Data Analysis and Preliminary model Development for an Odour Detection System based on the Behaviour of Trained Wasps

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Zhongkun Zhou

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Abstract of a thesis submitted in partial fulfilment of the

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Data Analysis and Preliminary model Development for an Odour

Detection System based on the Behaviour of Trained Wasps

By Zhongkun Zhou

Microplitis croceipes, one of the nectar feeding parasitoid wasps, has been found to associatively learn chemical cues through feeding. The experiments on *M. croceipes* are performed and recorded by a Sony camcorder in the USDA-ARS Biological Control Laboratory in Tifton, GA, USA. The experimental videos have shown that *M. croceipes* can respond to Coffee odour in this study. Their detection capabilities and the behaviour of *M. croceipes* with different levels of coffee odours were studied. First, the data that are related to trained *M. croceipes* behaviour was extracted from the experimental videos and stored in a Microsoft Excel database. The extracted data represent the behaviour of *M. croceipes* trained to 0.02g and then exposed to 0.001g, 0.005g, 0.01g, 0.02g and 0.04g of coffee. Secondly, indices were developed to uniquely characterise the behaviour of trained *M. croceipes* under different coffee concentrations. Thirdly, a preliminary model and its parameters were developed to classify the response of trained wasps when exposed to these five different coffee odours. In summary, the success of this thesis demonstrates the usefulness of data analysis for interpreting experimental data, developing indices, as well as understanding the design principles of a simple model based on trained wasps.

Keywords: Microplitis croceipes, insect behaviour, Tracker, video images analysis, Mathematical model

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Abbreviations

Agricultural Research Service (ARS)

Electro-antennograms (EAGs)

Georgia (GA)

Microsoft (MS)

Open Source Physics (OSP)

United States of America (USA)

United States Department of Agriculture (USDA)

Visual Basic for Applications (VBA)

Chapter 1: Introduction

1.1 Introduction

Many animals are known to have a remarkable ability to detect, recognize and locate target materials based on olfactory, visual and other cues. However, the capacity of their natural system remains poorly understood and under-utilised, other than a long and rich history of using dogs' keen sense of smell for detecting and monitoring drugs, cadavers, bombs, contraband, etc. Emerging information regarding the chemical detection capabilities of insects has revealed the potential for developing detection strategies utilizing insects, which are portable, cheap to reproduce and easy to use.

Microplitis croceipes, one of the black nectar-feeding parasitoid wasps, have been found to associatively learn chemical cues through feeding. The experiments on *M. croceipes* are performed and recorded by camcorder in the USDA-ARS Biological Control Laboratory in Tifton, GA, USA. The experimental videos have shown that *M. croceipes* can respond to the test chemical cues. To better understand their detection capabilities, the behaviour of a single *M. croceipes* wasp with different levels of chemical concentrations is studied. To study the effect of concentration level on wasp behaviour, it is necessary to extract data that are related to the wasp behaviour from the experimental videos, to analyse those data and investigate the relationships between the data and chemical concentration levels. Finally, a preliminary model could be built and utilised for chemical detection based on those information.

1.2 Motivation of study in the thesis

The basic motivation of this research is to investigate the connection between single trained wasp's searching response and the target odour concentrations. When a hand trained *Microplitis croceipes* is placed in the presence of a target odour, it will individually exhibit several different behaviours, such as coiling, head sticking, anntenating, and restricted searching (Wäckers et al., 2002, Olson et al., 2003). Humans can interpret these behaviours visually to indicate whether the response is positive (odour detected) or negative (odour not detected). However, it is really difficult from human observation to visually quantify a wasp's response to a target odour. In fact, the connection between wasp's response and different levels of target odour needs to be studied. A simple stochastic model (D. Kulasiri and W. Verwoerd, 2002) is built based on the experimental data to simulate the wasp's behavioural response under several target odour concentrations. This is a useful technique for developing objective measures of the behavioural response to varying concentrations of test odorants.

1.3 Research objectives

Throughout this study, the major theme is to investigate the relationship between single trained *M. croceipes* and different level of target odours, and to build a simple stochastic model which will be used to predict the level of target odour by simulating the single *M. croceipes*' behaviour under different level of target odours. The specific objectives of this study are:

- To extract data from video files and build up a database to store and analyse those data.
- To develop indices to uniquely characterise the behaviour of wasps under different target odour concentrations.

- To develop a simple model based on stochastic calculus equation $dX = a \cdot Xdt + b \cdot dw$ (X is variable, a and b are the parameters to be determined in this project; *dw* is increments of the standard Wiener process which is normally distributed with a unit variance) and parameter estimate processes for governing differential equation.

- Establish the parameter and model validation process and analyse the accuracy of the model.

- To identify the gaps of our current knowledge of the model.

1.4 Outline of the thesis

In the current chapter, an introduction of chemical detecting Technologies that utilize M. croceipes is provided, which leads to the motivation for the thesis. In chapter two, literature review covering the fields touched in this thesis is given. In chapter three, experiment procedure of M. croceipes, data preparation and variable exploration is given. In chapter four, the wasp's behaviours are analyzed and the variables extracted from these behaviours are given. In chapter five, the model parameters from stochastic calculus equations are estimated. In chapter six, the model framework is developed based on the mathematical equations. In chapter seven, the model simulation results are compared with the actual values, and the limitation of model is discussed. Finally, in chapter eight, a retrospective look at the overall implications of this work is provided, as well as the contributions of the thesis and directions for future research.

Chapter 2: Literature review and discussion

Since the thesis embraces several disciplines, the literature review discussed here covers the field of chemical detecting, biology species and computer modelling issues. Section 1 gives the introduction of traditional chemical detecting technology utilizing human olfactometry, training canines, and electronic olfaction. Section 2 provides the biological background of Microplitis croceipes, which include physiology, chemotaxis and research history on Microplitis croceipes. Section 3 gives the details of a successfully made computer vision system that utilizes a new biological species to detect target substances. Section 4 gives the relevant information of computer software used in this research.

2.1 Chemical sensing technologies

Historically, tracking illegal substances and detecting explosives are very important applications for detecting volatile chemicals. Detection of volatile compounds can also be a good method for detecting other organic materials, such as aflatoxin in peanuts and corn (Rains et al., 2003A). Traditional methods of detecting volatile chemicals are human olfactometry, training canines, and electronic olfaction (Gardner and Bartlett, 1998). In those methods, humans and dogs are the most sensitive; but also subjective and costly (Gardner and Bartlett, 1998). Because of security concerns and agriculture needs, volatile detection is being used to lower the cost and increase the efficiency of chemical screening.

Many electronic devices have been developed to reduce the cost and improve the reliability of volatile detection. The electronic devices are designed from simple to complex. The simple designs are inexpensive relative to training and maintaining a

canine, are very specific and sensitive to low chemical concentrations. On the other hand, they may detect a wider range of volatiles but lack sensitivity (Gardner and Bartlett, 1998; Dickinson, 1998). The complex electronic designs are relatively inexpensive, but are much less sensitive than human olfaction. The complex output is also difficult for the user to interpret.

Dr. Glen C. Rains (University of Georgia, US) has been carrying out experiments on parasitic wasps (Microplitis croceipes) that respond to odours from plants and can be trained to detect chemicals (Rains et al., 2003A). By having a sensor that makes use of trained parasitic wasps, the detection of chemicals at very low concentrations (ppb) could be improved. It is hypothesized that the use of wasps could prove to be more accurate than the current detection methods.

2.2 Microplitis croceipes

2.2.1 Physiology

Microplitis croceipes (Cresson) (Hymenoptera: Bracibudae) are larval parasitoids of heliothis virescens (tobacco budworm) and Helicoverpa zea (corn earworm). They are black nectar feeding wasps, approximately 10-12 mm in length and 2-3 mm in width, with a yellowish abdomen. The males are haploid and have antennae approximately the length of their body. The females are diploid and possess antennae approximately ½ the length of the male antennae. *M. croceipes* are facultative pathogenic, laying facultatively arrhenotokous eggs; *M. croceipes* larvae will develop as male if the egg is unfertilized and female if fertilized (Daly et al., 1998).

2.2.2 Research history on Microplitis croceipes

M. croceipes' ability to detect volatile chemicals has been widely studied. In 1988, Lewis and Tumilinson first discovered that a parasitoid wasp named Microplitis croceipes could associatively learn chemical cues from its host and food (Lewis and Tumilinson, 1988). These wasps can be trained to search chemical cues in various environments. Bioassays in a wind tunnel proved that *M. croceipes* can selectively seek out the plants by tracking the volatile chemicals released from the plant, based on their physiological state such as needs for reproduction or food (De Moraes et al., 1998). The volatile chemicals emitted from the plant were isolated and identified, then used for further investigation of *M. croceipes*' chemical detection ability. Further experiments have shown *M. croceipes* was able to selectively detect the odours at low concentrations when presented with the emitted volatiles without the plants (Olson et al., 2003).

The US Department of Agriculture (USDA) investigated the breadth of the chemicals detectable by *M. croceipes* and their limits of detection. Using wind tunnel trials as the standard testing method, *M. croceipes* was successfully trained and tested with a wide array of chemicals, including those that they might not normally encounter in their natural habitat. The chemicals ranged from common food stuffs, such as chocolate and coffee (Takasu and Lewis, 1993), to a wide array of aliphatic, ketones, aldehydes and 2,4 and 2,6 dinitrotoluene (Olson et al., 2003, Pare and Tumlinson, 1997). The detection capabilities of *M. croceipes* offered a possible method for detecting and tracking potential illegal or harmful substances instead of using canine olfaction and electronic noses. The method with which to best exploit the abilities of *M. croceipes* was uncertain. Several options included harvesting the antennae and measuring their activity with electro-antennograms (EAGs), tracking the wasps once released into the environment, and allowing a confined group of wasps to report the detection of a target odour.

In order to seek out additional behaviour of the trained wasps, many experiments that use the trained wasps to search out target odour was performed and the behaviour of trained wasps were studied. If the wasp is allowed to smell the target odour while feeding, it will flick its antennae (which is known as antennating behaviour) when exposed to the target odour again. If the wasp is allowed to smell the target odour while stinging a host, it will be contracting the abdomen in a stinging fashion (which is known as coiling behaviour) when exposed to the target odour again. If a trained wasp was exposed to the target odour which is emitted from a point source, it will rotate its body and antennate in a small area (which is known as area restricted searching behaviour) around that point source. However, if the target odour was emitted from a hole that is large enough for a wasp to fit into it, the wasp will generally enter the hole in search of the odour's source. (Olson et al., 2003; rains et al., 2002; Rains et al., 2001; Rains et al., 2000). Based on these behaviours and the abilities of *M. croceipes*, a whole organism sensor that utilize *M. croceipes* to detect target odour was made. Monitoring trained wasp's head sticking, body spinning and hole entering behaviours are used as the method to measure the absence or presence of the target odour. Area restricted searching is exhibited more quickly than head sticking and may be a quick, reliable, and easily measurable response (Utley et al., 2004). However, it is necessary to investigate the relationship between single wasp's behaviours and the concentration of target odour. Currently, the wasp sensor only measures whether the target odorant is present. By examination of the wasp behaviour, we may be able to detect other characteristics of the target odorants, such as concentration. It is helpful for development of organism sensor that utilizes *M. croceipes* to detect the target odour.

2.2.3 Chemotaxis

M. *croceipes*' life cycle depends on its keen ability to track volatile odours from plants

and hosts (Olson, et al., 2003, Pare and Tuminson, 1997). To find both hosts and foods, *M. croceipes* must track favourable feeding and breeding conditions over long distances through the use of chemical cues (Lewis and Tumlinson, 1988). In nature, female wasps use chemical cues to first locate plants where host organisms are feeding by tracking the volatiles emitted by both the plant and host's frass. After locating the plant, they need to determine the location of the host larvae. The larvae themselves are relatively odour free and therefore camouflaged. However, the larvae must feed to grow and, when they do, they give away their location. The saliva of the larvae enters the open wound of the plant causing the plant to begin to produce the volatile, which is not only from the wound but from the entire plant canopy. The insect is repelled by this volatile (Pare and Tumlinson, 1999). Interestingly, the wasps are not repelled by this volatile, but instead they use these chemical cues to locate the larvae. The odours emitted by the plant are dependent on its type, health, soil conditions, and the type of insect feeding on it (Tumlinson et al., 1999). Therefore, *M. croceipes* must possess outstanding learning and detection capabilities with many variables affecting the possible volatile emissions.

The volatiles that *M. croceipes* could learn and respond to are not limited to naturally occurring volatiles produced by plants or hosts. *M. croceipes* can also learn to recognize a range of chemical structures such as cyclic and aliphatic ketones, aliphatic aldehydes and alcohols, and aromatic hydrocarbons (Wäckers et al., 2002, Olson et al., 2003). *M. croceipes* can learn to associate these distinct odours with separate behaviours and will seek out the odours that they believe will lead to food or host, depending on their physiological state. Wind tunnel trials have shown that hungry wasps trained to associate a target odour with food will choose to seek out the target odour over a control in order to feed. Wasps allowed to sting a host or antennate frass while exposed to a target odour will seek out the target odour in order to lay eggs (Wäckers and Lewis, 1994, Olson et al., 2003). In nature, the wasps are able to track the faint traces of odours by tracking upwind along an odour concentration gradient. If the wasp is to seek out the target odour, a concentration gradient needs to exist,

otherwise the wasp cannot track the odour to its source.

2.3 Computer vision system

Some computer vision systems that utilise insects to detect volatile chemicals have been successfully devised. However, a computer application that utilises *M. croceipes* and outputs chemical concentration of the target odorant has not been made, due to the lack of information of how *M. croceipes*' searching behaviours are modified by chemical concentrations.

Inscentinel Ltd. (Hertfordshire, UK) has successfully devised and marketed a system using honey bees (Apis mellifera [Hymenoptera: Linnaeus]) for trace vapor detection (http://www.inscentinel.com). The system can hold trained bees in a safe and controlled environment and can be operated in a range of external environmental conditions. Sample air is delivered to the bees for recognition of specific odours. The bees are held in a special cassette and specially designed hardware and image recognition software monitors and records detection by the bees, converting their response into an electronic form. The electronic output can be given in a simple yes/no, green light/red light form.

Another computer vision system with image analysis software (*Visual Cortex*) successfully and objectively quantified the searching behaviour of five trained female *M. croceipes* parasitoid wasps (S. L. Utley, G. C. Rains, W.J. Lewis. 2004). It is an open-air system, consisting of a camera, computer and software. The wasp hound was developed as a handheld instrument for the detection of volatile compounds [*Appendix A* – Computer Vision System]. The wasp hound consists of a ventilated area, a mounted camera, fixed light source, and test cartridge loading area. The device's air sampling method creates an odour gradient inside the device by slowly drawing outside air through the test cartridge. Five trained female *M. croceipes* are

placed inside the test cartridge so they can only walk without flying away. The mounted camera is used for observing wasps' behaviour during testing. Images that are captured by camera are transferred to the laptop PC and analyzed with an image analysis software called *Visual Cortex*. *Visual Cortex* was developed S.L Utley using National Instruments' LabView 6.1 and Parente's LabView Webcam Library. It is used to observe and analyse wasps area-restricted searching behaviour during chemical detection. The handheld system constructed during this study quickly detected the presence of 3-octanone (2.6×10^{-5} mol/L) in a background of corn within 25 s (Rains et al, 2006). Such a system has the potential to be utilized for the detection of target chemical odours within an environment containing a masking background.

For more details on the computer vision system refer to *Appendix A*.

2.4 Tracker

During the preliminary studies, the single wasp was tested with coffee odour and recorded by camcorder. In order to study the relationship between the wasp's behaviour and the coffee concentrations, data need to be extracted from video files (wasp experimental videos) to a Microsoft Excel datasheet. The *Visual Cortex* is not suitable for extracting data from the video file in my study. Therefore, the Tracker software is used.

Tracker is a video analysis package built on the Open Source Physics (OSP) Java framework. The features include object tracking with position, velocity and acceleration overlays and graphs, special effect filters, multiple reference frames, calibration points and line profiles for analysis of spectra and interference patterns (http://www.cabrillo.edu/~dbrown/tracker/). It was originally designed to be used in introductory college physics labs and lectures. It features automatic and manual curve-fitting and statistics for user-selected portions of any dataset.

The Tracker software has a friendly user interface that easily tracks the object position from a video image file. A wide variety of functionalities can help the user set up axes, coordinate system, plot graphs and tables of track-generated data.
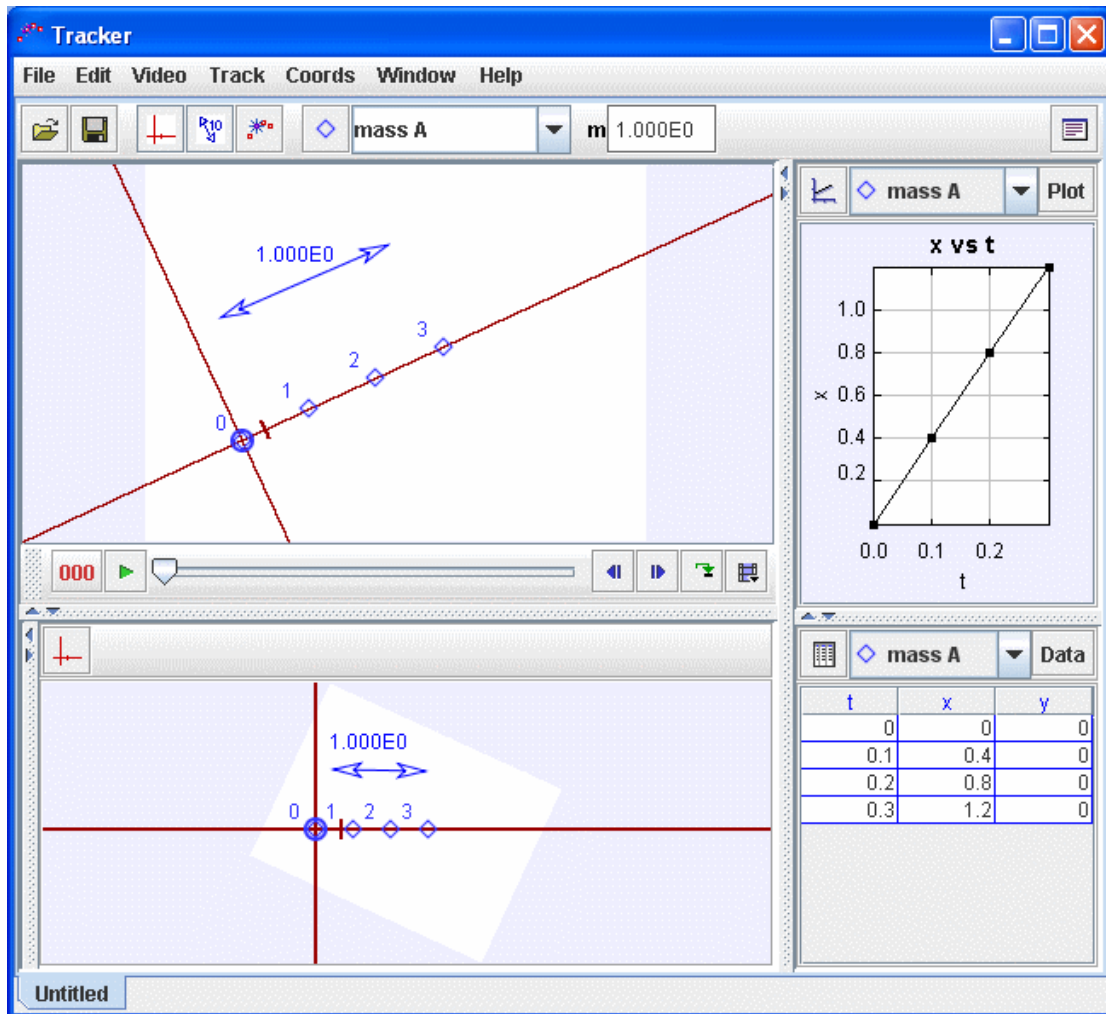
Figure 2.1: User interface showing the menu bar, tool bar and four of the split panes that have been opened. Four split panes are main video view, plot view, table view and world view. (From http://www.cabrillo.edu/~dbrown/tracker/).

For more details about how to use tracker in my study, refer to *Appendix B*.

Chapter 3: Experimental methodology

This chapter describes the methodology that is used in this research. Section 1 explains the biological experiment procedures that are implemented at Tifton, GA, USA. Section 2 gives the explanation of the data preparation procedures. Section 3 provides details of exploring variables and their calculation. In section 4, the variables are improved based on the previous variables in Section 3.

3.1 Experiments at Tifton

The data used in my study are video files that contain the behaviour of the wasp. Those videos are recorded during the experiments in the USDA-ARS Biological Control Laboratory in Tifton, GA, USA. All training procedures and experiments are performed at Tifton. They were performed under a fume hood, with a fluorescent ring light to lure escaped wasps.

3.1.1 Insects

M. croceipes are used for this study. The larval hosts used for rearing *M. croceipes* were *Heliothis zea* (Lepidoptera: Notuidae) as discussed by Lewis and Burton (1970). The breeding stock are provided with water and honey and kept in a Plexiglas cage (30 x 30 x 17 cm) at $28^{0}C$, 50-70% RH, and a L16:D8 photocycle. Test specimens were females, two days old, given only water from time of emergence and no oviposition experience.

3.1.2 Training procedure

In this study, female *M. croceipes* are conditioned to associate coffee odour with food through associative learning. All training procedures are performed under a fume hood in the USDA-ARS Biological Control Laboratory in Tifton, GA. A fluorescent ring light (Luxco Lamp Corp.) is placed in the fume hood to lure escaped wasps. For preliminary studies, 20 wasps were trained, but only 16 were tested, then approximately 300 wasps were trained for future analysis.

An odour delivery stage (Figure 3.1) was prepared for training each wasp. First, a filter disc was loaded with 0.02g coffee. Next, the filter disc was placed in a 200 ml glass jar which was then covered with a piece of aluminum foil. A piece of paper (2 x 2 mm) was placed in the centre of aluminum foil covering and saturated with 50% sucrose water solution. Lastly, a push pin was used to create six holes in a tight circular pattern around the sucrose-water-saturated filter paper approximately 10 minutes after the glass jar was closed with aluminum foil.
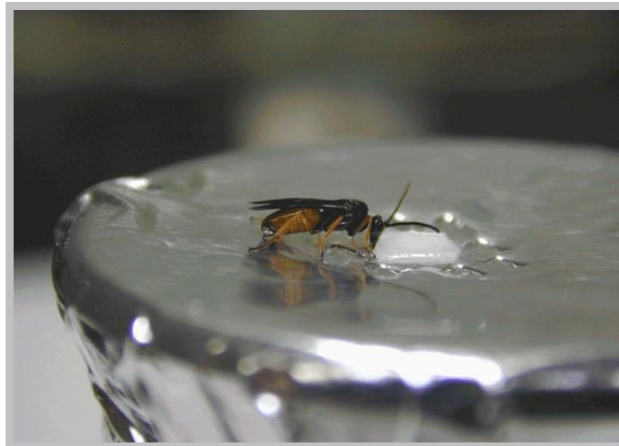
Figure 3.1:Odour deliver stage used during training.(From Glen C. Rains)

Female *M. croceipes* were captured and individually hand trained. These wasps were captured from their rearing cage and placed in separate vials. Each wasp was removed from its vial using a pair of forceps and individually allowed to feed on the sucrose

solution for ten seconds. The odorant emitted around the filter paper passed over their antennae while feeding. After feeding, each wasp was placed back in its vial. The process was repeated so each wasp was allowed to feed for three times, ten second intervals with approximately 60 seconds between each feeding (Tertuliano et al., 2004).

3.1.3 Test sample preparation

For preliminary studies, two different coffee sample preparations were used for the testing. One was 0.02g coffee sample, the other was 0.005g coffee sample. The researcher's hands were washed prior to creating the samples. First, a filter disc was loaded with 0.02 / 0.005g coffee. Next, the filter disc was placed in a 250 ml glass jar which was then covered with a piece of filter paper. There was a single hole in the middle of the filter paper where the odour emits. There were 2 circles centred on the hole. One was 25 mm in diameter and the other was 50 mm in diameter.

Subsequently, three more coffee sample preparations (0.001g, 0.01g and 0.04g coffee) were added for testing. The testing sample categories became 0.001g coffee, 0.005g coffee, 0.01g coffee, 0.02g coffee and 0.04g coffee.

3.1.4 Test procedure

Testing was performed under the same fume hood as was used in the training. The digital camcorder was placed on top of the test sample under the fume hood. During testing, all light sources within the room, except the overhead fluorescence room lights, were turned off or covered up, resulting in an average light intensity of 295lux at the top of the test sample. The digital camcorder was placed so that the tip of the

camera was approximately 3 cm above the top of the test sample. Trained wasps were placed on each test sample (0.001g, 0.005g, 0.01g 0.02g and 0.04g coffee) separately and recorded by camcorder.

3.2 Data record and preparation

The data was collected using the camcorder to record trained wasps (0.02g coffee) presented with 0.001g coffee, 0.005g coffee, 0.01g coffee, 0.02g coffee and 0.04g coffee odours. The original data from the camcorder was MPEG[1] movie file. They were grouped by five testing categories and stored in a computer hard drive. The Tracker software that is used in this study does not support MPEG files at this time; it only supports QuickTime[2] movies and AVI[3] movie files. In order to use the Tracker software to track the movement of the wasp from the data file, Blaze Media Pro[4] was used to change the format of the data file from MPEG to AVI format. Blaze Media Pro is a powerful, all-in-one, multimedia application supporting audio and video conversions. It performs two-way video conversions among AVI, MPEG, and WMV formats. The MPEG data files (record from camcorder) were converted to AVI format with a matching frame rate of 25 frames per second.

For more details on the steps of converting data files from MPEG to AVI refer to *Appendix C.*

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[1] MPEG (pronounced M-peg), which stand for Moving Picture Experts Group, is the name of family of standards used for coding audio-visual information (e.g., movies, video, music) in a digital compressed format. (http://www.mpeg.org/MPEG/index.html)

2 QuickTime is a multimedia architecture developed by Apple Computer for Mac OS, Mac OS X, Windows, and other platforms. It allows your computer to work with real-time movies, sounds, and high-quality compressed images. (http://www.apple.com/quicktime/)

[3] AVI (which typically end in the .avi extension) stands for Audio Video Interleave and is currently the most common file format for storing audio/video data on the PC.

[4] It is all-in-one multimedia software offering conversion, ripping, editing, recording, burning, playback, and much more. It is developed by Mystik Media Ltd. (http://www.blazemp.com)

3.3 Variables exploration and calculation

A single trained wasp was placed on the surface of the test sample and its movement was captured by camcorder, which is showed in Figure 3.5 (A). According to the video record, the wasp has three major movement behaviours on the surface of the sample. First, the wasp travels close to the centre hole (where coffee odour emits) or moves away from the centre by searching for the coffee odour. Secondly, the wasp travels around the centre point (centre hole where the coffee odours emits from), clockwise or anticlockwise. Thirdly, it rotated around itself, either clockwise or counter-clockwise. Therefore, this project explores the relationships between those three major behaviours and the different levels of coffee concentration as time changes. It is assumed for the short duration of this test that the odour concentration emitted through the centre hole remains constant. The three major behaviours lead to three variables R, θ, α. R is the distance between wasp's head and centre point as time changes (Figure 3.2). θ is the angle in radians between the line through the wasp's head to the origin(centre hole) and the horizontal line though centre point (X-axis) as time changes (Figure 3.3). α is the angle in radians between the wasp's body line (from wasp's head to abdomen) and the horizontal line though the centre point (positive X-axis) as time changes (Figure 3.4). Those three variables were calculated for every 0.2 seconds; using plane geometry (two-dimensional) formulae (refer to *Appendix D)* based on the video files.
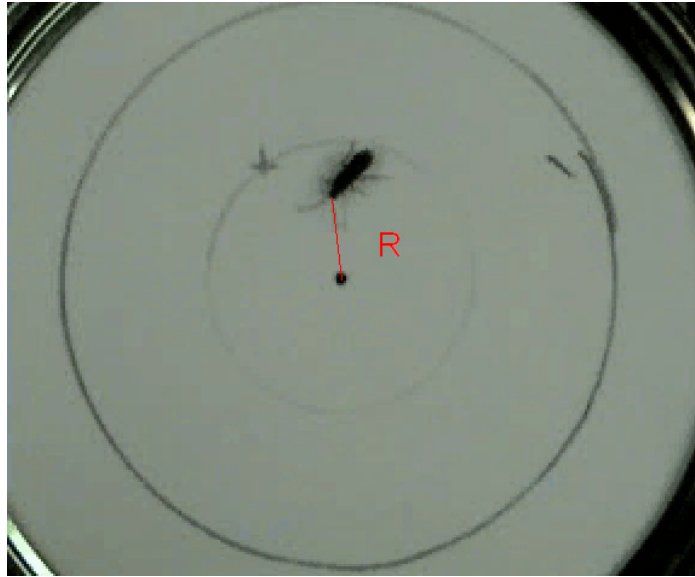
Figure 3.2: The variable R
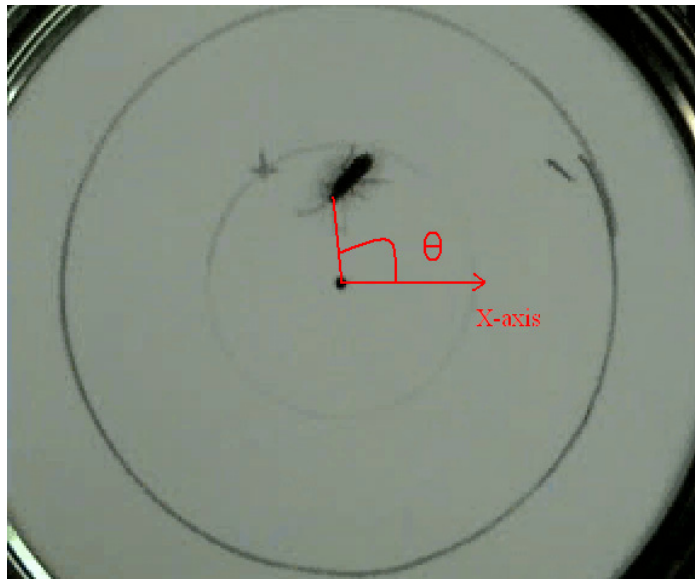
Figure 3.3: The variable θ
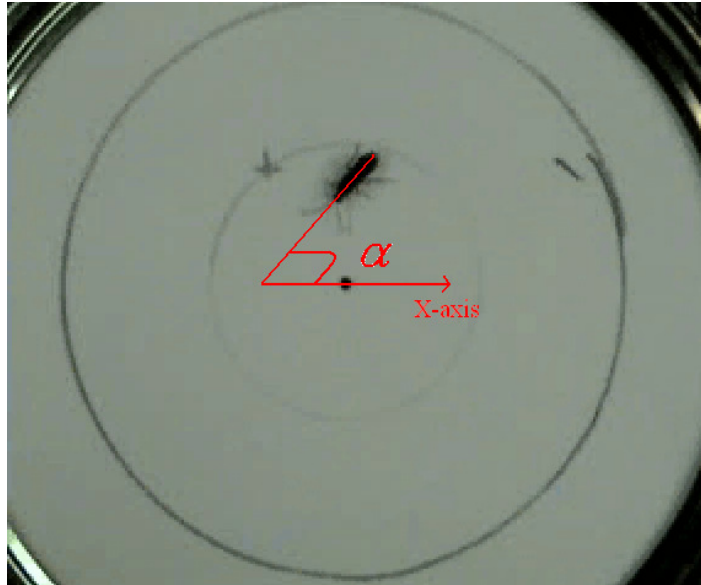
Figure 3.4: The variable α

The other variables could be used are the length of time that the wasp interrogates the centre hole, and the time at which the wasp exits the circle. But the major theme of this study is to investigate if there is a link between single trained *M. croceipes* and different level of target odours, and we use this information to build a simple model. Therefore, these two variables are not included in the model development in this study. However, these two variables could be useful for future development to improve the efficiency of the model.

Tracker 1.5.2 was used to open each of the AVI data files, which were converted from MPEG format using Blaze Media Pro software. Planimetric rectangular coordinates were set up and the centre hole (where the coffee odour emits from) was made to be the origin as showed in Figure 3.5 (B). Tracker was used to mark the positions of wasp's head and end of abdomen every 0.2 seconds and these positions (in X and Y coordinates) were copied into a Microsoft Excel data sheet (refer to *Appendix B*) . Those coordinates were used to calculate variables R, θ, α (refer to *Appendix D*).

Figure 3.5: (A) The wasp was placed on the test sample; the centre point is a hole where coffee odour emits from. (B) Planimetric rectangular coordinates are set up using Tracker software and the centre hole is origin.

Please refer to *Appendix B* for more details about how to use Tracker to track wasp's position (x and y data for head and abdomen) and to export that information into a Microsoft Excel data sheet.

The plane geometry formulas and other formulas used in the study will be showed in *Appendix D.*

3.4 Variables improvement and explanation

The variables were calculated and plotted using Microsoft Excel. However, it is difficult to imagine how the wasp travelled by looking at the graph. Three new variables were introduced to help understand how the wasp travelled. This helped to better understand the relationship between those variables (wasp behaviours) and the levels of concentration. Based on the previous variables (R, θ, α), new variables (ρ , γ and β) are described as follows:

$$\bullet \quad \rho = \frac{R_0 - R_n}{R_0} \qquad\qquad (3.1)$$

where R_0 is the initial distance between the wasp's head and the origin when the wasp is placed on the surface of the test sample. R_n is the distance between the wasp's head and the origin when time is increased by (N x 0.2) seconds. N = 0, 1, 2, 3…

$$\bullet \quad \gamma = \frac{\theta_n - \theta_0}{2\pi} \qquad\qquad (3.2)$$

where θ_0 is the initial angle in radian between the line through the wasp's head to the origin and the X-axis when the wasp is placed on the surface of the test sample. θ_n is the radian between the line through the wasp's head to the origin and the X-axis when time is increased by (N x 0.2) seconds. N = 0, 1, 2, 3…

$$\bullet \quad \beta = \frac{\alpha_n - \alpha_0}{2\pi} \qquad\qquad (3.3)$$

where α_0 is the initial angle in radian between the wasp's body line (from the wasp's head to abdomen) and the positive X-axis when the wasp is placed on the surface of the test sample. α_n is the radian between the wasp's body line and the positive X-axis when time is increased by (N x 0.2) seconds. N = 0, 1, 2, 3…

Instead of using the initial distance (from wasp's head to centre point) to observe the behaviour of the wasp, the variable ρ is used to see if the wasp moved toward the

centre, across the centre or away from centre. If the wasp moves toward the centre from where it is first placed, the value of ρ will be in the region (0, 1). If the wasp's head arrives at the centre, it will exhibit head sticking or hole entering at the centre hole and the value of ρ will become 1. If the wasp moves away from centre point, then the value of ρ will start to decrease from 1 to a negative value (when moving further away from the initial starting position).

The γ is used here to describe how many cycles around the centre point (0, 0) the wasp travelled during the test. The positive γ value means the wasp travelled counter-clockwise; the negative γ value means the wasp travelled clockwise. The integer value of γ means how many cycles the wasp travelled around the centre hole, for example, if $\gamma = -2.5$, the wasp travelled clockwise for 2 and half cycles.

The β is used here similar to γ, but to describe the number of the body rotations during the test. The positive β value means the wasp rotated counter-clockwise. The negative β value means the wasp rotated clockwise. The values of β is how many cycles the wasp has rotated about itself, for example, if $\beta = 2.5$, the wasp's body rotated counter-clockwise for 2 and half cycles.

Chapter 4: Data analysis of wasp behaviour

Based on information provided in the pervious chapter, the variables R, θ, α and the dimensionless variables ρ, γ, β are successfully computed and stored into MS Excel database. This chapter aims to provide detailed information on Excel database structure, the variable plots and the explanations on representative examples. Section 1 gives the details of the data structure in Excel database. Section 2 provides the conditions of the variable calculation. In Section 3, the representative example of variable plots are provided and explained. In Section 4, the major findings are given after the variable plots have analysed.

4.1 Data format in Excel database

In this research, the behavioural data of one hundred and fifty trained wasps are extracted from the experimental movie files and stored into Microsoft Excel database. The data are grouped by the testing conditions, and stored into five different Excel files, named as '001 coffee', '005 coffee', '01 coffee', '02 coffee' and '04 coffee'. Each of these Excel files contained approximately thirty wasp data and stored separately into the different Excel work sheet. A single sheet is named as a unique wasp number that contained this wasp's behaviour information. The indices used to calculate all the relevant variables that are listed in table 4.1.

Table 4.1: The indices that are used for variables calculation.

Title	Comment
t	Testing time, start from 0 until the wasp exit, increase by 0.2 seconds
x1	X coordinate of wasp's head
y1	Y coordinate of wasp's head
R	Variable R (refer to Figure 3.2)
ρ	Variable Rho (refer to Equation 3.1)
Theta	Variable Theta range from 0 to pi (refer to Figure 3.3)
Theta(0-2pi)	Variable Theta range from 0 to 2pi
N	N is the number of cycles the wasp moved towards clockwise/anticlockwise (refer to Appendix D)
Theta + N*2pi	Total angle of Theta
γ	Variable Gamma (refer to Equation 3.2)
x2	X coordinate of wasp's abdomen
y2	Y coordinate of wasp's abdomen
x3	X coordinate of origin (0,0)
y3	Y coordinate of origin (0,0)
x4	X coordinate of point (0,10)
y4	Y coordinate of point (0,10)
dx1	dx1 = x2-x1
dx2	dx2 =x4-x3
dy1	dy1 =y2-y1
dy2	dy2 =y4-y3
Alpha	Variable R range from 0 to pi (refer to Figure 3.4)
Alpha(0-2pi)	Variable Alpha range from 0 to 2pi
N	N is the number of cycles the wasp's body rotated towards clockwise/anticlockwise (refer to Appendix D)
Alpha + N*2pi	Total angle of Alpha
β	Variable Beta (refer to Equation 3.3)

4.2 Conditions of variable calculation

The variables ρ, γ and β for each wasp are calculated based on the formulas in chapter 3.4. The scatter plots of the variables ρ, γ and β against time for each wasp under five different test conditions are plotted out. These plots are used to help familiarize and analyse the relationship between the variables and the different concentration levels of the test samples.

The experiment testing time (from the wasp has been placed on the test sample until it exited the test sample) is slightly different for each wasp. It is also changed when the concentration levels of the test sample are changed. The wasp's testing time is increased sharply when the concentration levels of the test sample are increased. Most of the wasps' testing time is larger than 20 seconds. However, few of the wasps have a shorter testing time less than 20 seconds. Due to the different testing time, only the first 20 seconds' behavioural data is used for the variable analysis and the model implementation.

Ideally, all the wasps reach the centre point or find the test odour. However, some wasps do not reach the centre point (find the test odour). These wasps are not studied in my research and these data are not used for the model implementation.

4.3 Representative example of variable plots

Due to the large number of plots under each test condition, one representative is selected and discussed in this section as an example of others. The plot shows the variable changes from 0 seconds to 20 seconds (In Figure 4.1). The variable is calculated in every 0.2 seconds; all the data points are connected by smoothed lines

without marking the data points.

Figure 4.1: Scatter plot of variables for a selected wasp at 0.001g test concentration. (A) Variable Rho. (B) Variable Gamma. (C) Variable Beta.

(A)

Gamma

(B)

Beta
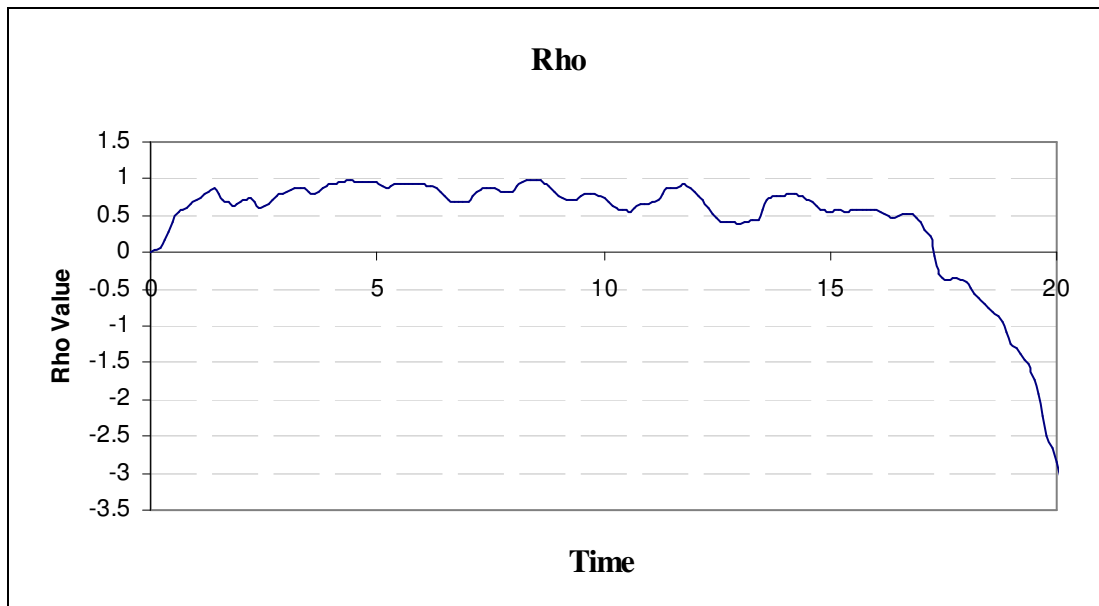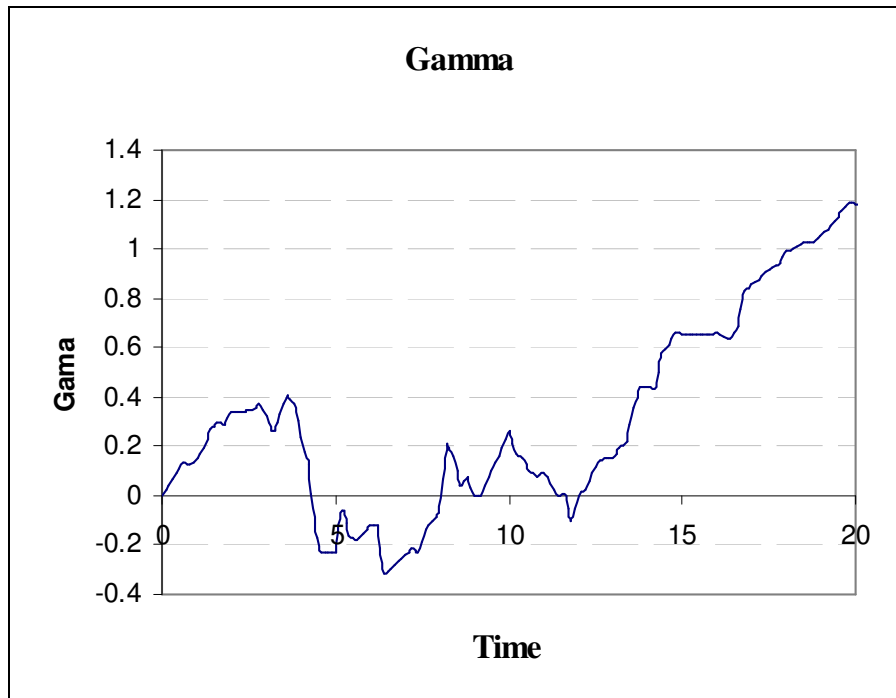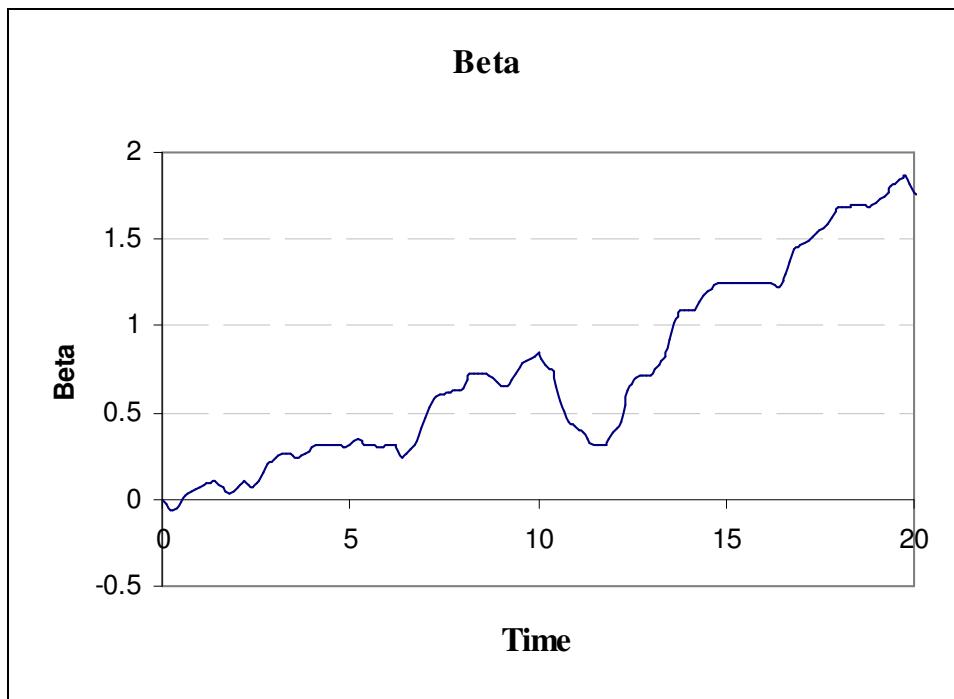
(C)

Figure 4.1(A) illustrates the distance changes between the wasp's head and the centre point (the odour-emitting point) as time changes. The graph shows that the wasp

moved to the centre in 4.4 seconds. The wasp then kept searching around the centre point until 8.7 seconds had elapsed. It moved slightly away from the centre point for 7.5 seconds (total now 16.2 seconds). Finally, the wasp is moved directly away from the centre point. On the other hand, the wasp's activity could be divided into three parts. The first part is the time taken by the wasp to reach the centre point; the second part is the time spent by the wasp at or near the centre point. The last part is the time between when the wasp moved away from the centre point until it either exited the surface of the test sample or 20 seconds had elapsed.

Figure 4.1(B) illustrates the number of cycles the wasp travelled around the centre point during the test as time changes. The graph shows that the wasp moved 0.4 cycles in a counter-clockwise direction in 3.6 seconds. Then it moved clockwise for 2.8 seconds. After that, it moved counter-clockwise until 20 seconds had elapsed. The graph also shows the wasp mainly moved in one direction and totally moved 1.2 cycles. In fact, there is a small oscillation that occurred between 0 seconds and 11.8 seconds. It means the wasp moved continuously in a counter-clockwise and clockwise direction every few seconds. In some cases, the wasp moved equally in both a counter-clockwise and clockwise direction as shown in Figure 4.2 (A). The slope of the graph also shows how fast the wasp moved in this direction. In fact, the slope changed when the test conditions change.

Figure 4.1(C) illustrates the number of the body rotations during the test as time changes. The graph shows that the wasp's body is mainly rotate counter-clockwise and totally rotated 1.8 cycles. The wasp moved in a clockwise direction between 10 seconds and 11.8 seconds. The slope of the graph shows the wasp's body rotated slowly counter-clockwise between 0 seconds and 6.4 seconds. Then it rotated more quickly counter-clockwise between 6.4 seconds and 10 seconds. After quickly reversing rotation (from 10 seconds to 11.8 seconds), the wasp rotated counter-clockwise direction until 20 seconds had elapsed. In some cases, the wasp is rotated equally in both directions as shown in Figure 4.2 (B). The slope of the graph

shows how fast the wasp is rotated in this direction. In fact, the slope changes when the test condition is changed.

(A)
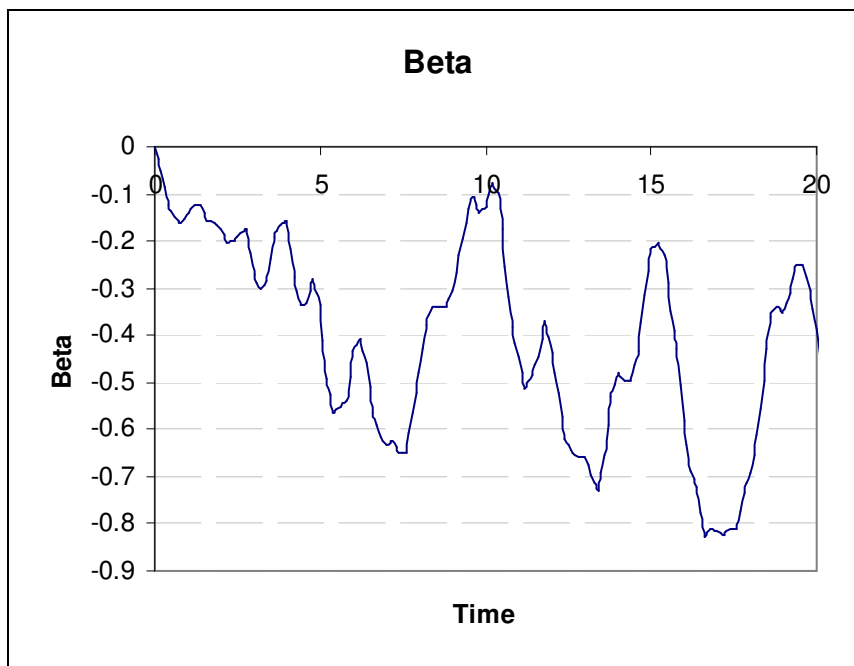
(B)

Figure 4.2: Scatter plot of variables for a selected wasp at 0.02g test concentration. (A) Variable Gamma. (B) Variable Beta.

4.4 Major findings after analysis of variables
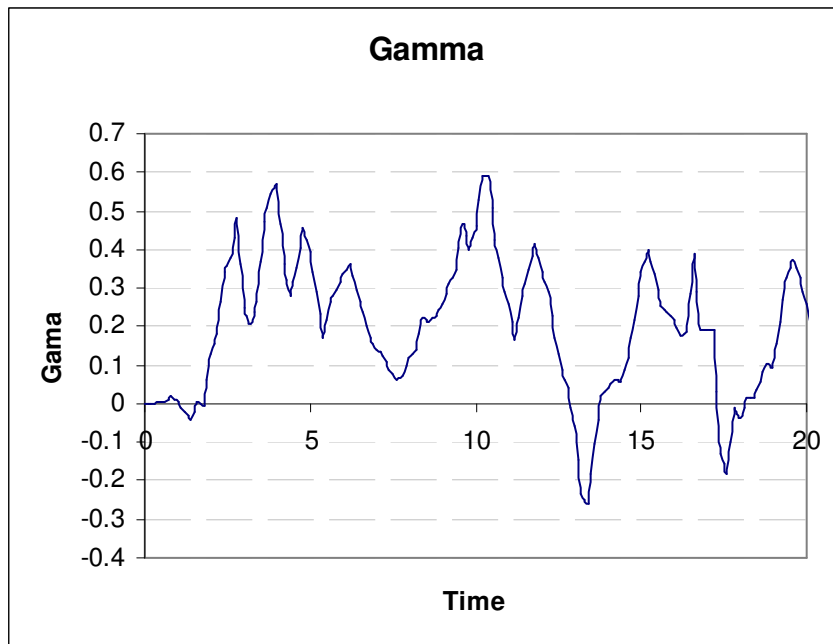
After comparing all the variable plots for five different test conditions, there are some major findings between the variables and the test sample levels.

Most of the wasps could reached the centre (the odour-emitting point) within five seconds. The time that wasp first reached the centre point is called the first resident time. This time is slightly different for the different concentration levels of the test sample. The wasp's first resident time for the 0.02g test is smaller than other; it may be because this concentration value is the same as the training concentration. The average first resident time is decreased when the test sample's concentration level is increased. As the test sample's concentration level increased, the total time that the wasp spend during the test is increased, which means the wasp is spent more time to search around the centre (the odour-emitting point) during the test and the number of times the wasp cross the centre (the odour-emitting point) is larger.

The mean value of γ and β are calculated over all wasps for each of the concentration levels as shown in Figure 4.3. The values on Figure 4.3 show the final positions averaged over all wasps in each concentration level.

Figure 4.3(A) illustrates mean value of variable γ (the number of cycles the wasp travelled around the centre) changes under the different test concentration levels. It shows that the average number of cycles the wasp travelled around the centre for the higher concentration levels is larger than low level concentrations.

Figure 4.3(B) illustrates mean value of variable β (the number of the body rotations) changes under the different test concentration levels. It shows that the average number of the body rotations for the lower concentration levels is larger than high level concentrations.
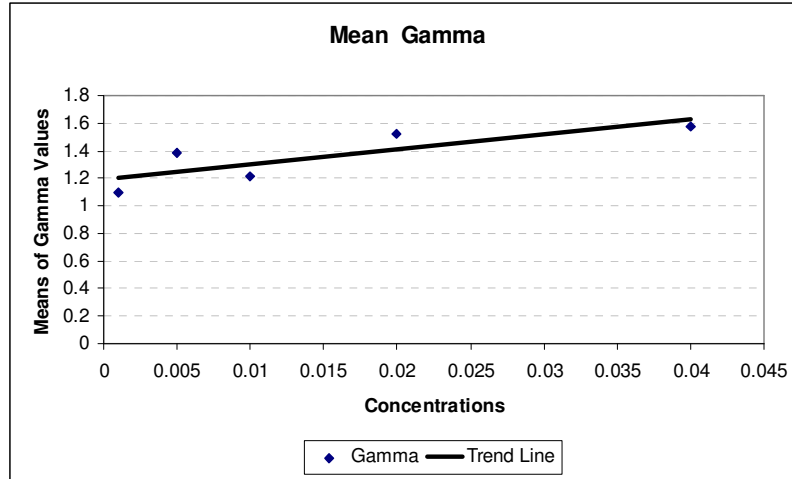
(A)
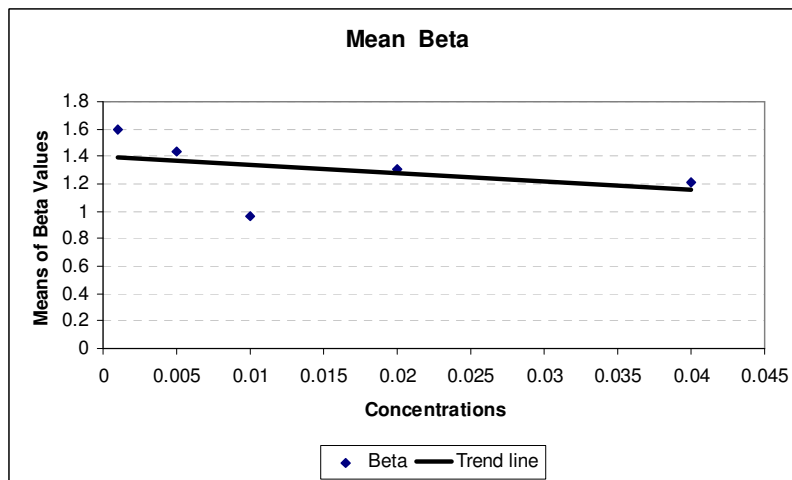
(B)

**Figure 4.3: Means of Gamma and Beta values for the different concentration
levels. (A) Mean Gamma Value. (B) Mean Beta Value.**

In this study, only one test sample's concentration is larger than the training
concentration (0.04g) test sample. The comparison is made between the 0.02g test
sample (same as the training concentration) and the 0.04g test sample (larger than the

training concentration). The variable plots are similar to each other. In general, we can hypotheses that the wasp may not be able to distinguish the difference between the concentrations when they have similar behaviours. It may be because that these two concentration do not have different levels of odour (they both saturate the air in the jar at equilibrium). It is presumable that when the test sample's concentration is saturated, the wasps are appeared to have the similar behaviours. However, more comparisons between the larger concentrations are necessary to investigate the effect of higher concentrations on wasp behaviour.

The mean values of γ and β recorded in Figure 4.3 are close to each other for each of the concentrations. It shows a very weak relationship (shallow slope) between γ and β, and this relationship is almost non-existent. This is not adequate for quantitative concentration sensing. A more elaborate model is developed in the next chapter.

Chapter 5: Stochastic model development and parameter estimation

In this chapter, a simple stochastic mathematical model $dX = a \cdot Xdt + b \cdot dw$ is introduced to simulate the wasp's behaviour. In this stochastic differential equation, X is the dimensionless variable that is discussed in chapter 3.4; a and b are the parameters to be determined in this chapter based on the experimental data; dw is the increments of the standard Wiener process which is normally distributed with a unit variance. The parameters are analysed in order to find out the hidden information between these parameters and the different test concentration levels. In Section 1, the stochastic mathematical model is described for three variables that are used in this study. In Section 2, the parameter estimation process is explained. In Section 3, the computed parameters are analysed and a new model equation is developed, which would lead to the final implementation of the model.

5.1 Stochastic mathematical model

Before establishing a realistic and reliable input-output function that could be used for the model development, we have to figure out what is the input and output, and how they relate to each other. The output is obvious; it is the test sample concentrations. However, the input is not obvious; it is related to the wasp's behaviour (or the improved variables) during the test. To estimate the input, we have to investigate how the wasp's behaviour changes when the concentration level of the test sample is changed. Therefore, a simple stochastic mathematical model is built to simulate the wasp's behaviour (or the improved variable changes) at the different concentration levels. The numerical solution of this model is solved by using the extracted data and the variable values. Ideally, by analysing the numerical solution of this model, we will be able to find out the unique characteristics of the different test samples. These

characteristics can be used as the input for the model.

Based on the knowledge of the analysed variables discussed in the previous chapter, a simple stochastic mathematical model is used in this study to simulate the wasp's behaviour (variables) changes. This mathematical model is based on a stochastic differential equation

$$dX = a \cdot Xdt + b \cdot dw \tag{5.1}$$

where X is the variable, a is the variable parameter, b is the standard Wiener process parameter, dw is the increments of the standard Wiener process which are normally distributed with a unit variance (Mean value is 0, Variance is Δt, Standard Deviation is $\sqrt{\Delta t}$).

Equation (5.1) is the general form used to describe how a variable changes. As discussed in Chapter 3.4, we have introduced three dimensionless variables ρ, γ and β in this study. Therefore, the stochastic differential equation for each of these variables is transferred into three different equations, each with their own parameters.

- For variable ρ, the stochastic differential equation is

$$d\rho = a \cdot \rho \cdot dt + b \cdot dw \tag{5.2}$$

where ρ is the variable used to describe the distance changes between the wasp and the centre point during the test, a is the parameter of variable ρ, b is the parameter of the standard Wiener process, and dw is the increments of the standard Wiener process.

- For variable γ, the stochastic differential equation is

$$d\gamma = c \cdot \gamma \cdot dt + d \cdot dw \tag{5.3}$$

where γ is the variable used to describe the number of cycles the wasp have travelled around the centre point, c is the parameter of variable γ, d is the parameter of the standard Wiener process, dw is the increments of the standard Wiener process.

- For variable β, the stochastic differential equation is

$$d\beta = e \cdot \beta \cdot dt + f \cdot dw \tag{5.4}$$

where β is the variable used to describe the number of the body rotations during the test, e is the parameter of variable γ, f is the parameter of the standard Wiener process, dw is the increments of the standard Wiener process.

In summary, the variables ρ, γ and β are described by these 3 stochastic differential equations with 6 parameters a, b, c, d, e and f. These parameters can be estimated from the experimental data that are extracted from the experiment video files. The parameters a, c and e are representing the spatial and angular velocities. The underlying idea is that these velocities change in response to odour concentration, rather than the static values that were tried in Section 4.4. The erratic nature of the observed movement as illustrated by Figure 4.1 and 4.2 is in this model ascribed to random perturbations superimposed on the regular velocity displacements. The parameters b, d and f are representing the amplitudes of such perturbations.

5.2 Parameter estimation for stochastic model

After defining the stochastic differential equations for each of the variables, their parameters should be estimated. The goal of parameter estimation is to find parameter values which give the model equation the best goodness of fit with the given measured data. This is also called the inverse problem. Normally, a model contains a set of unknown parameters. The parameter estimation processes could be extremely complex due to the large number of parameters.

For the current model, there are only two parameters as shown in equation (5.1). One is variable parameter a, the other one is standard Wiener process coefficient b. The coefficient b denotes the intrinsic noise coefficient which takes negligibly small values to include or exclude the intrinsic noise. The Wiener process is a random number generator to indicate the noise in the equation. In this study, Variance is 0.0125 (refer to Section 5.3.1) and Standard Deviation is 0.1118. Approximately, 85% of the dw values are less than 0.01; the other 15% of the dw values are between 0.01 and 0.03. Since, dw represents the noise in the equation and is far less than Xdt and dX, therefore the deterministic equation can be obtained by removing the terms involving dw, and consider the following part as a least square problem. The equation (5.1) now become

$$dX = a \cdot Xdt \qquad (5.5)$$

From the given data set Xi, and dXi (the extracted experimental data), where $i = 1..M$ ($M = total\_time / dt$), and the equation (5.5), we need to find the unknown parameter a.

The square error of model equation (5.5) is $\prod = \sum_{i=1}^{M} d_i^2 = \sum_{i=1}^{M} (dX_i - aX_i dt)^2$. In order

to reach the minimum square error, let the first derivative of $\prod$ be 0, we got equation

$$\frac{\partial \prod}{\partial a} = -2 \sum_{i=1}^{M} (dX_i - aX_i dt) X_i dt = 0 \tag{5.6}$$

Then we can easily compute the unknown parameter a from equation (5.6), and it is expressed in the form

$$\hat{a} = \frac{\displaystyle\sum_{i=1}^{M} X_i \cdot dX_i}{\displaystyle\sum_{i=1}^{M} X_i^2 \cdot dt} \tag{5.7}$$

where X_i is the experimental value of variables, $dX_i = X_{i+1} - X_i$, $dt = 0.2\,\text{sec}$ and $M = total\_time / dt = 20 / 0.2 = 100$.

Because equation (5.1) is the general form of the variable equation (5.2), (5.3) and (5.4), therefore the unknown parameters a, c and e in equation (5.2), (5.3) and (5.4) can be computed by using the same method and expressed as the same form of equation (5.7).

- For variable ρ, the stochastic differential equation is

$$d\rho = a \cdot \rho \cdot dt + b \cdot dw \tag{5.2}$$

and the solution of the parameter a is

$$\hat{a} \quad = \quad \frac{\sum\limits_{i=1}^{M} \rho_i \cdot d\rho_i}{\sum\limits_{i=1}^{M} \rho_i^{\,2} \cdot dt} \tag{5.8}$$

where ρ_i is the measured experimental value of variable ρ, $d\rho_i = \rho_{i+1} - \rho_i$, $dt = 0.2\,\sec$ and $M = total\_time / dt = 20 / 0.2 = 100$.

- For variable γ, the stochastic differential equation is

$$d\gamma = c \cdot \gamma \cdot dt + d \cdot dw \tag{5.3}$$

and the solution of the parameter c is

$$\hat{c} \quad = \quad \frac{\sum\limits_{i=1}^{M} \gamma_i \cdot d\gamma_i}{\sum\limits_{i=1}^{M} \gamma_i^{\,2} \cdot dt} \tag{5.9}$$

where γ_i is the measured experimental value of variable γ, $d\gamma_i = \gamma_{i+1} - \gamma_i$, $dt = 0.2\,\sec$ and $M = total\_time / dt = 20 / 0.2 = 100$.

- For variable β, the stochastic differential equation is

$$d\beta = e \cdot \beta \cdot dt + f \cdot dw \tag{5.4}$$

and the solution of the parameter e is

$$\hat{e} \quad = \quad \frac{\displaystyle\sum_{i=1}^{M} \beta_i \cdot d\beta_i}{\displaystyle\sum_{i=1}^{M} \beta_i^{\,2} \cdot dt} \tag{5.10}$$

where β_i is the measured experimental value of variable β, $d\beta_i = \beta_{i+1} - \beta_i$, $dt = 0.2\,\sec$ and $M = total\_time / dt = 20 / 0.2 = 100$.

The variable parameters are solved and successfully computed from the equations (5.8), (5.9) and (5.10). Since the increments of the standard Wiener process dw and its parameter are very small, we omit them and only focus on the variable parameter analyse.

5.3 Parameter analysis

The variable parameters a, c and e are computed based on equation (5.8), (5.9) and (5.10). These parameters are stored in a separate MS Excel database for analysis.

5.3.1 Parameter accuracy improvement

The initial conditions are $dt = 0.2\,\sec$ and $M = total\_time / dt = 20 / 0.2 = 100$. There are a total of 100 measured variable X values and dX values (from equation 5.7) that are used to calculate the variable parameters. One way to improve the parameter

accuracy is keeping the experiment time at a constant value (20 seconds in this study), and then, decrease the time interval dt (the time between data collecting), therefore the total number of measured variable X values and dX values are increased.

This leads to another question: how do we decide the value of dt in order to get the parameter value with a reasonable accuracy. As dt decrease, the number of the variable data (X and dX) used to compute the parameter will be increased sharply. This will increase the complexity of the parameter calculation. For instance, when dt = 0.001562, there will be 12802 variable X values and dX values used for parameter calculation. This will cause the calculation time to increase sharply.

In order to find out a suitable dt value, a simulation is carried out to examine the parameter changes under the different dt values. The experiment observation time is 20 seconds for all the wasps. The dt value is divided into eight different groups which start with 0.2 seconds and decrease by half each time. The dt values are 0.2 seconds, 0.1 seconds, 0.05 seconds, 0.025 seconds, 0.0125 seconds, 0.00625 seconds, 0.003125 seconds and 0.001563 seconds. The simulation results have shown that the parameter value (a or c or e) is increased when the time interval dt is decreased. In other words, the parameter value is increased when the amount of data that are used to calculate the parameter is increased. The experiment results have also shown that the parameter value is increased slowly as dt keeps decreasing, it will reach a stable point (stay constant) when dt is extremely small.

Figure 5.1 illustrates the relationship between the parameter a and the time interval dt for four randomly picked wasps at 0.02g coffee concentration. In this figure, Y axis is representing the parameter a values, the number 1 to 8 on X axis is representing eight different dt values, which are 0.2, 0.1, 0.05, 0.025, 0.0125, 0.00625, 0.003125 and 0.001563. Each joining line is representing the parameter a value changes for the same wasp as dt changes. For the same wasp, the parameter value is changed much more slowly when dt is less than 0.00125 seconds. It will reach its stable point when dt is less than 0.001563 seconds. The parameter value at dt = 0.0125 is very close to

the dt value at dt = 0.001563.
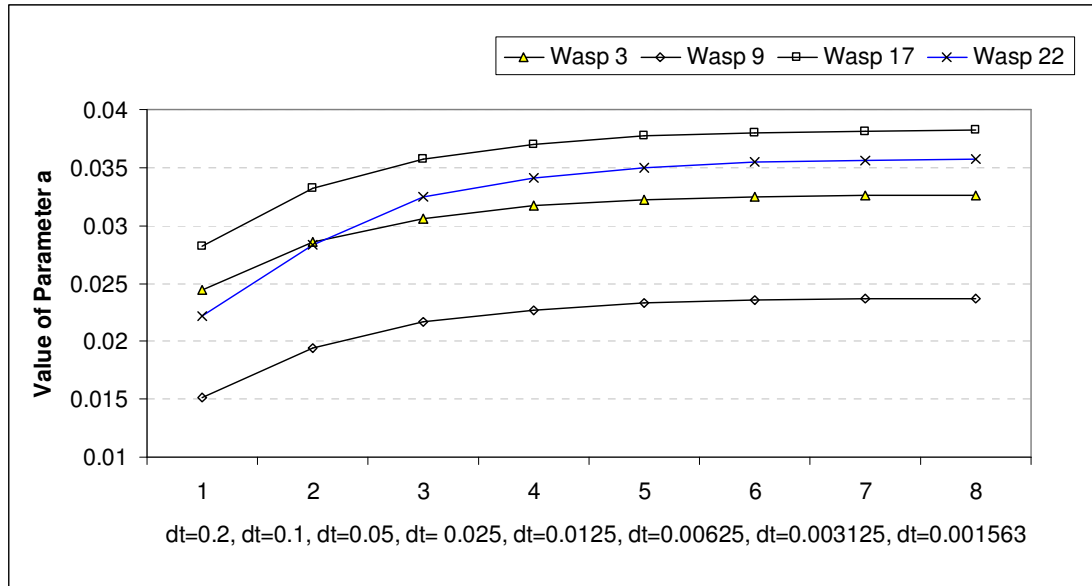
Figure 5.1: Scatter plot of the parameter a for four randomly picked wasps. Four wasps were randomly picked up and their parameter a values were calculated using 8 different dt values (from 0.2 to 0.001563).

After analysing the experiment results, it is decided to use dt = 0.0125 for all the parameter calculation and analysis. When dt = 0.0125 seconds, the parameter value is very close to the value we got from dt = 0.001562. However, the amount of data that are used to calculate the parameter value at dt = 0.0125 is eight times less than the amount of data used to calculate the parameter value at dt = 0.001562. The calculation time is much less than the time that dt = 0.001562 seconds as well.

A computer program is written in VBA programming language to compute and record the parameter a, c and e values at the different dt values. It is provided in Appendix E. After the parameter values have been computed using eight different dt values for all the wasps, the graphs of the parameter changes at 0.02g test concentration is plotted and provided as a example in Appendix E.

5.3.2 Linear regression analysis

The variable parameters a, c and e are computed at dt = 0.0125 sec and stored in a separate MS Excel file for statistical analysis. A regression analysis is carried out to examine the relationship between the parameters and the test concentration levels. The results have shown that the parameters a, c and e do not have a straightforward linear relationship with the test concentration levels. Most of the parameter values are spread into a similar range when the test concentration levels are increased, and they can not be classified. The scatter plots of parameters versus test concentrations are provided in Figure 5.2.

Figure 5.2(A) illustrates the distribution of the parameter a values under the different test concentrations. The graph shows that most of the parameter a values are in the same range from -0.06 to 0.4 when the test concentration is less than or equal to 0.01g. The parameter a values are spread from -0.06 to 0.6 when the test concentration is equal to the training concentration (0.02g). The parameter a values are in the small range from -0.06 to 0.2 when the test concentration is 0.04.

Figure 5.2(B) illustrates the distribution of the parameter c values under the different test concentrations. The graph shows that the parameter c values are in the range from -0.02 to 0.2 when the test concentration is less than or equal to 0.02g. The parameter c values are in the small range from -0.02 to 0.1 when the test concentration is 0.04g.

Figure 5.2(C) illustrates the distribution of the parameter e values under the different test concentrations. The graph shows that the parameter e values are all in the range from -0.03 to 0.18 except when the test concentration is 0.02g. The parameter e values are in the small range from -0.03 to 0.1 when the test concentration is 0.02g.

**Figure 5.2: Scatter plot of the parameters versus test concentrations. (A)
Parameter a. (B) Parameter c. (C) Parameter e.**
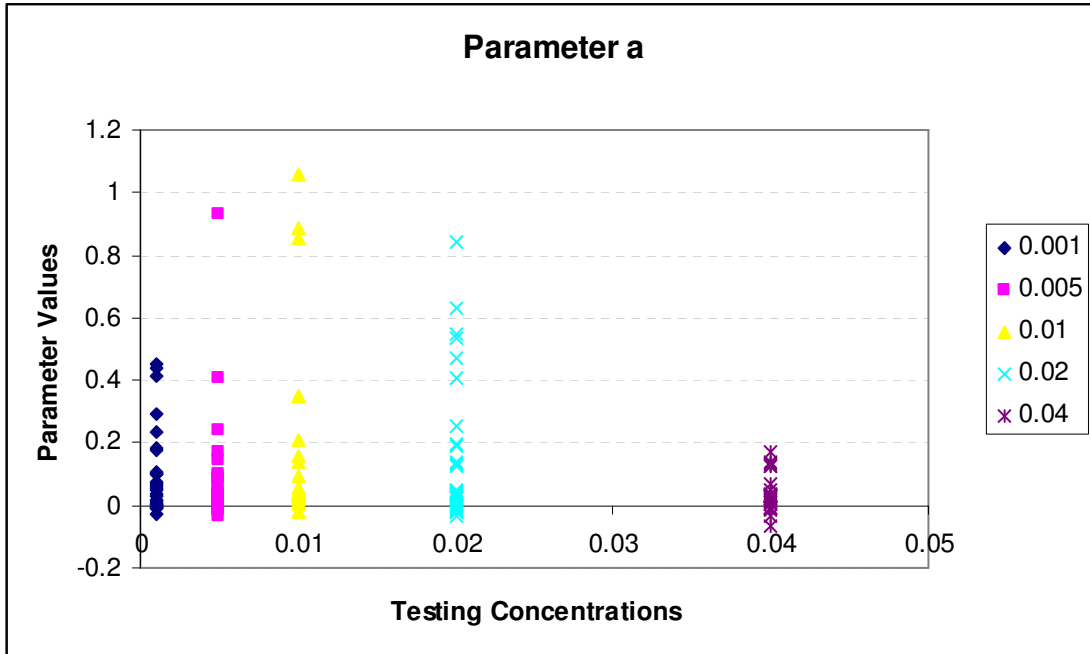
(A)
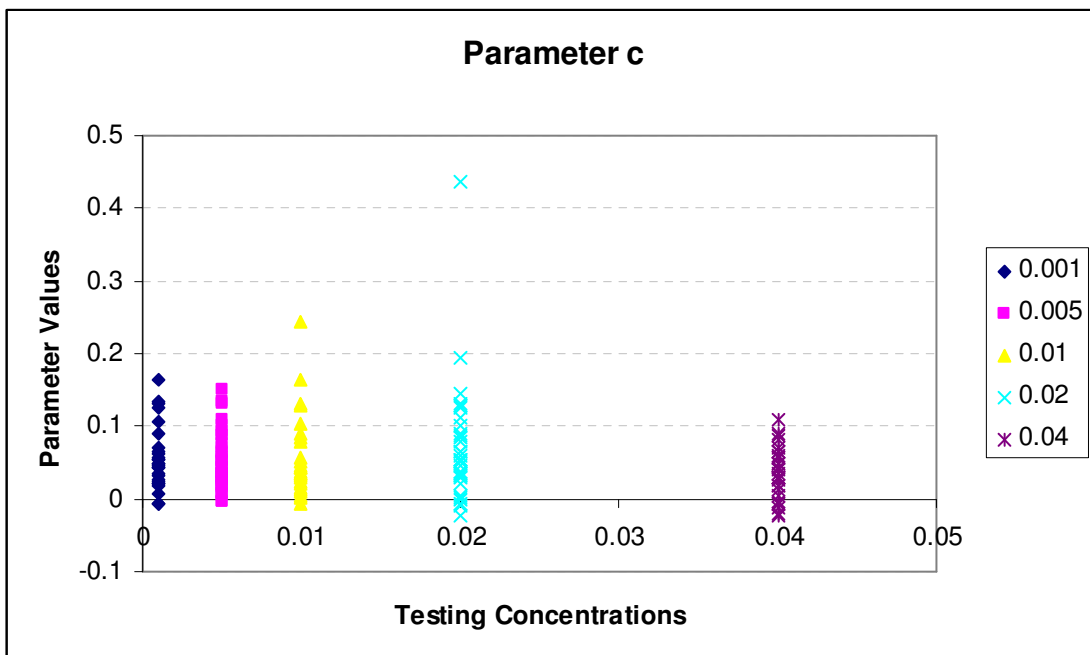
(B)
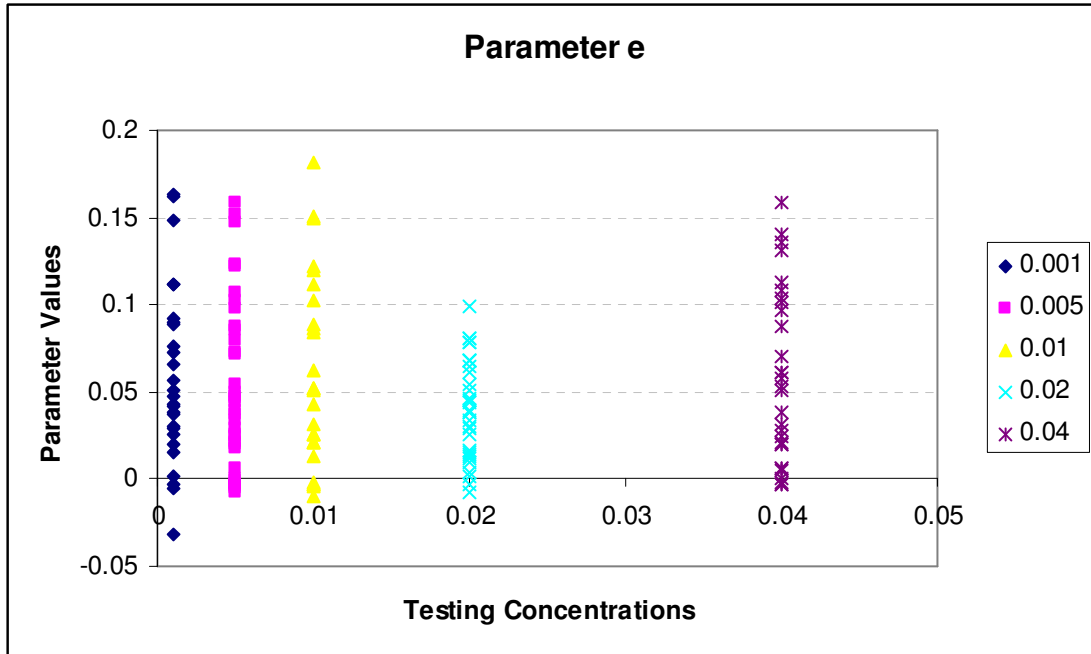
(C)

It is disappointing that the parameter a, c and e itself does not have a straightforward relationship with the test concentrations. Consider the real situation of a trained wasp's behaviour during the test; there are three variables that represent three different movements occurring at the same time during the test. Therefore, instead of studying them separately with the test concentrations, it is also necessary to investigate the relationship between the test concentrations and the combination of parameters a, c and e. There is most likely a connection between the test concentrations and the combination of the parameter a, c and e. Finding this information will lead us to a model that uses the combination of the parameters a, c and e as the independent input variables to get the test concentration as the dependent output variable.

5.3.3 New model equation and nonlinear regression analysis

In order to determine the connection between the parameters a, c and e, the study is carried out to investigate the relationship between parameter combinations. After analysis of many different parameter combinations' plots, it is found that $|a|$ and $e^{-\left|\frac{c-e}{a}\right|}$ have an exponential relationship for each of the test concentrations. Different combinations of a, c and e are also tested to check if they can provide better results. However, the test results shown that $e^{-\left|\frac{c-e}{a}\right|}$ provide better results than other combinations of a, c and e, such as $e^{-\left|\frac{a-e}{c}\right|}$. The scatter plots of $|a|$ versus $e^{-\left|\frac{c-e}{a}\right|}$ for each of the test concentrations are provided in Figure 5.3.

The Figure 5.3 illustrates the distribution of $e^{-\left|\frac{c-e}{a}\right|}$ values via $|a|$ changes for each test concentrations. The data distribution is slightly different from Figure 5.3(A) to Figure 5.3 (E). However, the general trend line of the data is similar to each other, which are all exponential functions.
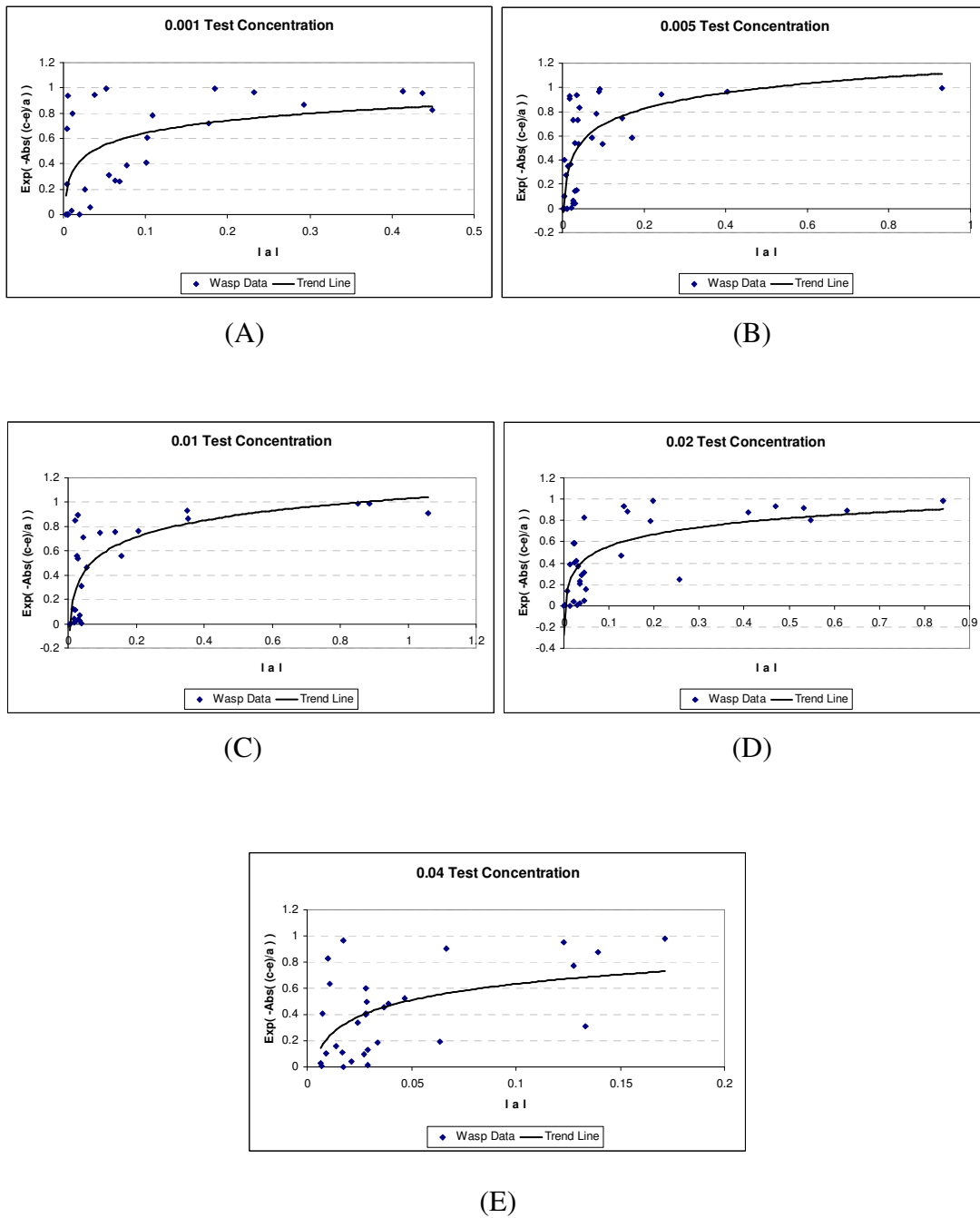
Figure 5.3: Scatter plot of $|a|$ **versus** $e^{-\left|\frac{c-e}{a}\right|}$ **for each of the test concentrations.**
(A) 0.001 test concentration. (B) 0.005 test concentration. (C) 0.01 test concentration. (D) 0.02 test concentration. (E) 0.04 test concentration.

Based on the study of the general exponential functions in Figure 5.3, a new model equation is found as showed below:

$$e^{-\left|\frac{c-e}{a}\right|} = 1 - \overline{\alpha} \cdot e^{-\overline{\beta}|a|}$$

(5.11)

where a, c and e are the known parameters from equation (5.8), (5.9) and (5.10). $\overline{\alpha}$ and $\overline{\beta}$ are the unknown parameters for equation 5.11.

Nonlinear regression analysis is performed to examine the new parameters in equation (5.11) for each of the test concentrations by using the MATHEMATICA software through an iterative process. The best fit parameters $\overline{\alpha}$ and $\overline{\beta}$ values and its Mean Square Error for each of the test concentrations are listed in table 5.1.

Test Concentration	$\overline{\alpha}$	$\overline{\beta}$	Mean Square Error
0.001	0.744068	6.28999	0.0915192
0.005	0.930106	16.2554	0.076745
0.01	0.905896	8.41131	0.0657437
0.02	0.878047	6.41053	0.052781
0.04	0.812218	8.59345	0.077577

Table 5.1: New parameters $\overline{\alpha}$, $\overline{\beta}$ and their mean square error values for each of the test concentrations.

Substitute the new parameters value from Table 5.1 into the equation (5.11), the new model equation for each of concentrations are solved and listed blow.

- For 0.001 Concentration, the model equation is

$$e^{-\left|\frac{c-e}{a}\right|} = 1 - 0.744068 \cdot e^{-6.28999|a|} \tag{5.12}$$

- For 0.005 Concentration, the model equation is

$$e^{-\left|\frac{c-e}{a}\right|} = 1 - 0.930106 \cdot e^{-16.2554|a|} \tag{5.13}$$

- For 0.01 Concentration, the model equation is

$$e^{-\left|\frac{c-e}{a}\right|} = 1 - 0.905896 \cdot e^{-8.41131|a|} \tag{5.14}$$

- For 0.02 Concentration, the model equation is

$$e^{-\left|\frac{c-e}{a}\right|} = 1 - 0.878047 \cdot e^{-6.41053|a|} \tag{5.15}$$

- For 0.04 Concentration, the model equation is

$$e^{-\left|\frac{c-e}{a}\right|} = 1 - 0.812218 \cdot e^{-8.59345|a|} \tag{5.16}$$

where a, c and e are the known parameters from equation (5.8), (5.9) and (5.10).

Chapter 6: Model development

In this chapter, the model is developed based on mathematical equation (5.11) and the function equations between its parameters ($\overline{\alpha}$ and $\overline{\beta}$) and the test concentrations. It will use the previous parameters a, c and e as the input variables, and output the relevant test concentration. In Section 1, the model equations are estimated and the model flow diagram is provided. In Section 2, the model equation validation process is carried out. In Section 3, the initial conditions and the interface of the model are provided.

6.1 Model equation estimation

Before implementing the model, the function between new parameters $\overline{\alpha}$ and $\overline{\beta}$ from equation (5.11) and the test concentrations must be found out. Based on the data in table 5.1, the scatter plot of the parameters $\overline{\alpha}$, $\overline{\beta}$ and the test concentrations are provided in Figure 6.1.

The Figure 6.1(A) illustrates the parameter $\overline{\alpha}$ changes when the test concentration is increased. The parameter $\overline{\alpha}$ and the test concentrations appeared to have a strong linear relationship apart from the $\overline{\alpha}$ value when the test concentration is 0.001 g, which is marked as a triangle in Figure 6.1(A).

The Figure 6.1(B) illustrates the parameter $\overline{\beta}$ changes when the test concentration is increased. The parameter $\overline{\beta}$ and the test concentrations appeared to have a quadratic polynomial relationship apart from the $\overline{\beta}$ value when the test concentration is 0.001

g, which is marked as a triangle in Figure 6.1(B).

(A)   (B)
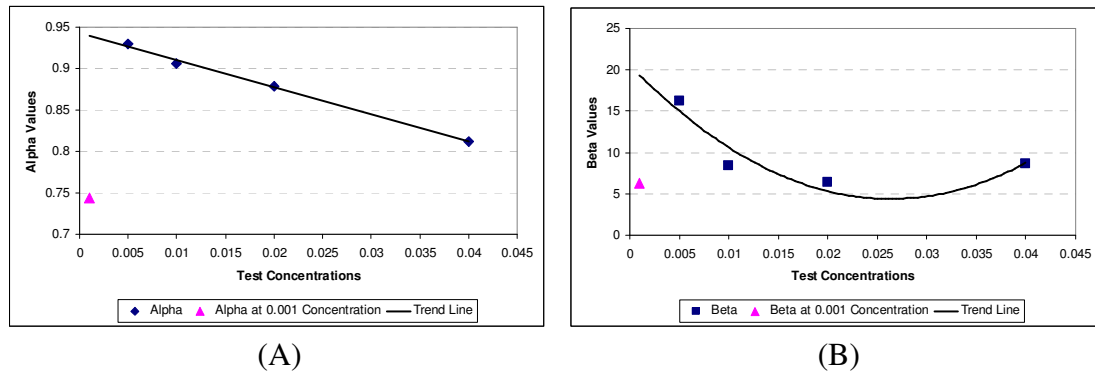
Figure 6.1: Scatter plot of the parameters versus the test concentrations with the trend line. (A) Scatter plot of parameter $\overline{\alpha}$. (B) Scatter plot of parameter $\overline{\beta}$.

Both Figure 6.1(A) and (B) show that the data trend could be described by the linear and the quadratic polynomial functions except when the test concentration is 0.001g. The reason for that may be because the test concentration is much smaller than the training concentration. Therefore, it is not detected by the wasp. If the wasp is not detecting anything, their behaviour is different. They are not searching. We assume that the wasp's behaviour is all similar to each other when the test concentration is much smaller than the training concentration (the test odour is not detected by the wasp).

Linear and nonlinear regression analysis is carried out to solve the linear and the quadratic polynomial functions as showed in Figure 6.1(A) and (B) by using the MATHEMATICA software.

The linear function used to model parameter $\overline{\alpha}$ is

$$\overline{\alpha} = f_1(Conc) = -3.2812 \cdot Conc + 0.9431 \tag{6.1}$$

where Conc is the test concentration with $0.005 < Conc \le 0.04$.

The quadratic polynomial function used to model parameter $\overline{\beta}$ is

$$\overline{\beta} = f_2(Conc) = 23363 \cdot Conc^2 - 1229.9 \cdot Conc + 20.568 \tag{6.2}$$

where Conc is the test concentration with $0.005 < Conc \le 0.04$.

The R square is also calculated to measure the relative prediction power of the equation (6.1) and (6.2). The R square value for equation (6.1) is 0.996, and the R square value for equation (6.2) is 0.8653, indicating both equation (6.1) and (6.2) are very good to predict the parameter values from the test concentrations.

Now, look at the equation (5.11) as showed blow

$$e^{-\left|\frac{c-e}{a}\right|} = 1 - \overline{\alpha} \cdot e^{-\overline{\beta}|a|} \tag{5.11}$$

where a, c and e are the parameters that were solved from the previous chapter. $\overline{\alpha} = f_1(Conc)$ and $\overline{\beta} = f_2(Conc)$ were also solved in equation (6.1) and (6.2).

Substitute equation (6.1) and (6.2) for $\overline{\alpha}$ and $\overline{\beta}$ into the equation (5.11), the equation become

$$e^{-\left|\frac{c-e}{a}\right|} = 1 - f_1(Conc) \cdot e^{-f_2(Conc) \cdot |a|} \tag{6.3}$$

Since the function $f_1(Conc)$ and $f_2(Conc)$ are solved, we are able to get the test concentration "Conc" from the known variables a, c and e.

The model is developed based on the equations (5.11) and the known parameters a, c, e. A computer algorithm is written by VBA programming language in MS Excel to convert the mathematical equations into a computer solvable problem. It is provided in Appendix F. The flow diagram of the model is summarized in Figure 6.2.
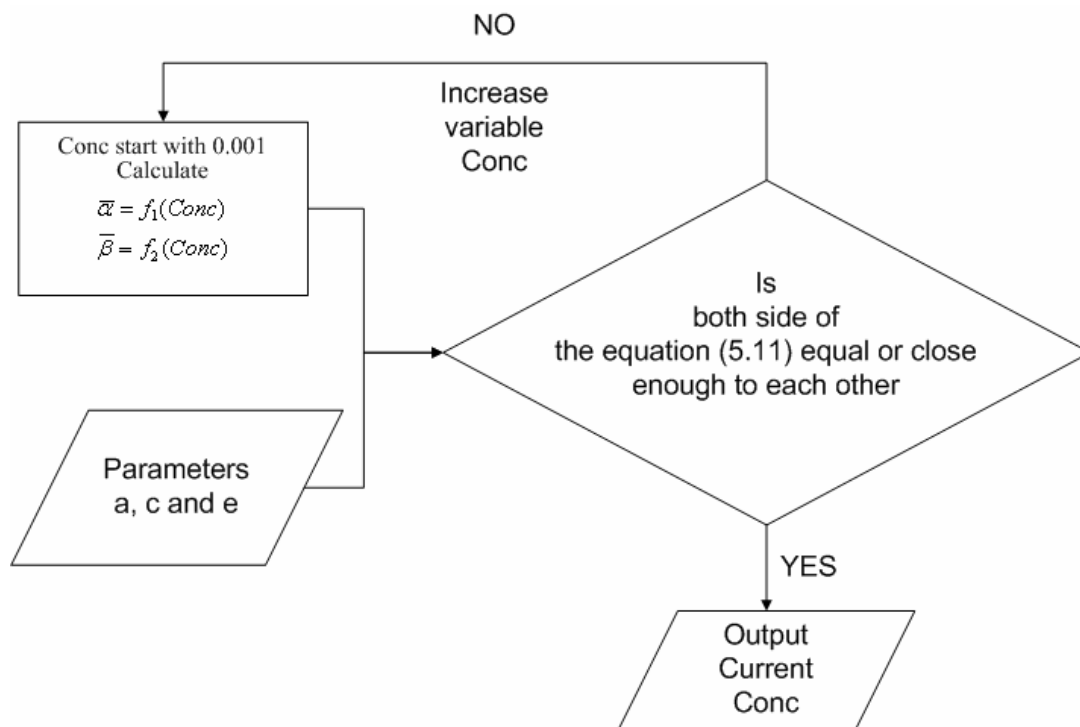
Figure 6.2: An overview of the Model process.

6.2 Model equation validation

The validation process is carried out to check the accuracy of the model and its equations (6.1), (6.2), (6.3). The following procedures are preformed to validate the model and fine tune the equations (6.1), (6.2) based on the equation (6.3).

First, four sets of the experimental data (a, c and e) are collected from each of the test concentrations (0.005, 0.01, 0.02 and 0.04). Each of the data set contains approximately 30 data. They are computed though the model and the computational results for both sides of the equation (6.3) are stored and compared. If they are equal or close enough to each other, it means these equations are accurate for the model. Otherwise, the equations (6.1), (6.2) will need to be fine tuned to make the equation (6.3) satisfied for all the experimental data set or most of the experimental data sets.

The equation used to validate the accuracy of equation (6.3) is

$$\frac{e^{-\left|\frac{c-e}{a}\right|}}{1-f_1(Conc)\cdot e^{-f_2(Conc)\cdot|a|}} \tag{6.4}$$

where $e^{-\left|\frac{c-e}{a}\right|}$ is the left side of equation (6.3), $1-f_1(Conc)\cdot e^{-f_2(Conc)\cdot|a|}$ is the right side of the equation (6.3).

Next, the initial parameters of the equations (6.1) and (6.2) were fine tuned by comparing the value of the equation (6.4) for all the data set. If most of the value are equal or close to 1, it means these equations are accurate for the model. Otherwise, the equations (6.1), (6.2) will need to be changed to make the value of the equation (6.4) close to 1 for all the experimental data sets or most of the experimental data sets.

Last, the equation (6.1) and (6.2) are determined when the equation (6.4) is given the best certifiable ratio from all the experimental data sets.

Equation (6.1) is the best equation that could be used in this study to describe the relation between the parameter $\bar{\alpha}$ and the test concentrations. It remained the same for the model implementation. Equation (6.2) is changed to give the best certifiable ratio from the experimental data set. The equation for the parameter $\bar{\beta}$ is changed to

$$\bar{\beta} = f_2(Conc) = 91249 \cdot Conc^2 - 2937.6 \cdot Conc + 28.662 \qquad (6.5)$$

where Conc is the test concentration with $0.005 < Conc \le 0.04$.

The computational results of equation (6.4) for all the data sets at four different test concentrations are plotted in Figure (6.3) after the equations (6.1) and (6.2) are determined. The graphs show that 64% to 76% of the data are equal or close to 1, which means they are satisfied by the equations (6.1) (6.3) and (6.5).

Figure 6.3: Computational results of the equation (6.4) for all the data sets at four different test concentrations. (A) 0.005g. (B) 0.01g. (c) 0.02g. (D) 0.04g.

(A) 0.005g

(B) 0.01g

(C) 0.02g

(D) 0.04g
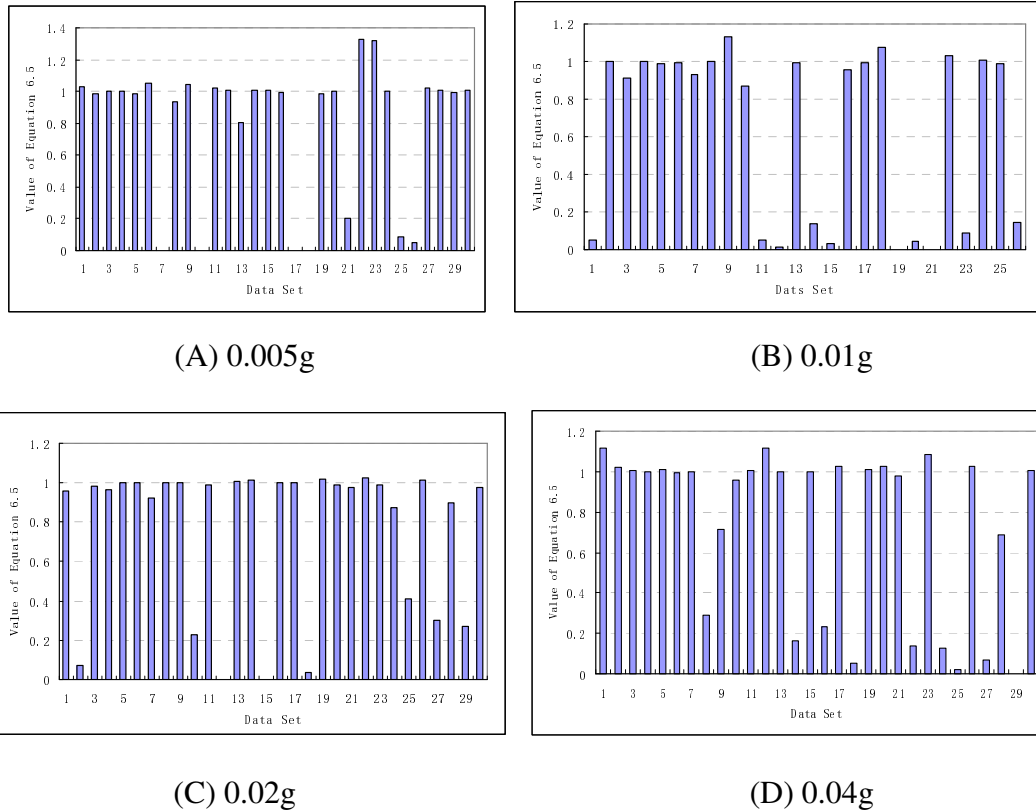
6.3 Initial conditions and the model interface

As described in the previous section, the model is developed based on the equations (6.1), (6.3) and (6.5). It is written by VBA programming language in MS Excel, and uses the parameters a, c, e as the input variables to predict the actual concentrations. The model is based on the study of the experimental data which are collected from four different test concentrations (0.005g, 0.01g, 0.02g and 0.04g). Therefore, the predicting range of the model is from 0.005g to 0.04g.

The interface of the model is provided in Figure 6.4. It has a friendly user interface that can easily process multiple rows of the input variables at the same time.

Input parameters				Model Output Concentration	Actual Concentration
a	c	e			
-0.012455	0.086289	0.073241	>>>>>	0.039999988	0.005
-0.002142	0.109676	0.079046		0.005	0.005
0.071494	0.064079	0.025799		0.008	0.005
0.002681	0.024469	0.047654		0.010000001	0.005
0.020618	0.020094	-0.000746		0.005	0.005
0.093831	0.02362	0.050495		0.006	0.01
0.055787	0.129038	0.086065		0.036999993	0.01
0.004102	0.009884	0.051642		0.010000001	0.01
0.028545	0.005082	0.102241		0.018000001	0.01
-0.021673	0.099824	0.029361		0.016000001	0.02
0.044054	0.029135	0.080212		0.021000002	0.02
0.192217	0.033279	0.078432		0.013000001	0.02
0.036069	0.144701	0.013854		0.020000001	0.02
-0.010119	0.02955	0.027604		0.039999988	0.04
-0.007254	0.063905	0.057379		0.039999988	0.04

Calculate Concentration

Figure 6.4: The interface of the model.

Chapter 7: Simulation results and discussion of the model

In this chapter, the model is tested by using the new wasp data that are collected from the experiment at various test concentrations. The model simulation results are compared with the actual results to find out the accuracy and limitation of the model. In Section 1, a discussion of the simulation results and a comparison with the actual values are given. In Section 2, a discussion of the model and its limitations are provided. In Section 3, the experimental data is re-simulated after lumping 0.02g and 0.04g behaviour data, and its results are analysed and discussed.

7.1 Simulation results

A total of 120 new data sets (not used for the model development) are tested by the model. Each data set included three parameters a c and e which are extracted from one wasp video at a particular concentration of the test sample. All the data sets are summarized in Table 7.1.

Dosage	Number of data
0.005g	30
0.01g	30
0.02g	30
0.04g	30
Σ	120

Table 7.1: Summary of the data sets

The model simulation results were not exactly equal to the actual dosage values. Most

of them were close to the actual dosage value. In this study, we use actual dosage value $+/-$ 0.005(25% of the training dosage) as the particular ranges to classify the simulation results. In fact, the classify range for 0.005, 0.01, 0.02 and 0.04 dosages are [0 – 0.01], [0.005 – 0.015], [0.015 – 0.025] and [0.035 – 0.045]. The model was able to roughly predict the actual dosage value. Approximately sixty to seventy percent of simulated results are close to actual dosage values, which are in the given range. The simulation results for each dosage are summarized and plotted in Figure 7.1.

The histogram in Figure 7.1(A) illustrates the simulation results for 0.005g dosage. The Figure shows 63.3% of the simulation results are close to 0.005, which are in the range of 0 to 0.01. The other 36.7% of the simulation results are larger than 0.005, which are in the range between 0.01g and 0.04g dosage.

The histogram in Figure 7.1(B) illustrates the simulation results for 0.01g dosage. The Figure shows 70% of the simulation results are close to 0.01, which are in the range of 0.005 to 0.015. The other 30% of the simulation results are larger than 0.01, which are in the range between 0.015g and 0.04g dosage.

The histogram in Figure 7.1(C) illustrates the simulation results for 0.02g dosage. The Figure shows 60% of the simulation results are close to 0.02, which are in the range of 0.015 to 0.025. The other 30% of the simulation results are either less or larger than 0.02. There is 13.3% of the simulation results are in the range of 0.005 to 0.015. There is 16.7% of the simulation results are in the range of 0.025 to 0.04.

The histogram in Figure 7.1(D) illustrates the simulation results for 0.04g dosage. The Figure shows only 20% of the simulation results are close to 0.04, which are in the range of 0.035 to 0.045. The other 80% of the simulation results are much less than 0.04. There is 13.3% of the simulation results are in the range of 0.025 to 0.035. There is 50% of the simulation results are in the range of 0.015 to 0.025. There is 16.7% of

the simulation results are less than 0.015.

In summary, the model can roughly predict the target concentration values in a particular range of 0.005 to 0.02. The actual rate is around 60% to 70%.

Figure 7.1: Statistic results of simulation and 5% error bar. (A) 0.005g. (B) 0.01g. (c) 0.02g. (D) 0.04g
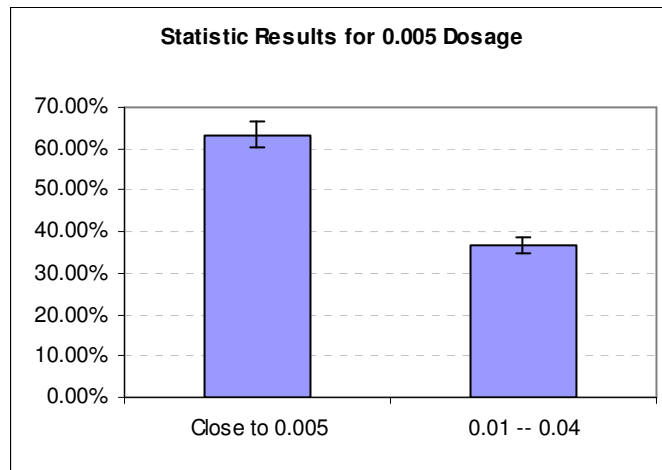
(A) 0.005g
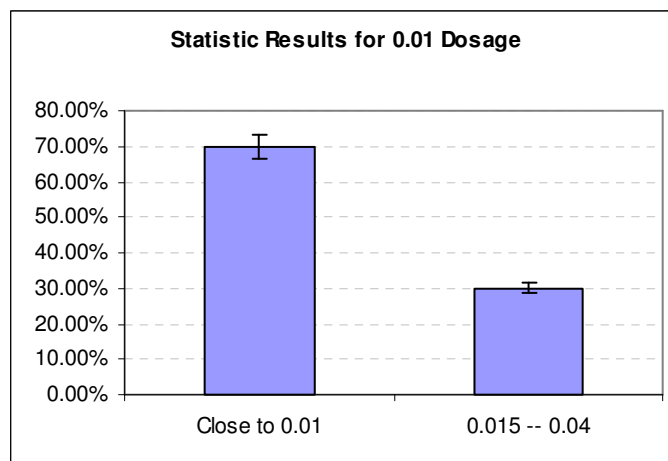
(B) 0.01g
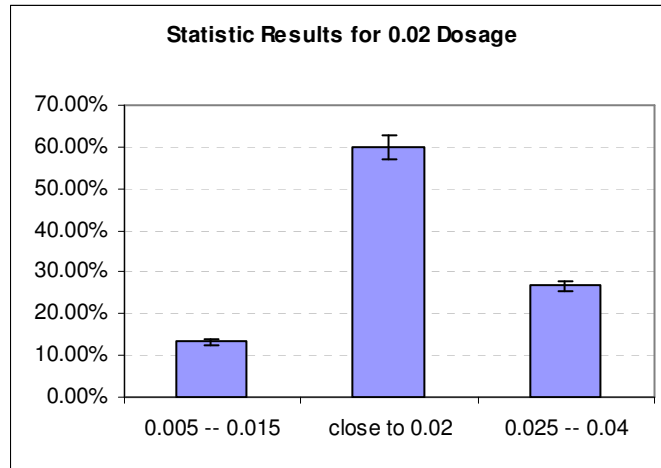
(C) 0.02g
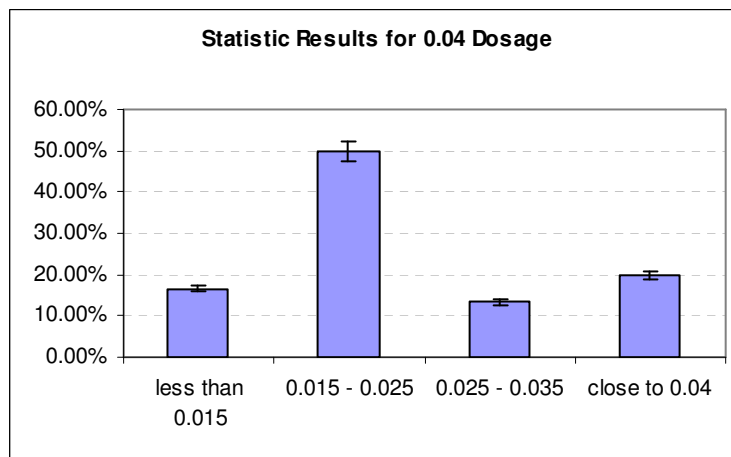
(D) 0.04g

7.2 Discussion of the Model and its limitations

In chapter three to six, a model has been presented that utilizes the behaviour of a single trained wasp to predict a target concentration value. This model has three unique input variables a, c and e which is computed based on three major behaviour of the trained wasp during the test experiment. It will output the target's actual concentration value based on these input variables.

The original predicting range of the model is from 0.005g dosage to 0.04g dosage.

However, through the analysis of the simulation results in the last section, it was shown that the model failed to predict the target's actual concentration value at 0.04g dosage (only 20% of the simulation results close to the actual dosage value). This may be caused by the lack of information on the experimental data that are higher than the training dosage or the actual volatile concentration at 0.04g of coffee is indistinguishable from 0.02g of coffee placed in the glass jars (coffee concentration is saturated at 0.02 g and thus unchanging as we place more coffee in the jar). In this study, we have analysed three different groups of the experimental data (0.001g, 0.005g, 0.01g dosage) which are less than the training dosage (0.02g dosage). On the other hand, we have only analysed one group of the experimental data (0.04g dosage) which is larger than the training dosage. It is necessary to analyse several group of the experimental data which are larger than the training dosage, e.g. 0.03g, 0.04g, 0.05g and 0.06g dosage, to improve the accuracy of the model at higher dosage in the future. However, if volatile concentrations at higher dosages are not different than the training dosage, then repeating tests at higher concentrations would not indicate any differences. Therefore, it is necessary to take concentration measurements for the different levels of coffee weights in the jars in the near future.

If the coffee concentration is saturated at 0.02g, it will remain unchanging as we place more coffee in the jar. This hypothesis is tested by lumping 0.04g behaviour data with 0.02g behaviour data, and see what happens to the prediction. The statistical analysis was performed and discussed in section 7.3.

In a word, the model can roughly predict the target concentration in a particular range of 0.005 to 0.02. The accurate rate of the model is 60% to 70%.

There are also some limitations to the model.

The simulation results are not exactly same as the actual values. Therefore we have to use a small range that is close to the actual value to decide if the simulation results are

acceptable. When the actual concentration values are very close to each other, it is much harder to decide the actual concentration values. For instance, when simulation result is 0.0075, it is hard for the user to decide whether the target's actual concentration is 0.005 or 0.01.

For this simple model, we have used a relevant large interval (actual value±0.005) to decide if the model output is correct. If the model output is in this range, we will count it as a correct output. However, this interval needs to be narrowed down as the model equations become more precise.

From the data analysis, we found that when target concentration is much smaller than training concentration, the wasp will exhibit similar behaviour. In other words, the parameter values for each wasp are very close to each other. We presume that the wasp will also exhibit similar behaviour when the target concentration is much larger than the training concentration. However, this is still need to be proved in the future based on the more experimental data.

Currently, the model is very simple. We expect that a more sophisticated model could be developed in the future as more data emerge from the experiments.

7.3 Discussion of the simulation results by lumping 0.04g behaviour data with 0.02g behaviour data

From analysing the simulation results of 0.04g coffee concentration (Figure 7.1D), it is found that the model is failed to predict the target's actual concentration value at 0.04g dosage (only 20% of the simulation results close to the actual dosage value). In fact, the model simulation results at 0.04g coffee concentration are very close to the simulation results at 0.02g coffee concentration (50% of the simulation results are between 0.015 and 0.025, 13.33% of the simulation results are between 0.025 and

0.035). Therefore, we presume that the coffee concentration is saturated at 0.02g dosage; it will remain unchanging as we place more coffee in the jar. This hypothesis is tested by lumping 0.04g behaviour data with 0.02g behaviour data, and see what happens to the model prediction.

Nonlinear regression analysis is performed to examine the new parameters in equation (5.11) for each of the test concentrations after lumping 0.04g behaviour data with 0.02g behaviour data. The best fit parameters $\overline{\alpha}$ and $\overline{\beta}$ values and its Mean Square Error for each of the test concentrations are listed in table 7.2.

Test Concentration	$\overline{\alpha}$	$\overline{\beta}$	Mean Square Error
0.005	0.930106	16.2554	0.076745
0.01	0.905896	8.41131	0.0657437
0.02 and 0.04 together	0.839621	7.44285	0.0647226

Table 7.2: New parameters $\overline{\alpha}$, $\overline{\beta}$ and their mean square error values for each of the test concentrations after lumping 0.02g and 0.04g dosage data.

Followed the model equation estimation process in Section 6.1, the new parameter function for $\overline{\alpha}$ and $\overline{\beta}$ are determined and listed blow.

The linear function used to model parameter $\overline{\alpha}$ is

$$\overline{\alpha} = f_1(Conc) = -6.1174 \cdot Conc + 0.9632 \tag{7.1}$$

where Conc is the test concentration with $0.005 < Conc \le 0.04$.

The quadratic polynomial function used to model parameter $\bar{\beta}$ is

$$\bar{\beta} = f_2(Conc) = 98131 \cdot Conc^2 - 3040.8 \cdot Conc + 29.006 \qquad (7.2)$$

where Conc is the test concentration with $0.005 < Conc \le 0.04$.

All the behaviour data are re-simulated through the new model which is built based on equation (7.1) and (7.2). The statistical analysis of the simulation results is performed to compare the prediction changes. The simulation results for each dosage are summarized and plotted in Figure 7.2.

The histogram in Figure 7.2(A) illustrates the new simulation results for 0.005g dosage. The Figure shows 56.7% of the simulation results are close to 0.005, which are in the range of 0 to 0.01. The other 43.3% of the simulation results are larger than 0.005, which are in the range between 0.01g and 0.04g dosage. Compared with Figure 7.1(A), the model prediction is decreased by 6.6% (from 63.3% to 56.7%).

The histogram in Figure 7.2(B) illustrates the new simulation results for 0.01g dosage. The Figure shows 63.3% of the simulation results are close to 0.01, which are in the range of 0.005 to 0.015. The other 36.7% of the simulation results are larger than 0.01, which are in the range between 0.015g and 0.04g dosage. Compared with Figure 7.1(B), the model prediction is decreased by 6.7% (from 70% to 63.3%).

The histogram in Figure 7.2(C) illustrates the simulation results for 0.02g and 0.04g dosage. The Figure shows 53.3% of the simulation results are close to 0.02, which are in the range of 0.015 to 0.025. Only 11.7% of the simulation results are close to 0.04, which are in the range of 0.035 to 0.04. There is 28.3% of the simulation results are in the range of 0.005 to 0.015. There is 10% of the simulation results are in the range of 0.025 to 0.035. Compared with Figure 7.1(C), the model prediction is decreased by

6.7% (from 60% to 53.3%).

In summary, the model prediction is slightly decreased by 6.7% after lumping 0.04g behaviour data with 0.02g behaviour data. It can still roughly predict the target concentration values in range of 0.005 to 0.02. The accurate rate of the model is 54% to 64%. In other words, the statistical analysis has proved that the coffee concentration is most likely to be saturated at 0.02g dosage. However, it is necessary to double check this conclusion by taking concentration measurements for the different levels of coffee weights in the jars in the near future.

Figure 7.2: Statistic results of simulation and 5% error bar after lumping 0.04g behaviour data with 0.02g behaviour data. (A) 0.005g. (B) 0.01g. (c) 0.02g and 0.04g

(A) 0.005g

(B) 0.01g

Statistic results for 0.02 and 0.04 Dosage
(total 60 data)
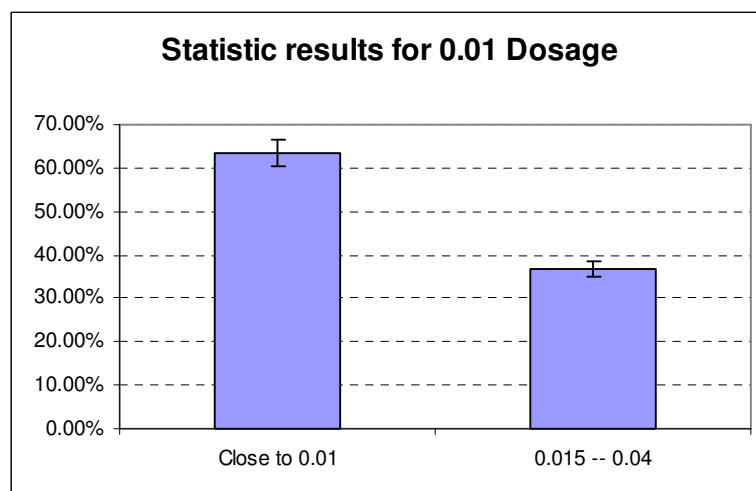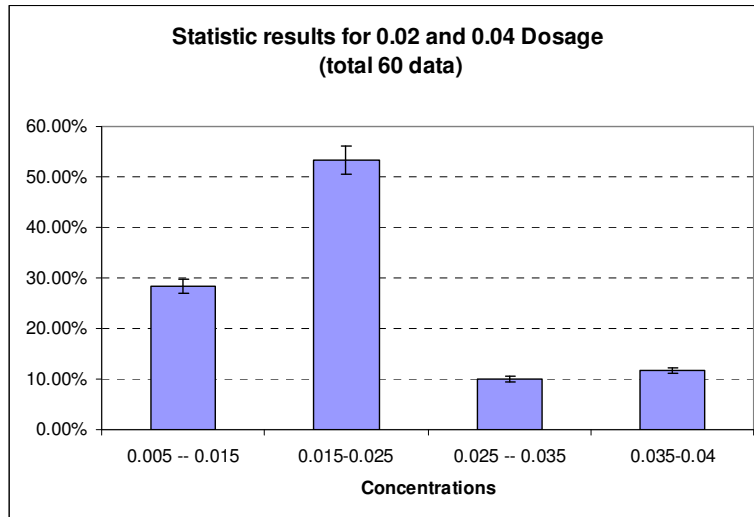
(C) 0.02g and 0.04g

Chapter 8: Conclusions and future outlook

The overall goal of the thesis was to investigate the hidden information between the single trained *M. croceipes* and the different levels of the target odour. In this thesis, a simple model that utilizes the behaviour of the single trained *M. croceipes* to predict the target's actual concentration has been developed. The simulation results have shown that the model can roughly predict the target concentration values in a particular range. We now give an overview of what we have achieved and main findings of the research, future directions that can follow on from the current step to improve the model, and finally, a conclusion.

8.1 Overview of research

The focus of the work were (1) to extract the useful data from the experimental video files of the single trained *M. croceipes*, and store in database for future analyse, (2) analyse these data and develop a model that utilize the behaviour of the single trained *M. croceipes* to predict the actual target concentrations. The purpose of the model were (1) to verify the current knowledge about chemical sensing technology that utilize the behaviour of the single trained *M. croceipes* to predict the concentration of the target odour, (2) to increase our confidence in understanding the model system and to develop a more sophisticated model in the future based on the knowledge of the current model system.

The biological experiment process is described. After a careful interpretation of the wasp's behaviour, three variables were decided and improved with a detailed explanation (Chapter 3). These improved variables are successfully computed and stored into MS Excel database. The general findings are given after analysing the

variables and their plots (Chapter 4). The stochastic model was built for each of the improved variables and its parameters were estimated. A new model equation was found after the parameter analysis (Chapter 5). The mathematical model was then converted into a computer solvable model. The model was built up (Chapter 6).

Finally, the model was extensively tested and compared with validation data (Chapter 7). The findings of the model are summarized into the following three aspects. (1) The model has the ability to predict the concentration value of the target odour in a particular range. Approximately 60% to 70% of the simulation results were close to the actual dosage value. (2) Through the analysis of the simulation results, it was found that a deficiency existed in the simulation results. The model equations need to be improved so that the simulation results become more accurate to the actual value. (3) The model predicting range could be improved by analysis of more experimental data that are tested under a wide range of concentrations.

8.2 Contributions of the research

The contributions of this thesis through modelling and analysing the behaviour of the trained wasps can be summarized into the following points.

- To extract data from the experimental video files of the trained *M. croceipes* and build up a database to store and analyse those data.
- To investigate the hidden information between the trained *M. croceipes* and various target dosages.
- To develop a simple model that could be used to predict the actual target dosage.

8.3 Limitation and suggestion for future directions

In the new area of modelling the behaviour of the trained wasp to predict the target's actual concentration, our work merely touches the surface of modelling the complex behaviours of the trained wasp. There are several directions in which to extend and improve the model presented in the thesis.

- The current model required the dosage of the detecting target in its predicting range which is from 0.005 to 0.02. This is a small range. In fact, there are still a large number of the experimental data that need to be tested under a wide range of the test dosage. They also need to be analysed and added into the current model. A more complete model could enhance its predicting range to solve a real world problem.

- This is a simple model. The model simulation results are not exactly the same as the actual values. They are in a relevant small range that is close to the actual value. Therefore, to improve the accuracy of the simulation results, the stochastic differential equations that are determined from the analysis of the experimental data are required to be improved in the future study.

- In this research, the model has three input variables which are extracted from the trained wasp's behaviours. It is possible that there are more variables that could be found based on the analysis of the trained wasp's behaviour. This could be used to help improve the model equations.

- Other important consideration is how the wasp's behaviour is affected by background odours mixed with the trained odour. In this study, the wasp is trained to a single odour and tested to that odour. In future, the wasps need to be trained to several compounds and tested to these odours.

- The quality of the food source (sugar water) used to train the wasp need to be examined in future. Lower and higher levels of sugar in sugar water may also affect the wasp's behavioural response.

- In this research, the wasp is tested to a point source (single hole where the odour emits). It is necessary to find out whether there is a difference in wasp's behaviour if the odour source is more dispersed, for example, the odour is emit from 5 or 6 holes.

- Three distinct behaviours are examined in this research. There may be more than a dozen distinct behavioural movements that could be used to measure the wasp's behaviours, including antennal movements. Therefore, much better video recordings are required to capture more subtle behaviours and use those as data for the model development.

8.4 Conclusion

The study of modelling the trained wasp's behaviour under several target concentration levels, and utilizing it to predict the target's actual concentration is a challenging topic. Our understanding of the dynamics and functions of the underlying biological sensing processes has been hampered by the complexity of the system. However, mathematic modelling has the potential to assist in understanding such processes. In this thesis, we have shown how stochastic models can be built using mathematical knowledge based on the trained wasp's behaviour information and how the model can be used to predict the concentration of the target odour.

Although our investigation focuses on a small subset of specific problems, there is indeed a large array of challenging and exciting biological experiments waiting to be approached and explored. It is our hope that the model presented in this thesis will

help other mathematicians to develop a more sophisticated model in the future.

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Appendix A – Computer Vision System

Components of computer vision system (developed by S. L. Utley, G. C. Rains and W.J. Lewis) was listed in following figures.
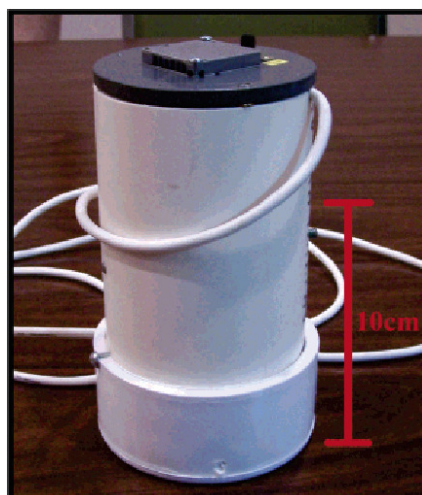
Figure A1: The Wasp Hound integrates a computer vision system into a portable handheld detector. The enclosure provides consistent lighting, cartridge placement, and air flow. (From Utley, S.L., G. C. Rains, W. J. Lewis, 2004.)
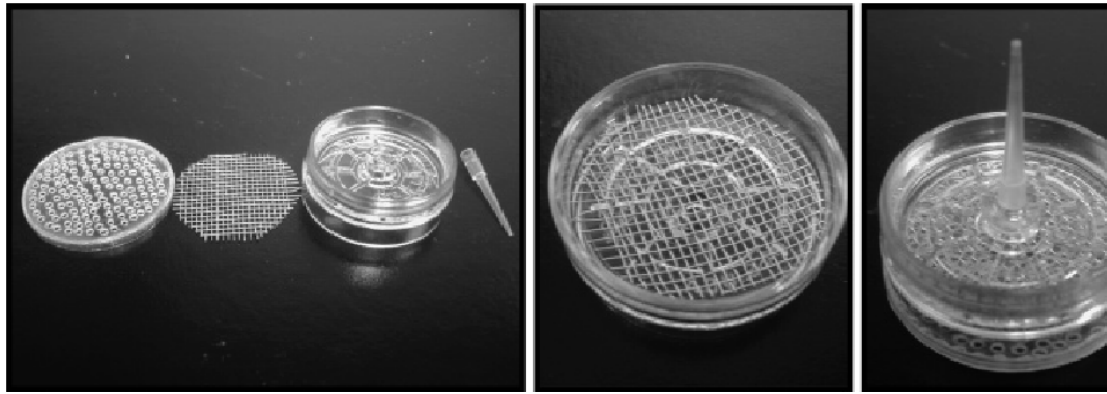

(A)   (B)

Figure A2: Mounting of camera and LED within Wasp Hound. (A) The Logitech QuickCam was suspended within the PVC body by three bolts 2.5 cm above the test cartridge top. (B) An LED was mounted within the Wasp Hound as the main source of illumination. (From Utley, S.L., G. C. Rains, W. J. Lewis, 2004.)

(A) (B) (C)

Figure A3: Test cartridge. (A) The test cartridge was composed of top from a Millipore PetriSlide, a wire mesh disk, part of a Millipore aerosol analysis monitor, and a FinTip Pipet tip. (B) The mesh disk was placed in the body of the cartridge to prevent *M. croceipes* from escaping through the bottom. (C) The top fit onto the body and prevented *M. croceipes* from flying away while providing adequate ventilation, and the pipet tip was inserted into the bottom to direct air samples into cartridge. (From Utley, S.L., G. C. Rains, W. J. Lewis, 2004.)
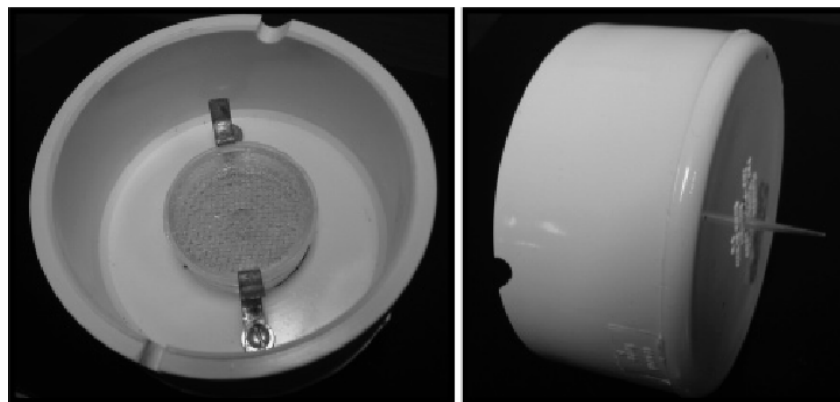
(A) (B)

Figure A4: Test cartridge placement within the Wasp Hound. (A) During testing the test cartridge was placed within the cap and secured by two clips. (B) the pipet tip protruded through the bottom of the cap. (From Utley, S.L., G. C. Rains, W. J. Lewis, 2004.)

Appendix B – Use Tracker 1.5.2 to Track wasp's movement

To use Tracker the user must first download and install Java 5, QuickTime 7 and Tracker1.5.2 (or higher version) in the order listed below.

1. Java 5: Download the most recent Java 5 installer from http://java.sun.com/j2se/downloads.html. The JRE (Java Runtime Environment) is all that is needed unless one is a Java developer. Double-click the installer and follow the instructions.

2. QuickTime 7: Download the most recent standalone QuickTime 7 installer from http://www.apple.com/quicktime/download/standalone.html. It is not necessary to purchase QuickTime Pro. Double-click the installer and follow the instructions. QuickTime for Java is automatically installed.

3. Tracker: Download Tracker.jar (version 1.5.2 or higher version) from Tracker's Home page at http://www.cabrillo.edu/~dbrown/tracker/.

Using Tracker, the following steps will allow the user to get the necessary data for variable calculation.

1. Double-click on Tracker.jar to open Tracker1.5.2. Use the Open button or File→Open menu item to open the wasp video file.

2. Click the inspector button at the right end of the player (Figure B1) to display the clip inspector. The clip inspector shows thumbnail images of the start and end frames along with the current video clip settings. In addition, there are fields for setting the mean time Dt between video frames (important for high-speed or time-lapse videos) and the play rate as a percent of normal playback speed. Set the Start frame at the number of the frame when the wasp was placed on the test sample. Set the Step size to 5 which is equal to 0.2 seconds (the video rate is 25 frame per second)

Figure B1: the video clip inspector button

3. Display the axes by click the **axes button** on the toolbar (or from **Tracks→ axes → Visible**). The axes show the location of the origin and direction of the positive x-axis of the coordinate system. The origin is at the intersection of the axes and the positive x-axis is indicated by a tick mark near the origin. Select and drag or nudge the origin to a desired location which is the centre hole in the main video view (click the right mouse button and choose suitable **Zoom in** option, will improve the accuracy of matching the origin and centre hole point).

4. Display the tape measure by clicking the Tape Measure button on the toolbar (or from **Tracks→ Tape measure → Visible**). Use the cycle on the test sample (refer to chapter 4.1.3) to calibrate the video image. First set the two ends of the tape measure on circle and through the centre (the tape measure represents the diameter now). Then double-click the readout and enter the known distance, such as 25 (unit: mm) in this project.

5. Now, the tracker is ready to mark the positions of the wasp's head and abdomen for every 0.2 seconds. Create a new track by clicking Point Mass button on the toolbar (or from **Tracks→ New → Point Mass**). The Track Control panel will appear on the main window (Figure B2). Every track is identified by its name, colour, footprint (visible shape) and description. Newly created tracks are assigned default values for the first three properties that depend on the type of track. For example, a point mass might initially be called "mass A" and be drawn as a blue diamond. A track's name, footprint and

colour are displayed on the toolbar when the track is selected. To change the default values, select the track and enter a new name in the editable name field or click the footprint button and choose a new footprint or colour. Now, shift-click on the wasp's head on video frame to mark it and the video frame will move to next frame (5 frames after the current frame as we set up in step 2). The new track is created to mark the position of wasp's abdomen after all the positions of wasp's head were marked. Hint: Unselect **Visible** option will make easy to mark the wasp's head or abdomen when the wasp was move slightly. By select **Mark by Default** option, it will allow the user to mark the wasp's position by left clicking the mouse instead of shift-clicking.
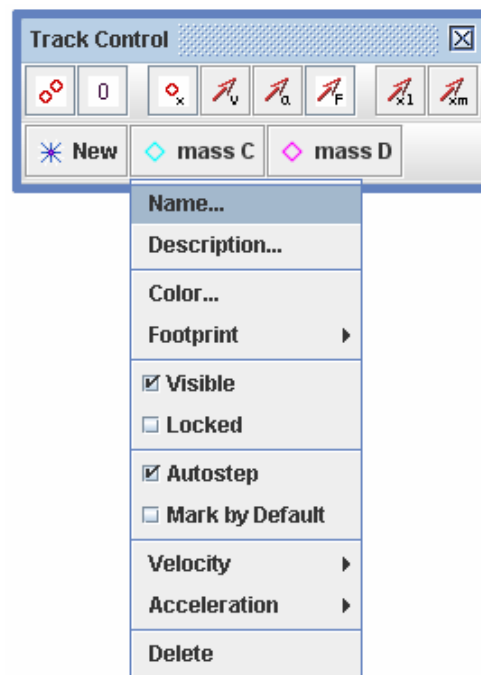
Figure B2: The Track Control panel.

6. Select **Window→ Right View** to open the data-table view, which displays a table of a track's data after each step were marked. Select the track (the wasp's head or abdomen) from the dropdown list on data-table view toolbar. Select the data columns included in the table by clicking the Data button and

checking those of interest. Time is always included. Here the X-comp, Y-comp and theta are also needed.

7. Click and drag in the data table to select cells. Currently the user have to reselect the track to unselect all cells. Right-click the table and select **Copy** from the popup menu to copy the selected cells to the clipboard. If no cells are selected, the entire data-table will be copied. Now, open Microsoft Excel and paste the copied data in a sheet. It will be used to calculate the variables later on.

Appendix C – Video Format Converting

Blaze Media Pro is capable of performing two-way video conversions among AVI, MPEG-1, MPEG-2, WMV, Multi-Page TIFF, and FLIC formats. The following steps will allowed the user to convert a video file from MPEG format to AVI format.

1. Open Blaze Media Pro software, click **Conversion** button in the main window, and then select **Video Converter**.

2. Click **Edit**, select **Add** or click the **Add Files to List** button.

3. Select the video files on hard drive that need to convert. (Tip: the user can select more than one file by pressing the Ctrl key while clicking the files or pressing the Shift key and using the arrows to select a range of files. To remove files from the list, select those files, and then, select Remove from the Edit menu or click the Remove Files button.)

4. Select the files, using the Move Up and Move Down options on Edit menu to put those files in a right process order.

5. Choose an Output Format from the dropdown list which is AVI for my study, and choose the output options as showed in Figure C1. (Tip: If the hard disk space is large enough, Choose **Full uncompressed** as AVI Codec Option. It will give good video quality but very large AVI video size after convert. If the hard disk space is an issues, Choose **Cinepak Codec by Radius** as AVI Codec Option. It will give acceptable video quality and much smaller video size than full uncompressed. **Cinepak Codec by Radius** is chosen as AVI Codec options for my study. At this point, the user can decide whether or not to click the Overwrite Existing Files option.)
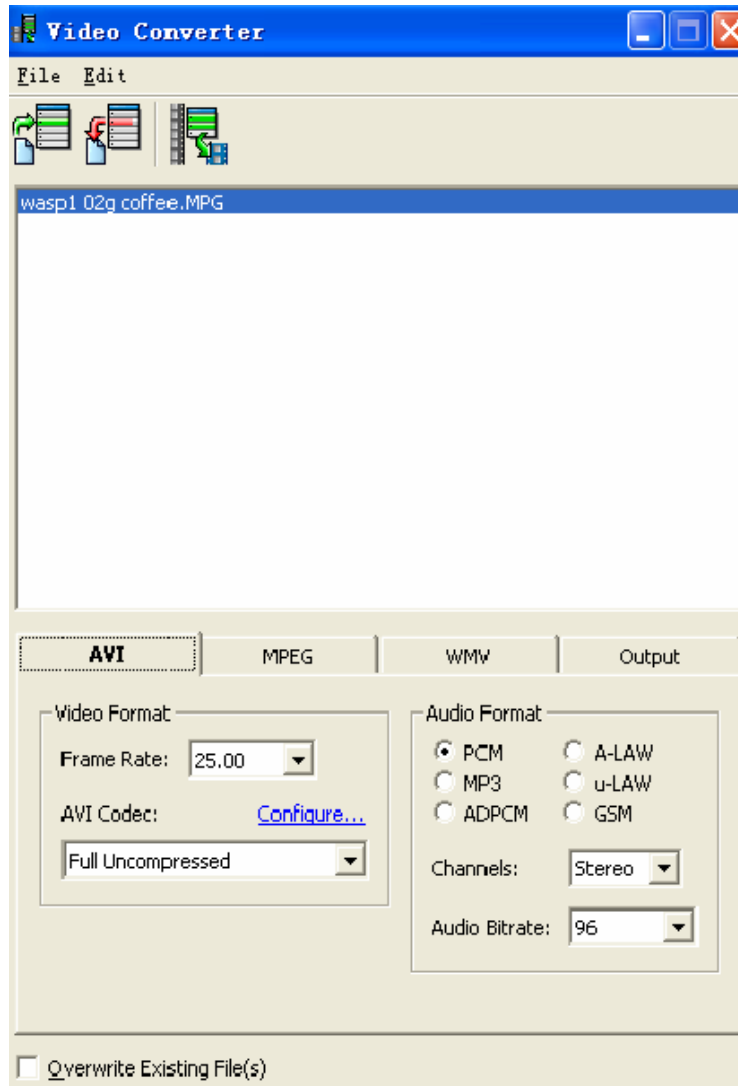
Figure C1: Video Converter Frame.

6. Click Convert button. (Tip: When you click Convert, you can set the codec and
    format of the output file. In cases where the conversion does not work for a
    certain file or does not produce the results you expected, you can try to use a
    different conversion method by clicking File, and then select Enable Alternate
    Conversion Method. After selecting this option, you must click the Convert
    button to convert the file.

Appendix D – Plane Geometry Formula used in the study

The wasp have three major behaviours lead to three variables R, θ, α in the study.
The R is the distance between the wasp's head and the centre point as time changes.
The R is calculated by using point to point distance formula in 2-D plane. If there are
two points A(a, b) and B(c, d) in 2-D rectangular coordinates as showed in Figure D1,
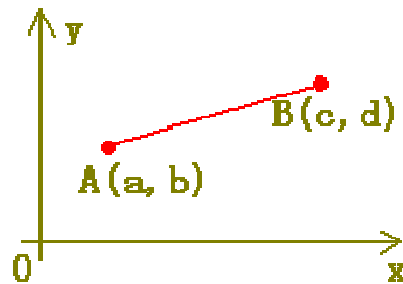
Figure D1: Two point with their coordinates in

the distance formula is $d = \sqrt{(c-a)^2 + (d-b)^2}$. In my study, point A will be the
origin point (0, 0), point B will be the coordinates of the wasp's head.

The θ is the angle in radian between the line through the wasp's head to the origin
and the horizontal line though the centre point (X-axis) as time changes, which is
show in Figure D2.
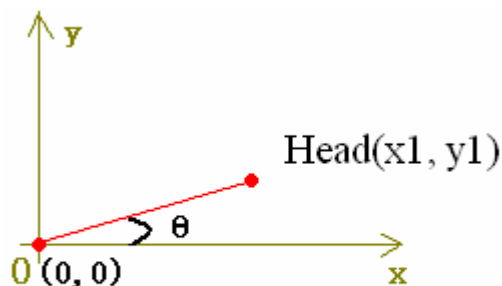
Figure D2: Angle theta between the wasp's head and the X-axis.

If the wasp was move anticlockwise, the formula and its condition are:

- When x1>0 and y1>0, $\theta = \arcsin(y1/\sqrt{x1^2 + y1^2}) + N*2\pi$.

- When x1<0 and y1>0, $\theta = \pi - \arcsin(y1/\sqrt{x1^2 + y1^2}) + N*2\pi$.

- When x1<0 and y1<0, $\theta = \pi + \arcsin(|y1|/\sqrt{x1^2 + y1^2}) + N*2\pi$.

- When x1>0 and y1<0, $\theta = 2\pi - \arcsin(|y1|/\sqrt{x1^2 + y1^2}) + N*2\pi$.

- Note: N is the number of cycles the wasp moved towards anticlockwise. N=0, 1, 2, 3…

If the wasp was move clockwise, the formula and its condition are:

- When x1>0 and y1>0, $\theta = -[2\pi - \arcsin(y1/\sqrt{x1^2 + y1^2})] - N*2\pi$.

- When x1<0 and y1>0, $\theta = -[\pi + \arcsin(y1/\sqrt{x1^2 + y1^2})] - N*2\pi$.

- When x1<0 and y1<0, $\theta = -[\pi - \arcsin(|y1|/\sqrt{x1^2 + y1^2})] - N*2\pi$.

- When x1>0 and y1<0, $\theta = -\arcsin(|y1|/\sqrt{x1^2 + y1^2}) - N*2\pi$.

- Note: N is the number of cycles the wasp moved towards clockwise. N=0, 1, 2, 3…

The α is the angle in radian between the wasp's body line (from the wasp's head to its abdomen) and the horizontal line though the centre point (positive X-axis) as time changes. A formula is used to calculate the angle between those 2 lines (the wasp's body line and the positive X-axis) for every 0.2 seconds.

There are two lines (line1[p1, p2]; line2[p3, p4]), the vertexes of first line are p1(x1, y1) and p2(x2, y2), which represent the wasp's head and its abdomen. The vertexes of second line are p3(x3, y3) and p4(x4, y4), which represent two vertexes on X-axis. Here point (0, 0) is used for (x3, y3) and point (10, 0) is used for (x4, y4) in my study. Vector a = p2 –p1, vector b = p3 – p4. From the dot product formula

$a \cdot b = |a| \cdot |b| \cdot \cos(\alpha)$, we have $\cos(\alpha) = \dfrac{a \cdot b}{|a| \cdot |b|} \Rightarrow \alpha = ar\cos(\dfrac{a \cdot b}{|a| \cdot |b|})$. Because of

$a \cdot b = (p2 - p1) * (p3 - p4)$ and $|a| * |b| = |(p2 - p1)| * |(p3 - p4)|$, The angle formula is

$$\alpha = ar\cos\frac{(dx1 * dx2 + dy1 * dy2)}{\sqrt{(dx1 * dx1 + dy1 * dy1) * (dx2 * dx2 + dy2 * dy2)}}$$, where dx1 = x2-x1, dx2

=x4-x3, dy1 =y2-y1, dy2 =y4-y3.

If the wasp's body was rotated anticlockwise, the formula and its condition are:

- $\alpha = ar\cos\dfrac{(dx1 * dx2 + dy1 * dy2)}{\sqrt{(dx1 * dx1 + dy1 * dy1) * (dx2 * dx2 + dy2 * dy2)}} + N * 2\pi$

- Note: N is the number of cycles the wasp moved towards anticlockwise. N=0, 1, 2, 3…

If the wasp wasp's body was rotated clockwise, the formula and its condition are:

- $\alpha = ar\cos\dfrac{(dx1 * dx2 + dy1 * dy2)}{\sqrt{(dx1 * dx1 + dy1 * dy1) * (dx2 * dx2 + dy2 * dy2)}} - N * 2\pi$

- Note: N is the number of cycles the wasp moved towards anticlockwise. N=0, 1, 2, 3…

Appendix E – Parameter Accuracy Analysis

E1. The VBA program that used to compute parameter values under different dt values.

E1.1 Parameter a

```
Sub parameter_a()

    ' This is the main program for calculating parameter a values under different dt values.
Column A and B must be the time and variable values at dt = 0.2 sec
    ' The parameter will be calculated print out at same worksheet

    ' set up the column variable A, B, C, D...
    Dim A, B As Integer
    A = 1: B = 2    ' the column number

    Dim J, K, L, M, N, O, P, Q As Integer
    J = 10: K = 11: L = 12: M = 13: N = 14: O = 15: P = 16: Q = 17

     ' Set up for loop to calculate parameter value under eight different dt    values.
dt is decrease half by half.
    For I = 1 To 8

    ' Print out the title of each column...
    Cells(1, J).Value = "t"
    Cells(1, K).Value = "p-value"
    Cells(1, L).Value = "d-p"
    Cells(1, M).Value = "p-square"
    Cells(1, N).Value = "det(t)"
    Cells(1, O).Value = "numerator"
    Cells(1, P).Value = "denominator"
    Cells(1, Q).Value = "parameter=numerator/denominator"
    '-------Calculate time------
    Dim row1 As Integer
        row1 = 2

    Do
```

```
        Cells(row1 * 2 - 2, J).Value = Cells(row1, A)
        If Cells(row1 + 1, A) <> "" Then

        Cells(row1 * 2 - 1, J).Value = (Cells(row1, A).Value + Cells(row1 + 1, A).Value) / 2
        End If
row1 = row1 + 1
Loop Until (Cells(row1, A).Value > 20 Or Cells(row1, A).Value = "")


'---------Calculate p----------
Dim row2, rows As Integer
    row2 = 2: rows = 2

    For firstrow = 2 To row1 - 1
    Cells(rows, K).Value = Cells(row2, B)
    rows = rows + 1

    If Cells(row2 + 1, B).Value <> "" Then
    Cells(rows, K).Value = (Cells(row2, B).Value + Cells(row2 + 1, B).Value) / 2
    rows = rows + 1
    End If



    row2 = row2 + 1
    Next firstrow


'----------Calculate d-p column----------
Dim row3 As Integer
    row3 = 2

Do

    If Cells(row3 + 1, K).Value <> "" Then
    Cells(row3, L).Value = Cells(row3 + 1, K).Value - Cells(row3, K).Value
    End If

row3 = row3 + 1
Loop Until (Cells(row3, J).Value > 20 Or Cells(row3, J).Value = "")


'-----------Calculate p-square value----------
Dim row4 As Integer
    row4 = 2
```

```vba
Do

    If Cells(row4, L).Value <> "" Then
    Cells(row4, M).Value = Cells(row4, K).Value ^ 2
    End If


row4 = row4 + 1
Loop Until (Cells(row4, J).Value > 20 Or Cells(row4, J).Value = "")




'----------Calculate det(t)----------
Dim row5 As Integer
    row5 = 2


Do

    If Cells(row5 + 1, J).Value <> "" Then
    Cells(row5, N).Value = Cells(row5 + 1, J).Value - Cells(row5, J).Value
    End If


row5 = row5 + 1
Loop Until (Cells(row5, J).Value > 20 Or Cells(row5, J).Value = "")




'----------Calculate numerator value of equation (5.7) ----------
Dim row6 As Integer
    row6 = 2


Do

    If Cells(row6, L).Value <> "" Then
    Cells(row6, O).Value = Cells(row6, K).Value * Cells(row6, L).Value
    End If


row6 = row6 + 1
Loop Until (Cells(row6, J).Value > 20 Or Cells(row6, J).Value = "")




'----------Calculate denominator value of equation (5.7) ---------
Dim row7 As Integer
    row7 = 2


Do
```

```vb
        If Cells(row7, M).Value <> "" Then
        Cells(row7, P).Value = Cells(row7, M).Value * Cells(row7, N).Value
        End If


    row7 = row7 + 1
    Loop Until (Cells(row7, J).Value > 20 Or Cells(row7, J).Value = "")



    '----------Calculate a which is SUM(top)/SUM(bottom)-----------
        Dim row8 As Integer
        row8 = 2
        Dim top, bottom As Single
        top = 0: bottom = 0

    Do
      If Cells(row8, O).Value <> "" Then
      top = top + Cells(row8, O).Value
      End If


      If Cells(row8, P).Value <> "" Then
      bottom = bottom + Cells(row8, P).Value
      End If



    row8 = row8 + 1
    Loop Until (Cells(row8, J).Value > 20 Or Cells(row8, J).Value = "")
    Cells(2, Q).Value = top / bottom



'Shift the column to next parameter value
A = A + 9: B = B + 9
J = J + 9: K = K + 9: L = L + 9: M = M + 9: N = N + 9: O = O + 9: P = P + 9: Q = Q + 9

Next I
End Sub
```

E1.2 Parameter c


```vb
Sub parameter_c()

    ' This is the main program for calculating parameter c values under different dt values.
```

Column A and B must be the time and variable values at dt = 0.2 sec
    ' The parameter c will be calculated print out at same worksheet

    ' set up the column variable A, B, C, D...
    Dim A, B As Integer
    A = 1: B = 2   ' the cloumn number

    Dim J, K, L, M, N, O, P, Q As Integer
    J = 10: K = 11: L = 12: M = 13: N = 14: O = 15: P = 16: Q = 17

    ' Set up for loop to calculate parameter value under eight different dt   values.
dt is decrease half by half.
    For I = 1 To 8

    ' Print out the title of each column...
    Cells(1, J).Value = "t"
    Cells(1, K).Value = "gama-value"
    Cells(1, L).Value = "d-gama"
    Cells(1, M).Value = "gama-square"
    Cells(1, N).Value = "det(t)"
    Cells(1, O).Value = "numerator"
    Cells(1, P).Value = "denominator"
    Cells(1, Q).Value = "parameter=numerator/denominator"
  '-------Calculate time------
  Dim row1 As Integer
        row1 = 2

    Do

        Cells(row1 * 2 - 2, J).Value = Cells(row1, A)
        If Cells(row1 + 1, A) <> "" Then

        Cells(row1 * 2 - 1, J).Value = (Cells(row1, A).Value + Cells(row1 + 1, A).Value) / 2
        End If
    row1 = row1 + 1
    Loop Until (Cells(row1, A).Value > 20 Or Cells(row1, A).Value = "")

    '---------Calculate p----------
    Dim row2, rows As Integer
        row2 = 2: rows = 2

        For firstrow = 2 To row1 - 1
        Cells(rows, K).Value = Cells(row2, B)

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```vba
        rows = rows + 1


        If Cells(row2 + 1, B).Value <> "" Then
        Cells(rows, K).Value = (Cells(row2, B).Value + Cells(row2 + 1, B).Value) / 2
        rows = rows + 1
        End If



        row2 = row2 + 1
        Next firstrow



'----------Calculate d-p column----------
Dim row3 As Integer
        row3 = 2

Do

        If Cells(row3 + 1, K).Value <> "" Then
        Cells(row3, L).Value = Cells(row3 + 1, K).Value - Cells(row3, K).Value
        End If

row3 = row3 + 1
Loop Until (Cells(row3, J).Value > 20 Or Cells(row3, J).Value = "")



'-----------Calculate p-square value----------
Dim row4 As Integer
        row4 = 2

Do

        If Cells(row4, L).Value <> "" Then
        Cells(row4, M).Value = Cells(row4, K).Value ^ 2
        End If

row4 = row4 + 1
Loop Until (Cells(row4, J).Value > 20 Or Cells(row4, J).Value = "")



'----------Calculate det(t)----------
Dim row5 As Integer
        row5 = 2
```

```
Do

    If Cells(row5 + 1, J).Value <> "" Then
    Cells(row5, N).Value = Cells(row5 + 1, J).Value - Cells(row5, J).Value
    End If

row5 = row5 + 1
Loop Until (Cells(row5, J).Value > 20 Or Cells(row5, J).Value = "")



'----------Calculate numerator value of equation(5.7)----------
Dim row6 As Integer
    row6 = 2

Do

    If Cells(row6, L).Value <> "" Then
    Cells(row6, O).Value = Cells(row6, K).Value * Cells(row6, L).Value
    End If

row6 = row6 + 1
Loop Until (Cells(row6, J).Value > 20 Or Cells(row6, J).Value = "")



 '----------Calculate denominator value of equation (5.7) ---------
 Dim row7 As Integer
    row7 = 2

Do

    If Cells(row7, M).Value <> "" Then
    Cells(row7, P).Value = Cells(row7, M).Value * Cells(row7, N).Value
    End If

row7 = row7 + 1
Loop Until (Cells(row7, J).Value > 20 Or Cells(row7, J).Value = "")



'----------Calculate a which is SUM(top)/SUM(bottom)-----------
    Dim row8 As Integer
    row8 = 2
    Dim top, bottom As Single
    top = 0: bottom = 0
```

```
Do
    If Cells(row8, O).Value <> "" Then
    top = top + Cells(row8, O).Value
    End If

    If Cells(row8, P).Value <> "" Then
    bottom = bottom + Cells(row8, P).Value
    End If


row8 = row8 + 1
Loop Until (Cells(row8, J).Value > 20 Or Cells(row8, J).Value = "")
Cells(2, Q).Value = top / bottom


'Shift the cloumn to next parameter value
A = A + 9: B = B + 9
J = J + 9: K = K + 9: L = L + 9: M = M + 9: N = N + 9: O = O + 9: P = P + 9: Q = Q + 9

Next I
End Sub
```

E1.3 Parameter e


```
Sub parameter_e()

    ' This is the main program for calculating parameter e values under different dt values.
Column A and B must be the time and variable values at dt = 0.2 sec
    ' The parameter will be calculated print out at same worksheet

    ' set up the column variable A, B, C, D...
    Dim A, B As Integer
    A = 1: B = 2    ' the column number

    Dim J, K, L, M, N, O, P, Q As Integer
    J = 10: K = 11: L = 12: M = 13: N = 14: O = 15: P = 16: Q = 17

    ' Set up for loop to calculate parameter value under eight different dt    values.
dt is decrease half by half.
    For I = 1 To 8

    ' Print out the title of each column...
```

```vb
    Cells(1, J).Value = "t"
    Cells(1, K).Value = "beta-value"
    Cells(1, L).Value = "d-beta"
    Cells(1, M).Value = "beta-square"
    Cells(1, N).Value = "det(t)"
    Cells(1, O).Value = "numerator"
    Cells(1, P).Value = "denominator"
    Cells(1, Q).Value = "parameter=numerator/denominator"


'-------Calculate time------
  Dim row1 As Integer
       row1 = 2


   Do


      Cells(row1 * 2 - 2, J).Value = Cells(row1, A)
      If Cells(row1 + 1, A) <> "" Then


      Cells(row1 * 2 - 1, J).Value = (Cells(row1, A).Value + Cells(row1 + 1, A).Value) / 2
      End If
row1 = row1 + 1
Loop Until (Cells(row1, A).Value > 20 Or Cells(row1, A).Value = "")


  '---------Calculate p----------
  Dim row2, rows As Integer
      row2 = 2: rows = 2


      For firstrow = 2 To row1 - 1
      Cells(rows, K).Value = Cells(row2, B)
      rows = rows + 1


      If Cells(row2 + 1, B).Value <> "" Then
      Cells(rows, K).Value = (Cells(row2, B).Value + Cells(row2 + 1, B).Value) / 2
      rows = rows + 1
      End If


      row2 = row2 + 1
      Next firstrow


  '----------Calculate d-p column----------
  Dim row3 As Integer
      row3 = 2


   Do
```

```vba
    If Cells(row3 + 1, K).Value <> "" Then
    Cells(row3, L).Value = Cells(row3 + 1, K).Value - Cells(row3, K).Value
    End If


row3 = row3 + 1
Loop Until (Cells(row3, J).Value > 20 Or Cells(row3, J).Value = "")

'-----------Calculate p-square value----------
Dim row4 As Integer
    row4 = 2

Do

    If Cells(row4, L).Value <> "" Then
    Cells(row4, M).Value = Cells(row4, K).Value ^ 2
    End If


row4 = row4 + 1
Loop Until (Cells(row4, J).Value > 20 Or Cells(row4, J).Value = "")

'----------Calculate det(t)----------
Dim row5 As Integer
    row5 = 2

Do

    If Cells(row5 + 1, J).Value <> "" Then
    Cells(row5, N).Value = Cells(row5 + 1, J).Value - Cells(row5, J).Value
    End If


row5 = row5 + 1
Loop Until (Cells(row5, J).Value > 20 Or Cells(row5, J).Value = "")

'----------Calculate numerator value of equation (5.7) ----------
Dim row6 As Integer
    row6 = 2

Do

    If Cells(row6, L).Value <> "" Then
    Cells(row6, O).Value = Cells(row6, K).Value * Cells(row6, L).Value
    End If
```

```
        row6 = row6 + 1
    Loop Until (Cells(row6, J).Value > 20 Or Cells(row6, J).Value = "")


     '----------Calculate denominator value of equation (5.7) ---------
     Dim row7 As Integer
         row7 = 2

    Do

        If Cells(row7, M).Value <> "" Then
        Cells(row7, P).Value = Cells(row7, M).Value * Cells(row7, N).Value
        End If


    row7 = row7 + 1
    Loop Until (Cells(row7, J).Value > 20 Or Cells(row7, J).Value = "")


    '----------Calculate a which is SUM(top)/SUM(bottom)-----------
        Dim row8 As Integer
        row8 = 2
        Dim top, bottom As Single
        top = 0: bottom = 0

    Do
        If Cells(row8, O).Value <> "" Then
        top = top + Cells(row8, O).Value
        End If


        If Cells(row8, P).Value <> "" Then
        bottom = bottom + Cells(row8, P).Value
        End If


    row8 = row8 + 1
    Loop Until (Cells(row8, J).Value > 20 Or Cells(row8, J).Value = "")
    Cells(2, Q).Value = top / bottom



' Shift the column to next parameter value
A = A + 9: B = B + 9
J = J + 9: K = K + 9: L = L + 9: M = M + 9: N = N + 9: O = O + 9: P = P + 9: Q = Q + 9

Next I
End Sub
```
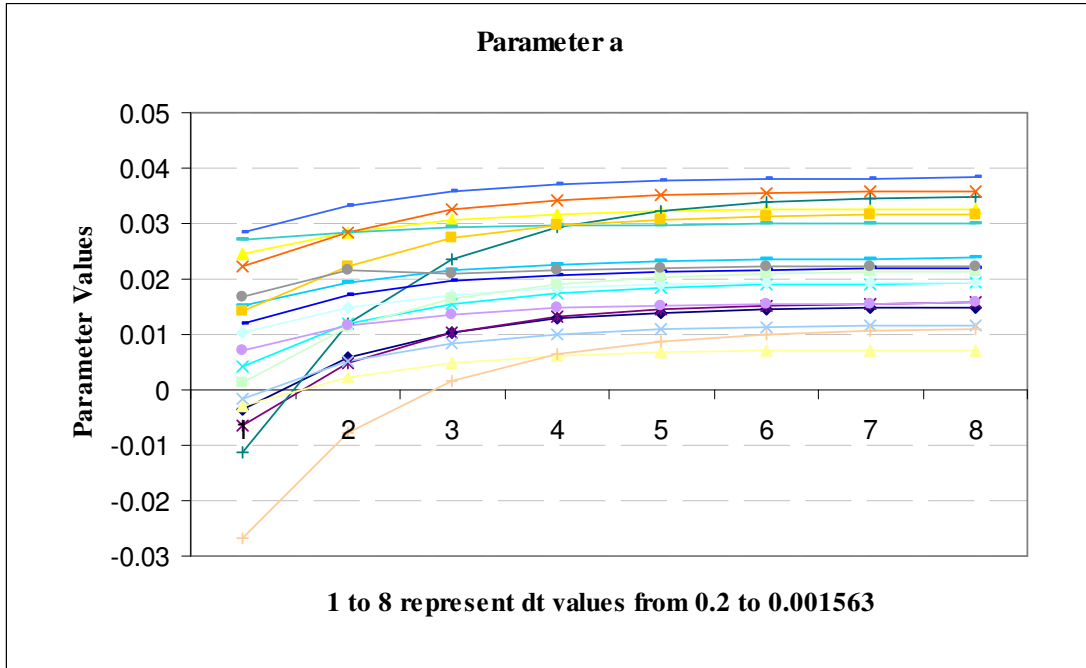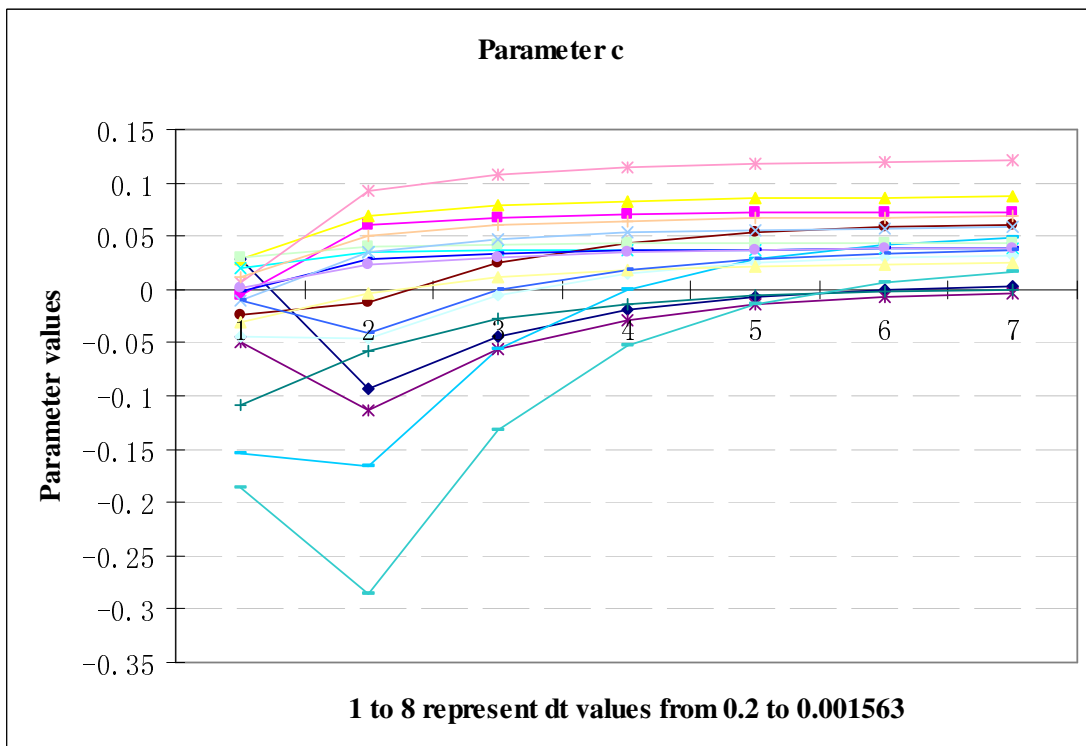
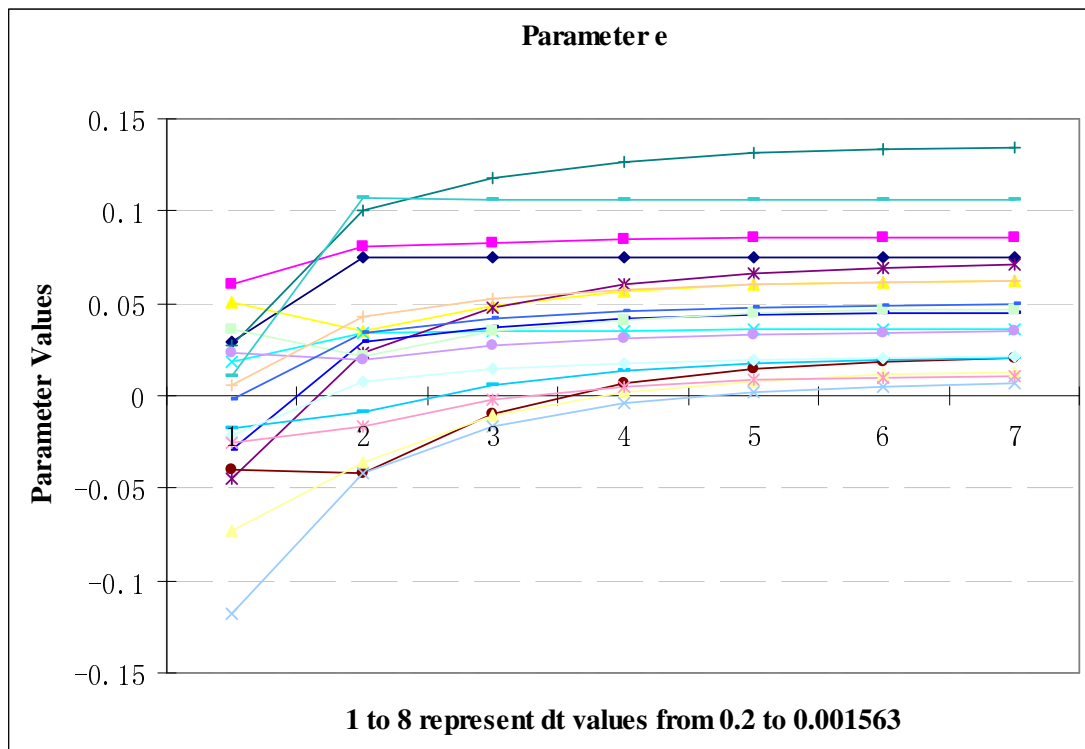**E2. Plot of parameter changes for all wasps at 0.02 test condition using different dt values.**

E2.1 Parameter a

E2.2 Parameter c

E2.3 Parameter e

Parameter e

Note: the value from 1 to 8 on X-axis are represent eight different dt values, which are 0.2 second, 0.1 second, 0.05 second, 0.025 second, 0.0125 second, 0.00625 second, 0.003125 second and 0.001563 second.

Appendix F – Model Algorithm

Private Sub CommandButton1_Click()

'This is main program to calculate test concentration by using parameters a, c, e
'and mathematical model equation   (6.1), (6.2), (6.4) from Chapter six of my thesis

    ' set up the column variable A, B, C, D...
    Dim A, B, C, E, Row As Integer
    A = 1: B = 2: C = 3: E = 5 ' the cloumn number

    For Row = 3 To 125 Step 1

    'get parameter values from cells
    Dim parameter_a, parameter_c, parameter_e As Single
    parameter_a = 0: parameter_c = 0: parameter_e = 0
    parameter_a = Cells(Row, A).Value
    parameter_c = Cells(Row, B).Value
    parameter_e = Cells(Row, C).Value

    'set the starting concentration
    Dim Conc As Single

    'set left and right side of equation (6.4) be 0
    Dim Left_side, Right_side As Single

    'Calculate left side of equation (6.4)
    Left_side = Exp(-Abs((parameter_c - parameter_e) / parameter_a))

    Dim m As Integer
    m = 3
    Dim min1, min2, current_conc As Single
    min1 = 0: min2 = 0: current_conc = 0

    Dim new_alpha, new_beta, new_right_side As Single
    new_alpha = 0: new_beta = 0: new_right_side = 0

    'Set up for loop let concentration from 0.001 increase to 0.045 by 0.001 each time
    For Conc = 0.001 To 0.045 Step 0.001

    'Set new alpha and new beta variable...

```vba
        Dim alpha, beta As Single
        alpha = -3.2812 * Conc + 0.9431
        beta = 91249 * Conc * Conc - 2937.6 * Conc + 28.662

        'Calculate right side of equation (6.4)
        Right_side = 1 - alpha * Exp(-Abs(parameter_a) * beta)


        'Calculate the minimun difference between left and right side of euqation (6.4)
        min1 = Abs(Right_side - Left_side)

        If (Conc = 0.001) Then
        min2 = min1
        current_conc = Conc
        Else
          If (min2 > min1) Then

          min2 = min1
          current_conc = Conc
          End If

        End If


        Next Conc


        'Output the simulation reslut in column E of the same row
          Cells(Row, E).Value = current_conc

          Next Row

End Sub
```