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# KOMUNIKASI SINGKAT

# Preservation technique to identify *Bactrocera dorsalis* complex (Diptera: Tephritidae) based on image analysis

## Teknik pengawetan untuk identifikasi *Bactrocera dorsalis* kompleks (Diptera: Tephritidae) berdasarkan analisis citra

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**ABSTRACT**

Fruit flies (*Bactrocera dorsalis* (Hendel)) are insect pests of many fruits and vegetables. Yield losses due to this pest can reach 100%, and many may be unaware that fruit flies are the trigger for several disease attacks on crops such as fungi and bacteria. This study aimed to identify the most appropriate preservation technique for morphological identification of *B. dorsalis* by image analysis. Images were taken with the Nikon DSF12 Trinocular Microscope. The methods used varied by trapping period (short-term and long-term) and types of preservatives (ethanol and propylene glycol). Specimens were obtained from Bandung and Sumedang Regency. Results demonstrated that ethanol-based preservation was the most appropriate to acquire the abdominal image of *B. dorsalis* obtained via short-term trapping, meanwhile a propylene glycol-based preservation was suggested for specimens trapped using longer-term methods.

**Key word:** fruit flies, identification, preservation technique

**ABSTRAK**

Lalat buah (*Bactrocera dorsalis* (Hendel)) merupakan serangga hama pada banyak buah dan sayuran. Kehilangan hasil akibat hama ini bisa mencapai 100%. Tidak banyak yang tahu bahwa lalat buah merupakan pemicu beberapa serangan penyakit pada tanaman, seperti cendawan dan bakteri. Penelitian ini bertujuan untuk mengidentifikasi teknik pengawetan yang paling tepat untuk identifikasi morfologi *B. dorsalis* melalui analisis citra. Gambar diambil dengan Mikroskop Trinocular Nikon DSF12. Metode yang digunakan didasarkan pada periode penangkapan (jangka pendek dan jangka panjang) dan jenis pengawet (etanol dan propilen glikol). Spesimen diperoleh dari Kabupaten Bandung dan Sumedang. Hasil penelitian menunjukkan bahwa pengawetan berbasis etanol adalah yang paling tepat untuk memperoleh citra perut *B. dorsalis* yang diperoleh dalam perangkap jangka pendek, sedangkan pengawetan berbasis propilen glikol disarankan untuk spesimen jangka panjang.

**Kata kunci:** identifikasi, lalat buah, teknik pengawetan

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## INTRODUCTION

Insects play an important role in most ecosystems, often as pests, where they represent the main cause of yield losses in crop productivity (Dalal & Singh 2017). Fruit flies (*Bactrocera dorsalis* complex) (Diptera: Tephritidae) are one of the most common and destructive pests of many economically important fruits and vegetables.

In order to control fruit flies and prevent damage to crops, an effective method is needed to detect, identify and monitor them. Taxonomic identification is a crucial step, followed by study and understanding of the insect's role in the environment, in order to implement the most effective control method. However, such identification first requires determining the best and most appropriate techniques for collecting and preserving fruit fly specimens.

One simple and common fruit fly trap is baited with the pheromone methyl eugenol (ME). This method is a targeted and very effective way to collect and also control insect populations (Epsky et al. 2008). Only male fruit flies are attracted to ME, and continuous and ongoing trapping using ME will eventually decrease the male fruit fly population, reducing mating success.

Preservation methods are used to prevent collected specimens from decaying, also necessary in order to undertake taxonomic identification. Chemical preservatives must be able to keep specimens intact and undamaged, over the needed duration. Ethanol, formalin, ethylene, and propylene glycol are commonly used for preservation (Braun et al. 2009).

The success of specimen collection protocols depends on both the number of fruit flies trapped, and their preservation for identification. This makes it possible to develop and use automatic identification methods based on image processing (Faria et al. 2014) the results of which can inform and improve pest management strategies (Epsky et al. 2008). Conversely, use of inappropriate trapping or preservation techniques can result in faulty analyses and adoption of inappropriate and ineffective pest management strategies. Our objective in this study was to identify the most appropriate preservation method to allow for morphological identification of *B. dorsalis* by image analysis.

## METHODOLOGY

Samples were collected from agricultural areas at Bandung Regency and Sumedang Regency during February to August 2019. The experiment of preservation was done at the Entomology Laboratory, School of Life Sciences and Technology, Institut Teknologi Bandung (ITB). Adult males of *B. dorsalis* were collected using traps baited with methyl eugenol. Each trap consisted of a transparent water bottle (perforated at two sides) containing a cotton ball infused with 0.25 ml of methyl eugenol hanging from a string. These traps were set in the middle of a cultivated field of chili plants, approx 30 cm above the soil surface. Trapping was carried out from 06.00 to 11.00 AM, then all fruit flies caught were put into a sample tube. To assess whether differences in trapping period and types of fixatives for specimen preservation affected the success of image analysis, the experiment included two treatments with each replicated 4 times. To accurately understand the effect of treatment options on image analysis, two different trapping methods were used: hanging traps for 24 hours (one-day collection); and hanging traps for 7 days with collection occurring only on the seventh-day. In addition, specimens collected were subject to different treatments were in the lab:

Treatment A, short-term collection: traps set out for 1 day

A1: 10 insects were put into 10 ml of 70% (v/v) ethanol for 1 day, then steamed using hot water;

A2: 10 insects were put into 10 ml propylene glycol for 1 day, then steamed using hot water;

A3: 10 insects were put into 10 ml propylene glycol for 1 day, followed by 10 ml 70% (v/v) ethanol for 5 minutes, then steamed using hot water;

A4: 10 insects were put into 10 ml propylene glycol for 1 day, steamed using hot water, then put into 10 ml 70% (v/v) ethanol for 5 minutes.

Treatment B, long-term collection: traps set out for 7 days

B1: 10 insects were put into 10 ml of 70% (v/v) ethanol for 1 day, then steamed using hot water;

B2: 10 insects were put into 10 ml propylene glycol for 1 day, then steamed using hot water;

B3: 10 insects were put into 10 ml propylene

glycol for 1 day, followed by 10 ml 70% (v/v) ethanol for 5 minutes, then steamed using hot water;

B4: 10 insects were put into 10 ml propylene glycol for 1 day, steamed using hot water, then put into 10 ml 70% (v/v) ethanol for 5 minutes.

All treatments were repeated four times and took place at room temperatures between 27–28 °C, and hot water temperatures at 70–80 °C. To reduce bias, observations and measurements were performed by two people independently. Images of treated specimens were taken under the trinocular microscope using Nikon ds-fi2, Nikon smz745T.

## RESULTS AND DISCUSSION

Ethanol is the most universally used preservative for insects, included in various methods. Aside from keeping specimens well-preserved, ethanol is less costly that other chemical preservatives (Schauff 2001; King & Porter 2004). Although widely used, it is not currently the standard preservative used to prepare specimens for image analysis. However, ethanol in general and in this study has been shown to be not suitable for preserving specimens obtained using long-term collection protocols (Treatment B) as it produces several undesirable effects, such as decolorization of specimen tissues, over time. Conversely, propylene glycol is the most common preservative for specimens caught in long-term trapping (7–14 days) and does not have the undesirable effects found with ethanol. Propylene glycol is also effective in preserving specimens for molecular purposes, DNA extraction, and PCR amplification (Vink et al. 2005).

Specimens obtained during short-term trapping are generally in good condition (Figure 1A–1D, Figure 2A–2D). The bodies are fresh and not fragile when handled. On the other hand, some specimens collected via long-term trapping were ruptured (white intersegmental gaps present) on the tergite (Figure 1E–1H) due to DNAse activity, microorganisms, and autolysis. Autolysis is the breakdown of body tissue by the lysis enzyme (inside the lysosome organelle in the cells making up the insect's body tissue). (King & Porter 2004). Fixation of specimens in preservatives enables the cell contents to remain intact and prevent autolysis.

One-day old specimens in our experiment reacted differently when treated with ethanol vs propylene glycol. Specimens preserved in propylene glycol were more rigid than specimens preserved in ethanol (Table 1). Ethanol is an oil-based preservative that can force cell tissue to release its water content (thereby causing tissue to shrink and lose shape), while glycol is an excellent water absorber, belonging to the hydroxyl group so that glycol form bonds similarly to water molecules (allowing tissues to maintain their shape) (Thomas 2008). Nonetheless, specimens that have been immersed in ethanol tend to return to their original shape once they are removed from the ethanol.

Different preservative agents alter specimen shape and tissue qualities in ways that impact the quality of images taken by a trinocular microscope. Specimens we collected that were preserved only in ethanol (treatment A1 and B1) tended to produce a clear image without light reflection (Figures 1A and 1E). Similarly, clear images without light reflection were also usually obtained from specimens subjected to treatments A4 and B4 -which combined ethanol with propylene glycol treatments, clear images were also usually obtained without light reflection-- (Figures 1D and 1H). This is likely due to the fact that when an insect is immersed in ethanol its tissue becomes saturated (King & Porter 2004), but due to the volatility of ethanol any residue on the specimen is quickly vaporized and therefore does not reflect the light, resulting in a clearer image. Conversely, in treatments A2, A3, B2, and B3 (insects preserved with propylene glycol), the images are not as clear due to light reflection (Figures 1A, 1C, 1F, and 1G). Propylene glycol is commonly used as a humectant or moisturizer, a solvent and a preservative, reducing evaporation to maintain moisture. Therefore unlike ethanol, a residue of the preservative fluid remains on the specimen, reflecting light, and interfering with the image-taking process. Use of propylene glycol as a preservative can reduce the quality of images due to light reflection off of the preservative residue, whereas ethanol tends not to have this effect. In addition to this, propylene glycol has other non-desirable qualities as well: it takes longer to dry specimens preserved in propylene glycol solution;

**Table 1.** Image analysis of *Bactrocera dorsalis* preserved in different treatment

| Treatment | | Image | Location | Details |
|---|---|---|---|---|
| Treatment A: Short-term collections | Insects were put into of 70% (v/v) ethanol for 1 day, then steamed using hot water. |  | Sumedang | - Insect in good condition with no wrinkles or shrinkage |
| | Insects were put into propylene glycol for 1 day, then steamed using hot water. |  | Sumedang | - Insect in good condition wrinkled/ shrunken abdomen.<br>- Light reflection present in the image. |
| | Insects were put into propylene glycol for 1 day, followed 70% (v/v) ethanol for 5 minutes, then steamed using hot water. |  | Sumedang | - Insect in good condition wrinkled/ shrunken abdomen.<br>- Light reflection present in the image. |
| | Insects were put into propylene glycol for 1 day, steamed using hot water, then put into 70% (v/v) ethanol for 5 minutes. |  | Bandung | - Insect in good condition with no wrinkles or shrinkage. |
| Treatment B: Long-term collections | Insects were put into of 70% (v/v) ethanol for 1 day, then steamed using hot water. |  | Sumedang | - Ruptured tergite.<br>- No wrinkles or shrinkage light reflection present in the image. |
| | Insects were put into propylene glycol for 1 day, then steamed using hot water. |  | Sumedang | - Ruptured tergite.<br>- Wrinkled/shrunken abdomen.<br>- Light reflection present in the image. |
| | Insects were put into propylene glycol for 1 day, followed by 70% (v/v) ethanol for 5 minutes, then steamed using hot water. |  | Sumedang | - Ruptured tergite.<br>- Wrinkled/shrunken abdomen. |
| | Insects were put into propylene glycol for 1 day, steamed using hot water, then put into 70% (v/v) ethanol for 5 minutes. |  | Bandung | - Ruptured tergite.<br>- No wrinkles or shrinkage. |

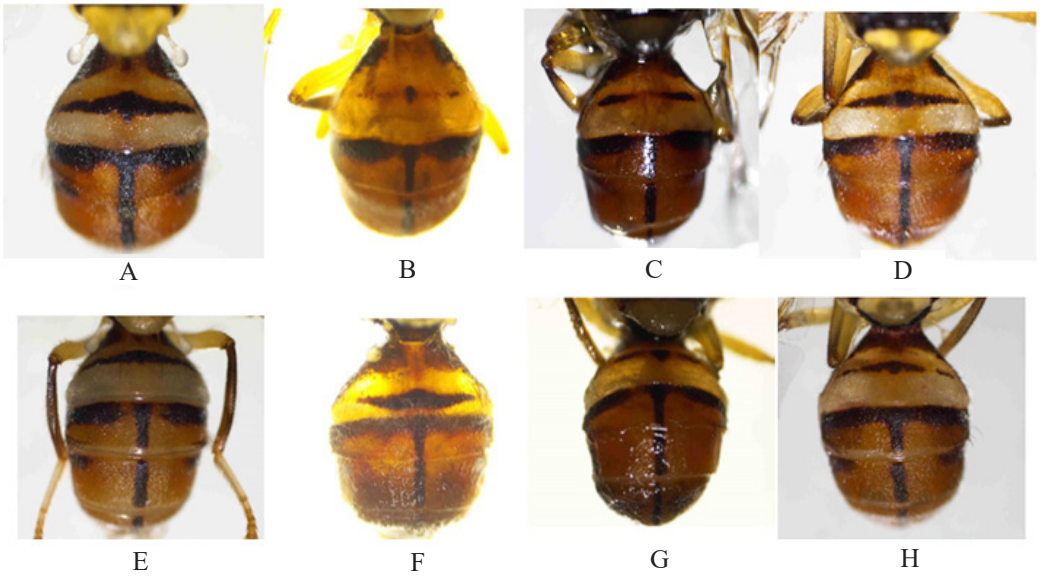


**Figure 1.** Image of an abdominal segment of *Bactrocera dorsalis* subjected to different treatments. A–D: short-term collections (A: ethanol; B: propylene glycol; C: propylene glycol, ethanol, hot water; D: propylene glycol, hot water, ethanol) and E–F: long-term collection (A: ethanol; B: propylene glycol; C: propylene glycol, ethanol, hot water; D: propylene glycol, hot water, ethanol).

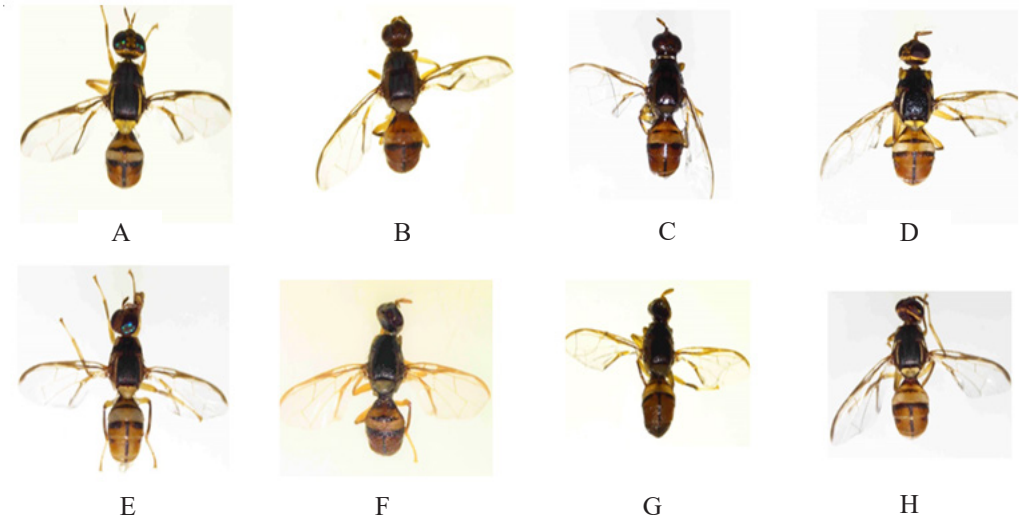


**Figure 2.** Image of *Bactrocera dorsalis* body preserved in different treatment. A–D: short-term collections (A: ethanol; B: propylene glycol; C: propylene glycol, ethanol, hot water; D: propylene glycol, hot water, ethanol) and E–F: long-term collection (A: ethanol; B: propylene glycol; C: propylene glycol, ethanol, hot water; D: propylene glycol, hot water, ethanol).

and the solution causes the body to wrinkled the abdomen to shrink.

For the reasons above, we recommend adopting a short-term trapping system and treating specimens with ethanol before imaging. The use of ethanol as a preservative agent for short-term specimens will prevent light reflection which can hinder image analysis. Nonetheless, long-term trapping methods are still a viable alternative. For specimens obtained using long-term collection methods, preservation with propylene glycol is more appropriate because propylene glycol is not easily volatilized and can maintain specimen color better than ethanol. Regardless of which trapping and preservation method is used, however, several other measures are still required for specimens prior to imaging, including steam exposure and short-term treatment with 70% ethanol.

In conclusion, a short-term trapping method is preferable for insect specimens intended for study and image analysis, and such specimens should be preserved in 70% ethanol before taking images. On the other hand, preservation using propylene glycol is considered best for older specimens collected via a long-term trapping method. Regardless, all specimens should be immersed in ethanol in advance of imaging to prevent light reflection.

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