

# An image analysis system developed for evaluation of *Coleomegilla maculata* larvae's behavior

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Vigneault, C., Roger, C., Hui, K.P.C. and Boivin, G. 1998. **An image analysis system developed for evaluation of *Coleomegilla maculata* larvae's behavior.** Can. Agric. Eng. 40:055-060. A method based on machine vision was developed to replace manual observations required in a study of prey discrimination of the twelvespotted ladybird beetle (*Coleomegilla maculata*) larvae. The system recorded the movement of the II or IV instar larva while the beetle was in contact with a group of 36 cabbage looper (*Trichoplusia ni*) eggs, half of which had been parasitized by *Trichogramma evanescens* before the test. Since the beetle larvae are approximately 20 to 35 times larger than the eggs, the system was programmed to locate the eggs and to follow the **movement of the larval head**. A three step procedure was developed to detect the larval head position with less than 0.1% of error. Results recorded by the system were more objective than those obtained visually. The developed method and different parameters used within this method are presented in this paper. **Keywords:** machine vision, insect tracking, biological control, *Trichogramma evanescens*, *Trichoplusia ni*, prey discrimination, host discrimination.

Une méthode basée sur la vision numérique a été développée pour remplacer les observations manuelles, nécessaires dans une étude de discrimination de proies chez les larves de la coccinelle maculée (*Coleomegilla maculata*). Ce système enregistre les déplacements des coccinelles aux stades larvaires II ou IV dans un groupe de 36 oeufs de fausse-arpenteuse du chou (*Trichoplusia ni*); la moitié de ces oeufs étant parasités par des trichogrammes (*Trichogramma evanescens*) avant l'essai. Puisque les larves de coccinelles sont approximativement 20 à 35 fois plus grandes que les oeufs le système a été programmé pour localiser les oeufs et suivre le mouvement de la tête de l'insecte. Une procédure divisée en trois étapes a été développée pour détecter la position de la tête de la larve avec une erreur de moins de 0.1%. Les résultats obtenus par le système sont plus objectifs que ceux obtenus visuellement. La méthode développée et les paramètres utilisés lors de la conception de cette méthode sont présentés dans ce papier.

## INTRODUCTION

Most beneficial insects cannot reduce all by themselves a pest population below the economic threshold. In biological control, the simultaneous utilization of several entomophagous insects may provide a better control of pests. Nevertheless, introduction of multiple-beneficial insects may increase the number of interactions between these species. Some of these interactions might become harmful if the species are not **complementary to each other** in resource exploitation. This type of competition between beneficial insects has been frequently invoked to explain why some species failed to control pest(s)

successfully (Mackauer 1990). Therefore, it is necessary to evaluate interactions between species before any release.

In North America, three lepidopterous pests are found in cruciferous crops, namely *Artogeia rapae*, *Plutella xylostella* and *Trichoplusia ni*. Losses caused by these pests are significant. For example, the cabbage looper (*Trichoplusia ni*) can eat up to 65 cm<sup>2</sup> of foliage during its development (Stewart and Jacques 1994). In a recent research project, two beneficial insects were chosen to reduce the cabbage looper population. The eggs of this pest can be controlled by *Trichogramma evanescens*, a small wasp that oviposits in lepidopterous eggs and consequently kills them. In addition, the twelvespotted ladybird beetle (*Coleomegilla maculata*) was also chosen to reduce eggs and larvae populations of the cabbage looper. *C. maculata* is a generalist predator which exhibits prey preferences. If *C. maculata* prefers eggs which are not parasitized, this could provide a more complete control on the cabbage looper population. The ability of the twelvespotted ladybird beetle to distinguish between unparasitized cabbage looper eggs and those parasitized by *T. evanescens* was therefore studied.

The tedious work of studying insect behavior has generally been achieved by collecting data manually (Vet et al. 1983; Frazer and McGregor 1994). Geers et al. (1991) used an image analysis system to examine animal behavior, which demonstrated the great potential inborn within machine vision to replace manual observations. However, only a very few computerized systems developed for insect tracking have been reported (Allemand et al. 1994). Vigneault et al. (1997b) examined several commercially available object tracking systems and concluded that they were not well adapted for insect behavior evaluation. Therefore, an image analysis system aimed at studying minute insects was developed by Vigneault et al. (1997a). The same system was modified and used to evaluate the prey preference of the twelvespotted ladybird beetle larvae.

The objective of the present work was to develop an image analysis system to evaluate some behavioral parameters of the twelvespotted ladybird beetle larvae required in the evaluation of this organism for a biological control program. This system has to be able to monitor the head position of a ladybird beetle



larva in a matrix of eggs. It has to be programmed to automatically record the sequence of egg visits done by the larva, the number of contacts each egg received, and the cumulative time of contact for each egg.

## MATERIALS and METHODS

### Preliminary preparation of eggs

Both II and IV instars larvae of the ladybird beetle were studied in the laboratory. They measured approximately 4200 x 1300  $\mu\text{m}$  and 6300 x 1400  $\mu\text{m}$ , respectively. In each test, a larva was placed on a glass plate within a group of 36 cabbage looper eggs. The cabbage looper eggs have shapes of ellipsoids and each egg measures approximately 430  $\mu\text{m}$  in length and 590  $\mu\text{m}$  in diameter. Half of the eggs had been parasitized by *T. evanescens* eight days before the test. The 36 eggs were disposed vertically and their circular surface areas were captured by the camera. The cabbage looper eggs were initially placed on a moist glass plate and once the water evaporated a thin sticky layer composed of proteins was formed between the eggs and the plate. The 36 cabbage looper eggs thus remained slightly glued. In addition, the eggs were arranged as a matrix, having 6 eggs on each row and column (Fig. 1). Positions of the parasitized and unparasitized eggs inside the matrix were pre-defined and were recorded by an operator before the test. Distances of 4 and 8 mm were maintained between the eggs for the II and IV instars larvae, respectively (Fig. 2). These distances were determined based on the insect searching behavior observed during preliminary tests (unpublished data). Each test lasted 60 minutes.

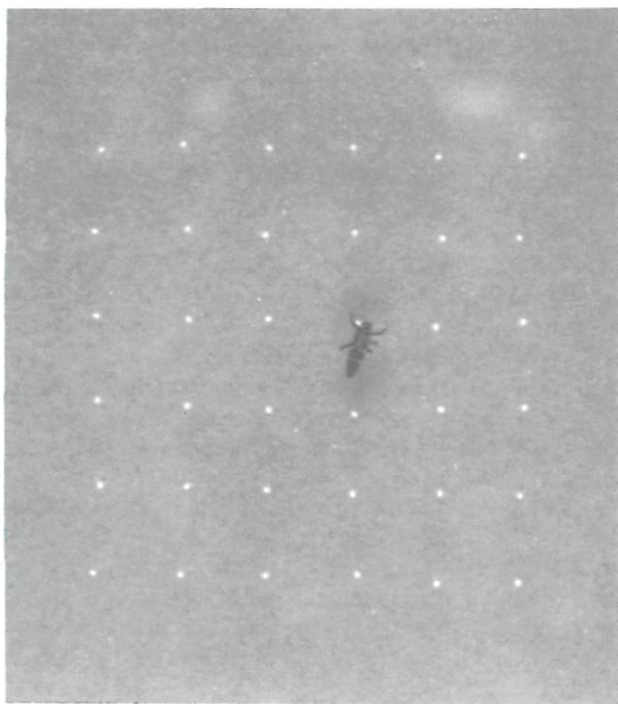


Fig. 1. A IV instar larva of the twelvespotted ladybird beetle within a matrix of 36 cabbage looper eggs.

### The image analysis system

The image analysis system consisted of a background light source, a light diffuser, a CCD video camera, a VHS video cassette recorder, two video monitors, and an IBM-AT compatible microcomputer. The light source, diffuser, and camera were covered by an opaque curtain to eliminate the effects of ambient light on larvae and images captured by the camera. Each component of this system is commercially available.

The light source consisted of a circular neon light tube which provided light from the back of the image. An acrylic plate was used as a diffuser to distribute light evenly inside the field of vision. Thirty images were captured by the video camera each second. The original image was displayed on a video monitor which allowed the operator to center the matrix of eggs properly inside the field of vision. The processed image was then shown on the second video monitor. The video signal produced by the camera was recorded by a cassette recorder, permitting analyses of images to be processed either immediately or later. In the study of the ladybird beetle, all images were analyzed in real time. Video signals were also transmitted to the computer and were digitized at a rate of 30 images/s. Digitization of the images was accomplished by an Oculus-300 board (Coreco Inc., St. Laurent, QC) installed inside the computer. The digitizing board divided an image of 83 by 79 mm into 512(H) x 484(V) square pixels, measuring 163  $\mu\text{m}$  on each side. Each pixel was described by its horizontal and vertical position coordinates and its light intensity measured in gray level. With 8 bits of resolution per pixel, gray level was ranked from 0 to 255 (black to white, respectively).

### Contrast stretching for captured images

After the glass plate containing moth eggs was centered inside the field of vision, the system was activated. The computer first determined the average background gray level by scanning a section of the original image which did not contain any eggs. The system then increased the contrast of the subsequent images by making use of this average background gray level (Jain 1989). Without affecting the results obtained in the analyses, this procedure allowed the operator to distinguish better the different objects presented on the second monitor. The average background gray level of the original image before contrast stretching was equal to  $110 \pm 4$ .

### Identification of eggs

The operator specified the following parameters to the system: number of eggs, test duration, threshold gray level, minimal projected surface area of an object to be recognized as an egg, length of the border used to surround the eggs, and minimal difference in gray level between the larva and the background used to recognize an object as larva. Next, the computer started to determine the position of each egg. Threshold gray level used to identify an egg was fixed to 150 after the contrast stretching. This threshold value corresponded to the gray level value located half-way between the background gray level and the minimum gray level of the egg images. The system first identified all objects which had a gray level below this threshold level, traced the contour, and calculated the projected surface area of each object by using a method developed by Vigneault et al. (1992). Objects which had a projected surface

area larger than 3 pixels were then considered as eggs by the system and displayed on the second monitor. Finally, the computer located the four extremities ( $X_{min}$ ,  $X_{max}$ ,  $Y_{min}$ , and  $Y_{max}$ ) of each egg contour. A border was added to these four extremities to create a rectangular zone around each egg (Fig. 2). When the head of an insect entered into an egg zone, the larva was considered in contact with the egg. Depending on the size of the larval body, different borders were used. A border of 7 pixels was added to each extremity of the II instar larva whereas a border of 10 pixels was used for the IV instar larva during preliminary tests (unpublished data). The lengths of these borders in which an insect was considered to be in contact with an egg were determined. All eggs displayed on the second monitor were replaced by small rectangles. The operator could verify the positions of each egg and correct any existing error. The 36 egg zones were then numbered by the computer. A value of 0 was allocated to empty areas between the eggs and values of 99 were used to define the exterior zone of the square eggs matrix (Fig. 2). The limits of each of the 38 zones were memorized by the system. Egg contacts made by a larva were counted by comparing the larval head position and the boundaries of the egg zones.

80 x 80 mm square fence  
surrounding the egg matrix

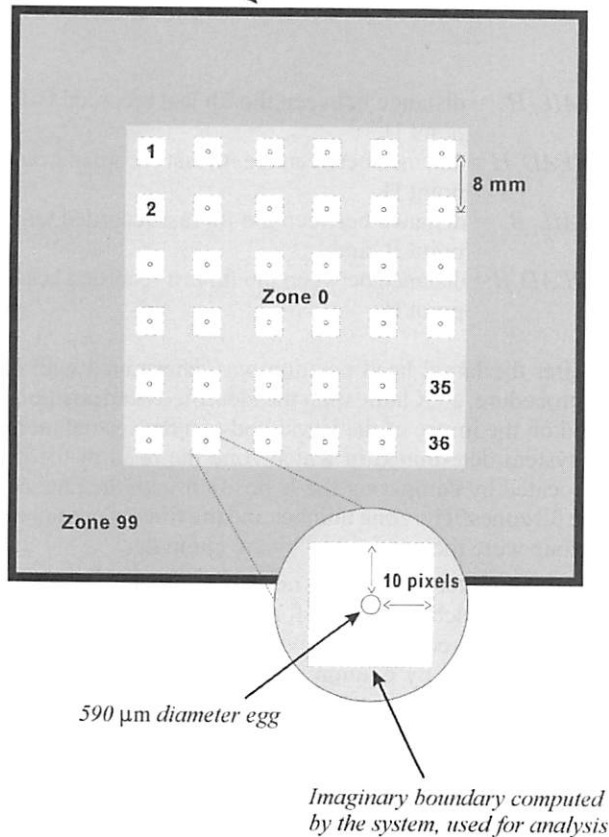


Fig. 2. Arrangement of the egg matrix and zones in the study of the IV instar larvae's behavior.

### Image subtraction

Before placing the larva into the camera's field of vision and initiating actual insect tracking, a square fence was placed and centered around the matrix of eggs to restrain the insect to the interior of the field (Fig. 2). Square fences of 60 x 60 mm and 80 x 80 mm were used for the II and IV instars larvae, respectively.

A reference image was then scanned by the system. Subsequent digitized images were subtracted pixel by pixel from this reference image (Vigneault et al. 1997b). Only subtracted images were used for insect tracking. This subtraction procedure allowed the system to discriminate the insect from the eggs and the fence. After image subtraction, everything shown on the second monitor, including the matrix of eggs and the fence, became black. Bright points were presented only if a great difference in gray level existed between pixels of the reference image and the current image. Consequently, when a larva was introduced into the field, a brilliant object corresponding to the position of the insect appeared on the second monitor. Image subtraction proceeded at a rate of 30 images/s. However, the computer could analyze only six subtracted images/s in real time. This limitation was due to the calculation time required to identify and verify the larval head position.

### Insect tracking

When a larva was released inside the square fence, the system's chronometer started running. The larval position was established by scanning from left to right at each 5 rows on full screen. The computer stopped tracking once it encountered a pixel with a gray level  $\geq 140$ ; this gray level was approximately half way between the screen background gray level and the maximum gray level of the pixels forming an insect. The technique of Vigneault et al. (1992) was used to trace the contour of this object and to calculate its perimeter. If the object had a perimeter  $\geq 5$  pixels, it was considered to be an insect and all the pixels which delimited the contour (contour pixels) of this insect were then memorized by the system.

A visit was counted only when the head of a larva entered in one of the 36 egg zones, because the projected body surface areas of the II and IV instars larvae were approximately 20 and 35 times larger than the cabbage looper egg. Localization of the larval head therefore became essential for this study. The insect head had to be located at one of the extremities of the larval body. However, the presence of legs increased the number of body extremities from 2 to 8 depending on how the legs were positioned.

Three steps were involved in the localization of the larval head (Fig. 3). First, after the presence of the insect was detected and all its contour pixels were traced by the system, the computer calculated the sum of all the distances between one particular contour pixel and the other contour pixels. This calculation was done for each contour pixel. Since the beetle larval tail is narrower than the head, the contour pixel which had the largest sum of distances generally corresponded to the insect's tail position. When the larval tail was located, the computer identified the contour pixel which was located at least 10 pixels ( $\approx 1/2$  length of the larval body) away from the larval tail and had the largest sum of distances. This pixel was considered to be the larval head by the system.



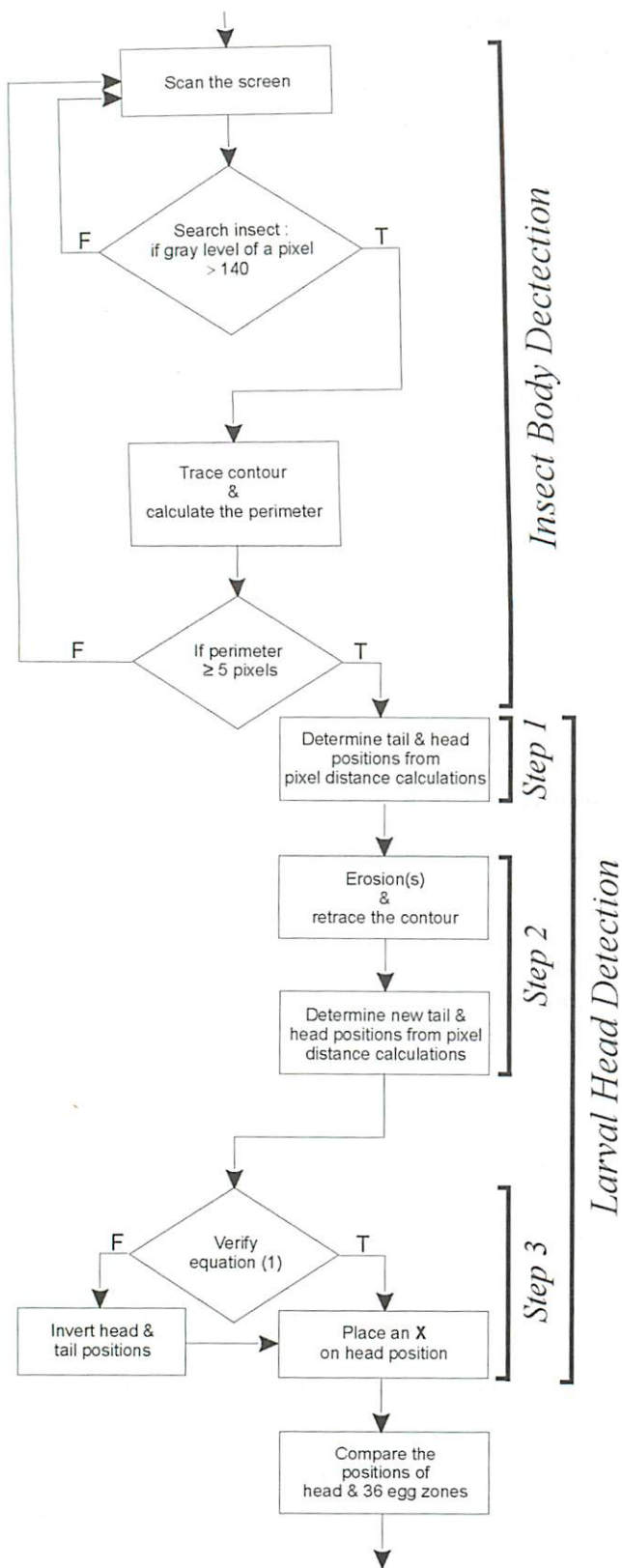


Fig. 3. Basic steps involved in the insect tracking process.

The second step involved a reduction in the number of extremities, by elimination of insect legs on the larval image using an erosion technique. This technique consisted of erasing the original insect contour by giving a gray level value of zero to all the contour pixels and retracing another new contour around the insect. This erosion technique was developed specifically for this application and is much more efficient to erase larvae legs than the erosion technique described by Jain (1989), which slowly attenuates the gray contrast of an object contour. One and two erosions were required for the II and IV instar larvae, respectively, because of the difference in body size. An example of the different shapes of contours before and after two erosions are shown in Fig. 4, using an IV instar larva. After one or two erosions, a new insect contour was then traced. New larval tail and head positions were also identified using the same sum of distance technique.

The third step consisted of comparing the head and tail positions identified in step two with the last five recorded larval head and tail positions. This procedure was used to eliminate the error of inversion between the larval head and tail. If the insect head and tail identified by the system were located at points H and B, respectively, the larval head was actually located at H only if Eq. 1 was valid or the larval head was located at B.

$$\sum_{i=1}^5 (TAIL_i H - HEAD_i H) \geq \sum_{i=1}^5 (TAIL_i B - HEAD_i B) \quad (1)$$

where:

$TAIL_i H$  = distance between the  $i$ th last recorded tail and point H,

$HEAD_i H$  = distance between the  $i$ th last recorded head and point H,

$TAIL_i B$  = distance between the  $i$ th last recorded tail and point B, and

$HEAD_i B$  = distance between the  $i$ th last recorded head and point B.

After the larval head position was determined with the 3 step procedure, an X indicating the identified head position was placed on the image of the larval body on the second monitor. The system determined in which zone the head of the insect was located by comparing the X position with the boundaries of the 38 zones. The zone number and the time of entrance into that zone were then registered by the computer.

The computer retrieved a new image from the digitizing board, from which it searched for the insect in a square of 60 x 60 pixels centered on the previous larval head position. By scanning column by column at every 5 rows in this smaller research area, the computer could relocate the insect faster. In cases where the insect body was not found in this square, the system retrieved another new image and proceeded with the same scanning inside the new square. After two new images, if the insect body position was still unknown, the system retrieved a third new image and searched for the insect on the full screen. Once the insect body was found, the computer retraced the insect body contour, re-determined the head larval position, and relocated the insect among the 38 zones.

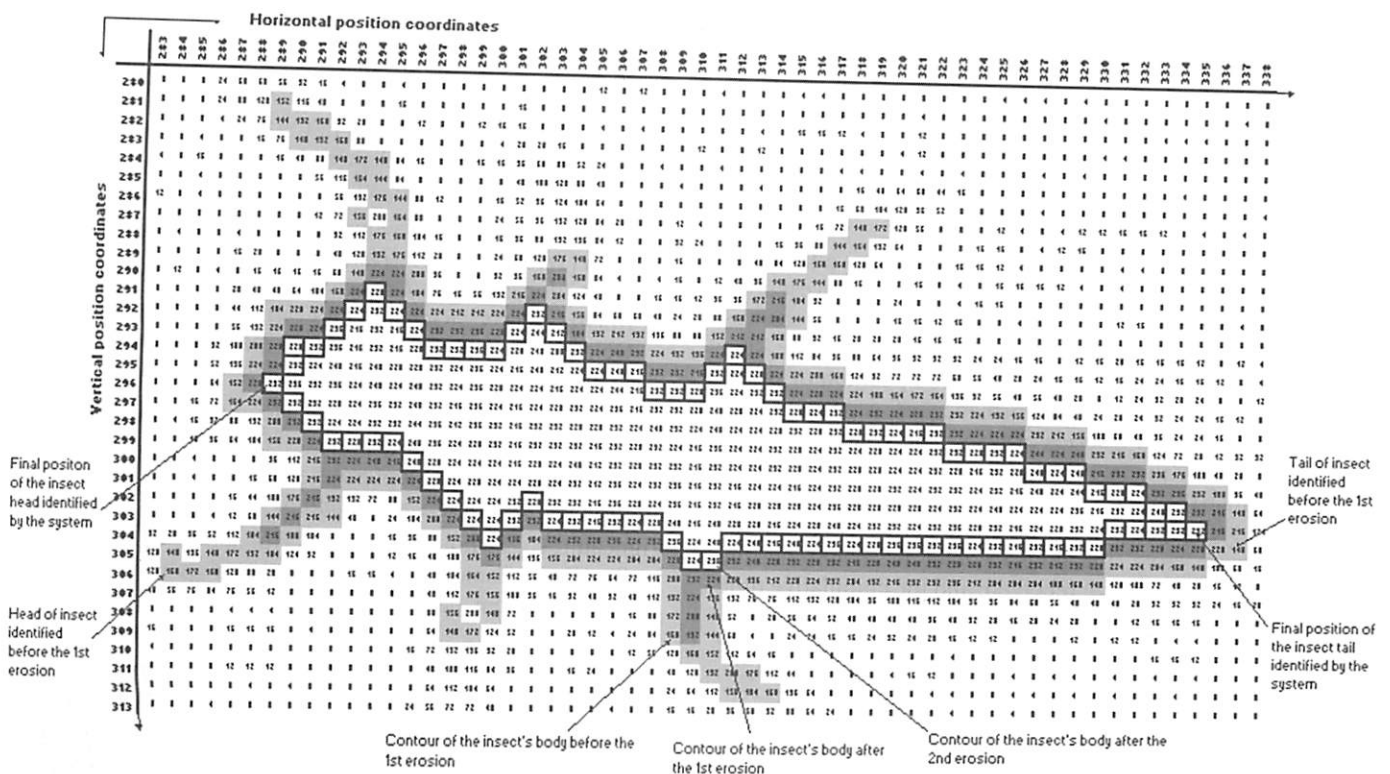


Fig. 4. Erosion technique used in digitized image of a IV beetle instar larva retrieved from a video cassette.

By comparing the previous zone numbers where the insect was located, the system determined if the insect had changed its location. If the two zone numbers were different, the new zone number where the insect head was located and the time of entrance in that zone were recorded. A sonic signal was also sent by the system to notify the operator that a change of zone had occurred. During the test, a list indicating the order of zone visits and the time of entrances in different zones were given by the computer.

At the end of each test, the computer calculated the number of contacts each egg received and the cumulative time of contacts for each egg. A final report containing the sequence of zone visits, the time of entrance into each zone, the total number of egg contacts and the cumulative contact time was presented to the operator. Several options were also added to the program, which allowed the operator to choose another new reference image or to terminate the analysis at any moment during the test.

#### Evaluation of system performance

A series of tests was performed during the development of the system to verify the performance of each of the three steps involved in the larval head localization procedure. For each step of the procedure, a total of 10,000 images (1000 images/insect x 10 insects) were retrieved by the system to quantify its percentage of error. During each test, the processed image containing the larval body was displayed on the second monitor. An X representing the larval head position determined by the computer was shown on the same monitor simultaneously. An operator compared the position of the X visually with the real larval head position and counted the

number of errors. The system stopped automatically after every 1000 images in this series of tests.

### RESULTS and DISCUSSION

In the first step of the larval head localization procedure, the system was able to position approximately 90% of the larval heads accurately. Two types of errors were observed. Most often, the system assigned another insect body extremity as the larval head, which was generally due to the presence of a leg located near the larval head. In other cases, the positions of the head and tail were inverted by the system.

In the second step, utilization of the erosion technique reduced significantly the number of errors due to the presence of legs. Fewer than 13 errors per 1000 images were found and the head was correctly identified in 98.7% of the occasions. Inversion of larval head and tail was the most common error observed, due to the fact that the shape of the insect body changed in some of its displacements. This gave the system an impression that the larval head was narrower than the tail and hence errors occurred.

The verification process of step three increased the rate of success to greater than 99.9%. Errors occurred solely when the insect moved very rapidly in circles and the identified head position came across the recorded tail positions in less than 5/6 of a second. The error was automatically corrected on the following analyzed image, which was 1/6 of a second later. This means that any egg contact done during this type of displacement would be considered as lasting only 1/6 of a second. Omission of such a short egg visit did not affect the



results obtained in the insect behavior analyses, since only egg visits which lasted more than 3 s were considered (unpublished data). Thus, an error of less than 0.1% was considered negligible. Results obtained at the end of step three were considered more than satisfactory.

### CONCLUSION

A method based on an image analysis system was adapted to automate observations required in a study of the twelvespotted ladybird beetle's behavior. The objectivity of observations was increased by eliminating human intervention. A three step procedure was developed to detect the head position of insect on its larval body and the performance of each step was evaluated separately by a series of tests. The system was able to locate accurately the larval head with an acceptable rate of error smaller than 0.1%. Several parameters were automatically calculated and recorded by the system: the order of zone visits done by a beetle larva, the time to enter in each zone, the number of contacts each egg received, and the cumulative time of contact for each egg, based on the positions of the larval head and the egg zones. This system successfully assisted in the evaluation of *Coleomegilla maculata* larvae's behavior.

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