DEVELOPMENT OF A PORTABLE, HANDHELD DEVICE UTILIZING COMPUTER VISION AND MICROPLITIS CROCEIPES FOR THE DETECTION OF VOLATILE CHEMICALS

by

SAMUEL L. UTLEY

(Under the Direction of Glen C. Rains)

ABSTRACT

*Microplitis croceipes* (Cresson) (Hymenoptera: Braconidae), a parasitoid wasp, are very keen odor detectors capable of being trained to respond to target odors. The feasibility of creating a portable, handheld volatile odor detector that utilizes *M. croceipes* as the chemical sensor was investigated. First, a computer vision system consisting of a laptop, web camera, and software package (*Visual Cortex*) was assembled. The system was able to measure the crowding behavior of five female *M. croceipes* hand trained to detect 3-octanone. Further, the system was able to distinguish between the crowding response of trained *M. croceipes* exposed to 0.5mg and 0.1mg of 3-octanone and a control within 20 seconds. Second, the computer vision system was packaged as a portable, handheld device and tested. The system was able to discriminate between the crowding response of trained *M. croceipes* when exposed to 3-octanone from all other treatment/training combinations (control, 3-octanone, Myrcene/Trained, Untrained).

INDEX WORDS:  *Microplitis croceipes*, chemical detection, computer vision, *Visual Cortex*, *Wasp Hound*, 3-octanone, Myrcene, insect behavior
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B.S.B.E., The University of Georgia, 2004

A Thesis Submitted to the Graduate Faculty of The University of Georgia in Partial Fulfillment of the Requirements for the Degree

MASTER OF SCIENCE

ATHENS, GEORGIA

2004
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August 2004
ACKNOWLEDGEMENTS

I would like to acknowledge Glen Rains for his outstanding academic and professional guidance, support, and friendship. I would like to thank Joe Lewis for lending the expertise and facilities that made this project possible. I would like to thank Moukaram Tertuliano for his patience and expertise on experimental design and M. croceipes. The craftsmanship of Dewayne Dales was indispensable during the design and crafting of the many Wasp Hound prototypes. Last, I would like to acknowledge the unwavering support provided by Phil and Dot Utley and Barb Crompton.
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Chemical Sensing Technologies

Detecting volatile chemicals is becoming a leading method of non-invasive searching. Historically, the detection of volatiles has been very important in tracking illegal substances and detecting explosives, but it also has been shown to be a viable means of detecting other organic materials (Rains et al., 2003A). With the advancing needs of precision agriculture and homeland security, efforts are being made to lower the costs and increase the efficiency of screening through the use of volatile detection. Traditional methods of detecting volatile chemicals include human olfactometry, training canines, and electronic olfaction (Gardner and Bartlett, 1998). Of these, humans and dogs are the most sensitive, however both can be subjective and costly (Gardner and Bartlett, 1998). Many electronic devices have been developed in response to the cost and reliability associated with volatile detection and range in design from simple (e.g. metal oxide doped transistors) to complex (e.g. array of polymer coated sensors analyzed using neural networks). The simple designs are relatively inexpensive but are normally very specific and sensitive to low concentrations, or they detect a wider range of volatiles but lack sensitivity (Gardner and Barlett, 1998; Dickinson, 1998; Börjesson et al., 1996). More elaborate electronic nose designs are inexpensive relative to training and maintaining a canine, but are about 100 times less sensitive than human olfaction (Raman and Gerhardt, 1997; Sarig, 2000), and the user is left to interpret the complex output (Rains et al., 2001).
**Microplitis croceipes**

**Physiology**

*M. croceipes* (Cresson) (Hymenoptera: Braconidae) 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 1/2 the length of the male antennae. *M. croceipes* are facultative parthogenic, laying facultatively arrhenotokous eggs; *M. croceipes* larvae will develop as male if the egg is unfertilized and female if fertilized (Daly et. al., 1998).

**Background**

*M. croceipes* ability to detect and respond to chemical cues has been extensively studied. During wind assays it was found that *M. croceipes* can selectively seek out plants offering needed resources based on their physiological state (i.e. needs for reproduction or food) by tracking the volatile chemicals released from the plants’ canopies (De Moraes et al., 1998). The volatiles emitted from the plant canopy were isolated and identified to further investigate *M. croceipes*’ ability to detect odors. When presented with the emitted volatiles without the plants, *M. croceipes* was able to selectively detect the odors at low concentrations (Olson et al., 2003, Pare and Tumlinson, 1997). The US Department of Defense subsequently requested that the USDA investigate the breadth of chemicals detectable by *M. croceipes* and their limits of detection. The detection capabilities of *M. croceipes* offered a possible alternative to the use of canine olfaction and electronic noses for detecting and tracking potentially illegal or harmful substances. Using wind tunnel trials as the standard testing method, *M. croceipes* was successfully trained and tested to 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 vanilla (Takasu and Lewis, 1993) to a wide array of aliphatics, ketones, aldehydes, and 2,4 and 2,6 dinitrotoluene (a byproduct of trinitrotoluene degradation)(Olson et al., 2003, Pare and Tumlinson, 1997, Smid et al., 2002). The method in which to best harness 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 odor. EAGs were successfully performed and yielded valuable information on the physiology and structure of the antennae (Park et al., 2001, Smid et al., 2002, Park et al., 2002, Ochieng et al., 2000), but the ability of *M. croceipes*’ olfaction system to interpret the electrical activity within the antennae greatly surpasses that of artificial neural networks and other analytical techniques. Additionally, the antennae were prone to desiccation once removed and were generally rendered useless within 1-2 hours. Confining live *M. croceipes* not only removed the difficulties associated with tracking free-roaming wasps but also those associated with interpreting EAGs and preserving detached antennae. Confining *M. croceipes* resulted in the investigation of alternative observable behaviors outside the realm of in-flight selection. Once trained to associate stinging a host with an odor, *M. croceipes* would exhibit a coiling behavior when exposed to the target odor. If trained to associate feeding with an odor, *M. croceipes* would exhibit area restricted searching, head sticking, and hole entering at the point source of the target odor (Olson et al., 2003; Rains et al., 2002; Rains et al., 2001; Rains et al., 2000). With knowledge of these behaviors and the abilities of *M. croceipes*, investigations were made into the applications of utilizing *M. croceipes* as a whole organism sensor. Previous prototypes for devices using a whole organism wasp sensor have utilized confined, trained *M. croceipes* while
monitoring their head sticking and hole entering behaviors as the method of reporting the absence or presence of a target odor (Olson et al., 2003; Rains et al., 2002; Rains et al., 2001; Rains et al., 2000). However, area restricted searching is exhibited more quickly than head sticking and may be a quick, reliable, and easily measurable response.

**Chemotaxis**

The life cycle of *M. croceipes* relies on its keen ability to track volatile odors from plant canopies and hosts such as short, straight carbon chains, aromatic hydrocarbons, and complex multi-ring sesquiterpenes (Olson, et al., 2003, Pare and Tumlinson, 1997). To find both hosts and nectar, *M. croceipes* tracks favorable feeding and breeding conditions over long distances through the use of chemical cues (Lewis and Tumlinson, 1988). In the wild, 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 frass up wind. After locating the plant, they determine the location of the host larvae. The larvae themselves are relatively odor 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 production of insect repellant type volatiles (Paré and Tumlinson, 1999) from not only the wound but from the entire plant canopy. Interestingly, the wasps are not repelled, but instead they use these chemical cues to locate the larvae. The odors emitted by the plant are dependent on its type, health, soil conditions, and the type of insect feeding on it (Tumlinson et al., 1999). With so many variables affecting the possible volatile emissions, *M. croceipes* must possess outstanding learning and detection capabilities.

*M. croceipes*’ adaptive responsiveness is not limited to naturally occurring volatiles produced by plant or hosts. This species can learn to recognize a diverse 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 odors with separate behaviors and will seek out the odors 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 odor with food will choose to seek out the target odor over a control in order to feed. Wasps allowed to sting a host or antennate frass while exposed to a target odor will seek out the target odor in order to lay eggs (Wäckers and Lewis, 1994, Olson et al., 2003).

The success experienced with training wasps to seek out target odors in flight bioassays led to the search for additional behaviors that may be exhibited. It was found that if allowed to smell an odorant while feeding, a wasp would exhibit antennating (flicking the antennae in a searching manner) when next exposed to the same odor. If allowed to sting a host while in the presence of an odor, a wasp would exhibit a coiling behavior (contracting the abdomen in a stinging fashion) when next exposed to the same odor. When a trained odor is emitted from a point source, a wasp will exhibit an area restricted searching behavior (spinning and antennating in a small area) around that source. If a learned odor is emitted from a hole large enough for a wasp to fit into, the wasp will generally ignore its phototropic and anti-geotropic tendencies and enter the hole in search of the odor’s source (Olson et al., 2003; Rains et al., 2002; Rains et al., 2001; Rains et al., 2000).

**Sensitivity**

Both *M. croceipes* and the Cyranose 320 are extremely sensitive odor detectors. The Cyranose 320 (Cyrano Sciences, Pasadena, CA, ~$8,000) is a 32-channel electronic nose based on Caltech’s swelling polymer technology (http://cyranosciences.com/products/cyranose.html)
and uses a built in pump to pass air samples over its polymer sensors. When trying to detect the presence of 3-octanone and Myrcene, *M. crociepes* is 74 and 94 times more sensitive, respectively, than the Cyranose 320 (Rains et al., 2003B). In nature the wasps are able to track the faint traces of odors by tracking upwind along an odor concentration gradient. If the wasp is to seek out the target odor, a concentration gradient needs to exist. If a gradient does not exist, the wasp cannot track the odor to its source.

**Computer Vision**

The subject of computer vision is a vast field integrating the principles of electrical, computer, and optical engineering (Upchurch & Thai, 2002 personal communication). Its application varies but the guiding principle behind computer vision is to allow for automated visual inspection. Much like the human visual system, a minimal computer vision system consists of a camera (eye) for acquiring images and an electronic or computer device (brain) for processing those images.

Each image acquired must be captured in or converted to a digital format before it can be processed in a computer. An image is digitally represented by small sections called pixels (picture elements). The amount of pixels used to represent a single image is dependent upon the camera’s detector or frame grabber. Each pixel has an intensity value associated with it. For example, a pixel within an image of 8 bit resolution can take on a value from 0 to 255 (2^8-1). In an 8 bit grayscale image, 0 is black, 255 is white, and everything in between is a shade of gray. So, each picture is digitized by turning it into a two-dimensional array (x, y coordinates) of values.

Some images taken do not make use of the full resolution available. For example, an 8 bit image with no pixels that takes on the value of 0 and/or 255 does not take advantage of the full
scale resolution offered by the 8 bits. By normalizing the pixel values, the full image resolution can be taken advantage of allowing for greater contrast. Take for instance, an 8 bit image containing 4 pixels with values:

<table>
<thead>
<tr>
<th></th>
<th>100</th>
<th>230</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>75</td>
<td>200</td>
</tr>
</tbody>
</table>

Figure 1.1: Example 4 pixels image with 8 bit resolution. The full-scale resolution of the image is not being utilized.

There is a clear edge within this image that separates it into a right (large values) and left (small values) side. The contrast between the two halves of the image can be increased by normalizing the pixel values using Equation 1.1 (Upchurch & Thai, 2002 personal communication).

\[
P'(x,y) = (2^N-1) * \left[ \frac{(P(x,y) - P_{\text{min}})}{(P_{\text{max}} - P_{\text{min}})} \right]
\]

Equation 1.1

Here \( P'(x,y) \) is the normalized pixel value calculated for location \((x,y)\) using the image resolution \(N\), the current pixel value at location \((x,y)\), and the maximum and minimum pixel value within the image. The normalized pixel values are:

<table>
<thead>
<tr>
<th></th>
<th>41</th>
<th>255</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>205</td>
</tr>
</tbody>
</table>

Figure 1.2: Normalized 4 pixel image. By normalizing the image shown in Figure 1.1, the full-scale resolution of the image can be utilized in order to create greater contrast. There is now more contrast between the right and left side, potentially allowing for easier processing.

A larger image contrast allows for easier edge detection and object identification through segmentation. For example, a grayscale image can be converted to a binary image through binary
segmentation with a user-defined threshold. All pixels of value lower than that of the threshold are forced to zero (black); all pixels of value higher than the threshold are forced to 1 (white). This binary image can be used to quickly classify each pixel as being a member of one of two classes (e.g. background or objects of interest).

Figure 1.3: Histogram data showing the frequency at which each pixel value occurs. Segmentation thresholds are generally selected where the frequency count dips drastically (i.e. ~70 and ~192).

Grayscale value frequency data provided by histograms offers valuable information when selecting segmentation thresholds. Thresholds for creating good binary images are often selected within “valleys” of the frequency data. Additionally, histograms inherently offer total and member class pixel counts (Figure 1.3).

If any of these processing techniques are to be applied only to a select region of interest (ROI), a mask must be applied to the image. Like a stencil, a mask covers any part of an image
to be ignored and exposes the area to be viewed, studied, modified, etc. Mask application allows for focusing the processing time and efforts only on the ROI.

**NI-LabVIEW**

National Instruments’ LabVIEW is a development suite based on the $G$ programming language for easily creating software for signal, data, and image acquisition and processing (LabVIEW User Manual, 2004). Through a drag and drop process users are able to quickly create their own graphical user interfaces (GUIs) and code. Each file created is known as a Virtual Instrument (VI), and a VI embedded inside another VI is called a subVI. The creation of subVIs is crucial to creating easily scalable and readable code.

Every VI is comprised of two parts, the Front Panel and the Block Diagram. The Front Panel is where the user interface is created. Knobs, gages, sliders, graphs, etc. are created by simply dragging, dropping, and resizing them on the Front Panel. For each control and indicator created on the Front Panel, a node is automatically created for it in the block diagram. The block diagram contains the code for the VI. Like the Front Panel, each function is placed within the Block Diagram through a drag and drop process (it is possible to create nodes which contain text based code).

A wide variety of extra functionalities can be added to the base LabVIEW installation through add-ons. Some add-on packages can include analysis, connectivity, motion control, control design, personal digital assistant (PDA), and application building toolkits. Additionally, National Instruments offers a Vision Development Module for image processing tasks (currently not ported for PDA’s). The Vision Development Module allows a user to easily incorporate masking, normalizing, segmentation, and image histograms into a VI.
CHAPTER 2
MONITORING OF *MICROPLITIS CROCEIPES* CROWDING BEHAVIOR USING A
COMPUTER VISION SYSTEM

\[\text{__________________________________________}\]

\[\text{1 Utley, S.L., G.C. Rains, and W.J. Lewis. To be submitted to Transactions of the ASAE.}\]
Abstract

*Microplitis croceipes* (Cresson) (Hymenoptera: Braconidae) are parasitoid wasps capable of being trained to respond to target odors. One such response is known as area restricted searching, and several wasps exhibiting area-restricted searching within the same area is known as crowding. A computer vision system consisting of a laptop, web camera, and software package (*Visual Cortex*) was assembled to objectively quantify the crowding behavior of *M. croceipes*. The system was able to measure the crowding behavior of five female *M. croceipes* hand trained to detect 3-octanone. Further, the system was able to distinguish between the crowding response of trained *M. croceipes* exposed to 0.5mg and 0.1mg of 3-octanone and a control within 20 seconds.

Introduction

*Microplitis croceipes*, hand trained to and placed in the presence of a target odor, can individually exhibit several distinct behaviors including: coiling, antennating, head sticking, and area restricted searching (Olson et al., 2003, Takasu & Lewis, 1993, Wäckers et al., 2002). Humans can interpret these behaviors as either indicating a positive (odor detected) or negative (odor not detected) response by visual assessment. However, it is very difficult for humans alone to objectively quantify a wasp’s response to an odor. A system is needed to objectively make quantifiable measurements of the behavioral responses.

Previous successful attempts at developing a volatile odor detector have exploited hand trained, 2 day old starved wasps, and their desire to seek out food while ignoring their phototropic and anti-geotropic instincts (Tertualiano et al., 2004). The *M. croceipes* movement down into a dark hole while seeking out the odor source was detected using an infrared beam. Their response was quantifiable both in time and number. A response was considered positive if
the wasps entered the hole within 10 seconds, and 88% of the conditioned females exhibited a positive response when presented with the target odor. However, large response variability reduced the reliability and robustness of any potential applications.

In an attempt to improve the response of trained *M. croceipes*, area restricted searching behaviors were investigated as a quicker, more reliable and detectable behavioral response. When multiple wasps are contained together and individually respond with area restricted searching, an emergent group behavior called crowding occurs. Crowding, easily observable with the human eye, lends itself to be detected and quantified through the use of digital image analysis. Digital image analysis offers the potential of not only determining if and when the wasps respond, but also the concentration of odor through the amount of crowding.

Digital image acquisition and analysis can be performed easily with National Instruments’ LabVIEW. LabVIEW is a graphical software development package. Its graphical coding environment allows for quick and easy code development for graphical user interfaces, port communications, image processing, and signal acquisition and analysis. Coupled with Peter Parente’s LabVIEW Webcam Library (http://www.cs.unc.edu/~parente/labview/index.shtml), LabVIEW easily manages input from a Logitech QuickCam (web camera) and subsequent image processing.

**Objectives**

The objective of this study is to explore the viability of utilizing a computer vision system for objectively quantifying the behavioral responses of *M. croceipes*, hand trained to detect 3-octanone, in the presence and absence of the target odor.
**Materials and Methods**

**Computer Vision System**

A computer vision system was created in order to objectively observe the crowding behavior of *M. croceipes* (Figure 2.1). It is an open-air system, consisting of a camera, testing stage, computer, and software.

![Figure 2.1](image.png)

Figure 2.1: An open air computer vision system was created for observing the crowding behavior of female *Mircoplitis croceipes* within a test cartridge. (A) The system was used under a fume hood and consisted of a camera, testing stage, computer, and software. (B) The distance from the tip of the camera to the test cartridge top was 1 inch.

**Hardware**

The computer vision system consists of two major hardware components: a camera and a portable laptop computer. A Logitech QuickCam (~$80) is used to acquire images of the insects, though a Logitech QuickCam Pro 4000 has also been successfully used with the software. Any computer with the processing capabilities to handle the software and a Universal Serial Bus (USB) port can be used. For this study, a Sony Vaio laptop computer (Model: PCG-GRT100, Processor: 2.4GHz Pentium 4, RAM: 512MB, OS: Microsoft Windows XP Home) was used to save and/or process the images acquired with the QuickCam. The Logitech camera interfaces with the computer through USB.
Software

Software that allows a user to utilize the hardware to acquire and process images was developed in house using National Instruments’ LabVIEW 6.1 and Parente’s LabVIEW WebCam Library. The software has been named Visual Cortex (Figure 2.2). Visual Cortex gives a user the power to perform several tasks related to observing and analyzing insect crowding behavior including: taking single snapshots, capturing time stamped still pictures, extracting time variant information from still pictures using image processing, and capturing and analyzing insect behavior in real-time.

Figure 2.2: Visual Cortex front panel. Visual Cortex was created in house using National Instrument’s LabVIEW 6.1 and Peter Parente’s LabVIEW WebCam library. Visual Cortex was used for the acquisition and processing of the images of the wasps’ behavioral responses. Visual Cortex allows a user to save settings, take single snapshots, capture a series of time-stamped images, process those time-stamped images, and acquire response curves in real time.

There are several basic image manipulation and analysis techniques forming the core process that allows Visual Cortex to analyze the crowding behavior of insects. These techniques include: masking, normalizing, creating histograms, and binary segmentation (see Chapter 1) (Figure 2.3). The core process is utilized in both the time stamped still and real-time image processing features. The captured image is first masked using a 320x240 pixel mask of the user’s choice in order to select a region of interest (ROI).
Figure 2.3: Image processing sequence. The original image (A) is masked with a 320x240 pixel mask of the user’s choice (B). The mask is given an offset to select a ROI for processing (C). The values of the pixels within the ROI are normalized and then processed using binary segmentation (D).

The pixel data within the ROI is normalized and segmented resulting in a binary image. The amount of black pixels within the ROI are counted and divided by the total number of pixels within the ROI. The time variant percent black pixel data is integrated using the Trapezoidal Rule (Equation 2.1) to produce time variant integration curves. The integration filters the data allowing for easier interpretation of the image analysis results.

\[
I_1 = I_0 + \frac{h}{2} (y_0 + y_1) \quad \text{Equation 2.1}
\]

- \( I_1 \) = Current Integration Value
- \( I_2 \) = Previous Integration Value
- \( h \) = current time \((t_1)\) – previous time \((t_0)\)
- \( y_1 \) = % Black Pixel Value for \( t_1 \)
- \( y_0 \) = % Black Pixel Value for \( t_0 \)

For immediate results, a user would utilize the real-time image analysis sub-VI (Figure 2.4). This subVI allows a user to capture and process images in real-time. The activity is quantified and displayed graphically on screen. At this point, the user is left to manually input positive response conditions; however incorporation of a calibration routine to automatically determine those conditions will soon be introduced. When a positive response is recognized, the user is alerted (Appendix A-1, B-1).
Figure 2.4: Real-Time.VI front panel. Visual Cortex allows a user to acquire images, analyze them, and graph the resulting data in real time using Real-Time.VI.

If the user prefers to record the wasp behavior and analyze it later, a sub-VI has been created to capture time stamped still shots of the wasp every $\approx 250$ms (some variation is present in the capture interval lengths due to computer latency) (Figure 2.5). The images are saved to a directory recorded in the global.inf file. The last 10 directories are recorded. This sub-VI was used extensively in this study so that records of the wasp behavior could be filed (Appendix A-2, B-2).

Figure 2.5: Capture-Stills.VI front panel. The Capture-Stills VI allows a user to capture a series of time-stamped images and save them to a local drive on a computer.
To analyze the captured time stamped still shots, a sub-VI has been created to process them (Figure 2.6). This sub-VI allows a user to browse a data disk for recorded images and set masking and threshold parameters. Additionally it records the number and percentage of black pixels within the ROI, and the resulting cumulative integration for the time variant stills to a file of the user’s choice (Appendix A-3, B-3).

![Figure 2.6: Process-Stills.VI front panel. Time-stamped still images can be analyzed using the process-stills VI.](image.png)

Global parameters are set using a settings sub-VI accessible from the main menu of *Visual Cortex* (Figure 2.7). The settings sub-VI allows for modification of camera, masking, and threshold parameters contained in the global.inf file. The global.inf file allows for the easy loading of parameters into all of the other sub-VI’s.
Figure 2.7: Settings.VI front panel. Camera, segmentation, and image mask settings can be globally changed using the settings VI.

For users who would like to take a single picture, a sub-VI has been created to save single snapshots from the camera (Appendix A-4, B-4).

**Experimental Procedure**

**Insects**

*M. croceipes* were used for this investigation. The larval hosts used for rearing were *Heliothis zea* (Lepidoptera: Notuidae) as described by Lewis and Burton (1970). The breeding stock was provided with water and honey and kept in a Plexiglass cage (30 x 30 x 17 cm) at 28°C, 50-70% r.h., and a L16:D8 photocycle. Test specimens were females, 2 days old, given only water from time of emergence and no oviposition experience.

**Training Procedure**

Through associative learning, 7 female *M. croceipes* were conditioned to associate 3-octanone (C₈H₁₆O, Vapor Pressure: 1.5 mmHg) with food. All training procedures were
performed under a fume hood in the USDA-ARS Biological Control Laboratory in Tifton, GA. A fluorescent ring light (Luxco Lamp Corp.) was used to lure wasps in the event of escape. Since escapes were common, 7 wasps were trained, but only 5 were tested per group.

An odor delivery stage (Figure 2.8) was prepared for each group trained. First, a Whatman filter disc (Whatman Int. Ltd., Maidstone, England, Cat. No. 1103323, Grade 3, 2.3 cm) was loaded with a 10 μL aliquot of 3-octanone/dichloromethane (1:16) solution and allowed to evaporate for 1 minute. Next, the filter disc was placed in a glass Petri dish (KIMAX USA, 1.7 x 5.3Ø cm) which was then covered with a piece of aluminum foil (12 x 12 cm). The head space within the covered dish was allowed to build for 1 minute, during which a piece of filter paper (2 x 2 mm) was placed in the center of the aluminum foil covering and saturated with 50% sucrose water solution. Last, a push pin was used to create 6 holes in a tight circular pattern around the sucrose water saturated filter paper.

Seven *M. croceipes* females were captured and individually hand trained. The 7 wasps were captured from their rearing cage and placed in separate vials. In order, each wasp was removed from its vial using a pair of forceps and individually allowed to feed on the sucrose solution for 10 seconds. The odorant emitted around the filter paper passed over their antennae while feeding (3-octanone at ~5.5 ppm). After feeding, each wasp was placed back in its vial. The process was repeated so each wasp was allowed to feed for three, 10 second intervals with approximately 60 seconds between each feeding (Tertuliano et al., 2004)
Figure 2.8: Odor delivery stage used during training. A filter disc was placed in a glass Petri dish and covered with aluminum foil. A piece of filter paper soaked in sucrose solution was placed in the center of the foil, and holes were made around it to allow the wasps to feed while smelling the target odor.

**Sample Preparation**

Three corn sample preparations were used for testing. The preparations were named blank, control, and test. Corn was utilized as a background odor potentially giving insight into the capabilities of utilizing *M. croceipes* for the detection of toxins present in large grain stores. The researcher’s hands were washed with soap (Sparkleen 1, Fisherbrand Scientific Co., Pittsburgh, PA) and water prior to creating all samples. The mouth of the jar was covered with a 12 x 12cm piece of aluminum foil and shaken for 15 seconds, subsequently creating small dimpling in the foil covering. Blank corn samples consisted of a 240mL Mason jar with 150mL (120 grams) of whole kernel feed corn. Control samples were created from existing blank samples. The foil covering of the blank sample was removed and a Whatman filter disc was placed on top of the corn using a pair of forceps; the filter disc was pushed to the bottom of the corn using a separate pair of forceps before recovering the jar. After shaking, the sample was set aside to allow the head space over the corn to build for 5 minutes. Test samples were created from control samples. A Whatman filter disc was loaded with an aliquot of 3-octanone/dichloromethane solution on a glass dish and allowed to dry for 1 minute. The foil
covering of the control sample was removed, and the glass dish was used to drop the disc onto the top of the corn. A separate pair of forceps was used to push the odorous filter disc to the bottom of the corn before recovering the jar. After shaking, the sample was set aside to allow the head space over the corn to build for 5 minutes.

Cartridge Preparation

Cartridges were observed while empty and while containing five *M. croceipes*. An empty cartridge placed over a blank corn sample was defined as a blank treatment. A cartridge containing five wasps placed over a control corn sample was defined as a control treatment. A cartridge containing five wasps placed over a test corn sample was defined as a test treatment.

The cartridge was composed of 3 parts (Figure 2.9). The body of the cartridge was part of a Millipore Aerosol Analysis Monitor. The top was a lid for a Millipore PetriSlide™ modified to fit the body and thoroughly perforated with small holes to allow for sufficient ventilation. A wire mesh disc was placed in the bottom of the body to prevent the wasp from escaping out through the inlet.

![Figure 2.9: Test cartridge. (A) The test cartridge was composed of a top from a Millipore PetriSlide™, a wire mesh disc, and part of a Millipore Aerosol Analysis Monitor. (B) The mesh disc 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.](image)
Before using, each cartridge was thoroughly cleaned with soap and water and dried. After drying, cleaning was continued by sweeping a 10 L/min air stream for approximately 15 seconds over all surfaces of the cartridge.

Wasps were placed individually into the cartridge. The cartridge was placed upside down in a clean area under the fume hood. Each wasp was removed from its vial using a pair of forceps and gently scraped off the forceps into the cartridge using the body of the container.

**Testing**

*Visual Cortex* was used for comparing the behavior of 5 hand-trained female *M. croceipes* when presented with the air from the headspace of the prepared corn samples. Data was taken for 3 different concentrations of 3-octanone masked with a background odor of whole kernel corn. The quantities of 3-octanone/dichloromethane solutions used to impregnate the filter disc were: 10uL of a 1:16 solution (~0.5mg of 3-octanone; 5.5ppm), 2uL of a 1:16 solution (~0.1mg of 3-octanone; 1.1ppm), and 10uL of a 1:842 solution (~0.01mg of 3-octanone; 111ppt). For each concentration, 5 replications of blank, control, and test treatments were recorded. During this study, a blank was defined as a cartridge with no wasp over a blank corn sample. A control was defined as a cartridge containing 5 wasps over a control corn sample. A test was defined as a cartridge containing 5 wasps over a test corn sample.

Testing was performed under the same fume hood as training. The laptop, camera, and samples were all placed under the fume hood. The laptop was placed in the front corner of the fume hood with the camera mount positioned approximately 15cm to the left of the keyboard. 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
cartridge. The Logitech QuickCam was placed so that the tip of the camera was approximately 2.54 cm (1 in) above the top of the test cartridges (i.e. 1.4 cm = 125 pixels).

The following procedure was used to obtain the blank, control, and test run pictures for each set of wasps:

1) Turned on the fume hood and the fluorescent ring light.
2) Washed hands with soap and water.
3) Set out 5 pieces of 8.5 x 11 in printer paper beneath fume hood to divide up work space (area for setting out vials, odor preparation area, training area, testing area, and area for setting clean objects on)
4) Positioned laptop and camera beneath hood.
5) Turned on laptop and connected camera.
6) Removed 7 female wasps for testing and placed beneath hood.
7) Hand trained all 7 wasps.
8) Made sure all light sources but overhead fluorescent room lights were off/blocked.
9) Washed hands.
10) Cleaned cartridge and set beneath hood.
11) Prepared blank corn sample.
12) Poked hole in aluminum foil covering with a 7 penny nail.
13) Quickly centered empty (blank) cartridge over new hole.
14) Quickly centered jar and cartridge beneath camera.
15) Used Visual Cortex to capture still pictures of cartridge every ≈ 250 ms for 60 seconds.
16) Removed cartridge from top of aluminum foil and placed in clean area.
17) Washed hands.
18) Prepared control corn sample.
19) Placed sample in clean area.
20) Placed 5 female wasps in cartridge.
21) Repeated steps 12-16 using control corn sample.
22) Prepared test corn sample
23) Placed sample in odor preparation area.
24) Washed hands.
26) Removed cartridge and placed it in odor preparation area.
27) Discarded foil covering and jar contents in wastebasket.
28) Washed cartridge, and all glassware.

Analysis

All pictures collected were analyzed with Visual Cortex’s Process Stills function (process-stills.vi, Appendix A-3, B-3). For each set of pictures, a black 320 x 240 pixel TIF image containing a centered 125 pixel diameter white circle was used as a mask. The image was given an X and Y offset to center the mask’s white circular area over the inlet of the cartridge using setmask.vi (Appendix A-7, B-7) accessible within process-stills.vi. The ROI set by the mask corresponded to a 1.4 cm diameter circular region. A lower threshold of 70 was used for binary segmentation (pixels values < 70 forced to 0 and > 70 forced to 1). Visual Cortex provided data describing the amount of black pixels within the ROI. The number of pixels, the percent of total pixels, and the integration of the time variant percent of total pixels that were black within the ROI were calculated for each set of pictures.
Initial analysis revealed large variations between the 15 blank treatments suggesting that some response curves may be inherently offset more than others due to significantly larger numbers of black pixels within the ROI not representing wasp body mass (i.e. background noise). To remove the effects of these variances, the control and test treatment data was calibrated. From each set of images corresponding to single tests, one image was selected in which the wasps contained within the cartridge were not searching within the ROI. This image was used to measure the amount of black pixel noise (not representing wasp body mass) present within the ROI throughout the 60 second test period. The image was masked, normalized, and segmented like all the other images processed during this study. The percent of the total black pixels within the ROI was recorded for each image selected and analyzed and then used to create calibration curves for each treatment. Since the lighting within the test area and cartridge positioning would not have changed during the 60 second testing period, it was assumed that the same amount of black pixels within the ROI not contributing to the measurement of the crowding response would have remained constant throughout all the images for that single treatment. The time values recorded for each test were copied to a spreadsheet and the percent black pixel values extracted from their corresponding calibration images were copied next to them, repeating the value for each time. The data was then integrated using the Trapezoidal rule function within LabVIEW to create 20 new time-variant integration value curves to be used for calibration. The newly created calibration curves were then subtracted from their corresponding treatment response curves.

Microsoft’s Excel was used to compile, average, and graph the approximately 256 (some variation existed due to computer latency) integration values and their corresponding time stamps for the five replications per treatment within each concentration. The standard deviation
was calculated for the integration values whose corresponding time stamps averaged out to approximately a multiple of five seconds (excluding zero). Confidence intervals were calculated using the resultant standard deviation values, an $\alpha = 0.05$, and $n = 5$.

An ANOVA statistical analysis of the data was performed using a general linear model (SAS). There were 3 dosage levels (0.5mg/5.5ppm, 0.1mg/1.1ppm, 0.01mg/111ppt), 3 treatments (blank, control, test), 5 replications of each dosage/treatment pair (15 total), and 12 observations from each replication (times stamps close to multiples of 5 seconds) to create a total of 540 observations analyzed with the general linear model (GLM). The 15 blank treatment replications (180 observations) were analyzed to determine if each was statistically the same. The remaining 30 calibrated replications (15 controls, 15 tests) were analyzed by dosage and next by treatment to determine if either factor had significant effect on the mean response.

Results & Discussion

A total of 45 replications yielding 540 observations were collected and analyzed (Table 2.1).

Table 2-1: Treatment layout. Blank (no odor, no wasps), control (no odor, 5 wasps), and test (3-octanone, 5 wasps) treatments were each replicated five times for the 0.5mg, 0.1mg, and 0.01mg dosage levels. Twelve observations occurring at multiples of 5 seconds were extracted from each replication.
Comparison of Blank Treatment Replications

There were significant differences between the 15 blank replications (d.f.=14, n=180, P < 0.0001). These results indicate the amount of black pixels measured within the ROI of the empty cartridges varied significantly, suggesting that the physical properties of the cartridges and/or lighting were variable.

Variability may have been caused by several factors including: dimpling in the aluminum foil covering and non-uniformity of cartridge tops, mesh bottoms, and lighting. Corn samples were shaken for 15 seconds after being covered, and the corn striking the covering caused dimpling in the foil. This dimpling created diffuse reflection that may have not been uniform between corn samples. The cartridge tops, made from Millipore PetriSlide™ coverings, were modified by drilling holes in them and removing the excess material from their edges. This was done by hand and non-uniformity in their construction is certain. Many of the edges of the drilled holes blocked the camera’s view of the cartridge bottom; therefore variability in their placement would have caused non-uniform blocking of camera’s view. Additionally, the mesh discs placed in the bottom of the cartridge body were metal and discolored by small amounts of oxidation through repeated washings. Discoloration may have been substantial enough to cause some of the pixels representing mesh in the images acquired to have a value lower than the segmentation threshold (in our study, LT=70). During testing all sources of lighting excluding the overhead room lights were covered. The overhead lights did not change location and it is assumed that their output was consistent over the test period. It is doubtful that the lighting conditions caused the large variability in the blank replications, but it is possible.

The adaptation of this system to a handheld device would require the development of an automated calibration method or controlling for physical cartridge and lighting differences. For
example, a molding or other reproducible manufacturing method would reduce the physical
differences between cartridges, currently modified individually by hand. A custom cartridge
design would dispose of the need for the mesh bottom used to prevent _M. croceipes_ from
crawling out the bottom. The cartridge would need to be made from Teflon, a hard plastic, or
other material that is fairly non odor absorbing. Additionally, enclosing the system and using a
fixed light source would reduce possible variability in lighting and shadows. However, for this
study, the cartridge/lighting variability was removed from the control and test treatments by
calibrating each image set before statistical comparisons were made.

*Effects of Treatment and Dosage on Response*

The control and test treatments (180 observations) were calibrated (see Analysis section
of Methods and Materials) and analyzed to determine the effects of treatment and dosage on the
mean response (average integration values over 60 second test period) (Figures 2.10, 2.11, 2.12).
Figure 2.10 shows the control and test treatment mean responses grouped by dosage. The errors
bars were calculated using \( n = 5 \) and \( \alpha = 0.05 \) for each treatment per dosage. Figure 2.11 shows a
different grouping of the same data in Figure 2.10; dosage responses are grouped by treatment
and error bars were calculated using \( n = 5 \) and \( \alpha = 0.05 \) for each dosage per treatment. The
response of the _M. croceipes_ groups over the 60 second test period can be seen in Figure 2.12.
The controls for all dosages were tightly grouped and were similar to the test treatment at the
0.01mg (111ppt) dosage. The test treatments at the 0.5 (5.5ppm) and 0.1 mg (1.1ppm) dosages
were both significantly different from all other treatment/dosage pairs after, at most, 20 seconds.
Errors were calculated using \( n = 5 \) and \( \alpha = 0.05 \) for each treatment per dosage.
Figure 2.10: The mean response for each treatment per dosage level and 95% confidence intervals. The means of the Controls are not significantly different from each other or the Test treatment at 0.01mg (~111ppt). The means of the Test treatments at 0.5 and 0.1 mg, 5.5 and 1.1 ppm respectively, are not significantly different from each other but are different from the Control treatments and the Test treatment at 0.01mg.

Figure 2.11: The mean response for each dosage per treatment and 95% confidence intervals. The means of the Controls are not significantly different from each other or the Test treatment at 0.01mg (111ppt). The means of the Test treatments at 0.5 (5.5ppm) and 0.1mg (1.1ppm) are not significantly different from each other but are different from the Control treatments and the Test treatment at 0.01mg.
Figure 2.12: Means and 95% confidence intervals for all treatments at all dosage levels. Both the 0.1mg (1.1ppm) (A) and 0.5mg (5.5ppm) (B) Test treatments were measured as significantly different from the 0.01mg (111ppt) Test (C) and all of the Controls (D, E, F) after 20 seconds.

Effects of Control and Test Treatments on Response

Five groups of *M. croceipes* (5 individuals per group) received both control and test treatments using 0.5mg of 3-octanone. The behavioral response of *M. croceipes* at the 0.5mg (5.5ppm) dosage level was significant across treatments (d.f.=1, n=120, P < 0.0001). The mean response of the test treatment (2.7638) was significantly higher than that of the control treatment (0.4763). The time (d.f=11, n=120, P < 0.0001) and treatment*time interaction (d.f=11, n=120, P < 0.0001) effects were also both significant, indicating that the integration values were dependent on both treatment and elapsed time. The system was able to detect a significant difference in the test and control treatment responses in ≈ 10 seconds (Figure 2.13).
Figure 2.13: Means and 95% confidence intervals for calibrated Control (B) and Test (A) treatments at the 0.5mg of 3-octanone dosage level. There was significant difference between treatments after \( \approx 10 \) seconds.

Similar results were obtained with five groups of *M. croceipes* (5 individuals per group) receiving both control and test treatments using 0.1mg of 3-octanone. The behavioral response of *M. croceipes* at the 0.1mg (1.1ppm) dosage level was significant across treatments (d.f.=1, n=120, P=0.0002). The mean response of the test treatment (3.5822) was significantly higher than that of the control treatment (0.6117). The time (d.f=11, n=120, P < 0.0001) and treatment*time (d.f=11, n=120, P < 0.0001) effects were also both significant, indicating that the 120 integration values (12 Obs./Rep. for 5 test and 5 control reps.) were time and treatment dependent. The system was able to detect a significant difference in the test and control treatment responses in \( \approx 10 \) seconds (Figure 2.14).
The system was able to quantify the behavior of the trained wasps in such a way as to successfully distinguish between the crowding behavior exhibited when presented with the target odor at the 0.5mg and 0.1mg levels and the individual searching behaviors exhibited when presented with only the odor of the corn. When looking at individual dosages, a significant difference in the two treatments was detectable in as little as 10 seconds (Figures 2.13 and 2.14). When the results from the dosages are pooled, a significant difference between the tests and controls was detectable in about 20 seconds (Figure 2.11).

The behavioral response of *M. croceipes* at the 0.01mg (111ppt) dosage level was not significantly different across treatments (d.f.=1, n=120, P = 0.3100). However, the time (d.f=11, n=120, P < 0.0001) and treatment*time interaction (d.f.=11, n=120, P=0.0498) effects were both significant at $\alpha = 0.05$, indicating that the 120 integration values (12 Obs./Rep. for 5 Test and 5
Control Reps.) were time dependent (Figure 2.15). At this dosage, it appears that the odor concentration was too low to elicit a crowding behavior strong enough for the system to detect as significantly different from the control, or the wasps were unable to detect the odor.

![Figure 2.15: Means and 95% confidence intervals for calibrated Control (B) and Test (A) treatments at the 0.01mg of 3-octanone dosage level. There was no significant difference between responses to treatments.](image)

Effects of 0.5mg, 0.1mg, and 0.01mg Dosages on Treatment Response

Fifteen groups of *M. croceipes* (5 individuals per group) received control (corn odor only) treatments before receiving test treatments at one of the three dosages. Dosage had no significant effect on *M. croceipes* response to the Control treatment (12 Obs/Rep, 5 Reps, 3 Doses) (d.f.=2, n=180, P=0.7159). Dosage*time interaction effects were not significant (d.f.=22, n=180, P=1.0), but time effects were (d.f.=11, n=180, P < 0.0001), indicating that the integration values were affected by time but not by what test treatment dosage they preceded (Figure 2.16).
Dosage grouping had no significant effect on response to Control treatment. These results imply that the groups of wasps exhibited similar searching behaviors. No group spent significantly more or less time within the ROI that any other group, allowing for the assumption that test treatment results were not biased by the normal searching behavior of the trained *M. croceipes*.

Dosage did have a significant effect on *M. croceipes* response to the test treatment (12 Obs/Rep, 5 Reps, 3 Doses) (d.f.=2, n=180, P=0.0005). The 0.1mg (3.5822) and 0.5mg (2.7639) response means were not significantly different from each other, but they were both significantly different from the 0.01mg response mean (1.0254). Both time (d.f=11, n=120, P < 0.0001) and the dosage*time interaction (d.f=22, n=120, P < 0.0001) significantly affected the integration values (Figure 2.17).
Figure 2.17: Means and 95% confidence intervals for calibrated Test treatments at (A) 0.1mg, (B) 0.5mg, and (C) 0.01mg dosage levels. The response mean of the Test treatment at the 0.01mg dosage level was significantly lower than at 0.1 and 0.5mg.

The system was not able to distinguish between responses to dosages that were significantly different from the controls. Therefore at this point it appears the concentration of the target odor can not be inferred from the magnitude or slope of the response curve. More investigation needs to be made into the abilities of the computer vision system in inferring target odor concentration levels and its sensitivity.
CHAPTER 3

HANDHELD INSTRUMENT UTILIZING INSECT BEHAVIOR MONITORING SYSTEM

AND WHOLE ORGANISM WASP SENSOR

\[^{1}\]

\[^{1}\]Utley, S.L., G.C. Rains, and W.J. Lewis. To be submitted to Transactions of the ASAE.
Abstract

The University of Georgia’s department of Biological and Agricultural Engineering has created a computer vision system that can objectively quantify the crowding response of 5 female Microplitis croceipes (Cresson) (Hymenoptera: Braconidae). A portable, handheld volatile odor detector (the Wasp Hound) that utilizes this computer vision system and M. croceipes as the chemical sensor was created. The Wasp Hound was able to collect air from the head spaces of corn samples prepared within the lab. Coupled with UGA’s Visual Cortex, the Wasp Hound was able to discriminate between the crowding response of trained M. croceipes exposed to 3-octanone from all other treatment/training combinations (control, 3-octanone, Myrcene/ Trained, Untrained).

Introduction

More traditional methods of volatile detection include utilizing human and canine olfactation, GC-MS, and the electronic nose (Rains et al., 2003A). However, a new field is emerging around harnessing the keen senses of other biological organisms.

The U.S. Army Center for Environmental Health Research (USACEHR) has devised a method of using bluegill sunfish (Lepomis macrochirus) for monitoring a broad range of toxins in water (http://usacehr.detrick.army.mil/envsen2.html). The aquatic biomonitor uses mounted electrodes to monitor electric signals generated in the water by the movement of the fish. When six or more of the eight parameters are detected as abnormal, the system initiates an alert. The system responds within an hour to most chemicals at toxic levels. This aquatic biomonitor is currently being implemented in a New York City reservoir.

Research by APOPO at the Sokoine University of Agriculture in Tanzania has led to the development of a successful regiment for training African Giant Pouched rats (Cricetomys
*gambianus*) to non-destructively detect landmines and accurately detect Tuberculosis, subsequently saving many lives (http://www.apopo.org/). The rats are capable of residual explosive scent tracing (REST) and direct detection of buried mines. The rats can be brought samples for identification or taken out and led through suspected mine fields. This research has led to further investigation into using rats for the detection of Tuberculosis in sputum (coughed up matter) samples. The rats have shown success in discriminating between positive and negative sputum samples without the need for expensive test equipment. Using rats to perform these tasks may have significant impact on TB control in low income countries.

Insentinial 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/). Using image recognition software, Insentinial can monitor the activity of honey bees inside their patented cassettes. The system’s electronic output can notify a user of the presence of a single target odor.

Like the bees utilized by Insentinial Ltd., trained *Microplitis croceipes* can be used to identify target odors (Wäckers et al., 2003, Olson et al., 2003, Tertuliano et. al., 2004). *M. croceipes* are capable of learning a wide spectrum of chemical odors in association with both food and host. When presented with the trained odor again, these parasitoid wasps will exhibit foraging or oviposition behaviors in response. One such foraging behavior is area restricted searching in which *M. croceipes* will scan a small area while antennating in search of the target odor point source (Wäckers et al., 2002). Multiple wasps exhibiting area restricted searching collectively demonstrate a crowding behavior. The crowding behavior can be monitored and quantified objectively using image processing techniques offered in the *Visual Cortex* (Utley et al., 2004) software package.
Objectives

The objective of this study was to investigate the effectiveness of a handheld instrument (Wasp Hound) utilizing a whole organism wasp sensor (5 containerized female M. croceipes) coupled with Visual Cortex, and to determine if this system can accurately measure the crowding behavior of M. croceipes when exposed to a target odor.

Materials and Methods

Handheld Design

A handheld instrument called the Wasp Hound was developed for the detection of volatile odors (Figure 3.1, Appendix C). 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 allows for the creation of an odor gradient inside the device by slowly drawing outside air through the test cartridge. The mounted camera is used for observing insect behavior under consistent lighting.

Figure 3.1: Wasp Hound. The Wasp Hound integrates a computer vision system created by UGA-BAE into a portable handheld enclosure. The enclosure provides consistent lighting, cartridge placement, and air flow.
The *Wasp Hound* contains electrical components for acquiring images, air sampling, and lighting the interior of the enclosure. All powering and communication for the *Wasp Hound* is done through a USB (Universal Serial Bus) connection. Images are acquired using a Logitech QuickCam with lighting provided by a white 2300mcd LED (Digikey, CMD333UWC) and a current limiting 46Ω resistor. Ventilation is provided by a flat unidirectional CPU fan (DigiKey, P11086-ND) whose speed is variable between “Purge” (12.18 Lm⁻¹, 0.43cfm) and “Test” (<12.18 Lm⁻¹, 0.43cfm) speeds through the use of a single pole double throw (SPDT) switch and a 56Ω current limiting resistor. Stranded wire (24 AWG) and Molex connectors (DigiKey, WM2510-ND, WM2533-ND, 16-02-0102, WM2517-ND) are used to make all electrical branching from the USB cable (Appendix D).

**Enclosure**

The *Wasp Hound* enclosure keeps all components positioned statically and allows for consistent uniform lighting (Figure 3.2, Appendix C). Body and cap are made from PVC which is a hard plastic a fairly odor non-absorbent. The body is made of a 15.72cm (6 3/16”) long piece of 7.62cm (3”) schedule 40 PVC pipe. The PVC body contains 3 through holes on 3 of its quadrants for bolts that suspend the camera inside. The holes on opposing sides are located 7.66cm (3 1/64”) from the top; the third hole is located 8.85cm (3 31/64”) from the top. A 4th hole, drilled halfway through the body wall, is located 3.81cm (1 1/2”) from the top for a bolt used to properly align the cap. The bottom of the body was capped with a 7.62cm (3”) flat-bottomed PVC (polyvinyl chloride) cap (Genova Plumbing Products, #70153), notched for consistent placement. The center of the inside of the cap was bored out to a depth of 0.32cm (1/8”) and diameter of 3.97cm (1 9/16”). A 0.40cm (5/32”) diameter through hole was made in
the center of bore. Two custom made clips are located on each side of the bore and are used to hold a test cartridge in place. The clips are made from soft metal and are each held in place by one screw. The top of the body is capped with a flat, circular piece of 0.64cm (0.25”) thick plastic. The plastic top is held in place with two screws, and washers provide the 0.08cm (1/32”) spacing between it and the body. A 2.30cm (29/32”) diameter hole is cut in the top of the top to allow for mounting of the ventilation fan. A 0.79cm x 1.59cm (5/16” x 5/8”) hole is cut 2.06cm (13/16”) from the center of the top to allow for mounting of the SPDT switch. Inside the body of the Wasp Hound, a custom made bracket is mounted 0.64cm (1/4”) from the bottom for holding the LED in place.

Figure 3.2: Mounting of camera and LED within Wasp Hound. (A) The Logitech QuickCam was suspended within the PVC body by three bolts 1 inch above the test cartridge top. (B) An LED was mounted within the Wasp Hound as the main source of illumination.

Software

Communication with the Wasp Hound was through the Visual Cortex interface (Utley et al., 2004). All image acquisition and analysis was performed using this software.

Test Cartridge Design

A cartridge was needed to containerize insects used for testing (Figure 3.3). The cartridge needed to be adequately sized to allow for sufficient movement of 5 female M. croceipes wasps.
but not so large as to diminish their timely responsiveness. Additionally the cartridge had to be well ventilated and transparent so the interior could be monitored and illuminated.

Finding a transparent and appropriately sized cartridge off-the-shelf was difficult, therefore a mixture of parts were modified and used in the design. The cartridge was composed of 3 parts. The body of the cartridge was part of a Millipore Aerosol Analysis Monitor. The top was a lid for a Millipore PetriSlide™ modified to fit the body and thoroughly perforated to allow for ventilation. A wire mesh disc was placed in the bottom of the body to prevent the wasp from escaping out through the inlet.

![Figure 3.3: Test cartridge. (A) The test cartridge was composed of a top from a Millipore PetriSlide™, a wire mesh disc, part of a Millipore Aerosol Analysis Monitor, and a FinTip Pipette tip. (B) The mesh disc 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 pipette tip was inserted into the bottom to direct air samples into the cartridge.](image)

**Experimental Procedure**

*Insects*

*M. croceipes* were used for this investigation. The larval hosts used for rearing were *Heliothis zea* (Lepidoptera: Notuidae) and the procedures as described by Lewis and Burton (1970) were followed. The breeding stock was provided with water and honey and kept in a Plexiglass cage (30 x 30 x 17 cm) at 28 °C, 50-70% r.h., and a L16:D8 photocycle. Test
specimens were females, 2 days old, given only water from time of emergence and no oviposition experience.

**Chemicals**

The chemicals used in this investigation were 3-octanone and Myrcene. 3-octanone \( (C_8H_{16}O, \text{Vapor Pressure: } 1.5 \text{ mmHg}) \) has a minty smell and is a compound found in many fungal pathogens. Myrcene \( (C_{10}H_{16}, \text{Vapor Pressure: } 2.01 \text{ mmHg}) \) has a pine smell and is released by cotton plants when fed on by cotton bollworms (Rains et. al., 2001). Both chemicals are irritants of the eyes, mucous membranes, and upper respiratory tract, and may be lethal if ingested in large quantities (Sigma-Aldrich Corporation).

Both chemicals were diluted using dichloromethane \( (CH_2Cl_2, \text{Vapor Pressure: } 350 \text{ mmHg}) \) as the solvent. When an aliquot of solution was allowed to evaporate, the dichloromethane evaporated quickly leaving behind the less volatile solute (Rains et. al., 2001).

**Training Procedure**

Five female *M. croceipes* were conditioned to associate 3-octanone with food. All training procedures were performed under a fume hood in the USDA-ARS Biological Control Laboratory in Tifton, GA (Figure 3.4). A fluorescent ring light (Luxco Lamp Corp.) was placed in the fume hood to lure wasps in the event of escape.

An odor delivery stage was prepared for each group trained. First, a Whatman filter disc (Whatman Int. Ltd., Maidstone, England, Cat. No. 1103323, Grade 3, 2.3 cm) was impregnated with a 10 uL aliquot of 3-octanone/dichloromethane \((1:16)\) solution and allowed to dry for 1 minute. Next, the filter disc was placed in a glass Petri dish (KIMAX USA, 1.7 x 5.3Ø cm) that was in turn covered with a piece of aluminum foil \((12 \times 12 \text{ cm})\). The head space within the covered dish was allowed to build for 1 minute, during which a piece of filter paper \((2 \times 2 \text{ mm})\)
was placed in the center of the aluminum foil covering and saturated with 50% sucrose solution. Last, a push pin was used to create 6 holes in a tight circular pattern around the sucrose water saturated filter paper.

Figure 3.4: Training, sample preparation, and testing area. All procedures were performed beneath a fume hood to ensure proper ventilation and minimize cross contamination from odors.

Five *M. croceipes* females were captured and individually hand trained using the feeding regiment set forth by Olson et. al (2003). The five wasps were captured from their rearing cage and placed in separate vials. In order, each wasp was removed from their vial using a pair of forceps and individually allowed to feed on the sucrose solution for 10 seconds. The odorant emitted around the filter paper passed over their antennae while feeding. After feeding each wasp was placed back in her vial. The process was repeated so each wasp was allowed to feed for three, 10-second intervals with approximately 60 seconds between each feeding.

**Sample Preparation**

Three corn sample preparations were used for testing. Each preparation contained either corn and an odorant (test) or corn alone (control and blank). Corn was utilized as a background odor potentially giving insight in to the capabilities of utilizing *M. croceipes* for the detection of
toxins present in large grain stores. The researcher’s hands were washed with soap (Sparkleen1, Fisherbrand Scientific Co., Pittsburgh, PA) and water prior to creating all samples. The mouth of the jar for all samples were covered with a 12 x 12 cm piece of aluminum foil and shaken for 15 seconds, subsequently creating small dimpling in the foil covering. Blank corn samples were created by filling a 240mL Mason jar with 150mL (120g) of whole kernel feed corn. Control corn samples were created from blank samples. The foil covering of the blank sample was removed and a clean Whatman filter disc was placed on the corn surface. A pair of forceps was used to push the disc to the bottom of the corn before recovering and shaking. Test corn samples were created from either existing control samples or from scratch. A Whatman filter disc was impregnated with an aliquot of 3-octanone/ dichloromethane (1:16) or Myrcene/dichloromethane (1:16) solution on a glass dish and allowed to evaporate for 1 minute. The glass dish was used to drop the disc onto the surface of the corn and a separate pair of forceps was used to push it to the bottom of the corn before covering the jar. After shaking, samples were set aside to allow the head space over the corn to build for 5 minutes. A total of 10 blanks, 15 controls, 10 tests containing Myrcene, and 10 tests containing 3-octanone were created.

Cartridge Preparation

Five female Microplitis croceipes were confined in a cartridge during testing. Before using, each cartridge was thoroughly cleaned with soap and water. After drying, cleaning was continued by sweeping a 10 L/min air stream for approximately 15 seconds over all surfaces of the cartridge.

Wasps were placed individually into the cartridge. The cartridge was placed upside down in a clean area under the fume hood. Each wasp was removed from its vial using a pair of forceps and gently scraped off the forceps into the cartridge using the body of the container.
A Fintip™ plastic pipette tip was inserted into the bottom of cartridge so that the large end of the pipette tip fit snugly into the inlet of the cartridge. This was done so that when the cartridge was placed inside the Wasp Hound a tip was available to penetrate the samples’ foil coverings. The tip channeled all airflow directly from the corn sample to the inlet of the cartridge (Figure 3.5).

Figure 3.5: 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 pipette tip protruded through the bottom of the cap.

Testing

Data was collected using the Wasp Hound and Visual Cortex with both untrained and trained wasps presented with 3-octanone and Myrcene odorants. Empty containers over blank corn samples were also tested to check for the uniformity of cartridges and lighting. The crowding behavior of *M. croceipes* was observed under 3 conditions:

1) Untrained wasps presented with control sample, then 3-octanone test sample
2) Untrained wasps presented with control sample, then Myrcene test sample
3) Wasps trained to 3-octanone presented with control sample, then Myrcene test sample, then 3-octanone test sample

Images of the behavioral responses of untrained wasps when presented with either odor were collected, to determine if *M. croceipes* has a natural attraction to either 3-octanone or Myrcene.
Also during this time the 10 blank replications were performed. The following steps were taken to investigate conditions 1 and 2:

1) Turned on fume hood.

2) Set out 6 pieces of clean 8.5 x 11 in paper under the fume hood to provide space for:
   setting out vials and cages, setting down clean objects, training, testing, and preparing 3-octanone, and Myrcene odorants.

3) Setup laptop under fume hood and opened Capture Stills function of Visual Cortex.

4) Attached *Wasp Hound* to the USB port of computer and allowed to purge until needed.

5) Collected 5 *M. croceipes* females individually in vials and set beneath hood.

6) Washed hands.

7) Prepared blank corn sample.

8) Placed sample in clean area.

9) Prepared empty cartridge and set beneath fume hood.

10) Inserted Fintip™ pipette tip into bottom of cartridge.

11) Switch *Wasp Hound* fan to Test speed.

12) Secured cartridge in *Wasp Hound*.

13) Turned off fume hood.

14) Cut off 10 mm of the pipette tip off with clean scissors.

15) Placed handheld on foil covering of jar allowing remaining tip to penetrate foil.

16) Captured still pictures every $\approx 250\text{ms}$ for 60 seconds.

17) Placed handheld to the side.

18) Turned on fume hood.

19) Removed test cartridge and placed in clean area.
20) Washed hands.
21) Prepared control corn sample.
22) Placed sample in clean area.
23) Using dedicated set of forceps, placed wasps in cartridge.
26) Washed hands.
28) Turned handheld fan speed to Purge.
29) Removed jar’s foil covering
30) Disposed of foil and jar contents in wastebasket.
31) Removed cartridge from handheld and disposed of wasps.
32) Cleaned all materials used with soap and water.
33) Heat-dried metal and glassware.
34) Air-dried all other materials.

Further data was collected to determine if hand trained M. croceipes exhibited a unique behavior when presented with a target odor and if prior exposure to odorous materials affects their ability to detect the target odor and respond distinctly. The following testing method was used to investigate condition 3:

1) Steps 1-6 of previous method.
2) Hand trained wasps to associate 3-octanone with sucrose solution.
3) Steps 20-30 of previous method using Myrcene as the odorant and pipette tip in cartridge.
4) Removed cartridge from handheld and placed in a clean and empty cage.
5) Recaptured wasps in vials
6) Washed and dried hands
7) Using dedicated set of forceps, placed wasps in cartridge.
8) Switched Wasp Hound fan to Test speed.
9) Secured cartridge in Wasp Hound.
10) Steps 25-34 of previous method using 3-octanone as the odorant pipette tip in cartridge.

Analysis

All pictures collected were analyzed with Visual Cortex’s Process Stills function (process-stills.vi, Appendix A-3, B-3). For each set of pictures, a black 320 x 240 pixel TIF image containing a centered 125 pixel diameter white circle was used as a mask. The image was given an X and Y offset to center the mask’s white circular area over the inlet of the cartridge using setmask.vi (Appendix A-7, B-7) accessible within process-stills.vi. The ROI set by the mask corresponded to a 1.40cm diameter circular region. A lower threshold of 70 was used for binary segmentation (pixels values < 70 forced to 0 and > 70 forced to 1). Visual Cortex provided data describing the amount of black pixels within the ROI. The number of pixels, the percent of total pixels, and the integration of the time variant percent of total pixels that were black within the ROI were calculated for each set of pictures.

Initial analysis revealed large variations between the 10 blank treatments (empty cartridges over corn containing no additional odor) suggesting that some response curves may be inherently offset more than others due to significantly larger numbers of black pixels within the ROI not representing wasp body mass (background noise). To remove the effects of these variances the control and test treatment data was calibrated. From each set of images corresponding to single tests, one image was selected in which the wasps contained within the
cartridge were not searching within the ROI. This image was used to measure the amount of black pixel noise (not representing wasp body mass) present within the ROI throughout the 60 second test period. The image was masked, normalized, and segmented like all the other images processed during this study. The percent of the total black pixels within the ROI was recorded for each image selected and analyzed and then used to created calibration curves for each treatment. Since the lighting and cartridge positioning would not have changed during the 60 second testing period, it was assumed that the same amount of black pixels within the ROI not contributing to the measurement of the crowding response would have remained constant throughout all the images for that single treatment. The time values recorded for each test were copied to a spreadsheet and the percent black pixel values extracted from their corresponding calibration images were copied next to them, repeating the value for each time. The data was then integrated using the Trapezoidal rule function within LabVIEW to create 35 new time-variant integration value curves to be used for calibration. The newly created calibration curves were then subtracted from their corresponding treatment response curves.

Microsoft’s Excel was used to compile, average, and graph the approximately 256 (some variation existed due to computer latency) integration values and their corresponding time stamps for all calibrated replications (untrained wasps: ten controls, five Myrcene tests, five 3-octanone tests; trained wasps: five controls, five Myrcene tests, five 3-octanone tests). The standard deviation was calculated for the integration values whose corresponding time stamps averaged out to approximately a multiple of five seconds (excluding zero). Confidence intervals were calculated using the resultant standard deviation values, an α = 0.05, and n = 5.

An ANOVA statistical analysis of the data was performed using a general linear model (SAS). Observations were taken from the 15 groups (5 individuals per group) of *M. croceipes*
divided unevenly into 2 levels of training (10 groups untrained, 5 groups trained). The untrained
groups were further divided into 2 sets (5 groups per set). One untrained set received control and
Myrcene treatments. The other untrained set received control and 3-octanone treatments. Prior to
each control treatment, measurements were taken from an empty cartridge over a blank corn
sample (i.e. blank treatment). The 5 groups of trained *M. croceipes* received control and Myrcene
and 3-octanone test treatments. Each treatment was replicated 5 times. The 10 blank treatment
replications (120 observations) were analyzed to determine if each was statistically the same.
The remaining 35 calibrated replications (15 controls, 10 Myrcene tests, 10 3-octanone tests)
were analyzed by training and by treatment to determine if either factor had significant effect on
the mean response.

**Results & Discussion**

A total of 45 replications containing 540 observations were collected and analyzed (Table
3.1).

Table 3-1: Treatment layout. Blank (no odor, no wasps) and control (no odor, 5 wasps)
treatments were replicated ten times each using untrained *M. croceipes*. Myrcene (Myrcene, 5
wasps) and 3-octanone test treatments (3-octanone, 5 wasps) were each replicated five times
using untrained *M. croceipes*. Control, Myrcene, and 3-octanone treatments were replicated five
times each using *M. croceipes* trained to detect 3-octanone. Twelve observations occurring at
multiples of 5 seconds were extracted from each replication for statistical analysis.

<table>
<thead>
<tr>
<th></th>
<th>Blank</th>
<th>Control</th>
<th>Myrcene</th>
<th>3-octanone</th>
<th>Σ</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Training</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Untrained</strong></td>
<td>10 Reps</td>
<td>10 Reps</td>
<td>5 Reps</td>
<td>5 Reps</td>
<td>30 Reps</td>
</tr>
<tr>
<td></td>
<td>12 Obs/Rep</td>
<td>12 Obs/Rep</td>
<td>12 Obs/Rep</td>
<td>12 Obs/Rep</td>
<td>360 Obs</td>
</tr>
<tr>
<td></td>
<td>120 Total</td>
<td>120 Total</td>
<td>60 Total</td>
<td>60 Total</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Obs</td>
<td>Obs</td>
<td>Obs</td>
<td>Obs</td>
<td></td>
</tr>
<tr>
<td><strong>Trained</strong></td>
<td>0 Reps</td>
<td>0 Reps</td>
<td>5 Reps</td>
<td>5 Reps</td>
<td>15 Reps</td>
</tr>
<tr>
<td></td>
<td>0 Obs/Rep</td>
<td>0 Obs/Rep</td>
<td>12 Obs/Rep</td>
<td>12 Obs/Rep</td>
<td>180 Obs</td>
</tr>
<tr>
<td></td>
<td>0 Total Obs</td>
<td>0 Total Obs</td>
<td>60 Total Obs</td>
<td>60 Total Obs</td>
<td></td>
</tr>
<tr>
<td><strong>Σ</strong></td>
<td>10 Reps</td>
<td>15 Reps</td>
<td>10 Reps</td>
<td>10 Reps</td>
<td>45 Reps</td>
</tr>
<tr>
<td></td>
<td>120 Obs</td>
<td>180 Obs</td>
<td>120 Obs</td>
<td>120 Obs</td>
<td>540 Obs</td>
</tr>
</tbody>
</table>
Comparing Blank Cartridge Measurements

There were significant differences between the 10 individual blank treatment replications (d.f.=9, n=120, P < 0.0001). These results indicate the amount of black pixels measured within the ROI of the empty cartridges varied significantly, suggesting that the physical properties of the cartridges and/or lighting were variable.

Variability may have been caused by non-uniformity of the cartridge tops and mesh bottoms. It is not suspected that lighting was variable. The test area was enclosed and a fixed light source was used. The cartridge tops, made from Millipore PetriSlide™ coverings, were modified by drilling holes in them and removing the excess material from their edges. This was done by hand and non-uniformity in their construction is certain. Many of the edges of the drilled holes blocked the camera’s view of the cartridge bottom; therefore variability in their placement would have caused non-uniform blocking of camera’s view. Additionally, the mesh discs placed in the bottom of the cartridge body were metal and discolored some through repeated washings. Discoloration was caused by small amounts of oxidation. Discoloration may have been substantial enough to cause some of the pixels representing mesh in the images acquired to have a value lower than the segmentation threshold (in this study, LT=70).

An automated calibration method or uniform cartridges are needed. For example, a molding or other reproducible manufacturing method would reduce the physical differences between cartridges, currently modified individually by hand. A custom cartridge design would dispose of the need for the mesh bottom used to prevent *M. croceipes* from crawling out the bottom. However, for this study, the cartridge variability was removed from the control and test treatments by calibrating each image set before statistical comparisons were made.
Effects of Treatment and Training on Response

The control and test treatments (420 observations) were calibrated (see Analysis section of Methods and Materials) and then analyzed to determine the effects of treatment and training on the mean response (average integration values over 60 second test period). Figure 3.6 shows the mean responses exhibited by untrained and trained *M. croceipes* grouped by treatment. The errors bars were calculated using \( n = 5 \) and \( \alpha = 0.05 \) for each state of training per treatment. Figure 3.7 shows a different grouping of the same data in Figure 3.6; treatments are grouped by training, and error bars were calculated using \( n = 5 \) and \( \alpha = 0.05 \). The response of the *M. croceipes* groups over the 60 second test period can be seen in Figure 3.8. The response of *M. croceipes* trained to detect 3-octanone in the presence of the target odor was significantly different from all other treatment/training pairs after 25 seconds.

![Figure 3.6](image-url)

Figure 3.6: The mean response for training per treatment. The means of the controls are not significantly different from each other or the Myrcene test treatment of trained *M. croceipes*. The means of the Myrcene test treatments for trained and untrained wasps are not significantly different. The means of the Myrcene and 3-octanone test treatments for untrained wasps are not significantly different. However the mean integration value of the 3-octanone test treatment for wasps trained to detect 3-octanone is significantly different from all other treatment/training pairs.
Figure 3.7: The mean responses for treatments per training. Data from Figure 3.6 arranged by treatments per training.

Figure 3.8: Means and 95% confidence intervals for treatments of trained (A, F, G) and untrained (B, C, D, E) *M. croceipes*. The mean response of *M. croceipes* trained to detect 3-octanone receiving 3-octanone test (A) treatments was measured as significantly different from untrained wasps receiving 3-octanone (B) and Myrcene (C) test and control (D, E) treatments, trained wasps receiving Myrcene test (F) and control (G) treatments after 25 seconds.
Comparing Response of *M. croceipes* Groups Receiving Control Treatment

Fifteen groups of *M. croceipes* received control treatments. Five groups received prior training to 3-octanone and ten did not. The behavioral response of *M. croceipes* receiving the control treatment was not significantly different within or across training (d.f.=2, n=180, P <0.8025). The mean responses of the untrained wasp (0.8670 and 0.7778) were not significantly different from each other or the mean response of *M. croceipes* trained to detect 3-octanone and receiving the same treatment (0.8316) (Figure 3.9). Similar results were shown by Utley et al. (2004). This suggests that any cohort or day differences that existed did not influence or bias the amount of time *M. croceipes* spent within the ROI.

![Figure 3.9: Means and 95% confidence intervals for calibrated no odor treatment responses for *M. croceipes* trained (C) and not trained (A & B) to 3-octanone. Training had no significant effect on response.](image-url)
Effects of Treatment on Response of *M. croceipes* Trained to 3-octanone

Five groups of *M. croceipes* trained to detect 3-octanone received control, Myrcene, and 3-octanone treatments, in order. The behavioral response of *M. croceipes* trained to detect 3-octanone was significantly different across treatments (d.f.=2, n=180, P < 0.0001) (Figure 3.10).

![Figure 3.10: Means and 95% confidence intervals for calibrated 3-octanone (A), Myrcene (B), control (C) treatment responses of *M. croceipes* trained to detect 3-octanone. The mean response to 3-octanone was significantly larger than those of Myrcene and control treatments. There was no significant difference between the Myrcene and control treatment responses.]

The mean response of the trained wasps exposed to 3-octanone (3.0698) was significantly larger than that of trained wasps exposed to Myrcene (1.1337) or corn alone (0.8316). However no significant difference existed between the mean response to Myrcene and control treatments.

Both time (d.f.=11, n=180, P <0.0001) and the treatment*time interaction (d.f.=22, n=180, P < 0.0001) had significant effects. Trained *M. croceipes* showed interest in the Myrcene, but the amount of crowding elicited by the 3-octanone was significantly greater than both the Myrcene and control treatments within 15 seconds.
Effects of Treatment on Response of Untrained M. croceipes

Ten groups of untrained *M. croceipes* received control and test treatments, in order. Five groups were presented with 3-octanone during their test treatment, and five groups were presented with Myrcene. The behavioral response of untrained *M. croceipes* was significantly different across treatments (d.f.=3, n=240, P=0.0033) (Figure 3.11).

![Figure 3.11: Means and 95% confidence intervals for calibrated 3-octanone (A), Myrcene (B), and control (C & D) treatment responses of untrained *M. croceipes*. There was no significant difference between 3-octanone and Myrcene treatment responses. There was no significant difference between control treatments. Both mean responses of the untrained wasps exposed to Myrcene and 3-octanone were significantly different from the mean response of the control treatment.](image)

The mean responses of untrained wasps exposed to 3-octanone (1.8190) or Myrcene (1.4537) were not significantly different, but they were both significantly different from the mean responses of both control treatments (0.8670 and 0.7778). The control treatment responses were not significantly different. Both time (d.f.=11, n=180, P <0.0001) and the treatment*time
interaction (d.f.=33, n=180, P < 0.0001) had significant effects. Again, the groups were not biased in the amount of time spent searching within the ROI, but did show a curiosity in the strong odorants though no prior training or exposure had been experienced. The curiosity may have been exasperated by their state of hunger, but further investigations need to be done to determine if their curiosity is correlated to their physiological condition.

Effects of Training to 3-octanone on Response of M. croceipes Presented with 3-octanone

Ten groups of wasps were exposed to 3-octanone. Five groups had received prior training to 3-octanone and five groups were untrained. The behavioral response of *M. croceipes* exposed to 3-octanone was significantly different across training (trained vs. untrained) (d.f.=1, n=120, P= 0.0059) (Figure 3.12).

![Figure 3.12: Means and 95% confidence intervals for calibrated 3-octanone treatment responses for trained (A) and untrained (B) *M. croceipes*. Trained *M. croceipes* exhibited more crowding when exposed to 3-octanone that did untrained wasps.](image-url)
M. croceipes trained to detect 3-octanone had a significantly higher mean response (3.0698) when presented with 3-octanone than did untrained wasps (1.8190) receiving similar treatment. Both the time (d.f.=11, n=120, P < 0.0001) and treatment*time interaction (d.f.=11, n=120, P < 0.0001) effects were significant. Untrained M. croceipes exhibited a natural curiosity towards 3-octanone, but trained wasps exhibited significantly more crowding.

**Effects of Training to 3-octanone on Response of M. croceipes Presented with Myrcene**

Ten groups of wasps were exposed to Myrcene. Five groups had received prior training to 3-octanone and five groups were untrained. The behavioral response of M. croceipes exposed to Myrcene was not significantly different across training (d.f.=1, n=120, P = 0.1732). Wasps trained to detect 3-octanone had a similar mean response (1.1337) when presented with Myrcene to untrained wasps (1.4537) receiving similar treatment. Both the time (d.f.=11, n=120, P < 0.0001) and treatment*time interaction (d.f.=11, n=120, P < 0.0010) effects were significant. The response to Myrcene was not significant across training, however it is possible that further data collection may reveal that prior training curbs curiosity to a non-target odor as shown in Figures 3-7 and 3-13.
Figure 3.13: Means and 95% confidence intervals for calibrated Myrcene treatment responses for *M. croceipes* trained (A) and not trained (B) to 3-octanone. Training had no significant effect on response.
CHAPTER 4

CONCLUSIONS

A computer vision system with image analysis software like Visual Cortex can be successfully utilized to objectively observe and quantify the crowding behavior of five trained female *M. croceipes* parasitoid wasps. The computer vision system constructed to prove this concept was able to distinguish between the strong crowding behavior exhibited by trained *M. croceipes* presented with their target odor and the random searching observed during the control treatment within 15 seconds. Such a system integrated into a portable handheld device such as the Wasp Hound has the potential to be utilized for the detection of target chemical odors within an environment containing a masking background odor such as corn. The handheld system constructed during this study can quickly detect the presence of 3-octanone within 25 seconds.

The Wasp Hound and Visual Cortex systems are still in their infancy and some modifications need to be made before implementation in field trials. The system’s capabilities of detecting other odors, its sensitivity, and cartridge design standardization need to be investigated further. Porting the program to a more mobile platform such as a personal digital assistant (PDA) would improve the mobility and reduce the cost (currently ~$1,100) of the entire system. Additionally, program development should be continued to make Visual Cortex more feature rich.

Future work may include investigating the use of vector analysis in interpreting the movement of individual *M. croceipes*. It is possible that *M. croceipes* exhibit behavioral movements that occur before crowding. As of now the Wasp Hound offers the ability to detect
target odors very quickly. 3-octanone was detected by trained wasps in approximately 25 seconds. Understanding behavioral or physiological changes temporally closer to the output of *M. croceipes*’ brain will allow for increased performance when detecting their response to target odors. Future work in recording and mapping the search behaviors of individuals or a group of *M. croceipes* may lead to methods for more quickly detecting target odors through behavior monitoring.
REFERENCES


Upchurch, B.L., and C. Thai. 2002 Personal communication.


APPENDIX A

VISUAL CORTEX VI FLOW DIAGRAMS
Appendix A-1: Real-Time VI Flow Diagram

START
   Read previous settings from global.inf
   Initialize Camera
   Camera Initialized?
      True
      Open global.inf for writing.
      Allow user input for mask path, mask offset, and threshold range
      Replace old setting with new user input
      setup_images.vi (grabs, normalizes, masks, and segments, image)
      Autoscale graph using test duration input
      Wait 100ms
      Stop?
         True
         FALSE
      FALSE
      Begin Processing?
         True
         Close global.inf
         Get Current Time -> T1
         process_images.vi (same as setup_images.vi, but extracts pixel information)
         Use current % black pixel information and previous % black pixel information (0 the first time) and T1 and time image captured in integration using Trapezoidal Rule
         Put time stamp, integration value, # black pixels, % black pixel information in array
         Graph all data collected so far
         Wait 10ms
         Last Time Stamp >= Test Duration?
            True
            Add test data to array
            Run another test?
               True
               FALSE
               Save Data?
                  True
                  Save all test results and settings used to file
                  Close Camera
                  STOP
               FALSE
               Run another test?
               TRUE
               FALSE
               Save Data?
                  TRUE
                  Close Camera
                  STOP
               FALSE
               Run another test?
               TRUE
               FALSE
               Save Data?
                  TRUE
                  Close Camera
                  STOP
               FALSE
               Run another test?
               TRUE
               FALSE
               Save Data?
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                  Close Camera
                  STOP
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               Run another test?
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               Save Data?
                  TRUE
                  Close Camera
                  STOP
               FALSE
               Run another test?
               TRUE
               FALSE
               Save Data?
                  TRUE
                  Close Camera
                  STOP
               FALSE
               Run another test?
               TRUE
               FALSE
               Save Data?
                  TRUE
                  Close Camera
                  STOP
               FALSE
               Run another test?
               TRUE
               FALSE
               Save Data?
                  TRUE
                  Close Camera
                  STOP
               FALSE
               Run another test?
               TRUE
               FALSE
               Save Data?
                  TRUE
                  Close Camera
                  STOP
               FALSE
               Run another test?
Appendix A-2: Capture-Stills.VI Flow Diagram

Start
- Get Current VI's Path
  - Strip Path
  - Strip Path
  - Build Path Using Previous Output From Strip Path and "config\global.inf"
    - Open Config Data File "global.inf"
      - Set CNTR=0
      - Read config file section "Recent Dirs" and extract value of "CNTR" Key
        - Add value (a path) to an array (array of paths)
          - FALSE
            - CNTR>=10?
              - TRUE
                - Camera Initialized?
                  - TRUE
                    - WebCam Grab.vi
                      - WebCam Picture.vi
                        - WebCam Get Color Table.vi
                          - WebCam Flat to Picture.vi
                            - Display Picture
                              - Wait 25ms
                                - FALSE
                                  - Exit? OR Capture?
                                    - TRUE
                                      - FALSE
                                        - WebCam Grab.vi
                                          - WebCam Picture.vi
                                            - WebCam Get Color Table.vi
                                              - WebCam Flat to Picture.vi
                                                - Display Picture
                                                  - Wait 25ms
                                                    - FALSE
                                                      - Exit? OR Capture?
                                                        - TRUE
                                                          - FALSE
                                                            - WebCam Grab.vi
                                                              - WebCam Picture.vi
                                                                - WebCam Get Color Table.vi
                                                                  - WebCam Flat to Picture.vi
                                                                    - Display Picture
                                                                      - Wait 25ms
                                                                        - FALSE
                                                                          - Exit? OR Capture?
                                                                            - TRUE
                                                                             - FALSE
                                                                                 - WebCam Grab.vi
                                                                                       - WebCam Picture.vi
                                                                                         - WebCam Get Color Table.vi
                                                                                           - WebCam Flat to Picture.vi
                                                                                             - Display Picture
                                                                                               - Wait 25ms
                                                                                                 - FALSE
                                                                                                   - Exit? OR Capture?
                                                                                                     - TRUE
                                                                                                       - TRUE
                                                                                                         - Exit?
                                                                                                          - FALSE
                                                                                                              - WebCam Grab.vi
                                                                                                                - WebCam Picture.vi
                                                                                                                  - WebCam Get Color Table.vi
                                                                                                                    - WebCam Flat to Picture.vi
                                                                                                                      - Display Picture
                                                                                                                            - Wait 25ms
                                                                                                                                - FALSE
                                                                                                                                    - Exit? OR Capture?
                                                                                                                                       - TRUE
                                                                                                                                            - FALSE
                                                                                                                                                - WebCam Grab.vi
                                                                                                                                                       - WebCam Picture.vi
                                                                                                                                                           - WebCam Get Color Table.vi
                                                                                                                                                                - WebCam Flat to Picture.vi
                                                                                                                                                                                 - Display Picture
                                                                                                                                                                                                 - Wait 25ms
                                                                                                                                                                                                                     - FALSE
                                                                                                                                                                                                                         - Exit? OR Capture?
                                                                                                                                                                                                                                           - TRUE
                                                                                                                                                                                                                                               - TRUE
                                                                                                                                                                                                                                                   - Exit?
                                                                                                                                                                                                                                                                       - FALSE
                                                                                                                                                                                                                                                                               - WebCam Grab.vi
                                                                                                                                                                                                                                                                                                   - WebCam Picture.vi
Exit? OR Stop? OR Diff >= Duration?

FALSE

Exit?

FALSE

TRUE

Change Value of Exit Button To False

Close Camera.vi

Init CNTR=0

Write Config File Section "Recent Dirs" with Value of Path array into key of "CNTR"

FALSE

CNTR >= size of dir path array size?

TRUE

Close Config File
Appendix A-3: Process-Stills.VI Flow Diagram

Set Process Images False | Set Stop Processing False | Set Exit False

Init Array of Null Path

Process Last Captured?

TRUE

Open ..\config\global.inf
Extract path of last captured pictures form configglobal.inf
Make Process Images Button Visible
Close global.inf

FALSE

Browse?

TRUE

explore_data.vi
Make process images button visible
Wait 100ms

Exit?

FALSE

Process?

FALSE

Get names of all files ending in *.bmp form first folder from explore_data.vi output
Put names of files in order
Build array of file paths

setmask.vi
Use output of setmask.vi to set properties of mask
Open indexed image in array
Display image
Normalize image
Mask image
Display masked image
Apply segmentation to masked image
Display segmented image

1 2 3 4
The variable "Pic" refers to the rectangular coordinate cluster and flat image array associated with a picture.
Appendix A-5: Setup-Images.VI Flow Diagram

START

Input image memory locations, webcam image, mask path, X and Y offset, segmentation thresholds, and errors

Give Mask X and Y Offset

Grab WebCam Image

Make WebCam Image Grayscale

Stretch.vi (Normalizes Grayscale Image)

Overlay Mask Onto Grayscale Image

Segment ROI

Output Color, Masked Grayscale, and Binary Segmented Images in Cluster and Picture Data Format, and error

STOP
Appendix A-6: Process-Images.VI Flow Diagram

START

Input image memory locations, webcam image, mask path, X and Y offset, segmentation thresholds, and errors

Give Mask X and Y Offset

Grab Webcam Image

Make Webcam Image Grayscale

Stretch.vi (Normalizes Grayscale Image)

Overlay Mask Onto Grayscale Image

Segment ROI

Extract total pixel in ROI, and total black pixels in ROI information from binary image using IMAQ Histogram

Calculate % black pixels in ROI

Output Color, Masked Grayscale, and Binary Segmented Images in Cluster and Picture Data Format, time picture was acquired, # Black pixels and % Black pixels in ROI, and error

STOP
Appendix A-7: Setmask.VI Flow Diagram

START

Open ..\config\global.inf
Read mask path
Read mask offsets

Open image

Open mask
Offset Mask
Apply mask to image
Display masked image
Wait 100ms

Done?

FALSE

TRUE

Write changes to offsets, and mask selection to global.inf
Close global.inf

STOP
Appendix A-8: Explore-Data.VI Flow Diagram

START

- Init Past_Path with Null Path
- Get Current Path
- Set Column Headers For Listbox

- Strip Path
- Strip Path

Build Path Using Stripped Path and "data"
Store in Path_Current

Path_Current NOT EQUAL TO Path_Past?

- Display Path_Current
- Get lists_and_symb.vi
- Fill Listbox With Item Names
- Fill Listbox With Item Symbols

Path_Past = Path_Current

- Up Level?

- Item in ListBox Double Clicked?
  - Get Item Number Clicked
  - Index Listbox Items Using Item Number
  - Build path using listbox item value and path

- Stripped Path Empty?
  - Pass Path Before Stripping
  - OK?

- Are There Any SubDirs?

OK?

- Get Highlighted Item

STOP
APPENDIX B

VISUAL CORTEX VI LABVIEW BLOCK DIAGRAMS
Appendix B-1: Real-Time.VI LabVIEW Block Diagram
Appendix B-3: Process-Stills.VI LabVIEW Block Diagram
Appendix B-4: SnapShot.VI LabVIEW Block Diagram
Appendix B-5: Setup-Images.VI LabVIEW Block Diagram
Appendix B-6: Process-Images.VI LabVIEW Block Diagram
Appendix B-7: SetMask.VI LabVIEW Block Diagram
APPENDIX D: Wasp Hound Electrical Schematic