Assessing Pesticide Effect on Honey Bees Using an Intelligent Imaging System

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Abstract

Since colony collapse disorder (CCD) has occurred at different countries in the world and has affected the honey bee population and agricultural productions, a better understanding of honey bee’s activity is important not only in the context of the individual forager but also in regard to the whole colony. This research has aimed to develop an efficient imaging system for honey bee behavior monitoring and analysis. In order to monitor honey bees individually, a special designed circular tag was glued onto the thorax of honey bees. The label image extracted from video frames could further recognized by optical character recognition based on support vector machine (SVM) to identify the individual honey bee. Through collecting and analyzing individual honey bee’s in-and-out behavior, different group activity patterns can be studied. Experiments were carried out to investigate the effect of pesticide on the honey bee’s behavior using the developed imaging system. The experimental results reveals that the flight duration probability density functions of the pesticide treated group and of the control group were significantly different. This indicates that pesticide could have adverse effect on the foraging behavior of honey bees.

Keywords: Honey bee, Monitoring, CCD, Pesticide effect, Image processing

1 Introduction

Honey bees are social insects and are the most economical efficient pollinators for agricultural crops in the world. As colony collapse disorder (CCD) has occurred at different countries, the abrupt disappearance of honey bees could significantly affect the agricultural productions (Oldroyd, 2007). Researchers have been trying to study honey bees behavior to figure out the causes of CCD. The activities of foragers flying in and out of the beehive and the waggle dance provide the food source information. Furthermore, studying these activities helps to understand population behavior of the whole colony. Human observation on the in-and-out beehive activities is not only labor-intensive but also encounters the difficulty to recognize individual honey bee. There are several automatic honey bees monitoring system developed. The passive radio frequency identification (RFID) system has been shown as an effective method (Decourtye et al., 2011; Henry et al., 2012; Streit, Bock, Pirk, & Tautz, 2003). However, there are shortcomings for the RFID approach: the heavy weight of the tag and the electromagnetic waves effect on the honey bee regular behaviors. To overcome these issues, our previous study (Chen, Yang, Jiang, & Lin, 2012) proposed an imaging system that
automatically records honey bee in-and-out activities with minimum interference to its regular behavior. A unique label is attached onto the thorax of each forager. The imaging system can identify each tagged bee and record their time of entries and exists. By collecting the in-and-out behavior of individual honey bees, the group foraging behavior can be analyzed. For this research, experiments were performed using this system and the group behaviors subject to pesticide treatment were studied. The objective of this research was to investigate and quantify the pesticide effect on the honey bee foraging behaviors.

2 Materials and methods

2.1 Hardware and software

The imaging system design is shown in Figure 1. The infrared CCD camera (DMK31AU03, The Imaging Source Europe GmbH, Germany) was positioned at the top of the bee passageway to record the honey bee activities. The illumination was provided by the infrared LEDs which located at the left and right hand side. A desktop computer was connected to the camera and used to process video frames acquired. From the video images, the label on the thorax of honey bees was identified and the times of entries and exits were recorded. The graphical user interface was developed with Borland C++ Builder 6.0. The program libraries included are Open Sourced Computer Vision (OpenCV 1.0) and LIBSVM v2.89 (Chang & Lin, 2011), which were used for image processing and character recognition respectively. The back-end data processing was running with an Intel Core 2 Duo 2.1 GHz processor and a 4 GB random access memory (RAM) on Microsoft Windows 7.

2.2 Labelling

In order to monitor honey bees individually, a paper tag is glued to the thorax of the honey bee for identification as shown in Figure 2. The designed tag is circular with a diameter of 3 mm. The outside part is a black circle, and a pair of character symbols and a black dot for orientation purposes inside the circle. The letter Q was excluded from the character sets because of the easy misidentification with letter O. The circular tags were graphed and edited in Microsoft® Word. A large-quantity of tags was manufactured in the form of waterproof stickers. The Consolas font (5-point font-size and bold weight) and a blank character (2-point font-size) were used to separate the two characters on each tag.

The labelling procedure is based on our previous work (Chen et al., 2012; Wu, 2013) and can be divided into three main steps as shown in Figure 3. For the first step, the honey bees are placed in a freezer of about 5°C for 3 – 4 minutes to lose their mobile capability. Secondly, putting honey bees on a fixture device and removing the thorax hair for labelling preparation. Finally, a drop of UV light curable adhesive is placed onto the thorax and then the tag was placed on top of the glue. A UV light is illuminated on the tag to bond it to the thorax securely.

2.3 Honeybee Monitoring Procedure

Figure 4 shows the image processing procedure of honey bee tag identification. First, the circular Hough transform (Kimme, Ballard, & Sklansky, 1975) was applied to detect tag position and segmented the tag from the background image, as shown in Figure 4A and Figure 4B. Figure 4C is the binarized tag image. The areas of three blocks (two characters and one positioning dot) were calculated. The positioning dot has the smallest area. The regularization was applied to correct the angle of tag image by the positioning dot's orientation. The character image blocks were extracted (Figure 4D). The contrast of character images were enhanced by histogram equalization as shown in Figure 4E. Each character extracted from the image is identified using the optical character recognition technique based on support vector machine (SVM). The final result is shown on the GUI as Figure 4F.

In this study, we used SVM method for character identification. The software library we used was LIBSVM 2.89 (Chang & Lin, 2011). We first used the CCD camera to collect a large number of tag images for training. Processing tag images with the procedure described above
generated the left-oriented and right-oriented character images. Four hundred images from letter A to Z (excluding Q) were manually selected and resized to 20 x 20 pixels to be the model training database. The linear kernel function was selected for speed consideration. The character A was encoded as 1, B as 2, and so on. Y was encoded as 24, and Z as 0. Using LIBSVM to construct a multiple classification model and 25 (character A to Z, excluding Q) binary classification model. Taking A as a binary model for example, we selected 400 images of A as positive sample which were encoded as 1, and we used other letters B-Z with 16 images each (a total of 384) as negative samples which were encoded as -1. These procedures were repeated for all characters to construct each letter model for right-oriented and left-oriented character.

2.4 Pesticide Effect Experiments

Experiments were carried to investigate the effect of pesticide of foraging behaviors of honey bees using the developed imaging system. The honey bees used in this study were Italian honey bees, known as *Apis mellifera ligustica* (Sanzhi Township, Taipei County, Taiwan). The bees were bred by the insect neurobiology laboratory in the Department of Entomology at National Taiwan University. Bees were randomly selected and captured from the existing beehive and divided into groups of 50 individuals. There were 50 foragers for the control group and 50 for the pesticide treated group. Each bee was glued with a unique paired-characters tag. The pesticide treated group was fed with contaminated sugar syrup while the control group was not. The pesticide we used was imidacloprid (Bayer Cropscience AG, Leverkusen, Germany) and it was diluted to a final concentration of 500 \(\mu\)g/liter in 50% sucrose solution. Two duplicated rounds of experiment were carried out with the same settings. Foragers were fed with 1 \(\mu\)l syrup with imidacloprid concentration of 500 \(\mu\)g/liter. Both groups were then released back to their original beehive after labelling. The whole operation time including labelling and feeding for each group took about 3~4 hours. These continuous monitoring experiments were conducted for over a period of 13 days.

3 Results and Discussions

In our experiments, the labelled honey bee activities can be tracked by the image system. From the individual foraging activities we can determine the group foraging activities. Figure 5 shows honey bee daily foraging in-and-out pattern across the monitoring period for both groups. In general, the foraging activity level was not high at Day 1, the day we labelled the honey bees, and resumed to their normal foraging activity at the next day. The maximum activity level was reached at the 2\(^{nd}\) or the 3\(^{rd}\) days. Figure 5 shows that the maximum activity level (179 flights a day) was reached at the 3\(^{rd}\) day in the first round (Table 1). In the second round, 175 flights a day were reached at the 2\(^{nd}\) day (Table 2). After reaching the peak activity level, at the next day detected activities were 69 and 55 flights a day for first round and second round, respectively. The activity level dropped at a rate of about 1/3 for both rounds which is similar to previous studies (Chen et al., 2012; Decourtye et al., 2011). However, we can see that the control group’s activity level remained at a higher level across the 13 day period while no observation was found at the last few days for the pesticide treatment group. Although, in both rounds the number for foraging activities of the pesticide treatment group was less than the control group as shown in Table 1 and Table 2, the daily foraging flight patterns illustrated in Figure 5 for both groups are very similar. The daily foraging activities began from around 6 am in the morning and steadily increased to its maximum level at midday. This foraging pattern matches with the ones reported in previous studies (Chen et al., 2012). In second round, the 8\(^{th}\) day started to rain until to the 13\(^{th}\) day, and the accumulated rainfall at the 8\(^{th}\) day was 18 mm, 9\(^{th}\) day was 52 mm, and 10\(^{th}\) day was 4mm. The high activity level showed at the 10\(^{th}\) day might be affect by the 8\(^{th}\) and 9\(^{th}\) day raining. Figure 6 plots the flight time duration distribution histogram, which shows the distribution of how long the honey bee takes in each foraging flight. From the histogram, the probability density function of flight time duration was calculated for each group. The probability density functions of flight time duration in both groups were close to the exponential distribution. A Kolmogorov Smirnov test was applied to the probability density function of each round to test
whether the control group and the pesticide treatment group are from the similar distribution or not. The results implied that in both rounds of experiment, the probability density function of flight time duration in the control group and the treated group were significant different where $p < 0.001$. The average flight time duration also shows a difference. The control group has short flight time duration than the pesticide treatment group. In first round, the average flight time was $20.9\pm24.8$ minutes and $28.6\pm30.0$ minutes for the control and the treated group respectively. In second round, it was $11.3\pm16.1$ minutes and $21.3\pm21.0$ minutes respectively.

4 Conclusions

The experimental results of pesticide effect on honey bee foraging behavior demonstrate that this imaging system is feasible and can effortlessly record foraging behavior details of individual honey bee. The imidacloprid pesticide concentration of 500 µg/liter has a significant effect on the flight time duration of the treated honey bees. Further experiments in necessary to investigate the threshold level of pesticide concentration that will not give an adverse effect on the honey bees. The developed imaging system thus can be used as an efficient tool to quantitatively investigate pesticide effect and shed light on possible causes for the colony collapse disorder mystery.

5 References


Figure 1: The imaging system consists of two infrared LED light sources, one infrared CCD camera, and an acrylic passageway. The personal computer is connected to the CCD camera to record the honeybee in-and-out activities.

Figure 2: The character symbol tag was glued on the thorax of honey bees.

Figure 3: Honey bee labelling processes.

Figure 4: Honey bee identification procedure: (A) Using circle detection to find tag position. (B) Extracting tag image. (C) Binary tag image. (D) Regulazing tag image and segmenting character image. (E) Character image normalization. (F) SVM classification result.
Figure 5: The daily foraging flight pattern over 13-day period for both sets of experiment.
Figure 6: The probability density function of flight time duration

Table 1. The number of daily foraging flights for adult foragers in the first round

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Table 2. The number of daily foraging flights for adult foragers in the second round

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