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Patterns of Fluorescence Associated with Citrus Peel Defects*

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Abstract

Unshu citrus were sorted by fluorescence imaging in a commercial packinghouse and undamaged-appearing unshu that had been rejected by the packinghouse due to fluorescence appearing on their peel were studied. We examined the various visible patterns, based upon fluorescence and microscopic images, to provide a categorization of physical reasons for the observed fluorescence. The categorization classes were: 1) slight physical damage: thin scar, hole and flow, shrunken at calyx; 2) rubbing against decayed fruits; 3) green spots; and 4) rind puffing. The percentage of observation for each of the four classes was 22 %, 15 %, 42 % and 21 %, respectively. Storage of the classes indicated that, except for the green spot class, the injured area expanded quickly and caused the fruits to rot within a week.

[Keywords] citrus defect, fluorescence image, fluorescence pattern, ultraviolet illumination, machine vision, digital microscope

I Introduction

With concerns about food quality and safety for the consumer, machine vision technology has been widely applied recently in the agro-industry sector. The combination of machine vision and image processing techniques is increasingly useful in the fruit industries because traditional visual inspection is labor intensive and prone to human errors and variability (Li *et al*., 2011). In Japan different cooperative societies use citrus grading systems to sort fruits by inspecting parameters such as color, size, shape, bruises and internal qualities. However, during post-harvest processing the detection of defective citrus fruits is still one of the major problems for grading facilities because of variability in defect pattern, size, position and color. Researchers worldwide are trying to explore suitable methods to detect defects and diseases related to citrus and as a consequence a number of techniques have been studied based on using machine vision systems. (Aleixos *et al*., 2002; Blasco *et al*., 2007; Kurita *et al*., 2009; López-García *et al*., 2010; Qin *et al*., 2008).

Most citrus species accumulate substantial quantities of flavonoid substances, that fluorescence under ultraviolet (UV) light (Kondo *et al*., 2009; Benavente-Garcia, *et al*., 1993; Castillo, *et al*., 1992). The peel of the citrus fruits fluorescence when the peel oil is released by some defects and can become visible when exposed to UV (Uozumi *et al*. 1987; Latz and Ernes, 1978). Tangeretin, a polymethoxylated flavone that is a component of the peel oil (Swift, 1967), fluoresces under UV light and is likely the source of the yellow fluorescence visible from damaged or decayed oranges in black light rooms. In a recent study Blasco *et al*., (2007) examined the use of UV-induced fluorescence as a part of a multispectral analysis to identify defects in citrus caused by the green mould. In another study Slaughter *et al*., (2008) evaluated the feasibility of using machine vision and long wave UV fluorescence to detect and separate freeze-damaged oranges.

In our prior research we have evaluated the ability of a machine vision system based on UV-induced fluorescence imaging to examine fruits on the packing line to detect defective citruses (Kondo *et al*., 2009, Kurita *et al*., 2009, Momin *et al*., 2010, Ogawa *et. al*., 2011). Kurita *et al*., (2009) proposed a machine vision system consisting of a pair of white and UV LED lighting devices and a color CCD camera for the orange fruit grading task. Kondo *et al*., (2009) found that the fluorescent substance extracted from the peel of mandarin oranges had the basic structure of flavones and it was quite possibly heptamethylflavone. The excitation and fluorescent wavelengths of the substance were 360 to 375nm and 530 to 550nm, respectively. Momin *et al*., (2010) proposed an algorithm for detecting fluorescent area of citrus fruits using fluorescence imaging. Ogawa *et al*., (2011)

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demonstrated the detection of rotten areas on fluorescent images of mandarin oranges. However during processing numerous lots of fruits using fluorescence imaging we noticed that apart from rotten fruits, mainly caused by biotic factors, there were some fruit that fluoresced and were detected by the imaging system even though their external appearance was fresh or normal to the naked eye. Some of the fluorescing areas did not have the characteristic appearance of decay lesions but will develop damage during storage and the fruits will decline in quality. Elimination of these fruit early in the packing process not only improves the overall quality of the fruit lot but also acts to limit the spread of peel oils through the rest of the packing line (Obenland *et al*., 2010). It is important to understand and explore the factors which are responsible for causing fluorescence of such fruit yet no research has been done to examine this. The detection of unseen injuries or defects in citrus by fluorescence using an automated system with machine vision and UV illumination will provide packinghouse managers and growers with the tool needed to eliminate poor quality fruits. Identification of causal reasons for fluorescence may also help both growers and packinghouses take necessary steps to reduce peel damage as well as to provide guidelines on how to manage fruit with areas of peel fluorescence to maintain blemishes on the peel at lowest possible levels. Thus, the objective of this work was to identify the physical causes for the fluorescent signatures that caused the fruits to be rejected and then what the significance of these fluorescent types was to fruit quality.

II Materials and Methods

Unshu oranges (*Citrus reticulate* Blanco var. *unshu)* harvested in late November and early December 2011 were used for evaluating the fluorescent imaging-based machine vision system in the Kawakami citrus grading facility (Ehime prefecture, Japan). The system illuminates the citrus on a moving conveyor with UV and white LED lighting panels and develops fluorescence and color images in a computer vision unit. The schematic diagram of the image acquisition system of one of the six camera set-ups is shown in the Fig. 1. During inspection every fruit passed through two sets of machine vision boxes each equipped with three cameras and twelve images of each were captured, six fluorescence and six color. Both fluorescence and color images were acquired simultaneously by the system as illustrated in Fig. 2. Then the resulting fluorescence images were transferred to the control unit for post-processing and the color images were used to detect fruits color, shape, size, etc. Based on the defect detection algorithm described in Ogawa *et al.*, 2011 and Momin *et al.*, 2010 for defective citrus with minor modification, they were sorted out automatically by the system from the conveyor.

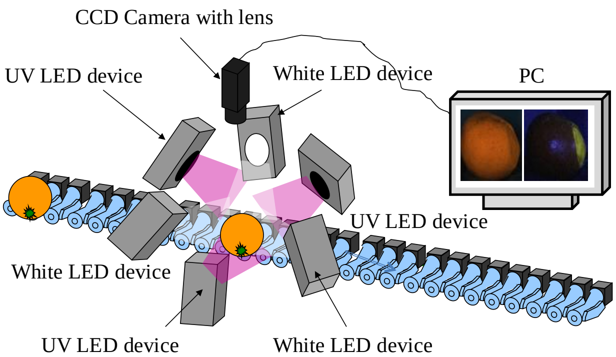

Fig. 1 Layout of the double LED image acquisition system

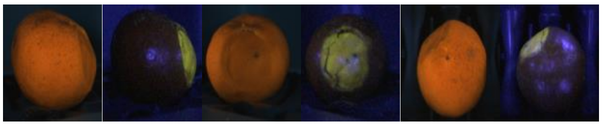

Fig. 2 Acquired color and fluorescence images of one fruit passing through one unit

After testing numerous lots of fruit (100 t approx.) we examined the sorted fruit to check whether the system identified only defective (rotten and damaged) fruit or fruit that were undamaged to the eye under visible light as well. We found that among sorted fruits there were about 20% that appeared undamaged to the naked eye (Fig. 3). These fruit had some peel areas that fluoresced but the fluorescence differed in appearance from that observed for rot or lesions. These same fruits would not have been culled by the packinghouse workers when examined visually. From the collected samples of the culled fruits 330 (35 kg approx.) were randomly selected and tested in the laboratory to identify some possible reasons behind the fluorescence emissions.

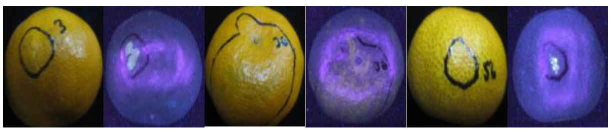

Fig. 3 Examples of fresh-appearing fruit sorted during processing (fluorescing from marked area)

In this experiment the color images were acquired using a color camera (Canon IXY digital 25 IS, Canon Inc. Japan). The fluorescence images were acquired using an UV illumination system composed of four fluorescent lamps (UV-A band light) that emitted light at 315-400 nm with the peak at 350 nm. For uniform illumination as well as to obtain more illumination on the object scene, the incident light angle (θ) was maintained between 50° and 55°. The VGA format camera (VCC-8350CLTS, CIS Corp. Japan) of 8 bit gray levels fitted with a C mount lens of 6 mm focal length was placed 15 cm above the scene, which provides a field size of 13 cm x 13 cm. The distance between center of a fruit and the fluorescence lighting panel was maintained at about 13 cm. As the fluorescence intensity of rotten or injured regions of citrus was much lower than the color signals of fresh parts, it was necessary to adjust the camera operation parameters such as shutter speed, gain, and gamma correction to obtain a good quality image and Table 1 shows the adjusted parameters. After adjusting the camera parameters then images were captured with a resolution of 0.27 mm/pixel and size of 512 x 480 pixels using the camera and a capture board (MTPCI-TL2, Micro-Technica Co., Japan) plugged into a computer. Then data were transferred to a computer to examine the fluorescence areas.

Table 1 Camera operation parameters

Lighting panel	Parameters			
	Shutter speed (sec)	Iris	Gain (dB)	Gamma correction
UV light	1/60	1.4	12	0.45

After identifying the fluorescence regions of each sample, then we used a digital microscope (DigiScope II v2™, Chronos, Taiwan) to capture enlarge color and fluorescence images of the affected areas for closer examination. To our knowledge this is the first time a digital microscope was introduced as an image acquisition system to acquire fluorescence images of citrus. The principal specifications of the digital microscope system included image editing and measurement software, a fiber optic cable, a camera unit with 1/3.2 inches image sensor, "A" roller for adjusting focus, a zoom lens with magnification from 10x to 230x with a 17 inch LCD display, eight white adjustable LEDs mounted at tip of the camera unit, an illumination roller and a metal stand. The color images were acquired using the built-in illumination system. Two fluorescent lamps of the same specifications mentioned above were used to acquire fluorescence images. The distance between microscope tip and object plate was 6 cm and distance between center of fruit and fluorescent lamp was 8 cm. During image acquisition the target objects were oriented to make sure the fluorescing areas were towards the camera and after calibrating the camera parameters (Table 2) the images were captured with a 1600 x 1200 pixel resolution and saved as images in a compressed BMP format. Fig. 4 represents the schematic layout of the UV fluorescence and digital microscope image acquisition systems.

Table 2 Camera calibrating parameters

Lighting panel	Parameters			
	Brightness	Contrast	Saturation	Gamma
UV light	123	25	20	86

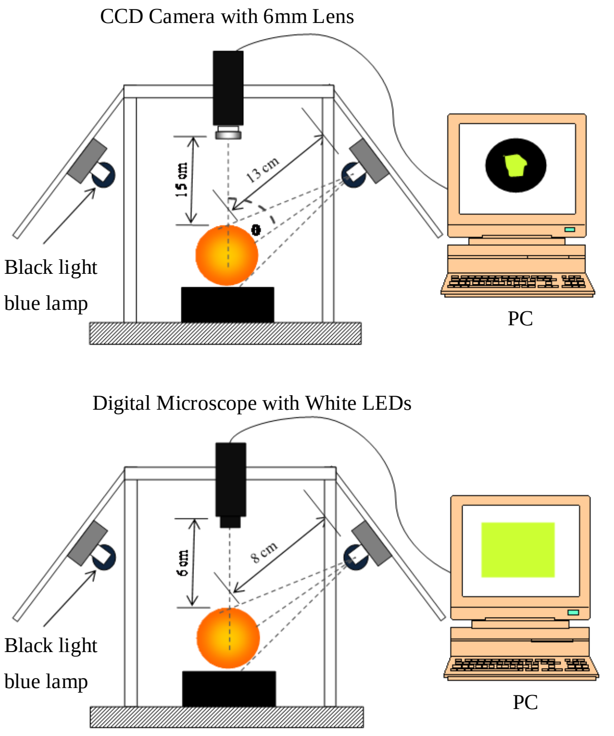

Fig. 4 Schematic representation of UV (up) and Digital Microscope image acquisition systems (bottom)

III Results and Discussion

1. Classification of possible reasons of fluorescence

In this study 330 fruits were randomly selected for further examination in the laboratory from a group of fruits that had been culled using UV fluorescence from the packingline but that did not have damage readily obvious to the naked eye under visible light. Some of the samples showed large areas of

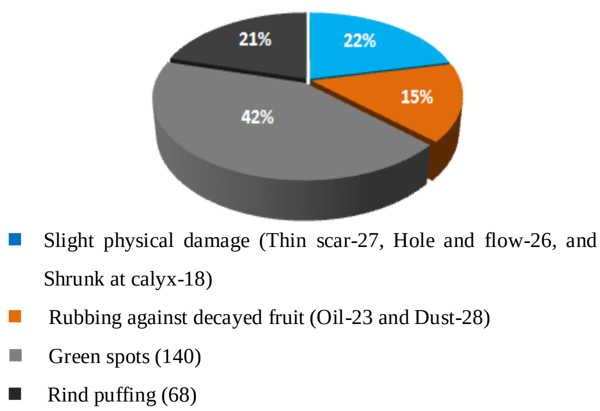

Fig. 5 Percent of observation of each fluorescence pattern

fluorescence but the majority of fruits had at least a small area of fluorescence. Based on the appearance of fluorescence noted from images captured by UV illumination and digital microscopy the selected samples were classified into four classes as shown in Fig. 5. Details are individually discussed below.

(1) Slight physical damage

This pattern of fluorescence was observed among one-fifth of the samples and was further classified into the following three peel defect classes: “thin scar,” “hole and flow” and “shrunk at calyx”. These types of injuries are really difficult to sort by the packinghouse workers as they are hard or impossible to see. Those unseen injuries can weaken peel structure and later lead to visible damage. The incidence of this kind of pattern could be caused by a variety of preharvest and postharvest injuries induced by thinning, pruning, spraying, picking, and the packing and sorting process. To avoid such unseen and tiny defects it is important to have recourse to technical knowledge, proper orchard management practices and postharvest processing.

(a) Thin scar

Thin scar likely occurred due to mechanical equipment during various cultural practices of orchard management. Mechanical damage such as the rough handling of the fruit with knife or cutter during picking often can cause thin scratches or minor wounding on the fruit surface which can collapse the oil cells and induce strong fluorescence when excited by UV light as shown in the Fig 6. This type of blemish was observed mainly on the side or top portion near the calyx of the fruit and the lowest pixel size of such scar was found to be around 150, i.e the minimum scratch length was around 6 mm measured from the processed fluorescence image captured by the UV illumination system.

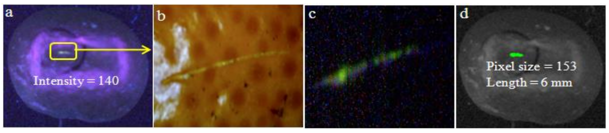

Fig. 6 Examples of thin scar fluorescence area: (a) fluorescence image captured by UV illumination, (b) and (c) color and fluorescence images of fluoresced areas captured by digital microscope, (d) processed fluorescence image

(b) Hole and flow

It is also evident that sometimes tiny holes or punctures were created on the peel and destroyed oil glands. The factor having a likely contribution in causing this type of blemish was the wind as it may cause fruits to rub against tree thorns, or twigs and cause damage to develop. In addition sometimes very small wounds or punctures were created by brushing equipment when citrus were brushed at the beginning of the line during the sorting process. We observed that the brushes used in the packinghouse were coarse with dense and hard bristles and each bristles diameter was found about 0.45 mm. After creating the hole or leakage in the citrus peel, the oil comes out and can fluoresce under UV light. The oil then spreads around the injured area and gradually the peel oil flows down and extends the fluorescence areas. From Fig. 7 it is seen that the fluorescence was fairly evident near to the hole and then the intensity of fluorescence gradually decreased. We noted that the smallest hole created on the peel that was sorted by the system during processing was about 3 mm in diameter.

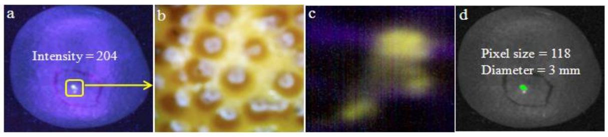

Fig. 7 Examples of hole and flow fluorescence with the corresponding color image

(c) Shrunk at calyx

Shrinking or squeezing around the calyx of fruit sometimes may cause appearance of fluorescence. In this study we observed in 25 percent of the fruits with slight physical damage the incidence of blemishes caused by shrinking. From Fig. 8 it is seen that although we cannot see any sign of disruption or damage of oil cells in the color image, under UV light it shows strong fluorescence characteristics. The cause of shrinking is not well known and it is difficult to determine the precise reason for the fluorescence due to this disorder. This damage may possibly be caused by excess gravity force or excess pulling force during manual harvest.

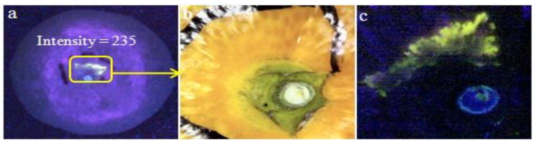

Fig. 8 Examples of shrunken at calyx fluorescence with the corresponding color image

(2) Rubbing against decayed fruits

Immediately after harvesting all citrus are packed in containers and transported to the packinghouse for postharvest processing. In the containers there are some rotten citrus caused by different fungal organisms. Also, severely damaged areas on these fruits contain large amounts of essential oils and during moving, due to pressing or collision some pieces of peel are easily smashed or detached. Some of the oil then becomes transferred to the surrounding uninjured fruits. From Fig. 9 it is seen that the non-decayed fruits were affected both by oil (top images) and decay spores (bottom images) of the rotten peel and the intensity of fluorescence of the affected areas almost has the same characteristic appearance as rotten fruits under UV illumination. These fruit, however, were not removed by the workers. It is also apparent (Fig. 9 (d)) that in both cases the fluorescence disappeared just after washing the affected areas.

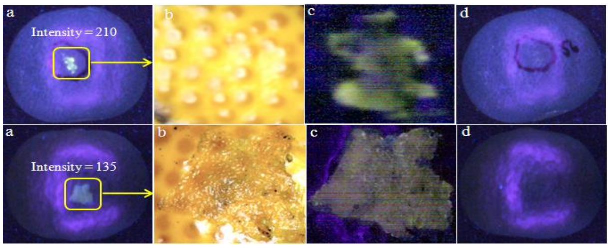

Fig. 9 Examples of fluorescence caused by contact with decayed fruit

(3) Green spots

Almost half of the samples with slight damage had this type of appearance. We observed that the outward appearance of this group of fruits were a combination of yellowish-green color and the fluorescence appeared only from the greenish parts though no sign of collapsed oil glands or blemishes was observed. The appearance of fluorescence looks scattered and intensity of fluorescence was relatively low in comparison to other fluorescent patterns observed (Fig. 10), but is detected by the system and the fruits were removed from the conveyor belt. The green spots are due to the presence of chlorophyll in that region of the peel, although the exact causes of this are not well understood. However, it is believed by the citrus industry that it can be caused by a variety of factors, including cultural practices, climate, nutritional deficiencies, abnormal maturation, insect damage, and lack of sufficient sun exposure.

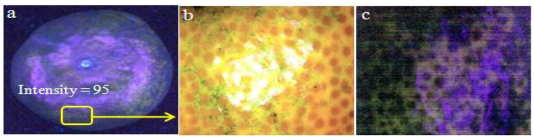

Fig. 10 Examples of green spots fluorescence area

(4) Rind puffing

It was observed that rind puffing also had a major contribution in causing blemishes. Rind puffing is a physiological disorder characterized by separation between peel and pulp. It is related to the disintegration of the deepest cell layers of the albedo tissue that gives rise to aerial spaces (Kuraoka, 1962). The cause of puffing has been related to the water exchange regulation through the peel (Kawase *et al.*, 1981). Harty and Anderson (1995) reported that keeping fruits on the trees to allow acid reduction carries the risk of rinds becoming excessively puffy. These puffy skin areas are very sensitive to damage caused by rubbing against branches, leaves or fruits during windy periods or hitting other fruits during picking, packing and transporting. Though they had not been normally culled from the pack line by the workers they were visible under UV illumination and detected by the system. Fig. 11 revealed that a group of oil glands has collapsed due to this type of injury that happened sometime in the past. These injured areas show small depressions on the peel that fluoresce under UV illumination.

Fig. 11 Examples of rind puffing fluorescence area

(5) Chemical effects

Finally, according to the citrus industry representatives, prior to harvest the spraying of agricultural chemicals can sometimes cause the display of fluorescence on the peel; for example the application of copper or mancozeb to prevent infection from melanose, brown spot, and brown rot. Extension of the season and early harvesting both may cause various physiological disorders and to control and prevent these disorders treatments are applied. Such rind disorders associated to the on-tree storage of fruits are prevented by adding GA (gibberellic acid) and those related to early harvest controlled by spraying ethephon (Agusti *et al.*, 2002). The pattern of fluorescence from such chemical applications looks like a splash of bluish color when excited by UV light as shown in Fig. 12 observed on early variety citrus. This fruits would be classified by the machine vision system as being of a lesser quality and would not be worth as much. However during experimentation in this time, we did not observe such pattern in the tested samples.

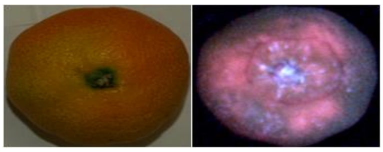

Fig. 12 Example of bluish fluorescence area

2. Storage effects

The samples that were included in the studies were stored for 15 days at 15ºC temperature and humidity around 80% in cardboard containers to observe the deterioration pattern of the fruits. The work showed that, in case of slight physical damage fluorescence types, the injured area caused by "thin scar” and “hole and flow” become noticeable and expanded quickly around the injured area, causing the fruit to rot within four to seven days as shown in the Fig. 13 (a) and (b), respectively. However, though it took some time to become visible, the shrunken calyx area could be observed to decay by day 10 (Fig. 13 (c)). For fruits that had been detected following contact with decayed fruits, it was noticed that, though there were no immediate blemishes the contacted area became weakened and visible over time. It took about a week to become rotten as shown in the Fig 13 (d), though it depends on how much area was affected during contact with rotten fruits. In citrus with puffing the oil cells in the depressed areas become dehydrated and flattened, and gradually formed brownish to black lesions (Fig. 13 (e)) that tended to coalesce and produce larger depressions of the affected areas. It was seen that they also became rotten in less than a week. During the observation period we observed some greenish spot areas become soft and apparent as shown in Fig. 13 (f) although these areas did not decay. As a result the removal of these fluorescent types may not be as critical as for the types previously mentioned. However, considering the cosmetic look of the fruits and for long term storage it may be better to remove such citruses.

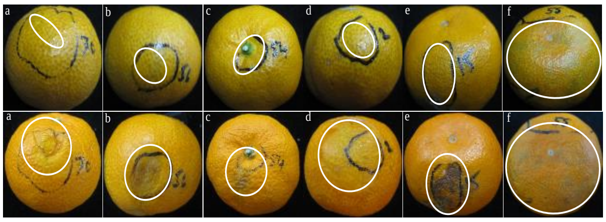

Fig. 13 Unseen injury areas top row images, became visible after storage bottom row images

In addition in the same condition we put one rotten fruit inside of fresh fruits in another cardboard container to examine the effects of rotten on fresh and as well how long it takes to spread the rotten fruit peel oils to deteriorate the quality of the fresh one. It is apparent that after one week the fresh fruits mostly adjacent with the rotten one, were affected and damaged. The damage continues to develop and spread day by day in other fruits and on the 15 day we observed almost 50% fresh fruits among 75 in the container were recorded severely rotten .

Fig. 14 a. rotten fruit, b. the rotten one surrounded by fresh fruits, and c. damage spread to the fresh

IV Conclusions

In this study, using UV illumination and digital microscopy, we observed some possible reasons why fluorescence is present in fruits that appear to be undamaged under visible light. It is apparent that beyond biotic causes (green or blue mould pathogens, insect, mite, or disease, micro-organisms), there are sometimes defects associated with various types of physical damage that fluoresce. In addition, the presence of the fluorescence on the peel would be likely related with the presence of green spots, puffed skin and contact with decaying fruits. The categorization results of fluorescence patterns of this study could be useful for packinghouse operators in the commercial citrus grading industry to make the entire automated system more efficient. The findings can help packinghouse members to answer grower`s questions as to why fruit are downgraded and enable the growers to understand different types of unseen injuries so that they can minimize losses in future. By understanding how the injury occurred both sides can set criteria for out-of-standard fruits and take possible remedies to reduce the occurrence those fluorescence types. This research also demonstrated that digital microscope imaging could be used to categorize visible fluorescence in addition to UV illumination-based fluorescent imaging techniques. Further experimentation is needed for other citrus varieties to verify the reasons for the fluorescence as well as to extend the research to find some reasonable remedies.

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