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Non-Destructive Quarantine Technique- Potential Application of Using X-ray Images to Detect Early Infestations Caused by Oriental Fruit Fly (*Bactrocera dorsalis*) (Diptera: Tephritidae) in Fruit 【Research report】

非破壞性檢疫技術—X光影像偵測東方果實蠅於水果早期危害之應用【研究報告】

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Abstract

In this study, we tested the possibility of using X-rays to examine internal injuries of various fruit. Fruit containing high amounts of water have been deemed unsuitable for X-ray imaging. However, with the aids of digitized X-ray imaging analysis, we are now able to examine internal injuries that produce differences from the homogeneity of normal fruit. Various kinds of fruit were implanted with the eggs of the Oriental fruit fly, *Bactrocera dorsalis*, and, the X-ray images of the fruit were examined after various time periods. The digitalized X-ray images showed that this technique can detect injuries caused by *B. dorsalis* at as early as 3 days after implantation of eggs in some fruits. Our results demonstrate that the current technique is a useful tool for the non-destructive inspection of internal injuries of fruit, something which cannot be determined solely with the naked eye.

摘要

本研究利用X光透視影像檢視水果的內部情形，以測試其應用在偵測檢疫害蟲的可行性。由於水果內部具有高水分含量，通常並不適用於X光檢測，然而藉助數位化影像分析，受害水果的內部危害情形可以與正常水果的均質情形有所區辨。我們以東方果實蠅 (*Bactrocera dorsalis*) 的卵植入多種不同類別的水果，於植卵後不同天數檢視水果內部的X光影像，並進行影像分析。水果受到蟲害亦可以與正常部位區辨，有些類別的水果在果實蠅孵化後三天即可偵測出受害情形。此項研究結果顯示X光透視影像應用於偵測檢疫害蟲具有可行性，且此種非破壞性技術可以有效偵測水果內部的受害情形，達到目視檢測無法達成的效果。

Key words: X-ray, non-destructive inspection, image analysis, fruit, Oriental fruit fly

關鍵詞: X光、非破壞性檢疫、影像分析、水果、東方果實蠅

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Non-Destructive Quarantine Technique- Potential Application of Using X-ray Images to Detect Early Infestations Caused by Oriental Fruit Fly (*Bactrocera dorsalis*) (Diptera: Tephritidae) in Fruit

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ABSTRACT

In this study, we tested the possibility of using X-rays to examine internal injuries of various fruit. Fruit containing high amounts of water have been deemed unsuitable for X-ray imaging. However, with the aids of digitized X-ray imaging analysis, we are now able to examine internal injuries that produce differences from the homogeneity of normal fruit. Various kinds of fruit were implanted with the eggs of the Oriental fruit fly, *Bactrocera dorsalis*, and, the X-ray images of the fruit were examined after various time periods. The digitalized X-ray images showed that this technique can detect injuries caused by *B. dorsalis* at as early as 3 days after implantation of eggs in some fruits. Our results demonstrate that the current technique is an useful tool for the non-destructive inspection of internal injuries of fruit, something which cannot be determined solely with the naked eye.

Key words: X-ray, non-destructive inspection, image analysis, fruit, Oriental fruit fly

Introduction

The World Trade Organization (WTO) has enhanced international business, while it has also reduced trading boundaries

among countries. In addition, the potential risk of importing pests has increased as well. Quarantine is the last defense against an unwanted imported disaster. The importance of developing non-destructive

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inspection techniques for imported agricultural products cannot be over emphasized. Tiny pests in fruit, some of the major agricultural products traded, make inspection very difficult, especially since most of the pests are larvae and normally hide themselves inside the fruit. Nowadays, quarantine inspection still mainly relies on traditional visual examinations, i.e. randomly sampling a preset ratio of fruit and then checking any surface injury by further dissection. The heavy load of inspections and the limited duration that fresh fruit is quarantined restricts the quality of the inspection and forfeits the chance of an accurate and effective quarantine program.

It is well known that X-rays can penetrate most materials. In addition to applications in industry and for medical examinations, X-rays have long been used in airport security systems by customs agents to check passengers' luggage. Although Yuasa (1926) indicated the importance of the application of X-rays in plant quarantine, instances of using X-rays for plant quarantine have seldom been reported. The poor penetration of X-rays in materials with high water content may have been the reason that its application in plant quarantine was limited. Hence, previous studies of applying X-rays to agricultural products mainly focused on X-ray irradiation quarantine treatments (Follett and Lower, 2000; Follett and Sanxter, 2002, 2003; Follett and Armstrong, 2004) and on dry or lower water-containing materials, e.g., checking seed quality with soft X-ray radiography (Singh and Benerjee, 1968; Benerjee, 1971; Singh, 1975; Lammertyn *et al.*, 2003) and for detecting hidden infestation of crop plants (Fesus, 1972; Wadhi, 1983; Chavagnat, 1985; Kim and Schatzki, 2001; Karunakaran *et al.*, 2003). Pioneering work by Reyes *et al.* (2000) enabled the use of X-ray images to diagnose the mango seed weevil in intact mango fruit by evaluating distinct features

of weevil-infested mango seeds.

Nevertheless, X-ray has not been proven to be a feasible technique for detecting pest injury in the soft parts of fruit. In the present study, we took X-ray images of different fleshy simple fruits, most of which are commonly traded fruit. Fruit with various internal structures were used to qualitatively evaluate the potential of using X-rays, to reveal internal injuries caused by pests or physical impingement. X-ray images taken from fruit infested by the Oriental fruit fly were digitized for further processing in order to investigate the distinct features of injuries caused by the pests in the soft parts of the fruit.

Materials and Methods

Fruit

Various kinds of fleshy fruit were used for the experiments covering the major types of simple fruits, including berry (tomato, *Lycopersicon esculentum* Mill), hesperidium (orange, *Citrus sinensis* (L.) Osbeck cv. Lir Cheng or *Citrus sinensis* var. *liucheng*), pome (apple, *Malus pumila* Mill; pear, *Pyrus communis* L.), and drupe (peach, *Prunus persica* (L.)). Fruits were purchased from local markets. For each kind of fruit, individual fruit of a similar size were selected for the following experiments.

Simulation of infested fruit

Various species of fruit flies in difference continents have been listed as important quarantine pests, and detection of their presence in the imported commodities has become an important task (Wu *et al.*, 2004). The Oriental fruit fly, *Bactrocera dorsalis* (Hendel), is a serious local pest and was selected to simulate the pest infestation of fruit. Flies were supplied by the Insect Physiology and Biochemistry Laboratory of the Department of Entomology, National Chung Hsing University, Taichung, Taiwan.

Larvae of *B. dorsalis* were fed an artificial diet based on that described by Chiu (1978); and adults were maintained on a mixture of sugar: yeast extract: peptone of 3: 1: 1. The rearing environment was maintained at $28 \pm 1^\circ\text{C}$ with a 12-h light and 12-h dark cycle. Flies were sexually mature 10 days after emerging under these incubation conditions. A plastic film container with guava juice inside and about 20 pin-holes in the bottom served as an egg collector. Fresh fly eggs were collected with this collector on the top of the raising cage for 2 h immediately before egg implantation into the fruit.

All pest-infested fruit were imitated by implanting fresh Oriental fruit fly eggs under the skin of the fruit. Three pieces of fruit of each kind were used for each treatment; two were used to imitate pest-infestation, and the other one was used as a control. Each individual fruit was divided into three areas (basal, middle, and terminal), and each area was divided into four parts. In each part, a pin-hole was created by piercing with a sterilized glass capillary tube, with a diameter of 1 mm, 3 mm down from the surface of the fruit. For the imitation treatment, each of the pin-holes was implanted with one egg only. In total, 12 eggs were implanted in each individual piece of fruit. After implanting the egg, each of the pin-holes was immediately covered with a piece of surgical tape ($5 \times 5\text{-}10$ mm, 1530-0, 3M Co., USA). For the fruit that served as a control, no eggs were implanted into the 12 pin-holes, but the holes were still covered with surgical tape. The fruit were then stored in an incubator at a temperature of $24 \pm 1^\circ\text{C}$. The experimental treatments described above were carried out every day for 21 days. X-ray images of fruit were taken on the 21st day. After making the X-ray images, the fruit were then dissected, and carefully examined to locate the implanted eggs, any hatched larvae, and the injury

they had caused to the fruit.

X-ray photography

All X-ray images of the fruit were taken on a medical X-ray system (Philips Diagnost 97) with digital spot imaging in a clinical X-ray inspection room of the Department of Radiology, China Medical University Hospital. The conditions of photography were adjusted to 40-50 kVp, and 12 mAs, depending on the fruit being examined.

Image digitization and analysis

The X-ray films were digitized using a desktop computer scanner (620ST AcerScan, Acer, Taiwan). Since the brightness of the X-ray image is proportional to the objective density, the intensity of the brightness of the digitized images was analyzed with a MATLAB (vers. 7.0, The MathWorks, Inc., USA) program. Changes in objective density were expressed using density contours and pseudo-colors, and therefore density changes caused by internal injuries of the fruit were detectable.

Results

X-ray photography revealed density changes inside the fruit. The best contrast of images revealing internal injuries caused by larvae was when the voltage of the X-ray source was around 45-50 kVp (with an exposure time of 0.1 s).

Density changes of the X-ray images were expressed by density contours as well as pseudo-colors. Because the eggs of the Oriental fruit fly were implanted just under the skin of the fruit, the X-ray images showed that most of the density changes developed from the outer parts of the fruit images. The earliest day that the internal injury caused by fly larvae could be detected by X-ray images depended on the type of fruit (Table 1). Consequently, the results are interpreted below in which internal injuries could be detected by X-ray according to each type

Table 1. Best conditions of X-ray intensity at a 100- μ s exposure time and the earliest time of detectable injury on X-ray images after implanting eggs of the Oriental fruit fly into various fruit

Fruit type	Intensity (kVp)	Time / earliest infestation was detectable after egg implantation (day)
Pome (apple)	45	7
Pome (pear)	45	3
Drupe (peach)	45	4
Berry (cherry tomato)	40	6
Hesperidium (orange)	50	6

of fruit. In contrast, no cases showed surface injuries which could be detected by the naked eye, until the fruit were dissected and carefully examined.

Pomes: apple and pear

The internal structure of pomes and drupes are not complex, and the internal density of both fruits is evenly distributed except for the area near the core. Therefore, any internal injuries of these fruit could be clearly revealed by X-ray imaging. Figure 1 is an X-ray image of an apple into which Oriental fruit fly eggs had been implanted for 7 days, and this is the earliest time the internal injury of the apple could be detected by either X-ray image or dissection. No surface injury was observed (Fig. 1A). A tunnel created by the fly larva was detected by X-ray imaging (Fig. 1B), and it exactly matched the internal injury found when the apple was subsequently dissected (Fig. 1C). Figure 2 shows two pears which were infested by Oriental fruit fly larvae; one had eggs implanted for 3 days (Fig. 2A, C, E) and the other for 6 days (Fig. 2B, D, F). The X-ray images revealed internal injuries as early as 3 days after egg implantation into the

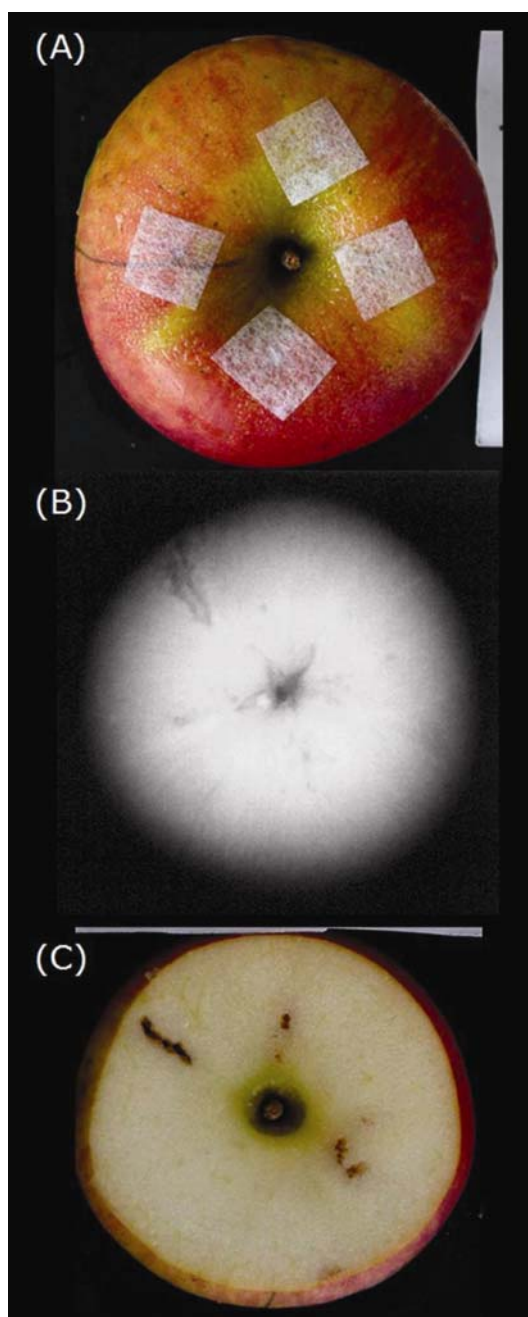


Fig. 1. Seven days after Oriental fruit fly eggs were implanted, an apple showed no outer damage (A) while an X-ray image (B) revealed signs of tunnels which closely matched the internal injuries (C). The four white patches in (A) are surgical tapes used to cover the pin-holes after egg implantation.

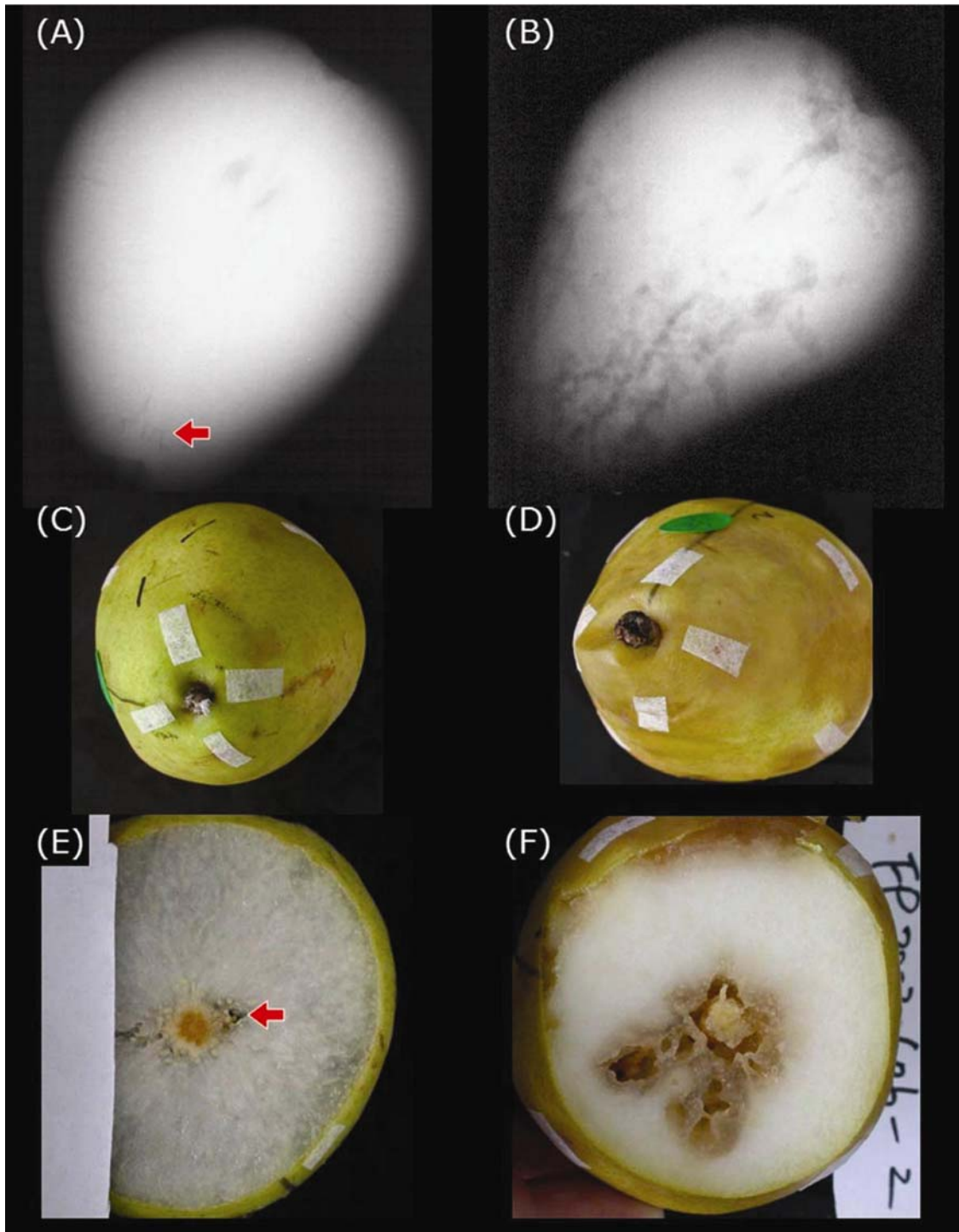


Fig. 2. Images of pears at different times of infestation. Three days after eggs were implanted, X-ray images (A) successfully revealed subtle signs of injury (red arrow) which were not detectable from the surface (C) and which closely matched the internal injuries (E). More-obvious signs of injury shown as tunnels in the X-ray image (B) were found 6 days after egg implantation, and serious internal injuries could be seen (F), while no obvious outer damage was revealed (D).

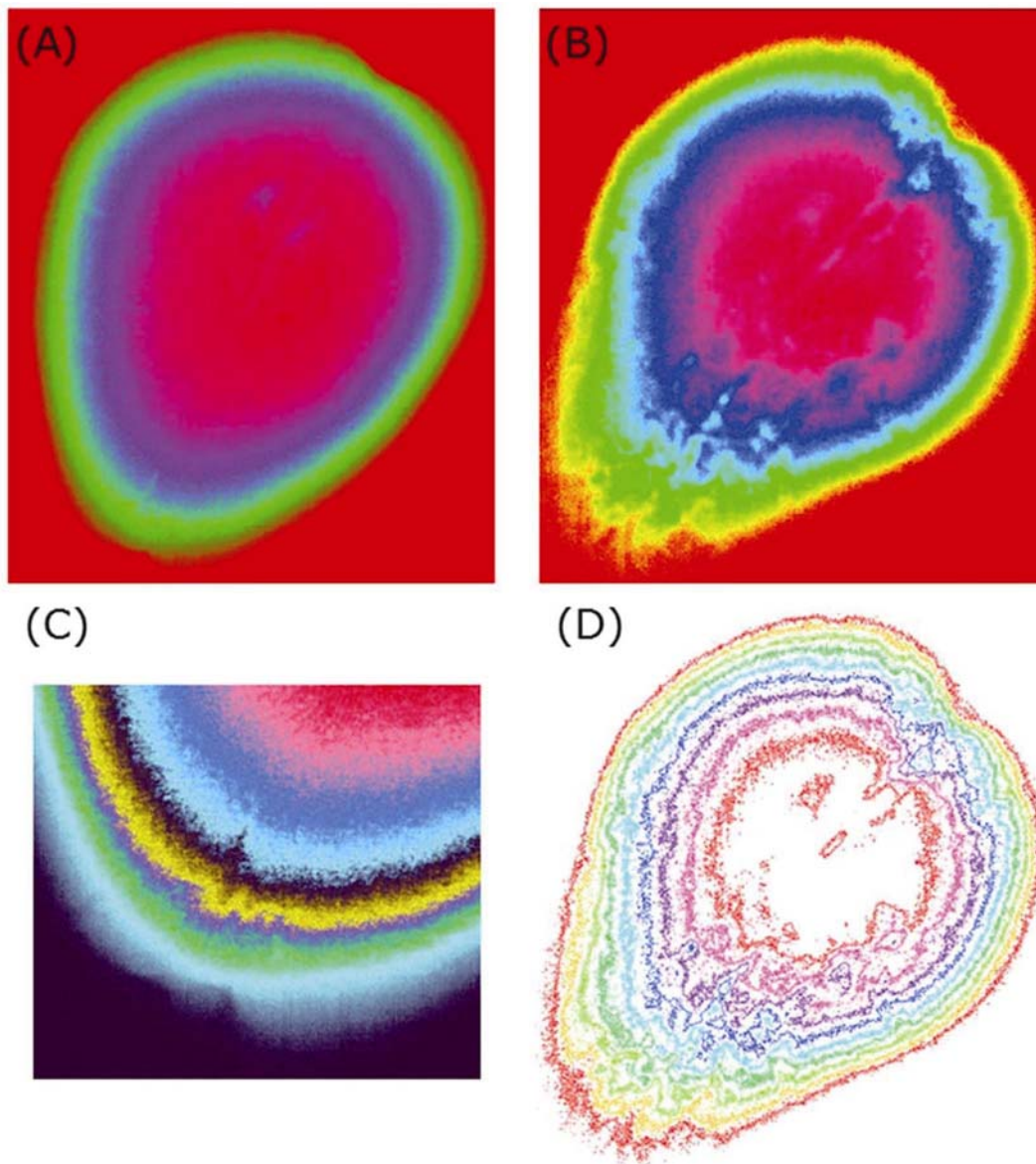


Fig. 3. X-ray images in Fig. 2 after image analysis for density changes. Picture (A) is the image analyzed from Fig. 2A, and (C) showed enlargement of the infested area. Pictures (B) and (D) were derived from Fig. 2B, and density changes are expressed as pseudo-colors and contour lines, respectively. The infested area revealed obvious distortion compared to the smooth contour curves of uninfested area.

pears. The red arrows in Fig. 2A, E indicate slight injury detected by the X-ray image, and the contour analysis of the density changes showed uneven contour lines in this area (Fig. 3A & C) while those of the healthy part were smooth. In

the pear infested for 6 days, the internal injuries were more serious than those of the pear infested for 3 days, and many tunnels were easily detected on the X-ray image (Fig. 2B). The contour lines of the serious internal injuries are not continuous

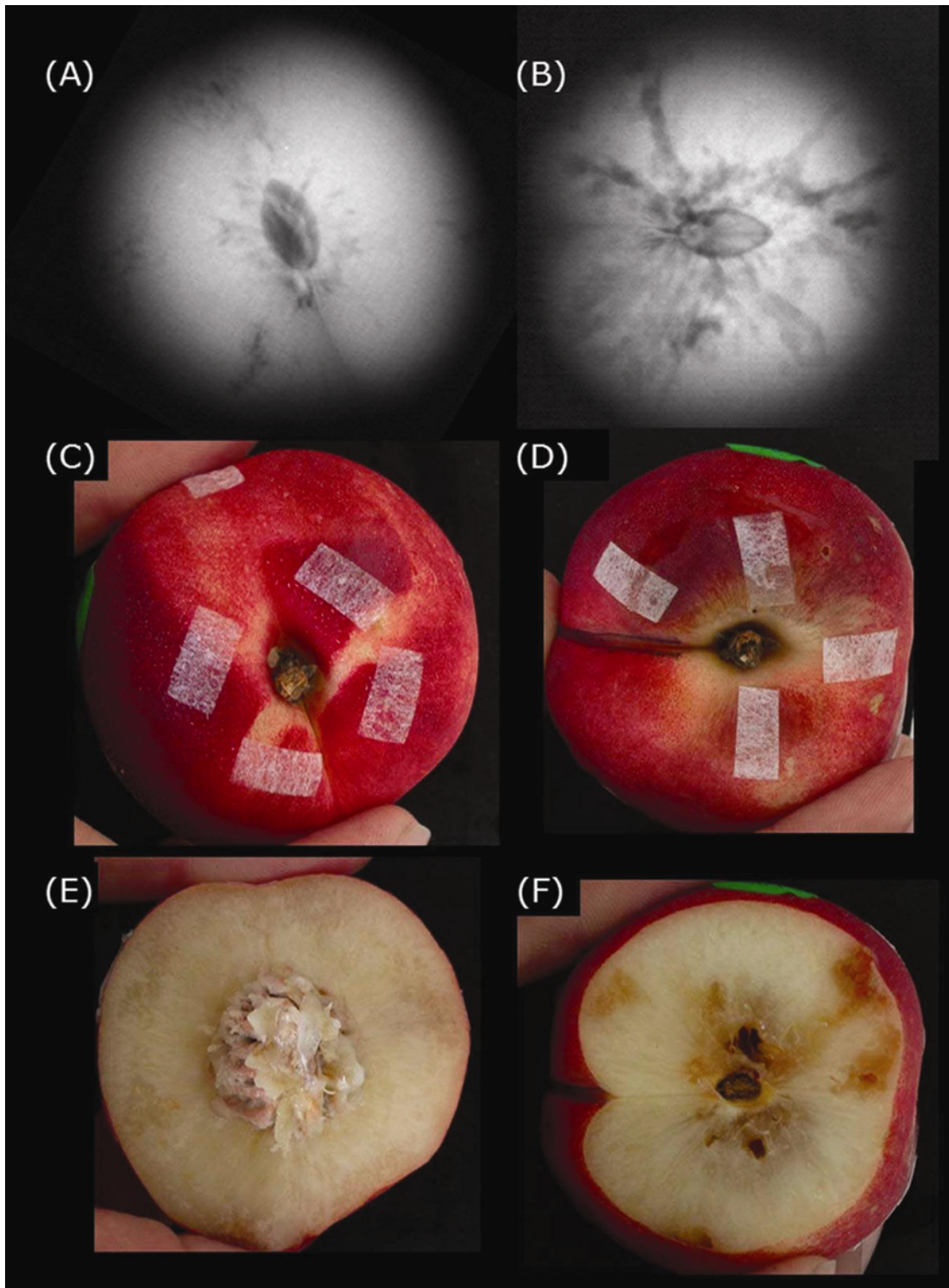


Fig. 4. Photos of peaches 4 (A, C, and E) and 6 (B, D, and F) days after egg implantation. (A) and (B), X-ray images showing signs of injury; (C) and (D), outer surface revealing no abnormalities; (E) and (F), internal injuries revealed after cutting open the peach.

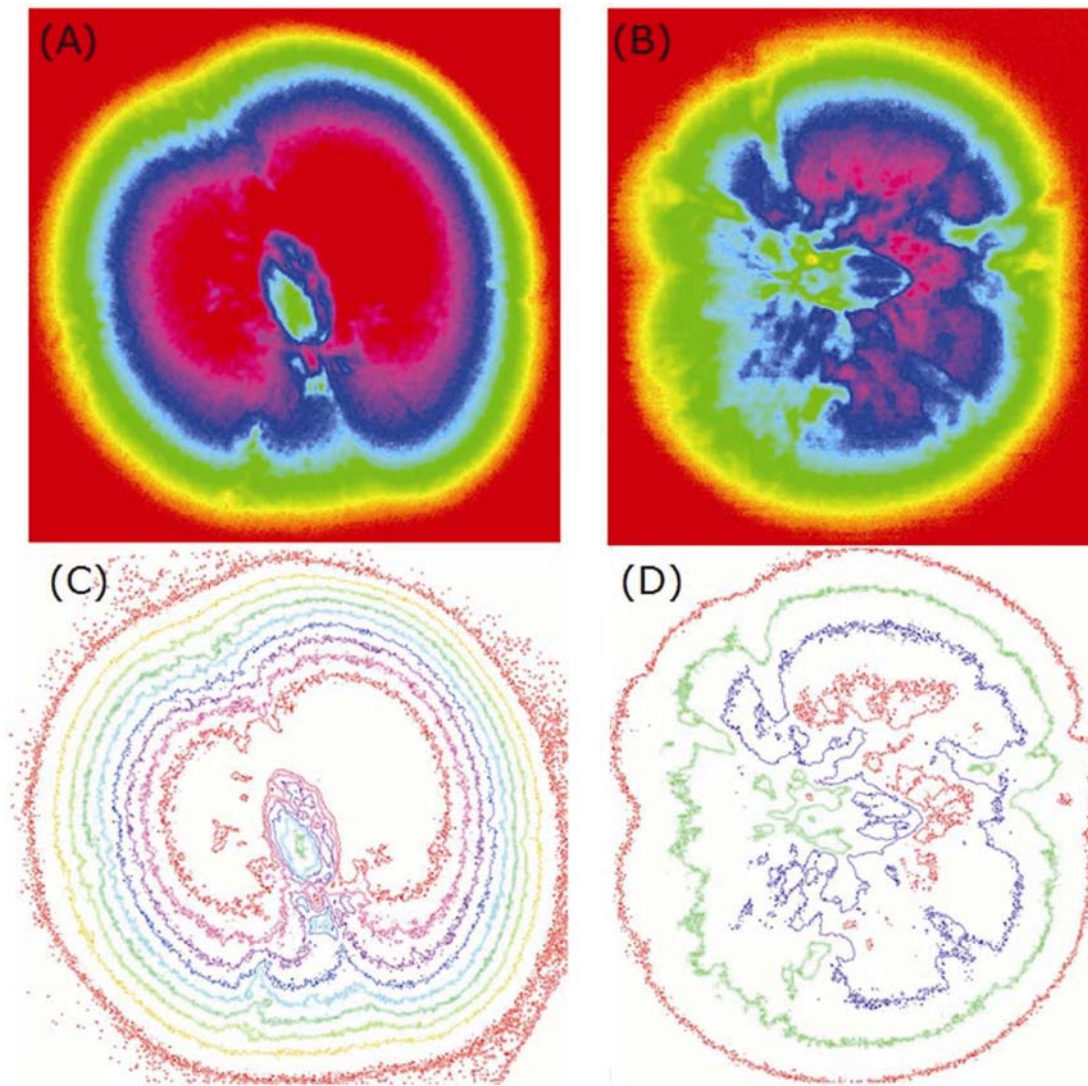


Fig. 5. After image analysis, X-ray images of peaches in Fig. 4A are shown in (A) and (C) with density changes expressed as pseudo-colors and contour lines, respectively. Similarly, (B) and (D) were derived from Fig. 4B, and show obvious injury symptoms.

(Fig. 3B, D), which greatly differs from the smoother lines shown in Fig. 3A, C. The best intensity condition for X-ray photography of the apple and pear was 45 kVp.

Drupe: peach

Similar to the pome, the best intensity for X-ray photography for peaches was 45 kVp. Figure 4 shows two peaches which

had been infested by Oriental fruit fly larvae. One had eggs implanted for 4 days (Fig. 4A, C, E) and the other for 6 days (Fig. 4B, D, F). The X-ray images revealed internal injuries at as early as 4 days after egg implantation into the peaches. In the peach infested for 6 days, the internal injuries were more serious than those of the peach infested for 4

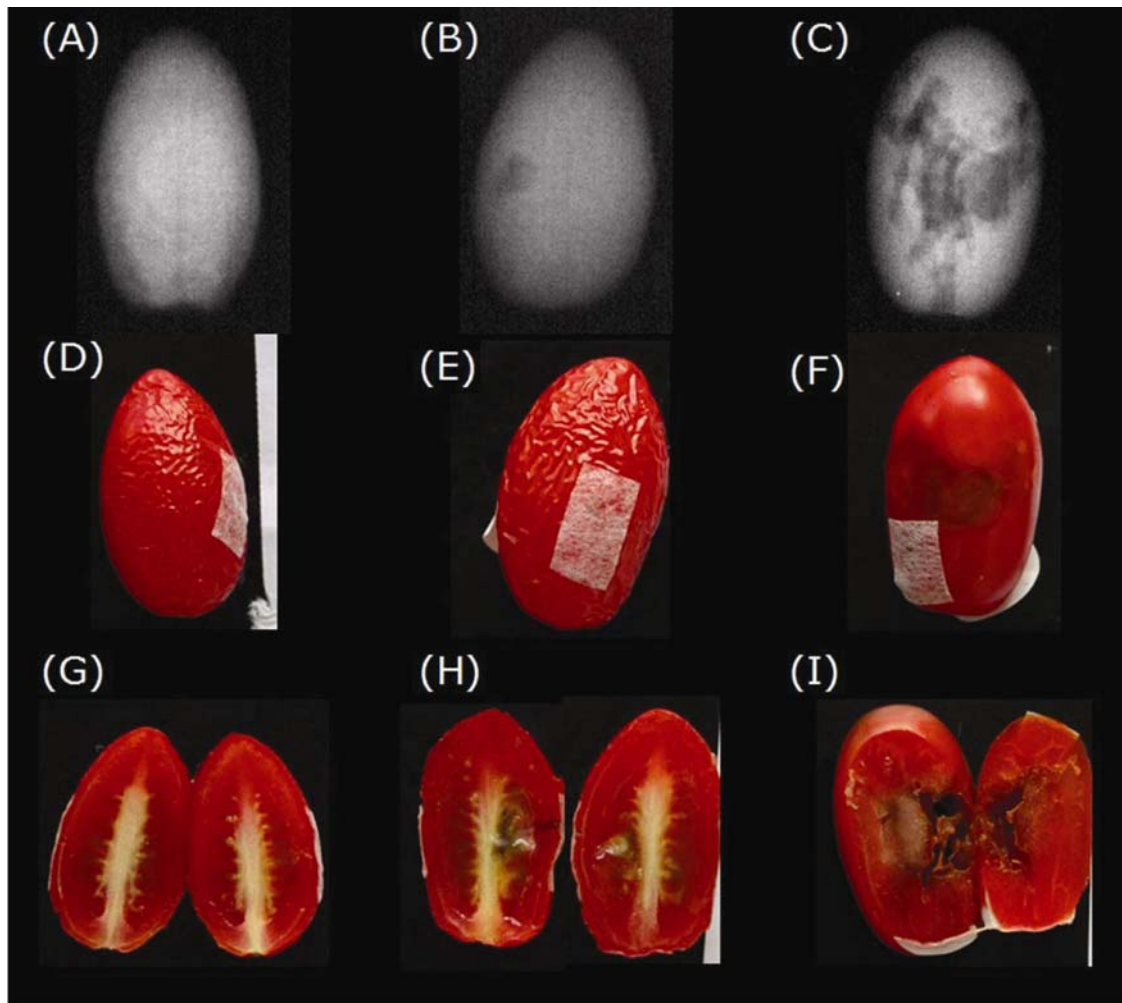


Fig. 6. Pictures of cherry tomatoes 6 and 17 days after egg implantation shown in the middle (B, E, and H) and right (C, F, and I) columns, respectively. The tomato with no eggs implanted for 6 days which served as the control is shown in the left column (A, D, and G). (A)-(C), X-ray images; (D)-(F), pictures showing the fruit surface in visible light; (G)-(I), internal injuries after cutting the tomato in half. Signs of internal injuries were obviously enhanced on the X-ray images over time.

days, and many tunnels were easily detected on the X-ray image (Fig. 4B). Similar to the pears shown in Fig. 2, no surface injury was observed on either of the peaches shown in Fig. 4. The contour lines were not smooth, and a V-shaped pattern was evident in the parts where the internal injuries were found (Fig. 5).

Berry: cherry tomato

With the same exposure time (0.1 s), we found that the best conditions for X-ray photography of cherry tomatoes required stronger power and therefore the voltage was increased to 50 kVp (Fig. 6). Internal injuries to the tomatoes were detected by X-ray images and dissection on the 6th day after egg implantation (Fig. 6B), even though the surface of the fruit appeared intact. A spot on the X-ray

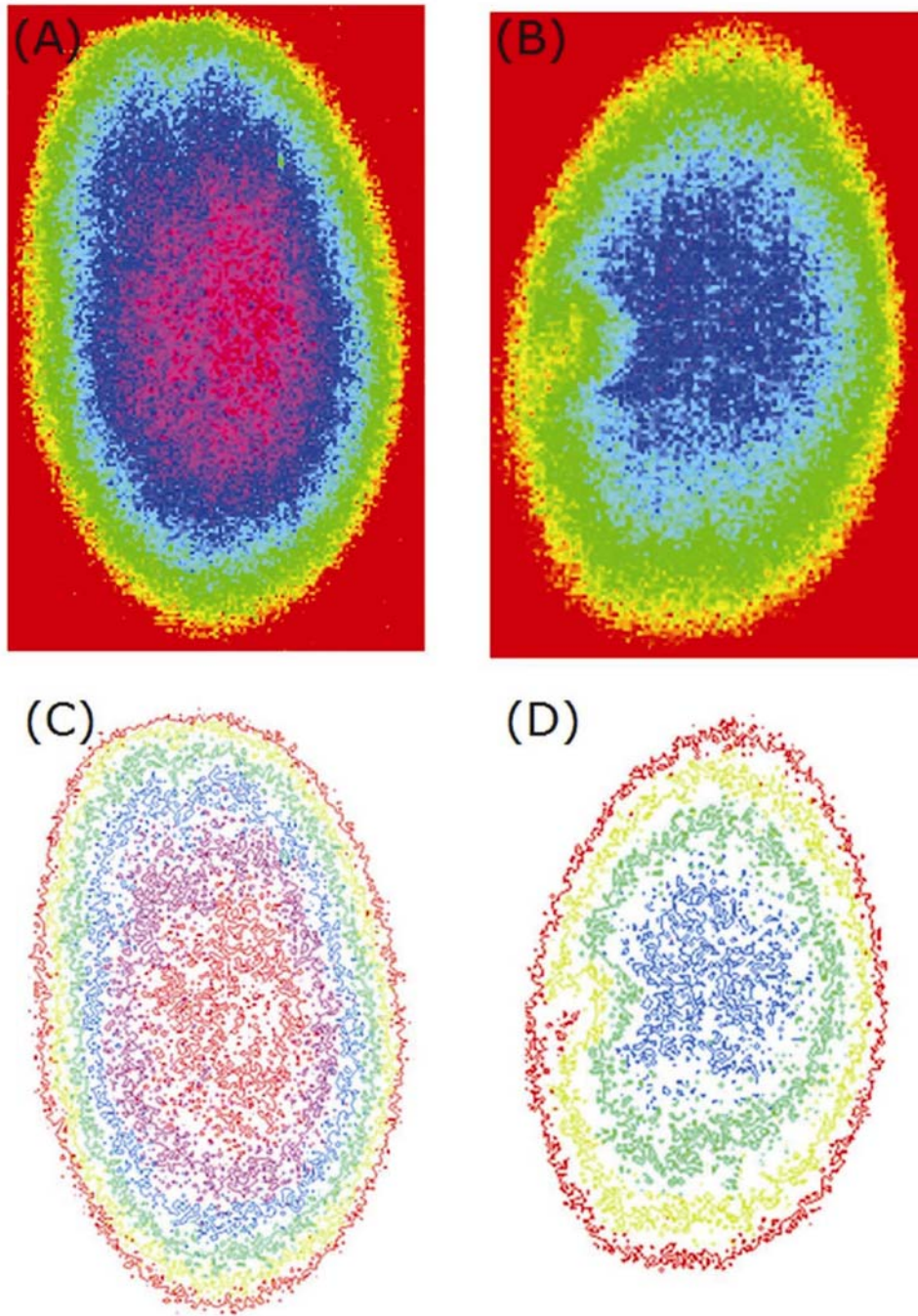


Fig. 7. After image analysis, X-ray images of cherry tomatoes in Fig. 6A are shown in (A) and (C) with density changes expressed using pseudo-colors and contour lines, respectively. Similarly, (B) and (D) were derived from Fig. 6B and show obvious injury symptoms.

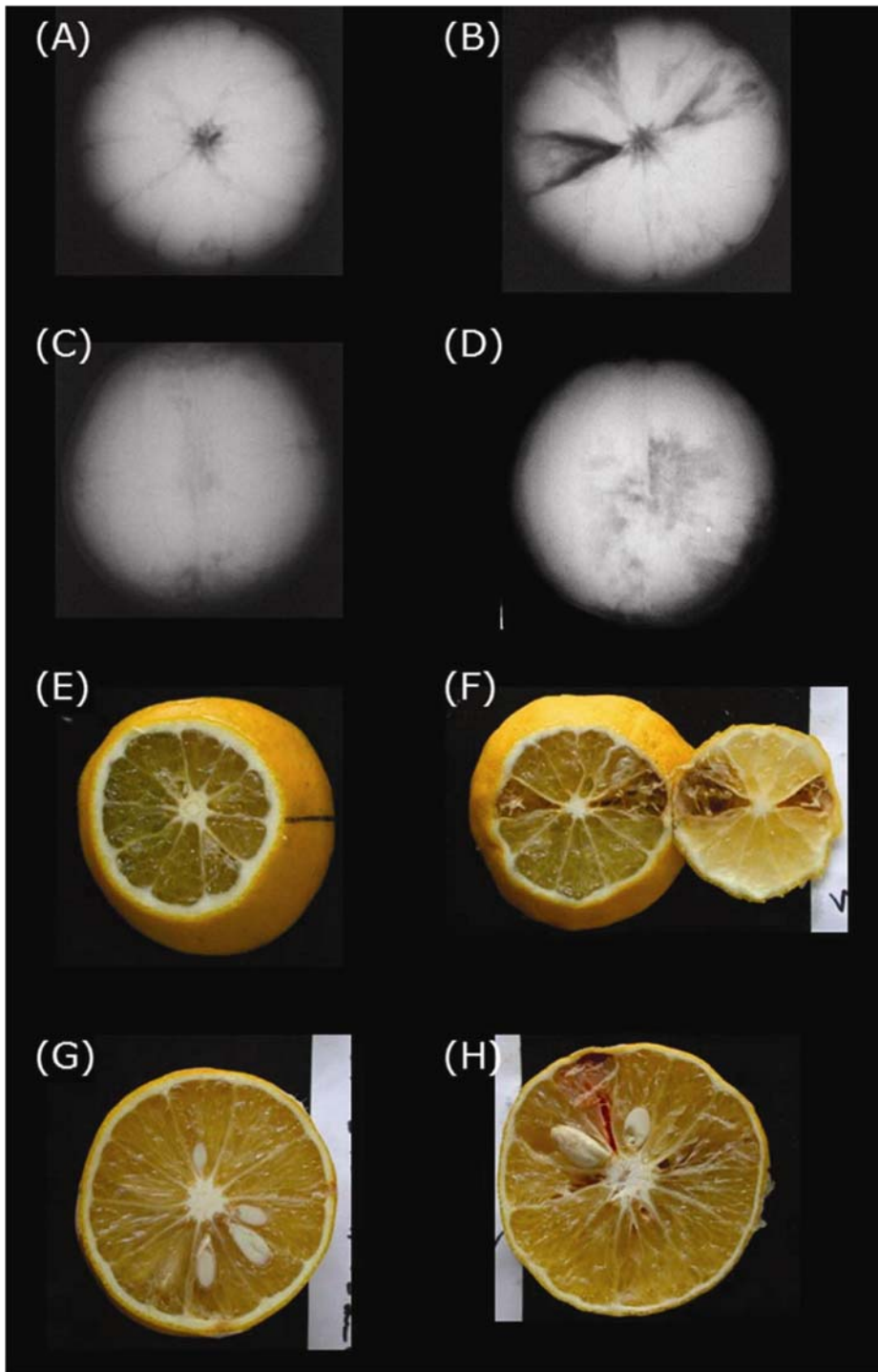


Fig. 8. Photographs of oranges 6 (A, C, E, and G) and 17 (B, D, F, and H) days after egg implantation. (A) and (B), X-ray images from the top view; (C) and (D), X-ray images from the side view; (E)-(H), fruit cut in different areas showing the internal injuries.

image indicated the injured part caused by fly larvae, and this did not show up on the X-ray image of the control (Fig. 6A). More-serious internal injuries and more-extensive tunnels were observed on the X-ray image on the 17th day after egg implantation (Fig. 6C). The injuries could be observed by closely examining the tomatoes without X-ray, but still no remarkable surface injuries could be observed (Fig. 6F). The contours and pseudo-colors of the density changes inside the infested tomatoes revealed uneven lines (Fig. 7B, D), as described previously for the pome and the drupe.

Hesiperidium: Orange

The best intensity for X-ray photography of oranges was the same as for tomatoes, i.e., 50 kVp (Fig. 8). Because the inside of an orange is divided into segments, the initial internal injuries caused by the fly larvae were confined by the segments. Fly larvae were first detected by X-ray images of an orange into which eggs had been implanted for 6 days. Since the internal structures are more complicated than those of the fruits described above, and the segments provide a strong contrast on X-ray images, a top view of the X-ray image did not provide reliable clues for locating the internal injuries (Fig. 8A). In contrast, a side-view X-ray image gave a clear indication of the location of the injury (Fig. 8C). The contour lines and pseudo-colors of the density changes on the side-view X-ray image also clearly showed the location of the injuries, but the top view was unsuitable for determining the location of the injuries (Fig. 9A & C). When slicing on orange, it was not easy to determine either the extent of internal injuries or the presence of larvae due to the juicy contents (Fig. 8E, G). For those oranges which had eggs implanted for 17 days, the internal injuries were serious and were easily diagnosed by locating brown tissue (Fig. 8F, H). Both the X-ray

images and examination of slices of seriously infested oranges showed that the injuries were not confined by the segments (Fig. 8B, D, F, H). The contour of density changes derived from the top-view X-ray image of the orange (with eggs implanted for 17 days) showed broken, uneven lines (Fig. 9B, D), indicating that the fruit had been seriously infested by fly larvae.

Discussion

Since the aim of this study was to evaluate the quality of X-ray photography as an inspection method of pest infestation in fruit for quarantine purposes, the results presented in this paper certainly confirm the possibility of applying this technique. The traditional quarantine inspection of fruit simply consists of visually examining the surface of a piece of fruit, and looking for any surface injury caused by pests, before it is suspected of being infested, and thus requiring further dissection and identification of the pest. As we have demonstrated in this paper, some important pests such as the Oriental fruit fly, melon fruit fly, apple maggot, Queensland fruit fly, and Mediterranean fruit fly, deposit their eggs underneath the skin of a fruit, and it is almost impossible to find the tiny surface injury created by the oviposition. With the aid of X-ray penetration, images of the internal injuries caused by the pest can be used for image analysis and further screening of the fruit including dissecting the fruit and identifying the pest. Although injuries to fruit created by an insect's ovipositor may be too tiny to be detected by X-ray image analysis, tunnels created by larvae in infested fruit provide a good contrast on the images and are easily detected. The shape of the injury tunnels obviously differs from the internal structures of the fruit, and, in addition to the contrast of the image, the density contours showed remarkable uneven

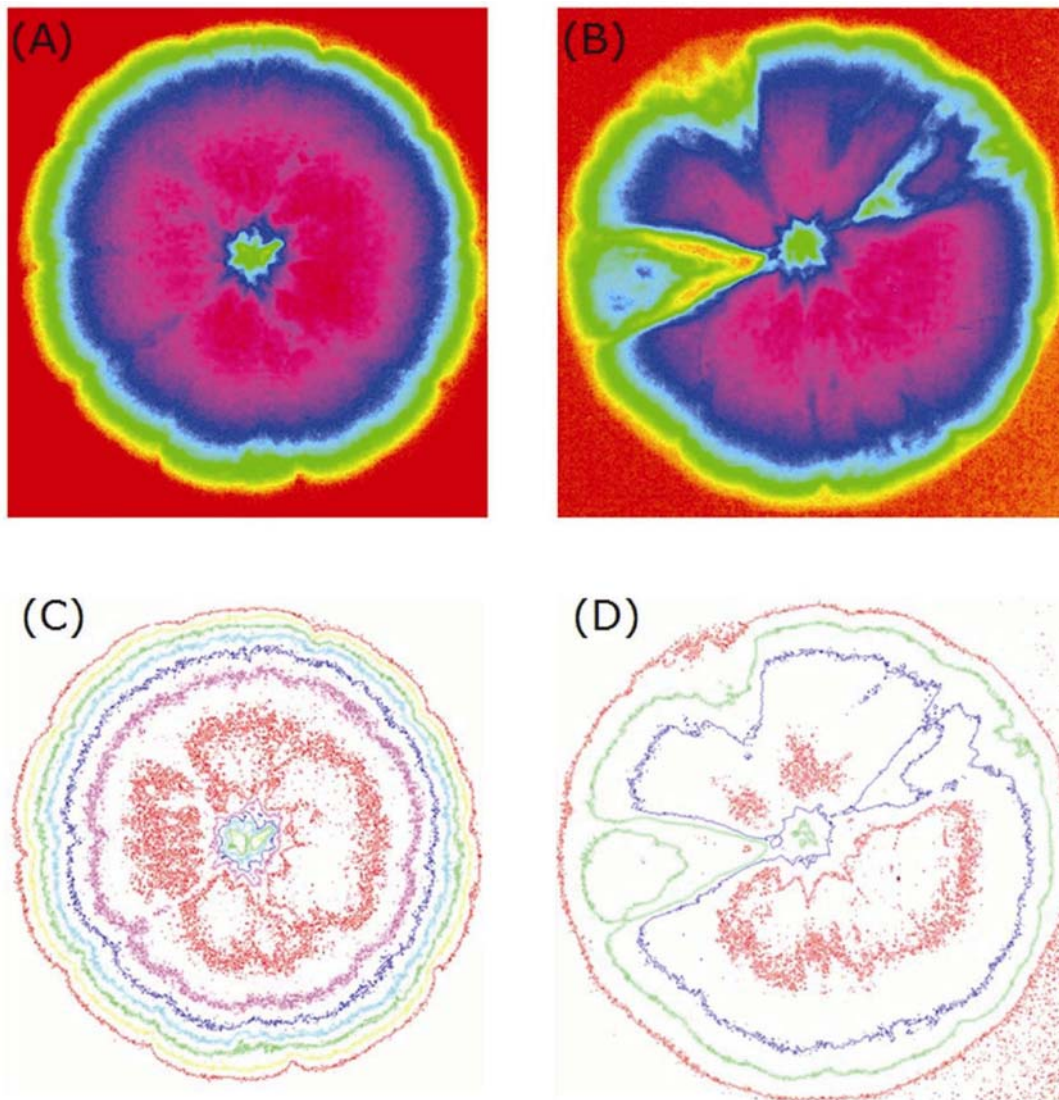


Fig. 9. After image analysis, X-ray images of the orange in Fig. 8A are shown in (A) and (C) with density changes expressed using pseudo-colors (A) and contour lines (C), respectively. Similarly, (B) and (D) were derived from Fig. 8B and show obvious injury symptoms.

lines or broken areas, indicating that the areas had been damaged by pests and the density had changed. More-advanced programming of image processing in order to detect injury tunnels, based on changes to the density of the flesh of the fruit, will improve detection success. Further development of this technology may offer an efficient and accurate inspection tool

to determine infestations for imported commodities and to sort clean fruit prior to export.

According to the X-ray images, the earliest time for which internal injuries of fruit could be detected varied for the different fruit species. Previous studies showed that the egg stage of the Oriental fruit fly is dependent on the host fruit

(Harris *et al.*, 1991; Sugayama *et al.*, 1998; Vargas *et al.*, 2000; Papadopoulos *et al.*, 2002), and differences among the earliest times at which internal injury could be detected were probably due to this reason. In this study we did not examine the duration of the egg stage in the different fruits, so the earliest time the internal injury was detected would not necessarily be the first day on which the larvae hatched. In some fruit such as the pear and peach, the internal injuries were detected as soon as the fly eggs hatched, since the eggs had only been implanted for 3-4 days; but for other fruit, such as the apple, tomato, and orange, the internal injuries were detected on the 6-7th days after egg implantation. Further investigation into determining the first day on which the larvae hatch in a host fruit will verify the time lag. Another purpose of this study was to provide fundamental information for developing possible non-destructive inspection technology in the future. Because there are no X-ray facilities specifically designed for the inspection of quarantined fruit, using hospital equipment for making the X-ray images of fruit was the most convenient option. Although the results presented in this paper are from the first trial using X-rays to examine internal injuries to fruit caused by pests, through this study and with advanced medical X-ray photographic facilities, information such as the photon energy required for various fruits is now available. This should be quite helpful for future designs, especially information that the voltage of the X-ray source for small fruit used in this study was 40-50 kVp, which produces about $1.3409-1.8252 \times 10^{-28}$ MeV·s. This energy range is within the so-called soft X-ray spectrum, and is very safe as far as producing free radicals is concerned.

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非破壞性檢疫技術—X光影像偵測東方果實蠅於水果早期危害之應用

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摘 要

本研究利用 X 光透視影像檢視水果的內部情形，以測試其應用在偵測檢疫害蟲的可行性。由於水果內部具有高水分含量，通常並不適用於 X 光檢測，然而藉助數位化影像分析，受害水果的內部危害情形可以與正常水果的均質情形有所區辨。我們以東方果實蠅 (*Bactrocera dorsalis*) 的卵植入多種不同類別的水果，於植卵後不同天數檢視水果內部的 X 光影像，並進行影像分析。水果受到蟲害亦可以與正常部位區辨，有些類別的水果在果實蠅孵化後三天即可偵測出受害情形。此項研究結果顯示 X 光透視影像應用於偵測檢疫害蟲具有可行性，且此種非破壞性技術可以有效偵測水果內部的受害情形，達到目視檢測無法達成的效果。

關鍵詞：X 光、非破壞性檢疫、影像分析、水果、東方果實蠅。