

**Risks to Birds from Pesticide-treated Seed and the Possible Role of  
Ultraviolet Reflection in Seed Colour Preferences and Repellent Strategies.**

by

Graham K. Smith

A thesis submitted to the Faculty of Graduate Studies and Research in partial  
fulfillment of the requirements of the degree of Master of Sciences.

Carleton University

Ottawa, ON. Canada

**© Graham K. Smith, 2006**



Library and  
Archives Canada

Bibliothèque et  
Archives Canada

Published Heritage  
Branch

Direction du  
Patrimoine de l'édition

395 Wellington Street  
Ottawa ON K1A 0N4  
Canada

395, rue Wellington  
Ottawa ON K1A 0N4  
Canada

*Your file* *Votre référence*  
*ISBN: 978-0-494-16503-4*  
*Our file* *Notre référence*  
*ISBN: 978-0-494-16503-4*

**NOTICE:**

The author has granted a non-exclusive license allowing Library and Archives Canada to reproduce, publish, archive, preserve, conserve, communicate to the public by telecommunication or on the Internet, loan, distribute and sell theses worldwide, for commercial or non-commercial purposes, in microform, paper, electronic and/or any other formats.

The author retains copyright ownership and moral rights in this thesis. Neither the thesis nor substantial extracts from it may be printed or otherwise reproduced without the author's permission.

**AVIS:**

L'auteur a accordé une licence non exclusive permettant à la Bibliothèque et Archives Canada de reproduire, publier, archiver, sauvegarder, conserver, transmettre au public par télécommunication ou par l'Internet, prêter, distribuer et vendre des thèses partout dans le monde, à des fins commerciales ou autres, sur support microforme, papier, électronique et/ou autres formats.

L'auteur conserve la propriété du droit d'auteur et des droits moraux qui protègent cette thèse. Ni la thèse ni des extraits substantiels de celle-ci ne doivent être imprimés ou autrement reproduits sans son autorisation.

---

In compliance with the Canadian Privacy Act some supporting forms may have been removed from this thesis.

Conformément à la loi canadienne sur la protection de la vie privée, quelques formulaires secondaires ont été enlevés de cette thèse.

While these forms may be included in the document page count, their removal does not represent any loss of content from the thesis.

Bien que ces formulaires aient inclus dans la pagination, il n'y aura aucun contenu manquant.

  
**Canada**

## ABSTRACT

The first component of this project investigated the potential use of avian colour preference towards strategies of reducing bird exposure to pesticide-treated seed. Emphasis was given to incorporating the full capabilities of avian perception, namely perception of ultraviolet wavelengths. Colour preference was determined through both field and laboratory studies. A consistent preference for red seed was demonstrated throughout the study. This is a concern because red is also a popular colourant used on seed to signify the presence of seed-applied pesticides. The addition of ultraviolet reflection proved to have minimal effect on results.

The second component of this study was geared towards performing risk assessments of birds foraging on pesticide-treated seed. A field study was conducted to collect data on relevant wild bird species, which is critical to performing a more realistic risk analysis. Risk was characterized using both a deterministic, and probabilistic assessment strategy. The results of these lower-tiered analyses strongly suggested that some pesticide active ingredients used in Canada present a serious risk to birds. Based on these results, a more thorough evaluation of these chemicals is warranted.

## ACKNOWLEDGEMENTS

This research was conducted at the National Wildlife Research Centre of the Canadian Wildlife Service. Funding was supplied by the Canadian Wildlife Service and a National Science and Engineering Council (NSERC) discovery grant to Dr. P. Mineau.

Firstly, I would like to thank my supervisor Dr. Pierre Mineau, and research committee members: Drs. Céline Boutin, Tom Sherratt, and Jules Blais for contributing their knowledge and general guidance throughout this project.

I am extremely indebted to Bill Pearce, Douglas Parks, Jocelyn Souigny and other employees at the Animal Research Division of Health Canada for donating their facilities and time. Their attitudes and generosity were truly remarkable. The laboratory study may not have been possible without their help.

John Kibbee and Gao Sun of Gustafson Partnership in Guelph, ON were also extremely helpful during the preparatory phase of this project. Their expertise and assistance in the development of seed-colour treatments for this project were invaluable.

There are a number of additional people whose contribution to this project I am grateful for:

All the local farmers who permitted me to use their lands for conducting field studies. Sarah Dauncey and Clare Morrision for their assistance in both field and laboratory work. My father, for his assistance and skill towards the construction of field and laboratory apparatus, and Brian Collins, for his continual assistance on statistical matters.

## TABLE OF CONTENTS

<b>CHAPTER 1: SEED COLOUR PREFERENCE AND THE INFLUENCE OF AVIAN ULTRAVIOLET LIGHT PERCEPTION .....</b>	<b>1</b>
<b>1.1.1 STUDY OBJECTIVES.....</b>	<b>1</b>
<b>1.1.2. INTRODUCTION .....</b>	<b>2</b>
1.1.2.1 Current knowledge on UV vision and foraging .....	2
1.1.2.2 Reasons for colour avoidance by birds.....	8
1.1.2.2.1 Neophobia.....	8
1.1.2.2.2 Aposematism .....	10
1.1.2.2.3 Cryptic detection.....	12
1.1.2.3 Seed treatments.....	13
1.1.2.4 Seed treatment colouration .....	14
<b>1.2 LABORATORY STUDY .....</b>	<b>15</b>
<b>1.2.1 MATERIALS AND METHODS.....</b>	<b>15</b>
1.2.1.1 Test subjects.....	15
1.2.1.2 Housing.....	16
1.2.1.3 Diet.....	17
1.2.1.4 Seed treatments.....	17
1.2.1.5 Reflection measurements.....	19
1.2.1.6 Test setup .....	19
1.2.1.7 Habituation .....	20
1.2.1.8 Test procedure.....	21
1.2.1.9 Endpoints / Analysis .....	22
<b>1.2.2 RESULTS.....</b>	<b>23</b>
1.2.2.1 Validation of testing apparatus and food. ....	23
1.2.2.2 UV discrimination ability .....	24
1.2.2.2.1 1 <sup>st</sup> seed chosen.....	24
1.2.2.2.2 Preference direction .....	25
1.2.2.2.3 Within-visible colour.....	26
1.2.2.2.4 Effect of taste .....	26
1.2.2.2.5 Effect of day.....	27
1.2.2.3 Colour preference analysis - UV-reflection considered.....	27
1.2.2.4 Colour preference analysis – UV-reflection ignored.....	29
<b>1.2.3 DISCUSSION.....</b>	<b>30</b>
1.2.3.1 Neophobia.....	30
1.2.3.2 Colour Preference.....	31
1.2.3.3 UV Reflection .....	31
<b>1.3 FIELD STUDY.....</b>	<b>33</b>

<b>1.3.1 MATERIALS AND METHODS.....</b>	<b>33</b>
1.3.1.1 Location of sites .....	33
1.3.1.2 Test setup .....	33
1.3.1.3 Seed Treatments .....	34
1.3.1.4 Test procedure.....	35
1.3.1.5 Endpoints .....	36
<b>1.3.2 RESULTS.....</b>	<b>37</b>
1.3.2.1 Bird visitation.....	37
1.3.2.1.1 Platform stations .....	38
1.3.2.1.2 Effect of Day .....	38
1.3.2.1.3 Effect of Position.....	39
1.3.2.1.4 Colour Analysis.....	40
1.3.2.2 Consumption.....	42
1.3.2.2.1 Effect of day.....	43
1.3.2.2.2 Effect of position .....	44
1.3.2.2.3 Colour analysis .....	44
1.3.2.2.4 Additional observations .....	45
<b>1.3.3 DISCUSSION.....</b>	<b>46</b>
1.3.3.1 Colour preference .....	46
1.3.3.2 UV reflection.....	48
1.3.3.3 Neophobia.....	49
<b>1.4 SUMMARY.....</b>	<b>51</b>
<b>1.4.1 COMPARISON OF FIELD AND LABORATORY RESEARCH .....</b>	<b>51</b>
<b>1.4.2 GENERAL DISCUSSION.....</b>	<b>54</b>
1.4.2.1 The need for UV incorporation in avian studies.....	54
1.4.2.2 UV light perception by birds .....	55
1.4.2.3 UV perception and experimental design .....	57
<b>1.5 CONCLUSIONS.....</b>	<b>59</b>
<b>1.5.1 COLOUR PREFERENCE .....</b>	<b>59</b>
<b>1.5.2 IMPLICATIONS FOR SEED TREATMENTS .....</b>	<b>61</b>
<b>CHAPTER 1 TABLES .....</b>	<b>64</b>
<b>CHAPTER 1 FIGURES.....</b>	<b>68</b>
<b>CHAPTER 1 PHOTOGRAPHS .....</b>	<b>86</b>

<b>CHAPTER 2: ASSESSING BIRD EXPOSURE TO PESTICIDE-TREATED SEED</b> .....	<b>87</b>
<b>2.1 INTRODUCTION</b> .....	<b>87</b>
<b>2.1.1 SEED TREATMENTS</b> .....	<b>87</b>
<b>2.1.2 SEED TREATMENT TOXICITY</b> .....	<b>88</b>
<b>2.1.3 FACTORS AFFECTING AVIAN EXPOSURE</b> .....	<b>90</b>
2.1.3.1 Factors of seed availability .....	90
2.1.3.1.1 Environmental.....	90
2.1.3.1.2 Agricultural .....	91
2.1.3.2 Seed residue .....	92
2.1.3.3 Bird-dependent factors .....	92
2.1.3.3.1 Avoidance .....	92
2.1.3.3.2 Regurgitation .....	93
2.1.3.3.3 Feeding rate.....	93
2.1.3.3.4 Seed de-husking.....	94
2.1.3.3.5 Bird presence.....	95
2.1.3.3.6 Crop seed preference.....	95
2.1.3.3.7 Meal size .....	96
<b>2.2 OBJECTIVE</b> .....	<b>97</b>
<b>2.3 MATERIALS AND METHODS</b> .....	<b>98</b>
<b>2.3.1 FIELD STUDY</b> .....	<b>98</b>
2.3.1.1 Bait stations.....	98
2.3.1.2 Food .....	99
2.3.1.2.1 Non-test seed .....	99
2.3.1.2.2 Test seed .....	99
2.3.1.3 Test procedure.....	100
2.3.1.4 Endpoints .....	100
<b>2.3.2 DETERMINISTIC RISK ASSESSMENT</b> .....	<b>101</b>
2.3.2.1 Active ingredients.....	101
2.3.2.2 Exposure Calculations.....	102
2.3.2.2.1 Acute exposure estimation.....	102
2.3.2.2.2 Chronic exposure estimation.....	102
2.3.2.3 Toxicity endpoints .....	104
2.3.2.3.1 Acute .....	104
2.3.2.3.2 Chronic.....	104
2.3.2.4 Risk quotients .....	106
<b>2.3.3 PROBABILISTIC RISK ASSESSMENT</b> .....	<b>106</b>

2.3.3.1 Toxic endpoints.....	108
2.3.3.2 Exposure calculations.....	109
2.3.3.2.1 Active ingredient per seed.....	109
2.3.3.2.2 Seeds consumed.....	110
2.3.3.3 Probability of death calculations.....	111
<b>2.4 RESULTS.....</b>	<b>112</b>
<b>2.4.1 BIRD VISITATION .....</b>	<b>112</b>
2.4.1.1. Effect of location .....	112
2.4.1.1.1 Species diversity.....	112
2.4.1.1.2 Visit frequency .....	113
2.4.1.2 Effect of field type and margins.....	113
2.4.1.3 Effect of crop type (test seed) .....	114
2.4.1.3.1 Species diversity.....	114
2.4.1.3.2 Visit frequency .....	114
2.4.1.4 Effect of time .....	114
2.4.1.5 Seed consumption .....	115
2.4.1.5.1 Proportion with consumption.....	115
<b>2.4.2 DETERMINISTIC RISK ASSESSMENT.....</b>	<b>116</b>
2.4.2.1 Acute Dose.....	116
2.4.2.2 Chronic Dose .....	117
2.4.2.2.1 Complete diet.....	117
2.4.2.2.2 Proportion of daily food intake.....	118
<b>2.4.3 PROBABILISTIC RISK ASSESSMENT.....</b>	<b>119</b>
2.4.3.1 Meal size .....	120
2.4.3.1.1 Corn .....	120
2.4.3.1.2 Barley, wheat, and oat.....	121
2.4.3.2 Maximum feeding bout .....	122
2.4.3.2.1 Corn .....	122
2.4.3.2.2 Barley, wheat and oat.....	122
2.4.3.3 Effect of colour on risk .....	123
<b>2.5 DISCUSSION.....</b>	<b>124</b>
<b>2.5.1 FIELD STUDY.....</b>	<b>124</b>
<b>2.5.2 ACUTE ASSESSMENTS .....</b>	<b>126</b>
2.5.2.1 Deterministic assessment.....	126
2.5.2.2 Probabilistic assessment.....	126
2.5.2.3 Deterministic vs. probabilistic comparison.....	130
<b>2.5.3 CHRONIC ASSESSMENT.....</b>	<b>132</b>

<b>2.6 CONCLUSIONS.....</b>	<b>133</b>
<b>CHAPTER 2 TABLES .....</b>	<b>135</b>
<b>CHAPTER 2 FIGURES.....</b>	<b>156</b>
<b>CHAPTER 2 PHOTOGRAPHS .....</b>	<b>161</b>
<b>APPENDICES .....</b>	<b>162</b>
Appendix 1: Field Study Sites.....	162
Appendix 2: Latin names and abbreviations for birds encountered in the field studies .....	163
Appendix 3: Risk assessment data tables .....	164
Appendix 4: General Linear Models .....	166
<b>LITERATURE CITED.....</b>	<b>171</b>

## LIST OF TABLES

- Table 1.1. Percentage of each pigment component used to create final seed treatment colours. .... 64
- Table 1.2. Results of replicated G-tests conducted on the observed frequencies for each colour during the first 20 seeds selected. Separate G-tests were calculated for UV present and UV absent conditions (N=24 and 20 respectively). .... 64
- Table 1.3. Observed ranking of colour preferences for both filter types, determined from analysis of first five seeds selected for each bird. Significant differences were determined using pairwise G-test analysis and adjusted levels of significance. Significant differences are denoted by superscript letters (abc). .... 65
- Table 1.4. Summary of visits to each platform site, organized by species. \*Sparrows denotes the sum of visits by Savannah and Song sparrows. Refer to appendix 2 for latin and common names to species abbreviations. .... 65
- Table 1.5. Mean visitation occurring at each colour for all species (a), and for the six bird species comprising the majority of visits (b). Significant differences at  $p=0.05$  are indicated by superscripted letters. Two values not having the same letter are sig. Different. No letters indicates no sig. differences. .... 66
- Table 1.6. Consumption decrease occurring on colour treated seed for each day of testing, relative to Day zero (consumption on natural sunflower seed). Values represent average consumption decrease at each platform position. Each mean decrease was derived by comparing to consumption values on day zero and were specific to each platform position and site location. .... 67
- Table 1.7. Mean consumption values for each colour. Statistical significance (at  $p<.05$ ) calculated on the log-transformed consumption values are indicated by the presence/absence of similar superscripted letters (absence of similar letters denotes significance). .... 67
- Table 2.1. Active ingredients used for risk assessment. All 17 active ingredients are available for use as seed treatments in Canada. .... 135
- Table 2.2 This table summarizes the observed presence of each species on bait stations by location (a), and by seed type (b). A checkmark denotes birds were observed at the location, but does not denote seed(s) were consumed. .... 136
- Table 2.3. The number of visits with consumption (visits), maximum seed intake per visit (max), and mean seed intake per visit (mean) for all crops. Soybean was not included because no visits with consumption were recorded. Only

species that were observed foraging on at least one crop type are included .....	137
Table 2.4. The following 3 tables are a summary of calculated Acute RQs derived from comparing a theoretical ingested dose, based on maximum feeding bouts to HD <sub>5</sub> values. The absence of data denotes that the a.i. is not intended for use on that crop. RQ<0.01 = minimal risk, RQ = 0.1 – 0.99 = moderate risk, RQ>1.0 = high risk.....	138
Table 2.5. The following 3 tables are a summary of chronic RQs derived from theoretical assumption of birds foraging solely on treated seed for an extended period, compared to body weight adjusted reproductive LOEC values. ....	141
Table 2.6. The following 3 tables are a summary of chronic RQs calculated using a daily a.i. doses determined by applying a 'dilution factor' based on maximum feeding bouts, and compared to body weight adjusted LOEC values .....	144
Table 2.7. The following tables summarize the results of a probabilistic risk assessment. A non-zero P(death), at the 50th percentile is considered high risk. P(death)>0.5, 50% of the time is considered to present very high risk of poisoning. Moderate risk is defined as P(death)>0, 10% of the time (and P(death)=0, 50% of the time). A scenario missing values at both percentiles denotes minimal / negligible risk. ....	147
Table 2.8 A comparison of probabilistic risk analyses done with and without incorporating the potential exposure reduction occurring from colour treatment. This analysis represents the foraging scenarios of Blue Jays (BLJA) and Red-winged Blackbirds (RWBL) foraging on diazinon-treated corn.....	155

## LIST OF FIGURES

- Figure 1.1 Reflectance spectra for each test seed colour from 300 – 750nm. In each figure the bold line represents the UV-absorbing form. The UV-reflecting form is denoted by the solid line. a.) blue seed, b.) green seed, c.) red seed..... 68
- Figure 1.2. Transmission spectra of the two filter types. The solid line represents transmission through the ACLAR<sup>®</sup> filter (UV+ condition). The bold line represents the ACLAR<sup>®</sup> filter (UV+ condition). Seemingly erratic transmission of ACLAR<sup>®</sup> at the lowest wavelength (325nm) is noise occurring as the sensitivity limitation of the spectrometer is approached..... 69
- Figure 1.3 The absolute difference between the number of UV-reflecting, and UV-absorbing seed selected during each trial. The ACLAR<sup>®</sup> filter creates UV+ illumination (Lexan<sup>®</sup> creates UV-). Whiskers denote one standard error from the mean. .... 70
- Figure 1.4 The mean frequency of selection for each colour in UV+ conditions (ACLAR<sup>®</sup>), and UV- (Lexan<sup>®</sup>) conditions. Frequencies are based on the entire 20 seeds selected. N=24 for ACLAR<sup>®</sup>, N=20 for Lexan<sup>®</sup>. Whiskers denote one standard error from the mean. .... 71
- Figure 1.5. The average frequency for each colour during the first five (left) and last five (right) seeds under ACLAR<sup>®</sup> (a) or Lexan<sup>®</sup> filtration (b). Whiskers denote one standard error from the mean. N=24 ACLAR<sup>®</sup> for , N=20 for Lexan<sup>®</sup> ..... 72
- Figure 1.6 The total pooled frequencies for each visible colour (UV-reflecting and UV-absorbing forms combined), for the first 5 seeds selected in each trial. .73
- Figure 1.7. Numbering system used to identify positioning on platforms, relative to camera location..... 74
- Figure 1.8. the frequency of visitation from each bird species visiting platforms during the entire test period. Latin and common names for species abbreviations are given in appendix 2..... 75
- Figure 1.9 The mean number of visits occurring on each day of testing. Whiskers denote one standard error from the mean..... 76
- Figure 1.10 Mean frequency of visitation to each position, organized by platform location. Whiskers represent one standard error from the mean..... 77
- Figure 1.11 The mean frequency of visitation to each platform position during the entire test period. Whiskers denote one standard error from the mean. .... 78

Figure 1.12. Cumulative frequency of visitation to each colour for the entire study period. Visitation values were summed for the first colour in which seeds were removed for a given visit.....	79
Figure 1.14. The effect of day on mean consumption at all platform locations. Site I was excluded due to squirrel consumption. Whiskers represent one standard error from the mean.....	81
Figure 1.15. The mean consumption occurring at each position (within platform for all sites. Whiskers denote one std. error from the mean.....	82
Figure 1.16. The mean consumption occurring at each platform position, organized by location. Whiskers represent one standard error from the mean.....	83
Figure 1.17. The overall mean consumption occurring at each colour for all platforms. Whiskers represent one standard error from the mean. ....	84
Figure 1.18. Comparison of total visits to each colour on field platforms from larger species (BLJA, COGR, RBGB, RWBL)(left), and all other species (right). ....	85
Figure 2.1 The average number of visits occurring over the two hour observational period. The 120min period is broken into 20min intervals. Whiskers denote one standard error from the mean.....	156
Figure 2.2a-e. The following five figures illustrate the proportion of visits by each species with consumption (light grey / lower portion of the bar) and without consumption (dark grey / upper portion of the bar), for each crop tested....	157
Figure 2.3. Examples of actual output from the probabilistic risk assessment. 2.3a. is the output for the maximum feeding bout of Red-winged blackbirds foraging on diazinon-treated corn (very high risk). 2.3b. illustrates the predicted risk for the maximum feeding bout of Song sparrows foraging on triticonazole-treated barley (high risk).....	160

## **CHAPTER 1: SEED COLOUR PREFERENCE AND THE INFLUENCE OF AVIAN ULTRAVIOLET LIGHT PERCEPTION**

### **1.1.1 STUDY OBJECTIVES**

Knowledge of the ability of birds to see ultraviolet (UV) light has been known for over 30 years now. Following this discovery in 1972 (Huth & Burkhardt 1972; Wright 1972), numerous species were subsequently tested for their ability to perceive UV light. It is now generally accepted that most birds possess this capability (excluding nocturnal species)(Bennett & Cuthill 1994). However, only within the past decade have we finally begun to grasp how this ability is utilised for foraging. Surprisingly, the majority of research concerning colour preferences or colour-mediated behaviours still does not take this into consideration. It may be argued that this is analogous to attempting to make generalisations on colour vision in humans without using the colour red (for example). Generally, UV light has been taken into consideration only when it is the primary focus of a study.

Certain characteristics of UV light suggest that it has the potential to be of significant importance for avian foraging. The proportion of UV wavelengths relative to visible light are highest during dawn and dusk (Endler 1993), which are also peak activity times for birds. Additionally, ultraviolet-reflecting materials are relatively rare in agricultural environments (foliage and soils have varying, but low levels of reflection). Exploitation of these characteristics may be useful in

developing new, or enhancing existing methods of deterring birds from foraging on pesticide-treated seeds.

The objective of this research was to incorporate the full capabilities of avian vision into an attempt to discern colour preferences, and shed additional light on the response of birds to UV-reflection in a foraging context. A laboratory study was first conducted to assess colour preferences, including UV-reflecting colours, under controlled conditions. A colour preference experiment utilizing the complete avian visual spectrum was also conducted in the field to gain similar insights on wild bird species. This was the first study attempting to identify avian colour preferences considering UV reflection using wild birds in the field. The importance of these results and their implications to reducing bird exposure to pesticide treated seeds is also discussed.

## **1.1.2. INTRODUCTION**

### **1.1.2.1 Current knowledge on UV vision and foraging**

Despite being aware of avian UV vision since the early 1970's, its role in avian behaviour has remained relatively unexplained. Only recently have we begun to develop an understanding of the function that this ability serves for birds. The unique characteristics of UV wavelengths (high scattering, polarization) suggest they can be used for specific visually-mediated tasks. A large body of evidence has been building which suggests that birds are using UV cues for orientation,

and signalling (as in mate choice). However, the extent and manner in which UV cues are used in avian foraging still requires further investigation.

In 1982, Dietrich Burkhardt found that many fruits contained a waxy bloom that is reflective in the UV (Burkhardt 1982). Using UV photography he demonstrated that these blooms significantly increased the conspicuousness of the fruits in contrast to the UV absorbing leaves. He suggested that this reflectance could assist birds in locating them, thereby promoting more efficient seed dispersal. It was also noticed that this waxy layer could easily be rubbed off, greatly reducing the UV reflectance. This discovery would prove to be very useful in future UV foraging experiments.

The first studies to demonstrate the use of UV wavelengths by birds for foraging were published in 1995 (Viitala et al. 1995). The UV reflecting properties of urine (Desjardins et al. 1973) was shown to be utilised by Eurasian Kestrels (*Falco tinnunculus*) to detect areas of high vole (*Microtus agrestis*) density (Viitala et al. 1995). In the lab, kestrels were shown to have the ability to detect vole urine trails only when UV wavelengths were present. Field studies revealed that the kestrels spent more time hunting in areas in which vole urine trails had been artificially applied. Similar foraging techniques have since been shown in the great grey shrike (*Lanius excubitor*) (Probst et al. 2002), and the rough-legged buzzard (Koivula & Viitala 1999). Interestingly, the nocturnal Tengmalm owl (*Aegolius funereus*) was shown not to use these cues during its foraging (Koivula

et al. 1997). This is consistent with the belief that UV vision is a trait belonging to diurnal birds only.

Invertebrates constitute a very important aspect of the diet for many birds.

Church *et al.* (1998b) tested the foraging ability of blue tits (*Parus caeruleus*) on two Lepidopteran species, the winter moth (*Operophtera brumata*), and cabbage moth (*Mamestra brassicae*). Both caterpillar species were tested on their natural backgrounds, in the presence and absence of UV light. Neither prey nor background reflected greatly in the UV, however there was a greater contrast in the UV between the cabbage moth and its background. Their results showed that blue tits were consistently able to find the first prey item more quickly in the presence of UV, and these results were more pronounced for the higher contrasting cabbage moths. However, this disparity between UV-present and UV-absent conditions was not so pronounced in successive trials as birds appeared to begin using other search tactics (throughout this document UV+ and UV- will be used to denote UV-present and UV-absent illumination conditions respectively). Despite this observation, Church *et al.* concluded their results suggest UV cues are used when birds forage for cryptic prey. Later reviews of this study advise against making this conclusion (Cuthill et al. 2000). Others have expressed that this increased initial latency (for UV- conditions), could be the result of abnormal illumination conditions (Honkavaara et al. 2002).

Siitari *et al.* (1999) used redwings (*Turdus iliacus*) to test the idea that the UV reflectance of certain fruits serves to signal the ripeness of fruits to potential (UV sensitive) seed dispersers. For this study they used bilberries (*Vaccinium myrtillus*), a fruit with a removable (by rubbing off) UV reflective waxy bloom. Adult redwings showed a preference for intact bilberries (UV bloom present) over bilberries with UV bloom removed, in UV+ conditions. When UV illumination was removed (via filter), birds consumed the same amount, but no preferences were exhibited. The same experiment was conducted on naive redwing chicks, and no preferences were shown for either light condition. These results indicate that UV light has the ability to affect foraging behaviour in redwings, but these UV dependant behaviours do not appear to be innate (Honkavaara *et al.* 2002).

A very similar study was conducted by Siitari and Viitala (2002), using Black Grouse (*Tetrao tetrix*). Their experiments used two different morphs of black bilberries, one with a UV reflective bloom and another without. The grouse showed similar foraging behaviour to the adult redwings in Siitari *et al.* (1999). These tests showed strong evidence that a Galliforme species possesses UV vision, despite its shortest wavelength sensitive cone typically having a  $\lambda_{max}$  at ~420nm (Cuthill *et al.* 2000).

Although these two studies show strong evidence that UV cues are used in foraging by frugivorous birds, other studies have found evidence for the contrary. Willson and Whelan (1989) tested the effects of waxy blooms on detection and

preferences by wild birds and found no relation. Similarly, Allen and Lee (cited in Honkavaara et al. 2002) also found no link between UV reflectant waxy blooms and bird preferences. It is quite possible that the use of UV cues for foraging is both species-specific and context-dependent.

In a recent study, Church *et al.* (2001) studied the effects of UV wavelengths on frequency-dependent foraging by Zebra Finches (*Taeniopygia guttata*). White and red millet seeds were presented at 9:1, or 1:9 ratios, and foraging behaviour was monitored under several light filters. It was found that the direction of frequency-dependent preferences could be changed from apostatic (over predation of more common prey type) in UV- conditions, to anti-apostatic (over predation of more rare prey type), when UV was included. The use of filters to omit light of wavelengths corresponding to the  $\lambda_{\max}$  of the three cone receptors in the visible range had no effects on foraging behaviour. These results were somewhat of a surprise since both seed types and background reflected primarily longer wavelengths. Church *et al.* postulated that the observed effects might be the result of a change in quantal flux which only occurred in the experiment involving UV filters. However, Bennett *et al.* (1996) found no effects of brightness when testing the effects of UV wavelengths on mate choice in Zebra Finches.

It had been shown by Church *et al.* (1998a) that some caterpillars (e.g. grey shoulder knot) appearing cryptic on their natural leafy backgrounds in the visible

wavelengths, are quite conspicuous in the UV range. The suggestion was put forth that this UV conspicuousness might be advertising unpalatability to potential avian predators. Lyytinen *et al.* (2001) used this idea to test whether UV reflection functions as an aposematic cue (the use of a conspicuous colour to signal unpalatability) to Great Tits (*Parus major*). Slices of almond were glued under small sheets of paper (1.0 X 1.5cm) which had been treated with either an UV-reflecting or absorbing colour mixture. Birds were trained to forage by turning over these sheets of paper to access the almond pieces. Preys were presented to birds under UV+ present or UV- illumination on a non UV reflecting black background. From these experiments it appeared that UV reflectance did not signal unpalatability, and in some cases appeared to confer palatability. This is consistent with earlier studies on avian foraging on waxy blooms of fruits. Additionally, Lyytinen *et al.* (2001) also concluded that birds were unable to use UV reflectance in avoidance learning. However, it is quite possible that the pale colours achieved by their methods were not strong enough for this type of association to form. Previous studies have shown that colours must be highly conspicuous to serve as aposematic signals (Lindström *et al.* 1999).

With the exception of Lyytinen *et al.* (2001), who conducted several pairwise presentations of UV-reflecting and UV-absorbing prey of a given human visible colour (hereafter referred to simply as colour), no other studies have attempted to explore colour preferences using UV-reflecting prey.

Since avian vision is so highly developed, it is not surprising that colour has the potential to play such a large role in so many aspects of avian behaviour. Colour can convey a great deal of information to birds, and this information can be exploited in attempts to minimise their exposure to pesticide-treated seeds. The colour of food may cause birds to avoid it because it looks unusual or it may indicate that certain foods are dangerous/unpalatable. Conversely, the colour of potential prey may serve to enhance its camouflage rendering it cryptic and therefore less likely to be eaten.

### **1.1.2.2 Reasons for colour avoidance by birds**

#### **1.1.2.2.1 Neophobia**

The avoidance of novel (unfamiliar) stimuli (e.g. sights, sounds, flavours, smells, etc.) is referred to as neophobia (Greig-Smith 1987a). Neophobic responses present a source of confounding variables and can result in misleading interpretation in research on bird repellency. Some of the results presented in a study by Greig-Smith and Rowney (1987) are evidence of this occurrence. Birds were given food with a blue dye and a chemical causing unpleasant sensations (e.g. methiocarb, tannic acid). It was expected the birds would make an association of the colour and unpleasantness and therefore continue to avoid the colour upon removal of the aversive chemical. This is in fact what was observed, however the control group which had never received an aversive chemical (only the colour) continued to display avoidance of the dyed-blue food for the same

time period. This potentially neophobic response in the control birds prevented Greig-Smith and Rowney from being able to attribute the observed avoidance to learnt aversion of a colour.

The avoidance of novel flavours and colours may be relevant in preventing agricultural damage by birds. However Reidinger and Mason (1983) state that exploiting neophobia is unreliable as a deterrent mechanism, due to observations that birds can habituate rapidly to novel substances. However, a field experiment by Marples *et al.* (1998) using Blackbirds (*Turdus merula*) and Robins (*Erithacus rubecula*) revealed some birds do avoid food which was novel in colour alone, for months at a time. Once sampled, these birds tended to consume the novel prey in subsequent encounters.

If neophobic responses to unnatural colours have the potential to cause avoidance of otherwise palatable food for periods of more than a month then it may be possible to use this behaviour for crop protection from birds. Studies have revealed UV reflecting foods are not all that uncommon to frugivorous birds (e.g. the UV-reflection of many berry types Burkhardt 1982). However, this type of reflection may not be as prevalent in the environment or on food sources for granivorous birds. If this is in fact the case, it may be reasonable to expect that such a food would result in strong neophobic avoidance.

#### **1.1.2.2.2 Aposematism**

Aposematic signals (usually referring to colouration) are those which serve to warn the unpleasant properties of prey to potential predators (Sillen-Tullberg 1983; Campbell & Lack 1985). The use of various sensory cues to indicate unpleasant properties of repellents to birds (discussed earlier), are in fact acting as aposematic signals. Most studies have shown that the conspicuousness of the aposematic prey is directly related to the effectiveness of the warning signal. Gittleman and Harvey (1980) presented unpalatable prey to chicks on either cryptic or conspicuous backgrounds. Their results revealed that chicks learned to avoid conspicuous unpalatable food more readily. A similar experiment in 2001 with young chicks demonstrated that the degree of contrast (conspicuousness) between prey colouration and background is proportional to the strength of the avoidance response (Gamberale-Stille 2001). It is possible that the degree of contrast against the presentation background resulted in a more intense warning signal and consequently the creation of a strengthened avoidance response. There is also evidence that the novelty of prey colouration can aid in the development of associations to unpalatability (Schlenoff 1984).

The low level of UV reflection of most soil substrates would likely cause food with this property to appear very conspicuous in the field. In conjunction with the idea that UV-reflecting granivorous foods are quite rare, it seems likely that such a food would have great potential to serve as an aposematic signal when used in combination with an unpleasant agent.

A study conducted by Lyytinen *et al.* (2001) attempted to assess the ability of UV reflection to serve as an aposematic signal. From their results, they concluded that UV reflection does not have the potential to serve as an aposematic signal to birds, and that UV seems to, in fact, indicate palatability. It is possible that , some of the methods used in this study had a confounding influence on the results. Firstly, birds (Great tits) were trained to forage by overturning pieces of paper to unveil almond slices. The paper was treated with colour and a UV reflectant or absorbant on the upper side. This unnatural presentation may have reduced the selectivity of the birds' foraging. Secondly, the choice of colours by the researchers may not have been suitable for the tests conducted. It is believed that certain colours may be more effective than others as warning colours (Harvey & Paxton 1981). Lyytinen *et al.* chose not to test the colour red, knowing that it has the potential to serve as a warning colour. On the other hand green was chosen to be tested in accordance with UV, on the basis that "in nature green is usually an indicator that something is edible". The authors may have been somewhat naive with this generalisation. In relation to many fruits, green often indicates that fruit is unripe, and therefore not suitable for consumption. Additionally, in the field study on neophobia by Marples *et al.* (1998) green food was avoided more than any other colour tested. A study on coloured grain preferences by Brunner and Coman (Brunner & Coman 1983) also observed an avoidance of green (and blue) seeds (more than black, yellow, and red seeds). There is also the distinct possibility that the intensity of the

stimuli (UV reflection and visible colour intensity) was not strong enough to elicit aposematic signalling (see above).

#### **1.1.2.2.3 Cryptic detection**

Although not a true form of avoidance, reducing the possibility of detection through cryptic colouration can produce the same effects from a prey's perspective. Many plant species use this strategy to prevent their seeds from being preyed upon. That is, they produce seeds that are cryptic on the surrounding substrate (usually dull yellows and browns). This is likely the result of selective pressure from generations of predation by post-dispersal seed predators (Nystrand & Granström 1997). Nystrand and Granström (1997) examined the rate of foraging by finches (*Fringilla coelebs* and *F. montifringilla*) on four natural forest substrates that differed in overall colour. Their results showed a higher level of foraging according to increased disparity between seed and substrate colours (dark seeds consumed more on pale surfaces, and vice versa). Furthermore, a study by Getty and Pulliam (1993) on white-throated sparrows (*Zonotrichia albicollis*) foraging for seeds on various substrates demonstrated that sparrows forage according to areas of greater food detectability, not necessarily where they are the most abundant.

It may be possible that by making seeds more cryptic, the level of foraging on pesticide-treated seeds could be reduced. Attempts to do so must take into account the fact that birds are able to see UV as well, a concept that has often

been ignored in many avian foraging experiments. Therefore a bird-cryptic seed must have levels of UV reflection that corresponds to reflection of the intended substrate.

Attempts to monitor the effectiveness of this strategy can be misleading at times due to formation of what is often loosely referred to as a 'search image'. For example, a study was conducted on chicks by Dawkins (1971) examining rates of foraging on cryptic and conspicuous grains. Chicks often appeared unable to detect cryptic grains at first, but this ability eventually developed, seemingly the result of a central change in the birds' perception. This type of perceptual change seems to be an explanation for the study discussed earlier (Church et al. 1998b) on the use of UV cues when foraging for cryptic prey. In this experiment blue tits (*Parus caeruleus*) seemed to increase their ability to detect prey in successive trials in the absence of UV cues (cryptic prey situation). The authors suggest that among other possibilities, this change in ability may be explained by a change in search tactics (e.g. formation of a search image).

### **1.1.2.3 Seed treatments**

The pre-sowing application of various chemicals to seeds is a favoured method of pesticide application. Historically, avian exposure to these chemicals via the consumption of treated seed has been responsible for numerous incidents of avian mortality. Consequently, the development of methods to prevent birds from foraging on these toxic seeds has been the focus of much research. Past

studies have explored visual, auditory, pyrotechnic, tactile, chemosensory, physiologic, or physical methods of repelling birds (for references see Mason & Clark 1992).

The responses of birds to various colours have been exploited in efforts to develop new bird repellents. Some of the more promising strategies have involved the addition of aversive chemicals to seed, with hopes of preventing consumption or developing an association to the treated seed forcing birds to forage elsewhere. Methiocarb has been one chemical which has received attention in the past for use in this context. Simply adding a colour to methiocarb treated seed, Nelms and Avery (1997) were able to maintain the same level of avoidance while decreasing application rates by 75%. This finding has implications for alleviating some of the costs associated with chemical repellent applications (costs often prove to be prohibitive for many promising repellent strategies). The benefits of sensory cue addition were also demonstrated by Greig-smith and Rowney (1987). Their experiments also demonstrated that birds can develop aversions to repellent-treated seed more readily when it is paired with a colour.

#### **1.1.2.4 Seed treatment colouration**

Currently, the Federal Insecticide, Fungicide and Rodenticide Act (FIFRA) in the United States, and the Seeds Act (SA) in Canada require all seed treated with a pesticide to be dyed or stained to indicate that they are unsuitable for non-

planting purposes. It is the responsibility of seed treatment manufacturers to add the appropriate colourant when pesticides are applied to the seed. Typically canola / rapeseed are dyed blue, while corn and other cereals are given red colouration. These colours were selected because they are generally unnatural, and render the seeds conspicuous in comparison to natural seed.

This study is primarily focused on identifying colour preferences of birds to various colours. Of particular interest is the response of birds to UV reflecting food. The search to find new, or enhance existing means of repelling birds from pesticide-treated seed continues to be the focus of many researchers. The use of colours for this purpose has been researched in the past, but the discovery of UV perception gives cause to revisit this prospect. Although the response of birds to various colours did not weigh heavily when deciding current pesticide-treated seed colouration, the discovery of a highly avoided colour would be difficult to ignore. Unfortunately, the role of crypsis and aposematic avoidance will not be addressed in this study. However, these areas should be thoroughly revisited with consideration of the complete avian visual spectrum.

## **1.2 LABORATORY STUDY**

### **1.2.1 MATERIALS AND METHODS**

#### **1.2.1.1 Test subjects**

All aspects of husbandry and experimental procedures were reviewed and approved by the Health Canada, Carleton University, and Canadian Wildlife

Service (Ontario region) animal care committees prior to commencement. Zebra Finches (*Taeniopygia guttata*) were used as the test species for all experiments. Birds were purchased directly from a breeder in Montreal, QC with the assistance of a local pet store ("Critter Jungle", Ottawa, ON). The average age of birds when received was estimated to be approximately 3-5 weeks, based on communication with the breeder and physical characteristics. Birds were housed and tested at the Animal Resource Division facilities of Health Canada, located in Tunney's Pasture, Ottawa, ON. To assist with identification, birds were immediately fitted with numbered plastic leg bands upon their arrival at the Health Canada facilities. The entire holding period (including habituation and testing) occurred from August to October, 2005. At the conclusion of experiments, birds were returned to the breeder.

#### **1.2.1.2 Housing**

Zebra Finches were housed and tested in a ~3.0 x ~6.0m room under a 14:10 light: dark photoperiod, illuminated by four paired banks of four foot cool-white fluorescent lamps (non UV-emitting). Birds were housed in cages measuring 60 by 30 by 45cm (w x l x h). The number of birds in each cage depended on their involvement in ongoing tests and/or habituation. When involved in testing, birds were housed in pairs, otherwise the number of birds per cage ranged from two to five. Cages were organized to minimize stress and aggression. Handling was kept to an absolute minimum to further reduce stress. Whenever possible,

efforts were made to avoid mixed-gender cages. Each cage was equipped with several perching dowels, swing(s), and artificial vegetation.

#### **1.2.1.3 Diet**

Birds were provided a mineral enriched commercial finch mixture (Hagen®) via side-mounted hoppers. Water was provided in shallow water baths on the cage floor (dually serving as drinking water and for bathing). Water baths were rinsed and water replenished daily. Cuttlebone, and oyster shell grit were provided *ad libitum*. A vitamin and mineral supplement, Prime®, was mixed with the regular diet twice per week to ensure birds were successfully achieving nutritional requirements.

#### **1.2.1.4 Seed treatments**

Previous exploratory experiments determined that Browntop millet (*Urochloa ramosa*) was a favoured food type by Zebra Finches, and was used as the seed type for these tests. Seed treatments were created with the assistance of Gustafson Partnership, a commercial seed treatment manufacturer, at their laboratory facilities (Guelph, ON). The objective of the seed treatments was to develop seeds that differed only in the UV range for several colours (therefore indistinguishable by the human eye). Additionally, the chemicals used had to be non-toxic, and UV-reflecting treatments had to reflect at levels adequate for avian perception as determined by previous experiments. For this experiment, UV-

reflecting, and UV-absorbing treatments of red, blue and green were created (UV-reflecting seeds of a specific colour will hereon be referred to as 'colour'+UV (e.g. red+UV), UV-absorbing seeds will simply be referred to by their colour in the human-visible spectrum (e.g. red)). Solfort<sup>®</sup> coloured pigments (green FG, red 2B, and blue FG) used for commercial seed treatment colouration, were provided by Gustafson and used to create base colours for each treatment. Calcium carbonate ( $\text{CaCO}_3$ ) and titanium dioxide ( $\text{TiO}_2$ ) were added to manipulate the UV-reflecting and UV-absorbing properties respectively. The acute oral, rat  $\text{LD}_{50}$  for  $\text{CaCO}_3$  is 6450 mg/kg and >7500 mg/kg for  $\text{TiO}_2$  (avian  $\text{LD}_{50}$ s could not be found for either of these compounds in publication). It should be noted that  $\text{CaCO}_3$  is used as human food additives and  $\text{TiO}_2$  as a whitening agent in toothpaste; also,  $\text{CaCO}_3$  is supplied to pet birds as an additive in dietary seed mixtures, oyster shells (for grit) and cuttlebone.

Final seed treatments were created using the compounds and amounts described in table 1.1. The variation in the amounts of pigments used for each treatment is a result of varying 'hiding powers' of the pigments used, a consequence of particle size and refractive index of each compound. The differential hiding power of  $\text{CaCO}_3$  and  $\text{TiO}_2$  required seed treatment application rates to be adjusted accordingly. All UV-absorbing ( $\text{TiO}_2$ ) treatments were applied at 1.5g treatment powder / 100g seed. UV-reflecting ( $\text{CaCO}_3$ ) treatments were applied at 3.0g/100g. Seeds were pre-weighed in a ~500ml glass jar, and the appropriate amount of treatment powder was added. The mixture was

shaken vigorously for 5min, or until it appeared all powder had adhered uniformly to the seed.

#### **1.2.1.5 Reflection measurements**

Colour treatments were verified after application to seed using an Ocean Optics™ USB2000 spectrometer configured for use in the visible and near-UV (referred to in this document as UV, referring to 300-400nm wavelengths) waveband, with OOIBase32 operating software. Illumination was provided using a pulsed xenon (PX-2) light source, and samples were measured via a 600µm reflection probe. The probe was mounted from below at a 45° sample interface 'looking' through a UV-transparent quartz slide. All UV-reflecting treatments produced a reflection of at least 40% at the 360nm wavelength (within the range of maximal UV sensitivity for Zebra Finches, (Bowmaker et al. 1997). See figure1.1 for reflection spectra of test seed.

#### **1.2.1.6 Test setup**

To minimize handling and stress to the birds, tests were conducted without removal from their regular holding cages. The entire cage was moved to the testing area. A cage divider was inserted to physically separate the bird being tested from its cage mate. In order to reduce stress and provide a better foraging environment, the test bird and companion bird were able to maintain visual contact (through the divider) at all times. Illumination was via a 1000W metal

halide (Hortilux™) lamp suspended above the cage, providing the most similar relative solar spectrum compared to solar irradiance (including UV wavelengths)(see photo 1.1).

Two light-filters were alternately placed over the cage to manipulate the light environment during tests. To eliminate wavelengths below 400nm (UV), a ~3mm thick, 0.45 x 0.60m Lexan® sheet was used. Lexan® is manufactured by General Electric primarily as an impact-resistant substitute to glass. In addition to absorbing wavelengths below 400nm, Lexan® absorbs a small proportion of wavelengths in the visible spectrum. As a result, removal of the Lexan® filter may affect sensory contribution of visible wavelengths to colour perception (intensity of visible light decreased). To compensate for this, a second filter was constructed consisting of three layers of ACLAR® polyfluorine film (each 38µm thick; type 22 A, Honeywell Inc.). ACLAR® is a transparent film manufactured for use in moisture sensitive packaging (e.g. pharmaceuticals). A unique property of this film is that it transmits light evenly throughout both the visible and UV wavebands. The ACLAR® filter was substituted for the Lexan® filter to maintain a consistent light intensity in the visible range, in both UV+ and UV- conditions (figure 1.2).

#### **1.2.1.7 Habituation**

To alleviate bird stress during testing, birds were gradually introduced to the test setup in a step-wise manner prior to any involvement in experiments. There

were three steps involved in the habituation process: 1) test light only, 2) test light + divider + filter (alternating each day), 3) full test setup (test light + divider + video camera). During all steps, birds were presented with untreated test seed on the regular testing grid (described in 1.2.1.8). Birds were given up to 10 minutes to consume at least 20 seeds each day. Once both birds demonstrated they were habituated to their surroundings (indicated by consuming >20 seeds on consecutive days), they were introduced to the next step. All food was removed from the cages at the onset of light (before birds have an opportunity to forage) on days when birds were being habituated to increase hunger when attempting to habituate. Water was also removed during habituation trials (because of obstruction with presentation of seeds on the cage floor).

#### **1.2.1.8 Test procedure**

As in habituation, all trials were conducted shortly after the onset of light. Food and water were removed before birds had an opportunity to forage to ensure adequate hunger when being tested. Seeds were presented on a 96-square grid with a black foam background to eliminate background contrast as a confounding factor to seed selection. For each trial, an equal number of seeds (16 each) of each color (UV-reflecting and UV-absorbing forms of blue, green, and red) were arranged on the grid according to a randomly generated pattern, determined using Microsoft Excel. Each grid was placed into a tray and slid into the cage from below, substituting the regular litter tray. Trials were halted once the test bird had consumed >20 seeds, or until 10 minutes had elapsed. During trials, the

bird not being tested (companion bird) simultaneously foraged on natural browntop millet on the cage floor to promote a more natural foraging situation. All trials were video recorded using a digital camcorder, viewing the test grid through a tinted window mounted on the side of the cage.

#### **1.2.1.9 Endpoints / Analysis**

Video recordings of trials were reviewed, and various endpoints observed. The seed color, order of selection and location of each individual seed were recorded and used for analysis. Statistical analyses were carried out on observed frequencies using Chi-square tests and various G-tests. A replicated G-test was used to compare frequencies of selection between colours when all 20 seeds are considered (Sokal & Rohlf 1994). This test allows for the identification of deviations from uniform foraging within birds ( $G_{TOTAL}$ ), and the degree to which deviations are correlated between birds ( $G_{HETEROGENEITY}$ ), in addition to assessing whether overall frequencies on colours differ from a uniform distribution ( $G_{POOLED}$ ). Replicated G-tests were not possible when considering only the first five seeds selected because expected frequencies for each colour and bird were less than one (expected frequencies of at least five are recommended for replicated G-tests). In this scenario only  $G_{POOLED}$  was calculated (on the summed frequencies for each colour). Comparisons between colours were performed using results of  $G_{POOLED}$  tests and comparing to Bonferroni-adjusted significance levels. We acknowledge the frequencies of selection for various colours (for each bird) are not entirely independent, as is assumed by these

statistical methods. However, it was felt that these analyses remained the most suitable for these data.

## **1.2.2 RESULTS**

All trials in which birds did not consume at least 20 seeds were omitted from analysis. This cut-off was selected based on observations that birds would rarely consume more than 30 seeds, and a hunger-motivated bird consuming less than 20 seeds could likely be an indication that birds were experiencing stress. Of the 29 trials conducted under the ACLAR<sup>®</sup> filter, five failed to achieve the 20 seed criterion. Under Lexan<sup>®</sup> filters, 20 of 29 reached criterion and were used in the analysis.

### **1.2.2.1 Validation of testing apparatus and food.**

Because these experiments are dependent on the visual, and gustatory sensory perception of the birds, it is important they were conducted in a manner that allowed for confirmation of successful manipulation of their sensory environment. This is the primary reason for the use of filters altering the light environment during each test. The pigments used for the colour treatment of test seeds were created to prevent possible discrimination between UV-reflecting and UV-absorbing seeds of a given colour in UV- conditions. This was verified by first comparing the two forms of each colour visually, then comparing the similarity of their seed reflectance spectra in the visible waveband.

Visual comparison of either form of a given colour is useful for confirming colour similarity only to a certain degree. Because colour is a property determined by the sensory system, our visual perception should not be used to make final conclusions about colour similarity, as seen by birds. Measurements of reflection spectra provided the ability to compare the similarity of the information (percent wavelength reflection) which will be used for colour perception by the birds. Additionally, these measurements allowed us to verify the presence and magnitude of the differences between UV-reflecting UV-absorbing seed within the UV range. To ensure these differences were perceivable by Zebra Finches under our testing illumination, several experiments were performed.

### **1.2.2.2 UV discrimination ability**

#### **1.2.2.2.1 1<sup>st</sup> seed chosen**

When considering only the first seed chosen in each trial, birds appeared to be unable to discriminate between the two seed types when the ACLAR<sup>®</sup> filter was applied. Under these conditions, UV-reflecting and UV-absorbing seeds were both selected first on ten occasions, during the 20 successful trials. Although more UV-absorbing seeds were selected as the first seed under Lexan<sup>®</sup> filtration (UV- illumination), this difference was not significant ( $\chi^2 = 0.67$ ,  $df=1$ ,  $p = 0.414$ ). Of the 24 successful trials conducted under ACLAR<sup>®</sup> filtration, the first seed selected was UV-reflecting on 10 occasions (UV-absorbing was chosen first on 14 occasions).

The lack of a significant difference between the two seed types, does not necessarily denote a perceptual inability to discriminate between them. It is very possible that the reason for the insignificant difference is driven by an equal, or lack of preference for all seed colours being tested.

#### **1.2.2.2 Preference direction**

For the purpose of assessing the ability of birds to perceive differences in the UV reflectance of our colour-treated seeds, the direction of the preference is not important (i.e. whether birds preferred or avoided UV reflection). We were concerned only with the removal of any preference which is dependent on UV reflectance (through the use of a Lexan<sup>®</sup> filter). This was analyzed by calculating the absolute difference between the number of UV-reflecting and absorbing seeds chosen for each bird (for the entire 20 selections), and comparing the mean value of all birds for each filter type. The mean difference between the number of UV-reflecting and UV-absorbing seed selected, slightly increased when UV wavelengths were removed, however this increase was not significant (mean of 3.11 to 3.78, paired t:  $p=0.483$ ) (figure 1.3). This seems to suggest birds were able to discriminate just as easily between seed types within our UV-conditions, as when UV wavelengths were present. However, the absence of a significant difference under UV+ conditions makes this type of comparison difficult. The use of the Lexan<sup>®</sup> filter eliminates the UV-wavelengths and *should*

have rendered discrimination between UV-reflecting and UV-absorbing forms impossible.

#### **1.2.2.2.3 Within-visible colour**

Researchers have not yet achieved a consensus on the method by which birds perceive UV wavelengths. Research on avian vision has found evidence for birds perceiving UV light achromatically, however there is also a large body of evidence which supports chromatic UV perception (contributing to colour vision). Analysing the differences in the frequency of selection of UV-reflecting versus UV-absorbing seed consumed is more likely to differ between filter types if UV is achromatically perceived. Alternatively, if UV light is being used for colour vision, analysing the difference between either form of each colour is more applicable. This analysis revealed that the presence of UV wavelengths had no effect on differences between forms of each colour. The mean difference between the colours was 2.00 (seeds) for both filters (n=72 for ACLAR<sup>®</sup>, n=60 for Lexan).

#### **1.2.2.2.4 Effect of taste**

It was also a possibility that bird preferences may change through the course of the trial. This can be a result of several factors. Of special concern, was the possibility that birds might begin changing their preference due to the taste of the different chemicals used in the seed treatments (CaCO<sub>3</sub> and TiO<sub>2</sub>). If this were occurring, a shift in preference towards or away from a particular seed type

would likely be evident. The number of seeds consumed for each type (UV-reflecting and –absorbing) as the trial progressed (from 1<sup>st</sup> seed to 20<sup>th</sup>) did not change for either filter type. A Chi-square comparison of the frequency of UV-reflecting seed chosen as first seed against the frequency selected as the 20<sup>th</sup> seed was non significant:  $\chi^2 = 0.4$ ,  $p=0.527$ ).

#### **1.2.2.2.5 Effect of day**

Each bird was tested once with each filter type. The sequence in which each bird was exposed to either filter (ACLAR<sup>®</sup> or Lexan<sup>®</sup> first) was assigned randomly. The possibility that experience acquired during the first trial may have affected performance during the second trial was considered.

A comparison was done by analysing the change in seed selection frequencies on the basis of UV reflection. The mean difference between the number of UV-reflecting and UV-absorbing seed for all birds decreased from 4.00 to 3.18 ( $n=17$ ) from day 1 to day 2. This difference was not significant  $p=0.409$  (paired t test). This selectivity decrease is maintained when the data are partitioned on the basis of filter type.

#### **1.2.2.3 Colour preference analysis - UV-reflection considered**

Figure 1.4 illustrates the apparent unselective foraging which results when the entire trial of 20 seeds is considered for all birds. The results of a  $G_{\text{POOLED}}$  test

conducted on the summed frequency for all birds confirms that observed frequency did not differ from an even distribution in either UV+ or UV- conditions (table 1.2). For both illumination conditions,  $G_{TOTAL}$  and  $G_{HETEROGENEITY}$  were highly significant, indicating that birds were often preferring certain colours over others (indicated by significant  $G_{TOTAL}$ ), but these preferences were inconsistent between birds (indicated by significant  $G_{HETEROGENEITY}$ ).

Although birds did not shift their preferences on the basis of UV-reflection as the trial progressed, plotting the selection frequency for each colour for the first five, and last five seeds under ACLAR<sup>®</sup> filtration reveals an apparent change in selectivity (figure 1.5a and b). G-test analysis (non-replicated due to expected frequencies  $\ll 5$  for each colour) on the summed frequencies of these colours confirms that selectivity decreased substantially with progression of the trials. There is a highly significant deviation from uniform (random) selection of colours during the first five seeds selected ( $G=28.681$ ,  $p<0.001$ ), contrasting with a non significant deviation observed during the last five seeds selected ( $G=2.372$ ,  $p=0.796$ ). The same analysis on the first five seeds under the Lexan<sup>®</sup> filter had marginally significant results ( $G=10.473$ ,  $p=0.063$ ). For some birds the observed loss in selectivity may be in part due to depletion of favoured seed colours. This would likely only be a factor for birds displaying very strong preferences because 16 seeds of each colour were available on the testing grid (no bird consumed all seeds of a given colour during a trial). Due to this finding, only data from the first five seeds selected during each trial were used to infer on colour preferences.

On the basis of our observed frequencies, using the ACLAR<sup>®</sup> filter, the preference ranking was: red+uv > red > green+uv > green > blue > blue+uv. A similar ranking was achieved when the Lexan<sup>®</sup> filter was applied: red+uv > red > blue+uv > green+uv > green > blue (see Table 1.3 for the presence of significance). The only notable change when the UV wavelengths were removed, is an increased selection for blue+uv seeds.

#### **1.2.2.4 Colour preference analysis – UV-reflection ignored**

Given that Lexan<sup>®</sup> was used to provide UV- conditions, it is more appropriate to pool frequencies for both forms of each colour when considering results of these trials. This grouping results in a marked preference for red seed. The other two colours, green and blue are equally favoured (but less than red). When frequencies observed under ACLAR<sup>®</sup> filtration are also pooled, the preferred colour is again red, followed by green, then blue (figure 1.6).

In UV- conditions, G-test results indicate the three pooled colours deviate significantly from random foraging ( $G = 7.999$ ,  $p=0.018$ ). A comparison between colours using Bonferroni adjusted levels of significance for calculated G-test values shows overall selection for red to be significantly greater than blue and green ( $p=0.019$  and  $0.013$  respectively). Under UV+ conditions, the pooled colours again deviate highly from expected values if foraging randomly ( $G=24.762$ ,  $p<0.001$ ). Comparisons of the frequencies for three pooled colours

indicates that blue was selected significantly less than both green and red ( $p=0.002$  and  $<0.001$  respectively)(using Bonferroni adjusted levels of significance and G-test values).

### **1.2.3 DISCUSSION**

Our results suggest that UV reflection plays little role in the granivorous colour preferences of captive Zebra Finches. Of the three colour pairs, only UV reflecting blue differed significantly from its non-reflecting counterpart (UV-absorbing blue). The minimal presence of UV-dependent differences made it difficult to derive additional information from using the Lexan<sup>®</sup> filter (UV-illumination). However, removal of UV light was successful in eliminating the significant difference that existed in the one circumstance where UV-dependent preference was suggested (blue and blue+UV).

#### **1.2.3.1 Neophobia**

Neophobic avoidance of the colour treated seeds was not prevalent in this study. Several birds did not consume any seed during their test, which *could* have been a neophobic response. However, these birds were generally displaying signs of stress during the trials, and it was assumed this was the reason for their poor performance. An argument could be made that the documented colour preferences are actually differential colour-mediated neophobic responses. This notion is supported by the rapid deterioration of preferences once alternative seed types were sampled. True colour preferences should not wane so quickly,

as we observed. A more accurate assessment of colour preference would involve repeated long term trials to cause the confounding effects of neophobia to become irrelevant. Unfortunately, this was not possible given the limitations of our set-up and facilities.

### **1.2.3.2 Colour Preference**

Birds did not display an initial avoidance for seed on the basis of UV reflection. This is consistent with the results of a sequential pairwise preference experiment done by Lyytinen et al (2001). When considering the different colours, analysis of frequencies of the first seed selected revealed that under UV+conditions, no birds consumed blue+UV as their first seed, which could be evidence for an innate avoidance of this colour. Any preference / avoidance exhibited in the early stages of each trial were generally short-lived. Through the course of each trial, birds became increasingly less selective. These captive birds were very willing to sample the various prey types, and the absence of negative consequences (e.g. bad taste) appeared to promote further sampling. Although capable of determining the absence of avoidance, the short duration of these experiments does not allow for inferences on the presence of avoidance.

### **1.2.3.3 UV Reflection**

Although the removal of UV light had only little effect on the ranking of colour preferences, it appeared to influence the strength of those preferences. This is

supported by the finding that the highly significant difference among summed frequencies (for all birds) under UV-present conditions did not persist once UV wavelengths were removed.

Furthermore, the presence of UV wavelengths may have assisted birds in discriminating amongst the colours. When UV was present, there was a significant difference in preference for the three pooled colours: red > green > blue (pooled refers to addition of both forms of a given visible colour). Removal of UV wavelengths resulted in an equalization in preference between blue and green (red remained strongly preferred). Because the addition of CaCO<sub>3</sub> and TiO<sub>2</sub> resulted in pale visible colours, blue and green were not as obviously distinguishable as red for the test seed (see spectra, fig. 1.1). It appears that UV wavelengths might have been helpful in distinguishing amongst colours in the visible spectrum.

## **1.3 FIELD STUDY**

### **1.3.1 MATERIALS AND METHODS**

#### **1.3.1.1 Location of sites**

Research was conducted using various study sites for a four month period extending from May through August, 2005. Study sites were located on the field margins of agricultural sites near the towns of Barrhaven and Carp, ON (a list of field sites is available in appendix 1). Agricultural lands were used for this study because it was conducted in succession with another experiment for which these landscapes were a requirement. Furthermore, conducting this study on agricultural lands made this data more relevant when speaking of implications for seed treatments (data was gathered on agriculturally relevant species). Feeding platforms were erected at ten different locations. Nine platforms were used for testing (one was dismantled due to low bird activity). Special attention was given to ensure platforms were erected in areas with high bird activity, and in areas where they would receive full, direct sunlight for the morning hours. Only one platform was erected on a given field, to reduce the likelihood of birds visiting more than one station.

#### **1.3.1.2 Test setup**

Six feeding platforms were assembled, each measuring 0.50 x 0.75m. Once testing had concluded at a site, the platform was reassembled at another location. Each platform had removable dividers which, when inserted formed six

equal compartments. Each compartment had a hole cut into the floor to hold a seed container. Seed containers were shallow (~5cm deep) clear plastic dishes which were spray painted black on the outside, so birds were unable to see through when foraging. The six platform compartments were numbered to assist in setup and observations (figure 1.7). A steel fencing pipe was forced into the ground using a handheld post-pounding device. Approximately 1.5m of piping remained above ground, which was inserted securely into the base of the platform. As a result of depredation by raccoons early in our study, all platform piping received a generous application of mechanical grease once erected.

Approximately 3.0m from the platform feeder, a small camera housing was placed ~2.0m above ground in the same manner. The video camera was fully concealed within this housing during tests. A small tinted window on the housing permitted the camera to record events on the platform without detection by the birds (see photo 1.2).

### **1.3.1.3 Seed Treatments**

We tried various seed types before settling on black-oil sunflower as the most appropriate for our purposes. Black-oil sunflower is a preferred/accepted seed type by most granivorous birds foraging in the areas of our study. Additionally, the black-oil sunflower shell received our pigments very well when treated (ie. some seeds, like safflower, are too polished for pigments to adhere well).

The same seed treatment colours, preparation, and application rates were applied for this experiment as was used for the laboratory colour preference trials (refer to figure 1.1 pigment spectra).

#### **1.3.1.4 Test procedure**

Immediately following their setup, platform feeders received generous amounts of natural black-oil sunflower in each of the six compartments. These compartments were replenished as needed (typically every two days), for at least two weeks to ensure maximum bird activity at the feeder before testing. Camera housings were in place for at least three days before testing.

To ensure adequate direct sunlight (and sufficient UV irradiance), experiments were not conducted before 10:00am (before this time, the angle of the sun often prevented direct sunlight on the platforms). Also, tests were only conducted on days with minimal cloud cover and without precipitation. Tests were conducted for seven days at each station. On the first day of testing (day 0), 20.0g of natural seed was placed in each of the six compartments and visits recorded for two hours. The remaining seed in each compartment was then weighed. Before weighing, all broken shells, feces, and debris were removed by hand and by winnowing the finer material.

On days 1 through 6, colour treated seed was used for testing. It was necessary to repeat colour testing for six days at each location since six colours were being

tested according to a latin square design. The employment of a latin design ensured that each colour was randomly assigned to a different position (with the platform) each day, and neighbouring colours were therefore randomised. Although random, this design places limitations on each colour so that positions and neighbouring colours are not repeated. The six colours of seed tested were UV-reflecting and UV-absorbing forms of blue, red, and green. Following a thorough cleaning of the platform, each pre-weighed (20.0g) container of seed was placed in the appropriate compartment, and birds were allowed to forage freely for two hours. Each trial was video recorded using a digital camcorder, placed in the camera housing. After two hours elapsed, trials were halted and remaining seed collected for weighing. The remaining whole seeds left on the floor of each compartment were collected into the appropriate containers to be included in weighing. Between trials, platforms were replenished with natural black-oil sunflower.

#### **1.3.1.5 Endpoints**

Each trial was two hours in duration and was recorded using digital camcorders. After dubbing to VHS format, trials were analysed for: species, sex (if identifiable), order of consumption (by colour), and amount of seed consumed (recorded as either: seed handling, swallowing, or removal depending on species). Seeds were also weighed following trials and daily consumption values for each colour were derived for analysis. Daily recordings of the solar spectra were taken, and weather conditions noted. Colour preference analysis will be

separated into consumption results (from daily measurement of seed disappearance), and visitation results. For visitation results, a 'visit' was recorded as any instance in which seed was consumed (determined using options described above).

## **1.3.2 RESULTS**

### **1.3.2.1 Bird visitation**

For the entire study period, a total of 2434 bird visits were recorded at the platforms during tests. Sixteen different species were observed at the platforms. Six species comprised over 90% of observed visits: Black-capped Chickadee, Blue Jay, Common Grackle, Rose-breasted Grosbeak, Red-winged Blackbird, White-breasted Nuthatch (see figure 1.8 for visit frequencies by species, and appendix 2 for common names and abbreviations of species). Together, Black-capped Chickadees and Blue Jays accounted for greater than 50% of visits. Three sparrow species were observed visiting the platforms (White-crowned, Savannah, and Song). Our ability to discriminate between Savannah and Song sparrows was often compromised by both video camera resolution and platform design. As a result, observations on these two species were pooled into one group, denoted as 'sparrows'.

#### **1.3.2.1.1 Platform stations**

The number of species visiting each station ranged from as few as five (sites A, B, I) to thirteen species (site E)(Table 1.4). The mean visit frequency over the six day testing period was 271 visits. Site B was the most frequently visited, experiencing nearly twice as many visits (607) as the next highest station.

Despite having one of the lowest numbers of different species, the high activity at this station was solely the result of frequent visitation by black-capped chickadees (494 total visits over all six days). Not surprisingly, the species noted earlier as comprising over 90% of visits, were also the species (excluding white-breasted nuthatch) which occurred at the highest number of platforms (Rose-breasted grosbeak observed at 8 of 9 sites, black-capped chickadee, blue jay, and common grackles observed at 7 of 9).

#### **1.3.2.1.2 Effect of Day**

Despite the two weeks of pre-baiting at each site, as trials progressed from the first day of colour testing through the sixth day, an overall steady increase in the frequency of bird visits was recorded (figure 1.9). The mean visitation frequency on the final day of testing was 60.67 visits, a more than two-fold increase from day 1 (27.56). Consumption values were log-transformed to better fit data to normality for the purposes of analysis (appendix 4). A general linear model (GLM) was used to isolate the effect of day from other factors (platform location, position within platform, and seed colour). Using this model, day was seen to

have a highly significant effect on the number of visits with seed removal ( $p < 0.001$ ).

#### **1.3.2.1.3 Effect of Position**

The six positions within each platform were numbered for reference during colour pattern development (latin design), and to ensure consistency in seed presentation and collection. The position to the far left (from the cameras point of view) was designated as #1, and the remaining positions were number from left to right (figure 1.7). The setup we used for this study (separate camera housing and platform), resulted in certain positions being in potentially better locations for bird visitation. Positions 1, 2, and 3 were frequently closest to vegetative cover, from which birds were likely to approach and retreat to. Additionally, the corner positions (1, 3, 4, 6) may have been more likely to be the site of initial approach to the platform. As a consequence of this, it seemed likely that certain positions would have a significant effect on bird visitation.

Figure 1.10 shows the frequency of visitation for each site. Position 1 is often the most frequently visited location, but a strong site dependence is evident.

Knowledge of the individual sites and the location of nearby vegetative cover explains most of this site dependent variation. The vegetative cover at sites C and H was directly behind the platform, bordering positions 1, 2, and 3. It is very likely that the pronounced disparity between positions 1, 2, 3 and 4, 5, 6 is the result of this arrangement. Similarly, positions 1 and 4 were highest at the site I,

where cover was located on the left side of the platform. The mean visitation for all sites is illustrated in figure 1.11.

An analysis of variance using the same GLM model and nesting platform location within platform position to isolate the effects on consumption indicates a highly significant effect of within-platform position on bird visitation ( $p < 0.001$ ).

#### **1.3.2.1.4 Colour Analysis**

For this analysis, a 'visit' to a colour required that seed(s) of that colour were consumed/removed. Only the first colour visited for each bird was used for colour preference analysis. The frequency in which each colour was visited as the first choice, totalled for the entire study is illustrated in figure 1.12. The most frequently visited colour was red, followed by red+uv, blue+uv, blue and green+uv, then green. The confounding effects of site, day-of-test, and within-platform position were considered when testing for significant effects of colour by using a nested GLM model. To ensure data followed an approximate normal distribution, visit values were log transformed (after adding one to adjust for several zero values). Using this method, colour was shown to have a highly significant effect on visit frequency ( $p < 0.001$ ) when totalled for all species. Significant differences were calculated using Tukey's pairwise post-hoc test, and are summarized in table 1.5a.

The same analysis was performed on each of the six major species in this study (Black-capped Chickadee, Blue Jay, Common Grackle, Rose-breasted Grosbeak, Red-winged Blackbird, White-breasted Nuthatch). The total visits to each colour for these species are shown in figure 1.13. Only visits by Black-capped Chickadees and Blue Jays appeared to have been affected by seed colour ( $p=0.007$  and  $<0.001$  respectively). For both of these species UV-absorbing red (red) and red+uv were the most preferred seed colours. Blue Jays and Black-capped Chickadees were also the two species which recorded at platforms most frequently, which may suggest that our analysis was not capable of finding significance at lower visitation rates. Interestingly, the trends (although not significant) were much different for the remaining four species (table 1.5b). Red was *not* the most preferred colour (according to visit frequency) for any of these species. When the GLM was used to compare the effect of colours for individual species, the increased number of zero values caused a larger deviation from the assumption of normality. Due to the large sample sizes, it was concluded that results of these analyses remained valid (also, no non-parametric alternatives exist for performing nested analyses such as these).

The UV-reflection of seeds did not have a significant effect on visitation frequency for any of the species visiting the platforms ( $p=0.464$  for all species). For no species was a significant difference in visitation found between the two reflecting forms of a given colour. When UV reflection is ignored (colours are grouped according to their human visible colour, Black-capped Chickadees and

Blue Jays remain the only species experiencing a significant effect of colour on visitation. For both species, a highly significant preference for red seed (over blue and green) is exhibited. Additionally, a marginally significant effect ( $p=0.059$ ) of colour on visit frequency was found for Common Grackles.

### **1.3.2.2 Consumption**

Following each two hour test, all remaining seed was collected, weighed, and the mass of seed consumed (g) for each colour was calculated. The mean consumption for a given colour during tests was 1.32g, and the max consumption for a single colour was 13.34g (blue+uv, day 6, site K). The overall amount of seed being consumed during tests varied greatly from site to site. The site experiencing the least consumption was Ross, where a mean consumption recorded at each position was 0.42g. Site C experienced a mean consumption rate of 2.75g at each position, which was the highest mean rate of seed removal observed during this study.

Analysing variation in the amount of consumption occurring on each colour is valuable for monitoring overall preference for birds visiting the platforms.

Unfortunately, this design does not allow us to attribute consumption levels to individual birds or species. Consequently, the results from this component of our analysis gives unequal weighting to species based upon the consumption potential (size) of birds feeding on a given colour. Of the birds visiting platforms, Red-winged Blackbirds, Common Grackles, and Mourning Doves have the ability

to consume large quantities of seed during a single visit, effectively removing the effects of several visits by smaller birds (eg. Black-capped chickadees and White-breasted Nuthatches, typically remove only one seed per visit). This was precisely what we observed in the case of the maximum recorded visit of 13.34g. This level of consumption was achieved from 38 visits by several larger bird species (Common Grackles, Blue Jays, and Mourning Doves). In contrast, the highest number of visits to a given position in one test day was 54, at site B. Despite the high frequency of visits, only 3.39g of seed was consumed at this position. This can easily be attributed to the observation that visits were mostly from Black-capped Chickadees, which generally remove only one seed before retreating to cover. Because consumption analysis is generally more sensitive to the preferences of larger species visiting the platforms, it is necessary to re-explore the confounding influence of other factors, namely that of day-of-test, and colour position.

The consumption results for the site I were omitted due to occasional visitation by squirrels, consuming large quantities of seed.

#### **1.3.2.2.1 Effect of day**

The amount of seed consumed during tests consistently increased as trials were repeated for six days (figure 1.14). Untreated black-oil sunflower was presented for no less than two weeks prior to testing to ensure bird visitation/consumption had stabilized. Mean consumption decreased dramatically when colour seed

was presented on the platforms, indicated by day 0 consumption in figure 1.14 (day 0 indicates consumption of black-oil sunflower immediately prior to the first test day). During testing, day-of-test had a significant effect on consumption ( $p < 0.001$ ) according to an ANOVA (GLM used to account for additional influence of colour, site and position).

#### **1.3.2.2.2 Effect of position**

The position on the platform in which colours were arranged appeared to have a less pronounced influence on consumption (than on bird visits)(figure 1.15). As discussed in the case of bird visitation, the effect of position is assumed to be a consequence of the location of nearby cover from which birds approach/retreat to. With only a few exceptions (e.g. site E), the location of this cover is not as apparent on a site-by-site basis (figure 1.16). The results of the GLM ANOVA show position (nested by site) to have a marginally significant effect on consumption levels ( $p = 0.062$ ).

#### **1.3.2.2.3 Colour analysis**

The mean consumption per colour for all sites is illustrated in figure 1.17. UV-absorbing red had the highest mean consumption at 1.94g per trial. The lowest mean consumption per trial was 0.97g, occurring on the UV absorbing green seed. Since consumption on the platforms was affected by factors in addition to colour, a nested GLM was used on the log-transformed consumption values to

test for significance (refer to appendix 4). This model accounted for the influence of day-of-test, site, and position within the platform (dependent on site). Colour was found to have a highly significant effect on consumption ( $p < 0.001$ ). A Tukey post-hoc test was used for pair wise comparisons (table 1.7). The level of consumption measured for the UV-absorbing red seed was significantly greater than all other colours, except UV-reflecting blue (the second most preferred colour). At a 0.1 significance level, the differences in the level of consumption between the UV-reflecting and UV-absorbing forms of red and blue become significant. Despite significant differences between the two forms of blue and red (at  $p = 0.1$ ), a significant effect of UV reflection was not found using this GLM ( $p = 0.694$ ). Although UV reflection did appear to have an effect in two of three possible colours, the preferences were in opposite directions, which is likely why the analysis does not suggest it to be an influencing factor.

Combining the six test colours into three seed types on the basis of their human visible colour (ignoring UV reflection) maintains the significant effect on consumption ( $p = 0.012$ ). At a 0.05 level of significance, red was significantly more preferred than green ( $p = 0.009$ ) (Tukey post-hoc test).

#### **1.3.2.2.4 Additional observations**

On all platforms, bird visitation typically declined over the course of the two hour testing period. On many occasions, the final 45 min were without any visitation (following frequent visits at the beginning of the trial). This could be an indication

that birds were experiencing aversive effects of the pigments used to colour-treat the test seed (e.g. bad taste). However, earlier Zebra Finch laboratory testing, and the observed increase in consumption associated with day-of-test suggests otherwise.

On several occasions, unusual behaviour was observed on the platforms. Several species were observed exhibiting rapid 'tremor-like' shaking for periods of a few seconds. Other birds were recorded maintaining a tilted body, wings spread out, motionless position for periods often longer than a minute. In one instance, a Black-capped Chickadee rolled off the platform divider upon which it was perched after holding this position for a prolonged period. Once birds began to move again, they typically preened for a few seconds before leaving the platform. Our original concern was that these behaviours resulted from our colour treatment compounds. However, a Common Grackle was recorded exhibiting this behaviour during its first visit to the platform that day (first Common Grackle recorded), which seems to suggest an alternative explanation. Furthermore, the same treatments were used during laboratory colour preference tests without any observations of abnormal behaviour or any other side effects.

### **1.3.3 DISCUSSION**

#### **1.3.3.1 Colour preference**

The results of this field study suggest that of the colours tested, granivorous wild birds tend to prefer red coloured seed. This finding is supported by analysis of

both visitation and consumption data. The results derived from analysis of these data types produced similar conclusions. However, the decision to approach these data separately was warranted. While it is true that consumption cannot occur without visitation, the amount of consumption which occurs during visits may fluctuate depending on preferences. If a bird were to visit the red seed once, and consume 30 or so seeds, there would likely be little need for repeated visits (depending on bird size and hunger). Relying on visitation data alone, would cause the importance of this visit to be diminished. Another bird with the same preference for red, which visited the platform 30 times, consuming red on each visit, would have a more substantial impact on results. The agreement between the results of both data types further strengthens inferences made from this study.

The foraging situation described above was frequently observed during the trials. On most occasions, smaller bird species (Black-capped Chickadee, White-breasted Nuthatch) would consume only a single seed per visit, usually removing the seed for consumption under the protection of nearby vegetative cover. Larger birds (Blue Jay, Red-winged Blackbird, Mourning Dove, etc.) would more frequently consume large amounts of seed during a single visit, sometimes consuming from several colours during a visit. The difference in foraging habits between large and small bird species suggests that results of consumption analysis will be more sensitive to large birds, while visitation analysis will be more effective at conveying preferences from smaller species.

Our study indicates a preference for red seed when all participating species are collectively analysed. As mentioned, Black-capped Chickadees and Blue Jays represent over half of the total visits for which seeds were removed. The significant preference for red seed over blue and green was very evident for these two species. Of the other four species comprising the bulk of the remaining visits, no significant preferences were found. Consequently, it may not be appropriate to extend this finding to all wild granivorous species.

### **1.3.3.2 UV reflection**

Of major interest in this study was observing how wild birds respond to UV-reflecting colours in comparison to UV-absorbing (non-reflecting) colours on granivorous foods. The results of visitation analysis indicate that UV-reflection had no effect on observed avian colour preferences. The grouping which is evident when ranking the mean visit frequencies of each colour (red > red+uv > blue+uv > blue > green+uv > green) suggests that the wavelength reflection in the human visible range is the greatest determinant of colour preference.

When the results of consumption analysis are considered, the effect of UV reflection on colour preference becomes less clear. For both blue and red, the difference in consumption levels between either form (UV-reflecting vs UV-absorbing) was statistically significant (at  $p=0.1$ ). For red seed, the UV-reflecting form was preferred, while UV-absorbing seed was preferred for blue. This

observation serves to further confirm that the chemicals used to manipulate UV reflection in this study are not aversive to the birds in other ways (e.g. taste). As mentioned, the results of consumption analysis are likely to be more indicative of the preferences of larger species visiting the platforms. The differences between UV-reflecting forms found in consumption data (but not visitation), could likely be attributed to this notion. However, a plot of the total visitation by the four major larger species participating in this study (Blue jays, Common Grackles, Rose-breasted Grosbeaks, Red-winged Blackbirds) to each colour does not deviate from the visitation results for all species enough to account for these differences (figure 1.18). Although larger species did not visit UV-reflecting blue, and UV-absorbing red more often, they consumed more during their visits to those colours.

### **1.3.3.3 Neophobia**

The frequency of bird visitation increased significantly over the six days in which trials were repeated (figure 1.9). All platforms were assembled and supplied with natural seed for at least two weeks prior to the commencement of testing and the introduction of coloured seed. This time period was given to allow bird visitation to stabilize by ensuring local birds had adequate opportunity to discover and familiarize themselves with the platform. As a result, any change observed in consumption and visitation associated with increasing day can be safely attributed to the introduction of coloured test seed. The observed pattern of a strong, initial avoidance of the test seed, diminishing after repeated encounters suggests that the avoidance is of a neophobic nature.

All bird species visiting the platforms displayed an initial avoidance of the coloured seed. The number of visits with consumption on the first day of testing was typically only a fraction of those occurring on any other day. However, the degree to which birds avoided the test food was highly variable between species. No visits with consumption were recorded with Red-winged Blackbirds and Common Grackles on the first day of testing. Likewise, Rose-breasted Grosbeaks did not visit any platforms before the fourth day of testing, despite being present at eight of the nine locations.

It was not surprising that the frequency of visits in which consumption was recorded was dramatically less on the first day of testing. However, the reduction in total visits (with or without consumption) on the first day was less expected. A probable explanation is that birds were simply not returning to platforms after finding them filled with unfamiliar seed. However, some species did not even visit platforms once coloured seed was introduced (e.g. Rose-breasted Grosbeaks). For these species it seems that individuals may have observed the contents of the platform from a distance, and not been compelled to investigate. Alternatively, there may have been some social learning occurring as individuals observed the behaviour of other birds approaching the platforms. Indeed, the reaction of some birds upon discovering coloured seed upon their (assumedly) first encounter was often quite dramatic (esp. Northern cardinals and Common Grackles). Birds were often observed attempting to hover over the platforms

while trying to assess the unfamiliar seed, or repeatedly retreating a few meters before approaching again.

## **1.4 SUMMARY**

### **1.4.1 COMPARISON OF FIELD AND LABORATORY RESEARCH**

Although the colour selectivity of Zebra Finches used during the lab study diminished very quickly, the initial preferences exhibited were surprisingly similar to the results found from the field-visit data. Both of these results found a grouping of preferences based on colours in the human visible spectrum, indicating very little influence of UV wavelengths. In both studies, red was preferred more than blue or green seed. Additionally, the preference direction between the two forms of each colour was not consistent (UV-reflecting form was not consistently preferred or avoided). The consumption data derived from the field study was less correlated with the findings of the lab experiments. Although neither lab nor field-consumption data found a significant overall effect of UV reflection on colour preference, both found occasional significant differences in preference between two forms of a given colour (blue seed in the lab, red and blue (at  $p=0.1$ ) in field-consumption).

The experimental design used for laboratory testing was constrained by the facilities and housing environment. Consequently, it was difficult to assess the presence or magnitude of avoidance (neophobic or otherwise) to the coloured test seed from our results. Generally, Zebra Finches did not hesitate to forage

on test seed once tests had begun, suggesting that no neophobic avoidance was present. The absence of this avoidance could be a product of the unrealistic situation created in the lab where birds were food-deprived, and were presented only coloured seed (no other options are obviously apparent). Furthermore, captive Zebra Finches have likely had no reason to exercise caution when sampling new foods. For these birds, the negative experiences associated with foraging have been extremely minimal. This is in stark contrast to wild birds, which must be more 'conscious' of what they are consuming and learn to identify and avoid foods with negative effects. The disparity in the level of avoidance and persistence of preferences between wild birds and captive Zebra Finches can likely, at least partially, be attributed to these past experiences.

The ability of birds to perceive UV wavelengths has been generally accepted as a capability of all diurnal birds (including Zebra Finches). Despite this, there was still concern that our captive Zebra Finches, which were likely raised in indoor environments without UV irradiance, and probably the offspring of many generations in such an environment would have a compromised ability to see in the UV. It is possible that the absence of UV light during development may lead to impaired visual perception (Smith et al. 2002). Despite the significant differences observed between some of the UV-absorbing and UV-reflecting paired colours, it is possible that this ability was diminished. This could explain the minimal difference in results between UV-present and UV-absent conditions in the lab.

Our Zebra Finches proved to be adequate for providing results which could be cautiously extended to wild birds. It is possible that birds which have been bred for many generations in a controlled environment (i.e. chickens and pet bird species) may become less representative of wild species, and this should be considered when making inferences from experimental results. The experiments conducted in this study were focused on acquiring information on colour preferences by birds using the full capabilities of the avian visual system, and providing further insights on the role of UV vision in foraging. Although some colour preference might be innately present in birds, most are developed from experiences (Rowe & Johnsen 2004). The lack of such experiences in captive-reared birds makes it extremely unlikely that their response to colours would be similar to those of wild species. However, if UV light is perceived achromatically, and therefore more relevant in cryptic prey detection, previous experience is less critical and captive birds would likely produce results which are adequate for translating to wild species.

Although the results of our field and laboratory research were similar, due to the nature of these experiments it is not possible to conclude whether this was coincidental, or if Zebra Finches are in fact good representatives of wild species. The variation found in the colour preferences of different species suggests that they are strongly species dependent. Consequently, one species should not be used as an indication of all species (captive or otherwise). The results of Zebra

Finch colour preference experiments should be used only to infer on the potential role of UV light in avian foraging, and how UV light is perceived by birds.

## **1.4.2 GENERAL DISCUSSION**

### **1.4.2.1 The need for UV incorporation in avian studies**

The importance of visual stimuli on avian behaviour has been recognized for many years. Consequently there has been extensive research dedicated towards its understanding. One of the most cited publications on avian colour preference pertaining to granivorous foraging was that of Larry Pank (1976). Pank tested colour preference on two passerine species (Varied thrush, *Ixoreus naevius*, and Oregon junco, *Junco hyemalis*) and a galliforme (California quail, *Lophortyx californicus*). The UV reflection of the various pigments were not considered in his study, but blue, red, and green were included amongst the 8 colours he tested. There was very little consistency in the order of colour preferences between species in his results, in particular pertaining to red, blue and green. However, in none of Pank's experiments did blue and green differ significantly. This correlates well with the results of our laboratory experiments in UV-absent conditions (Pank's experiments were conducted under cool-white fluorescent lighting, which do not emit UV). Our findings in the lab, suggesting that addition of UV light resulted in the development of a significant difference between blue and green, strongly illustrates the need for consideration of UV light when researching on visually mediated avian behaviour.

The inconsistencies encountered in Pank's research are not uncommon among attempts to determine colour preference in granivorous birds. Colour preferences were established for Steller's Jays (*Cyanocitta stelleri*) feeding on coloured peanuts (Slaby & Slaby 1977). The order of preference was determined as: red > yellow > blue > green. Northern bobwhites (*Colinus virginianus*) were used as the test species in another attempt to discern colour preference (Mastrota & Mench 1995). The results of this experiment were nearly the complete opposite: blue > green > yellow > red. It should be noted that none of these experiments took UV wavelengths into consideration (reflection of prey or illumination).

Our experiments are the first to explore avian colour preference of several UV-reflecting and UV-absorbing colours. The only published research incorporating UV light into colour preference, was done by A. Lytinen et. al in 2001.

However, these experiments did not allow for comparison between colours (only between UV-reflecting and UV-absorbing forms of a given colour).

#### **1.4.2.2 UV light perception by birds**

The mechanism by which birds perceive UV light has been the focus of many studies in recent years. The discovery of UV sensitive cone receptors in avian retinæ seemed to suggest that UV light contributed to colour vision (Chen et al. 1984). Depending on the neural coding of these cone receptors in the avian eye, one possibility is that UV light provides a fourth spectral colour in avian colour

vision. If this were the case, input from UV receptors could combine with input from the other three types of cone receptors to produce several colours which are non-comprehensible to humans (Smith et al. 2002).

The blue, green, and red seed chosen for our study correlates with the peak sensitivities of the three established cone receptor types in the avian eye. If the aforementioned scenario existed, the addition of UV reflection to either colour (blue, green, or red) should produce colours very different (to the birds eye) than the non UV-reflecting colours. Our results do not appear to support the existence of this mechanism. If the presence of UV reflection caused the perception of additional colours, it is logical that we would observe frequent instances where significant differences would be found between UV-reflecting and UV-absorbing forms of a colour. This occurrence was found in our study (blue seed in the lab and red seed in field-consumption data), however, not frequently enough to suggest the perception of additional colours. Furthermore, the grouping of visible colours when preferences are ranked from field-visitation, and from UV+ laboratory data gives little indication of an influence of UV reflection in colour perception.

The alternative explanation is that, instead of contributing to colour perception, UV light provides achromatic input akin to a perception of brightness. This type of input can be beneficial in several ways, the most prominent of which seems to be in the detection of cryptic prey types (Church et al. 1998b; Probst et al. 2002).

This study was designed to observe how birds respond to colours (with special attention to UV), and not necessarily detection ability. Consequently, test seed was always presented in a highly conspicuous manner, and detection ability was not a factor when birds were foraging.

#### **1.4.2.3 UV perception and experimental design**

It was not possible to create UV- conditions in our field study. Consequently, inferences on the ability of birds to perceive differences in the UV reflection of seeds were difficult to ascertain. However, the significant differences found in the consumption data between colours on the basis of UV-reflection are good evidence that UV-differences were perceivable by birds. Additionally, as with our laboratory experiments, the UV-reflection we were able to achieve on the seeds, and the intensity of UV wavelengths present during our trials were much higher than other successful studies involving avian perception to UV light. Thus, it should be safe to assume our conditions were sufficient to induce UV-mediated responses if possessed by wild birds.

A large proportion of the research exploring UV light and avian foraging has focused on the consumption of fruit. The 'UV-reflecting' fruits used in these experiments often reflect considerably less in the UV-range than we were able to achieve in this study. At 350nm, example approximate reflectance levels of UV-reflecting prey were: berries reflecting 10-15% (Honkavaara et al. 2002), bilberries at <10% (Siitari et al. 1999; Siitari & Viitala 2002). For insect prey, UV

light had an effect on prey detection rates when the prey-background difference was less than 10% at 350nm (Church et al. 1998b). Even though these experiments had relatively low reflection values, coupled with generally less UV illumination, they were still able to identify an effect of UV light on avian foraging behaviour.

The only avian foraging experiment which had UV prey reflectance levels approaching those achieved in our experiments was that of Lyttinen *et al.* (2001). Their UV reflecting stimuli ranged in reflection from approximately 25-35% at 350nm. As in our experiments, Lyttinen *et al.* used calcium carbonate and titanium dioxide to create their UV-reflecting and UV-absorbing prey. However, the prey itself (almond slices) were not altered. Instead, birds were required to find prey on the basis of coloured stimuli (almonds were glued to the underside of colour-treated paper). Despite less illuminance, birds were able to make foraging decisions on the basis of UV reflection.

No other studies pertaining to UV colour preferences and avian foraging have used prey which reflected as highly in the UV waveband as this one.

Surprisingly, with the exception of consumption data from the field, we had difficulty demonstrating a clear effect of UV on colour preference, or even that birds were able to perceive the large difference between our UV+ and UV- prey. Most of the studies mentioned previously used UV filters and the birds' abilities to detect prey in the presence or absence of UV light as a basis for their

experiments. Any discovered effect indirectly confirmed successful manipulation of the sensory environment (i.e. Church et al. 1998b). Alternatively, a taste aversive chemical was sometimes used which causes birds to choose prey on the basis of UV reflection where a preference may not have otherwise existed (Lyytinen et al. 2001).

Our attempts in the laboratory to utilize a cryptic-detection experiment for the purpose of confirming successful manipulation of the sensory environment encountered difficulties. Additionally, our method of seed treatment (powder coating) did not facilitate the addition of an aversive chemical (attempts to do so resulted in a loss of UV reflection). Lyytinen *et al* (2001) were able to treat their prey with an aversive chemical without influencing UV reflection because the seeds themselves were not powder coated. Exploratory experiments also suggested birds were able to discriminate between UV-reflecting and UV-absorbing seeds in our experimental setup. These early experiments also gave mixed results on the influence of UV-reflection on colour preferences (using different birds). In several circumstances, a pronounced preference for UV-reflecting food items was identified.

## **1.5 CONCLUSIONS**

### **1.5.1 COLOUR PREFERENCE**

We conducted two studies aimed at exploring avian colour perception, with special attention to UV influence in granivorous birds. The first study was

conducted inside the laboratory, and permitted a more controlled lighting environment. Specifically, the presence or absence of UV light could be manipulated allowing for simpler inferences on its influence towards colour preferences. In the field, a modified version of the same test was conducted. Obtaining data from wild birds living in agricultural areas was the focus of the field study because of our interest in applying the results towards methods of reducing bird exposure to pesticide treated seed.

The captive Zebra Finches used in this study readily sampled unfamiliar seed colours. With the absence of negative consequences, existing colour preferences quickly waned to undetectable levels. Since colour preference is largely a consequence of previous experience, caution should be exercised when using these or similar data to make inferences on wild birds.

In the presence of UV light, our experiments consistently found a preference for red seed. The favourability of the remaining two colours (green and blue) was less consistent in their ranking. In the laboratory, addition of UV light resulted in a significant difference between blue and green seed, appearing to aid birds in discriminating between these colours. Our results give little evidence that addition of UV reflection produces additional colours perceivable to birds. The grouping of colours observed in the lab, and to a lesser degree in the field, lend additional support to the notion that UV light is perceived achromatically.

### 1.5.2 IMPLICATIONS FOR SEED TREATMENTS

It has repeatedly been shown that birds prefer natural seed over artificially coloured seed, when offered a choice between the two (Pank 1976; Slaby & Slaby 1977; Brunner & Coman 1983; Mastrota & Mench 1995). However, alternate food choices will not always be readily available for birds foraging on agricultural fields. It has been shown by Getty and Pulliam (1993) that birds will choose to forage in areas where food is highly detectable over areas of high availability, which is often the scenario following agricultural planting.

Furthermore, avoidance of novel food colouration is often a short-lived neophobic response and is likely not a reliable method of reducing avian exposure to pesticide-treated seed (Reidinger & Mason 1983). The trend towards increasing platform visitation with day-of-test is evidence of this behaviour. However, it should be noted that this effect was highly variable between species. Some birds were extremely hesitant to forage on coloured seed for the entire 6 days of testing (e.g. Rose-breasted Grosbeaks), while other displayed very little regard for novel colouration (e.g. Black-capped chickadees).

Although birds may possess some unlearnt aversions to certain typically aposematic colours, most colour preferences are the result of previous experience (Rowe & Johnsen 2004). Once a previously avoided colour is sampled, and no negative consequences are experienced (ie. bad taste), birds will likely continue consuming seed of that colour (Marples et al. 1998). This is precisely what appeared to be occurring during our laboratory trials. Any colour

preference initially displayed by the birds quickly dissipated, as birds continually sampled less preferred colours without consequence.

Our study repeatedly demonstrated that red is a preferred colour for granivorous birds (of those colours tested). This was unexpected since the colour red is rarely seen in nature on a food source for granivorous birds. Additionally, the natural occurrence of this colour is often in the context of an aposematic signal, increasing the likelihood that wild birds would have made a negative association to this colour. The widespread use of this preferred colour as a colourant for cereal seed treatments, including applications of the highly toxic insecticide diazinon (see chapter 2) gives cause for concern. Not only is this colour highly conspicuous on a soil background, it appears to be a preferred colour (relative to other novel colours).

The role of colour in reducing exposure to pesticide treated seed will continue to be at its maximal effect when it is used in the context of a sensory cue associated with a bird repellent. Several studies have explored this concept, with very effective results (i.e. Avery et al. 1994). Surprisingly, very little emphasis is given to the colour of the visual cue, seemingly granting the assumption that all colours will perform equally. Unfortunately, exploring this concept was beyond the scope of this study. There is very little evidence from our trials that birds respond to UV reflecting colours any differently than other colours.

Consequently, UV reflectance need not be considered in developing bird repellent strategies for seed treatments.

## CHAPTER 1 TABLES

Table 1.1. Percentage of each pigment component used to create final seed treatment colours.

Pigments	Colours					
	Blue+UV	Red+UV	Green+UV	Blue	Red	Green
Calcium carbonate	99.5	98.0	96.0			
Titanium dioxide				98.5	95.0	90.0
Blue	0.5			1.5		
Red		2.0			5.0	
Green			4.0			10.0

Table 1.2. Results of replicated G-tests conducted on the observed frequencies for each colour during the first 20 seeds selected. Separate G-tests were calculated for UV present and UV absent conditions (N=24 and 20 respectively).

<i>UV present</i>	G	df	P value
Total	227.00	120	< 0.001
Heterogeneity	216.79	115	<0.001
Pooled	10.22	5	0.07
<i>UV absent</i>	G	df	P value
Total	195.56	100	< 0.001
Heterogeneity	189.91	95	<0.001
Pooled	5.65	5	0.341

Table 1.3. Observed ranking of colour preferences for both filter types, determined from analysis of first five seeds selected for each bird. Significant differences were determined using pairwise G-test analysis and adjusted levels of significance. Significant differences are denoted by superscript letters (abc).

	ACLAR <sup>®</sup>	Lexan <sup>®</sup>
Least preferred	Blue + UV <sup>a</sup>	Blue <sup>a</sup>
	Blue <sup>b</sup>	Green <sup>ab</sup>
	Green <sup>bc</sup>	Green + UV <sup>ab</sup>
	Green + UV <sup>bc</sup>	Blue + UV <sup>ab</sup>
	Red <sup>bc</sup>	Red <sup>ab</sup>
Most preferred	Red + UV <sup>c</sup>	Red + UV <sup>b</sup>

Table 1.4. Summary of visits to each platform site, organized by species. \*Sparrows denotes the sum of visits by Savannah and Song sparrows. Refer to appendix 2 for latin and common names to species abbreviations.

	A	B	C	D	E	H	I	K	L
AMGO					10	2		1	3
BCCH		494	10		44	40	158	32	29
BLJA			266	3	18	79	43	96	63
AMCR					1	3			
DOWO					14		2		1
EUST				5					
COGR	66	45	20	47	4			53	62
HOFI				12					5
MODO	7			1				13	
NOCA			5	3	3			7	
RBGB	1	44	1	13	21	63	15	3	
RWBL	98	16	25	39	121				10
*sparrows	23	8			8			8	18
WBNU					36	31	56	1	
WCSP					6				
no. species	5	5	6	8	13	6	5	9	9
Total visits	195	607	327	123	286	218	274	214	191

Table 1.5. Mean visitation occurring at each colour for all species (a), and for the six bird species comprising the majority of visits (b). Significant differences at  $p=0.05$  are indicated by superscripted letters. Two values not having the same letter are sig. Different. No letters indicates no sig. differences.

a.

	All species
BLUE	5.42 <sup>a</sup>
BLUE+UV	6.73 <sup>ab</sup>
GREEN	5.42 <sup>a</sup>
GREEN+UV	5.10 <sup>ab</sup>
RED	9.46 <sup>c</sup>
RED+UV	7.65 <sup>bc</sup>

b.

	BCCH	BLJA	COGR	RBGB	RWBL	WBNU
BLUE	1.33 <sup>ab</sup>	1.3 <sup>c</sup>	0.57	0.41	0.52	0.39
BLUE+UV	1.37 <sup>ab</sup>	1.82 <sup>bc</sup>	0.65	0.3	0.96	0.48
GREEN	1.07 <sup>ab</sup>	1.39 <sup>c</sup>	0.5	0.44	0.57	0.28
GREEN+UV	0.91 <sup>b</sup>	1.46 <sup>c</sup>	0.65	0.54	0.63	0.26
RED	2.67 <sup>a</sup>	3.41 <sup>a</sup>	0.44	0.46	0.78	0.37
RED+UV	2.61 <sup>ab</sup>	2.69 <sup>ab</sup>	0.33	0.37	0.39	0.24

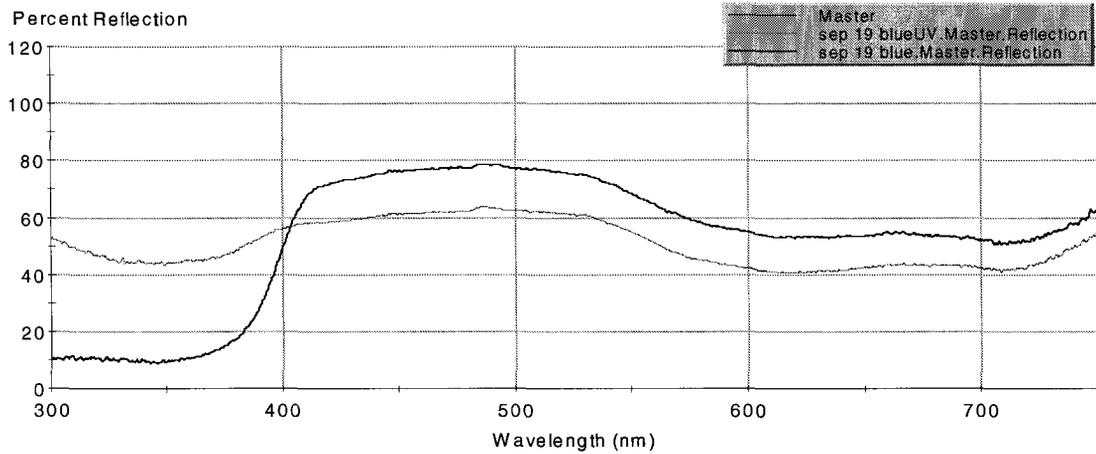
Table 1.6. Consumption decrease occurring on colour treated seed for each day of testing, relative to Day zero (consumption on natural sunflower seed). Values represent average consumption decrease at each platform position. Each mean decrease was derived by comparing to consumption values on day zero and were specific to each platform position and site location.

Day of test	N	Mean decrease (g)	Mean decrease (%)	Standard Dev
1	48	3.83	0.81	0.36
2	48	3.44	0.75	0.29
3	48	3.40	0.76	0.39
4	48	2.98	0.63	0.29
5	48	2.86	0.52	0.58
6	48	2.44	0.34	0.95

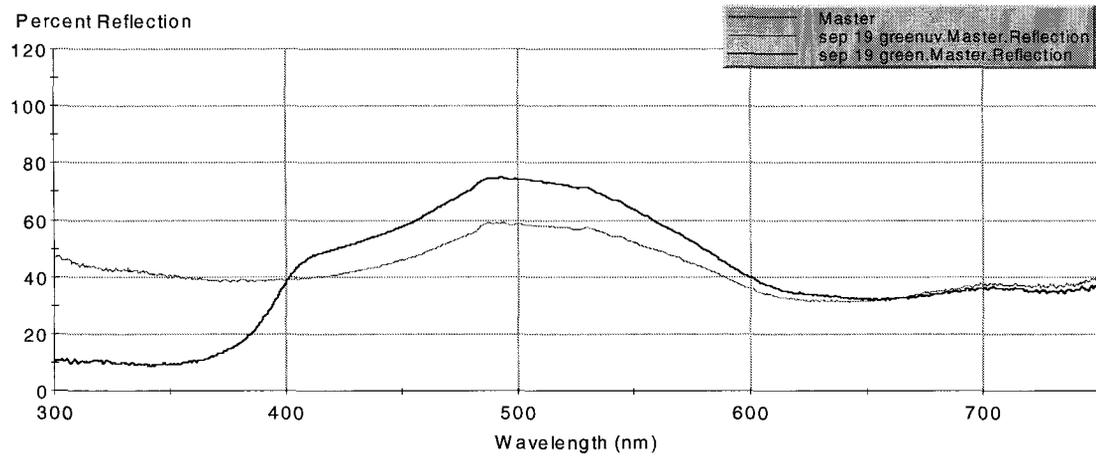
Table 1.7. Mean consumption values for each colour. Statistical significance (at  $p < .05$ ) calculated on the log-transformed consumption values are indicated by the presence/absence of similar superscripted letters (absence of similar letters denotes significance).

	All species
BLUE	1.04 <sup>a</sup>
BLUE+UV	1.64 <sup>ac</sup>
GREEN	0.97 <sup>a</sup>
GREEN+UV	1.18 <sup>a</sup>
RED	1.94 <sup>bc</sup>
RED+UV	1.17 <sup>a</sup>

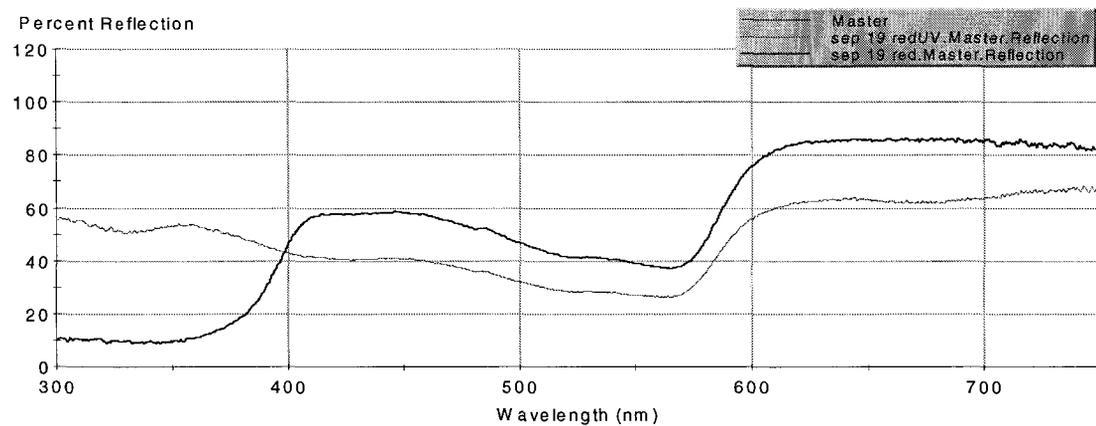
## CHAPTER 1 FIGURES



a.



b.



c.

Figure 1.1 Reflectance spectra for each test seed colour from 300 – 750nm. In each figure the bold line represents the UV-absorbing form. The UV-reflecting form is denoted by the solid line. a.) blue seed, b.) green seed, c.) red seed.

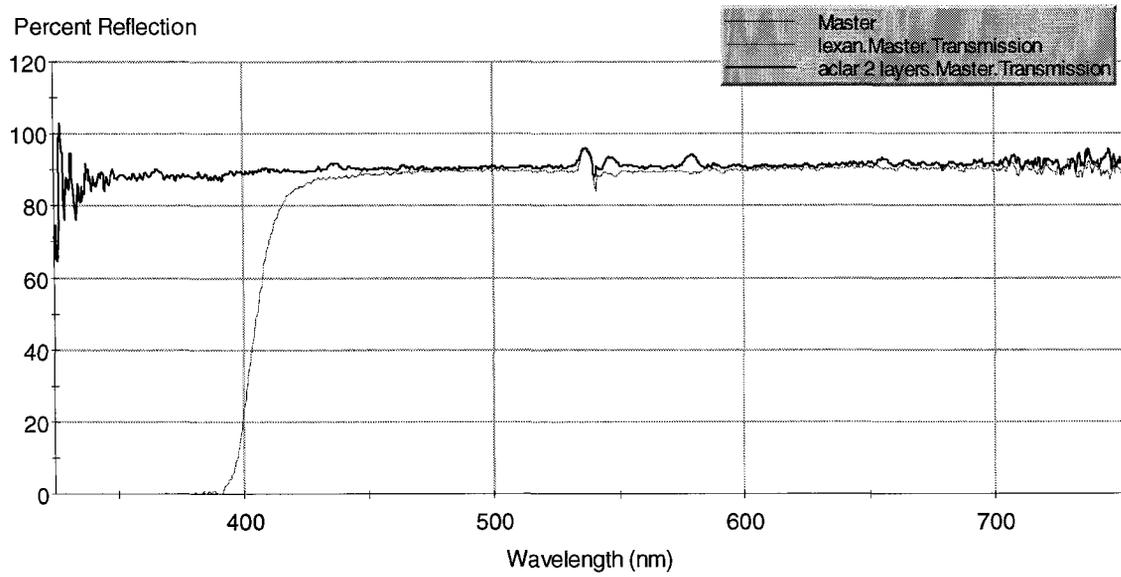


Figure 1.2. Transmission spectra of the two filter types. The solid line represents transmission through the ACLAR<sup>®</sup> filter (UV+ condition). The bold line represents the ACLAR<sup>®</sup> filter (UV+ condition). Seemingly erratic transmission of ACLAR<sup>®</sup> at the lowest wavelength (325nm) is noise occurring as the sensitivity limitation of the spectrometer is approached.

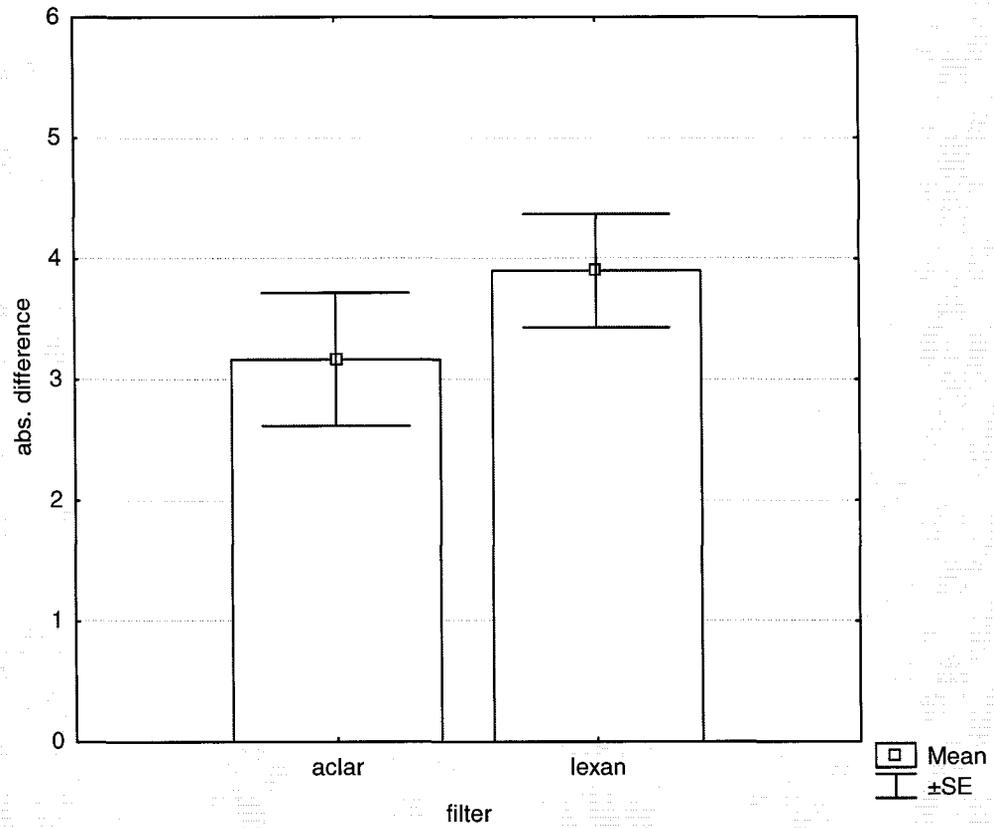


Figure 1.3 The absolute difference between the number of UV-reflecting, and UV-absorbing seed selected during each trial. The ACLAR<sup>®</sup> filter creates UV+ illumination (Lexan<sup>®</sup> creates UV-). Whiskers denote one standard error from the mean.

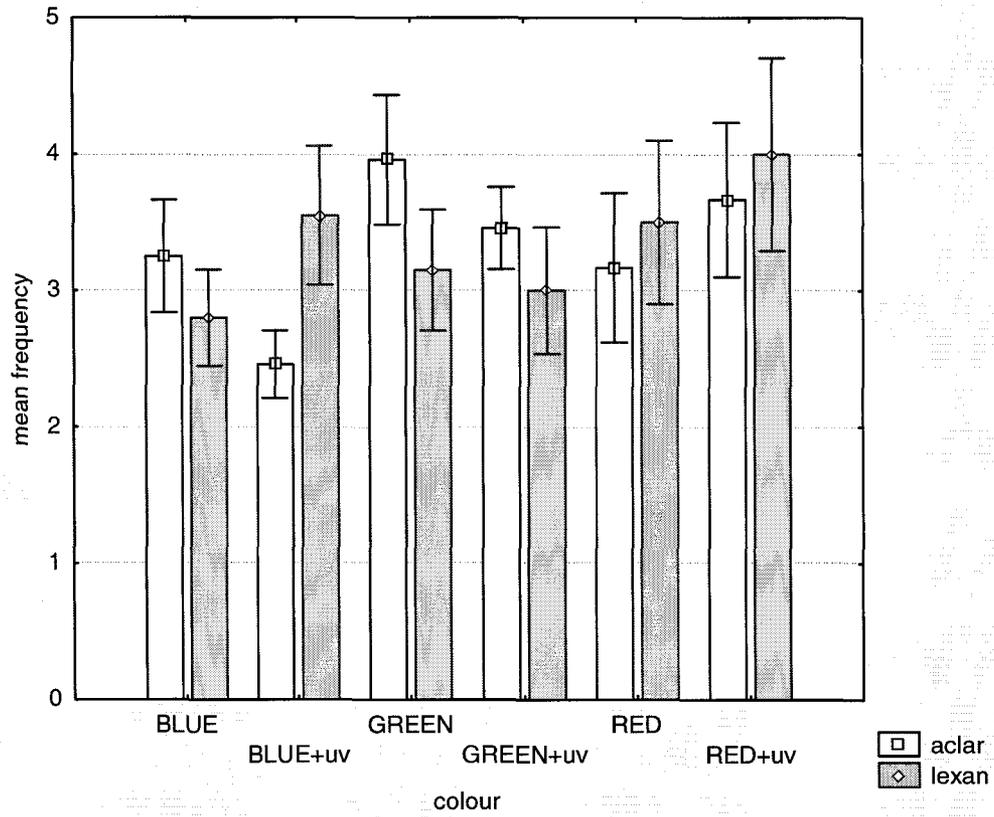
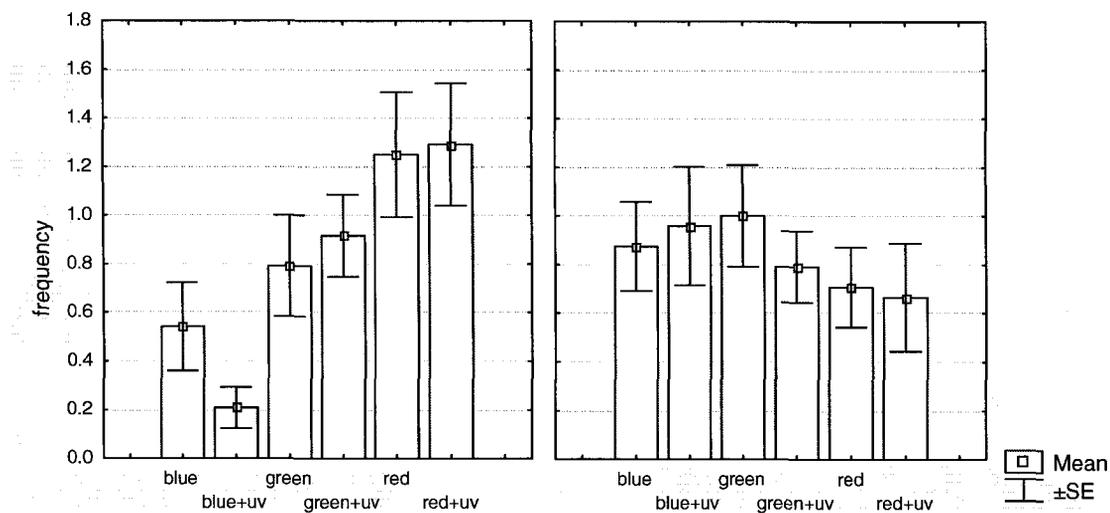
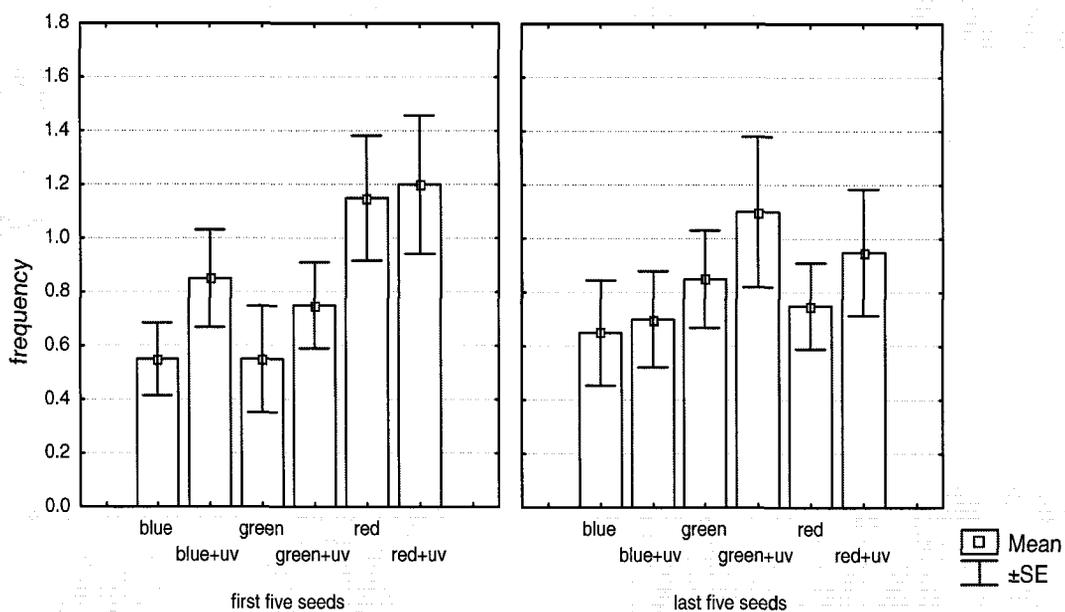


Figure 1.4 The mean frequency of selection for each colour in UV+ conditions (ACLAR<sup>®</sup>), and UV- (Lexan<sup>®</sup>) conditions. Frequencies are based on the entire 20 seeds selected. N=24 for ACLAR<sup>®</sup>, N=20 for Lexan<sup>®</sup>. Whiskers denote one standard error from the mean.



a



b.

Figure 1.5. The average frequency for each colour during the first five (left) and last five (right) seeds under ACLAR® (a) or Lexan® filtration (b). Whiskers denote one standard error from the mean. N=24 ACLAR® for , N=20 for Lexan®.

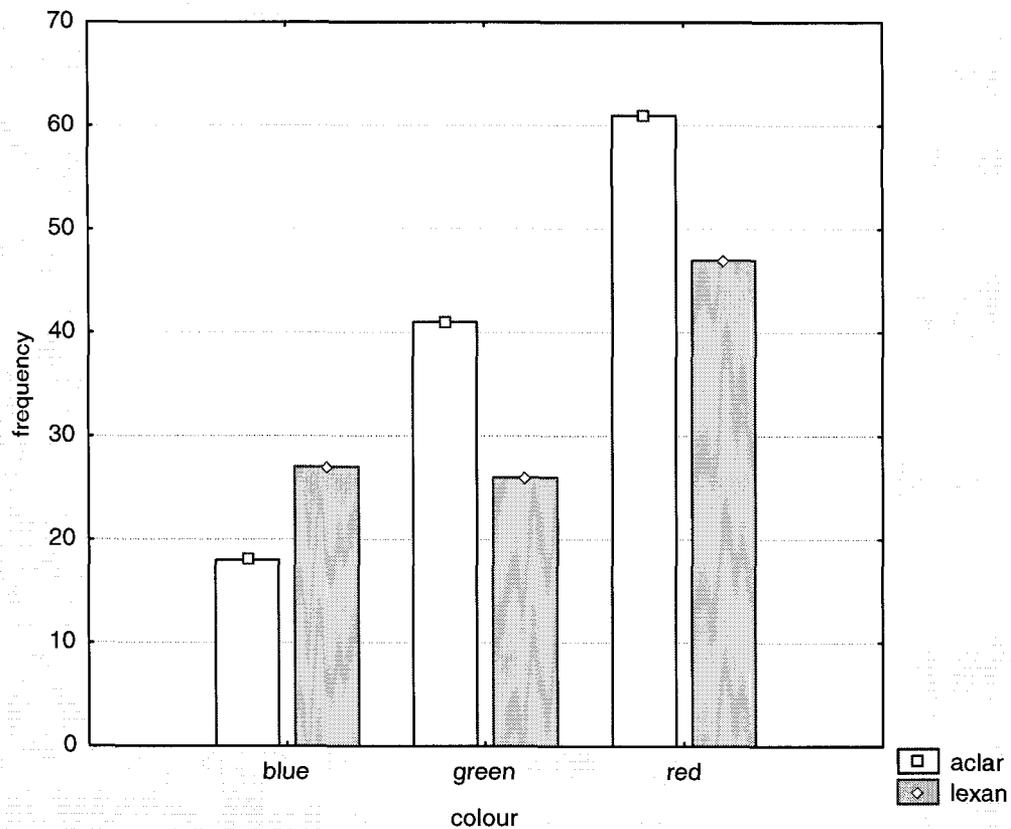


Figure 1.6 The total pooled frequencies for each visible colour (UV-reflecting and UV-absorbing forms combined), for the first 5 seeds selected in each trial.

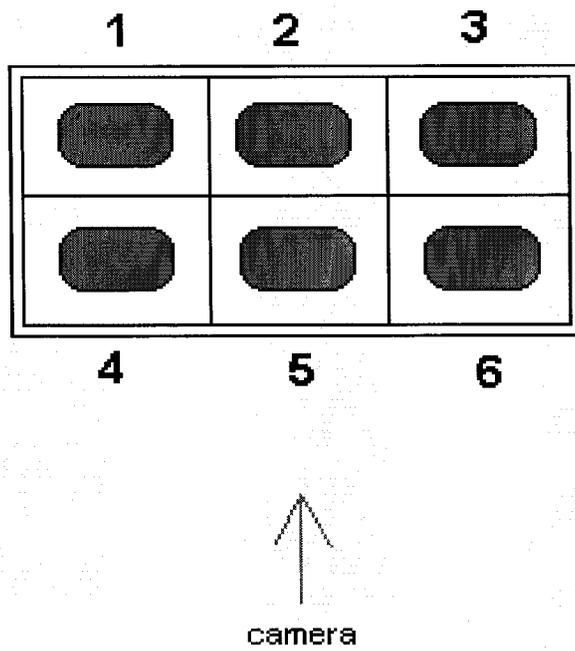


Figure 1.7. Numbering system used to identify positioning on platforms, relative to camera location

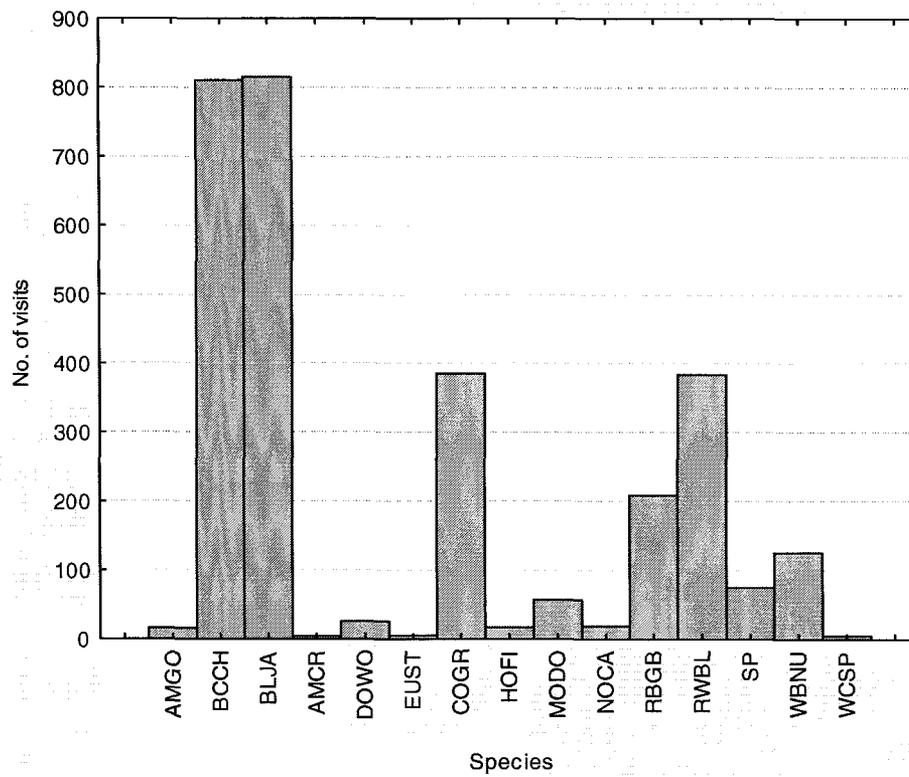


Figure 1.8. the frequency of visitation from each bird species visiting platforms during the entire test period. Latin and common names for species abbreviations are given in appendix 2.

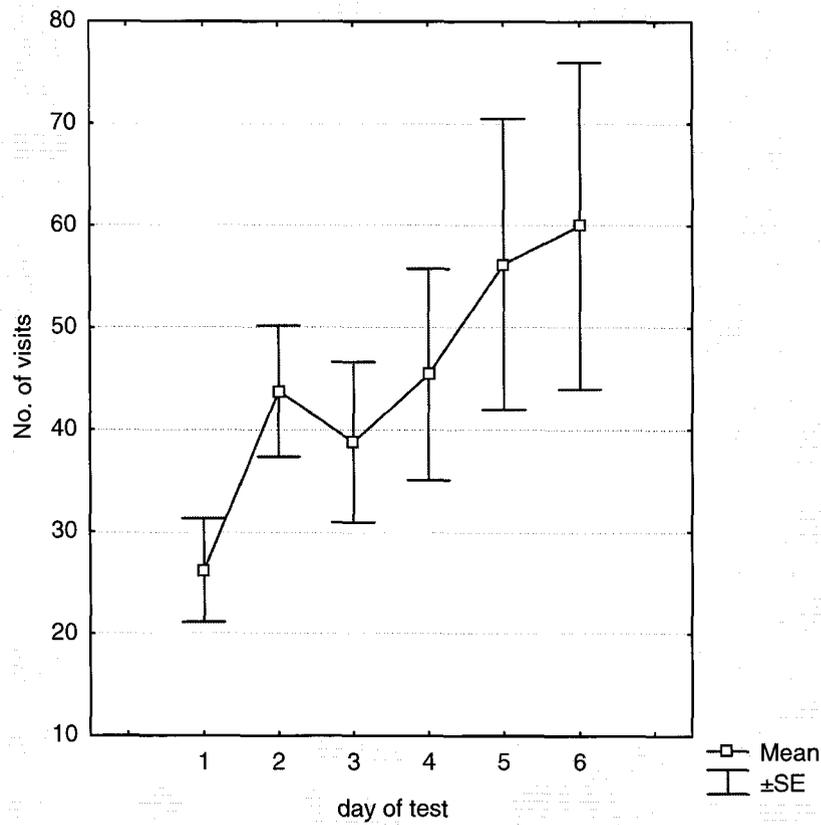


Figure 1.9 The mean number of visits occurring on each day of testing. Whiskers denote one standard error from the mean.

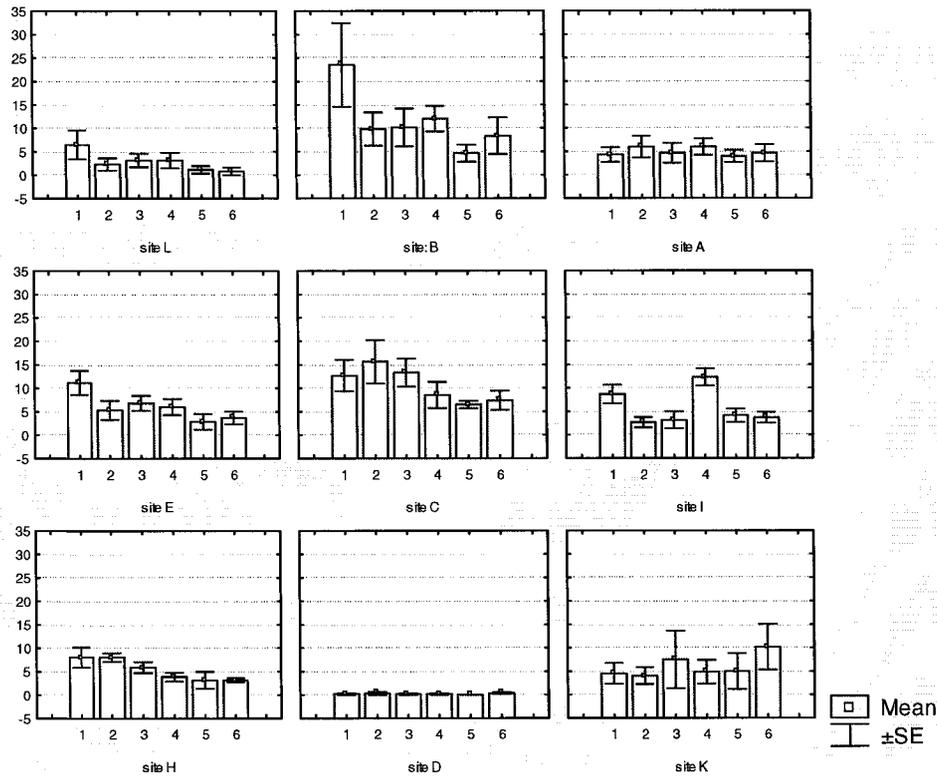


Figure 1.10 Mean frequency of visitation to each position, organized by platform location. Whiskers represent one standard error from the mean.

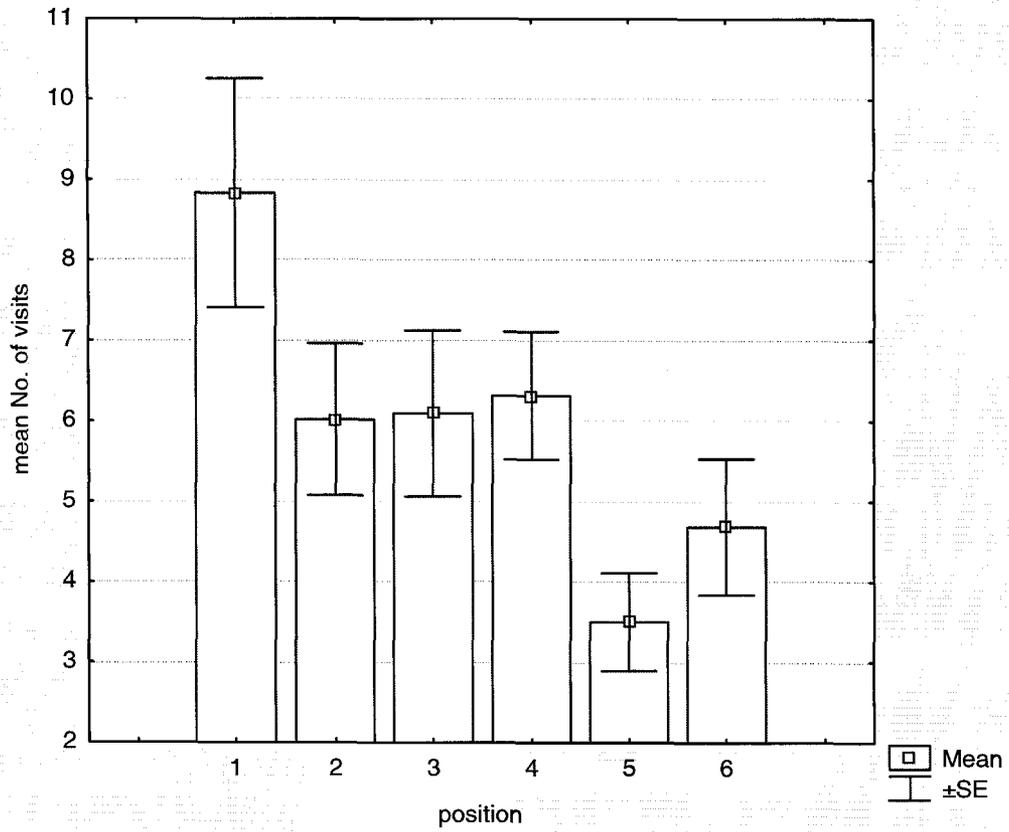


Figure 1.11 The mean frequency of visitation to each platform position during the entire test period. Whiskers denote one standard error from the mean.

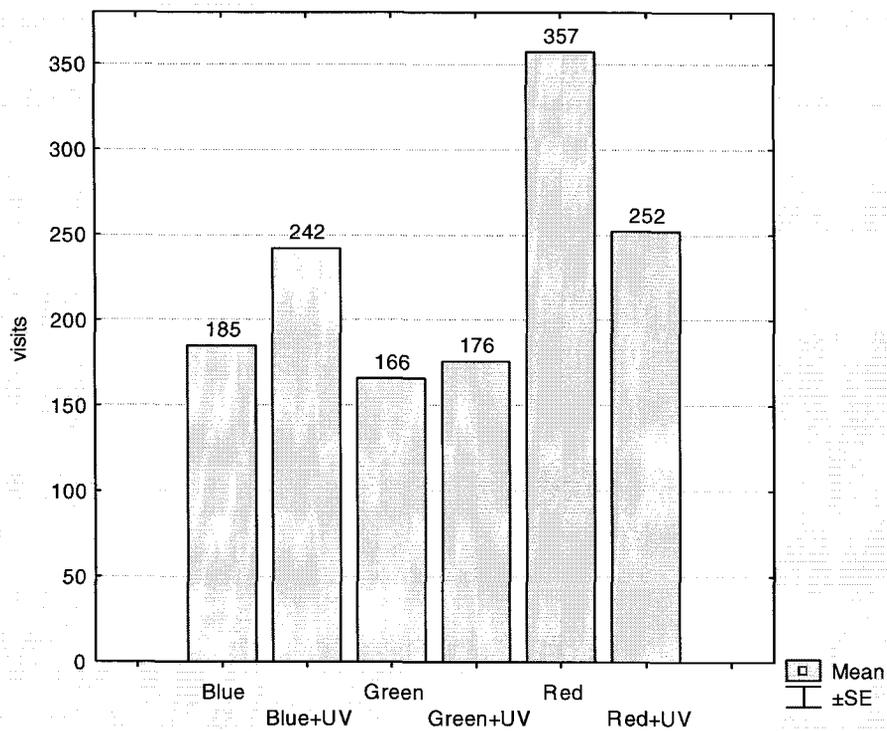


Figure 1.12. Cumulative frequency of visitation to each colour for the entire study period. Visitation values were summed for the first colour in which seeds were removed for a given visit.

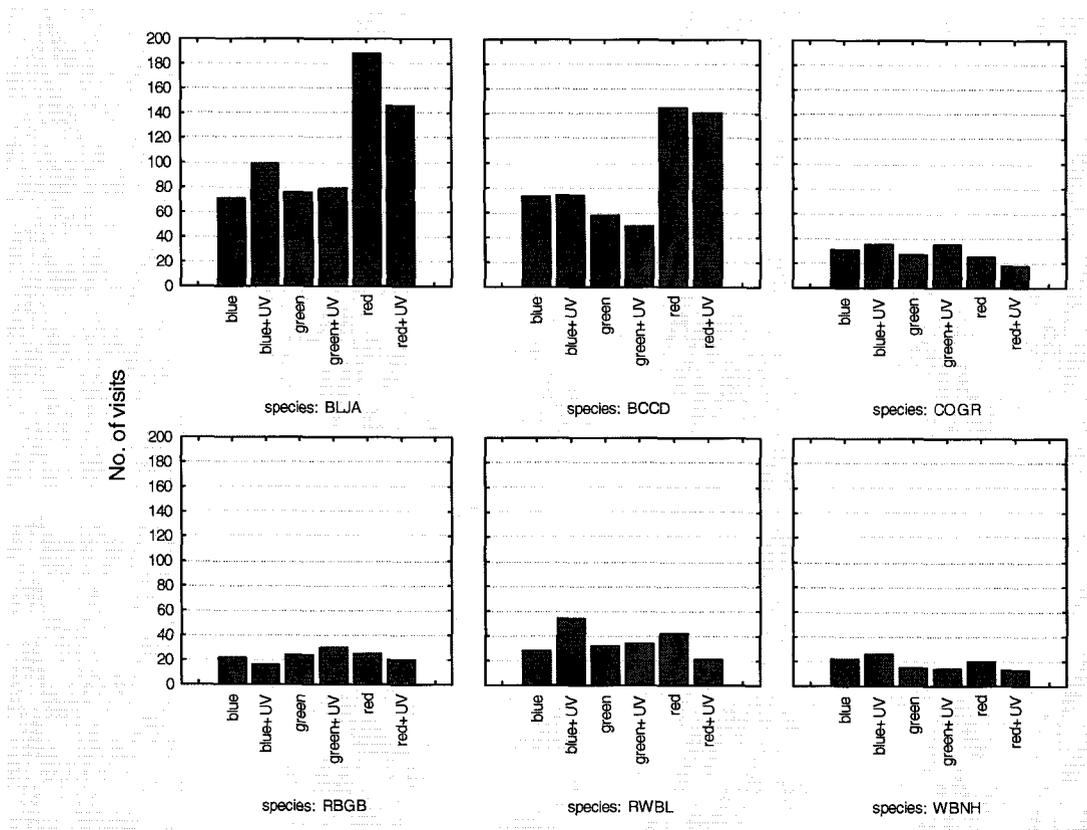


Figure 1.13. The number of visits to each colour for each of the six major species observed visiting platforms. Data includes only the first colour consumed during each visit.

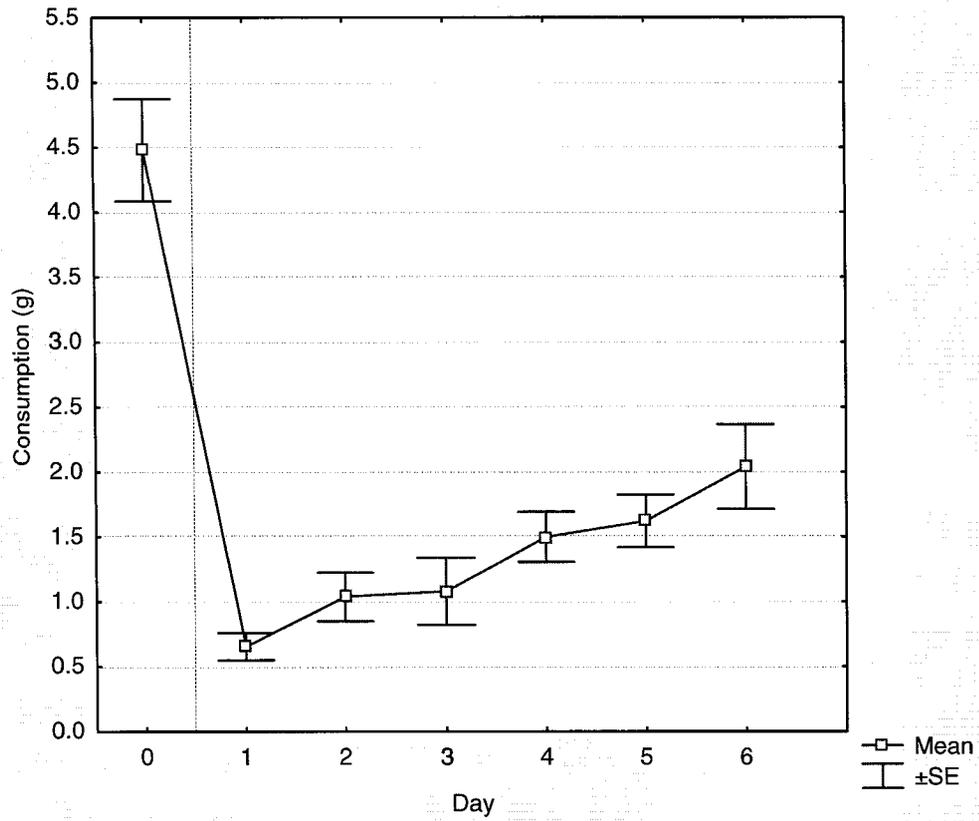


Figure 1.14. The effect of day on mean consumption at all platform locations. Site I was excluded due to squirrel consumption. Whiskers represent one standard error from the mean.

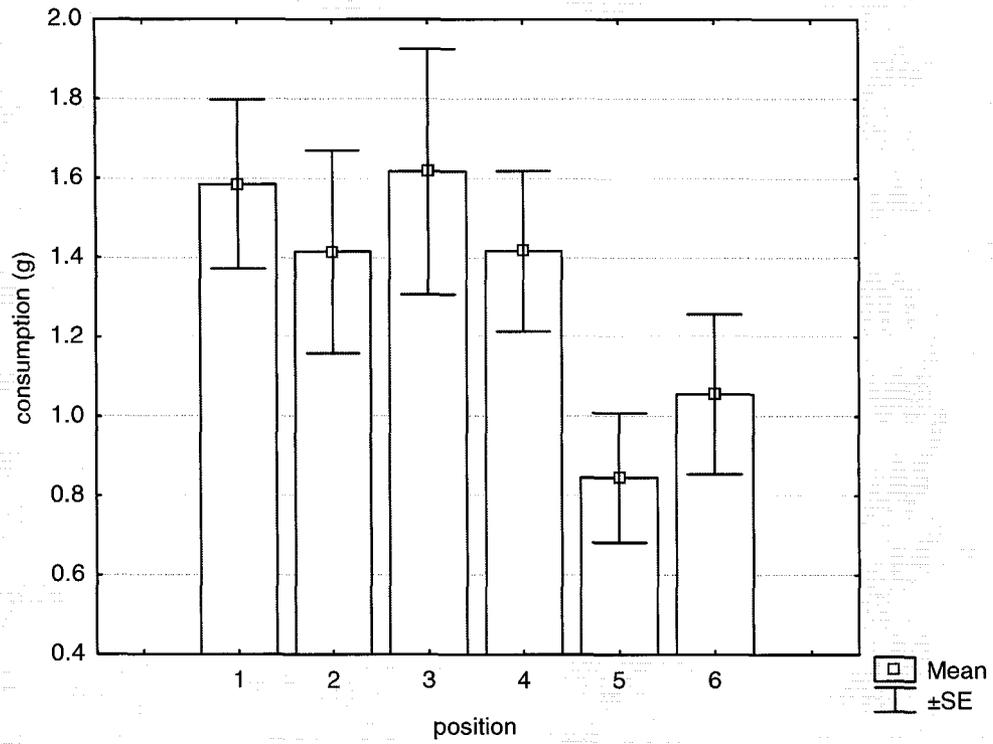


Figure 1.15. The mean consumption occurring at each position (within platform for all sites). Whiskers denote one std. error from the mean.

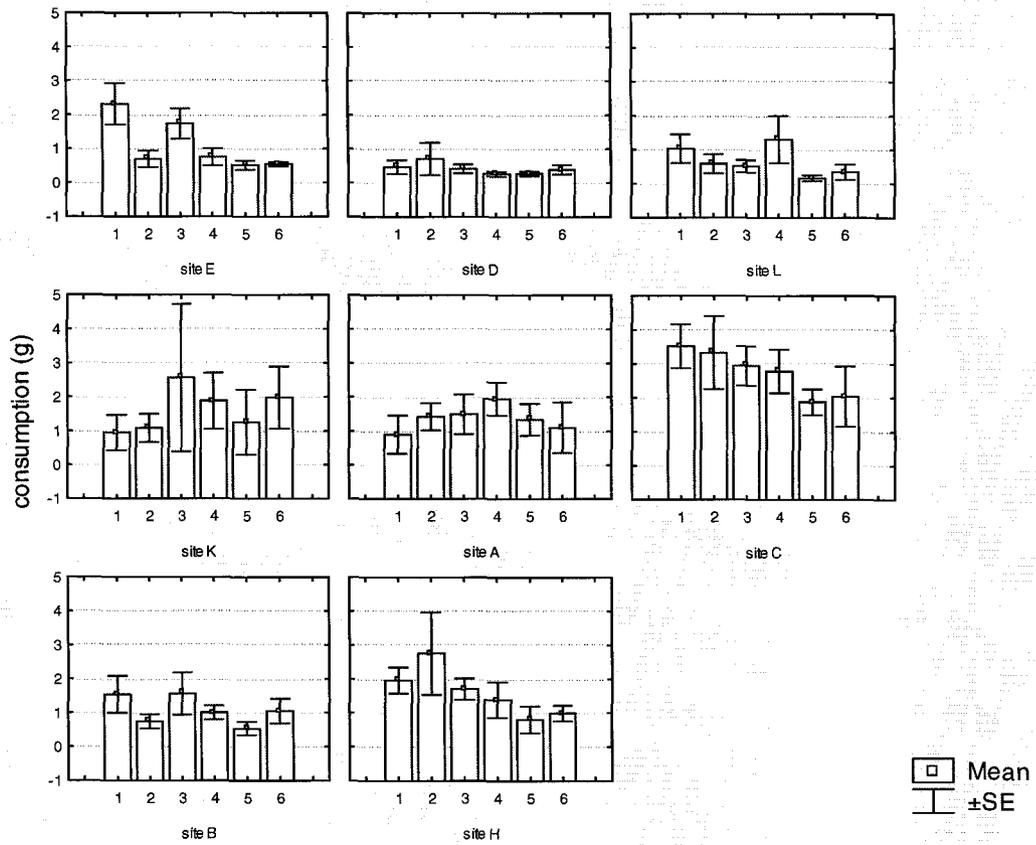


Figure 1.16. The mean consumption occurring at each platform position, organized by location. Whiskers represent one standard error from the mean.

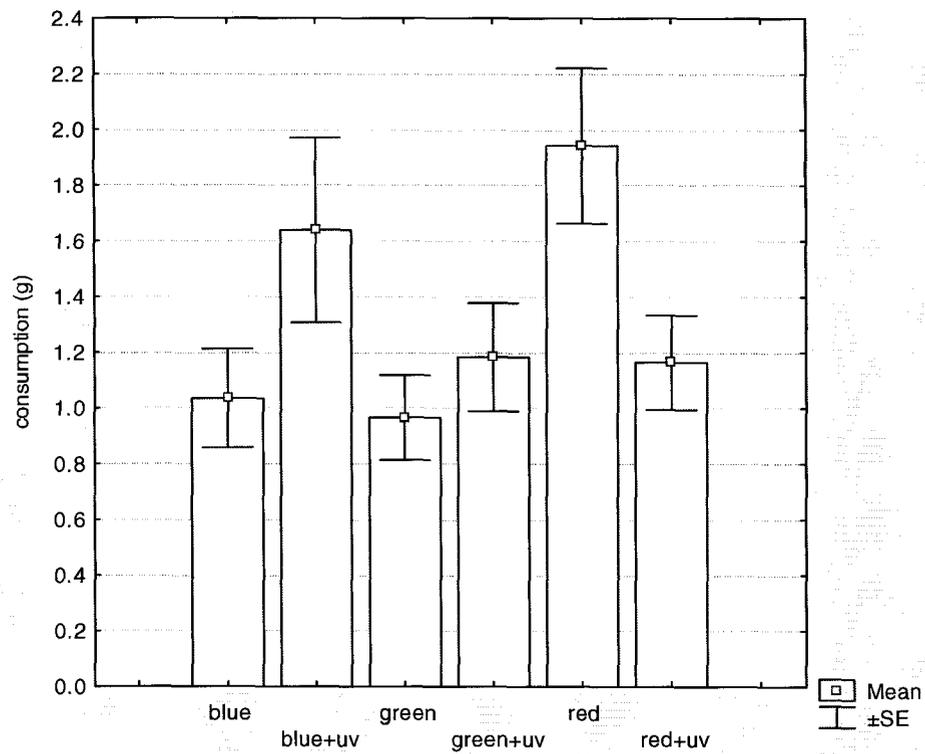


Figure 1.17. The overall mean consumption occurring at each colour for all platforms. Whiskers represent one standard error from the mean.

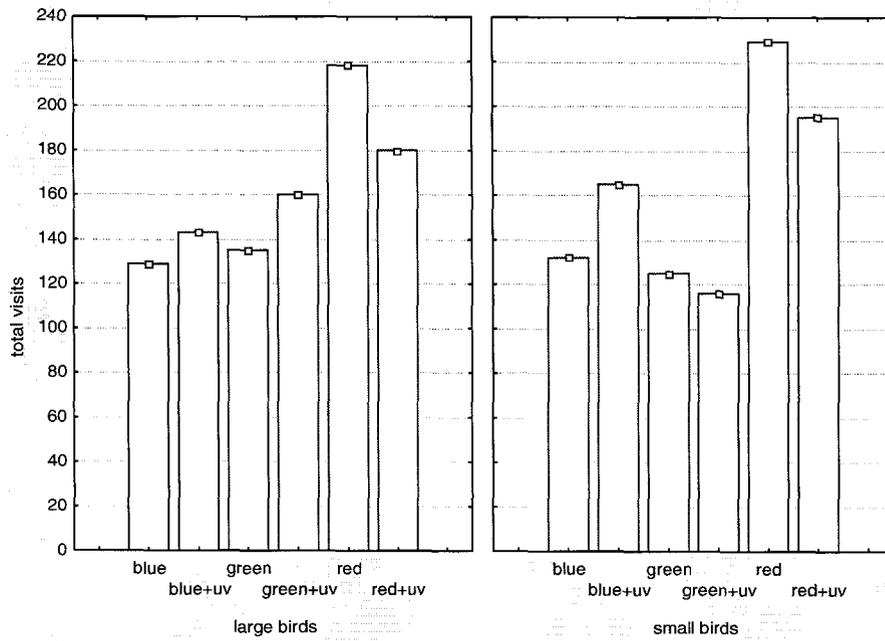
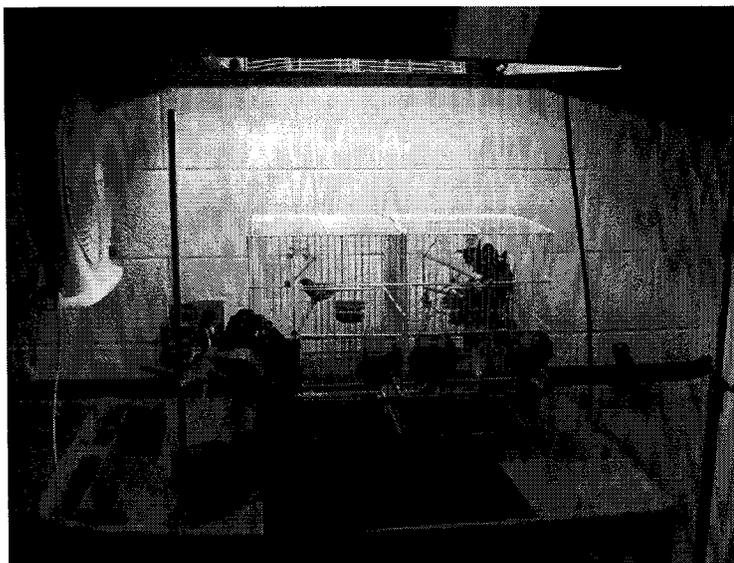


Figure 1.18. Comparison of total visits to each colour on field platforms from larger species (BLJA, COGR, RBGB, RWBL)(left), and all other species (right).

## CHAPTER 1 PHOTOGRAPHS



Photograph 1.1 The laboratory testing setup, equipped with video camera and full-spectrum illumination provided from above. A fan (on left) is used to prevent cage from heating during test.



Photograph 1.2 Sample platform setup (site H), showing platform in foreground and camera housing (in background).

## **CHAPTER 2: ASSESSING BIRD EXPOSURE TO PESTICIDE-TREATED SEED**

### **2.1 INTRODUCTION**

#### **2.1.1 SEED TREATMENTS**

The treatment of seeds with various chemicals (fungicides, insecticides, etc.) provides a potentially efficient method of pest management. Seed treatments deposit the active ingredient where it is most needed, cutting down on wastage, and minimising the need for spraying while reducing operator exposure to potentially harmful chemicals (Prosser et al. 1998). However, these chemicals can pose serious risks to wildlife. Many species of birds forage in agricultural areas and may be exposed to dangerous levels of toxicants from seed treatments. A recent UK study stated that at the recommended application rate for fonofos (an organophosphorous insecticide), birds would easily exceed the lethal dose per day if feeding solely on treated seeds (Pascual & Hart 1997). Indeed, investigations of suspected pesticide poisonings reported in the UK Wildlife Incident Investigation Scheme have repeatedly confirmed that seed treatments do still cause lethal poisonings in the wild (Hart et al. 1999).

Historically, there have been several recordings of bird mortalities in the thousands, as well as declines of predatory species, mostly resulting from ingestion of mercurial and organochlorine seed treatments. Fortunately, most of these highly toxic pesticides have since been deregistered (in North America and

most of the world). Today, the application of pesticides to seeds before planting has become more popular than ever. Although mortality incidents still occur, they are rarely of the magnitude of those observed in the mid-1900's (Greig-Smith 1987b).

Mortality incidents appear to have declined substantially, even though many seed treatment chemicals used today remain very toxic to birds. It is unwise to assume that because we are not seeing as many dead birds in the field, avian mortality incidents are not occurring. A paper by Mineau and Collins (1988) cited several reasons, including poor detection during carcass searches and removal by scavengers, which explain why we are likely not detecting many current incidents of avian mortality from pesticide poisonings. It is also quite possible that these pesticides may still be exerting adverse effects on bird populations through sub-lethal effects (i.e. on reproduction).

### **2.1.2 SEED TREATMENT TOXICITY**

When a pesticide is undergoing review for registration, the Pest Management Regulatory Agency (PMRA) in Canada, and the United States Environmental Protection Agency (USEPA) require several measures of toxicity depending on the use patterns intended for the product in question. Due to the potential exposure of birds to toxic chemicals from the consumption of pesticide-treated seed, a number of avian toxicity tests are required when a chemical is being evaluated for this application. For acute measures of toxicity, an avian oral LD<sub>50</sub>

(median lethal dose) and a 5-day dietary LC50 (median lethal concentration) are determined. To assess more long term effects (usually 21 weeks), a Lowest Observable Adverse Effect Concentration (LOAEC) and/or No Observable Adverse Effect Concentration (NOAEC) is derived from an avian reproduction test. These tests are conducted within the laboratory according to standardized protocols, and are typically only carried out on one or two species: the Bobwhite Quail (*Colinus virginianus*) and the Mallard (*Anas platyrhynchos*), which serve to represent all avian species (the Japanese quail, *Coturnix japonica* is often used in Europe).

These lab-derived hazard levels are used to perform basic risk assessments by incorporating hypothetical measures of exposure. One strategy employed by the USEPA involves estimating the quantity of pesticide available to birds within a square foot of treated area, and comparing this value to the LD<sub>50</sub>. Another approach is to estimate the amount of toxicant ingested per day (using application rates) by a bird consuming only treated seed, and compare this value to the LD<sub>50</sub>. If these methods produce exposure levels >1/2 the LD<sub>50</sub>, it is assumed to present a serious risk to birds. (Avery et al. 1997). In other circumstances, a toxicity-exposure ratio (TER) is estimated, and values <10 are taken to suggest unacceptable risk (more common in Europe).

These risk assessment strategies are considered first tier evaluations and are intended to determine if worst-case scenarios for bird exposure to a given

chemicals could present an unacceptable level of risk. If such risks are predicted, additional risk analyses incorporating additional exposure factors are warranted. Estimating avian exposure to seed treatments is the most difficult aspect of this type of risk assessment. The number, and weighting of exposure factors have been, and continues to be the focus of numerous studies. Factors influencing avian exposure to pesticide-treated seed can be allocated into three groups: those influencing seed availability, residue-dependent factors, and bird-dependent factors.

### **2.1.3 FACTORS AFFECTING AVIAN EXPOSURE**

#### **2.1.3.1 Factors of seed availability**

##### **2.1.3.1.1 Environmental**

Weather and soil conditions are the two most influential environmental factors relating to bird exposure of pesticides on seeds. Heavy rain during planting season may dictate the type of planting used, (ie. switching to broadcast planting which is a not as efficient at seed incorporation)(Hart et al. 1999). Soil conditions, which are largely dependent on weather have been shown to cause up to a 13-fold difference in seed incorporation during planting (de Snoo & Luttik 2004). This finding was in agreement with an earlier study by Davis (1974) who found that exposed wheat seed was highest on fields planted shortly after rainfall.

### 2.1.3.1.2 Agricultural

The number of seeds left exposed on the soil surface is a product of several factors. The equipment used for sowing has been shown to have a significant effect on the percentage of exposed seed left on the soil (Maze et al. 1991). Similarly, the type of sowing (standard vs. precision drilling) will influence seed incorporation (Tamis et al. 1994). The density of exposed seed is generally highest at the ends of the field, where planting devices are lifted from the soil to accommodate turning. A recent UK study found surface seed density to be more than twice (2.1 times) as high at these field-ends, compared to values in field centre (Pascual et al. 1999d). The same research group also studied the effects of sowing depth on exposed seed densities. Manipulation of planting depth (to levels which did not hinder crop yield) was shown to have a highly significant effect on seed incorporation (Pascual et al. 1999c).

Seed spillage is a relatively common occurrence on agricultural fields. A study by Leeuw *et al.* (1995) recorded an average of 2 spills per field. Spills provide an easily detectable source of food, enabling birds to consume large quantities of pesticide in a short period of time. The rapid consumption of treated seed may cause birds to ingest a toxic dose before experiencing any ill effects of the pesticide, which may have occasioned avoidance from the seed (as seen with pigeons foraging on fonofos-treated seed (Pascual & Hart 1997)).

100% incorporation of seeds into the soil is rarely, if ever achievable. However, several measures can be taken by growers to minimise seed availability to birds. Increasing sowing depth, using proper post-sowing techniques, and minimizing seed spills can greatly reduce the potential for avian exposure to pesticide-treated seed (Pascual et al. 1999c; Pascual et al. 1999d).

### **2.1.3.2 Seed residue**

The amount of pesticide residue present on the seed before planting is highly variable between products. Depending on application rate and % active ingredient (a.i.) for each product, an extremely broad range of a.i. residue will be present on the seed before planting. Once seeds are planted, an immediate loss of residue is experienced. One study using fonofos found an average loss of 64.31% during the first day after planting, assumedly from abrasion during the planting process (McKay et al. 1999). On days following planting, a 2% residue decay was recorded. Similarly a study by Davis (1974) demonstrated a loss of up to 27% of dieldrin from treated wheat seed during the first day of planting, followed by a daily loss of 2-3% on subsequent days.

### **2.1.3.3 Bird-dependent factors**

#### **2.1.3.3.1 Avoidance**

On several occasions, the presence of pesticides on seed has been observed invoking an avoidance response in birds (Nelms & Avery 1997; Pascual & Hart 1997; Avery et al. 1997; McKay et al. 1999; Avery & Decker 1991; Avery et al.

1994). It is not always apparent what properties are triggering the avoidance response. Possibilities include taste, sensory irritation or post-ingestional illness. In the case of fonofos, it appears the latter is causing the avoidance because of the frequent occurrence of regurgitation (Pascual & Hart 1997).

#### **2.1.3.3.2 Regurgitation**

Regurgitation following the ingestion of a pesticide has been observed in several instances. In several cases the regurgitation of fonofos-treated seed prevented death in feral pigeons (*Columbia livia*), despite the birds ingesting more than the median lethal dose for this pesticide (Pascual et al. 1999b). In that study regurgitation was effective in expelling an average of 88% of the ingested treated-seed (in surviving birds). Despite the enormous potential for exposure reduction, the effects of regurgitation are generally not included in avian risk assessments (Hart & Thompson 1995). The intra- and inter-species variability in the extent of regurgitation is very high, and is likely responsible for their omission from most risk analyses (Hart & Thompson 1995; Pascual et al. 1999b).

#### **2.1.3.3.3 Feeding rate**

The presence of high densities of exposed treated-seed in fields (ie. seed spills) allows birds to ingest larger pesticide doses in a shorter period of time. Feeding rate is also influenced by social habits of various species. Studies have found that birds of the same species will consume significantly greater quantities of

seed when foraging in a group situation, than when alone (Clook & Hart 1998). The importance of feeding rate on avian mortality from pesticide ingestion has been suggested as the reason for discrepancies between lab derived toxicity measures and field results (Mineau et al. 1994). An increased feeding rate can result in a lethal pesticide dose before the development of an avoidance response or regurgitation.

Feeding rate may also be influenced by bird hunger and/or body condition. Pascual *et al.* (1999a) found that bird hunger and body condition could delay/override the onset of avoidance, causing birds to ingest toxic doses of pesticide.

#### **2.1.3.3.4 Seed de-husking**

Many bird species de-husk seed before consumption. By doing so, birds are removing the pesticide-laden surface, thereby reducing the amount of residue being ingested. The seed handling involved in de-husking does result in some residue ingestion. However, a study by Avery *et al.* (1997) found that actual residue intakes were 15-40% of theoretical intake (had de-husking not occurred) for several species. The presence of de-husking behaviour is assumed to be dependent on both bird size and bill morphology. Further studies are required to establish which species possess this behaviour, and for which crop types it is relevant (which crop seed is planted with/without husks).

#### **2.1.3.3.5 Bird presence**

Although bird presence in agricultural areas have been well documented (i.e. Birds In Agricultural Areas, (BIAA) online database), there is surprisingly little data available in North America on bird presence on agricultural fields *during planting*. The presence / absence of birds on mature or maturing crops later in the season cannot be used to make predictions about presence and foraging habits during planting due to large seasonal changes in diet experienced by most species (Bird & Smith 1964).

#### **2.1.3.3.6 Crop seed preference**

Studies have found that granivorous birds do prefer certain seed types more than others (Geis 1980). One of best sources of avian crop seed preference comes from Martin *et al.* (1951). In this publication, stomach and crop contents were examined and compiled from thousands of studies to better assess the dietary habits of birds (and other animals)(Martin et al. 1951). Corn, wheat, oat, and to a lesser degree barley, were found to be highly important crops to many bird species. Unfortunately, this research is only a snapshot of the time period from which the data were collected (early 1900's). When considering agricultural plants, the abundance of each species in the stomach contents of birds will be a reflection of farming efforts during that time period. Perhaps most importantly, the results also show that most typically granivorous birds are indeed foraging primarily on plant material during the planting season (May and June primarily).

However, it is not possible to determine how much of this plant material is newly planted seed or left over from previous harvests.

#### **2.1.3.3.7 Meal size**

Closely related to crop preference, is the amount of seed birds can be expected to consume in the field (meal size). Theoretical values of daily food intake are easily calculated using various documented equations (i.e. Nagy 1987).

However, actual data from the field on meal size for specific birds and crops is not well known, and would be highly valuable for risk assessments. This data would be particularly useful for performing more accurate measures of acute doses for given species and crops. For this very reason, a recent study was conducted in the UK to gather field data on local bird species, crop seed preferences, and meal size (Prosser & Hart 2005).

Knowledge of these two parameters (bird presence, and meal size (considered a function of crop seed preference)) are extremely important when trying to realistically assess the hazards presented to birds by certain pesticides (Avery et al. 1997). A highly toxic seed treatment may provide less of a hazard to birds if it is used only on less preferred crop types, or consumed only by larger birds. Similarly a moderately toxic seed treatment could be very hazardous if it is used on crop seed which is highly preferred by small birds.

The factors discussed here are some of the better established factors contributing to avian pesticide exposure from treated seed, and is by no means a complete list. The most rigorous attempts at risk analysis could not possibly focus on all factors of exposure. Even in these higher tiered assessments, factors must be weighted and incorporated based on their importance and reliability.

## **2.2 OBJECTIVE**

A recent study was carried out in the UK attempting to provide information on crop seed foraging and bird presence in agricultural fields to assist in local avian risk assessments (Prosser & Hart 2005). In realisation of a shortage of this type of information in North America, we conducted a similar study to provide information on these parameters, relevant to local avian fauna and which can be used for our own detailed risk assessments. Using these data, along with toxicity levels and estimated seed residue levels, we performed lower tier risk assessments using two different strategies. The first assessments used a deterministic model, incorporating field data for comparisons to acute and chronic measures of toxicity for assessing the risk presented to birds from specified seed treatments. Secondly, our field data were also incorporated into acute dose probability-based (probabilistic) risk assessments to strengthen findings from the deterministic assessments. Assessments were carried out on active ingredients (a.i.'s) which are registered for use on the main crops grown in eastern Canada.

## **2.3 MATERIALS AND METHODS**

### **2.3.1 FIELD STUDY**

This field study was carried out on farmland near Ottawa, ON during May, 2005. Research was conducted during this time so that results would coincide with peak planting activity. In April 2005, owners of selected local farms were contacted and asked for their consent in allowing their lands to be used for this study (a list of field sites is available in appendix 1).

#### **2.3.1.1 Bait stations**

Each bait station consisted of a 0.75 x 1.00m feeding surface, consisting of a wood frame and corrugated plastic flooring (photo 2.1, 2.2). The entire feeding surface was covered by burlap and anchored to the ground on each side using conventional plastic tent pegs. A video camera housing was constructed using a ~25cm diameter cardboard tube (50cm height). During testing, a digital camcorder was placed inside the tube on a suspended wood base, and positioned to view the feeding surface through a tinted window (8cm hole). A wooden top was constructed to be placed on the tube at all times. The camera housing was secured in place ~1m from the feeding surface by fastening to wooden stakes driven into the ground. Every effort was made to ensure the visual appearance of the bait stations did not change when testing was in progress. Stations were placed as close as possible to the cultivated portion of the field and in areas expected to have at least moderate bird activity. To

prevent birds from visiting more than one study site, only one station was generally assembled at each farm. Site A and J were on extremely large farms, allowing two stations to be placed on different fields (sites B, and K respectively).

### **2.3.1.2 Food**

#### **2.3.1.2.1 Non-test seed**

Once in place, each bait station was supplied with a generous amount of a mixture of wild bird seed (sunflower, millets, safflower, etc.), and each of the five test seed types (corn, barley, oat, wheat, soybean). Two weeks were allowed before tests were conducted to allow birds to discover and become familiar with feeding at the stations. Each station was attended frequently, and additional seed was supplied as needed during the initial two-week period as well as between test days (generally every two days, depending on station).

#### **2.3.1.2.2 Test seed**

Corn, soybean, wheat, oat, and barley seed were used for this study. These crops were chosen because they represent five of the more prevalently farmed local crops and are therefore relevant to local estimates of exposure to pesticides from seed treatments. Wheat, oat, and barley were purchased from a local agricultural supply store (Ross Agristore, Ashton, ON). Soybean and corn were purchased from Pride seeds. All seed used in this study was untreated, planting-grade crop seed.

### **2.3.1.3 Test procedure**

All tests were conducted for a two-hour period shortly after sunrise. During each test, only one of the five test seed types was present on the tray. Each location was tested once with each crop. The sequence in which each crop was tested, was randomly assigned for each location. On test day, all mixed seed was thoroughly removed from the feeding surface, by using a stiff-wired brush. The crop seed to be tested was then spread onto the feeding surface. A digital camcorder was placed in the camera housing and allowed to run for two hours (two hours was the maximum duration our cameras were operable). Following testing, the remaining crop seed was collected, and substituted for mixed seed.

### **2.3.1.4 Endpoints**

Following the tests, each digital tape was transferred to VHS format for later analysis. Each tape was viewed and data entered for the following endpoints: bird species, sex (where identifiable), duration of visits, number of seeds consumed during each visit, and other relevant notes on foraging behaviour (i.e. presence/absence of de-husking behaviour). The number of seeds consumed during each visit was recorded several ways, often dependent on the foraging behaviour of different species (and to a lesser extent, crop type). For example, Blue Jay consumption was recorded as the number of seeds swallowed because it was easily observable from their head movements.

## **2.3.2 DETERMINISTIC RISK ASSESSMENT**

Our deterministic risk assessment was performed using field data obtained from this study rather than the usual practice of assuming that birds consume treated seed only. Actual ingestion data from our observations enabled us to perform risk analyses using relevant bird species and consumption levels for four of the most prominent locally grown crops (corn, oat, wheat, barley). No analysis was performed on soybeans. We found that few, if any, birds were attracted to this seed type. Using observed values of maximum seed intakes (meal sizes) from the field, theoretical estimates of short-term (acute) and long-term (chronic) exposure were derived. These exposure levels were then compared to established measures of toxicity for each pesticide active ingredient (a.i.)

### **2.3.2.1 Active ingredients**

Information on suggested crops, application rates, and the percent concentration of active ingredient in pesticide formulated products were obtained for 32 currently registered seed treatments available for use in Canada (PMRA Electronic Label Search and Evaluation (ELSE), <http://eddenet.pmra-arla.gc.ca>). Seed treatments were selected with the intent to include the majority of active ingredients available in Canada for the seed types chosen. More than one product with the same active ingredient was selected when large variation was found in either percent a.i. or application rates, which was a frequent occurrence. In total, seventeen a.i.'s were chosen, and evaluated in this risk assessment.

The most common chemicals used for seed treatments are fungicides, and this is represented in the chemicals selected for this risk assessment (Table 2.1).

### **2.3.2.2 Exposure Calculations**

#### **2.3.2.2.1 Acute exposure estimation**

The amount of a.i. on each seed was estimated for each crop type by using product application rates, percent a.i., and measured seed weights. Pesticide product labels typically suggest application rates as the amount of pesticide for a given weight of seed. The units are frequently g/25kg seed, or ml/25kg seed. When the latter was offered, the specific gravity of the formulated product (PMRA, pers. comm.) was used to convert the amount of pesticide to grams (if unknown, a value of 1.0 was used as the specific gravity). Only the higher application rates were used to estimate exposure levels (refer to appendix 3 for calculations of a.i./seed).

$$\text{mg a.i. / seed} = \frac{\% \text{ a.i.} \times \text{high appl. rate}}{(25000 / \text{wt per seed (g)})}$$

#### **2.3.2.2.2 Chronic exposure estimation**

Chronic risk assessments were performed using two separate estimates of exposure. One estimate assumes that the total daily consumption of treated seed by each bird is equal to the observed maximum feeding bout for that species and crop (from field study). This is likely an underestimate of intake because, in reality, birds could return to the seed source for subsequent

feedings. However, it may be a reasonable scenario in cases where feed aversion has had a chance to develop between feeding bouts. Daily food intake (FI) was calculated using Nagy's (1987) equation for passerines, which incorporated knowledge of metabolic rates and dietary metabolizable energy

$$\text{FI (g/day)} = 0.398 \times \text{body weight}^{0.850} \text{ (g)}$$

Species-specific body masses were obtained from Dunning (1992). Where separate body masses were offered for each sex, the average value was used. For chronic risk assessments, exposure is evaluated as the dietary concentration of a toxicant (ppm). We estimated dietary concentrations for the a.i. in each pesticide using the following calculation:

$$\text{ppm} = \frac{\text{mg a.i.}}{\text{Kg diet}} = \frac{\% \text{ a.i.} \times \text{high appl. rate}}{(25 \times 1000)}$$

the denominator in this calculation (25 x 1000) converts the units of the application rate (g/25kg) to g/1000kg (equivalent to ppm). The mass (g) of seed consumed during the maximum feeding bout was divided by FI to derive the proportion of FI represented by the single meal. The resulting proportion was multiplied by the a.i. concentration in the seed to transform the single meal into a dietary concentration-equivalent. Chronic measures of toxicity are typically derived from tests where the entire diet consists of treated food. With this worst case assessment in mind, the dietary concentration for each active ingredient if birds fed solely on treated seed, was also used as an exposure estimate for comparison (100% of daily FI consisting of treated seed).

### **2.3.2.3 Toxicity endpoints**

#### **2.3.2.3.1 Acute**

To identify the risk of mortality associated with a single dose from feeding on treated seed, acute measures of toxicity were utilized. The 5% hazardous dose (HD<sub>5</sub>) was selected as a representative measure of acute oral toxicity to birds. Values for each active ingredient were extracted from Mineau *et al.* (2001)(available in appendix 3). The HD<sub>5</sub> is the median lethal dose (LD<sub>50</sub>) occurring at the 5% lower tail end of a sensitivity distribution constructed using available LD<sub>50</sub> values adjusted for compound-specific scaling of toxicity to body mass. This measure provides a conservative estimate of the LD<sub>50</sub>, which theoretically 'protects' 95% of bird species given the weight of birds typically seen in farmland (this is not protection in the true sense of the word because the HD<sub>5</sub> is still based on median lethal doses). For each species, the compound specific HD<sub>5</sub> was multiplied by its body weight (kg)(Dunning Jr. 1992) to obtain a species specific median lethal dose expressed as mg a.i. per bird.

#### **2.3.2.3.2 Chronic**

Our chronic risk assessment estimates the risk presented to birds from specific pesticides assuming birds forage repeatedly on treated seed over an extended period of time. The chronic oral toxicity of a pesticide is traditionally derived from 21-week avian reproduction tests using the Mallard or Bobwhite Quail. The

resulting endpoint is the lowest observable adverse effect concentration (LOAEC or LOEC). LOEC values were extracted from USEPA's one liner database, a summary of proprietary test data publicly available from the USEPA upon request. In situations where both Mallard and Bobwhite Quail were available, the lowest LOEC was used. When only open-ended (greater than) LOEC values were available, the higher value was used and the qualifier (>) was omitted. LOEC values are provided as the dietary concentration of an a.i. in parts per million (ppm)(available in appendix 3). The birds from which these values are derived (Mallard and/or Bobwhite Quail) are much larger than most birds observed foraging on crop seed in this study. Consequently, the dosage (mg a.i./kg body weight/ day) producing the observed reproductive effects must be adjusted on the basis of size-specific food intake rates to obtain an equivalent dosage for birds used in this risk assessment. This adjustment was calculated according to the equation:

$$\text{LOEC dosage (species of interest)} = \text{LOEC (ppm)} \times \frac{\text{Daily FI (kg) / kg body weight (Mallard or Bobwhite)}}{\text{Daily FI (kg) / kg body weight (species of interest)}}$$

where daily food intake, FI (kg) was calculated using Nagy's (1987) equations:

$$\text{Mallard and Bobwhite Quail} = 0.648 \times \text{body weight (g)}^{0.651} / 1000$$

$$\text{Species of interest (for Risk analyses)} = 0.398 \times \text{body weight (g)}^{0.850} / 1000$$

It should be noted that the calculated daily FI / kg body weight is roughly twice as high for the Bobwhite Quail, than for the Mallard. This disparity will result in higher RQ values when risk assessments are performed on ai's for which Bobwhite Quail LOEC values were used. Theoretically, this effect should be

compensated by lower Bobwhite-associated LOEC values (correlating to a Mallard-equivalent dose).

#### **2.3.2.4 Risk quotients**

To assess the level of risk presented by various a.i.'s applied as seed treatments, a risk quotient (RQ) was calculated by dividing the estimated exposure levels by the corresponding measure of toxicity. RQs were only calculated for scenarios where birds were observed feeding in the field study (ie. RQs calculated for Song Sparrows foraging on wheat, but not on corn). For acute assessments, the ingested a.i. (mg) was divided by the species (body weight) specific LD<sub>50</sub> (from HD<sub>5</sub> values). For chronic assessments, the estimated dietary intake concentration (ppm) for each crop was divided by the body weight adjusted LOEC dosage (mg ai/ kg diet). A pesticide was assessed as presenting minimal/negligible risk if the resulting risk quotient (RQ) was less than 0.099. Moderate risk was assigned if the RQ fell in the range of 0.1 – 0.99. A RQ value equal to or greater than 1.0 was taken to indicate that a pesticide presented a high risk to the particular species for the crop in question because exposure exceeded our chosen toxicity thresholds.

#### **2.3.3 PROBABILISTIC RISK ASSESSMENT**

A probabilistic risk assessment was carried out to better quantify the *acute* hazard posed to birds from pesticides which were of moderate or high risk, as indicated by our deterministic assessment. In addition to maximum feeding bouts (used in deterministic assessment), the probabilistic risk assessment was

performed utilizing the full range of observed meal sizes from the field data as well as distributions for other selected variables.

Probabilistic risk analyses have been becoming increasingly popular over the past two decades in recognition of the need to incorporate variability and acknowledge uncertainty in risk assessments. The use of probabilistic analysis is supported by USEPA for conducting pesticide related risk assessments (Thompson 2002). The construction of probability-based models consists of distribution assignment to some or all variables to represent sources of uncertainty. Monte Carlo numerical repeated sampling randomly assigns values to each variable according to their distributions, thus producing the full range of possible outcomes and the probability of each occurring (Warren-Hicks & Moore 1998). The main advantage of these risk assessments is that rather than producing a point estimate for comparison against a predefined risk threshold (deterministic analyses), risk assessors are able to make judgements on risk given the probability of an outcome which considers known sources of variability and uncertainty (Thompson 2002).

A spreadsheet model of the risk assessment calculations was designed using Microsoft® Excel. Where applicable, parameters were fitted, or assigned to the most appropriate distribution (described in following text) using Crystal Ball® 2000 software. This software was used to forecast the probability of individual

death for each species and specified a.i. by performing repeated Monte Carlo simulations of the risk model.

### **2.3.3.1 Toxic endpoints**

Body weight adjusted LD<sub>50</sub> values were used instead of HD<sub>5</sub> values (used in deterministic assessment) as the toxicity level for comparing to theoretical exposures. Studies have shown that LD<sub>50</sub> values do not scale with avian body weight at a 1:1 ratio (Mineau et al. 1996). Since most of the species in our study are very small (<100g), a simple weight adjustment of LD<sub>50</sub> values (from mg/kg to mg/bird) would typically result in an underestimation of risk. Using a database of observed LD<sub>50</sub> values for a large number of pesticide a.i.s (Environment Canada, unpublished), linear regressions of body weight against LD<sub>50</sub>s were attempted with the intent of applying the slope(s) of the best fit line as an a.i.-specific scaling factor. Unfortunately, not enough LD<sub>50</sub> values were available to perform a regression on most of the a.i.'s of concern here.

To remedy this problem, we constructed a distribution of extracted slope values from the existing database (available in Mineau et al. 2001). Values were removed for all regressions performed using less than five LD<sub>50</sub> values (N<5). Of the remaining data, only regressions with p<0.2 were included, leaving slope values for 40 a.i.'s. Using Crystal Ball<sup>®</sup>, these values were found to best conform to a logistic distribution with an average of 0.39 and scale of 0.27. This

distribution was inputted to our model and used to scale LD<sub>50</sub> to body weight as follows (*pers. comm.* B.T. Collins, Env. Canada statistician):

$$Y_{g0} = y_g + b(X_o - x_g)$$

Where:

$Y_{g0}$  = predicted log(LD<sub>50</sub>) of chemical  $g$  for species with log(body weight),  $X_o$

$y_g$  = mean log(LD<sub>50</sub>) of all values for chemical  $g$

$x_g$  = mean log(body weight) of all values for chemical  $g$

$b$  = logistic distribution of slope values (mean=0.39; scale 0.27)

Mean LD<sub>50</sub> values and associated body weights for each a.i. were extracted from an existing database (compiled from various sources).

### **2.3.3.2 Exposure calculations**

#### **2.3.3.2.1 Active ingredient per seed**

This assessment used the same a.i.'s as the deterministic assessment. For several a.i.'s there was very large variation in mg/seed as a result of variation in percent a.i. and suggested application rates based on labels for different formulated products. In order to represent this diversity of products, the maximum rate for each product was entered into the model with an equal probability of occurrence. This assumes that pesticides are always applied at

their maximum suggested rates (a common assumption in lower-tier risk assessments), and that each pesticide receives an equal market share.

#### **2.3.3.2.2 Seeds consumed**

Risk analyses were performed using two measures of seed consumption recorded in the field. The crop-specific maximum seed intake feeding bout for each species was applied to the model (as a constant) and used as one feeding scenario. When >5 individuals of a given species were observed foraging on a crop, an average meal size, and standard deviation was calculated and used in our model (table 2.3). It was assumed this data would follow a normal distribution around the mean (lower tails limited to zero). Using the application rate and either maximum intake, or a normally distributed meal size, a theoretical dose was predicted as: mg (a.i.)/kg (body weight) ingested.

The results of our earlier colour preference study consistently demonstrated a substantial decrease in consumption by wild birds when encountering coloured seed. Since colourants are also used on treated seed, this data was incorporated into two additional probabilistic assessments for RWBLs and BLJAs foraging on diazinon-treated corn seed to illustrate the potential influence of colour on exposure. The average percent decrease in consumption occurring with the introduction of coloured seed and associated standard deviation (day 1 in table 1.7) were entered into the probabilistic model as a normal distribution.

This value was multiplied by the number of seeds consumed to obtain meal sizes for colour treated seed, which were subsequently used to estimate exposure.

### 2.3.3.3 Probability of death calculations

The degree of risk posed to birds from each seed treatment is assessed from the resulting probability of death  $P(\text{death})$  forecasted from each simulation.  $P(\text{death})$  was calculated as follows (*pers. comm.* B.T. Collins, Env. Canada statistician):

$$P(x) = 1 - Z(x)[a_1t + a_2t^2 + a_3t^3] \text{ when } x \geq 0$$

$$\text{or } P(x) = -Z(x)[a_1t + a_2t^2 + a_3t^3] \text{ when } x < 0$$

where:

$$Z(x) = \frac{\text{Exp}(-x^2/2)}{(2\pi)^{1/2}}$$

and  $t = 1/(1 + px)$ ,

given  $x = \text{abs}(\text{standard deviate from LD}_{50})$

$$= \text{abs}([\log(\text{dose ingested}) - \log(\text{LD}_{50})] \times \text{slope})$$

and

$$p = 0.33267$$

$$a_1 = 0.4361836$$

$$a_2 = -0.1201676$$

$$a_3 = 0.9372980$$

The slope referred to in this equation is the probit slope associated with the individual  $\text{LD}_{50}$  tests. Ideally, average dose-response slopes should be derived

for each a.i. However, very few slope values were available for the a.i.'s of interest. As for the scaling factors earlier, we used all available slopes for all a.i.'s within the pesticide database assembled by Mineau et al. (2001) to create a distribution of possible values (N=207). Using Crystal Ball<sup>®</sup> distribution-fitting tools, slope values were determined to follow a lognormal distribution and were entered into the model as such (mean=5.87, std dev=3.84).

## **2.4 RESULTS**

### **2.4.1 BIRD VISITATION**

18 different bird species were recorded visiting the stations during the entire testing period (see table 2.2a and appendix 2 for a list of latin names and abbreviations). A total of 1798 visits were recorded at the stations. Of the birds recorded at the stations, seven species were responsible for over 95% of the total visits (BHCO, BLJA, COGR, RWBL, SAVS, SOSP, WCSP). The most frequently observed species was the Red-winged Blackbird, accounting for ~32% of visits (580 visits).

#### **2.4.1.1. Effect of location**

##### **2.4.1.1.1 Species diversity**

The number of different species visiting each station was highly dependent on study site (table 2.2a). Species diversity at each location ranged from as low as one (site F) to 11 (site C). The most ubiquitous species were the SAVS, SOSP,

and RWBL which were recorded at nearly all locations (recorded at 9, 8, and 8 locations respectively).

#### **2.4.1.1.2 Visit frequency**

A similar trend was observed for the number of visits occurring at the various stations. Not surprisingly, those stations which experienced a high level of species diversity, were generally visited more frequently than those with fewer species. Using a general linear model (GLM) to account for the potential influence of crop seed type, the location of bait stations was shown to have a highly significant effect on the frequency of visits ( $p < 0.001$ ). Visit data were log-transformed to meet assumptions of normality (refer to appendix 4).

#### **2.4.1.2 Effect of field type and margins**

The type of crop being planted in the field in which the bait station was placed did not appear to affect the number or types of birds present at the stations. Stations located on hay fields were responsible for the highest species diversity and visit frequency (site C), and also the lowest (site F had the lowest diversity, and site G had the lowest visitation). Birds visitation was more frequent at locations which had an abundance of cover (usually mature areas), and at stations which were highly conspicuous (eg. Site D).

### **2.4.1.3 Effect of crop type (test seed)**

#### **2.4.1.3.1 Species diversity**

There was minimal variation in the number of species recorded visiting each of the different crop types (table 2.2b). The total number of species ranged from 10 to 12 for each of the crops being tested. The most probable explanation for this is that birds were approaching stations with the intent of foraging on the mixed seed that was normally present at the stations. After encountering the crop seed, the number of return visits was expected to fluctuate depending on the favourability of the crop seed in question.

#### **2.4.1.3.2 Visit frequency**

The total number of visits occurring at each site was relatively consistent among crops, with the exception of soybean. Despite this exception, a GLM model designed to account for the highly significant influence of station location, confirms that crop seed had no effect on the frequency of bird visitation ( $p=0.365$ ).

#### **2.4.1.4 Effect of time**

Each crop was tested at each location for a two-hour period. To examine possible changes occurring over this period of time, the two hour period was divided into 6, 20 minute windows. A slight negative trend was generally observed when time is plotted against visit frequency using this method (figure

2.1). However, this trend was shown to be non-significant for all crops when analysed using a Kruskal-Wallis ANOVA (barley,  $p=0.988$ ; corn,  $p=0.725$ ; oat,  $p=0.718$ ; soybean,  $p=0.503$ , wheat,  $p=0.524$ ). For this analysis the first window (0-20 minutes) was omitted because it was assumed that visitation would be affected by the researcher's presence when introducing the seed at the beginning of the test.

#### **2.4.1.5 Seed consumption**

Birds were observed consuming crop seed during 640 of the 1798 recorded visits. Several species were recorded foraging on Barley, wheat, and oat seed (11, 10 and 8 species respectively). Corn appeared to be less preferred, experiencing consumption from only 4 species. No species were recorded consuming seed during soybean tests (summarized in table 2.3).

##### **2.4.1.5.1 Proportion with consumption**

The proportion of visits for which consumption was recorded provides an indication of the acceptability / preference for each crop seed. The order of seed preference determined by this method was: wheat > oat > barley > corn > soybean (figure 2.2).

## 2.4.2 DETERMINISTIC RISK ASSESSMENT

### 2.4.2.1 Acute Dose

The resulting acute dose RQs, obtained from dividing a theoretical a.i. dose (based on maximum observed intakes in the field) by previously established  $HD_5$  values (Mineau et al. 2001) are summarized in table 2.4. The majority of RQ values indicated a minimal risk to birds ( $RQ < 0.1$ ). However, moderate ( $RQ 0.1-0.99$ ), and high risk ( $RQ > 1.0$ ) were also frequently indicated.

Pesticide treatment of corn seed presented the highest risk to birds. All species observed feeding on corn (RWBL, MODO, MALL, BLJA) were found to be at a high risk of ingesting lethal amounts of certain a.i.'s. The highest RQs encountered from this study were from the scenario of Blue Jays feeding on diazinon-treated corn seed ( $RQ 26.6 - 33.2$ ). Diazinon, imidacloprid, and carboxin were assessed as posing a high risk when applied to corn seed, for all birds observed foraging on corn.

For the other three crops (barley, wheat, oat), the only occurrence of high risk predictions were from carboxin and thiram-treated seeds. Carboxin was responsible for presenting a high level of risk to birds most frequently. This was in part due to its  $HD_5$  value, but also to the fact it was the most widely used of the a.i.'s analysed (suggested for use on all four crop types).

Where multiple formulated products were considered for a given a.i., the variation in application rates did not typically translate to large differences in RQ assessment. However, a few exceptions were found. The most noticeable of which was for Blue Jays feeding on metalaxyl-treated corn. RQs were derived for three metalaxyl pesticides which were highly variable in their suggested application rates. The three resulting RQs (for Blue Jays) were 0.081 (minimal risk), 0.217 (moderate risk), and 1.26 (high risk). It should be noted that the minimal and high risks for metalaxyl were from the same product label. The disparity in associated risk arose from two application rates provided for this product. The application rate resulting in the higher RQ value was listed for 'seed planted for export only', and was considerably higher than the alternate suggested rate (for seed not intended for export).

#### **2.4.2.2 Chronic Dose**

##### **2.4.2.2.1 Complete diet**

Chronic RQs were calculated using dietary concentrations based on suggested maximum application rates for each a.i. and body weight adjusted reproductive LOEC dosages, as defined earlier. The complete diet chronic assessment assumes birds forage solely on treated seed for an extended period of time (e.g. on spills). RQ values for this risk assessment are summarized in table 2.5.

Only four active ingredients did not result in a prediction of high risk for any crop-bird foraging scenario (difenoconazole, fludioxonil, hexaconazole, tebuconazole). Pesticide-treatment of corn was most commonly associated with high risk predictions. Imidacloprid and thiram treatment of corn seed produced extremely high RQ values, occasionally greater than 100 for certain bird species. The highest risk scenario was for Red-winged Blackbirds feeding on thiram-treated corn. For other crop types, thiram and maneb were responsible for the highest RQ values (frequently above 50).

For most a.i.'s, the level of risk (minimal, moderate, or high) was not dependent on the crop or bird species being considered. Typically, those a.i.'s resulting in high RQ values did so for all foraging scenarios (exceptions: captan, metalaxyl, and thiamethoxam).

#### **2.4.2.2.2 Proportion of daily food intake**

Chronic RQ values were estimated using the calculated dietary concentration obtained from repeatedly ingesting a daily dose of treated seed equal to the maximum observed feeding bout for each species. This concentration was then divided by the established LOEC values (following transformation to body-weight specific LOEC daily dosage) to derive each RQ. Using this method, the vast majority of risk assessment scenarios indicated a minimal to moderate level of risk (table 2.6). Of the assessments determined to present a high risk, corn seed was the crop which most consistently presented a high risk to birds as a result of

pesticide application. Imidacloprid treatment of corn was the only a.i. to produce high RQ values for each foraging scenario assessed. Clothianidin, diazinon, and thiram were also determined to present a high risk to most bird species when applied to corn seed (metalaxyl and carboxin also has isolated occurrences of high risk). Blue Jays foraging on thiram-treated corn resulted in the highest RQ value for this assessment (RQ = 38).

With the exception of a few anomalous findings of high risk determined for carboxin and triticonazole, the majority of high RQ values for other crop types (barley, wheat, oat) were calculated for applications of maneb and thiram. These a.i.'s are applied to most (maneb) or all (thiram) crops being considered and were the two most commonly found to present a high risk to birds in this chronic assessment. For a.i.'s which are applied to more than one crop, the level of risk frequently varied between crops. This variation could be attributed to either observed intake rates, or variation in application rates between crop types.

### **2.4.3 PROBABILISTIC RISK ASSESSMENT**

The probabilistic risk model used distributions of several parameters to produce an estimate of P(death). Output for this risk assessment is summarized as P(death) at two percentiles (10% and 50%) from the forecasted distribution in a series of tables (2.7a-h). Results are interpreted at each percentile as: "10% (or 50%) of the time, for individuals of species 'x' foraging in this scenario, a greater than 'y' probability of death is predicted". For purposes of this assessment any

a.i. producing a  $P(\text{death})$  between 0 and 0.5, at least 50% of the time, was considered to present high risk to the species being considered. A  $P(\text{death})$  value greater than 0.5, at the 50<sup>th</sup> percentile was considered a prediction of very high risk. Moderate risk was assigned to a.i.'s resulting in a  $P(\text{death})$  greater than zero at the 10<sup>th</sup> percentile, but equalling zero at the 50<sup>th</sup> percentile (interpreted as  $P(\text{death})=0$ , >50% of the time. Minimal or negligible risk was given to scenarios where no  $P(\text{death})$  was registered at either percentile, which is interpreted as  $P(\text{death})=0$ , >90% of the time.

Figure 2.3 provides two examples of a  $P(\text{death})$  frequency distribution generated from this probabilistic risk assessment. 2.3a. illustrates the very high risk presented to Red-winged blackbirds foraging on diazinon-treated corn. This assessment resulted in a  $P(\text{death}) > 0.896$ , 50% of the time. Figure 2.3b illustrates a less hazardous scenario, presented to Song sparrows foraging on triticonazole-treated barley ( $P(\text{death}) > 0.25$ , 50% of the time (high risk).

### **2.4.3.1 Meal size**

#### **2.4.3.1.1 Corn**

Distributions of meal size for birds were possible for Blue Jays and Red-winged Blackbirds foraging on corn seed. According to this assessment, treatment of corn with diazinon and imidacloprid at suggested maximum application rates

presents a high risk to both of these species. The highest risk was predicted for diazinon, which had a  $P(\text{death}) > 0.4$ , 50% of the time.

#### **2.4.3.1.2 Barley, wheat, and oat**

No mortality was forecasted (at the 50% occurrence level) based on the predicted doses arising from field-derived meal sizes for larger species in this study (e.g. BHCO, BJLA, RWBL). However, for several a.i.'s, certain foraging scenarios resulted in moderate risk categorization.

For sparrow species (SOSP, SAVS, WCSP), relatively large meal sizes (relative to body weight), were reflected in  $P(\text{death})$  predictions. The highest  $P(\text{death})$  for birds foraging on barley, oat, and wheat was predicted for Song Sparrows foraging on the bactericide, TCMTB. For Barley, this scenario resulted in a predicted  $P(\text{death}) > 0.28$ , 50% of the time. The only scenarios that produced a predictions of high risk ( $P(\text{death}) > 0$ , 50% of the time), based on meal size distributions for these crops, were sparrows foraging on TCMTB-treated seed (SAVS on oat and wheat; SOSP and SAVS on barley).

### **2.4.3.2 Maximum feeding bout**

The use of maximum observed seed intakes (from field data) to perform this probabilistic risk assessment produces results which are suitable for comparison with our earlier deterministic risk assessment.

#### **2.4.3.2.1 Corn**

Nearly all foraging scenarios assessed for birds foraging on diazinon and imidacloprid-treated corn seed were determined to present very high risk. Based on the maximum observed seed intakes, Blue Jays were forecasted as being at the highest risk of poisoning from these a.i.'s ( $P(\text{death}) > 0.99$  and  $> 0.96$ , 50% of the time for diazinon and imidacloprid respectively). The remaining three species (MALL, MODO, RWBL), were also predicted as being at very high risk from diazinon, with a  $P(\text{death}) > 0.5$ , 50% of the time. The same risk categorizations hold true for imidacloprid, with the exception of the Mallard ( $P(\text{death}) > 0.47$ , 50% of the time). Thiram, carboxin, clothianidin, and thiamethoxam resulted in predictions of moderate risk for certain foraging scenarios.

#### **2.4.3.2.2 Barley, wheat and oat**

The only predictions of very high risk occurred from the scenarios of Savannah and Song Sparrows foraging on TCMTB-treated barley seed, and Savannah sparrows foraging on TCMTB-treated oat seed. Similar estimates of risk were predicted for birds foraging on treated barley, wheat, and oat seed. The three

sparrow species for which this risk assessment was performed (WCSP, SOSP, SAVS) received similar predictions of risk from these crop seeds. For each of these species several occurrences of moderate and high risk predictions were determined. The a.i.'s most commonly responsible for these predictions were fludioxonil, TCMTB, triticonazole, and hexaconazole.

The utilization of maximum feeding bout values resulted in higher predictions of P(death) for larger birds species in our study (relative to smaller species). For these species (BHCO, COGR, RWBL, MODO, BLJA), predictions of high risk presented from barley, wheat and oat seed were uncommon. The highest predicted risk for these scenarios was presented to Red-winged Blackbirds foraging on TCMTB-treated oat seed ( $P(\text{death}) > 0.34$ , 50% of the time). Other predictions of high risk to larger species were predicted for the same a.i.'s described affecting sparrow species. American Crows and Blue Jays were not predicted as being at high risk from any a.i.'s applied to these three crop types.

#### **2.4.3.3 Effect of colour on risk**

The decrease in consumption resulting from the presence of a colourant observed during field colour preference studies were incorporated into probabilistic risk assessments for Blue Jays and Red-winged blackbirds foraging on diazinon-treated corn seed. These were the only two species which foraged on corn seed to the extent that permitted distributions of meal size to be constructed. For both Blue Jays and Red-winged blackbirds, the probability of

death from one meal was reduced from greater than 0.4, 50% of the time, to nearly zero (50% of the time). An influence of similar magnitude was demonstrated on the analyses performed on maximum feeding bouts.

## **2.5 DISCUSSION**

### **2.5.1 FIELD STUDY**

This field study identified 17 different bird species foraging on agricultural lands near Ottawa, ON, Canada. These species do not necessarily constitute the entire list of species which will forage on locally planted crop seed, but can be considered representative of the more common agriculturally associated birds. Seven species accounted for 95% of visits to our bait stations, with Red-winged Blackbirds comprising roughly 32% of visits.

The timing of this field study proved to be very critical. As mentioned, this study was conducted during May, 2005 to correlate with peak planting activity. The same study was attempted the previous year (in June, 2004). During this first attempt, bird visitation was extremely rare, experiencing only occasional visits by sparrow species. For both studies the same crop types and many of the same locations were used. The only apparent explanation for the discrepancy between years appears to be that by June, most local bird species experience a shift towards a more proteinaceous (insectivorous) diet as their foraging efforts become focused on feeding young. Evidence of this seasonal shift in the diet of

agriculturally associated bird species has been well documented (Martin et al. 1951; Bird & Smith 1964).

Using videotape analysis of visitation to our bait stations we were also able to assess crop preferences of local bird species. As a consequence of our experimental design, the number of visits occurring at each crop type did not vary enough to derive crop seed preference. It was decided that the most accurate strategy for deriving preferences from our field data was to identify the proportion of visits to each crop type for which seeds were consumed. Using this method we determined that bird preference for the seed types used in our study were of the order: wheat > oat > barley > corn > soybean. Soybean was found to be extremely disliked by all birds observed during this study. For the entire duration of this study, no birds were observed consuming soybean seed.

In addition to bird presence and crop seed preference, field data of observed seed intakes were recorded. These three parameters were measured primarily in recognition that there is a current shortage of data of this nature in North America. These data are essential when attempting to perform more realistic assessments of potential risks to birds from pesticide-treated seed. Following this field study, two lower tier risk assessments were performed utilizing the newly acquired information, and targeting seed treatments available for use in Canada.

## **2.5.2 ACUTE ASSESSMENTS**

### **2.5.2.1 Deterministic assessment**

Deterministic assessments were conducted using a RQ derived from dividing theoretical acute doses of various a.i.'s by their respective HD<sub>5</sub> values. Results indicated that several currently registered seed treatments may present a high risk to certain species which were observed feeding on those crops. This risk assessment indicated that the a.i.'s used for treatment of corn were of the greatest concern (diazinon, imidacloprid, and to a lesser extent, captan). Using intake rates observed in the field, all birds consuming corn were determined as being at high risk of poisoning from various a.i.'s.

We also discovered that for the same a.i., the differences in suggested application rates on the labels of different formulated products can be sufficient to produce radically-different RQ values, alternating between minimal, moderate, and high risk of poisoning for a given feeding scenario (e.g. Blue Jays feeding on metalaxyl-treated corn). This was not expected and suggests that risk assessments need to include all registered products for a given a.i. and not merely a few representative products.

### **2.5.2.2 Probabilistic assessment**

In addition to the deterministic assessment, probabilistic assessments were performed to incorporate known sources of variability and acknowledge uncertainty in the parameters used for risk characterisation. Additional

calculations were included enabling risk to be assessed in terms of probability of death occurring under each foraging scenario. When this probabilistic assessment was performed using maximum observed feeding rates, diazinon and imidacloprid-treated corn seed was again forecasted as presenting the highest risk of poisoning.

The findings of high risk presented by diazinon seed treatments are not necessarily surprising. Evaluations of risks to birds from granular formulations have occasionally concluded that the risk of mortality from diazinon is as high as that presented by carbofuran (Hill & Camardese 1984). Carbofuran is a carbamate insecticide which is extremely toxic to birds, and has been responsible for numerous incidents of avian mortality. In recognition of the dangers presented to birds, most granular uses of carbofuran have since been phased out in the United States (Fite et al. 2006). Granular use of diazinon has also resulted in incidents of avian mortality. In 1988, the USEPA cancelled the use of diazinon on sod farms and golf courses because of numerous reports of bird kills (2002). The use of diazinon for seed treatments was also found to be of moderate to extremely high risk to birds in risk assessments conducted by the PMRA (2005). Their assessment found that one diazinon-treated seed could be enough to achieve a dose greater than the LD<sub>50</sub> for smaller bird species (e.g. Red-winged blackbirds, sparrows).

Our findings for imidacloprid seed treatments are not as supported by existing literature. Michael Avery researched the response of Red-winged Blackbirds foraging on imidacloprid-treated rice seed (Avery et al. 1993; Avery et al. 1994). He concluded that imidacloprid presents very little hazard to birds, and in fact has good potential for use as a bird repellent seed treatment. This conclusion was reached after observing that birds developed a very strong avoidance to treated seeds, assumedly a result of post-ingestional illness. On the other hand, bird intoxications from imidacloprid-treated seed are routinely reported in France (Terrier et al. 2002/2003,2004 as well as previous annual reports).

Probabilistic risk assessments were also performed using distributions of meal size (instead of maximum intake values). Although no feeding scenarios were predicted to present a very high risk of poisoning ( $P(\text{death}) > 0.5$ , 50% of the time), high risk was still predicted for a few a.i.'s. The highest predicted risk based on the full distribution of possible meal sizes occurred from the insecticides, diazinon and imidacloprid. Using a distribution of meal sizes reduced the level of risk predicted for larger birds much more than for smaller species (e.g. sparrows). This was because average meal size did not appear to scale with bird size. The reason for this unexpected result is not clear, but may be caused by differences in foraging behaviour. It is possible that larger birds made more trips (with less consumption per trip), and this behaviour was in contrast to smaller species.

Unfortunately it was not possible to identify individual birds during our field study. It is likely a safe assumption that individuals were returning to bait stations over the course of the two hour observation period. At one station, a Red-winged Blackbird was identifiable due to an injured leg (resulting in a very awkward gait). During the two hour observation period, this bird was recorded visiting the station eight times, consuming very small amounts of seed during each visit. Due to potential repeated visits it is very probable that using average meal size underestimates the ingested dose, upon which these risk assessments were performed. It is also likely that our maximum feeding bouts underestimated the amount of seed that birds might ingest in a seed-spill situation.

Our earlier colour preference study indicated that the addition of a colourant to seed caused a substantial decrease in consumption by wild birds (chapter 1). Acknowledging that all pesticide-treated seed is also colour-treated, the degree of avoidance observed in the field colour preference study was incorporated into two sample probabilistic assessments to demonstrate the possible effect this factor may have on probability of death estimates. The mean consumption decrease of greater than 80% from the field study resulted in a very large decrease in probability of death estimates for the two example species (RWBL and BLJA) foraging on diazinon-treated corn. From these results, it appears likely that colour may have the ability to reduce the level of risk substantially for many other foraging scenarios also. However, the true effect of colour is difficult to quantify for many reasons. The response of birds to colours is not only

species specific, but also changes (typically decreases) with repeated encounters (as the colour becomes less novel).

The presence of seed de-husking behaviour could also have a large impact on risk analyses when applicable. The wheat, oat, and barley seed used in our field study were presented without prior removal of the seed husks. The majority of species foraging on these seed types removed husks before consuming (typically determined by the amount of seed handling prior to consumption). Generally, all smaller species (e.g. sparrows) exhibited de-husking behaviour at every opportunity. For larger birds, this behaviour was more variable. Common Grackles and Red-Winged blackbirds were observed de-husking seeds most of the time, while Blue Jays and Mourning Doves rarely, if ever, attempted to de-husk. In addition to bird size, seed type also appears to factor into the occurrence of de-husking behaviour. Although Blue Jays did not attempt to de-husk during our crop preference field study, they were frequently observed removing seed shells when consuming sunflower seed during the colour preference study. Further research on the presence of this behaviour and its effects on pesticide residue exposure are important to confidently permit its consideration into higher-tiered risk assessments.

#### **2.5.2.3 Deterministic vs. probabilistic comparison**

According to our first level deterministic assessment, carboxin was determined to present a high risk of poisoning to birds. The probabilistic assessment predicted

minimal risks from this a.i. Likewise, the deterministic assessment of Blue Jays feeding on captan-treated corn resulted in a suggestion of high risk of poisoning. The same feeding scenario produced a minimal risk when examined using our probabilistic assessment ( $P(\text{death}) = 0.00$ , >90% of the time).

For several other a.i.'s, the probabilistic risk assessment greatly increased the associated level of predicted risk. This was especially true for the only bactericide considered in these assessments, TCMTB. For most feeding scenarios, our deterministic assessment suggested a minimal or marginally moderate risk of poisoning. Using the probabilistic assessment, TCMTB was frequently predicted as presenting a high risk of poisoning, for several feeding scenarios. A similar trend was noticed for fludioxonil, triticonazole, and hexaconazole.

The inconsistency between the two risk assessment strategies may have arisen from various sources. One of the largest differences between the two strategies is the toxicity endpoint used for comparison. The deterministic model uses  $HD_5$  values, while the probabilistic method attempts to use the entire distribution of possible  $LD_{50}$  values from dose-response slopes and scaling factors obtained from a large number of other pesticide active ingredients. There is some indication that this level of uncertainty may not be warranted for all pesticides assessed here. For example, the available data for diazinon suggests that larger-bodied birds may be more sensitive than smaller ones (a negative scaling factor)

which is in contrast to the average tendency for the majority of pesticides. It is likely that the probabilistic model in this case overestimates risk. Nevertheless, both the deterministic and the probabilistic approach gave similar results for this particular active ingredient.

### **2.5.3 CHRONIC ASSESSMENT**

In our chronic deterministic assessment, the proportion of daily food intake attained from the maximum feeding bout was incorporated and used as a 'dilution factor' for calculating a dietary concentration to compare to reproductive LOEC values. This chronic risk assessment assumes that birds ingest the amount of treated seed equal to the maximum feeding bout on a daily basis for an extended period of time. While it is likely that birds are capable of ingesting this amount each day and more following repeat visits, it is unlikely that this seed would be available for an extended period other than in a spill situation. However, the available literature suggests that seed spills are indeed frequent. Whatever the case, results of this assessment produced interesting results. In addition to the a.i.'s deemed to present acute risks to birds (e.g. diazinon, imidacloprid), other a.i.'s, most noticeably maneb and thiram, were consistently found to present a high chronic risk of causing reproductive effects. Although these a.i.'s were of minimal risk according to acute assessments, they may still present a serious risk of reproductive toxicity if a source of treated seed persists. This finding is supported by the USEPA, on the basis of findings from toxicity studies of Mallards foraging on thiram-treated seed (2004). It should be added that,

assuming the true reproductive LOEC is as variable among species as is acute toxicity, our assessment which relied on taking the minimum LOEC value measured from two species likely underestimates the reproductive risk of the a.i.s (Luttik et al. 2005). Also, the regulatory reproductive studies consider only a fraction of the possible reproductive toxicity endpoints that could be used (Mineau 2005).

## **2.6 CONCLUSIONS**

The results of our field study provide useful data on meal size and crop preference for relevant bird species that can be used when performing assessments of risks to birds from seed treatments. Two lower-level risk assessments were performed using field derived data. Results suggested that several a.i.'s used for seed treatments available in Canada present a high risk of poisoning to certain bird species. Based on our findings, a more rigorous risk assessment of certain a.i.'s, primarily the insecticides diazinon and imidacloprid is clearly warranted.

Several additional sources of exposure uncertainty have been identified from previous studies. Unfortunately many of them have not been researched well enough to permit their incorporation into risk assessments without adding an unacceptable level of uncertainty (e.g. pesticide loss during seeding, seed de-husking behaviour, social factors etc.). Further studies should attempt to clarify the contribution of those factors with the greatest potential to influence exposure.

For example, it is possible that a better understanding of the residue loss during the planting process, or presence of de-husking behaviour, which permits their incorporation into risk assessments could reduce the hazards presented by some of these a.i.'s below levels of concern.

Since all pesticide-treated seed is also treated with a colourant, it is expected that this would factor into consumption levels. However, the response of birds to coloured seed is another example of a factor which could potentially contribute to exposure, but has not been researched well enough to be incorporated into risk assessments. The results of our colour preference study demonstrated a consistent decrease in consumption on the first test day, following the introduction of novel seed. When this decrease was incorporated into our probabilistic risk analysis, a dramatic decrease in probability of death predictions were observed. Furthermore, the addition of colour to treated seeds may also contribute to the strength and development of avoidance responses for pesticides which have been shown to cause such effects (i.e. Avery et al. 1994).

## CHAPTER 2 TABLES

Table 2.1. Active ingredients used for risk assessment. All 17 active ingredients are available for use as seed treatments in Canada.

Active Ingredient	Pesticide Type
captan	fungicide
carboxin	fungicide
clothianidin	insecticide
diazinon	insecticide
difenoconazole	fungicide
fludioxonil	fungicide
hexaconazole	fungicide
imidacloprid	insecticide
maneb	fungicide
metalaxyl	fungicide
TCMTB	bactericide
tebuconazole	fungicide
thiamethoxam	fungicide
thiophanate-methyl	fungicide
thiram	fungicide
triadimenol	fungicide
triticonazole	fungicide

Table 2.2 This table summarizes the observed presence of each species on bait stations by location (a), and by seed type (b). A checkmark denotes birds were observed at the location, but does not denote seed(s) were consumed.

SITE	AMCR	BCCH	BHCO	BLJA	BRTH	COGR	DOWO	EUST	HAWO	HOSP	MALL	MODO	RWBL	SAVS	SOSP	TRSW	WBNU	WCSP	TOTAL SPECIES
A		✓		✓		✓		✓				✓	✓	✓	✓				8
B						✓							✓	✓					3
C			✓	✓		✓				✓	✓	✓	✓	✓	✓	✓		✓	11
D	✓		✓			✓		✓				✓	✓		✓				7
E			✓	✓		✓	✓	✓					✓	✓	✓		✓	✓	10
F														✓					1
G			✓										✓	✓	✓				4
J				✓	✓	✓			✓			✓	✓	✓	✓			✓	9
K			✓	✓								✓	✓	✓	✓			✓	7
L		✓	✓	✓										✓	✓				5

a.

	AMCR	BCCH	BHCO	BLJA	BRTH	COGR	DOWO	EUST	HAWO	HOSP	MALL	MODO	RWBL	SAVS	SOSP	TRSW	WBNU	WCSP	TOTAL SPECIES
CORN	✓		✓	✓	✓	✓					✓	✓	✓	✓	✓			✓	11
WHEAT			✓	✓		✓		✓		✓		✓	✓	✓	✓			✓	10
BARLEY	✓		✓	✓		✓	✓			✓		✓	✓	✓	✓	✓		✓	12
SOYBEAN		✓	✓	✓		✓				✓		✓	✓	✓	✓			✓	10
OAT			✓	✓		✓		✓	✓			✓	✓	✓	✓		✓	✓	11

b.

Table 2.3. The number of visits with consumption (visits), maximum seed intake per visit (max), and mean seed intake per visit (mean) for all crops. Soybean was not included because no visits with consumption were recorded. Only species that were observed foraging on at least one crop type are included

Species	BARLEY			CORN			OAT			WHEAT		
	visits	max	mean									
AMCR	2	10	4.00									
BHCO	20	42	10.30				12	11	3.75	13	17	6.85
BLJA	2	2	1.50	40	20	5.48	26	27	5.00	15	14	5.27
COGR	18	13	2.83				14	67	13.29	47	48	9.17
EUST										1	1	1.00
HOSP	4	2	1.25							7	4	1.71
MALL				1	92	92.00						
MODO	15	37	14.20	2	15	10.00	4	3	2.00	4	90	44.75
RWBL	34	33	3.97	10	5	2.2	88	71	9.71	63	53	10.22
SAVS	16	38	8.25				18	46	10.89	42	23	7.83
SOSP	19	52	17.63				15	15	5.53	32	27	5.13
TRSW	1	1	1.00									
WCSP	12	33	7.58				2	10	5.50	41	42	9.63

Table 2.4. The following 3 tables are a summary of calculated Acute RQs derived from comparing a theoretical ingested dose, based on maximum feeding bouts to HD<sub>5</sub> values. The absence of data denotes that the a.i. is not intended for use on that crop. RQ<0.01 = minimal risk, RQ = 0.1 – 0.99 = moderate risk, RQ>1.0 = high risk.

2.4a

active	WCSP		SOSP		SAVS		RWBL		MODO		MALL		HOSP		COGR		AMCR		BLJA		BHCO		
	C	O	C	O	C	O	C	O	C	O	C	O	C	O	C	O	C	O	C	O	C	O	
	B	W	B	W	B	W	B	W	B	W	B	W	B	W	B	W	B	W	B	W	B	W	
captan	0		0		0		0.47		0.74		0.51		0		0		0		1.39		0		
captan	0		0		0		0.63		0.98		0.69		0		0		0		1.86		0		
captan	0		0		0		0.84		1.3		0.91		0		0		0		2.47		0		
captan	0		0		0		0.8		1.25		0.87		0		0		0		2.35		0		
carboxin	0	1.95	0	4.09	0	0	6.74	6.38	10.5	0.14	7.29	0	0	0	0	3.02	0	0	19.8	1.78	0	1.28	
	8	7.87	17.7	7.08	18	6.15	3.7	4.59	2.15	4.03	0	0	0.51	0.79	0.73	2.08	0.16	0	0.16	0.89	6.11	1.91	
carboxin		2.03		4.26		0		6.66		0.15		0		0		3.15		0		1.86		1.34	
	10.4	10.3	23	9.24	23.5	8.02	4.83	5.99	2.8	5.26	0	0	0.66	1.03	0.95	2.72	0.2	0	0.21	1.16	7.97	2.49	
clothianidin	0		0		0		1.16		1.79		1.25		0		0		0		3.39		0		
diazinon	0		0		0		9.08		14.1		9.82		0		0		0		26.6		0		
diazinon	0		0		0		9.44		14.6		10.2		0		0		0		27.7		0		
diazinon	0		0		0		11.3		17.6		12.2		0		0		0		33.2		0		

2.4b

active ingredient	WCSP		SOSP		SAVS		RWBL		MODO		MALL		HOSP		COGR		AMCR		BLJA		BHCO			
	C	O	C	O	C	O	C	O	C	O	C	O	C	O	C	O	C	O	C	O	C	O		
	B	W	B	W	B	W	B	W	B	W	B	W	B	W	B	W	B	W	B	W	B	W		
difenoconazole	0		0		0		0.01		0.02		0.01		0		0		0		0.03		0			
	0.06	0.06	0.12	0.05	0.13	0.04	0.03	0.03	0.02	0.03	0	0	0	0.01	0.01	0.01	0	0	0	0.01	0.04	0.01		
difenoconazole	0		0		0		0.01		0.02		0.01		0		0		0		0.03		0			
	0.05	0.05	0.12	0.05	0.12	0.04	0.03	0.03	0.01	0.03	0	0	0	0.01	0.01	0.01	0	0	0	0.01	0.04	0.01		
fludioxonil	0		0		0		0		0.01		0		0		0		0		0.01		0			
hexaconazole																								
	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
imidacloprid	0		0		0		<b>6.29</b>		<b>9.76</b>		<b>6.8</b>		0		0		0		<b>18.4</b>		0			
imidacloprid	0		0		0		<b>2.69</b>		<b>4.17</b>		<b>2.9</b>		0		0		0		<b>7.87</b>		0			
maneb		0.07		0.15		0		0.24		0.01		0		0		0.11		0		0.07		0.05		
	0.21	0.16	0.47	0.15	0.48	0.13	0.1	0.1	0.06	0.08	0	0	0.01	0.02	0.02	0.04	0	0	0	0.02	0.16	0.04		
maneb		0.06		0.13		0		0.2		0		0		0		0.1		0		0.06		0.04		
	0.18	0.11	0.41	0.1	0.41	0.09	0.09	0.06	0.05	0.06	0	0	0.01	0.01	0.02	0.03	0	0	0	0.01	0.14	0.03		
metalaxyl	0		0		0		0.07		0.12		0.08		0		0		0		0.22		0			
metalaxyl	0	0.02	0	0.04	0	0	0.03	0.06	0.04	0	0.03	0	0	0	0	0.03	0	0	0.08	0.02	0	0.01		
	0.08	0.07	0.17	0.07	0.17	0.06	0.03	0.04	0.02	0.04	0	0	0	0.01	0.01	0.02	0	0	0	0.01	0.06	0.02		
metalaxyl	0	0.05	0	0.11	0	0	0.43	0.16	0.67	0	0.46	0	0	0	0	0.08	0	0	<b>1.26</b>	0.05	0	0.03		
	0.21	0.2	0.45	0.18	0.46	0.16	0.1	0.12	0.06	0.1	0	0	0.01	0.02	0.02	0.05	0	0	0	0.02	0.16	0.05		
TCMTB		0.07		0.14		0		0.22		0		0		0		0.1		0		0.06		0.04		
	0.2	0.16	0.44	0.14	0.44	0.12	0.09	0.09	0.05	0.08	0	0	0.01	0.02	0.02	0.04	0	0	0	0.02	0.15	0.04		

2.4c

active ingredient	WCSP		SOSP		SAVS		RWBL		MODO		MALL		HOSP		COGR		AMCR		BLJA		BHCO		
	C	O	C	O	C	O	C	O	C	O	C	O	C	O	C	O	C	O	C	O	C	O	
tebuconazole	0	0	0.01	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
thiamethoxam	0	0	0	0	0.19	0	0.29	0	0.2	0	0	0	0	0	0	0	0	0.55	0	0	0	0	
thiophanate-methyl	0.15	0.15	0.33	0.13	0.34	0.12	0.07	0.09	0.04	0.08	0	0	0.01	0.01	0.01	0.04	0	0	0	0.02	0.12	0.04	
thiram	0	0	0	0	0	0	0.03	0.04	0.04	0.03	0	0	0	0	0	0	0	0.08	0	0	0	0	
thiram	0	0.16	0	0.34	0	0	0.56	0.53	0.86	0.01	0.6	0	0	0	0	0.25	0	0	1.63	0.15	0	0.11	
thiram	0.66	0.65	1.46	0.58	1.48	0.51	0.31	0.38	0.18	0.33	0	0	0.04	0.06	0.06	0.17	0.01	0	0.01	0.07	0.5	0.16	
thiram	0	0	0	0	0	0	0.8	1.24	0.87	0	0	0	0	0	0	0	0	2.35	0	0	0	0	
triadimenol	0.03	0.03	0.06	0.03	0.07	0.02	0.01	0.02	0.01	0.01	0	0	0	0	0	0.01	0	0	0	0	0	0.02	0.01
trificonazole	0.11	0.11	0.25	0.1	0.25	0.09	0.05	0.06	0.03	0.06	0	0	0.01	0.01	0.01	0.03	0	0	0	0.01	0.08	0.03	

Table 2.5. The following 3 tables are a summary of chronic RQs derived from theoretical assumption of birds foraging solely on treated seed for an extended period, compared to body weight adjusted reproductive LOEC values.

2.5a

active ingredient	AMCR		BHCO		BLJA		COGR		HOSP		MALL		MODO		RWBL		SAVS		SOSP		WCSP	
	C	O	C	O	C	O	C	O	C	O	C	O	C	O	C	O	C	O	C	O	C	O
	B	W	B	W	B	W	B	W	B	W	B	W	B	W	B	W	B	W	B	W	B	W
captan					1.34						0.37		1.28		1.45							
captan					1.8						0.5		1.72		1.94							
captan					2.38						0.66		2.27		2.57							
captan					2.28						0.63		2.17		2.45							
carboxin				3.26	6.68	2.94		2.82			1.86		6.37	2.8	7.2	3.17		3.66		3.64		3.46
	2.3		3.26	3.26	2.94	2.94	2.82	2.82	3.49	3.49			2.8	2.8	3.17	3.17	3.66	3.66	3.64	3.64	3.46	3.46
carboxin				3.4		3.07		2.95					2.93		3.31		3.82		3.8		3.61	
	3		4.25	4.25	3.83	3.83	3.68	3.68	4.55	4.55			3.66	3.66	4.13	4.13	4.78	4.78	4.75	4.75	4.51	4.51
carboxin																						
clothianidin					10.1						2.79		9.59		10.8							
diazinon					12.4						3.45		11.8		13.4							
diazinon					12.9						3.59		12.3		13.9							
diazinon					15.5						4.3		14.8		16.7							

2.5b

active ingredient	AMCR		BHCO		BLJA		COGR		HOSP		MALL		MODO		RWBL		SAVS		SOSP		WCSP	
	C	O	C	O	C	O	C	O	C	O	C	O	C	O	C	O	C	O	C	O	C	O
	B	W	B	W	B	W	B	W	B	W	B	W	B	W	B	W	B	W	B	W	B	W
difenoconazole					0.39						0.11		0.37		0.42							
difenoconazole	0.6		0.85	0.85	0.77	0.77	0.74	0.74	0.91	0.91			0.73	0.73	0.83	0.83	0.96	0.96	0.95	0.95	0.91	0.91
difenoconazole					0.38						0.1		0.36		0.41							
difenoconazole	0.59		0.84	0.84	0.75	0.75	0.72	0.72	0.9	0.9			0.72	0.72	0.81	0.81	0.94	0.94	0.93	0.93	0.89	0.89
fludioxonil					0.34						0.09		0.32		0.37							
hexaconazole																						
hexaconazole	0.1		0.14	0.14	0.13	0.13	0.12	0.12	0.15	0.15			0.12	0.12	0.14	0.14	0.16	0.16	0.16	0.16	0.15	0.15
imidacloprid					<b>97.3</b>						<b>27</b>		<b>92.8</b>		<b>105</b>							
imidacloprid					<b>41.6</b>						<b>11.5</b>		<b>39.6</b>		<b>44.8</b>							
maneb			<b>84.6</b>		<b>76.4</b>		<b>73.4</b>						<b>72.9</b>		<b>82.4</b>		<b>95.2</b>		<b>94.7</b>		<b>89.9</b>	
maneb	<b>42.9</b>		<b>60.7</b>	<b>47.8</b>	<b>54.8</b>	<b>43.2</b>	<b>52.7</b>	<b>41.5</b>	<b>65.1</b>	<b>51.3</b>			<b>52.3</b>	<b>41.2</b>	<b>59.1</b>	<b>46.6</b>	<b>68.3</b>	<b>53.8</b>	<b>67.9</b>	<b>53.5</b>	<b>64.5</b>	<b>50.8</b>
maneb			<b>73.4</b>		<b>66.3</b>		<b>63.7</b>						<b>63.2</b>		<b>71.4</b>		<b>82.6</b>		<b>82.1</b>		<b>78</b>	
maneb	<b>37.2</b>		<b>52.7</b>	<b>31.9</b>	<b>47.5</b>	<b>28.8</b>	<b>45.7</b>	<b>27.7</b>	<b>56.4</b>	<b>34.2</b>			<b>45.3</b>	<b>27.5</b>	<b>51.3</b>	<b>31.1</b>	<b>59.2</b>	<b>35.9</b>	<b>58.9</b>	<b>35.7</b>	<b>55.9</b>	<b>33.9</b>
metalaxyl					0.82						0.23		0.78		0.88							
metalaxyl				0.34	0.31	0.31		0.3			0.09		0.29	0.29	0.33	0.33		0.38		0.38		0.36
metalaxyl	0.24		0.34	0.34	0.31	0.31	0.3	0.3	0.36	0.36			0.29	0.29	0.33	0.33	0.38	0.38	0.38	0.38	0.36	0.36
metalaxyl				0.93	<b>4.76</b>	0.84		0.81			<b>1.32</b>		<b>4.54</b>	0.8	<b>5.13</b>	0.91		<b>1.05</b>		<b>1.05</b>		0.99
metalaxyl	0.66		0.93	0.93	0.84	0.84	0.81	0.81	<b>1</b>	<b>1</b>			0.8	0.8	0.91	0.91	<b>1.05</b>	<b>1.05</b>	<b>1.05</b>	<b>1.05</b>	0.99	0.99



Table 2.6. The following 3 tables are a summary of chronic RQs calculated using a daily a.i. doses determined by applying a 'dilution factor' based on maximum feeding bouts, and compared to body weight adjusted LOEC values

2.6a

active ingredient	AMCR		BHCO		BLJA		COGR		HOSP		MALL		MODO		RWBL		SAVS		SOSP		WCSP	
	C	O	C	O	C	O	C	O	C	O	C	O	C	O	C	O	C	O	C	O	C	O
	B	W	B	W	B	W	B	W	B	W	B	W	B	W	B	W	B	W	B	W	B	W
captan					0.3						0.5		0.2		0.1							
captan					0.5						0.7		0.3		0.2							
captan					0.6						0.9		0.3		0.3							
captan					0.6						0.9		0.3		0.2							
carboxin				0.1	<b>1.7</b>	0.2		0.3			0.3		0.9	0.1	0.8	0.7		<b>1.1</b>		0.4		0.2
carboxin	0.1		0.6	0.2	0.1	0.8	0.8	0.2	0.4	0.7			0.2	0.4	0.4	0.5	<b>1.2</b>	0.5	<b>1.6</b>	0.6	0.7	0.7
carboxin				0.1		0.2		0.4						0.1		0.7		1.2		0.4		0.2
carboxin	0.2		0.8	0.2	0.2	0.2	0.9	0.3	0.6	0.9			0.3	0.5	0.6	0.6	<b>1.5</b>	0.7	2.2	0.8	1.0	0.9
carboxin																						
clothianidin					<b>2.6</b>						0.4		<b>1.4</b>		<b>1.7</b>							
diazinon					<b>3.2</b>						0.5		<b>1.7</b>		<b>1.3</b>							
diazinon					<b>3.3</b>						0.5		<b>1.8</b>		<b>1.4</b>							
diazinon					<b>4.0</b>						0.6		<b>2.2</b>		<b>1.6</b>							

2.6b

active ingredient	AMCR		BHCO		BLJA		COGR		HOSP		MALL		MODO		RWBL		SAVS		SOSP		WCSP	
	C	O	C	O	C	O	C	O	C	O	C	O	C	O	C	O	C	O	C	O	C	O
difenoconazole					1.0						0.1		0.5		0.5							
	0.4		0.2	0.5	0.4	0.2	0.2	0.5	0.1	0.2			0.5	0.9	0.1	0.1	0.4	0.1	0.5	0.2	0.2	0.2
difenoconazole					1.0						0.1		0.5		0.4							
	0.4		0.2	0.5	0.4	0.2	0.2	0.5	0.1	0.2			0.5	0.9	1.0	0.1	0.3	0.1	0.4	0.2	0.2	0.2
fludioxonil					0.9						0.1		0.5		0.4							
hexaconazole																						
	0.6		0.3	0.8	0.6	0.3	0.3	0.9	0.2	0.3			0.8	0.2	0.2	0.3	0.5	0.2	0.7	0.3	0.3	0.3
imidacloprid					25						3.7		13.7		1.3							
imidacloprid					1.7						1.6		5.8		4.5							
maneb			3.2		4.2		7.6						0.3		17.4		30		9.3		4.4	
	0.3		11.5	2.7	0.3	1.1	1.3	3.0	0.8	1.2			3.6	5.3	7.3	7.9	21.9	8.7	29	9.1	13.0	1.4
maneb			2.9		3.5		6.6						0.3		15.1		26		8.9		3.9	
	0.2		9.6	1.8	0.2	0.8	1.1	2.0	0.7	0.7			3.1	3.5	6.3	4.7	19.0	5.4	25	6.1	11.2	6.8
metalaxyl					0.2						0.3		0.1		0.9							
metalaxyl			0.1	0.8	0.2		0.4				0.1		0.4	0.1	0.3	0.7		0.1		0.4		0.2
	0.1		0.6	0.2	0.1	0.9	0.7	0.2	0.5	0.7			0.2	0.4	0.5	0.5	0.1	0.6	0.2	0.7	0.7	0.7
metalaxyl			0.4	1.2	0.4		0.8				0.2		0.7	0.4	0.5	0.2		0.3		0.1		0.5
	0.4		0.2	0.5	0.5	0.2	0.2	0.6	0.1	0.2			0.6	0.1	0.1	0.1	0.3	0.2	0.4	0.2	0.2	0.2

2.6c

active ingredient	AMCR		BHCO		BLJA		COGR		HOSP		MALL		MODO		RWBL		SAVS		SOSP		WCSP				
	C	O	C	O	C	O	C	O	C	O	C	O	C	O	C	O	C	O	C	O	C	O	C	O	
tebuconazole																									
	B	W	B	W	B	W	B	W	B	W	B	W	B	W	B	W	B	W	B	W	B	W	B	W	
		0.3		0.3		0.3		0.6						0.3		0.1		0.2		0.7				0.3	
	0.3		0.1	0.4	0.3	0.2	0.1	0.4	0.9	0.1	0.9	0.1		0.4	0.7	0.8	1.0	0.2	0.1	0.3	0.1	0.1	0.1	0.1	
tebuconazole																									
	0.2		0.7	0.2	0.2	1.0	0.9	0.3	0.6	0.9				0.2	0.4	0.5	0.6	0.1	0.7	0.2	0.8	0.9	0.8		
thiamethoxam																									
	0.3		0.1	0.4	0.3	0.2	0.2	0.5	0.1	0.2	0.9			0.3	0.8	1.0	0.1	0.3	0.1	0.4	0.1	0.2	0.2		
thiophanate-methyl																									
thiram																									
	0.2		9.1	2.9	0.2	1.2	2.0	3.1	0.7	1.7	4.0		14.5	0.2	1.9	1.4	17.6		5.5					2.7	
thiram																									
triadimenol																									
	0.1		0.5	0.2	0.1	0.6	0.6	0.2	0.4	0.6			0.2	0.3	0.3	0.4	1.0	0.5	1.3	0.5	0.6	0.6	0.6		
trificonazole																									
	0.2		0.8	0.2	0.2	1.0	0.9	0.3	0.6	0.9			0.2	0.5	0.5	0.6	1.5	0.7	2.0	0.8	0.9	0.9	0.9		

Table 2.7. The following tables summarize the results of a probabilistic risk assessment. A non-zero P(death), at the 50th percentile is considered high risk. P(death)>0.5, 50% of the time is considered to present very high risk of poisoning. Moderate risk is defined as P(death)>0, 10% of the time (and P(death)=0, 50% of the time). A scenario missing values at both percentiles denotes minimal / negligible risk.

Tables are segregated on the basis of crop, and the meal size used in the assessment (average or max), indicated on each table.

2.7a

**CROP = CORN: AVG MEAL**

	Percentiles	BLJA	RWBL
captan	50%	.	.
	10%	.	.
carboxin	50%	.	.
	10%	.	.
clothianidin	50%	.	.
	10%	.	.
diazinon	50%	0.421	0.470
	10%	0.988	1.000
difenoconazole	50%	.	.
	10%	.	.
fludioxonil	50%	.	.
	10%	.	.
imidacloprid	50%	0.24	0.29
	10%	0.91	0.99
metalaxyl	50%	.	.
	10%	.	.
thiamethoxam	50%	.	.
	10%	.	.
thiophanate-methyl	50%	.	.
	10%	.	.
thiram	50%	.	.
	10%	0.01	0.01

2.7b

<b>CROP = CORN: MAX FEEDING BOUT</b>					
	<b>Percentiles</b>	<b>BLJA</b>	<b>RWBL</b>	<b>MODO</b>	<b>MALL</b>
captan	50%	.	.	.	.
	10%	.	.	.	.
carboxin	50%	.	.	.	.
	10%	0.05	0.01	0.01	.
clothianidin	50%	.	.	.	.
	10%	0.01	.	.	.
diazinon	50%	0.992	0.896	0.877	0.651
	10%	1.000	1.000	1.000	0.997
difenoconazole	50%	.	.	.	.
	10%	.	.	.	.
fludioxonil	50%	.	.	.	.
	10%	.	.	.	.
imidacloprid	50%	0.96	0.77	0.73	0.47
	10%	1.00	1.00	1.00	0.95
metalaxyl	50%	.	.	.	.
	10%	0.02	.	.	.
thiamethoxam	50%	.	.	.	.
	10%	0.03	.	.	.
thiophanate-methyl	50%	.	.	.	.
	10%	.	.	.	.
thiram	50%	.	.	.	.
	10%	0.09	0.03	0.03	0.01

2.7c

<b>CROP = WHEAT: AVG MEAL</b>								
	<b>Percentiles</b>	<b>BHCO</b>	<b>COGR</b>	<b>RWBL</b>	<b>SAVS</b>	<b>SOSP</b>	<b>WCSP</b>	<b>BLJA</b>
carboxin	50%	.	.	.	.	.	.	.
	10%	.	.	.	0.01	.	.	.
difenoconazole	50%	.	.	.	.	.	.	.
	10%	.	.	.	0.06	0.03	0.03	.
fludioxonil	50%	.	.	.	.	.	.	.
	10%	0.01	.	0.03	0.59	0.36	0.30	.
hexaconazole	50%	.	.	.	.	.	.	.
	10%	0.02	.	0.03	0.68	0.42	0.41	.
maneb	50%	.	.	.	.	.	.	.
	10%	.	.	.	.	.	.	.
metalaxyl	50%	.	.	.	.	.	.	.
	10%	.	.	.	.	.	.	.
TCMTB	50%	.	.	.	0.01	.	.	.
	10%	0.05	.	0.08	0.80	0.62	0.58	.
tebuconazole	50%	.	.	.	.	.	.	.
	10%	.	.	.	.	.	.	.
thiamethoxam	50%	.	.	.	.	.	.	.
	10%	.	.	.	.	.	.	.
thiram	50%	.	.	.	.	.	.	.
	10%	.	.	.	.	.	.	.
triadimenol	50%	.	.	.	.	.	.	.
	10%	.	.	.	.	.	.	.
triticonazole	50%	.	.	.	.	.	.	.
	10%	0.01	.	0.03	0.57	0.38	0.33	.

2.7d

CROP = WHEAT: MAX FEEDING BOUT										
	Percentiles	BHCO	COGR	RWBL	MOD0	AMCR	WCSP	SOSP	SAVS	BLJA
carboxin	50%	.	.	.	.	.	.	.	.	.
	10%	.	.	0.02	0.01	.	0.05	0.04	0.03	.
difenoconazole	50%	.	.	.	.	.	.	.	.	.
	10%	.	0.01	0.11	0.05	.	0.25	0.21	0.15	.
fludioxonil	50%	.	.	0.01	.	.	0.04	0.03	0.01	.
	10%	0.11	0.13	0.61	0.41	.	0.85	0.79	0.69	0.01
hexaconazole	50%	.	.	.	.	.	0.01	.	.	.
	10%	0.14	0.17	0.70	0.49	.	0.90	0.86	0.78	0.01
maneb	50%	.	.	.	.	.	.	.	.	.
	10%	.	.	.	.	.	.	.	.	.
metalaxyl	50%	.	.	.	.	.	.	.	.	.
	10%	.	.	.	.	.	.	.	.	.
TCMTB	50%	.	.	0.17	0.06	.	0.38	0.32	0.24	.
	10%	0.22	0.26	0.83	0.62	.	0.97	0.95	0.90	0.03
tebuconazole	50%	.	.	.	.	.	.	.	.	.
	10%	.	.	.	.	.	.	.	.	.
thiamethoxam	50%	.	.	.	.	.	.	.	.	.
	10%	.	.	.	.	.	0.01	.	.	.
thiram	50%	.	.	.	.	.	.	.	.	.
	10%	.	.	0.01	.	.	0.02	0.02	0.01	.
triadimenol	50%	.	.	.	.	.	.	.	.	.
	10%	.	.	.	.	.	.	.	.	.
triticonazole	50%	.	.	0.01	.	.	0.04	0.02	0.01	.
	10%	0.11	0.14	0.63	0.42	.	0.86	0.80	0.72	0.01



2.7f

CROP = OAT: MAX FEEDING BOUT		BHCO	COGR	RWBL	MODO	WCSP	SOSP	SAVS	BLJA
Percentiles									
carboxin	50%	.	.	.	.	.	.	.	.
	10%	.	.	0.03	.	.	0.01	.	0.10
fludioxonil	50%	.	.	0.03	.	.	.	.	0.18
	10%	0.03	0.26	0.80	.	0.08	0.37	0.98	0.07
maneb	50%	.	.	.	.	.	.	.	.
	10%	.	.	.	.	.	.	0.01	.
metalaxyl	50%	.	.	.	.	.	.	.	.
	10%	.	.	.	.	.	.	0.01	.
TCMTB	50%	.	0.02	0.34	.	.	0.06	0.71	.
	10%	0.09	0.43	0.95	.	0.17	0.58	1.00	0.15
tebuconazole	50%	.	.	.	.	.	.	0.05	.
	10%	0.01	0.07	0.36	.	0.02	0.12	0.74	0.01
thiram	50%	.	.	.	.	.	.	.	.
	10%	.	.	0.02	.	.	.	0.07	.
triticonazole	50%	.	.	0.03	.	.	.	0.20	.
	10%	0.04	0.28	0.82	.	0.08	0.38	0.99	0.07

2.7g

<b>CROP = BARLEY: AVG MEAL</b>									
	<b>Percentiles</b>	<b>BHCO</b>	<b>COGR</b>	<b>RWBL</b>	<b>SAVS</b>	<b>SOSP</b>	<b>WCSP</b>	<b>HOSP</b>	<b>MODO</b>
carboxin	50%	.	.	.	.	.	.	.	.
	10%	.	.	.	.	0.21	0.01	.	.
difenoconazole	50%	.	.	.	.	.	.	.	.
	10%	.	.	.	.	0.02	.	.	.
fludioxonil	50%	.	.	.	.	0.02	.	.	.
	10%	0.08	.	.	0.82	0.98	0.25	0.06	.
hexaconazole	50%	.	.	.	.	.	.	.	.
	10%	0.06	.	.	0.86	0.97	0.29	0.08	.
maneb	50%	.	.	.	.	.	.	.	.
	10%	.	.	.	.	.	.	.	.
metalaxyl	50%	.	.	.	.	.	.	.	.
	10%	.	.	.	.	0.01	.	.	.
TCMTB	50%	.	.	.	0.05	0.28	.	.	.
	10%	0.18	.	0.01	0.96	1.00	0.45	0.13	.
tebuconazole	50%	.	.	.	.	.	.	.	.
	10%	.	.	.	.	.	.	.	.
thiamethoxam	50%	.	.	.	.	.	.	.	.
	10%	.	.	.	0.01	0.05	.	.	.
thiram	50%	.	.	.	.	.	.	.	.
	10%	.	.	.	0.03	0.10	.	.	.
triadimenol	50%	.	.	.	.	0.00	.	.	.
	10%	.	.	.	.	0.02	.	.	.
triticonazole	50%	.	.	.	.	0.02	.	.	.
	10%	0.07	.	.	0.82	0.98	0.23	0.06	.

2.7h

<b>CROP = BARLEY: MAX FEEDING BOUT</b>									
	<b>Percentiles</b>	<b>BHCO</b>	<b>COGR</b>	<b>RWBL</b>	<b>MODO</b>	<b>AMCR</b>	<b>WCSP</b>	<b>SOSP</b>	<b>SAVS</b>
carboxin	50%	.	.	.	.	.	.	0.01	.
	10%	0.03	.	0.01	.	.	0.04	0.24	0.14
difenoconazole	50%	.	.	.	.	.	.	.	.
	10%	.	.	.	.	.	.	0.03	0.01
fludioxonil	50%	0.01	.	.	.	.	0.01	0.25	0.12
	10%	0.59	.	0.31	0.07	.	0.71	0.99	0.95
hexaconazole	50%	.	.	.	.	.	.	0.07	0.02
	10%	0.63	.	0.34	0.07	.	0.75	0.99	0.96
maneb	50%	.	.	.	.	.	.	.	.
	10%	.	.	.	.	.	.	0.01	.
metalaxyl	50%	.	.	.	.	.	.	.	.
	10%	.	.	.	.	.	.	0.02	0.01
TCMTB	50%	0.14	.	0.03	.	.	0.23	0.76	0.59
	10%	0.79	0.01	0.49	0.14	.	0.89	1.00	1.00
tebuconazole	50%	.	.	.	.	.	.	.	.
	10%	.	.	.	.	.	.	.	.
thiamethoxam	50%	.	.	.	.	.	.	.	.
	10%	.	.	.	.	.	0.01	0.08	0.04
thiram	50%	.	.	.	.	.	.	.	.
	10%	0.01	.	.	.	.	0.02	0.13	0.08
triadimenol	50%	.	.	.	.	.	.	.	.
	10%	.	.	.	.	.	.	0.02	0.01
triticonazole	50%	.	.	.	.	.	0.01	0.25	0.11
	10%	0.59	.	0.31	0.07	.	0.70	0.99	0.96

Table 2.8 A comparison of probabilistic risk analyses done with and without incorporating the potential exposure reduction occurring from colour treatment. This analysis represents the foraging scenarios of Blue Jays (BLJA) and Red-winged Blackbirds (RWBL) foraging on diazinon-treated corn.

	Percentile	BLJA		RWBL	
		meal size dist'n	max intake	meal size dist'n	max intake
w/o colour	50%	0.421	0.992	0.470	0.896
	10%	0.988	1.000	1.000	1.000
w/ colour	50%	0.003	0.507	0.005	0.157
	10%	0.519	0.998	0.971	0.913

## CHAPTER 2 FIGURES

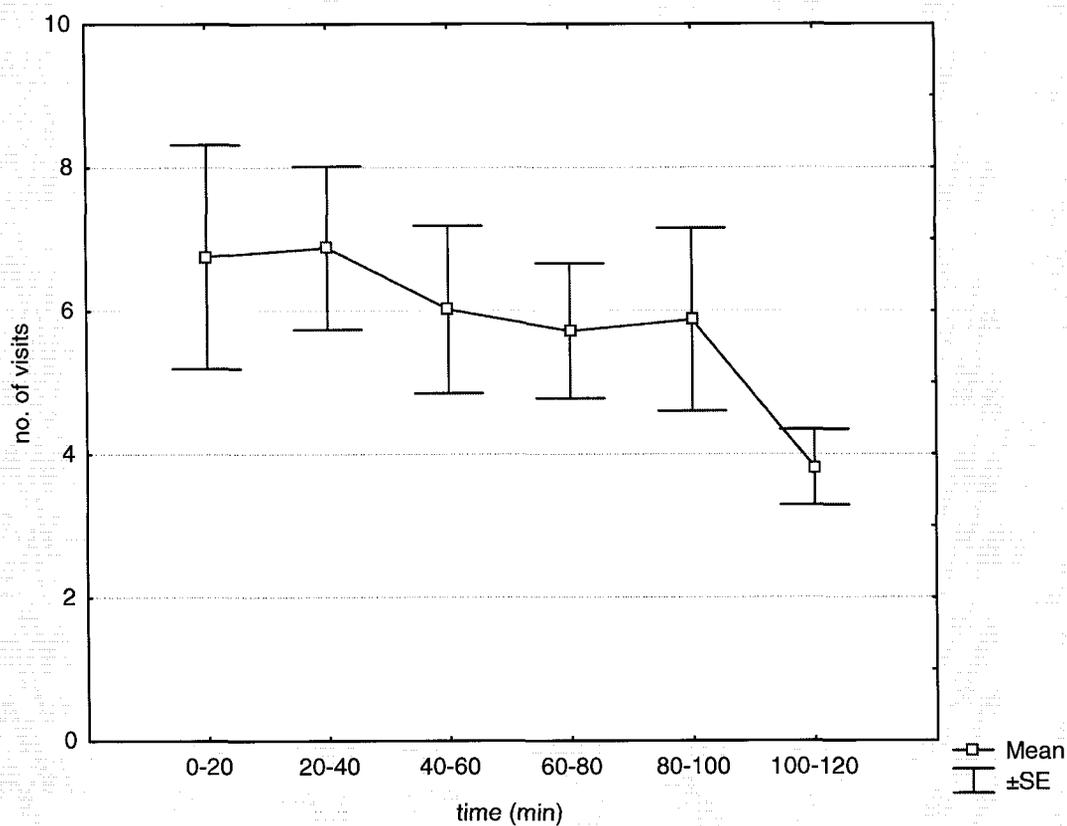
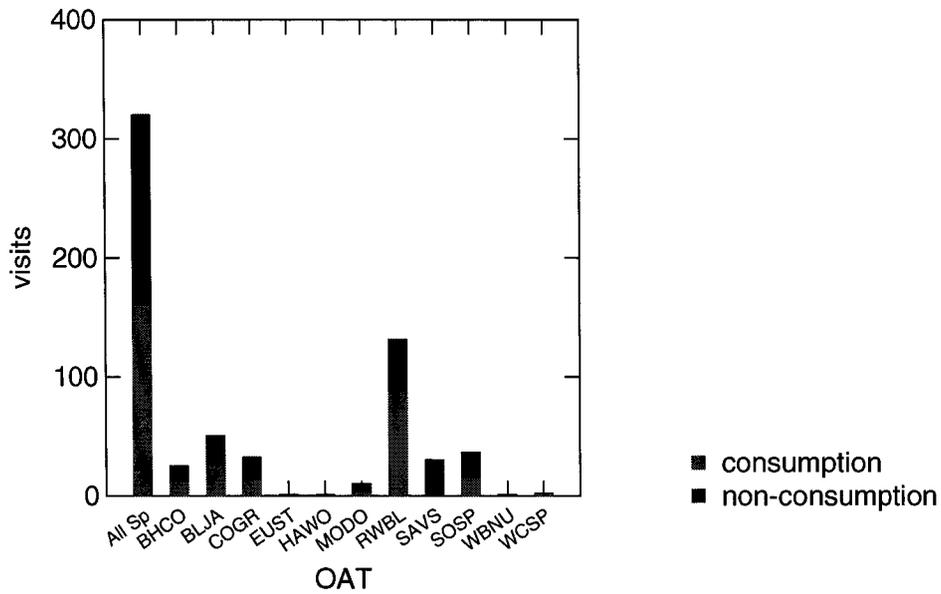
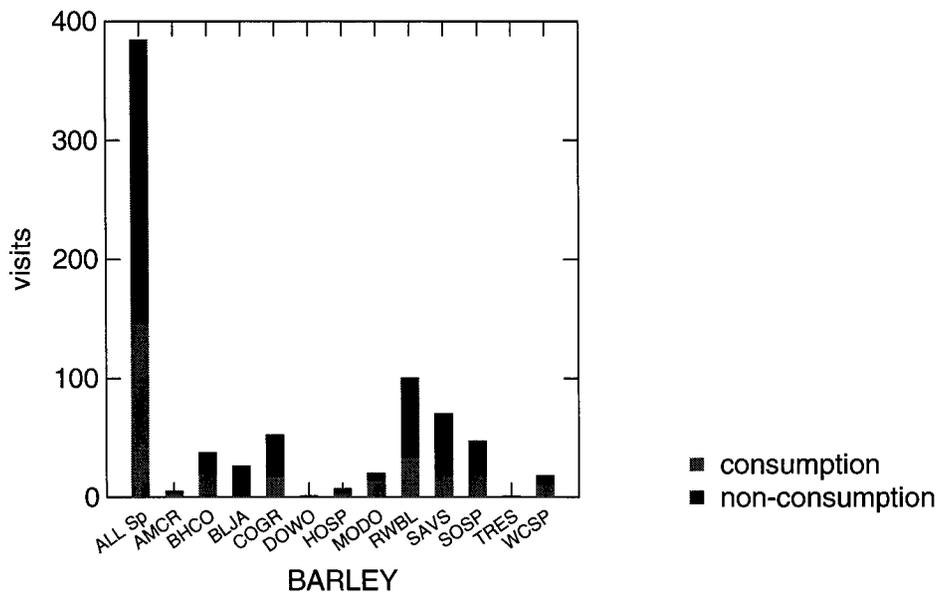


Figure 2.1 The average number of visits occurring over the two hour observational period. The 120min period is broken into 20min intervals. Whiskers denote one standard error from the mean.

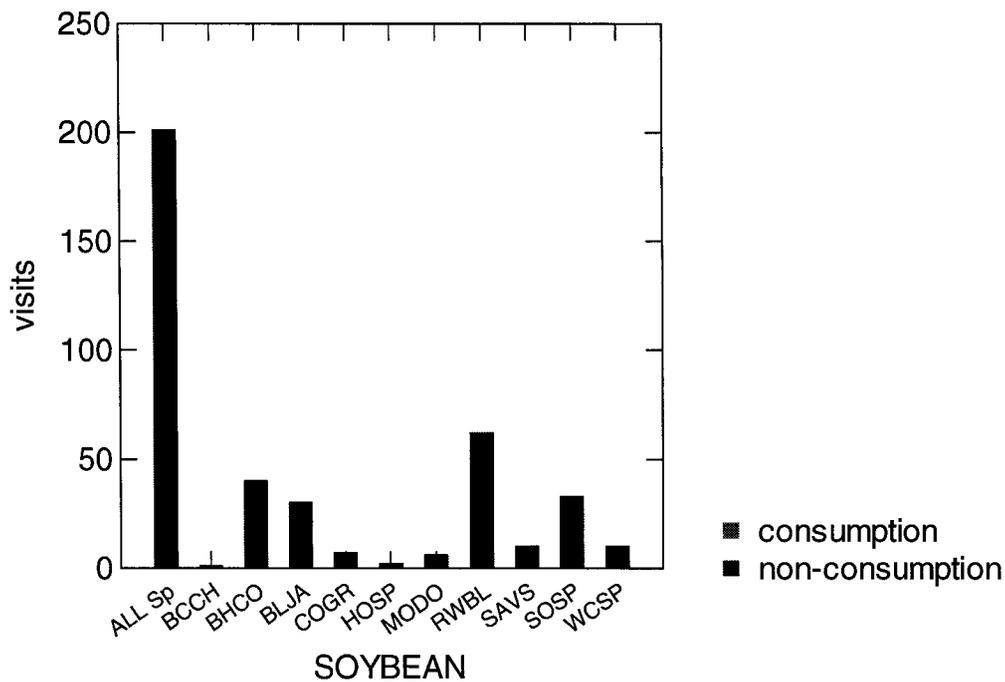
Figure 2.2a-e. The following five figures illustrate the proportion of visits by each species with consumption (light grey / lower portion of the bar) and without consumption (dark grey / upper portion of the bar), for each crop tested.



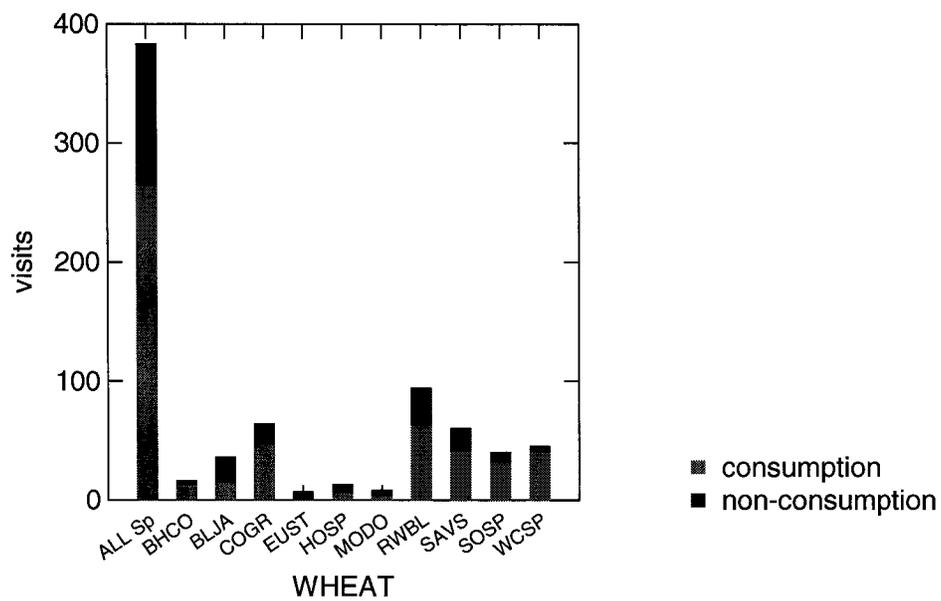
2.2a.



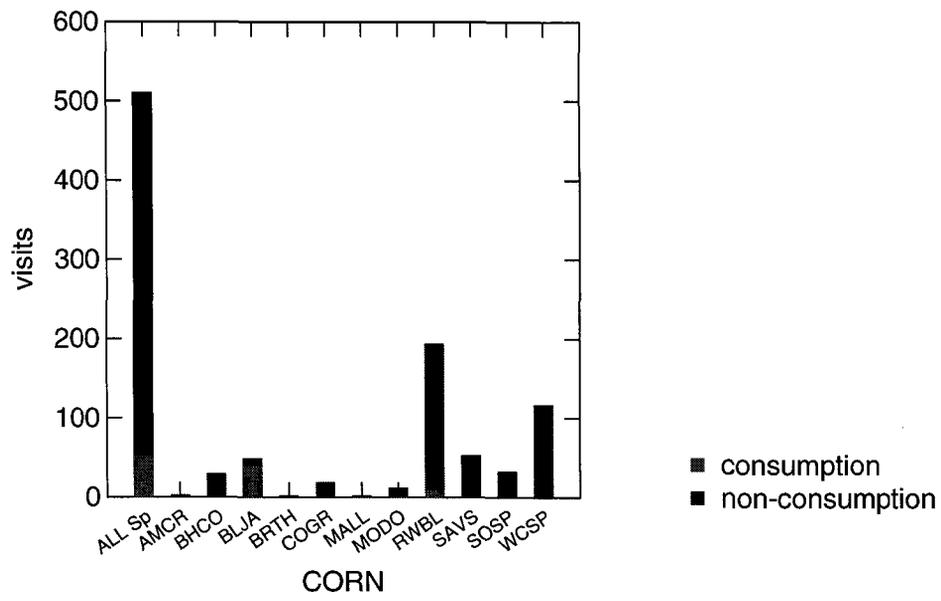
2.2b.



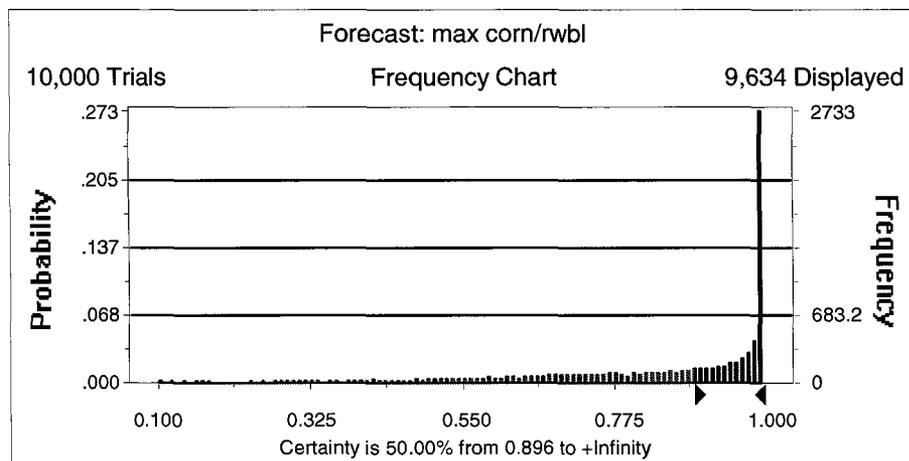
2.2c.



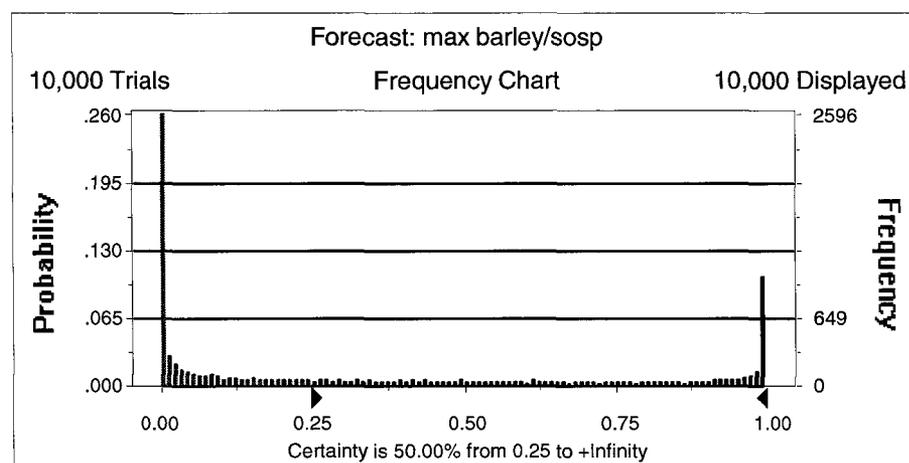
2.2d.



2.2e.



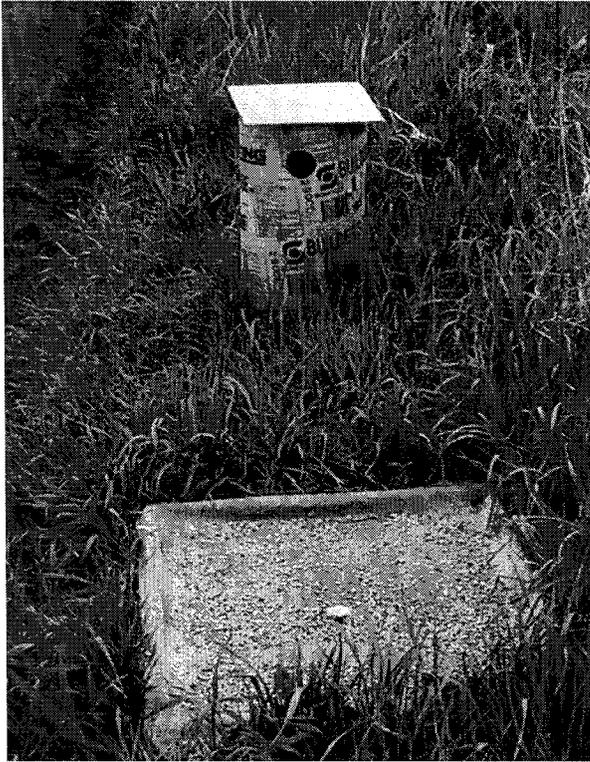
a.



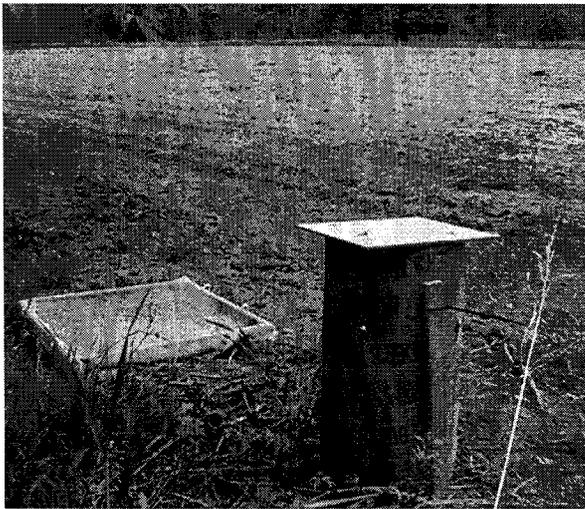
b.

Figure 2.3. Examples of actual output from the probabilistic risk assessment. 2.3a. is the output for the maximum feeding bout of Red-winged blackbirds foraging on diazinon-treated corn (very high risk). 2.3b. illustrates the predicted risk for the maximum feeding bout of Song sparrows foraging on triticonazole-treated barley (high risk).

## CHAPTER 2 PHOTOGRAPHS



2.1a



2.1b

Photograph 2.1 Sample bait stations used in the field. Locations are site L (a.) and site K (b.). The cylindrical structure was used to house the video camera during observations. The foraging surface consisted of a burlap-covered wood frame.

## APPENDICES

### Appendix 1: Field Study Sites

Study site locations:		
Site	Field type	Site description
Barrhaven and surrounding area		
A*	Corn	marshy field margin (fm.)
B*	Corn	grassy fm. Jock river nearby
C*	hay	grassy / shrub fm
D*	soybean	mature wooded fm.
E*	Corn (hay)	mature field edge, w/ creek
F	hay (oat)	grass + shrub f.m
G	hay	field edge. Marsh, mature forest
H*	hay	large neighboring woodlot
I*	oat	large neighboring woodlot
Carp and surrounding area		
J	Corn	field edge, nearby river, mature forest
K*	soybean	wooded fm
L*	hay (wheat / corn)	field edge, young forest

Field sites used for crop preference testing (chapter 2). Sites with asterisk (\*) were used for colour preference tests (chapter 1).

## Appendix 2: Latin names and abbreviations for birds encountered in the field studies

Species	Latin name	Abbreviation
American goldfinch	<i>Carduelis tristis</i>	AMGO
Black-capped chickadee	<i>Poecile atricapilla</i>	BCCH
Brown-headed cowbird	<i>Molothrus ater</i>	BHCO
Blue jay	<i>Cyanocitta cristata</i>	BLJA
Brown thrasher	<i>Toxostoma rufum</i>	BRTH
American crow	<i>Corvus brachyrhynchos</i>	AMCR
Downy woodpecker	<i>Picoides pubescens</i>	DOWO
European starling	<i>Sturnus vulgaris</i>	EUST
Common grackle	<i>Quiscalus quiscula</i>	COGR
Hairy woodpecker	<i>Picoides villosus</i>	HAWO
House finch	<i>Carpodacus mexicanus</i>	HOFI
House sparrow	<i>Passer domesticus</i>	HOSP
Mallard	<i>Anas platyrhynchos</i>	MALL
Mourning dove	<i>Zenaida macroura</i>	MODO
Northern Cardinal	<i>Cardinalis cardinalis</i>	NOCA
Red-winged blackbird	<i>Agelaius phoeniceus</i>	RWBL
Rose-breasted Grosbeak	<i>Pheucticus ludovicianus</i>	RBGB
Savannah sparrow	<i>Passerculus sandwichensis</i>	SAVS
Song sparrow	<i>Melospiza melodia</i>	SOSP
Tree swallow	<i>Tachycineta bicolor</i>	TRSW
White-breasted nuthatch	<i>Sitta carolinensis</i>	WBNU
White-crowned sparrow	<i>Zonotrichia leucophrys</i>	WCSP

### Appendix 3: Risk assessment data tables

Calculations of mg a.i./seed and ppm concentrations for each crop considered in risk assessments. Multiple calculations for the same a.i. represents different products.

Pesticide (a.i.)	% a.i. guarantee	Wheat high (ppm)	Wheat high (mg/ seed)	Barley high (ppm)	Barley high (mg/ seed)	Oats high (ppm)	Oats high (mg/ seed)	Corn high (ppm)	Corn high (mg/ seed)
captan	0.30	0.00	0.00	0.00	0.00	0.00	0.00	672.00	0.15
captan	0.18	0.00	0.00	0.00	0.00	0.00	0.00	900.00	0.20
captan	0.39	0.00	0.00	0.00	0.00	0.00	0.00	1192.55	0.27
captan	0.34	0.00	0.00	0.00	0.00	0.00	0.00	1139.00	0.26
carboxin	0.16	571.43	0.02	571.43	0.02	571.43	0.02	1298.70	0.29
carboxin	0.23	745.20	0.02	745.20	0.03	596.16	0.02	0.00	0.00
carboxin	0.67	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
clothianidin	0.48	0.00	0.00	0.00	0.00	0.00	0.00	2643.17	0.60
diazinon	0.06	0.00	0.00	0.00	0.00	0.00	0.00	300.00	0.07
diazinon	0.50	0.00	0.00	0.00	0.00	0.00	0.00	312.00	0.07
diazinon	0.11	0.00	0.00	0.00	0.00	0.00	0.00	374.00	0.08
difenoconazole	0.03	240.96	0.01	240.96	0.01	0.00	0.00	120.48	0.03
difenoconazole	0.17	235.95	0.01	235.95	0.01	0.00	0.00	117.98	0.03
fludioxonil	0.40	0.00	0.00	0.00	0.00	0.00	0.00	51.13	0.01
hexaconazole	0.01	15.75	0.00	15.75	0.00	0.00	0.00	0.00	0.00
imidacloprid	0.48	0.00	0.00	0.00	0.00	0.00	0.00	2970.24	0.67
imidacloprid	0.48	0.00	0.00	0.00	0.00	0.00	0.00	1268.72	0.29
maneb	0.32	1199.23	0.04	1522.11	0.07	2121.72	0.07	0.00	0.00
maneb	0.50	800.00	0.03	1320.00	0.06	1840.00	0.06	0.00	0.00
metalaxyl	0.32	0.00	0.00	0.00	0.00	0.00	0.00	369.62	0.08
metalaxyl	0.33	138.53	0.00	138.53	0.01	138.53	0.00	138.53	0.03
metalaxyl	0.32	380.08	0.01	380.08	0.02	380.08	0.01	2142.29	0.49
TCMTB	0.30	252.00	0.01	315.00	0.01	441.00	0.02	0.00	0.00
tebuconazole	0.31	32.95	0.00	32.95	0.00	32.95	0.00	0.00	0.00
tebuconazole	0.10	20.06	0.00	20.06	0.00	0.00	0.00	0.00	0.00

## Toxicity data used for risk assessments

Avian HD5 values used for acute deterministic assessment. Test species refers to species used for determination of reproductive LOEC values (used for chronic assessment).

Pesticide (a.i.)	Avian HD5 mg a.i./kg (Mineau et al. 2001)	EPA REPRO LOEC (ppm)	TEST SPECIES
captan	25.32	1000	bobwhite
captan	25.32	1000	bobwhite
captan	25.32	1000	bobwhite
captan	25.32	1000	bobwhite
carboxin	3.44	700	mallard
carboxin	3.44	700	mallard
carboxin	3.44	700	mallard
clothianidin	40.8	525	bobwhite
diazinon	0.59	87	mallard
diazinon	0.59	87	mallard
diazinon	0.59	87	mallard
difenoconazole	207	625	bobwhite
difenoconazole	207	625	bobwhite
fludioxonil	208	300	bobwhite
hexaconazole	391	250	bobwhite
imidacloprid	8.43	61	bobwhite
imidacloprid	8.43	61	bobwhite
maneb	345	100	mallard
maneb	345	100	mallard
metalaxyl	89	900	bobwhite
metalaxyl	89	900	bobwhite
metalaxyl	89	900	bobwhite
TCMTB	76.75	N/A	N/A
tebuconazole	347	110	bobwhite
tebuconazole	347	110	bobwhite
thiamethoxam	98.4	900	bobwhite
thiophanate-methyl	483	500	bobwhite
thiram	36.81	40	mallard
thiram	36.81	40	mallard
thiram	36.81	40	mallard
triadimenol	556	500	mallard
triticonazole	232	286	bobwhite

## Appendix 4: General Linear Models

### Colour preference study: Systat output for analysis of visitation using general linear model:

Dep Var: LOGPLUSONE N: 288 Multiple R: 0.884 Squared multiple R: 0.782

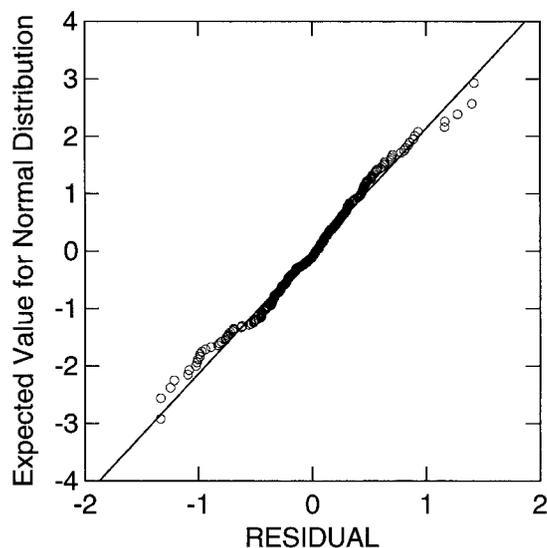
#### Analysis of Variance

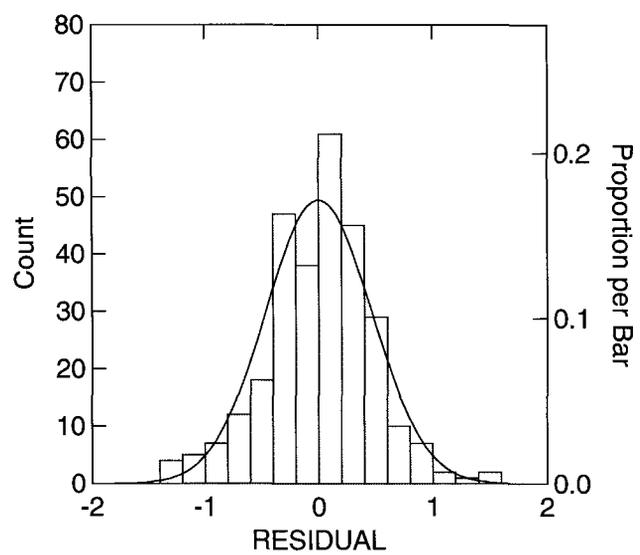
Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
COLOUR\$	15.361	5	3.072	9.672	0.000
LOCATION\$	43.293	7	6.185	19.472	0.000
DAY\$ (LOCATION\$)	125.202	40	3.130	9.855	0.000
POSITION\$ (LOCATION\$)	38.218	40	0.955	3.008	0.000
Error	61.937	195	0.318		

#### Tukey's post-hoc test

Matrix of pairwise comparison probabilities:

	blue	blue+uv	green	green+uv	red	red+uv
1	1.000					
2	0.268	1.000				
3	1.000	0.237	1.000			
4	0.736	0.975	0.697	1.000		
5	0.000	0.003	0.000	0.000	1.000	
6	0.027	0.941	0.022	0.548	0.068	1.000





**Colour preference study:  
Systat output for analysis of consumption using general linear model:**

Dep Var: LOGCONS N: 284 Multiple R: 0.820 Squared multiple R: 0.673

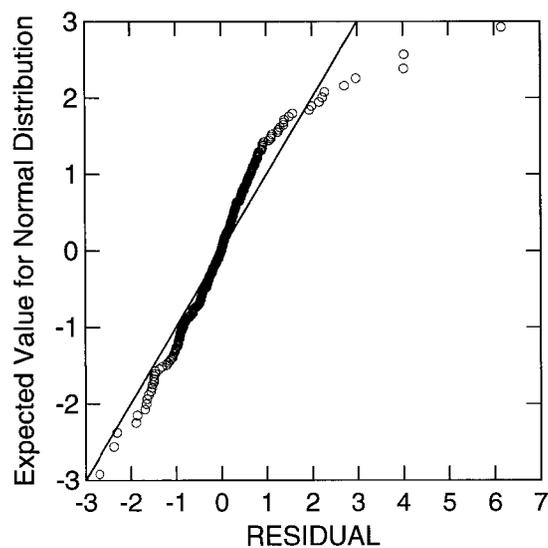
Analysis of Variance

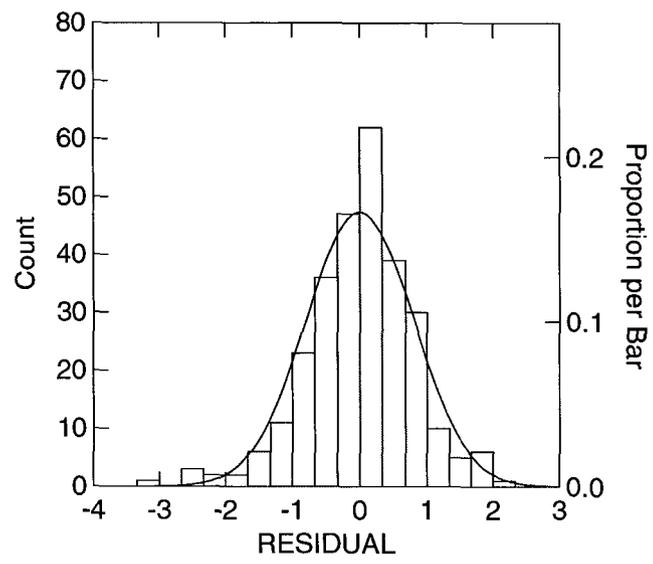
Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
COLOUR\$	23.555	5	4.711	4.995	0.000
SITE\$	117.641	7	16.806	17.820	0.000
DAY\$(SITE\$)	176.807	40	4.420	4.687	0.000
POSITION\$(SITE\$)	53.644	40	1.341	1.422	0.062
Error	180.129	191	0.943		

Tukey HSD Multiple Comparisons.

Matrix of pairwise comparison probabilities:

	blue	blue+uv	green	green+uv	red	red+uv
blue	1.000					
blue+uv	0.100	1.000				
green	1.000	0.046	1.000			
green+uv	0.988	0.371	0.940	1.000		
red	0.001	0.787	0.000	0.015	1.000	
red+uv	0.994	0.325	0.958	1.000	0.011	1.000





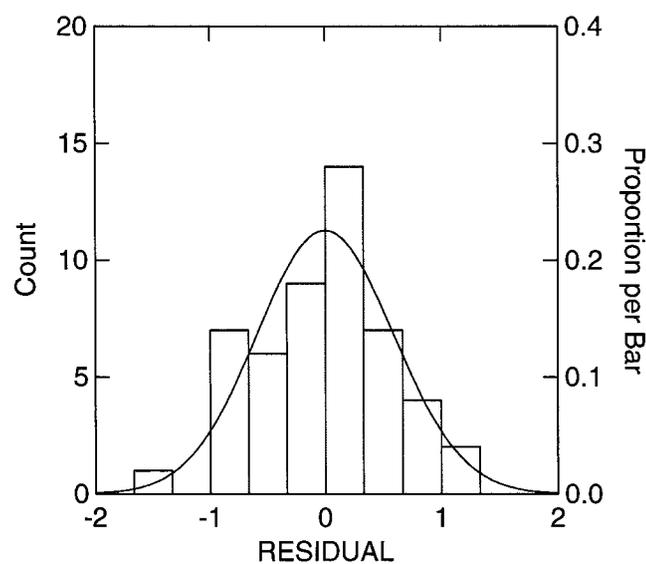
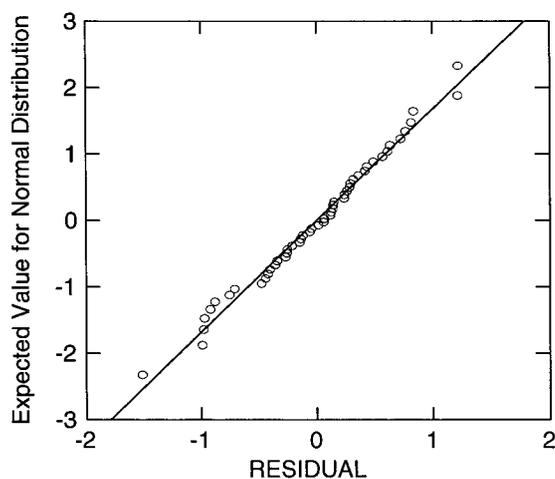
**Risk assessment study:  
Systat output for GLM analysis of log-transformed field visitation data.**

LOCATION\$ (10 levels)  
A, B, C, D, E, F, G, J, K, L  
CROP\$ (5 levels)  
barley, corn, oat, soybean, wheat

Dep Var: LOGVISITS N: 50 Multiple R: 0.797 Squared multiple R: 0.635

Analysis of Variance

Source	Sum-of-Squares	df	Mean-Square	F-ratio	P
LOCATION\$	27.528	9	3.059	6.454	0.000
CROP\$	2.111	4	0.528	1.114	0.365
Error	17.061	36	0.474		



## LITERATURE CITED

- Interim Reregistration Eligibility Decision for Diazinon. EPA 738-R-04-007. 2002. USEPA.  
Ref Type: Report
- Reregistration Eligibility Decision for Thiram. EPA 738-R-04-012. 2004. USEPA.  
Ref Type: Report
- Preliminary risk and value assessments of diazinon. REV2005-06. 2005. Pest Management Regulatory Agency.  
Ref Type: Report
- Avery, M.L. & Decker, D.G. 1991. Repellency of fungicidal rice seed treatments to red-winged blackbirds. *Journal of Wildlife Management* **55(2)**, 327-334.
- Avery, M.L., Decker, D.G. & Fischer, D.L. 1994. Cage and flight pen evaluation of avian repellency and hazard associated with imidacloprid-treated rice seed. *Crop Protection* **13**, 535-538.
- Avery, M.L., Decker, D.G., Fischer, D.L. & Stafford, T.R. 1993. Responses of captive blackbirds to a new insecticidal seed treatment. *Journal of Wildlife Management* **57**, 652-656.
- Avery, M.L., Fischer, D.L. & Primus, T.L. 1997. Assessing the hazard to granivorous birds feeding on chemically treated seeds. *Pesticide Science* **49**, 362-366.
- Bennett, A.T.D. & Cuthill, I.C. 1994. Ultraviolet vision in Birds: What is its function. *Vision Res.* **34**, 1471-1478.
- Bennett, A.T.D., Cuthill, I.C., Partridge, J.C. & Maier, E.J. 1996. Ultraviolet vision and mate choice in zebra finches. *Nature* **380**, 433-435.
- Bird, R.D. & Smith, L.B. 1964. The food habits of the red-winged blackbird, *Agelaius phoeniceus*, in Manitoba. *The Canadian Field-Naturalist* **78**, 179-186.
- Bowmaker, J.K., Heath, L.A., Wilkie, S.E. & Hunt, D.M. 1997. Visual pigments and oil droplets from six classes of photoreceptor in the retina of birds. *Vision Res.* **37**, 2183-2194.
- Brunner, H. & Coman, B.J. 1983. The ingestion of Artificially Coloured Grain by Birds, and its Relevance to Vertebrate Pest Control. *Aust Wildl. Res.* **10**, 303-310.
- Burkhardt, D. 1982. Birds, berries and UV - a note on some consequences of UV vision in birds. *Naturwissenschaften* **69**, 153-157.
- Campbell, B. & Lack, E. 1985. *A Dictionary of Birds*. Vermillion: Buteo books.
- Candy Rowe & Johnsen, A. 2004. Colour biases are a question of taste. *Animal Behaviour* **69**, 587-594.
- Chen, D.-M., Collins, J.S. & Goldsmith, T.H. 1984. The ultraviolet receptor of bird retinas. *Science* **225**, 337-340.
- Church, S.C., Bennett, A.T.D., Cuthill, I.C., Hunt, S., Hart, N.S. & Partridge, J.C. 1998a. Does lepidopteran larval crypsis extend into the ultraviolet? *Naturwissenschaften* **85**, 1-5.
- Church, S.C., Bennett, A.T.D., Cuthill, I.C. & Partridge, J.C. 1998b. Ultraviolet cues affect the foraging behavior of blue tits. *Proc. R. Soc. Lond. B* **265**, 1509-1514.

- Church,S.C., Merrison,A.S.L. & Chamberlain,T.M.M. 2001. Avian Ultraviolet vision and frequency-dependent seed preferences. *The Journal of experimental biology* **204**, 2491-2498.
- Clook,M.A. & Hart,A.D.M. Effects of seed treatments on birds. Contract PN0902. 1998. Central Science Laboratory, final report.  
Ref Type: Report
- Cuthill,I.C., Partridge,J.C., Bennett,A.T.D., Church,S.C., Hart,N.S. & Hunt,S. 2000. Ultraviolet vision in Birds. *Advances in the study of behavior* **29**, 159-214.
- Davis,B.N.K. 1974. Levels of dieldrin in dressed wheat seed after drilling and exposure on the soil surface. *Environmental Pollution* **7**, 309-317.
- Dawkins,M. 1971. Perceptual changes in chicks: another look at the 'search image' concept. *Animal Behaviour* **19**, 566-574.
- de Snoo,G.R. & Luttik,R. 2004. Availability of pesticide-treated seed on arable fields. *Pest Management Science* **60**, 501-506.
- Desjardins,C., Maruniak,J.A. & Bronson,F.H. 1973. Social rank in house mice: Differentiation revealed by ultraviolet visualization of urinary marking patterns. *Science* **182**, 939-941.
- Dunning Jr.,J.B. 1992. *CRC Handbook of Avian Body Masses*. Boca Raton, Florida: CRC press, Inc.
- Endler,J.A. 1993. The Color of Light in Forests and its Implications. *Ecological Monographs* **63**, 1-27.
- Fite,E., Randall,D., Young,D., Odenkirchen,E. & Salice,C. Reregistration eligibility science chapter for carbofuran.  
environmental fate and effects chapter. 2006. USEPA.  
Ref Type: Report
- Gamberale-Stille,G. 2001. Benefit by contrast: an experiment with live aposematic prey. *Behavioral Ecology* **12**, 768-772.
- Geis,A.D. Relative attractiveness of different foods at wild bird feeders. 233, 1-11. 1980. Washington, D.C., U.S. Department of the Interior, Fish and Wildlife Service. Special Scientific Report - Wildlife.  
Ref Type: Report
- Getty,T. & Pulliam,H.R. 1993. Search and prey detection by foraging sparrows. *Ecology* **74**, 734-742.
- Gittleman,J.L. & Harvey,P.H. 1980. Why are distasteful prey not cryptic? *Nature* **286**, 149-150.
- Greig-Smith,P.W. 1987a. Aversions of starlings and sparrows to unfamiliar, unexpected or unusual flavours and colours in food. *Ethology* **74**, 155-163.
- Greig-Smith,P.W. Hazards to wildlife from pesticide seed treatments. 127-134. 1987b. Lavenham, Suffolk. BCPC monograph No. 39: Applications to seeds and soil.  
Ref Type: Conference Proceeding
- Greig-Smith,P.W. & Rowney,C.M. 1987. Effects of colour on the aversions of starlings and house sparrows to five chemicals repellents. *Crop Protection* **6**, 402-409.

- Hart,A.D.M. & Thompson,H.M. 1995. Significance of regurgitation in avian toxicity tests. *Bull. Environ. Contam. Toxicol.* **54**, 789-796.
- Hart,A., Fryday,S., McKay,H., Pascual,J. & Prosser,P. Understanding risks to birds from pesticide-treated seeds. 1070-1087. 1999. Johannesburg: BirdLife South Africa. Proc. 22 Int. Ornithol. Congr. Durban. Slotow, R. H. and Adams, N. J.  
Ref Type: Conference Proceeding
- Harvey,P.H. & Paxton,R.J. 1981. The evolution of aposematic coloration. *OIKOS* **37**, 391-396.
- Hill,E.F. & Camardese,M.B. 1984. Toxicity of anticholinesterase insecticides to birds: technical grade versus granular formulations. *Ecotoxicology & Environmental Safety* **8**, 551-563.
- Honkavaara,J., Koivula,M., Korpimäki,E., Siitari,H. & Viitala,J. 2002. Ultraviolet vision and foraging in terrestrial vertebrates. *OIKOS* **98**, 505-511.
- Huth,H.H. & Burkhardt,D. 1972. Der spektrale Sehbereich eines Violettöhr-Kolibris. *Naturwissenschaften* **59**, 650.
- Koivula,M. & Viitala,J. 1999. Rough-legged buzzards use vole scent marks to assess hunting areas. *Journal of Avian Biology* **30**, 329-330.
- Koivula,M., Korpimäki,E. & Viitala,J. 1997. Do Tengmalm's owls see vole scent marks visible in ultraviolet light? *Animal Behavior* **54**, 873-877.
- Leeuw,J.d., Gorree,M., de Snoo,G.R., Tamis,W.L.M., van der Poll,R.J. & Luttik,R. Risks of granules and treated seeds to birds on arable fields. CML report 118, -131. 1995. Leiden, Leiden University.  
Ref Type: Report
- Lindström,L., Alatalo,R.V., Mappes,J., Riipi,M. & Vertainen,L. 1999. Can aposematic signals evolve by gradual change? *Nature* **397**, 249-251.
- Luttik,R., Mineau,P. & Roelofs,W. 2005. A review of interspecies toxicity extrapolation in birds and mammals and a proposal for long term toxicity data. *Ecotoxicology* **14**, 817-832.
- Lyytinen,A., Alatalo,R.V., Lindstrom,L. & Mappes,J. 2001. Can ultraviolet cues function as aposematic signals. *Behavioral Ecology* **12**, 65-70.
- Marples,N.M., Roper,T.J. & Harper,D.G.C. 1998. Responses of wild birds to novel prey: evidence of dietary conservatism. *OIKOS* **83**, 161-165.
- Martin,A.C., Zim,H.S. & Nelson,A.L. 1951. *American Wildlife and Plants. A Guide to Wildlife Food Habits*. New York: dover publication.
- Mason,J.R. & Clark,L. Nonlethal repellents: the development of cost-effective, practical solutions to agricultural and industrial problems. 15, 115-129. 1992. 15th Vertebrate Pest Conference.  
Ref Type: Conference Proceeding
- Mastrota,F.N. & Mench,J.A. 1995. Colour avoidance in northern bobwhites: effects of age, sex and previous experience. *Animal Behavior* **50**, 519-526.
- Maze,R.C., Atkins,R.P., Mineau,P. & Collins,B.T. 1991. Measurement of surface pesticide residue in seeding operations. *Transactions of the ASAE* **34**, 795-799.

- McKay,H.V., Prosser,P.J., Hart,A.D.M., Langton,S.D., Jones,A., McCoy,C., Chandler-Morris,S.A. & Pascual,J.A. 1999. Do wood pigeons avoid pesticide treated cereal seed? *Journal of Applied Ecology* **36**, 283-296.
- Mineau,P. 2005. A review and analysis of study endpoints relevant to the assessment of 'long term' pesticide toxicity in avian and mammalian wildlife. *Ecotoxicology* **14**, 775-799.
- Mineau,P., Baril,A., Collins,B.T., Duffe,J., Joerman,G. & Luttk,R. 2001. Pesticide acute toxicity reference values for birds. *Rev. Environ. Contam. Toxicol.* **170**, 13-74.
- Mineau,P. & Collins,B.T. 1988. Avian mortality in agro-ecosystems. methods of detection. In: *BCPC Mono*, pp. 13-27.
- Mineau,P., Collins,B.T. & Baril,A. 1996. On the use of scaling factors to improve interspecies extrapolation of acute toxicity in birds. *Regulatory Toxicology and Pharmacology* **24**, 24-29.
- Mineau,P., Jobin,B. & Baril,A. A critique of the avian 5-day dietary test (LC50) as the basis of avian risk assessment. 215, -23. 1994. Hull, Canadian Wildlife Service. Technical Report Series. Ref Type: Report
- Nagy,K.A. 1987. Field metabolic rate and food requirement scaling in mammals and birds. *Ecological Monographs* **57**, 111-128.
- Nelms,C.O. & Avery,M.L. 1997. Reducing bird repellent application rates by the addition of sensory stimuli. *International Journal of Pest Management* **43**, 187-190.
- Nystrand,O. & Granström,A. 1997. Post-dispersal predation on *Pinus sylvestris* seeds by *Fringilla* spp:ground substrate affects selection for seed color. *Oecologia* **110**, 353-359.
- Pank,L.F. 1976. Effects of seed and background colors on seed acceptance by birds. *Journal of Wildlife Management* **40**, 769-774.
- Pascual,J.A., Hart,A.D.M. & Fryday,S.L. 1999a. Effects of food restriction on body condition, food avoidance and risk of poisoning of captive feral pigeons from fonofos-treated seeds. *Archives of Environmental Contamination and Toxicology* **37**, 115-124.
- Pascual,J.A., Hart,A.D.M. & Fryday,S.L. 1999b. Incidence of lethal bird poisoning reduced by regurgitation of pesticide-treated food. *Environmental Toxicology and Chemistry* **18**, 247-253.
- Pascual,J.A., Hart,A.D.M., Saunders,P.J., McKay,H.V., Kilpatrik,J. & Prosser,P. 1999c. Agricultural methods to reduce the risk to birds from cereal seed treatments on fenlands in eastern England. I. Sowing depth manipulation. *Agriculture, Ecosystems and the Environment* **72**, 59-73.
- Pascual,J.A. & Hart,A.D.M. 1997. Exposure of captive feral pigeons to fonofos-treated seed in a semifield experiment. *Environmental Toxicology and Chemistry* **16**, 2543-2549.
- Pascual,J.A., Saunders,P.J., Hart,A.D.M. & Mottram,J. 1999d. Agricultural methods to reduce the risk to birds from cereal seed treatments on fenlands in eastern England II. Rolling and harrowing as post-sowing cultivations. *Agriculture, Ecosystems and the Environment* **72**, 75-86.
- Probst,R., Pavlicev,M. & Viitala,J. 2002. UV reflecting vole scent marks attract a passerine, the great grey shrike *Lanius excubitor*. *Journal of Avian Biology* **33**, 437-440.

Prosser,P. & Hart,A.D.M. 2005. Assessing the potential exposure of birds to pesticide-treated seeds. *Ecotoxicology* **14**, 679-691.

Prosser,P., Hart,A., Langton,S., McKay,H. & Cooke,A. Estimating the rate of poisoning by insecticide-treated seeds in a bird population. Contract PN0902, 1-22. 1998. Effects of Seed Treatments on Birds.

Ref Type: Report

Reidinger,R.F. & Mason,J.R. 1983. exploitable characteristics of neophobia and food aversions for improvements in rodent and bird control. In: *Vertebrate Pest Control and Management Materials, 4th Symp.* (Ed. by D.E.Kaukeinen), pp. 20-39. Philadelphia.

Schlenoff,D.H. 1984. Novelty: A basis for generalization in prey selection. *Animal Behaviour* **32**, 919-921.

Siitari,H., Honkavaara,J. & Viitala,J. 1999. Ultraviolet reflection of berries attracts foraging birds. A laboratory study with redwings (*Turdus iliacus*) and bilberries (*Vaccinium myrtillus*). *Proc. R. Soc. Lond. B* **266**, 2125-2129.

Siitari,H. & Viitala,J. 2002. Behavioral evidence for ultraviolet vision in a tetranoid species - foraging experiment with black grouse *Tetrao tetrix*. *Journal of Avian Biology* **33**, 199-202.

Sillen-Tullberg,B. 1983. The significance of coloration per se, independent of background, for predator avoidance of aposematic prey. *Animal Behaviour* **33**, 1382-1384.

Slaby,M. & Slaby,F. 1977. Color preference and short-term learning by Steller's Jays. *Condor* **79**, 384-386.

Smith,E.L., Greenwood,V.J. & Bennett,A.T.D. 2002. Ultraviolet colour perception in European starlings and Japanese quail. *The Journal of experimental biology* **205**, 3299-3306.

Sokal,R.R. & Rohlf,J.F. 1994. *Biometry*. San Francisco: Freeman.

Tamis,W.L.M., Gorree,M., de Leeuw,J., de Snoo,G.R. & Luttik,R. 1994. The number of exposed dressed seeds in the field; an outline for field research. *BCPC Monograph - Seed Treatment: Progress and Prospects* **57**, 471-476.

Thompson,K.M. 2002. Variability and uncertainty meet risk management and risk communication. *Risk Analysis* **22**, 647-654.

Viitala,J., Korpimäki,E., Palokangas,P. & Koivula,M. 1995. Attraction of kestrels to vole scent marks in ultraviolet light. *Nature* **373**, 425-427.

Warren-Hicks,W.J. & Moore,D.R.J. 1998. *Uncertainty Analysis in Ecological Risk Assessment*. Proceedings from the Pellston workshop on uncertainty analysis in ecological risk assesment. 23-28 August 1995 edn. Pellston, Michigan: Setac Press.

Willson,M.F. & Whelan,C.J. 1989. Ultraviolet reflectance of fruits of vertebrate-dispersed plants. *OIKOS* **55**, 341-348.

Wright,A.A. 1972. The influence of ultraviolet radiation on the pigeons' color discrimination. *Journal of the experimental analysis of behavior* **17**, 325-337.