move is determined, and then the individual moves forward that number of patches. The distance travelled is logged in the variable "distance-travelled", and the time spent active is logged in "time-active".

Expose-pesticide

If, after attempting to avoid pesticide residues via the "repel" submodel above, the individual ends a time step on pesticide residues, then the "expose-pesticide" submodel logs this. The counter for duration of pesticide exposure (exposure-duration) is updated by 1 minute, and cumulative toxicity (tox) is increased based on the pesticide concentration. Tox can never decrease, as we have assumed there is no scope for recovery or repair.

Reproduce

Every 1440 time steps (i.e. every 24 hours), adults are asked to check their fertility status (determined as they develop into adults). If the adult is a fertile individual, the next submodel check is to see whether the adult is at an ovipositing age, determined

by the “be-mature” variable. If the individual’s adult-age counter is higher than their be-mature value, they will oviposit. The number of eggs laid is determined by the repro-rate state variable, and this number is deposited on the patch the adult resides on at that moment. For each egg laid, the time to hatch (hatch-time) and egg viability (egg-via) are determined.

Lacewing development submodels:

Grow-adult

The submodel for development of pupae into adults firstly checks the age of the pupa. If their age is over the point at which that individual would develop into an adult (based on the sum of the state variables grow-to-adult, grow-to-pup, grow-to-l3 and grow-to-l1), the individual either develops into an adult, or dies, and this is based upon a randomly generated number (0 – 100): if it falls below pup_surv, the pupa becomes an adult; otherwise the pupa dies. This is based on development data published by Pappas et al. (2013), who demonstrated that 74% of larvae develop into adults.

grow-pupa

The submodel checks the age of third instar larvae (larvae-3): if their age is over the threshold for pupating (based on grow-to-l2, grow-to-l3 and grow-to-pup), then the larvae will develop into a pupa. The pupal stage is a combination of the pre-pupa and pupal stages as described by Pappas et al. (2013).

grow-l3

The submodel checks the age of second instar larvae (larvae-2): if their age is over the threshold for pupating (based on grow-to-l2 and grow-to-l3), then the larva will moult into a third instar larva.

grow-l2

The submodel checks the age of first instar larvae (larvae-1): if their age is over the threshold for moulting (based on grow-to-l2), then the larva will moult into a second instar larva.

hatch-egg

The submodel checks the age of the egg: if the age is over the threshold for hatching (hatch-time), then one of two events occur. If a randomly generated number (0 – 100) falls below the egg's viability value (egg_via), then the egg hatches, their age is reset to 0, and their state variables are defined. Otherwise, the egg fails to hatch and the individual dies.

Mite development submodels:

grow-adult

The submodel for development of deutonymphs into adults firstly checks the age of the deutonymphs. If their age is over the point at which that individual would develop into an adult (based on the sum of the state variables grow-to-adult, grow-to-deuto and grow-to-proto), the individual either develops into an adult, or dies. This is based upon a randomly generated number (0 – 100): if it falls below deuto-surv, the deutonymph becomes an adult; otherwise it dies. This is based on development data published by Gadino and Walton (2012), where survival to adulthood was 89.6%.

grow-deuto

The submodel checks the age of protonymphs: if their age is over the threshold for developing into a deutonymph (based on grow-to-proto + grow-to-deuto), then the individual develops into a deutonymph.

grow-proto

The submodel checks the age of mite larvae: if their age is over the threshold for developing into a protonymph (based on grow-to-proto), then the individual's breed is changed to protonymph.

hatch-egg

This submodel is identical to the submodel of the same name in the lacewing development submodels.

Death

The death submodel handles age- and pesticide-related mortality. Every time step, the adult-age of adults is checked against their natural-end; if adult-age exceeds natural-end, the adult dies. Once an hour (60 steps), individuals' accumulated toxicity (tox) is checked against their pesticide tolerance (tol). if tox exceeds tol, the individual dies.

Check-parameters (on setup only)

The check-parameters submodel only runs at the model initialisation, and checks the assigned development and reproduction parameter values. This was necessary due to instances where, for example, an individual's development to the next life stage was negative, meaning entire life stages were skipped. If values are extreme (i.e. outside the bounds of mean ± SD), then the submodel would draw the extreme values back within the expected range. An excerpt of code is below to provide an example of how this worked with *T. pyri* development.

```
to check-parameters
  ask larvae [
    if grow-to-proto < 3326.4 [
      set grow-to-proto (grow-to-proto + 705.6)
    ]
  ]
End
```

This code checked whether the development time for a mite larva to become a protonymph was below the mean – SD. If so, the code then reset the grow-to-proto value by adding the standard deviation (705.6) to the original value. We found that this step was only necessary for adjusting values at the lowest end of the scale, and that similar adjustment of values at the highest end of the scale did not deliver any further improvement to numbers. Therefore, we only implemented this on the lowest values. A more sophisticated method to eliminate extreme development times would be to truncate the applied distributions at the 2.5^{th} and 97.5^{th} percentiles.

5.3 Model testing

To ensure the model was performing as expected, we conducted a number of tests where the model was run on two "control" exposure landscapes: one 0% pesticide coverage, to mimic a water control, and one 100% pesticide coverage, to mimic the exposure landscape individuals would be exposed to in laboratory assays. Based on the framework for model "evaludation" devised by Augusiak et al. (2014a), we are conducting "model output verification", defined as the step of comparing data and patterns output by the model to the data and patterns that aided model design.

All analysis was undertaken in GraphPad Prism 7 (GraphPad Software Inc., La Jolla, California, US).

5.3.1 Control development and reproduction

We selected one dataset per species for parameterising development and reproduction, and selected datasets that investigated development rates at a range of air temperatures for future model development. For lacewings, we parameterised using data published by Pappas et al. (2013), based on development at 24°C and reproduction at 25°C; for mites we used data published by Gadino and Walton (2012) based on development and reproduction at 25°C. Both datasets published separate development rates for male and female individuals; in the current model iteration we only modelled females, so utilised the female-specific development data. For each species, we ran a single model simulation to test five factors: first development time from egg hatch to adulthood, survival to adulthood, adult longevity, total oviposition per lacewing, and infertility rates. Although parameters were the same irrespective of whether avoidance behaviour was exhibited by individuals, we ran simulations of lacewings and mites with and without avoidance behaviour to ensure the behaviour did not cause shifts in development or reproduction.

Model simulations were conducted on the 0% coverage (control) surface. Individuals were simulated one at a time to allow for a high resolution of data, with 200 individuals per avoidance behaviour. These were modelled from egg hatch, so lacewings started as first instar larvae, and mites started as larvae, through to death.

The model output total longevity, time to adulthood, time as an adult, and the number of eggs per female. If an individual died during the pupal stage, only total longevity would be returned by the model.

We summarised metrics in Tables 5.11 and 5.12, and conducted one-way ANOVAs to examine the variance in population means for development and reproduction parameters, comparing them against the datasets used to parameterise the model.

5.3.1.1 Lacewings

Simulated lacewing larvae take up to 2.5 days less time to emerge into adults compared to the literature data (Table 5.10); however, our modelled individuals displayed smaller confidence intervals around the averages, and these fell within the confidence intervals of the published data. Adult longevity and total oviposition observed in the modelled populations were very close to the published data (Table 5.11), with the largest difference in longevity being less than 0.01% off the published data, and the largest difference in oviposition being just 1.6% versus published data. Finally, infertility rates were also very close to the published data.

We conclude the development and reproduction in simulated lacewings is a good representation of the published data from laboratory studies, and requires no further refinement to improve the model functioning. Any variance seen between the populations with and without avoidance behaviour is due to model stochasticity.

Table 5.10 – Model output verification of *Chrysoperla carnea* development and reproduction in a control exposure landscape (0% coverage). Development times from larva to adult, adult longevity and total oviposition are presented as means with 95% confidence intervals (in parentheses) for simulated populations with and without avoidance behaviour; survival to adult and population infertility rates are also shown. Published data used to parameterise the model are presented and are based on female development times (Pappas et al., 2013). Modelled population data were derived from individual lacewings from first instar to death, with 200 runs per behaviour. All simulated data are derived from the same model run.

Lacewing population	**n**	**Mean time from larva to adult (days)**	**Survival to adult (%)**	**Mean adult longevity (days)**	**Mean total oviposition (eggs/female)**	**Infertility rate (%)**
Avoidance behaviour	200	25.7 (24.5 – 26.9)	76.5	54.6 (53.5 – 55.6)	705 (680 – 729)	11.8
No avoidance behaviour	200	24.4 (23.3 – 25.6)	74.5	54.0 (52.9 – 55.1)	686 (661 – 710)	10.7
Published data	20	27.0 (22.9 – 31.1)	74.0	54.1 (53.0 – 55.2)	697 (636 – 887)	10.0

Table 5.11 – Comparisons of *Chrysoperla carnea* mean development time to adulthood, adult longevity and total oviposition between simulated control populations exhibiting avoidance behaviour and no avoidance behaviour, and empirical data used to parameterise the model (Pappas et al., 2013). Mean differences and 95% confidence intervals (in parentheses) are presented. One-way ANOVA was conducted to test whether development parameters differed significantly between simulated populations and empirical data ($\alpha = 0.05$).

	Larva to adult (days)		**Adult longevity (days)**		**Total oviposition (eggs per female)**	
Comparisons	**Mean Differences**	***P***	**Mean Differences**	***P***	**Mean Differences**	***P***
Avoidance vs Published	-1.4 (-4.8 – 3.4)		-0.12 (-3.6 – 3.4)		7.4 (-58.3 – 73.1)	
No avoidance vs Published	-2.6 (-6 – 0.9)		0.47 (-3 – 4)		-11.8 (-77.6 – 54)	
Avoidance vs No avoidance	1.2 (-0.96 – 3.4)	0.16	-0.59 (-2.8 – 1.6)	0.81	19.2 (-24.1 – 62.5)	0.58

5.3.1.2 Mites

Table 5.12 summarises the mite model testing results. Simulated mites take about half a day longer to develop into adults compared to the literature data, with differences between simulated and literature populations significant (Table 5.13). This is likely due to our "check-parameter" submodel only being applied to prevent extreme low values. However, the increase in longevity of two days in simulated mites compared to literature data was not significant. Finally, simulated adult female mites produced on average three more eggs each compared to literature data. Confidence intervals around the means for simulated total longevity and oviposition are smaller than the confidence intervals relating to literature data. We did not compare population fertility rates as we did not have literature data for comparison.

In summary, overall development and reproduction in mites simulated in the PANTA model are good representations of the published data resulting from laboratory studies. There are some small significant differences in the larvae to adult development period rates in simulated mites. This could benefit from some

refinement; however, we are not concerned at this stage due to the total longevity values being in agreement with literature data.

Table 5.12 – Model output verification of *Typhlodromus pyri* development and reproduction in a control exposure landscape (0% coverage). Development times from larva to adult, total longevity and total oviposition are presented as means with 95% confidence intervals (in parentheses) for simulated populations with and without avoidance behaviour; survival to adult and population infertility rates are also shown. Published data used to parameterise the model are presented and are based on female development times (Gadino and Walton, 2012). Modelled population data were derived from individual lacewings from first instar to death, with 200 runs per behaviour. All simulated data are derived from the same model run.

Lacewing population	**n**	**Mean time from larva to adult (days)**	**Survival to adult (%)**	**Mean total longevity (days)**	**Mean total oviposition (eggs/female)**	**Infertility rate (%)**
Avoidance behaviour	200	5.6 (5.5 – 5.7)	92.5	47.1 (44.7 – 49.4)	42.8 (40.9 – 44.8)	97.8
No avoidance behaviour	200	5.7 (5.6 – 5.9)	89.5	46.8 (44.3 – 49.3)	44.3 (42.4 – 46.3)	97.2
Published data	24	5.2 (4.8 – 5.6)	89.6	45 (23.4 – 66.6)	43.7 (37.8 – 49.6)	-

Table 5.13 – Comparisons of *Typhlodromus pyri* mean development time to adulthood, total longevity and total oviposition between modelled control populations exhibiting avoidance behaviour and no avoidance behaviour, and published data (Gadino and Walton, 2012). Mean differences and 95% confidence intervals (in parentheses) are presented. One-way ANOVA was conducted to test whether development parameters differed significantly between simulated populations and empirical data ($\alpha = 0.05$).

	Larva to adult (days)		**Total longevity (days)**		**Total oviposition (eggs per female)**	
Comparisons	**Mean Differences**	***P***	**Mean Differences**	***P***	**Mean Differences**	***P***
Avoidance vs Published	0.4 (-0.01 – 0.81)		2.1 (-7.1 – 11.3)		3.4 (-3.4 – 10.2)	
No avoidance vs Published	0.55 (0.14 – 0.96)		1.8 (-7.4 – 11.0)		3.1 (-3.7 – 9.9)	
Avoidance vs No avoidance	-0.15 (-0.34 – 0.04)	**0.004**	0.3 (-3.9 – 4.5)	0.86	0.3 (-2.9 – 3.5)	0.50

5.3.2 Survival after 48 hours (lacewings)

We parameterised the mortality portion of the model based on survival data derived from Nasreen, Mustafa and Ashfaq (2005), which studied larval survival following exposure to acetamiprid residues at three levels (0.05%, 0.1%, 0.15%). The authors did not clearly report actual dose rates; in addition, "Raja", the formulated product assessed in the published study, is no longer on the market, so we have had to make some assumptions. The authors state that 0.1% corresponds to the recommended application rate; based on the current recommended application rate of 75 $g_{a.s.}$ ha^{-1}, we infer the following acetamiprid concentration rates: 0.05% = 37.5 g ha^{-1}; 0.1% = 75 g ha^{-1}; 0.15% = 112.5 g ha^{-1}. Respective mortalities reported by Nasreen and colleagues were 91.6%, 100% and 98.3% after 48 hours, with this trend not discussed or explained by the authors. We parameterised each of the treatments individually based on these mortality data.

To verify our model outputs, we generated a scenario where lacewings were exposed to 0% and 100% coverage exposure landscapes over 48 hours (2880 model steps).

Each run was conducted ten times per landscape, per pesticide concentration, with each run containing 20 lacewings at larval instar 1 (total simulated n = 200 per combination). As in the control development test, we modelled individuals exhibiting avoidance and no avoidance behaviour, to ensure responses were consistent. The model returned the number of surviving lacewings at the 48 hour point for each run.

Table 5.14 summarises this verification test. As the authors of the study we drew the parameterisation data from did not include information such as standard error with their results, we assumed that standard errors were 0 to allow us to conduct three one-way ANOVAs, one per acetamiprid treatment level. Table 5.15 summarises the mean differences between simulated populations showing avoidance behaviour and no avoidance behaviour, and the published data. There were no significant differences between survival in our simulated populations and the published data, therefore we conclude that, when lacewings have no choice in exposure to acetamiprid residues within the model, their survival is representative of the published data. No further refinement is necessary.

Table 5.14 – Model output verification of *Chrysoperla carnea* survival over 48 hours in a 100% coverage exposure landscape. Mean survival with 95% confidence intervals are presented for populations displaying avoidance behaviour and no avoidance behaviour, and published survival data (Nasreen, Mustafa and Ashfaq, 2005). Modelled survival data were derived from ten runs of 20 individuals over 48 hours (total simulated n = 200 per treatment, per behaviour). All simulated data are derived from the same model run.

	Control		**0.05% Acetamiprid**		**0.1% Acetamiprid**		**0.15% Acetamiprid**	
Lacewing population	**n**	**Mean Survival (%)**	**n**	**Mean Survival (%)**	**n**	**Mean Survival (%)**	**n**	**Mean Survival (%)**
Avoidance behaviour	200	100	200	8.0 (1.9 – 14.1)	200	2.0 (-0.5 – 4.5)	200	4.0 (1.2 – 6.8)
No avoidance behaviour	200	100	200	7.0 (2.2 – 11.8)	200	2.0 (0.2 – 3.8)	200	6.0 (1.9 – 10.1)
Published data[a]	20	100	20	8.4	20	0.0	20	1.7

[a] No confidence intervals or standard errors published by authors.

Table 5.15 – Comparisons of survival after 48 hours of exposure on a 100% coverage surface between simulated populations exhibiting avoidance behaviour and no avoidance behaviour and published data used to parameterise the model (Nasreen, Mustafa and Ashfaq, 2005). Mean differences and 95% confidence intervals are presented. One-way ANOVA was conducted to test whether development parameters differed significantly between simulated populations and empirical data ($\alpha = 0.05$).

	0.05% Acetamiprid		**0.1% Acetamiprid**		**0.15% Acetamiprid**	
Comparisons	**Mean Differences**	***P***	**Mean Differences**	***P***	**Mean Differences**	***P***
Avoidance vs Published	-0.4 (-20.9 – 9.7)		2.0 (-6.2 – 10.2)		2.3 (-10.8 – 15.4)	
No avoidance vs Published	-1.4 (-21.9 – 19.1)		2.0 (-6.2 – 10.2)		4.3 (-8.8 – 17.4)	
Avoidance vs No avoidance	1.0 (-7.7 – 9.7)	0.95	0.0 (-3.5 – 3.5)	0.82	-2.0 (-7.6 – 3.6)	0.54

5.3.3 Mortality after 7 days (mites)

Mite mortality when exposed to acetamiprid was parameterised using mortality data from EFSA (2016), where *T. pyri* protonymphs were exposed to acetamiprid at five concentrations ranging 10.66 – 100.0 g ha^{-1}. Mortality was reported after seven days exposure, with mortality ranging 32 – 95% in that time. Control mortality in the exposure experiment was 14%, though the model is parameterised using survival data used to parameterise development to adulthood (Gadino and Walton, 2012). To verify model outputs, we generated similar scenarios to those used to verify lacewing survival: mites were exposed to 0% and 100% coverage exposure landscapes over seven days (10080 model steps). Each run was conducted ten times per landscape, per pesticide concentration, with each run containing 20 mite larvae (total simulated n = 200 per combination). As with previous verification tests, we modelled individuals exhibiting avoidance and no avoidance behaviour. Each model run output the number of surviving mites after seven days of exposure.

Table 5.16 summarises the mortality test results, and Table 5.17 presents the mean differences between the simulated populations and the published data used for parameterisation. There were no significant differences in mortality between simulated and published data where mites were exposed to acetamiprid. We observed a large difference in control mortality: data published by EFSA reported control mortality of 14% whereas modelled populations reported mortality of 5.5 and 7%. We did not assess this difference because the purpose of the test was to verify mortality in relation to pesticide exposure. We also know that the difference is due to control mortality in modelled populations arising from development-related mortality, whereas control mortality from the laboratory study leading to the EFSA data included mortality and loss via escape and trapping in test arena boundaries.

Since there were no significant differences in simulated and published mortalities in relation to acetamiprid exposure, we conclude that their simulated mortality after seven days is representative of the published data.

Table 5.16 – Model output verification of *Typhlodromus pyri* mortality over 7 days in a 100% coverage exposure landscape. Mean mortality with 95% confidence intervals (in parentheses) are presented for populations displaying avoidance behaviour and no avoidance behaviour; published mortality data used to parameterise the pesticide responses are also presented (EFSA, 2016). Sample n corresponds to how many replicates were run, with each replicate containing 20 mites, both for simulated and literature data (total simulated n = 200 per treatment, per behaviour). All simulated data are derived from the same model run.

						Acetamiprid					
	Control		**10.66 g Ha^{-1}**		**18.66 g Ha^{-1}**		**32.65 g Ha^{-1}**		**57.14 g Ha^{-1}**		**100 g Ha^{-1}**	
Mite population	**n**	**Mean mortality (%)**	**n**	**Mean mortality (%)**	**n**	**Mean mortality (%)**	**n**	**Mean mortality (%)**	**n**	**Mean mortality (%)**	**n**	**Mean mortality (%)**
Avoidance behaviour	10	5.5 (1.9 – 9.1)	10	39.5 (32.3 – 46.7)	10	53 (44.9 – 61.1)	10	42 (35.4 – 48.6)	10	84.5 (78.6 – 90.5)	10	92 (87.8 – 96.2)
No avoidance behaviour	10	7 (3.2 – 10.9)	10	36.5 (30.9 – 42.1)	10	47 (42.5 – 51.5)	10	43.5 (36.5 – 50.5)	10	81 (73.9 – 88.1)	10	92 (88.5 – 95.5)
Published data[a]	5	14 (7.5 – 20.5)	3	31.7 (2.6 – 60.8)	3	51.7 (35.4 – 68.0)	3	43.3 (40.0 – 46.6)	3	85 (68.0 – 102.0)	3	95 (89.3 – 100.7)

Table 5.17 – Comparisons of *Typhlodromus pyri* mortality after 7 days of exposure on a 100% coverage surface between simulated populations exhibiting avoidance behaviour and no avoidance behaviour, and published data used to parameterise the model (EFSA, 2016). Mean differences are presented with 95% confidence intervals in parentheses. One-way ANOVA was conducted to test whether development parameters differed significantly between simulated populations and empirical data ($\alpha = 0.05$).

	Acetamiprid									
	10.66 g ha^{-1}		**18.66 g ha^{-1}**		**32.65 g ha^{-1}**		**57.14 g ha^{-1}**		**100 g ha^{-1}**	
Comparisons	**Mean Difference**	***P***	**Mean Difference**	***P***	**Mean Difference**	***P***	**Mean Difference**	***P***	**Mean Difference**	***P***
Avoidance vs Published	7.8 (-11.9 – 27.5)		1.3 (-15.1 – 17.7)		-1.3 (-16.3 – 13.7)		-0.5 (-17.0 – 16.0)		-3 (-11.9 – 5.9)	
No avoidance vs Published	4.8 (-14.9 – 24.5)		-4.7 (-21.1 – 11.7)		0.2 (-14.8 – 15.2)		-4 (-20.5 – 12.5)		-3 (-11.9 – 5.9)	
Avoidance vs No avoidance	3 (-10.4 – 16.4)	0.60	6 (-5.2 – 17.2)	0.40	-1.5 (-11.7 – 8.7)	0.93	3.5 (-7.7 – 14.7)	0.69	0 (-6.0 – 6.0)	0.67

5.3.4 Movement parameters

Movement was parameterised based on the distances walked and time spent active for each species (Table 5.4). To test whether the modelled individuals were behaving as expected, we conducted one test per species to allow for model output verification of distance walked and time active. We generated a scenario where individuals were simulated in the control landscape over 10 minutes (10 model steps) for *T. pyri*, and 15 minutes for *C. carnea*. As with previous model tests, we tested populations exhibiting avoidance and no avoidance behaviour. Each scenario was run 100 times, with each run containing one individual (total simulated n = 100 per behaviour, per species). After 10 or 15 minutes, the model returned total distance walked and time spent active.

Table 5.18 summarises the movement verification test. Table 5.19 summarises the mean differences in distance covered and time spent active, comparing values from simulated populations and either literature data in the case of *C. carnea*, or laboratory data from our own studies in the case of *T. pyri*. We observed no significant differences in the movement behaviour of simulated lacewings or mites in comparison with the parameterisation data. The largest deviation from parameterisation data was observed in the distance walked by simulated lacewings, with simulated populations covering on average an extra 8 cm in comparison to the published data. However, the means and confidence intervals fell within the published data confidence intervals, so we did not refine the behaviour further within the model.

We conclude that simulated movement behaviour for both lacewings and mites is behaving as expected, and that the movement within modelled landscapes is a good representation of observed movement in the two species.

Table 5.18 – Model output verification of movement behaviour in *Chrysoperla carnea* and *Typhlodromus pyri* in a control exposure landscape. Mean distances covered and time spent active over 15 minutes (for *C. carnea*) and 10 minutes (for *T. pyri*) are presented with 95% confidence intervals (in parentheses) for simulated populations displaying avoidance behaviour and no avoidance behaviour; data used to parameterise movement behaviour are also presented.

	Chrysoperla carnea			***Typhlodromus pyri***		
Population	**n**	**Distance walked (cm)**	**Time active (%)**	**n**	**Distance walked (cm)**	**Time active (%)**
Avoidance behaviour	100	139.2 (137.3 – 141.1)	47.0 (46.4 – 47.7)	100	52.4 (51.4 – 53.4)	75.5 (74.2 – 76.7)
No avoidance behaviour	100	137.1 (135.3 – 138.9)	46.4 (45.9 – 47.0)	100	51.7 (51.7 – 53.6)	74.6 (73.2 – 76.0)
Reference data	50[a]	131.1 (118.7 – 143.5)	46.0 (43.3 – 48.7)	24[b]	52.1 (38.7 – 65.5)	74.6 (59.4 – 89.8)

[a] – Reference data from Porcel, Cotes and Campos (2013)

[b] – Reference data from our own control mite movement observations (Chapter 3)

Table 5.19 – Comparisons of movement behaviour in *Chrysoperla carnea* and *Typhlodromus pyri* in a control exposure landscape. Mean differences with 95% confidence intervals are presented for distances covered and time spent active over 15 minutes (for *C. carnea*) and 10 minutes (for *T. pyri*) for simulated populations displaying avoidance behaviour and no avoidance behaviour, and data for parameterisation. One-way ANOVA P values from comparing the population means are also presented (α = 0.05).

	Chrysoperla carnea					***Typhlodromus pyri***				
		Distance walked		**Time active**			**Distance walked**		**Time active**	
Comparisons	**n**	**Mean difference (cm)**	**P**	**Mean difference (%)**	**P**	**n**	**Mean difference (cm)**	**P**	**Mean difference (%)**	**P**
Avoidance vs Published	100	8.1 (-0.78 – 16.9)		1.0 (-1.1 – 3.1)		100	0.5 (-5.5 – 6.5)		0.9 (-6.2 – 8.0)	
No avoidance vs Published	100	6.0 (-2.9 – 14.8)		0.4 (-1.7 – 2.5)		100	0.3 (-5.7 – 6.3)		0.1 (-7.0 – 7.2)	
No avoidance vs Avoidance	50[a]	-2.1 (-9.3 – 5.1)	0.10	-0.6 (-2.4 – 1.1)	0.46	24[b]	-0.25 (-4 – 3.5)	0.97	-0.8 (-5.2 – 3.6)	0.90

[a] – Reference data from Porcel, Cotes and Campos (2013)

[b] – Reference data from our own control mite movement observations (Chapter 3)

5.4 Results

5.4.1 Movement patterns

Figure 5.2 shows the difference in movement patterns in *C. carnea* and *T. pyri* individuals that avoid or do not avoid pesticide residues. When showing avoidance behaviour, individuals do not always avoid residues; however, their contact with them is reduced markedly due to the ability to sense unexposed surfaces.

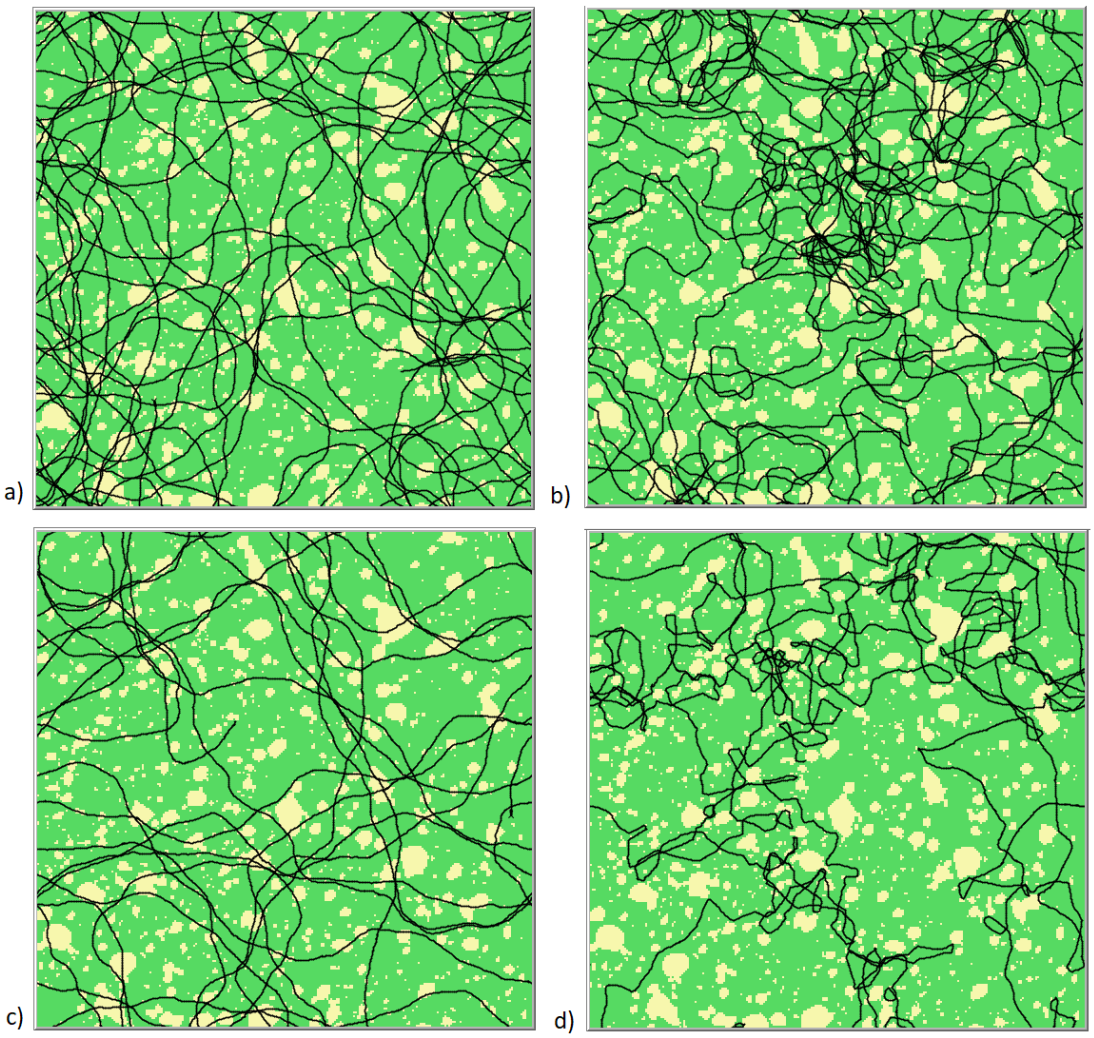

Figure 5.2 – Movement tracks of individual larvae after 2.5 hours of simulation in a heterogeneous pesticide exposure landscape. Acetamiprid residues are coloured yellow; unexposed surfaces are coloured green. a) *Chrysoperla carnea* larva not avoiding residues; b) *C. carnea* avoiding residues; c) *Typhlodromus pyri* larva not avoiding residues; d) *T. pyri* avoiding residues. Acetamiprid coverage in all images is 35%.

5.4.2 Simulation analysis methods

For the following simulation tests, we chose one acetamiprid concentration per species: 0.05% for *Chrysoperla carnea*, and 10.66 g ha^{-1} (which equates to 0.05% acetamiprid in 200 L water) for *Typhlodromus pyri*. We simulated movement of individuals on 24 exposure landscapes, all derived from pesticide spray patterns derived from a field study (Witton et al., 2018). These were then plotted as scatter graphs for visual comparison of trends when individuals showed avoidance behaviour or no avoidance behaviour. To quantify the effect of avoidance behaviour (and thus answer our research questions), we selected three exposure landscapes: the median (13.1%), the 25^{th} percentile (8.74%), and the 75^{th} percentile (18.5%) for surface coverage across the 24 samples that comprise a single apple tree from the aforementioned study. We then conducted multiple t tests to compare measures derived from populations that avoided residues, and those that did not. P values were adjusted for multiple comparisons using the Bonferroni-Dunn method ($\alpha = 0.05$).

5.4.3 *Chrysoperla carnea* survival

More lacewings tend to survive to adulthood when the larvae show avoidance behaviour (Figure 5.3). When unable to avoid residues, 1% (95% confidence intervals (CIs) [-0.96%, 2.96%]) of larvae survived to adulthood on a 24% covered surface with no survival to adulthood at any higher coverage level; by comparison, 4% (95% CIs [2.04%; 5.96%]) of larvae survived to adulthood on a surface with 46% acetamiprid residue coverage. When exposed to 100% coverage, no lacewings survived to adulthood (data not shown on graphs).

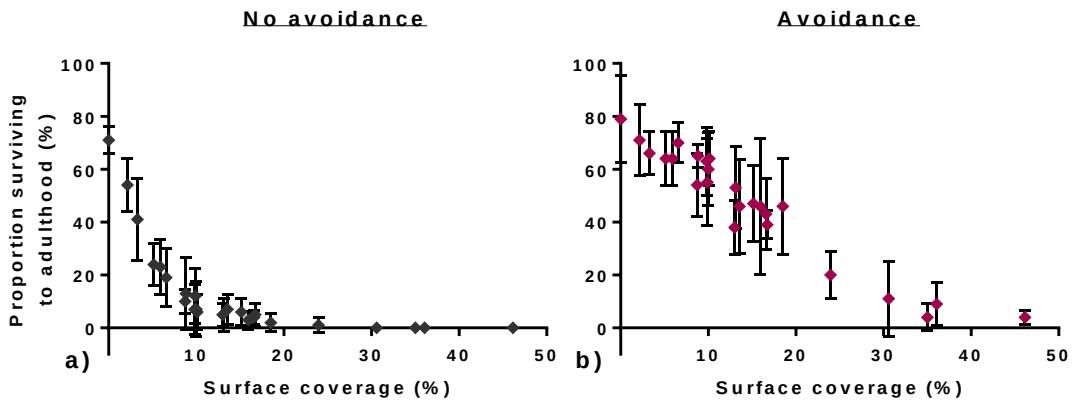

Figure 5.3 – Proportion of *Chrysoperla carnea* larvae surviving to adulthood when a) not avoiding acetamiprid residues (left) and b) avoiding acetamiprid residues (right). Survival to adulthood was simulated on 24 heterogeneous acetamiprid exposure landscapes with varying residue coverage levels. Acetamiprid concentration was set at 0.05%. Dots represent mean ± 95% confidence intervals generated by five simulations of 20 lacewings (total simulated n = 100 per coverage per behaviour).

When comparing survival to adulthood in the three example exposure landscapes, we found that avoidance behaviour led to significant increases in survival rates of 44% on the 8.7% coverage surface, 48% on the 13.1% coverage surface and 44% on the 18.5% coverage surface (Figure 5.4; Table 5.20).

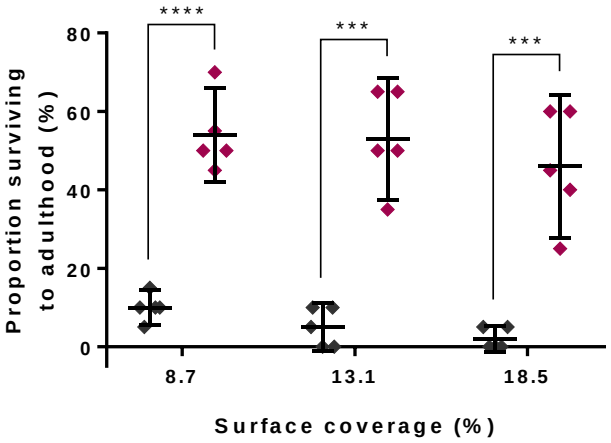

Figure 5.4 – Differences in survival to adulthood in *Chrysoperla carnea* individuals that do not avoid acetamiprid residues (grey dots, left), and individuals that do avoid acetamiprid residues (pink dots, right). Lines represent mean ± 95% confidence intervals. Each dot represents a model run with 20 lacewings. Brackets display statistical significance in comparison between behaviours (P values adjusted for multiple comparisons; *** = P < 0.001; **** = P < 0.0001). Total simulated n = 100 per behaviour, per coverage level.

Table 5.20 – Mean differences in survival to adulthood in *Chrysoperla carnea* individuals avoiding or not avoiding acetamiprid residues at three surface coverage levels. Comparison of no avoidance behaviour vs avoidance behaviour is shown with the standard error of the difference and adjusted P values resulting from multiple t tests. n = 100 per behaviour, per coverage level. Significant differences (α = 0.05) are highlighted in bold.

Surface coverage (%)	**Mean difference (%)**	***P***
8.7	44 (± 4.6)	**< 0.0001**
13.1	48 (± 6.0)	**0.0001**
18.5	44 (± 6.7)	**0.0005**

We explored the mean longevity of individuals simulated for the reproduction study (Section 5.4.2), and observed that avoidance behaviour improves the longevity of individuals, with lacewings exposed at all coverage levels living on average over eight days when they avoid residues, compared with longevity dropping below eight days at 18.5% coverage (Figure 5.5).

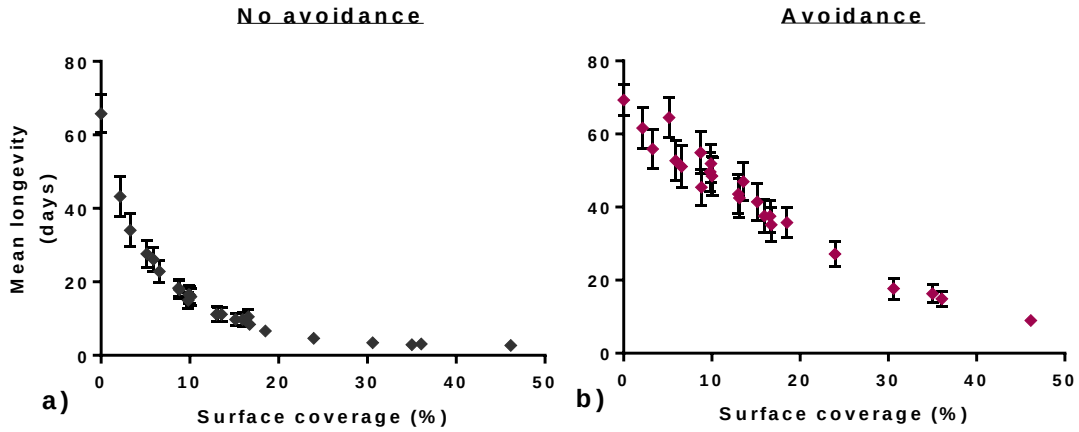

Figure 5.5 – Mean longevity of *Chrysoperla carnea* individuals when a) not avoiding acetamiprid residues (grey dots, left) and b) avoiding acetamiprid residues (pink dots, right). Data were generated by simulating individuals completing life cycles on 24 pesticide exposure landscapes with the acetamiprid concentration set at 0.05%. Dots represent mean ± 95% confidence intervals; *n* = 100 per behaviour, per coverage level.

When investigating the three representative coverage levels, we observed that avoidance behaviour on average increased lacewing longevity by over 32 days (Figure 5.6). Acetamiprid residue avoidance led to increased longevity of 36.6 days in the 8.7% coverage scenario, 31.5 days in the 13.1% coverage scenario, and 29.1 days in the 18.5% coverage scenario (Table 5.21).

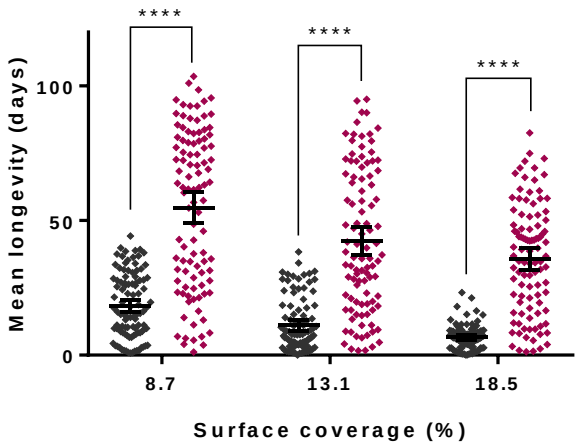

Figure 5.6 – Differences in longevity in *Chrysoperla carnea* individuals that do not avoid acetamiprid residues (grey dots, left), and individuals that do avoid acetamiprid residues (pink dots, right). Lines represent mean ± 95% confidence intervals. Each dot represents an individual lacewing. Brackets display statistical significance in comparison between behaviours (P values adjusted for multiple comparisons; **** = $P < 0.0001$). Total simulated *n* = 100 per behaviour, per coverage level.

Table 5.21 – Mean differences in longevity in *Chrysoperla carnea* individuals avoiding or not avoiding acetamiprid residues at three surface coverage levels. Comparison of no avoidance behaviour vs avoidance behaviour is shown with the standard error of the difference in parentheses, and adjusted P values resulting from multiple t tests. n = 100 per behaviour, per coverage level. Significant differences ($\alpha = 0.05$) are highlighted in bold.

Surface coverage (%)	Mean difference (days)	P
8.7	54.9 (± 3.2)	**< 0.0001**
13.1	31.5 (± 2.8)	**< 0.0001**
18.5	29.1 (± 2.1)	**< 0.0001**

5.4.4 *Chrysoperla carnea* reproduction

Lacewing adults laid more eggs when they avoided acetamiprid residues (Figure 5.7). In lacewings not exhibiting avoidance behaviour, reproduction was observed up to 13.6% surface coverage; in lacewings that did avoid residues, reproduction was apparent at every coverage level, though diminished. No eggs were laid by lacewings exposed to 100% coverage, as no individuals survived to adulthood (data not shown in graph).

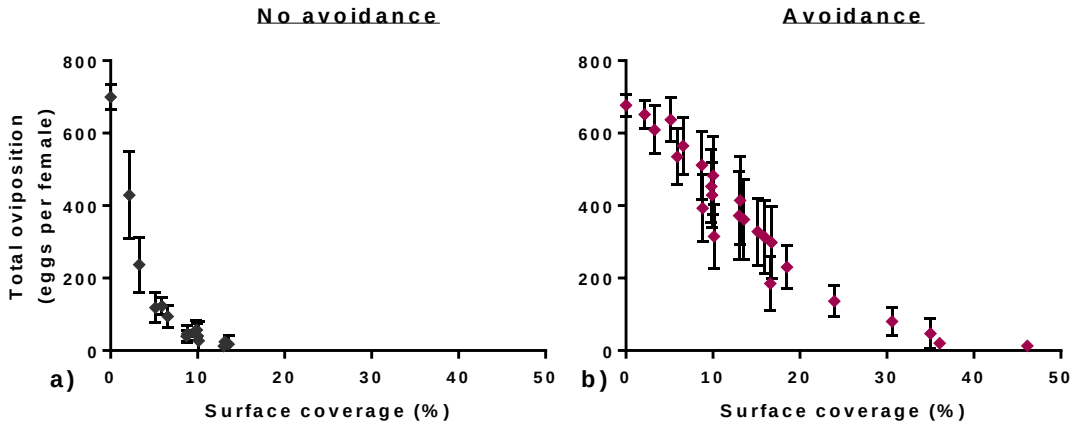

Figure 5.7 – Total oviposition per adult female *Chrysoperla carnea* when a) not avoiding acetamiprid residues (grey dots) and b) avoiding acetamiprid residues (pink dots). Oviposition was simulated on 24 pesticide exposure landscapes with the acetamiprid concentration set at 0.05%. Dots represent mean ± 95% confidence intervals generated by five simulations of 20 lacewings (total simulated n = 100 per coverage per behaviour; infertile individuals and individuals that did not survive to adulthood omitted from analysis).

When comparing oviposition at the three representative coverage levels, we found that avoidance behaviour led to a mean increase in oviposition of 472 eggs per female at 8.7% surface coverage (Figure 5.8, Table 5.22). At 13.3% coverage, we saw an increase in oviposition of 390.5 eggs per female; however, we could not test this due to only one female being reproductive at this coverage level. No reproduction was observed in females not avoiding residues at the 18.5% coverage level.

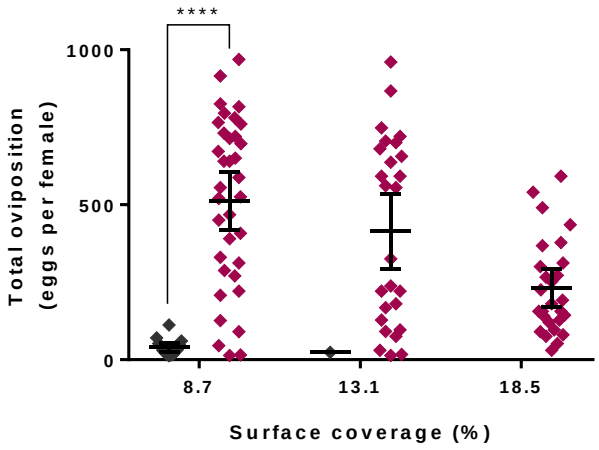

Figure 5.8 – Differences in total oviposition per female in *Chrysoperla carnea* individuals that do not avoid acetamiprid residues (grey dots, left), and individuals that do avoid acetamiprid residues (pink dots, right). Lines represent mean ± 95% confidence intervals. Each dot represents a single reproducing female; infertile females are excluded from analysis. Brackets display statistical significance in comparison between behaviours (**** = P < 0.0001). Comparisons are not shown for the other coverage levels due to lack of samples in the population not avoiding residues. 8.7% *n* = 35, 14; 13.1% *n* = 26, 1; 18.5% *n* = 27.

Table 5.22 – Mean differences in oviposition per female in *Chrysoperla carnea* individuals avoiding or not avoiding acetamiprid residues at three surface coverage levels. Comparison of no avoidance behaviour vs avoidance behaviour is shown with the 95% confidence intervals of the difference in parentheses, P value resulting from a Welch's t test. Significant differences (α = 0.05) are highlighted in bold.

Surface coverage (%)	Mean difference (eggs per female)	P
8.7	472 (377.4 – 566.5)	**< 0.0001**
13.1[a]	390.5	Not tested
18.5[b]	230.6	Not tested

[a] – Comparison not tested as only one lacewing reproduced in the no avoidance population
[b] – Comparison not tested as no lacewings reproduced in the no avoidance population

5.4.5 *Typhlodromus pyri* survival

No distinct trend was apparent in the proportion of mites surviving seven days of exposure to 10.66 g ha^{-1} acetamiprid when showing avoidance behaviour or not (Figure 5.9). At all coverage levels, mean mite survival was consistently above 70% irrespective of behaviour. At 100% coverage, 7-day survival averaged 72% across both avoiding and non-avoiding populations (95% CIs [67.5, 76.5]; data not shown on graph).

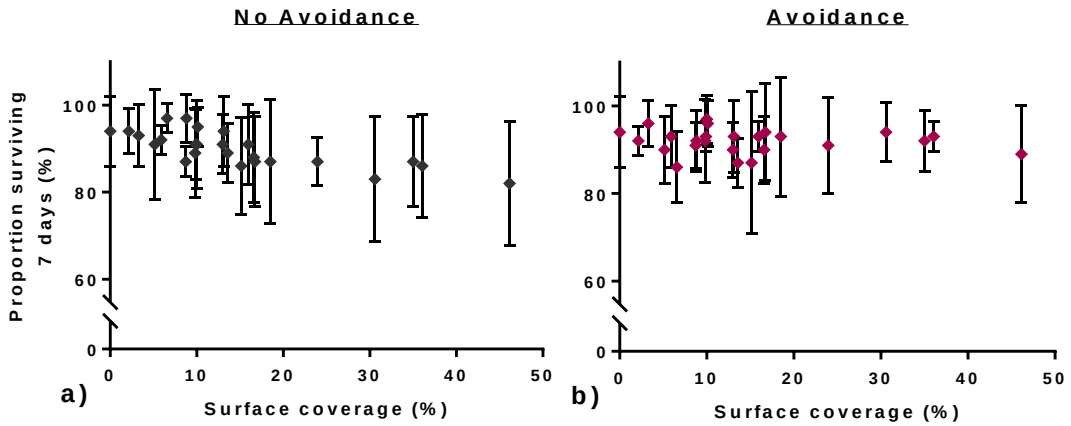

Figure 5.9 – Proportion of *Typhlodromus pyri* individuals surviving seven days of exposure when a) not avoiding acetamiprid residues (left) and b) avoiding acetamiprid residues (right). The seven-day survival assessments were simulated on 24 heterogeneous acetamiprid exposure landscapes with varying residue coverage. Acetamiprid concentration was set to 10.66 g ha^{-1}. Dots represent mean ± 95% confidence intervals generated by five simulations of 20 lacewings (total simulated n = 100 per coverage per behaviour).

When comparing survival after seven days of exposure in the three example exposure landscapes, we found no significant differences in survival when mites avoided residues at any coverage level (Figure 5.10, Table 5.23).

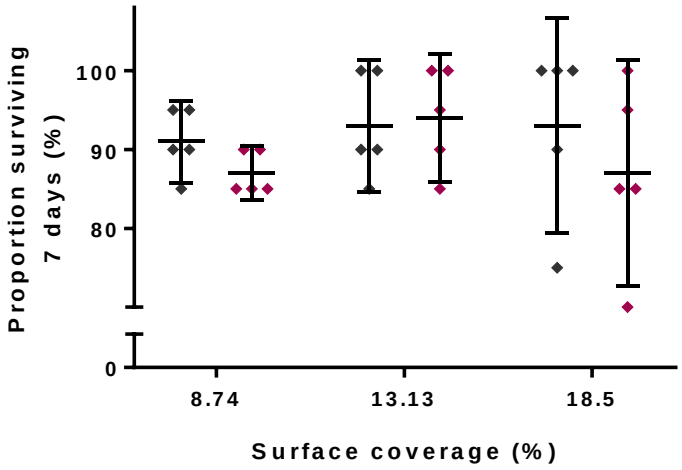

Figure 5.10 – Differences in survival over seven days in *Typhlodromus pyri* individuals that do not avoid acetamiprid residues (grey dots, left), and individuals that do avoid acetamiprid residues (pink dots, right). Lines represent mean ± 95% confidence intervals. Each dot represents a model run with 20 lacewings. Total simulated n = 100 per behaviour, per coverage level.

Table 5.23 – Mean differences in survival over seven days in Typhlodromus pyri individuals avoiding or not avoiding acetamiprid residues at three surface coverage levels. Comparison of no avoidance behaviour vs avoidance behaviour is shown with the standard error of the difference in parentheses, and adjusted P values resulting from multiple t tests. n = 10 × 20 individuals per behaviour, per coverage level.

Surface coverage (%)	Mean difference (%)	P
8.7	4 (± 2.2)	0.33
13.1	-1 (± 4.2)	> 0.99
18.5	6 (± 7.1)	> 0.99

We also explored the mean longevity of individual mites simulated for the reproduction study (Section 5.4.6), and observed that avoidance behaviour improves the longevity of individuals at higher coverage levels. Mites exposed at all coverage levels surviving on average over 40 days when they avoid residues, compared with

survival time dropping below 35 days at 23.95% coverage (Figure 5.11). In mites exposed to 100% coverage, mean longevity was 10.8 days (95% CIs [9.8, 11.8]; data not shown on graph).

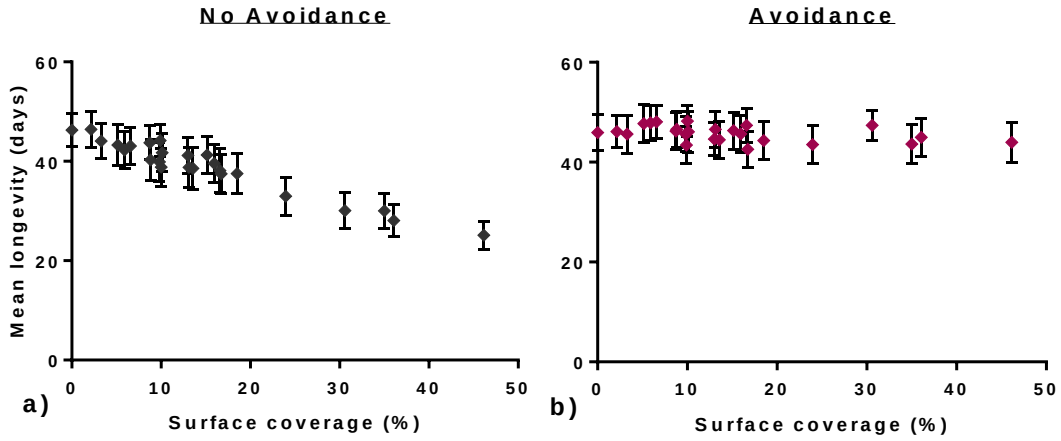

Figure 5.11 – Mean longevity of *Typhlodromus pyri* individuals when a) not avoiding acetamiprid residues (grey dots, left) and b) avoiding acetamiprid residues (pink dots, right). Data were generated by simulating individuals completing life cycles on 24 pesticide exposure landscapes with the acetamiprid concentration set at 10.66 g ha^{-1}. Dots represent mean ± 95% confidence intervals; *n* = 100 per behaviour, per coverage level.

When investigating the three representative coverage levels, we observed that avoidance behaviour on average increased mite longevity by over 7 days at 13.13% and 18.5% surface coverage (Figure 5.12). Acetamiprid residue avoidance had no significant impact on mite longevity at the 8.72% coverage level, at 13.13% avoidance behaviour increased longevity by 7.8 days, and avoidance behaviour improved longevity by 6.8 days in the 18.5% coverage scenario (Table 5.24).

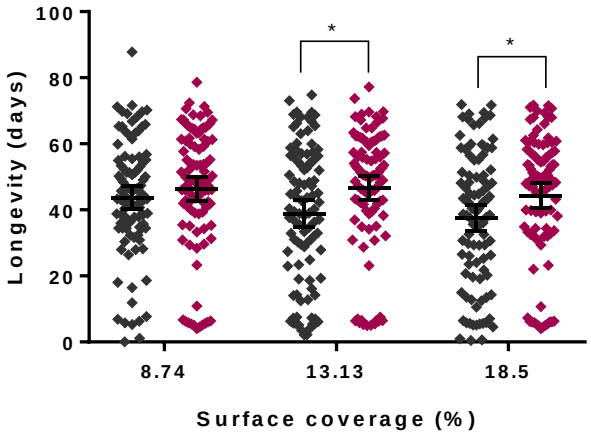

Figure 5.12 – Differences in longevity in *Typhlodromus pyri* individuals that do not avoid acetamiprid residues (grey dots, left), and individuals that do avoid acetamiprid residues (pink dots, right). Lines represent mean ± 95% confidence intervals. Each dot represents an individual mite. Brackets display statistical significance in comparison between behaviours (P values adjusted for multiple comparisons; * = $P < 0.05$). Total simulated *n* = 100 per behaviour, per coverage level.

Table 5.24 – Mean differences in longevity in *Typhlodromus pyri* individuals avoiding or not avoiding acetamiprid residues at three surface coverage levels. Comparison of avoidance behaviour vs no avoidance behaviour is shown with the standard error of the difference in parentheses, and adjusted P values resulting from multiple t tests. n = 10 × 20 individuals per behaviour, per coverage level. Significant differences (α = 0.05) are highlighted in bold.

Surface coverage (%)	Mean difference (days)	*P*
8.7	2.5 (± 2.6)	0.98
13.1	7.8 (± 2.8)	**0.02**
18.5	6.8 (± 2.8)	**0.04**

5.4.6 *Typhlodromus pyri* reproduction

Adult mites laid more eggs when they avoided acetamiprid residues (Figure 5.13). In mites not exhibiting avoidance behaviour, reproduction showed a downward trend with increasing acetamiprid coverage, with total oviposition dropping to 20 eggs per female at 30.6% coverage; however, total oviposition did not drop below 30 eggs per female in mites that avoided residues. In mites exposed to 100% coverage, mean oviposition was 4.1 eggs per female (95% CIs [3.4, 4.9]; data not shown on graph).

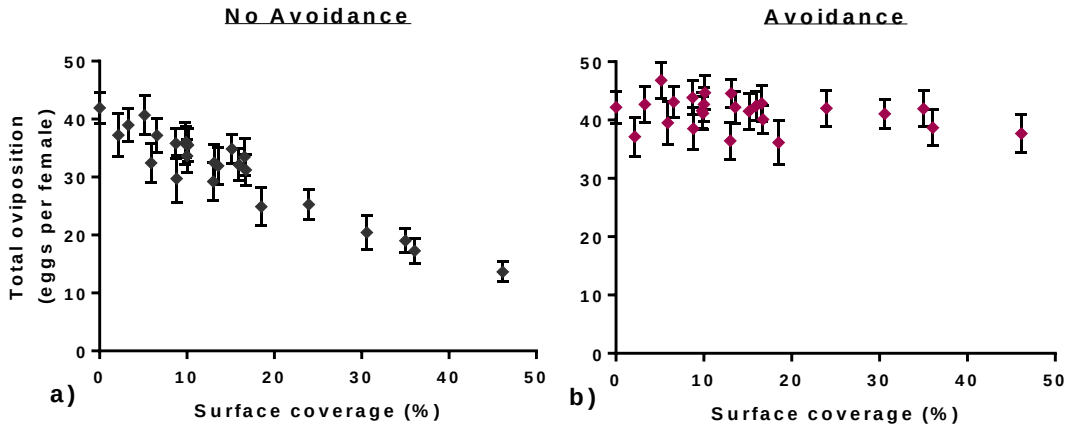

Figure 5.13 – Total oviposition per adult female *Typhlodromus pyri* when a) not avoiding acetamiprid residues (grey dots) and b) avoiding acetamiprid residues (pink dots). Oviposition was simulated on 24 pesticide exposure landscapes with the acetamiprid concentration set at 10.66 g ha^{-1}. Dots represent mean ± 95% confidence intervals generated by 10 simulations of 20 lacewings (total simulated n = 200 per coverage per behaviour; infertile individuals and individuals that did not survive to adulthood omitted from analysis).

When comparing oviposition at the three representative coverage levels, we found that avoidance behaviour led to a mean increase in oviposition of 8 eggs per female at 8.7% surface coverage (SE of difference = 1.96, t = 4.1; P = 0.0002; Figure 5.14). At 13.3% coverage, we saw an increase in oviposition of 12 eggs per female (SE = 2.01; t = 6.03; P < 0.0001), and at 18.5% oviposition increased by 13 eggs per female (SE = 2.06; t = 6.35; P < 0.0001).

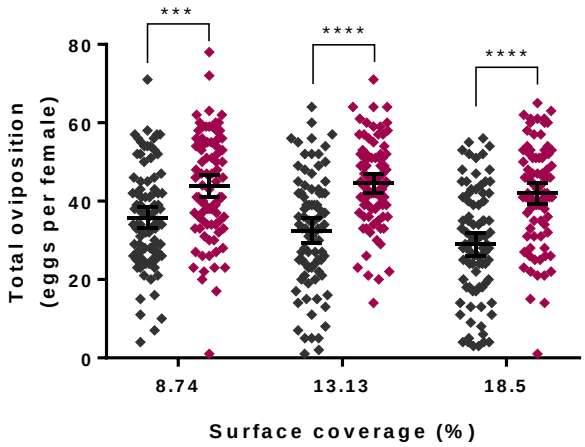

Figure 5.14 – Differences in total oviposition per female in *Typhlodromus pyri* individuals that do not avoid acetamiprid residues (grey dots, left), and individuals that do avoid acetamiprid residues (pink dots, right). Lines represent mean ± 95% confidence intervals. Each dot represents a single reproducing female; infertile females are excluded from analysis. Brackets display statistical significance in comparison between behaviours (*** = P < 0.001; **** = P < 0.0001). Comparisons not shown for the other coverage levels due to lack of samples in the no avoidance behaviour population. 8.7% *n* = 92, 87; 13.1% *n* = 83, 85; 18.5% *n* = 86, 86.

Table 5.25 – Mean differences in total oviposition in *Typhlodromus pyri* individuals avoiding or not avoiding acetamiprid residues at three surface coverage levels. Comparison of avoidance behaviour vs no avoidance behaviour is shown with the standard error of the difference in parentheses, and adjusted P values resulting from multiple t tests. n = 100 individuals per behaviour, per coverage level. Significant differences (α = 0.05) are highlighted in bold.

Surface coverage (%)	**Mean difference (eggs per female)**	***P***
8.7	8.0 (± 2.0)	**0.0002**
13.1	12.1 (± 2.0)	**< 0.0001**
18.5	13.1 (± 2.1)	**< 0.0001**

5.5 Discussion

5.5.1 Model output verification and current status

Having tested the PANTA model to verify outputs based upon longevity, reproduction and movement in control scenarios, and mortality in acetamiprid exposure scenarios, we were able to conclude that our model provides a good representation of lacewing and mite development, reproduction, movement and mortality when exposed to acetamiprid (Section 5.3). However, we have not yet covered several steps in model "evaludation". Two important steps to undertake are sensitivity analysis and output corroboration against data independent to those used for parameterisation (Augusiak et al., 2014b), though these steps would only be appropriate once the model's concept is developed further.

5.5.2 Model simulation results

In its current state, our PANTA model allowed us to investigate the indirect effects of pesticide exposure on non-target arthropods through the use of standard laboratory test data. Our simulations showed that avoidance of acetamiprid residues increased the survival of *Chrysoperla carnea* larvae to adulthood by an average of 45% (Figure 5.4). Acetamiprid avoidance also increased mean longevity by an average of 36 days when compared to lacewings that did not avoid residues (Figure 5.5). In *Typhlodromus pyri*, we observed no change in the trend in 7-day survival when mites avoided residues or not (Figure 5.9); though more mites appeared to survive 7 days when avoiding residues, we observed > 70% survival in all replicates, irrespective of behaviour, and the difference between means was not significant (Figure 5.10). This was likely a result of the relatively low mortality observed in the regulatory study at 10.66 g ha^{-1} (31.7%; SD ± 25.7%; EFSA, 2016). However, we did observe a change in longevity trend in mites at different coverage levels: a distinct downward trend in mean longevity in mites not avoiding acetamiprid was lost when mites avoided residues (Figure 5.11). When comparing longevity, we observed increases in longevity

of over one week (a 19% increase) in mites that avoided residues at 13.1% and 18.5% coverage levels (Figure 5.12).

Due to the improvements in longevity, we also observed increases in oviposition in both species when avoiding acetamiprid residues. Lacewing adults that avoided residues laid on average 430 more eggs – a 12 times increase – than those that did not avoid (Figure 5.8). We also observed oviposition at every acetamiprid coverage level in lacewings that avoided residues, whereas those that did not avoid residues failed to reproduce at coverage levels above 13% (Figure 5.7). Oviposition rates in *T. pyri* followed similar trends to mite longevity, where oviposition decreased with increasing acetamiprid coverage levels in individuals that did not avoid residues, but oviposition rates were more constant in mites that avoided acetamiprid (Figure 5.13). We detected an increase in oviposition of 11 eggs, or 36%, between mites that avoided residues and those that did not (Figure 5.14). Therefore, the trends we observed in oviposition were similar between species, though the size of increase when individuals avoided acetamiprid differed. This is likely a result of the lower mortality of acetamiprid at 10.66 g ha^{-1}, or roughly 0.05% concentration when diluted in 200 L, in mites than in lacewings.

In laboratory studies, mites laid on average 4.9 eggs per female (SD = 1.0) when exposed to 10.66 g ha^{-1} acetamiprid (EFSA, 2016). These data were not used to parameterise the model; however, our model produced a result of 4.1 eggs per female (95% CIs [3.4, 4.9]). We conclude our model functioned well in assessing indirect effects on reproduction in this instance. There were no comparable data for reproductive effects in *C. carnea*; however, EFSA (2016) reported no significant effects of acetamiprid residues on reproduction at 13 and 100 g ha^{-1}.

Beers and Schmidt-Jeffris (2015) suggested that bioassays that use homogeneously covered test arenas for the study of lethal and sublethal effects were likely to be overestimating the harmful effects of pesticide contact exposure on non-target arthropods. By comparing the results for both mites and lacewings in heterogeneous exposure scenarios (i.e. those below 100% coverage), to observations in populations

in a homogeneous exposure landscape, we can conclude that our findings support the hypothesis of Beers and Schmidt-Jeffris, as longevity, survival and reproduction all increased in lacewings and mites in heterogeneous exposure scenarios. This was observed even in individuals that did not avoid acetamiprid residues.

There is no acetamiprid LR_{50} published for *C. carnea*, with EFSA (2016) deeming the insecticide to have "no adverse lethal or sublethal effects" on the lacewing, though this contrasts with the study used to parameterise this model (Nasreen, Mustafa and Ashfaq, 2005). Conversely, the same EFSA assessment concluded that the 7-day LR_{50} for *T. pyri* was 30 g ha^{-1} (95% confidence intervals [24.6, 35.9 g ha^{-1}]), while an older assessment reported a 7-day LR_{50} of 18 g ha^{-1} (European Commission, 2004), meaning the lethal effects observed in laboratory studies are occurring at rates less than half of what is suggested for use in orchards. The study utilised for risk assessment does not quantify the actual residues mites were exposed to, nor the size of the test arena; however, the study was undertaken to Good Laboratory Practice principles and followed IOBC approved methods, so we trust that the pesticide application was conducted with accuracy and precision. Based on these LR_{50}s alone, we would expect the conclusion that acetamiprid posed an unacceptable risk to *T. pyri*; however, the risk assessment also included aged residue studies that allowed EFSA to conclude that lethal effects in *T. pyri* were short-lived, and that populations would recover sufficiently fast to not be affected at unacceptable levels (EFSA, 2016).

In Chapter 2, we calculated that 17% of the penconazole spray applied to an apple orchard was deposited on the upper surfaces of apple leaves when 250 L of spray was applied (Section 2.8). In apple crops, the suggested application rate for acetamiprid as an active substance is 75 g ha^{-1} (or 375 g formulated product; EFSA, 2016), and in the *T. pyri* mortality study this was applied in 200 L Ha^{-1}, giving a concentration of 0.375 mg mL^{-1}. The mortality study only reported concentrations of acetamiprid in solution (Nasreen et al., 2005), so we have inferred that this was also equivalent to being applied in 200 L Ha^{-1}. If 17% of a 200 L Ha^{-1} application landed on apple leaves within an orchard, then this would equate to 34 L, or 0.173 µL cm^{-2}_{leaf}. Based on this, we have converted the active substance field application rates to show their assumed concentrations in solution, and have then used this to estimate acetamiprid residues

on apple leaves in an orchard (Table 5.26). We also applied the same basis to the recommended application rate for acetamiprid, to allow discussion in the context of residues expected in orchards.

If acetamiprid was applied at a rate of 75 g ha^{-1} in 200 L in an orchard, then based on our field study, an average of 0.052 $\mu g_{a.s.}$ cm^{-2}_{leaf} would be deposited on apple leaves, equating to total leaf residues in the orchard of 12.77 g ha^{-1}_{ground}. This is based on an overall mean spray coverage of 15.6% when considering all samples from the field study, though mean coverage amongst the sample spray patterns within the PANTA model was 15.1%, depositing 0.22 µL cm^{-2}_{leaf}. Based on these calculations, we can infer that the effects we observed in our simulations when the acetamiprid rate was set to 10.66 $g_{a.s.}$ Ha^{-1} would be similar to what would be experienced in apple orchards if acetamiprid was applied at the field rate of 75 $g_{a.s.}$ Ha^{-1} in 200 L water. We conclude that, based on our model simulations, heterogeneous spray exposure leads to higher survival rates and thus increased longevity and reproduction. By combining model simulation results and our knowledge of spray patterns, we can also conclude that, if 17% of the acetamiprid applied in an orchard at recommended field rate lands on apple leaves, then a mortality rate close to the 32% observed at the 10.66 g ha^{-1} rate in laboratory studies would be expected within the orchard. However, to be able to make robust conclusions in this context, more information on typical spray patterns for acetamiprid would be necessary.

Table 5.26 – Acetamiprid application rates utilised in the PANTA model expressed as the laboratory application rate and their equivalent liquid concentration if applied at a rate of 200 L ha^{-1}. Test concentrations are then expressed as estimations of acetamiprid residues landing on apple leaves in one hectare of orchard and per unit of leaf area. Presented values are based on the assumption that 17% of a 200 L ha^{-1} pesticide spray application lands on apple leaves in one hectare of orchard. Application rates are based on mortality studies published for *Typhlodromus pyri* and *Chrysoperla carnea*.

	Laboratory study test concentrations		**Estimated residues on apple leaves in field**	
	Laboratory application rate ($g_{a.s.}$ Ha^{-1}_{ground})	**Spray mixture concentration at rate of 200 L Ha^{-1} ($mg_{a.s.}$ mL^{-1})**	**Apple leaf residues in orchard ($g_{a.s.}$ Ha^{-1}_{ground})**	**Apple leaf residues ($\mu g_{a.s.}$ cm^{-2}_{leaf})**
T. pyri 7 day mortality	10.66	0.053	1.815	0.007
study rates [a]	18.66	0.093	3.177	0.013
	32.65	0.163	5.558	0.023
	57.14	0.286	9.727	0.040
	100	0.500	17.02	0.069
C. carnea 48 hour	37.5	0.188	6.384	0.026
mortality study rates [b]	75	0.375	12.77	0.052
	112.5	0.563	19.15	0.078
Field application rate[a]	75	0.375	12.77	0.052

[a] – *EFSA (2016)*
[b] – *Nasreen, Mustafa and Ashfaq (2005)*

In summary, our model has shown that heterogeneous spray exposure leads to improvements in longevity and thus reproduction. In addition, we have shown that avoidance behaviour further increases longevity and reproduction, with the effect more noticeable when mortality rates are higher. These are clearly positive outcomes in terms of population consequences for the two modelled species. However, we cannot interpret these results too much as the model in its current state is too simple and not yet robustly tested.

5.5.3 Model limitations

The largest limitation to our PANTA model is that it is currently a very simple model design with no validation. Energy, food requirements, and toxic effects of acetamiprid on reproduction are not yet considered within the model, and for this model to be of any regulatory use, it would need to be improved to become a TKTD model. However, as we noted previously, TKTD at this stage would have introduced additional complexity and uncertainty that was unnecessary when developing the concept.

To be considered a successfully validated model, good quality data are required for model conceptualisation, parameterisation, calibration, and finally validation (Augusiak et al., 2014b). A major issue we encountered when developing and parameterising the model was that many of the relevant data in the present literature are not well suited to parameterising or testing individual-based models. Ideally, we would have generated our own data for pesticide effects on survival and reproduction to parameterise the model, but time constraints made this unfeasible. There is also a lack of consistency in data quantity, driven by some species receiving more research focus. *T. pyri* movement behaviour has been studied widely in many settings; the literature surrounding this was reviewed in Chapters 3 and 4. We conducted laboratory studies to complement the current research by studying movement behaviour changes at a high resolution and a small temporal scale, and then followed this up with studies to investigate avoidance behaviour. However, it was very difficult to find information on lacewing larva movement behaviour, as most

research effort into the movement of lacewings has focussed upon the long distances covered by adults (Chapman et al., 2006; Duelli, 1984b; Peter Duelli, 1980; Keulder and Van den Berg, 2013). Our movement data came from two studies, neither of which studied behaviour for more than 15 minutes. Conversely, data on *T. pyri* movement behaviour was more complete, and one study also offered insight into activity over a 24 hour period (Croft and Zhang, 1994). As a result of our decision to keep the PANTA model's function consistent for the two modelled species, our model assumes that movement and activity remains the same for every hour of the day, even though we know this not to be true, in particular for *T. pyri*. To accurately reflect movement behaviour in the model, we would need high resolution data from studying individuals over a long temporal scale, and also at different life stages. We fully discuss research needs in the next section (Section 5.5.4).

Another major limitation of the present model relates to spatial scale, and this is a concept that is a great challenge for modelling population consequences in a realistic environment. The current spatial scale of 26 cm^2 requires refinement as the water sensitive paper image from which the exposure patterns are derived only represented 2.6 cm^2. Each of the papers represented coverage on the upper surface of one leaf (Chapter 2), and we modelled the full life cycle of individuals on that one "leaf". However, *T. pyri* can complete their full life cycle on one apple tree (Zacharda, 1989), and spend time on spurs, twigs, and within the bark. Lacewings on the other hand remain within the tree canopy during larval stages, but as adults they disperse from the original habitat, flying for up to 40 km before landing in another habitat (P Duelli, 1980). Once they reach reproductive age, they then fly into habitats where they have detected the kairomones of prey, deposit eggs, and then take flight once again (Duelli, 1984b). Covering such a spatial scale would be highly complex, though the life history raises interesting questions: how would initial contact exposure as a larva then impact on populations in a regional landscape covering several kilometres? This could be answered with a great deal of effort placed on improving the spatial representation within the PANTA model.

5.5.4 Research needs

To successfully model the population consequences of pesticide exposure, and to investigate the potential for avoidance behaviour to mitigate pesticide effects, many studies would be necessary to inform the model development. Additionally, the studies would need to be undertaken in a particular way so that data are suitable for parameterising a model.

Two classes of studies are required: ecological studies, necessary for increasing the understanding of the ecology, development and behaviour of the test species; and toxicological studies, necessary for understanding the species-specific responses to a particular pesticide. The ecological studies would only need to be conducted once, and would need to fill gaps in the current literature: there are studies investigating the effects of food availability and temperature on development and reproduction for both *C. carnea* and *T. pyri*, so these would not need repeating. However, there are aforementioned gaps in the knowledge of movement and activity in both species, and these gaps would need to be filled before good quality ecological models could be developed for the two species. Toxicology studies would be repeated for every compound that would be tested in the model, and these would ideally follow a set protocol for identifying specific responses to a compound, in line with regulatory needs for knowing lethal and sublethal responses. These would be conducted at a range of concentrations, and, if relevant, temperatures and food availability rates.

A high priority for laboratory studies would be to undertake experiments that increase the community's knowledge of the effects of various contaminants on energy allocation in mites and lacewings (i.e. physiological modes of action). Understanding energy allocation would enable the application of TK/TD theory and allow for the development of a DEBtox model. The details necessary for modelling effects of compounds on organisms are well known for aquatic species (Ashauer et al., 2013; EFSA Panel on Plant Protection Products and their Residues, 2018); however, more work is required for terrestrial species. Of fifteen species listed as having known physiological modes of action, only one is a terrestrial non-target arthropod: the soil dwelling springtail *Folsomia candida* (Ashauer and Jager, 2018).

Another priority would be to consider movement behaviour of lacewings and mites in longer temporal scales. The availability of digital cameras and tracking systems such as EthoVision enable researchers to study behaviour for hours at a time, and this should be put to use to better understand movement behaviour. Studies should focus on understanding how mite and lacewing activity fluctuates over a 24-hour period, to discern whether there are diurnal patterns in larval behaviour. They should also investigate the movement behaviour effects over several hours using a repeated measures design to investigate whether the behavioural changes we observed are a temporary effect, whether there are long term impacts on movement, and also whether there is a point at which behaviour recovers to pre-exposure levels.

To ensure data arising from laboratory and field studies are of sufficient quality for parameterising a model, experiments would need to be designed with sufficiently large sample sizes as small samples risk inflating the effect of outliers, thus reducing the chances of a model being successfully validated (Augusiak, Van den Brink and Grimm, 2014). Studies would also need to be reported with a good level of detail. One specific problem we encountered when examining published studies was the lack of clear detail on sample sizes, or a lack of reporting standard deviation, standard error, or confidence intervals surrounding reported means. For understanding results, confidence intervals are a useful addition; however, for modelling purposes, provision of raw data would be ideal to allow modellers to fully understand and integrate the variance in responses. This would help avert issues regarding extreme values being generated, as previously discussed (Section 5.2.2.7).

To produce a realistic population-scale life cycle model, it would be necessary to include factors such as the developmental time of larvae to adults at different air temperatures and photoperiods. Both of these factors have been shown to be important for *C. carnea* development (Pappas et al., 2013; Fujiwara and Nomura, 1999; Joschinski, Kiess and Krauss, 2017) and *T. pyri* (Gadino and Walton, 2012; Hayes and McArdle, 1987), and the resolution of these data would allow implementation of the effects in a more developed version of our model. Development is also affected by the food choice and availability (Atlihan et al., 2004; Hayes and McArdle, 1987) and this would need to be considered to increase model realism.

Field studies would be necessary to maximise the model's realism on larger spatial scales. Surveys should be undertaken to develop understanding of mite and lacewing densities within orchards and should also consider on- and off-crop densities. This would enable extrapolation of individual-level effects identified within laboratory studies to landscape scales.

Finally, a population model should be able to operate on an annual cycle to determine cross-generational impacts on populations, both lethal and sublethal. For example, *C. carnea* are known to have three generations per year in France (Trouve et al., 2002), and two per year in the UK (Perry and Bowden, 1983). The model should be capable of covering this over several years; it would then be well placed to inform academia, industry and regulatory bodies of the long term population impacts upon the two species.

Chapter 6 – Overall discussion

6.1 Key findings

This research project had an overall aim to understand three topics: the spatial heterogeneity of pesticide exposure, pesticide avoidance behaviour in non-target arthropods, and the population consequences of exposure and avoidance behaviour. Additionally, this project aimed to improve the realism of regulatory risk assessment for NTAs. Below is a summary of the key findings.

The results arising from the spray coverage study demonstrate that pesticide residues can be successfully quantified on individual apple leaves with high levels of accuracy and precision. Furthermore, the data show correlation between measures of pesticide deposition derived from water sensitive paper and residues quantified analytically from leaves (Witton et al., 2018). Pesticide residues measured on apple leaves were found to be six times lower than residue values derived from estimates of liquid deposition on water sensitive paper, demonstrating that water sensitive paper overestimates pesticide residues when used on its own (Figure 2.3). Additionally, in the case of the crop spray event analysed, 42.6 L out of the 250 L applied to the orchard landed on apple leaves (i.e. 17%; Section 2.8). This value was based on liquid deposition measured on the water sensitive paper samples, and was scaled up through applying the leaf area index (LAI) concept to better estimate the overall proportion of spray landing on apple leaves. This was useful for informing the discussion of pesticide effects on non-target arthropods in crop systems.

The results from the behaviour studies show that movement behaviour of the predatory mite *Typhlodromus pyri* is significantly altered by the presence of low levels of deltamethrin residues. Deltamethrin residues on the entire arena led to no mites being inactive for the 10-minute observation period (Figure 3.4), which suggests deltamethrin causes irritation or hyperactivity. The effects on distance walked, angular velocity, meander and time spent active were all seen in mites exposed to

surface residues of 0.0004 – 0.004 $\mu g_{a.s.}\ cm^{-2}{}_{glass}$ when the product label rate of 8.75 $g_{a.s.}\ ha^{-1}{}_{orchard}$ would equate to apple leaf residues of 0.006 $\mu g_{a.s.}\ cm^{-2}{}_{leaf}$ (Tables 3.14 – 3.15). Therefore, we would expect to see these effects in mites residing in apple crops sprayed with deltamethrin. Mites also exhibited avoidance behaviour when exposed to fully treated arenas at these rates (Figure 3.6), meaning that we would also expect to observe avoidance behaviour in orchards sprayed at the recommended label rate and also at diluted rates. However, these effects were not carried over into the choice arena experiments, where, surprisingly, no clear avoidance of deltamethrin was observed; nor were behavioural changes when mites made contact with the residues (Figure 4.8).

In choice arenas, deltamethrin affected distances walked by mites, but the effect was only observed when considering the test arena as a whole (Figure 4.8). This suggests that the effect of deltamethrin on mite movement is not only observed when individuals are in direct contact with residues, indicating either that there is an effect when in close proximity to residues, or that effects continue for a period of time after moving to an unexposed surface. This means that mite movement would likely be affected by deltamethrin even in a heterogeneous residue exposure landscape, as refugia do not appear to alleviate deltamethrin effects.

A clear preference was observed for mites to spend less time on dimethoate treated surfaces when exposed to the residues in a choice arena, with mites spending approximately 65% of the observation time on untreated surfaces (Figure 4.6). This was an unexpected response as mites did not show avoidance behaviour in the full coverage experiments. However, it cannot be inferred whether effects observed in the laboratory would occur under field conditions, as dimethoate use is no longer permitted in fruit orchards and as such there are no published application rates.

Across all movement behaviours, angular velocity was the most sensitive behaviour in terms of effects of pesticide residues. In the full coverage study, angular velocity increased in mites exposed to all insecticides. This suggests that acetamiprid, deltamethrin and dimethoate all caused irritation in *T. pyri*, based on the definition by Wiles and Jepson (1994).

With behaviour responses varying greatly when comparing full coverage arenas and choice arenas, this work reinforces the premise put forward by Beers and Schmidt-Jeffris (2015) that full coverage arenas really are a worst case scenario for considering pesticide effects on non-target arthropods.

The simple individual-based model, PANTA, demonstrated that individual avoidance of acetamiprid residues increases survival, longevity and reproduction in the green lacewing *Chrysoperla carnea* and *T. pyri* (Section 5.4). The model also demonstrated that heterogeneous pesticide coverage increases survival and longevity. However, when conceptualising the model, it was learned that the premise of modelling population consequences at the landscape scale, with ecological realism, is a highly complex matter. Though the PANTA model is simplistic and is some way from regulatory application, it is a useful tool even in these early development stages for aiding the interpretation of experiments and would be useful for designing future experiments.

6.2 Novel method contributions

Throughout this project, novel analytical methods and ecotoxicological studies have been developed that hopefully will be utilised by researchers in the future. These contributions are summarised below.

There was a need for a conventional analytical method that could quantify pesticide residues on individual leaves. Previously reported methods had extracted and quantified residues from bulk samples of leafy vegetables (González-Rodríguez et al., 2008), while the most relevant method in terms of context analysed residues on apple leaves, with two leaves per sample (X.-M. Xu et al., 2008). The GC-MS method developed in this thesis is able to quantify the fungicide penconazole (applied as a formulated product) on individual apple leaves weighing on average 0.6 g (Witton et al., 2018). The method is both accurate and precise based on pesticide residue quantification benchmarks set out by the European Commission (1996), and has a limit of detection of 0.08 mg kg^{-1}, and limit of quantification of 0.26 mg kg^{-1}. None of the extracted leaf samples had residues below the LOQ, including the control leaf

samples that were taken before the spray was applied. Based on this, it can be concluded that this analytical method is effective at sampling very low levels of penconazole, and can be adapted for several other active ingredients.

In studying pesticide spray patterns in the crop environment, water sensitive paper was used to obtain visual representations of the spray pattern. Using digital analysis, it was possible to quantify the volume of liquid deposited on the paper through using DepositScan (Zhu et al., 2011); however, it was unknown as to whether these data could be used to accurately estimate pesticide residues. The software was used to determine liquid deposition based on area, and through knowing the penconazole concentration in the spray mixture, it was possible to estimate the penconazole deposits. Water sensitive paper residue estimates were found to be over six times higher than actual leaf residues; this was likely due to co-formulants causing droplets to spread on impact. However, by knowing the difference in actual residues on apple leaves, and estimated residues derived from water sensitive paper, a correction factor can be applied to the latter measure. This allows water sensitive paper to be used to provide a rapid, low cost method for estimating pesticide residues within a crop environment. This method has the potential to allow large sample sizes that would normally be prohibitively expensive and time consuming to analyse.

When developing methods for studying pesticide induced movement behaviour changes in mites, the review revealed an apparent route to poor reporting of effects: no papers reported actual pesticide residues from the test arenas used in their experiments. Additionally, the present style of documenting studies used in regulatory decision making does not clearly report actual residues, nor do they report measures that would allow scientists to estimate surface residues, such as volume of spray deposited on the test arena surface. Residues need to be quantified and reported to best understand any observed responses, so as part of this study methods were developed that involved test arena designs that allowed easy extraction of surface residues. ^{14}C-radiolabelled insecticidal active substances were used to develop a single method that demonstrated high recovery rates of 96% for acetamiprid and 95% for dimethoate, and acceptable recoveries of 79% for

deltamethrin (Section 3.3). The method is also easily adapted for low concentrations (Section 4.2.4).

The theme of unquantified pesticide residues in lethal and sublethal effect studies continued with the development of choice arenas. Chapter 4 outlined the existing methods and highlighted how many of these were inappropriate either for the study of mite behaviour, or for measuring residues. The method used for applying pesticide residues for full pesticide coverage experiments was adapted so that residues were on roughly half of the test arena surface, while still ensuring residue extraction was possible. This novel method balances the issues of having a homogenous test arena surface, so as to not affect mite movement, while also having only partial pesticide residue coverage and also being able to quantify the residues after observations were complete.

6.3 Future research needs

6.3.1 Potential for pesticides to attract

This project has focussed upon the premise of pesticides being repellent to non-target arthropods, with pesticide avoidance behaviour being a well-known phenomenon. However, the opposite response to repellence – attraction – is now receiving attention. A recent study into bee foraging behaviour found that flowers contaminated with neonicotinoids become disproportionately attractive to the bees, leading to the potential for reductions in colony size and function (Arce et al., 2018). The authors concluded that the attractiveness of pesticides to foraging bees should be incorporated into risk assessments, and this is a premise that should also be explored for other non-target species, especially in considering population- and ecosystem-scale consequences. No attraction to surface residues was observed in the studies for this thesis; however, the premise could easily be studied when researchers investigate avoidance behaviour, and so future studies could draw conclusions on both avoidance and attraction.

6.3.2 Interactive effects of environment and pesticide

Chapter 3 concluded that there was an interaction between exposure to dimethoate residues and air temperature in *T. pyri* adults, shown in increased angular velocity and meander in individuals with increasing concentration (Figure 3.3), but also increasing air temperature (Table 3.3). This indicated that one of two points were true: either dimethoate residues could increase mite sensitivity to environmental conditions, or higher air temperatures increased dimethoate toxicity in mites. The latter premise is reinforced by other studies (Glunt et al., 2013; Mansoor et al., 2015), with the former authors recommending that, as a result, testing regimes for investigating pesticide efficacy in malaria control should be improved to include a range of air temperatures. A similar recommendation is proposed for risk assessors: to improve the understanding of the effects of insecticides in non-target arthropods, laboratory assessments of pesticide effects should be undertaken at a range of air temperatures related to those the species would be exposed to in the natural environment. The studies should be done to complement the current IOBC standard tests (undertaken at 25°C), but at a range of additional temperatures relevant to the test species. Gadino and Walton (2012) studied the effect of temperature on a *T. pyri* population collected from orchards in Oregon, US, and found that the minimum and maximum development thresholds were 7.2 and 42.6°C respectively, with population growth occurring between 15 and 30°C. Therefore, studies should be undertaken at a range of temperatures within the species' limits. Generation of these data would allow better informed decisions on pesticide use, potentially allowing regulators to deliver more refined advice on when to spray particular active substances to minimise the effects on non-target arthropods.

6.3.3 Studies to increase ecological relevance

Future research would need to consider whether population fitness is affected by repeated exposures to both the same compound, and a range of compounds. A study involving the freshwater invertebrate *Gammarus pulex* found that the sequence of contaminants can affect survival (Ashauer et al., 2017), and this premise requires

investigation in non-target arthropods, especially as UK apple orchards received on average 16 spray treatments containing 32 products in 2016 (Garthwaite et al., 2017).

Any laboratory studies involving pesticide exposure should cover three points: they should study realistic residue levels, heterogeneous exposure patterns, and should build residue analysis into the experimental design. Residue levels should be considered in the context of what mites and lacewings would be exposed to in orchard systems, both on- and off-crop. Laboratory studies should then study these residue levels, but should also consider a range of pesticide coverage levels to maximise realism in the laboratory. Finally, as previously mentioned, few lethal and sublethal effect studies in the terrestrial ecotoxicology context quantify the residues test subjects are exposed to, and this is an important factor for understanding observed effects. It was demonstrated in Chapters 3 and 4 that the use of radiolabelled active substances allows for rapid quantification of residues; by knowing exactly what residues individuals were exposed to, it is possible to better understand responses. Quantifying residues would also improve the quality of data being implemented in the model, as any measurement or instrument errors would be known and dealt with appropriately, thereby reducing the chance of data quality being too low for modelling use (Augusiak et al., 2014b).

Although laboratory studies are useful for studying lethal and sublethal effects of toxicants on non-target arthropods in standardised test set ups (e.g. Candolfi et al., 2000), such studies lack ecological relevance. This can be improved by field studies, though these can be prohibitively expensive and difficult to interpret (Jänsch et al., 2006). Laboratory studies also lack realism. For one, they tend to only consider one route of exposure in NTAs, which is contact with dried residues, when field exposure can occur via contact with dry and wet residues, oral exposure, and overspray of an individual (EFSA, 2015). Some studies are starting to consider other routes by studying the effects of direct spray on individuals (e.g. Porcel, Cotes and Campos, 2011), though this has not yet filtered into regulatory studies used for risk assessment. Another way in which laboratory studies lack realism is through not investigating the effects of pesticides in heterogeneous exposure environments: the

work in Chapter 2 demonstrates that pesticide spray varies widely within a single apple tree, as well as between trees within the same orchard (Witton et al., 2018).

A pair of recent studies in the United States have found that, in many cases, the toxicity of pesticides can vary between different life stages, with larvae more resistant to some pesticides than adults, and vice versa (Amarasekare et al., 2016; Mills et al., 2016). The studies in this thesis were undertaken on one life stage, with *T. pyri* adult behaviour studied, and the population model for *C. carnea* was parameterised using toxicity data for one life stage. However, a combination of the findings from the two aforementioned studies and experiences of conceptualising the PANTA model (Chapter 5) lead to conclusions that more time and effort should be spent studying the varied toxicity of pesticides on different life stages of non-target arthropods. This recommendation would benefit the scientific community, and hopefully allow for the development of accurate models showing the long term population consequences of pesticide exposure. It would also benefit the pesticide risk assessors by allowing them to better consider the population consequences of pesticide exposure.

Finally, choice arena studies such as the one devised in Chapter 4 need to be extended to investigate what choices mites and lacewings make when presented with a contaminated food source. If an active substance is highly repellent to an individual, does it not feed, or come into contact with the pesticide to feed? This would be an important study to undertake if long term population effects are to be truly understood.

Although it would be beneficial to improve the ecological realism of tests for species such as *T. pyri*, the increased realism might not be appropriate for direct application in pesticide regulatory science. *T. pyri* is used widely as it is a sensitive species with fast generation times and is relatively easy to culture in laboratories. However, these traits may make it unsuitable for studying avoidance behaviour and the population implications of avoidance. Furthermore, there is a disconnect between laboratory and wild strains of *T. pyri* due to widespread wild resistance to pesticides. As such, it may be appropriate for *T. pyri* to remain as a species used for generalised ecotoxicology studies; behavioural research should focus on longer lived species such

as *Chrysoperla carnea* when considering the wider implications of avoidance of pesticide exposure.

6.4 Implications for pesticide manufacturers

Though the main research aims did not aspire to investigate or even consider pesticide efficacy, some conclusions cover efficacy and as such would be of use to pesticide manufacturers. Conclusions drawn in previous chapters surrounding the implications of heterogeneous pesticide coverage for non-target arthropods could be redirected to consider implications for pest species. A homogeneous surface coverage within a crop may prove to be most effective for pest control; however, if the substance is repellent to the target species, it may lead to control only where heterogeneous residues are found, and no population- or landscape-scale control as the repellence may simply displace pest populations to crop areas with less consistent protection. Similarly, a heterogeneous spray coverage would compromise efficacy by potentially providing refugia for pests to reside and forage without coming into contact with the insecticide intended to control the pest.

Based on the findings from this study, pesticide manufacturers may benefit from considering field application methods, with particular focus on whether specific active substances require total crop coverage to achieve optimal efficacy.

The study of dimethoate effects on *T. pyri* movement behaviour unexpectedly showed one of two outcomes: either air temperature increased sensitivity to dimethoate, or dimethoate exposure affected sensitivity to air temperature. The previous section highlighted the need for more study into interactions between pesticide exposure and environmental conditions, and this is something that also warrants further consideration in pesticide efficacy. By investigating effects at a range of temperatures, manufacturers may find that an active substance may demonstrate higher or lower efficacy in certain regions. This would be advantageous to manufacturers who could tailor the formulated products based on any interactive effects of temperature and effect, and could also reduce the risk of an active substance or product failing to be approved for use on grounds of insufficient efficacy in some regions.

6.5 Implications for regulators

Additional to the main research aims, this study also aimed to improve the realism of regulatory risk assessment of pesticides. By considering the effects of pesticide residues on movement behaviour in full and half coverage arenas, it is clear that homogeneous pesticide coverage could act as a worst case scenario for pesticide exposure in non-target arthropods, though this is not certain as heterogeneous coverage may also lead to greater residues where spray lands. Laboratory studies and modelling show that, even if non-target arthropods do not avoid residues, heterogeneous exposure patterns on leaves increase longevity. Therefore, it is recommended that a heterogeneous coverage study be added to follow the standard full coverage studies undertaken for product registration. Half coverage arena designs would allow avoidance and repellence to be quantified and may assist in bridging the gap between laboratory and field study outcomes. Half coverage studies could also reduce the necessity for field studies, which would then greatly reduce the costs associated with registering a pesticide.

As highlighted in the previous section, dimethoate and air temperature potentially interacted for *T. pyri*. Though these findings were highlighted from an efficacy perspective for pesticide manufacturers, the implications outlined above also apply to regulators. By considering pesticide efficacy on target pests and non-target effects at a range of temperatures, regulators will be better placed to make refined decisions on pesticide use, tailoring approvals to climatic zones where deleterious effects on non-target arthropods are reduced and target pest efficacy is increased. As an example, if *T. pyri* is more sensitive to an insecticide when air temperatures are above 25°C, regulators could limit usage of the insecticide in regions where air temperatures regularly exceed that level, but offer less restriction in areas where that temperature is rarely experienced.

Finally, the inclusion of validated, standardised individual-based population models into risk assessment processes would provide regulators with insight that cannot be gained from a laboratory or field study, and instead is currently only being learned

through experience or observation of the effects in crop systems over several years. By using an ecological model, long term impacts on non-target arthropod populations would be better understood. The modelling would potentially allow risk assessors to model a sequence of pesticide applications through a whole growing season, offering greater understanding of population recovery following each exposure. This could then be extrapolated over several years to gain a fuller understanding of population consequences over several years.

There is great potential for the use of individual-based modelling in risk assessment, and this has been recognised through the recent conclusion from EFSA that TK/TD models are ready to be used in aquatic contexts (EFSA Panel on Plant Protection Products and their Residues, 2018). As more studies are undertaken to develop our understanding of exactly how pesticides affect non-target arthropods, hopefully models will also be used in the terrestrial context.

Appendices

Appendix A – Measures of environmental conditions

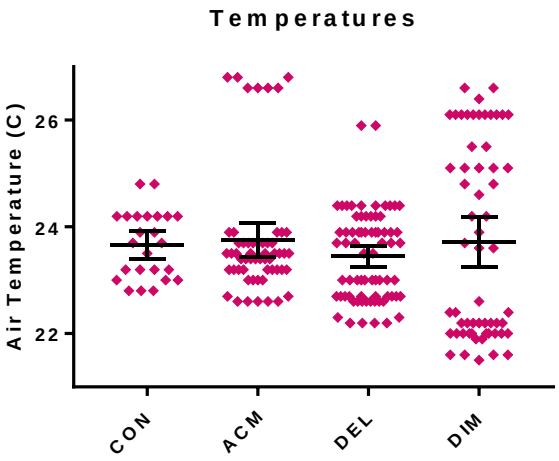

Figure A.1 – Spread of air temperatures measured throughout the full coverage arena study. Black line and whiskers represent mean ± 95% confidence intervals. CON = control; ACM = acetamiprid; DEL = deltamethrin; DIM = dimethoate. n = 24; 56; 71; 59.

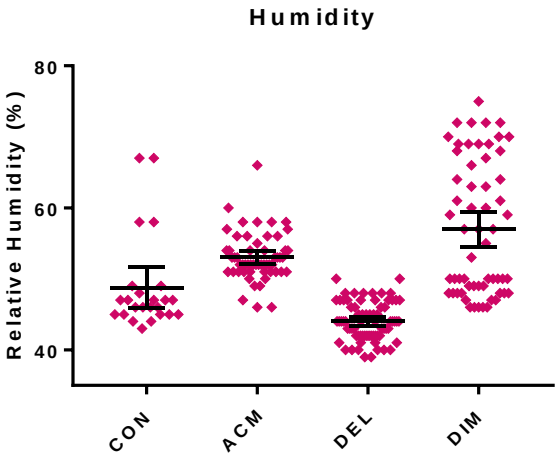

Figure A.2 – Spread of relative humidity measured throughout the full coverage arena study. Black line and whiskers represent mean ± 95% confidence intervals. CON = control; ACM = acetamiprid; DEL = deltamethrin; DIM = dimethoate. n = 24; 56; 71; 59.

Appendix B – PANTA lacewing model code (NetLogo)

```
;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;
;;;;;;;; SETTING BREEDS FOR MODEL ;;;;;;;;----------------------
;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;


patches-own [
  pesticide-patch? ; determines whether patch contains pesticide
  active-sub       ; defines the active substance (e.g. acetamiprid,
dimethoate)
  p-mortality      ; used to define the hourly mortality based on 48
hr tests. This accumulates and reduces the life of individuals
  patch-scent       ; patch "scent" to aid in avoidance of pesticide
patches
]

turtles-own [      ; the following are measures that are specific to
each lacewing
  exposure-duration          ; records how long individual has been
exposed in hrs/ticks
  tox                        ; the accumulated toxicity from walking
on contaminated surfaces derived from p-mortality. Eggs and pupae
residing on exposed surfaces end up with much higher levels due to
inability to avoid at that life stage
  tolerance                  ; tolerance to exposure. Adds randomness
to when individuals succumb to exposure but should all die after 48
hours cumulative exposure
  eggs-laid                  ; number of eggs laid by the individual
  distance-traveled           ; to record distance covered by each
lacewing. Velocity will be calculated from this
  time-active               ; to determine whether lacewing moves each
tick
  age                        ; individual's age in hours
  adult-age                   ; counts how long an adult has been an
adult. Used to work out natural death
  pup_surv                   ; probability of pupa hatching to adult
  repro-rate                   ; the individual's reproduction rate,
defined at creation and based on total oviposition @ 25C (Pappas et
al, 2013)
  infertility-chance        ; determines whether individual is fertile
or not (based on Pappas et al, 2013 @ 25C)
  fertile                    ; tells us whether individual is fertile
or not
  grow-to-l2                 ; the grow-to-X measures are defined for
each individual to allow for development processes to happen
  grow-to-l3                  ; within a range around an average time
at each stage. This set up allows the development to have some
stochasticity.
  grow-to-pup               ; All based on Pappas et al (2013) @ 25C.
  grow-to-adult
  be-mature                      ; the age at which an adult becomes
reproductively mature. Works like the grow-to-X measures.
```

```
  natural-end              ; used for varying when individual adults'
lives end naturally. Based on adult-age
  time-to-adult    ; records time to develop to adult (for model
checking)
]

breed [eggs egg]
breed [larvae-1 larva-1]
breed [larvae-2 larva-2]
breed [larvae-3 larva-3]
breed [pupae pupa]
breed [adults adult]
breed [dead-adults dead-adult]   ; used to check model operation only

eggs-own
[
  hatch-time   ; time to hatch to larvae. Temperature dependent
  egg_via      ; egg viability; probability the eggs will hatch
]

;;;;;;;;;;;;;;;;;;;;;;
;;;;;;;; SETUP ;;;;;;;;-------------------
;;;;;;;;;;;;;;;;;;;;;;

; acts as a reset and will do this when the "setup" button is pressed

to setup
  ca
  set-default-shape eggs "dot"
  set-default-shape larvae-1 "bug"
  set-default-shape larvae-2 "bug"
  set-default-shape larvae-3 "bug"
  set-default-shape pupae "dot"
  set-default-shape adults "butterfly"

  setup-patches   ; to generate model landscape
  setup-turtles
  reset-ticks
end

;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;
;;;;;;;; SETUP SUBMODELS ;;;;;;;;-------------------
;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;

;;;;;;;;;;;;;;;;;;;;;;;;;;;
;;; ENVIRONMENT SUBMODELS ;;;----------------
;;;;;;;;;;;;;;;;;;;;;;;;;;;

to setup-patches
  setup-env         ; Allows user to use drop down to select model
landscape from a series of artificially generated, and real exposure
landscapes. Real landscapes are derived from water sensitive paper
collected from field study (Witton et al, 2018)
  assign-patches    ; Re-colours the binary images used to generate
model landscape and establishes what is pesticide exposure and what
is not
```

```
  setup-contam
  diffuse-scent
end


to setup-env
; Various exposure landscapes to allow behaviour and life cycle to be
studied in different exposure landscapes.
; The files cover a range of coverage rates, but also one orchard
tree, and the off-crop exposure in the hedgerow.
  if file = "Avoidance Test 1" [import-pcolors "Images/Avoidance Test
1.png"]  ; artificial arena
  if file = "Avoidance Test 2" [import-pcolors "Images/Avoidance Test
2.png"]  ; artificial arena
  if file = "choice arena" [import-pcolors "Images/Choice Arena.png"]
; Replicates 50% coverage lab test arena
  if file = "control" [ask patches [set pcolor 3]]   ; Acts as the
control
  if file = "100% cover" [ask patches [set pcolor 1]]   ; Acts as 100%
cover control
; Real exposures
  if file = "1" [import-pcolors "Images/B-1-M-001-crop-binary.png"]
  if file = "2" [import-pcolors "Images/B-1-M-002-crop-binary.png"]
  if file = "3" [import-pcolors "Images/B-1-M-003-crop-binary.png"]
  if file = "4" [import-pcolors "Images/B-1-M-004-crop-binary.png"]
  if file = "5" [import-pcolors "Images/B-1-M-005-crop-binary.png"]
  if file = "6" [import-pcolors "Images/B-1-M-006-crop-binary.png"]
  if file = "7" [import-pcolors "Images/B-1-M-007-crop-binary.png"]
  if file = "8" [import-pcolors "Images/B-1-M-008-crop-binary.png"]
  if file = "9" [import-pcolors "Images/B-1-M-009-crop-binary.png"]
  if file = "10" [import-pcolors "Images/B-1-M-010-crop-binary.png"]
  if file = "11" [import-pcolors "Images/B-1-M-011-crop-binary.png"]
  if file = "12" [import-pcolors "Images/B-1-M-012-crop-binary.png"]
  if file = "13" [import-pcolors "Images/B-1-O-001-crop-binary.png"]
  if file = "14" [import-pcolors "Images/B-1-O-002-crop-binary.png"]
  if file = "15" [import-pcolors "Images/B-1-O-003-crop-binary.png"]
  if file = "16" [import-pcolors "Images/B-1-O-004-crop-binary.png"]
  if file = "17" [import-pcolors "Images/B-1-O-005-crop-binary.png"]
  if file = "18" [import-pcolors "Images/B-1-O-006-crop-binary.png"]
  if file = "19" [import-pcolors "Images/B-1-O-007-crop-binary.png"]
  if file = "20" [import-pcolors "Images/B-1-O-008-crop-binary.png"]
  if file = "21" [import-pcolors "Images/B-1-O-009-crop-binary.png"]
  if file = "22" [import-pcolors "Images/B-1-O-010-crop-binary.png"]
  if file = "23" [import-pcolors "Images/B-1-O-011-crop-binary.png"]
  if file = "24" [import-pcolors "Images/B-1-O-012-crop-binary.png"]

end


to assign-patches       ; used to recolour environment and assign
pesticide attributes
  ask patches [
    if pcolor > 2.5 [
      set pcolor 63
      set pesticide-patch? false
  ]
```

```
  ]
  ask patches [
    if pcolor <= 2.5 [
      set pcolor 44
      set pesticide-patch? true
  ]
  ]
  ask patches [
    ifelse pesticide-patch?
    [ set patch-scent 0 ]
    [ set patch-scent 100 ]
  ]
end


to setup-contam     ; used to establish toxicity of pesticide
contaminated patches
                  ; ACM based on Nasreen et al (poorly reported data)
if pesticide = "ACM 0.15%" [
    ask patches [
      if pesticide-patch? = true [
        set active-sub "acetamiprid"
        set p-mortality 0.0342   ; Mortality rate from 48h study made
into per hour value.
      ]
    ]
  ]
if pesticide = "ACM 0.1%" [
    ask patches [
      if pesticide-patch? = true [
        set active-sub "acetamiprid"
        set p-mortality 0.0347
      ]
    ]
  ]
if pesticide = "ACM 0.05%" [
    ask patches [
      if pesticide-patch? = true [
        set active-sub "acetamiprid"
        set p-mortality 0.0318
      ]
    ]
  ]

end

to diffuse-scent  ; only diffuses the pesticide scent on setup meaning
diffused scent should reach over pesticide patches
    diffuse patch-scent 0.5 ; diffuses 50% of pesticide scent to 8
neighbouring patches
end

;;;;;;;;;;;;;;;;;;;;;;;;
;;; POPULATION SETUP   ;;;-----------------------------------------
----
;;;;;;;;;;;;;;;;;;;;;;;;
```

```
to setup-turtles       ; sets up initial population of larvae based
on sliding scale
  create-turtles larvae-number [
    set breed larvae-1
    set color 0
    set size 5
    setxy random-xcor random-ycor
    set heading random 360
    set pup_surv (round random-normal 74 0)  ; this and all development
times below are based on Pappas et al 2013
    set repro-rate (round random-normal 14.78 2.95)
    set infertility-chance (round random-float 100)
    ;set grow-to-l1 (round random-normal 5760 3225.6) ; all updated
for 1 tick = 1 min
    set grow-to-l2 (round random-normal 5616 5184)
    set grow-to-l3 (round random-normal 4176 3225.6)
    set grow-to-pup (round random-normal 5616 3225.6)
    set grow-to-adult (round random-normal 17424 11520)
    set be-mature (round random-normal 9936 1281.6)
    set natural-end (round random-normal 77904 16128)
    set tolerance precision random-float 100 2
    check-parameters
    if movement-track = true [
      set pen-mode "down"
    ]
  ]
end

; this is all necessary to prevent development stage values falling
significantly out of range.
to check-parameters
  ask larvae-1 [
    if grow-to-l2 < 432 [
      set grow-to-l2 (grow-to-l2 + 5184)
    ]
  ]

  ask larvae-1 [
    if grow-to-l3 < 950.4 [
      set grow-to-l3 (grow-to-l3 + 3225.6)
    ]
  ]

  ask larvae-1 [
    if grow-to-pup < 2390.4 [
      set grow-to-pup (grow-to-pup + 3225.6)
    ]
  ]

  ask larvae-1 [
    if grow-to-adult < 5904 [
      set grow-to-adult (grow-to-adult + 11520)
    ]
  ]
```

```
  ask larvae-1 [
    if be-mature < 8654.4 [
      set be-mature (be-mature + 1281.6)
    ]
  ]
  ask larvae-1 [
    if be-mature > 11217.6 [
      set be-mature (be-mature - 1281.6)
    ]
  ]
  ask larvae-1 [
    if natural-end < 61776 [
      set natural-end (natural-end + 16128)
    ]
  ]
  ask larvae-1 [
    if natural-end > 94032 [
      set natural-end (natural-end - 16128)
    ]
  ]
  ask larvae-1 [
    if repro-rate < 11.83 [
      set repro-rate (repro-rate + 2.95)
    ]
  ]
  ask larvae-1 [
    if repro-rate > 17.73 [
      set repro-rate (repro-rate - 2.95)
    ]
  ]
end


;;;;;;;;;;;;;;;;;;;;;;;;;;;;;
;;;;;;;; GO PROCEDURE ;;;;;;;;;----------------------------------------
--------
;;;;;;;;;;;;;;;;;;;;;;;;;;;;;

to go
  if not any? turtles [stop]    ; model stops when no lacewings left
  ; alternative go procedure
  ; for model output verification and for testing fecundity
  if (not any? adults) and (not any? pupae) and (not any? larvae-3)
and (not any? larvae-2) and (not any? larvae-1) [stop]   ; model stops
when all surviving lacewings have moulted to adult

  ; for testing survival to adults
  ;if turtles = adults [stop]


  ask turtles                ; so lacewings age and develop
    [ set age (age + 1) ]   ; age is in mins/ticks
  ;test-rng
  move
  expose-pesticide
```

```
  if reproduction? = true
    [ reproduce ]

  ask adults
    [ set adult-age (adult-age + 1) ]

  ask pupae
    [ grow-adult ]

  ask larvae-3
    [ grow-pupa ]

  ask larvae-2
    [ grow-l3 ]

  ask larvae-1
    [ grow-l2 ]

  ;ask eggs
   ; [ hatch-egg ]

  death
  tick
end

to test-rng
  let a 0
  let b 0
  repeat 1000000 [
    set b random-normal 5760 3225.6
    if b > 8985.6 [set a a + 1]
  ]
  print a
end
;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;
;;;;;;;; LACEWING DEVELOPMENT ;;;;;;;;------------------------------
----------------
;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;

to grow-adult
  if age > (grow-to-l2 + grow-to-l3 + grow-to-pup + grow-to-adult) [
    ifelse random 100 < pup_surv [
      set breed adults
      set color 52
      set size 10
      set adult-age 0
      set time-to-adult age
  ]
    [die]
  ]
end

to grow-pupa
  if age > (grow-to-l2 + grow-to-l3 + grow-to-pup) [
    set breed pupae
    set color 7
```

```
    set size 10
  ]

end

to grow-l3   ; note that in literature dataset all Instar 2 make it
to Instar 3; this is coded in so that others can modify survival based
on their own datasets
  if age > (grow-to-l2 + grow-to-l3) [
    set breed larvae-3
    set color 11
    set size 10
  ]

end

to grow-l2
  if age > grow-to-l2 [
    set breed larvae-2
    set color 33
    set size 7
  ]

end

to hatch-egg   ; set up is "if at this age, if number is below egg
viability rate, hatch to larvae; otherwise die.
  ask eggs [
    if age > hatch-time [
      ifelse random-float 100 < egg_via [
      set breed larvae-1
      set age 0
      set color 13
      set size 5
      set heading random 360
      ]
      [die]
  ]
  ]
end

;-------------------------------------------------------------------
---------------

to reproduce ; allows oviposition only if old enough. Egg laying rate
is based on 697.4 across entire adult lifetime (Pappas et al 2013);
done daily.
  if ticks mod 1440 = 0 [
    ask adults [
      check-fertility
      if adult-age > be-mature [
        if fertile = "true" [
          set eggs-laid (eggs-laid + repro-rate)
          show eggs-laid
          hatch repro-rate [
            set breed eggs
```

```
            set color white
            set size 5
            set age 0
            set hatch-time round random-normal 5760 3225.6
            set egg_via precision (random-normal 75.1 3.3) 2 ; sets
the egg's chance of hatching at creation (Amarasekare and Shearer,
2013)
            set eggs-laid 0
            set tox 0
            set distance-traveled 0
            set exposure-duration 0
            set adult-age 0
            set pup_surv 0
            set repro-rate 0
            set infertility-chance 0
            set grow-to-l2 0
            set grow-to-l3 0
            set grow-to-pup 0
            set grow-to-adult 0
            set be-mature 0
            set natural-end 0

            ask eggs [
              if hatch-time < 2534.4 [
               set hatch-time (hatch-time + 3225.6)
              ]
            ]
            ask eggs [
              if hatch-time > 8985.6 [
                set hatch-time (hatch-time - 3225.6)
              ]
            ]

  ]
  ]
      ]
  ]
  ]
end

to check-fertility   ; based on infertility observed by Pappas et al.,
2013 and oviposition period from Amarasekare and Shearer (2013).
  ask adults [
    ifelse infertility-chance < 90
    [ set fertile "true" ]
    [ set fertile "false" ]
  ]
end

;-----------------------------------------------------------------
---------------

to move ; does not apply to eggs or pupae.

  ask larvae-1 [
    let active? random-normal 46 9.73   ; reset every minute
```

```
    repeat 60 [   ; movement is run for every second within the minute
      if random 100 < active? [
        rt random 30.6
        lt random 30.6
        repel
        let d random-normal 3.28 1.14  ; distance changed every second
        forward d   ; they move this proportion of a patch
        let ta 1   ; counts how many seconds they move
      set distance-traveled (precision (distance-traveled + d) 2)
      set time-active time-active + ta
  ]
  ]
  ]

  ask larvae-2 [
    let active? random-normal 46 9.73   ; reset every minute
    repeat 60 [   ; movement is run for every second within the minute
      if random 100 < active? [
        rt random 30.6
        lt random 30.6
        repel
        let d random-normal 3.28 1.14   ; distance changed every
second
        forward d   ; they move this proportion of a patch
        let ta 1   ; counts how many seconds they move
      set distance-traveled (precision (distance-traveled + d) 2)
      set time-active time-active + ta
  ]
  ]
  ]

  ask larvae-3 [
    let active? random-normal 46 9.73   ; reset every minute
    repeat 60 [   ; movement is run for every second within the minute
      if random 100 < active? [
        rt random 30.6
        lt random 30.6
        repel
        let d random-normal 3.28 1.14  ; distance changed every second
        forward d   ; they move this proportion of a patch
        let ta 1   ; counts how many seconds they move
      set distance-traveled (precision (distance-traveled + d) 2)
      set time-active time-active + ta
  ]
  ]
  ]

  ask adults [
    let active? random-normal 46 9.73   ; reset every minute
    repeat 60 [   ; movement is run for every second within the minute
      if random 100 < active? [
        rt random 30.6
        lt random 30.6
        repel
        let d random-normal 3.28 1.14  ; distance changed every second
        forward d   ; they move this proportion of a patch
```

```
        let ta 1   ; counts how many seconds they move
      set distance-traveled (precision (distance-traveled + d) 2)
      set time-active time-active + ta
  ]
  ]
  ]

end

;-------------------------------------------------------------------
---------------

to repel ; attracts lacewings away from pesticide, but only within a
90 degree cone around the lacewing's heading
  if avoidance? = true [
  ask larvae-1 [
    let scent-ahead patch-scent-at-angle 0
    let scent-right patch-scent-at-angle 45
    let scent-left patch-scent-at-angle -45
    if (scent-right > scent-ahead) or (scent-left > scent-ahead)
  [ ifelse scent-right > scent-left
    [ rt 45 ]
    [ lt 45 ]
  ]
]
    ask larvae-2 [
    let scent-ahead patch-scent-at-angle 0
    let scent-right patch-scent-at-angle 45
    let scent-left patch-scent-at-angle -45
    if (scent-right > scent-ahead) or (scent-left > scent-ahead)
  [ ifelse scent-right > scent-left
    [ rt 45 ]
    [ lt 45 ]
  ]
]
    ask larvae-3 [
    let scent-ahead patch-scent-at-angle 0
    let scent-right patch-scent-at-angle 45
    let scent-left patch-scent-at-angle -45
    if (scent-right > scent-ahead) or (scent-left > scent-ahead)
  [ ifelse scent-right > scent-left
    [ rt 45 ]
    [ lt 45 ]
  ]
]
    ask adults [
    let scent-ahead patch-scent-at-angle 0
    let scent-right patch-scent-at-angle 45
    let scent-left patch-scent-at-angle -45
    if (scent-right > scent-ahead) or (scent-left > scent-ahead)
  [ ifelse scent-right > scent-left
    [ rt 45 ]
    [ lt 45 ]
  ]
]
  ]
```

```
end

to expose-pesticide
  ask turtles [
    if pesticide-patch? = true [
      set exposure-duration (exposure-duration + 1)
      set tox (precision (tox + p-mortality) 3)
  ]
  ]
end

;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;
;;;;;;;;;;;;



;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;
;;;;;;;;;;;;

to death
  ask turtles [   ; step 1: natural death by coming to end of life
    if adult-age > natural-end [
      set breed dead-adults

    ]
   ]
  if ticks mod 60 = 0 [
  ask turtles [   ; step 2: death from exposure to pesticides
    if tox > tolerance [
        set breed dead-adults
    ]
    ]
  ]
end

;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;
;;;;;;;;;;;;

to-report coverage
 report precision ((count patches with [pcolor = 44] / count patches)
* 100) 2
end

to-report patch-scale
  report ("1 mm")
end

to-report mean-exposure
  report (mean [exposure-duration] of turtles)
end

to-report exposure
  report ([exposure-duration] of dead-adults)
end

to-report mean-toxicity
```

```
  report (mean [tox] of turtles)
end

to-report toxicity
  report ([tox] of turtles)
end

to-report distance-walked
  report ([distance-traveled] of turtles)
end

to-report activity
  report ([time-active] of turtles)
end

to-report patch-scent-at-angle [angle]
  let p patch-right-and-ahead angle 1
  if p = nobody [ report 0 ]
  report [patch-scent] of p
end

to-report eggs-per-female
  report count eggs
end

to-report tol
  report ([tolerance] of adults)
end

to-report count-lacewings
  report count turtles
end

to-report adult-longevity
  report [adult-age] of dead-adults
end

to-report dev-to-adult
  report [time-to-adult] of turtles
end

to-report fertility-status
  report [fertile] of dead-adults
end
```

Appendix C – PANTA mite model code (NetLogo)

```
;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;
;;;;;;;; SETTING BREEDS FOR MODEL ;;;;;;;;--------------------------------
;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;


patches-own [
  pesticide-patch? ; determines whether patch contains pesticide
```

```
  active-sub           ; defines the active substance (e.g. acetamiprid,
dimethoate)
  p-mortality        ; used to define the hourly mortality based on 48 hr
tests. This accumulates and reduces the life of individuals
  patch-scent      ; patch "scent" to aid in avoidance of pesticide patches
]

turtles-own [       ; the following are measures that are specific to each
lacewing
  exposure-duration          ; records how long individual has been exposed
in hrs/ticks
  tox                            ; the accumulated toxicity from walking on
contaminated surfaces derived from p-mortality. Eggs and pupae residing on
exposed surfaces end up with much higher levels due to inability to avoid at
that life stage
  tolerance                 ; tolerance to exposure. Adds randomness to when
individuals succumb to exposure but should all die after 48 hours cumulative
exposure
  eggs-laid                 ; number of eggs laid by the individual
  distance-traveled          ; to record distance covered by each lacewing.
Velocity will be calculated from this
  time-active               ; to determine whether lacewing moves each tick
  age                       ; individual's age in hours
  adult-age                  ; counts how long an adult has been an adult.
Used to work out natural death
  repro-rate                ; the individual's reproduction rate, defined at
creation and based on eggs per day @ 25C (Gadino and Walton, 2012)
  grow-to-proto               ; the grow-to-X measures are defined for each
individual to allow for development processes to happen
  grow-to-deuto             ; within a range around an average time at each
stage. This set up allows the development to have some stochasticity.
  grow-to-adult
  ;surv-to-adult             ; chance of surviving to adulthood derived from
figure 1 in Gadino and Walton (2012)
  larvae-surv               ; chance of surviving larval stage
  proto-surv                ; chance of surviving protonymph stage
  deuto-surv                ; chance of surviving deutonymph stage
  be-mature                ; the age at which an adult becomes reproductively
mature. Works like the grow-to-X measures.
  natural-end              ; used for varying when individual adults' lives
end naturally. Based on adult-age
  time-to-adult   ; records time to develop to adult (for model checking)
]

breed [eggs egg]
breed [larvae larva]
breed [protonymphs protonymph]
breed [deutonymphs deutonymph]
breed [adults adult]
breed [dead-adults dead-adult]   ; used to check model operation only

eggs-own
[
  hatch-time   ; time to hatch to larvae. Temperature dependent
  egg_via      ; egg viability; probability the eggs will hatch
]



;;;;;;;;;;;;;;;;;;;;;;
;;;;;;;; SETUP ;;;;;;;;----------------------------------------------
```

```
;;;;;;;;;;;;;;;;;;;;;;

; acts as a reset and will do this when the "setup" button is pressed

to setup
  ca
  set-default-shape eggs "dot"
  set-default-shape larvae "bug"
  set-default-shape protonymphs "bug"
  set-default-shape deutonymphs "bug"
  set-default-shape adults "bug"

  setup-patches   ; to generate model landscape
  setup-turtles
  reset-ticks
end

;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;
;;;;;;;; SETUP SUBMODELS ;;;;;;;;----------------------------------------
----
;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;

;;;;;;;;;;;;;;;;;;;;;;;;;;;
;;; ENVIRONMENT SUBMODELS ;;;-------------------------------------------
;;;;;;;;;;;;;;;;;;;;;;;;;;;

to setup-patches
  setup-env        ; Allows user to use drop down to select model landscape
from a series of artificially generated, and real exposure landscapes. Real
landscapes are derived from water sensitive paper collected from field study
(Witton et al, 2018)
  assign-patches    ; Re-colours the binary images used to generate model
landscape and establishes what is pesticide exposure and what is not
  setup-contam
  diffuse-scent
end


to setup-env
; Various exposure landscapes to allow behaviour and life cycle to be studied
in different exposure landscapes.
; The files cover a range of coverage rates, but also one orchard tree, and
the off-crop exposure in the hedgerow.
  if file = "Avoidance Test 1" [import-pcolors "Images/Avoidance Test 1.png"]
; artificial arena
  if file = "Avoidance Test 2" [import-pcolors "Images/Avoidance Test 2.png"]
; artificial arena
  if  file  =  "choice  arena"  [import-pcolors  "Images/Choice  Arena.png"]
; Replicates 50% coverage lab test arena
  if file = "control" [ask patches [set pcolor 3]]  ; Acts as the control
  if file = "100% cover" [ask patches [set pcolor 1]]   ; Acts as 100% cover
control
; Real exposures
  if file = "1" [import-pcolors "Images/B-1-M-001-crop-binary.png"] ; Real
exposures from the field study
  if file = "2" [import-pcolors "Images/B-1-M-002-crop-binary.png"]
  if file = "3" [import-pcolors "Images/B-1-M-003-crop-binary.png"]
  if file = "4" [import-pcolors "Images/B-1-M-004-crop-binary.png"]
  if file = "5" [import-pcolors "Images/B-1-M-005-crop-binary.png"]
  if file = "6" [import-pcolors "Images/B-1-M-006-crop-binary.png"]
  if file = "7" [import-pcolors "Images/B-1-M-007-crop-binary.png"]
```

```
  if file = "8" [import-pcolors "Images/B-1-M-008-crop-binary.png"]
  if file = "9" [import-pcolors "Images/B-1-M-009-crop-binary.png"]
  if file = "10" [import-pcolors "Images/B-1-M-010-crop-binary.png"]
  if file = "11" [import-pcolors "Images/B-1-M-011-crop-binary.png"]
  if file = "12" [import-pcolors "Images/B-1-M-012-crop-binary.png"]
  if file = "13" [import-pcolors "Images/B-1-O-001-crop-binary.png"]
  if file = "14" [import-pcolors "Images/B-1-O-002-crop-binary.png"]
  if file = "15" [import-pcolors "Images/B-1-O-003-crop-binary.png"]
  if file = "16" [import-pcolors "Images/B-1-O-004-crop-binary.png"]
  if file = "17" [import-pcolors "Images/B-1-O-005-crop-binary.png"]
  if file = "18" [import-pcolors "Images/B-1-O-006-crop-binary.png"]
  if file = "19" [import-pcolors "Images/B-1-O-007-crop-binary.png"]
  if file = "20" [import-pcolors "Images/B-1-O-008-crop-binary.png"]
  if file = "21" [import-pcolors "Images/B-1-O-009-crop-binary.png"]
  if file = "22" [import-pcolors "Images/B-1-O-010-crop-binary.png"]
  if file = "23" [import-pcolors "Images/B-1-O-011-crop-binary.png"]
  if file = "24" [import-pcolors "Images/B-1-O-012-crop-binary.png"]


end


to assign-patches      ; used to recolour environment and assign pesticide
attributes
  ask patches [
    if pcolor > 2.5 [
      set pcolor 63
      set pesticide-patch? false
  ]
  ]
  ask patches [
    if pcolor <= 2.5 [
      set pcolor 44
      set pesticide-patch? true
  ]
  ]
  ask patches [
    ifelse pesticide-patch?
    [ set patch-scent 0 ]
    [ set patch-scent 100 ]
  ]
end


to setup-contam    ; used to establish toxicity of pesticide contaminated
patches
                   ; ACM based on EU ACM Dossier (EFSA, 2016)
if pesticide = "ACM 10.66 g Ha" [
    ask patches [
      if pesticide-patch? = true [
        set active-sub "acetamiprid"
        set p-mortality 0.00315
      ]
    ]
  ]
if pesticide = "ACM 18.66 g Ha" [
    ask patches [
      if pesticide-patch? = true [
        set active-sub "acetamiprid"
        set p-mortality 0.00513
      ]
```

```
    ]
  ]
if pesticide = "ACM 32.65 g Ha" [
    ask patches [
      if pesticide-patch? = true [
        set active-sub "acetamiprid"
        set p-mortality 0.00430
      ]
    ]
  ]

if pesticide = "ACM 57.14 g Ha" [
    ask patches [
      if pesticide-patch? = true [
        set active-sub "acetamiprid"
        set p-mortality 0.00843
      ]
    ]
  ]

if pesticide = "ACM 100 g Ha" [
    ask patches [
      if pesticide-patch? = true [
        set active-sub "acetamiprid"
        set p-mortality 0.00943
      ]
    ]
  ]
end

to diffuse-scent    ; only diffuses the pesticide scent on setup meaning
diffused scent should reach over pesticide patches
    diffuse  patch-scent  0.5  ;  diffuses  50%  of  pesticide  scent  to  8
neighbouring patches
end

;;;;;;;;;;;;;;;;;;;;;;;;;
;;; POPULATION SETUP   ;;;-----------------------------------------------
;;;;;;;;;;;;;;;;;;;;;;;;;

to setup-turtles         ; sets up initial population of larvae based on
sliding scale
  create-turtles larvae-number [
    set breed larvae
    set color 0
    set size 5
    setxy random-xcor random-ycor
    set heading random 360
    ;set larvae-surv (round random-normal 93.9 0)  ; Survival rates derived
from Gadino and Walton (2012)
    ;set proto-surv (round random-normal 93.9 0)    ; Based on observations
at 25C
    set deuto-surv (round random-normal 89.6 0)   ;
    set repro-rate (round random-normal 1.4 0.48) ; Based on the eggs per
day value. Total fecundity in the thesis chapter can be used to validate.
Might need some rethinking due to small numbers.
    set grow-to-proto (round random-normal 1440 705.6)
    set grow-to-deuto (round random-normal 3024 705.6)
    set grow-to-adult (round random-normal 3024 705.6)
    set be-mature (round random-normal 3168 3456)    ; the SD might cause
issues so maybe refine to 3168
```

```
    set natural-end (round random-normal 64800 31104)
    set tolerance precision random-float 100 2
    check-parameters
    if movement-track = true [
      set pen-mode "down"
    ]
  ]
end

; this is all necessary to prevent development stage values falling
significantly out of range.
to check-parameters
  ask larvae [
    if grow-to-proto < 3326.4 [
      set grow-to-proto (grow-to-proto + 705.6)
    ]
  ]

  ask larvae [
    if grow-to-deuto < 734.4 [
      set grow-to-deuto (grow-to-deuto + 705.6)
    ]
  ]

  ask larvae [
    if grow-to-adult < 2318.4 [
      set grow-to-adult (grow-to-adult + 705.6)
    ]
  ]

  ask larvae [
    if be-mature < 0 [
      set be-mature (be-mature + 3456)
    ]
  ]
  ask larvae [
    if be-mature > 6624 [
      set be-mature (be-mature - 3456)
    ]
  ]
  ask larvae [
    if natural-end < 33696 [
      set natural-end (natural-end + 31104)
    ]
  ]
  ask larvae [
    if natural-end > 95904 [
      set natural-end (natural-end - 31104)
    ]
  ]
  ask larvae [
    if repro-rate < 0.92 [
      set repro-rate (repro-rate + 0.48)
    ]
  ]
  ask larvae [
    if repro-rate > 1.88 [
      set repro-rate (repro-rate - 0.48)
    ]
  ]
end
```

```
;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;
;;;;;;;; GO PROCEDURE ;;;;;;;;----------------------------------------------
-
;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;

to go
  if not any? turtles [stop]    ; model stops when all mites have died
  ; alternative go procedure
  ; for model output verification and for testing fecundity
  if (not any? adults) and (not any? deutonymphs) and (not any? protonymphs)
and (not any? larvae) [stop]   ; model stops when all surviving mites have
moulted to adult

  ; for testing survival to adults
  ;if turtles = adults [stop]


  ask turtles                ; so lacewings age and develop
    [ set age (age + 1) ]   ; age is in mins/ticks
  ;test-rng
  move
  expose-pesticide

  if reproduction? = true
    [ reproduce ]

  ask adults
    [ set adult-age (adult-age + 1) ]

  ask deutonymphs
    [ grow-adult ]

  ask protonymphs
    [ grow-deuto ]

  ask larvae
    [ grow-proto ]

  ;ask eggs
   ; [ hatch-egg ]

  death
  tick
end

to test-rng
  let a 0
  let b 0
  repeat 1000000 [
    set b random-normal 5760 3225.6
    if b > 8985.6 [set a a + 1]
  ]
  print a
end

;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;
;;;;;;;; LACEWING DEVELOPMENT ;;;;;;;;-----------------------------------
---------
;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;
```

```
to grow-adult
  if age > (grow-to-proto + grow-to-deuto + grow-to-adult) [
    ifelse random 100 < deuto-surv [
      set breed adults
      set color 52
      set size 10
      set adult-age 0
      set time-to-adult age
  ]
    [die]
  ]
end

to grow-deuto
  if age > (grow-to-proto + grow-to-deuto) [

    set breed deutonymphs
    set color 7
    set size 10
  ]
end

to grow-proto
  if age > (grow-to-proto) [

    set breed protonymphs
    set color 11
    set size 10
  ]
end

to hatch-egg   ; set up is "if at this age, if number is below egg viability
rate, hatch to larvae; otherwise die.
  ask eggs [
    if age > hatch-time [
      ifelse random-float 100 < egg_via [
      set breed larvae
      set age 0
      set color 13
      set size 5
      set heading random 360
      ]
      [die]
  ]
  ]
end

;-------------------------------------------------------------------------
--------

to reproduce ; allows oviposition only if old enough. Done daily.
  if ticks mod 1440 = 0 [
    ask adults [
      if adult-age > be-mature [
          set eggs-laid (eggs-laid + repro-rate)
          show eggs-laid
          hatch repro-rate [
            set breed eggs
            set color white
            set size 5
```

```
            set age 0
            set hatch-time round random-normal 4032 705.6
            set egg_via precision (random-normal 98.1 0) 2 ; sets the egg's
chance of hatching at creation (Amarasekare and Shearer, 2013)
            set larvae-surv 0
            set proto-surv 0
            set deuto-surv 0
            set repro-rate 0
            set grow-to-proto 0
            set grow-to-deuto 0
            set grow-to-adult 0
            set be-mature 0
            set natural-end 0
            ; tolerance is inherited from mother
          set distance-traveled 0
          set time-active 0
          set exposure-duration 0

            ask eggs [
              if hatch-time < 3326.4 [
               set hatch-time (hatch-time + 705.6)
              ]
            ]
            ask eggs [
              if hatch-time > 4737.6 [
                set hatch-time (hatch-time - 705.6)
              ]
            ]

  ]
  ]
      ]
  ]

end

;-------------------------------------------------------------------------
--------

to move ; does not apply to eggs

  ask larvae [
    let active-or-not random-normal 74.6 36.3   ; reset every minute
    if active-or-not < 38.3 [ set active-or-not (active-or-not) + 36.3]
    repeat 60 [   ; movement is run for every second within the minute
      if random 100 < active-or-not [
        rt random-normal 19.15 5.07
        lt random-normal 19.15 5.07
        repel
        let d random-normal 1.166 0.71  ; distance changed every second

        forward d   ; they move this proportion of a patch
        let ta 1   ; counts how many seconds they move
      set distance-traveled (precision (distance-traveled + d) 2)
      set time-active time-active + ta
  ]
  ]
  ]
  ask protonymphs [
    let active-or-not random-normal 74.6 36.3   ; reset every minute
    if active-or-not < 38.3 [ set active-or-not (active-or-not) + 36.3]
```

```
    repeat 60 [   ; movement is run for every second within the minute
      if random 100 < active-or-not [
        rt random-normal 19.15 5.07
        lt random-normal 19.15 5.07
        repel
        let d random-normal 1.166 0.71  ; distance changed every second

        forward d   ; they move this proportion of a patch
        let ta 1   ; counts how many seconds they move
      set distance-traveled (precision (distance-traveled + d) 2)
      set time-active time-active + ta
  ]
  ]
  ]
  ask deutonymphs [
    let active-or-not random-normal 74.6 36.3   ; reset every minute
    if active-or-not < 38.3 [ set active-or-not (active-or-not) + 36.3]
    repeat 60 [   ; movement is run for every second within the minute
      if random 100 < active-or-not [
        rt random-normal 19.15 5.07
        lt random-normal 19.15 5.07
        repel
        let d random-normal 1.166 0.71  ; distance changed every second

        forward d   ; they move this proportion of a patch
        let ta 1   ; counts how many seconds they move
      set distance-traveled (precision (distance-traveled + d) 2)
      set time-active time-active + ta
  ]
  ]
  ]
  ask adults [
    let active-or-not random-normal 74.6 36.3   ; reset every minute
    if active-or-not < 38.3 [ set active-or-not (active-or-not) + 36.3]
    repeat 60 [   ; movement is run for every second within the minute
      if random 100 < active-or-not [
        rt random-normal 19.15 5.07
        lt random-normal 19.15 5.07
        repel
        let d random-normal 1.166 0.71  ; distance changed every second

        forward d   ; they move this proportion of a patch
        let ta 1   ; counts how many seconds they move
      set distance-traveled (precision (distance-traveled + d) 2)
      set time-active time-active + ta
  ]
  ]
  ]
end

;-------------------------------------------------------------------------
--------

to repel ; attracts lacewings away from pesticide, but only within a 90
degree cone around the lacewing's heading
  if avoidance? = true [
  ask larvae [
    let scent-ahead patch-scent-at-angle 0
    let scent-right patch-scent-at-angle 45
    let scent-left patch-scent-at-angle -45
    if (scent-right > scent-ahead) or (scent-left > scent-ahead)
```

```
  [ ifelse scent-right > scent-left
    [ rt 45 ]
    [ lt 45 ]
  ]
]
    ask protonymphs [
    let scent-ahead patch-scent-at-angle 0
    let scent-right patch-scent-at-angle 45
    let scent-left patch-scent-at-angle -45
    if (scent-right > scent-ahead) or (scent-left > scent-ahead)
  [ ifelse scent-right > scent-left
    [ rt 45 ]
    [ lt 45 ]
  ]
]
    ask deutonymphs [
    let scent-ahead patch-scent-at-angle 0
    let scent-right patch-scent-at-angle 45
    let scent-left patch-scent-at-angle -45
    if (scent-right > scent-ahead) or (scent-left > scent-ahead)
  [ ifelse scent-right > scent-left
    [ rt 45 ]
    [ lt 45 ]
  ]
]
    ask adults [
    let scent-ahead patch-scent-at-angle 0
    let scent-right patch-scent-at-angle 45
    let scent-left patch-scent-at-angle -45
    if (scent-right > scent-ahead) or (scent-left > scent-ahead)
  [ ifelse scent-right > scent-left
    [ rt 45 ]
    [ lt 45 ]
  ]
]
  ]
end

to expose-pesticide
  ask turtles [
    if pesticide-patch? = true [
      set exposure-duration (exposure-duration + 1)
      set tox (precision (tox + p-mortality) 3)
  ]
  ]
end

;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;
;;;;;;

to death
  ask adults [   ; step 1: natural death by coming to end of life
    if adult-age > natural-end [
      set breed dead-adults

    ]
   ]
  if ticks mod 60 = 0 [
  ask turtles [   ; step 2: death from exposure to pesticides
    if tox > tolerance [
        die
```

```
    ]
    ]
  ]
end

;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;;
;;;;;;

to-report coverage
 report precision ((count patches with [pcolor = 44] / count patches) * 100)
2
end

to-report patch-scale
  report ("1 mm")
end

to-report mean-exposure
  report (mean [exposure-duration] of turtles)
end

to-report exposure
  report ([exposure-duration] of dead-adults)
end

to-report mean-toxicity
  report (mean [tox] of turtles)
end

to-report toxicity
  report ([tox] of turtles)
end

to-report distance-walked
  report ([distance-traveled] of turtles)
end

to-report activity
  report ([time-active] of turtles)
end

to-report patch-scent-at-angle [angle]
  let p patch-right-and-ahead angle 1
  if p = nobody [ report 0 ]
  report [patch-scent] of p
end

to-report eggs-per-female
  report count eggs
end

to-report tol
  report ([tolerance] of adults)
end

to-report count-mites
  report count turtles
end

to-report adult-longevity
  report [adult-age] of dead-adults
```

```
end

to-report dev-to-adult
  report [time-to-adult] of turtles
end
```

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