Short scientific report *Journal of Plantation Crops, 2008, 36 (1): 69-72*

Tracking the locomotion of coconut black headed caterpillar, *Opisina arenosella* and its parasitoid, *Goniozus nephantidis*

(Manuscript Received: 25-06-07, Revised: 17-10-07, Accepted: 07-02-08)

Keywords: Correspondence, edge detection, euclidian distance, larvae, locomotion, olfactory response, parasitoid

The ectoparasitoids, *Goniozus nephantidis* (Musebeck) (Hymenoptera: Bethylidae) is a gregarious ectoparasitoid of coconut black headed caterpillar, *Opisina arenosella* Walker (Oecophoridae: Lepidoptera). The host-location behavior of *G. nephantidis* is predominately performed by walking. Hence, the locomotor activity plays a major role in the life cycle of these insects. On locating its host *O. arenosella* larvae, they sting and permanently paralyze them and then lay eggs on their cuticle (Hardy *et al*., 1992).

Till recently, the locomotion and behavior of parasitoid and its host was done by manual recording. Manual observations are laborious and error prone due to observers fatigue. Various attempts had been made to address these problems and one such is automation of tracking insects. Diverse approaches like grids of infrared beams or Doppler radar have been employed in tracking (Lucas *et al*., 2002). Tracking multiple animals simultaneously increases experimental throughput and helps in the study of interactions between individuals. Voss and Zeil (1995) implemented a system that could track multiple flying wasps in an outdoor environment. Regions potentially containing wasps were found by detecting differences between consecutive frames, i.e. moving objects. Software for tracking animals have been developed by Noldus *EthoVision* (Lucas *et al*., 2002), but they offer only general purpose solutions.

Considering the above we chose to develop a technique rather than use a commercial package, as it suits our specific requirements and is cost effective. The technique developed was used to assess the olfactory response of the parasitoids using Y maze. The velocity and distance traveled by the parasitoid and its host were calculated to analyze orientation of the parasitoid to its host.

The cultures of *Opisina arenosella / Corcyra cephalonica* and *Goniozus nephantidis* were maintained in Entomology laboratory at Central Plantation Crops Research Institute (CPCRI), Kasaragod, Kerala, India. *G. nephantidis* culture maintained on *O.arenosella* was used for the study. The parasitoids were cultured by placing the adult female wasp and larvae of *O. arenosella* in a vial of 2.5 x 10 cm plugged with cotton. Drops of 50 % honey (in water) placed on the wax coated paper served as food. All the cultures were maintained at 25^0 C and 65 – 70 % RH with a constant dim light illumination.

The larval and parasitoid movements were recorded at 25^o C between 9:00 -16:00 h by the JVC TK C 1380 CCD camera, which in turn was connected to Pinnacle video capturing card in the CPU. The data was captured in 640 × 480 resolution, 30 frames per second and stored in MPEG format. Ten insects were used per replication. Four such replications were maintained.

The program was implemented using the functions of Image Processing Toolbox in MATLAB® Release14 (Dan Lee and Steve Eddins, 2003). The MATLAB script was run with a graphical user interface on a Dell Optiplex GX270, 2.99 GHz, Pentium IV Hyper Threading (HT) processor, with WINDOWS XP Professional (Service Pack 2) and 512 megabyte of memory.

After recording the insect movements in the MPEG video format, each experiment's video clip was converted into AVI format, 25 frames per second, 'truecolor' image type and 'indeo5' video compression using Adobe Premier Pro 1.5 software. Every colour (RGB) frame/image in the video clip was converted into grayscale image in the image pre processing stage. In the process of identifying foreground (insects) and background (other than insects) pixels, the linear combination [1] was used between current and next frame (Alan, 2000).

$$I(x, y) = \alpha * I_c(x, y) + \beta * I_n(x, y),\ 0 < \alpha, \beta < 1 \quad .....(1)$$

where $I(x, y)$ is linearly combined two dimensional discrete density function of an image, I_c and I_n were current and next frame in the video clip.

The resultant image $I(x, y)$ was used for detecting the edges of insects. Here we applied Canny edge detection method (Canny, 1986; Parker, 1997) to detect the insects. Canny assumed a step edge subject to white Gaussian noise. Hysteresis method was use to find the strength of the edge to produce the final edge of an insect. Digital morphological operator, binary dilation was used to dilate the image [2].

$$\text{Dilation} = I \oplus S_{el} = \{c \mid c = a + e, a \in I, e \in S_{el}\} \quad .....(2)$$

where I represented the image being operated on and S_{el} was a structuring element. The image holes were filled with digital morphological operator, fill [3].

$$\text{Fill} = I \oplus (S_{el}, I^c) \quad .....(3)$$

where I was an image containing only the seed pixel, which was any pixel know to be inside the region to be filled and I^c was the boundary image for the region to be filled. S_{el} was structuring element. After obtaining the filled image a threshold was set to obtain blobs. The marked each blob was represented insect.

From the each detected blob, the features centroid and orientation were computed Centroid was the centre of area of a blob. In the two dimensional case, the center of mass was given by the first order moments (I_{10}, I_{01}) [4], [5].

$$I_{10} = \sum\sum xI(x, y)/I_{00} \quad .....(4)$$

$$I_{01} = \sum\sum yI(x, y)/I_{00} \quad .....(5)$$

Euclidian distance was computed between the blobs in current and next frame to establish the correspondence between successive frames to trace of position of the blob over time.

The influence of feeding on olfactory response of *G.nephantidis* was assessed by choice method. The parasitoids (0 -12 h old) were fed / starved for 24 h. Their orientation to honey and host was assessed in Y maze (Y shaped glass tube). The choice of the parasitoids was assessed from the tracks of the images captured. Ten adults were assessed with four replications per treatment.

An automated method of tracking the *O. arenosella* larvae and *G. nephantidis* was developed. The background and foreground gray levels in the image were mixed and well distributed in range of 100 to 250 (Fig. 1a). The linear combination of frames aided to identify the background and foreground objects separately in the image by enhancing clarity of foreground objects and increasing brightness of uniform background (Fig. 1b). After applying the linear combination, the background pixels were distributed in the range of 235 to 255 gray levels in Fig. 1b. The foreground object pixels were distributed in the range of 190 to 234 gray levels in Fig. 1b.

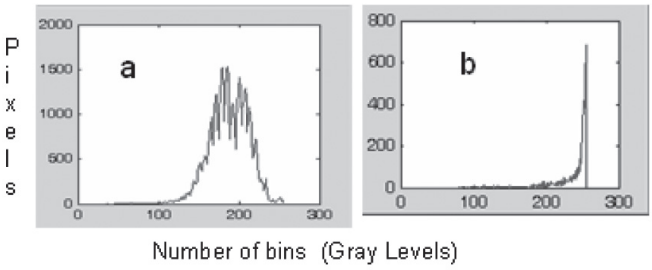

Fig. 1. a) Pixel distribution in the original image of *O. arenosella, C. cephalonica* and *G. nephantidis* b) Pixel distribution of the linearly combined images. The background converted into maximum gray level (white) and foreground pixels were distributed around the gray level 200.

The canny edge detection algorithm was applied on the linearly combined image to detect the insect edge (Fig. 2a). In this process spurious objects also appeared along with the insects. On edge detection, the image was made clear by eliminating spurious objects, by setting threshold 0.4 (Fig. 2b). After identifying the blobs the centroids were extracted for three insects. Alternative frames were taken for analysis as there was significant motion in continuous frames. The correspondence of extracted centroids between frames was made using Euclidian distance between centroids (Table 1). Centroids were then arranged in order of their correspondence and were stored in a vector. The matched centroids were plotted in a linear 2D graph with the aspect ratio of the original image to give the trace of each individual insect (Fig. 3). The plotted graph was

converted into a 2D image to calculate the exact length of the path. The length of the path was estimated by computing the number of pixels on the plotted image. The pixel number was converted into millimeters with the known measurements of petri dish.

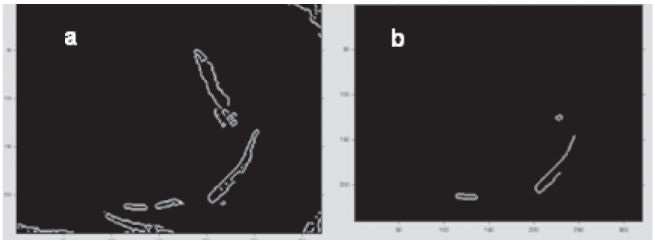

Fig. 2. a) The detected edges with spurious objects without threshold. b) Clear edges of *O. arenosella, C. cephalonica* and *G. nephantidis* with threshold 0.4

Table 1. The coordinates (x, y) of centroids of the insects after establishing their correspondence between frames.

Frame No.	Centroids of *G. nephantidis*		Centroids of *O. arenosella*		Centroids of *C. cephalonica*	
	x	y	x	y	x	y
F1	125	213	223	183	228	125
F3	125	213	222	185	221	119
F5	116	208	215	191	205	118
F7	104	198	213	194	210	117
F9	101	195	205	199	223	116
F11	94	187	205	199	214	132
F13	81	176	205	199	219	128
F15	67	166	193	207	221	131
F17	62	162	195	205	225	129
F19	60	155	183	211	225	124
F21	58	148	180	210	227	124
F23	57	143	180	210	225	127
F25	55	134	180	210	225	126

In a few frames only the head of the larva moves up, left or right with the larva staying in the same position. In this case, the centroid of the larva is displaced very slightly. Though there is no forward motion, the displacement of the centroid was computed.

The automated setup was used to study the movement of larvae and parasitoid. The results indicated that during the period under observation (5 min) the adult *G. nephantidis* travelled a distance of 299.75 ± 2.13 mm with an average velocity of 2.70 ± 0.16 mm/sec. In the case of *O. arenosella*, larvae they travelled a distance of 215.50 ± 2.217 mm with an average velocity of 1.30 ± 0.17 mm/sec. The rice moth *C. cephalonoica* travelled the least distance of 65.00 ± 2.54 mm with an average velocity of 0.7 ± 0.081 mm/sec. There was significant difference between the distance travelled and their velocity between *G. nephantidis,* its host *O. arenosella* and *C. cephalonica* (Fig. 3). During the host searching behavior the parasitoid has to move quickly and be efficient in identifying its host. This activity is clearly reflected in the track captured as *G. nephantidis* travelled 35 and 361 per cent more distance than *O. arenosella* and *C. cephalonica*, respectively (Table 2).

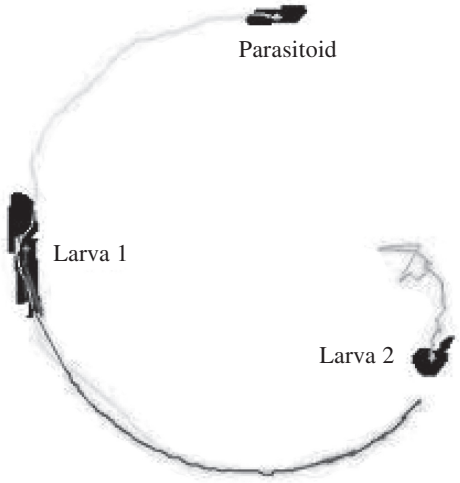

Fig. 3. Tracks of *G. nephantidis* (parasitoid), *O. arenosella* (larva1) and *C. cephalonica* (Larva2)

Table 2. Distance traveled by insects

Insects	Distance travelled (mm)	Average velocity (mm/sec)	Time taken for paralyzing (min)
G. nephantidis	299.75 ± 2.13	2.70 ± 0.16	—
O. arenosella	215.50 ± 2.217	1.30 ± 0.17	6.0 ± 0.7
C. cephalonica	65.00 ± 2.54	0.70 ± 0.081	2.47 ± 0.2
CD (p=0.05)	7.38	0.47	1.80

The time taken by the parasitoids to paralyze the larvae depended on the behavior of the larvae. *G. nephantidis* paralyzed *O. arenosella* in 6.0 min and *C. cephalonica* in 2.47 min. The time taken for *O. arenosella* was more, as they moved quickly when compared to *C. cephalonica* and they prevented *G. nephantidis* coming near them.

Automated observation using video tracking is particularly suitable for measuring loco motor behavior, expressed as spatial measurements (distance and speed etc.), that the human observer is unable to accurately estimate (Buresova *et al.,* 1986; Spruijt *et al.,* 1990, 1998). The ability to track multiple objects in an arena means that interactions between animals can be identified (Spruijt *et al.,* 1990). The researcher can rely on his technique to study parasitoid–host relationships.

The olfactory response of starved and fed *G. nephantidis* was assayed in Y maze. The orientation of the parasitoid to the arm having the odour of their choice was estimated by capturing the image of the parasitoids choosing the arms. The results indicate that the

parasitoids that were starved were attracted more to honey than its host or host products, while the parasitoids that were fed, oriented towards the host or host products. The decline in olfactory response in starved *G. nephantidis* to odour source was reported by Subaharan *et al.* (2005).

More than 63 per cent of the parasitoids oriented to the arm with *O. arenosella* hemolymph followed by 35 per cent to *O. arenosella* frass (Table 3). This orientation provides a cue for the source of kairomones that could be used to enhance the efficacy of the parasitoids. This study helps us to understand the behavior of parasitoids towards the host and design pest control measures.

Table 3. Olfactory response of *G. nephantidis* by choice assay

Treatment	Olfactory response of *G. nephantidis*	
	Fed	Starved
Honey	19 ^{c}	32 ^{a}
O. arenosella larvae	30 ^{c}	19 ^{cd}
C. cephalonica larvae	26 ^{cd}	17 ^{c}
O. arenosella frass	35 ^{b}	13 ^{e}
O. arenosella hemolymph	63 ^{a}	26 ^{b}
CD (p=0.05)	2.56	2.41

Means with same letter are not significantly different at p = 0.05 with DnMRT

Nutrient limitation on emergence or in field may indirectly affect fitness traits such as host finding, ability to overcome behavioral host defenses and the production of the paralyzing venom (Nakamatsu and Tanaka, 2003). The orientation of the starved parasitoids to honey clearly stresses the need to feed the parasitoids on emergence prior to field release as they would spend their time in host searching than food searching.

This study demonstrated the methodology using the digital image processing techniques to create automated system for tracking insects. This helps to develop real-time processing and analysis of motion of insects in sequence of captured images. Analyzing the results generated by the automated system would aid in devising pest management strategies. The system can also be used as a vital tool in the area studies involving the semiochemicals of parasitoid and its host.

Acknowledgement

The authors are grateful to Dr. George V. Thomas, Director, CPCRI for providing necessary facilities for the conduct of study.

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