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原著論文

An Experiment to Detect Apples Infested by the Peach Fruit Moth, *Carposina sasakii* Matsumura (Lepidoptera: Carposinidae), Using Near-Infrared Spectroscopy †1

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Abstracts

The potential of near-infrared spectroscopy to detect apples ('Fuji' cultivar) infested by the peach fruit moth, *Carposina sasakii*, was evaluated using a prevalent NIR device. A calibration equation was developed using both the NIR spectra and injury levels of the infested fruits. The equation predicted the injury levels of fruits using a simple correlation coefficient of 0.8868 and a standard error of prediction of 0.3688. At the level of estimates to discard ca 90% of infested fruits, 90.15% of the infested fruits and 39.24% of the uninfested fruits were excluded from the samples. Although its accuracy was not sufficient, the present NIR device has the potential to identify internally infested fruits.

Key words: *Carposina sasakii*, apple, near-infrared spectroscopy, non-destructive inspection, NIR

Introduction

The peach fruit moth, *Carposina sasakii* Matsumura, is considered the most serious pest of pome fruits in Japan, Korea, China, and Russia (Narita, 1986). After the mated females lay eggs on the fruits, the first-instar larvae tunnel toward the center of the fruit, and the mature larvae leave the fruits and seek cocooning sites for shelter on the ground (Kajita, Y. and H. Nakao, 1978; Narita and Takahashi, 1978; Narita, 1986). Infested fruits, which have traces and excreta of larvae inside them, are detested by Japanese consumers. It is difficult to detect all the infested fruits during the fruiting season and after harvesting because the surface of some infested fruits does not show any signs of infestation. Therefore, to prevent this infestation, the occurrence of *C. sasakii* has to be completely eliminated from the orchards.

Since the 1950s, Japanese growers have been spraying pesticides at two-week intervals during the fruiting season to control the occurrence of *C. sasakii* and the invasion of first-instar larvae into fruits (Narita, 1987). Since the 1980s, mating disruption has been applied as an alternative to pesticide sprays, to control *C. sasakii* in apple, peach, and

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Japanese pear orchards (Oku, 1986; Oku et al., 1989; Sato, 1992; Okazaki, 2000). The mating disruption technique is designed to interfere with pre-mating communications between female and male moths by permeating the atmosphere with synthetic female sex pheromones (Kydonieus and Beroza, 1982). Although the air-permeation technique becomes more efficient as the insect population level declines, infested fruits frequently appear due to accidental encounters between the female and male moths in orchards as well as the influx of gravid female moths from non-treated areas. In such situations, pesticides are sprayed to supplement the technique (Okazaki, 2000). These supplementary sprays increase the expenses incurred during the pest control. More than that, however, it is inconsistent with application policy of mating disruption, i.e. the reduction of pesticide sprays.

As an alternative to pesticide sprays, we propose an approach to exclude the infested fruits not only from the orchard but also after harvesting. Non-destructive detection of internal infestation by pests in apples may help minimize the occurrence of infested fruits in the market. A non-destructive inspection technique for quality management of agricultural products was developed at the US Department of Agriculture (USDA) in the 1960s (Iwamoto et al., 1994), and has been used for quality sorting of fresh fruits in Japan. The internal fruit quality, such as sugar content, total acidity, and water content is estimated by near-infrared (NIR) spectroscopy (Birth et al., 1985; Dull et al., 1989; Kawano et al., 1992). NIR spectroscopy has also been used successfully to detect insects hidden in individual wheat kernels (Dowell et al., 1998), parasitized weevils (Baker et al., 1999), and parasitized fly puparia (Dowell et al., 1999). If an adequate calibration is established for apples infested by *C. sasakii*, the infested apples can be excluded accurately during the quality-sorting process. As a result, complete exclusion of *C. sasakii* population will not be required, and the use of pesticide sprays will be reduced in orchards subjected to the mating disruption technique.

The present study investigated the availability of a prevalent NIR device for the detection of apples infested by *C. sasakii*. A calibration equation was developed from the spectral data and injury levels of infested fruits, and evaluated for its ability to detect the infested fruits accurately.

Materials and Methods

Sampling of the infested fruits was conducted in an apple orchard containing ca 200 trees in the experimental field at the Department of Apple Research, National Institute of Fruit Tree Science located in Morioka, Japan. Since pesticide sprays had not been used in this orchard for more than 10 years, almost all fruits were infested by *C. sasakii*. To obtain fruits with different injury levels, fenitrothion-fenvalerate was sprayed directly on each fruit of 14 trees ('Fuji' cultivar) using a hand sprayer on June 4, 2003, and on each fruit of another 14 trees on June 4 and 30, 2003. Conventional management practices were conducted to obtain high quality fruits during the fruiting season in this orchard, and 480 standard-sized fruits of the 'Fuji' cultivar were harvested on Nov 12, 2003.

Since the size and surface color of fruits affect the NIR transmittance spectra (Iwamoto, 1994), the mean diameter at the equator and red intensity (from 0 to 255, original values of null dimension of the sorting device) of the harvested fruits were measured using the fruit sorting device, "Color Grader®" (ACE04-1, Maki Manufacturing, Co. Ltd., Hamamatsu, Shizuoka, Japan). Fruits with extreme values for both these parameters were excluded from the analysis. Subsequently, the fruits were randomly separated into two sets, namely, calibration set and validation set. A calibration equation was developed from the analyses of the spectrum data and injury levels in the calibration set, and was evaluated against those of the validation set. Fruits from each set were cut into 12 pieces along their respective axes. The injury levels were determined based on the area of injury on both surface of each piece, because some adjoining surfaces showed different injury scores.

The injured region on the face of a piece was visibly scored into the following five ranks: 0, no injury; 1, less than a quarter of the face area was injured; 2, less than half of the face area was injured; 3, less than three quarters of the face area was injured; and 4, three quarters or more of the face area was injured.

The injury level of each fruit was determined by averaging the injured regions of 24 faces. Since the 'Fuji' cultivar usually has a water-core in its fruits, the occupancy of water-core on both faces of each piece was also scored in the same way as that for injury, and the water-core level of each fruit was determined in the same way to investigate the effects of the water-core levels on the accuracy of calibration.

Prior to determining the injury levels, the fruits were scanned for non-destructive fruit analysis, using an NIR device ("Fruits5®", FANTEC Research Institute, Kosai, Shizuoka, Japan) which is presently used to evaluate the sugar content of fruits, to obtain NIR transmittance spectra. In this device, fruits on a conveyor belt pass between halogen lamps that are located to illuminate the fruit on the belt from both sides. A detector located toward the bottom of the fruit receives light transmitted through the fruit (Fig. 1).

Using the program in the NIR device, the transmitted NIR spectra at 5 nm intervals from 650 nm to 1000 nm and injury levels of the fruits were analyzed. Relative absorbance was obtained by the following equation:

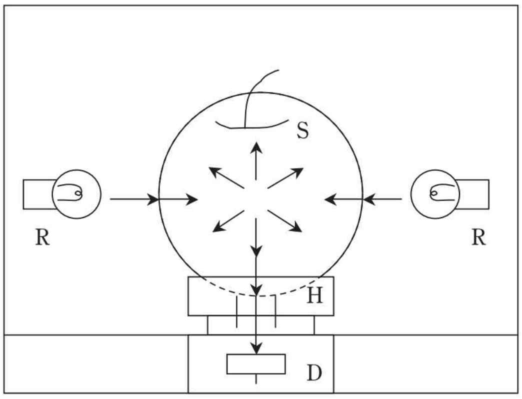

Fig. 1. Schematic illustration of the transmittance geometry in the scanning box of the prevalent NIR device. Fruits on a conveyor belt pass between halogen lamps that are located to light a fruit on the belt from both sides (5 lamps on each side), and the light passing through the fruit is received by a detector located toward the bottom of the fruit. Arrows show the light image. D: Detector; H: Holder on the conveyor belt; R: Radiation source, S: Sample.

$$As(\lambda) = \log(Ir(\lambda) / Is(\lambda))$$

where λ is the wavelength (nm), $As(\lambda)$ is the relative absorbance of samples at λ nm, $Ir(\lambda)$ is the intensity of light transmitted through the reference at λ nm, and $Is(\lambda)$ is the intensity of light transmitted through the samples at λ nm. The five most adequate wavelengths were selected by the technique, stepwise multiple linear regression, and the following equation was developed to estimate injury levels:

$$\text{Injury level} = K + K_1As(\lambda_1) + K_2As(\lambda_2) + K_3As(\lambda_3) + K_4As(\lambda_4) + K_5As(\lambda_5)$$

where K is the regression equation intercept and K_i is the regression coefficient of relative absorbance at λ_i nm.

Results and Discussion

As a result of the initial analysis, the calibration equation (Calibration 1) was developed as follows:

$$\text{Injury level} = 1.5456 - 25.66As(715\text{nm}) + 71.23As(730\text{nm}) + 20.13As(755\text{nm}) - 17.61As(800\text{ nm}) + 51.41As(825\text{ nm})$$

For the above equation, the multiple correlation coefficient was 0.8826 and the standard error of the calibration was 0.3732 (n=234) (Table 1, Fig. 2a). When the calibration equation was evaluated by samples of the validation set, visible scores of injury levels and predictive values of injury levels were correlated with a simple correlation coefficient of 0.8858 and a standard error of prediction of 0.3688 (n=234) (Fig. 2b). In the validation samples, the injury levels ranged from −0.36 to 0.84 in 79 of the uninfested fruits (white circles in Fig. 2b) and from −0.22 to 2.98 in 132 of the infested fruits (black circles in Fig. 2b). At the value of 0.37, 90.15% of the infested fruits

Table 1. Correlation coefficients of calibration equations

Calibration equation	Prop. water-core level[1]	Correlation coefficient[2]	SEC[3]	Correlation coefficient[4]	SEP[5]
Calibration 1	0.0	0.8826	0.3732	0.8858	0.3688
Calibration 2	0.2	0.8895	0.3343	0.8869	0.3392
Calibration 3	0.5	0.8844	0.3136	0.8832	0.3156
Calibration 4	0.7	0.8681	0.3269	0.8641	0.3303
Calibration 5	1.0	0.8290	0.3816	0.8285	0.3792

1: Proportion of water-core levels for adding the injured level of each fruit to compose the actual scores.
2: Multiple correlation coefficient when the calibration was developed.
3: Standard error of correlation for each multiple correlation coefficient.
4: Simple correlation coefficient between the actual injury levels and those estimated by calibration.
5: Standard error of prediction for each correlation coefficient.

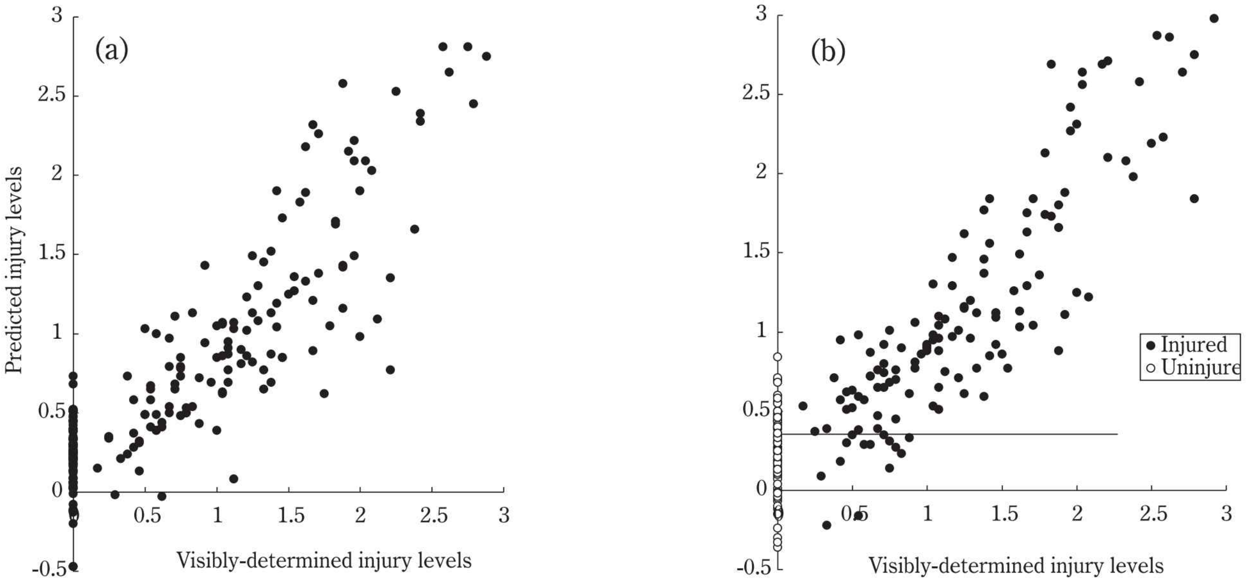

Fig. 2. Relationship between injury levels determined visibly and those predicted by the calibration equation (Calibration 1) that was developed by transmittance of five wavelengths (715, 730, 755, 800, and 825 nm). (a) Dot distribution of samples with a multiple correlation coefficient of 0.8826 and a standard error of calibration of 0.3732 (n=234) to establish the equation. (b) Dot distribution of samples with a simple correlation coefficient of 0.8868 and a standard error of prediction of 0.3688 (n=234) to evaluate the equation. White (○) and black (●) circles indicate uninjured and injured fruits, respectively. In the distribution of predicted values of injured and uninjured fruits, 39.24% of uninjured fruits are removed if 90.15% of injured fruits are discarded at the predicted value of 0.37 (horizontal line).

were excluded from the samples, and 39.24% of the uninfested fruits were also excluded (Table 2). Since the predicted injury levels of the uninfested fruits overlapped those of the infested fruits, the probability of detection using NIR was not high.

‘Fuji’ is a cultivar with much water-core, although its amount is influenced by weather conditions two month before harvesting. Water-core was contained in most samples in this study and may have affected the NIR measurement, because NIR absorption is small in the water-

Table 2. Proportion of uninfested fruits

Calibration equation	limit[1]	% injured fruits removed	% uninjured[2] fruits removed	Ref. Graph
Calibration 1	0.37	90.15%	39.24%	Fig2(b)
Calibration 2	0.48	90.91%	55.70%	Fig3(a)
Calibration 3	0.75	90.15%	63.29%	Fig3(b)
Calibration 4	0.92	90.15%	62.03%	Fig3(c)
Calibration 5	1.07	90.91%	70.89%	Fig3(d)

1: The level of estimates to discard ca 90% of infested fruits.
2: Proportion of uninjured fruits removed when ca 90% of injured fruits are removed by the calibration equation.

core. Apples with water-core showed higher transmittance of NIR than apples without water-core. Therefore, for the next analysis, water-core levels, which were multiplied by 0.2, 0.5, 0.7, and 1.0, were added to the injury levels to develop calibration equations with the injury-water-core (IWC) levels (Calibrations 2-5).

Each equation was developed with multiple correlation coefficients of more than 0.8 (Table 1), and predicted IWC levels correlated with visibly determined IWC levels, with simple correlation coefficients of more than 0.8 (Table 2). Of these five equations, Calibration 2 showed the highest correlation coefficients, a multiple correlation coefficient of 0.8895 with a standard error of calibration of 0.3343, and a simple correlation coefficient of 0.8869 with a standard error of prediction of 0.3392. However, the IWC levels of the uninfested fruits predicted by Calibration 2 ranged from

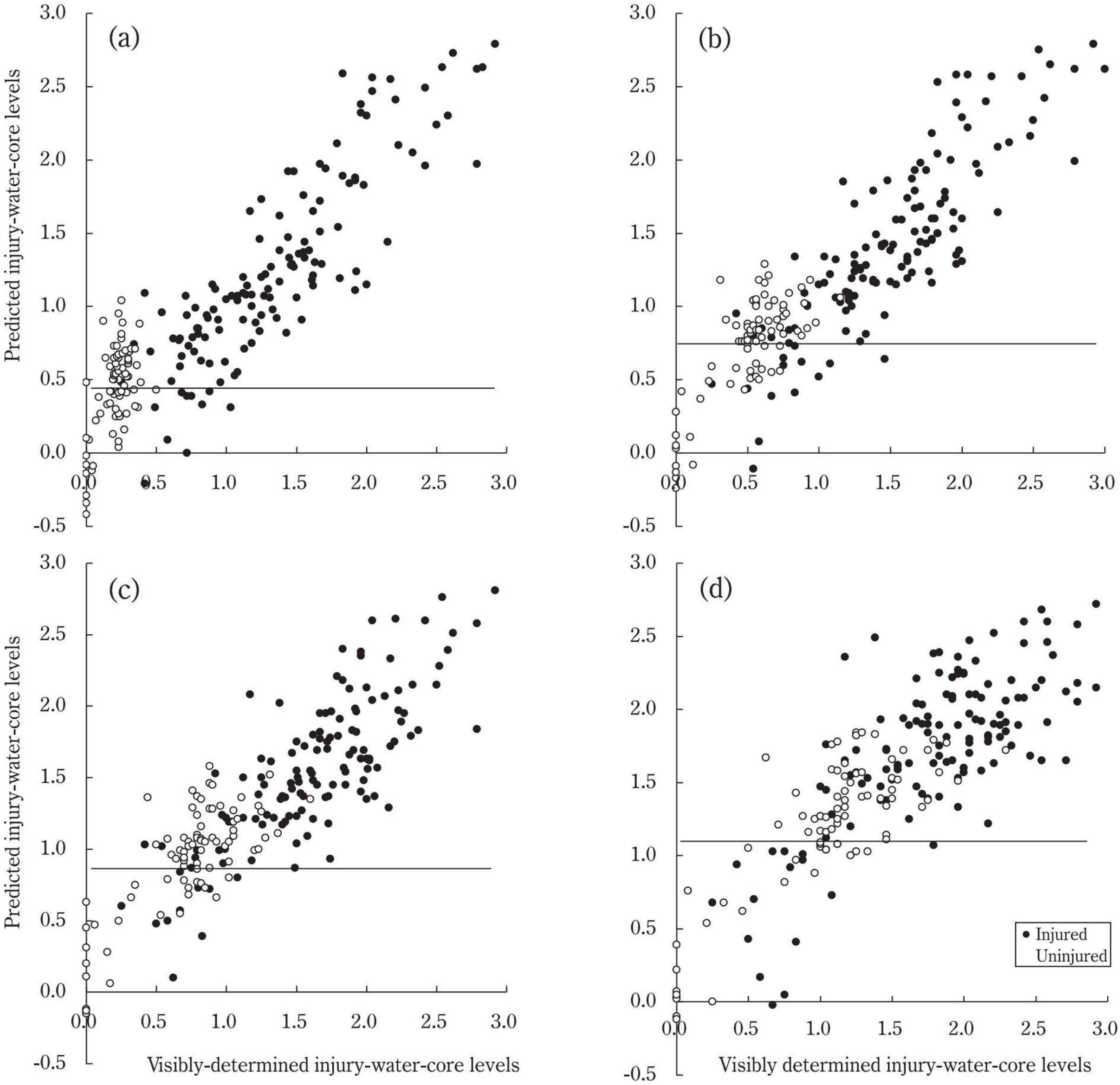

Fig. 3. Relationships between injury and water-core levels determined visibly and those predicted by the calibration equations (Calibration 2-5). All distributions are composed of validation samples. White (○) and black (●) circles indicate uninjured and injured fruits, respectively. Horizontal lines indicate the levels of predicted values when ca 90% of injured fruits are discarded. Correlation coefficient and its standard error of prediction, levels of prediction to exclude ca 90% of injured fruits, and the proportion of uninjured fruits excluded at the levels are presented in Table 2. Visible scores comprise injured fruits with (a) 0.2 times (20%) of water-core levels, (b) 0.5 times (50%) of water-core levels, (c) 0.7 times (70%) of water-core levels, and (d) 1.0 time of (100%) water-core levels.

−0.42 to 1.04 and overlapped those of the infested fruits (Fig. 3a).

At the value of 0.48, when 90.91% of the infested fruits were excluded from the samples, 55.70% of the uninfested fruits were also excluded (Table 2). In the same way, the IWC levels of the uninfested fruits predicted by Calibrations 3, 4, and 5 ranged from −0.24 to 1.29, −0.25 to 1.58, and −0.12 to 1.84, respectively, and overlapped the predicted levels of the infested fruits (Fig. 3b-3d). As a result, more than 60% of the uninfested fruits were excluded when ca 90% of the infested fruits were excluded based on each equation (Table 2).

The change in estimates of uninfested fruits (injury level = 0) caused by adding the water-core level to injury level may have improved the correlation between visibly-determined and predicted levels. As a whole, however, the addition of water-core estimates to injury estimates did not improve but rather hindered its accurate detection. In order to know the effect of water-core on the detection of injury, an apple cultivar without water-core has to be used to clarify the relationship between water-core and internal injury.

The previous cited examples of using this technology to detect insects involved only very small targets (Dowell et al., 1998; 1999; Baker et al., 1999), but apples are big fruits. Therefore, it is a major accomplishment that the NIR technology has the potential to detect apples infested by *C. sasakii*.

There are several reasons why infested apples were not detected accurately by the prevalent device and procedure of the NIR spectroscopy. Mechanically, the prevalent device collects NIR spectra from the bottom but not the top of apples (Fig. 1). So, NIR measurements may not provide information on the whole apple.

Statistically, frequency of injury levels was not normally distributed but may be distorted to disturb the regression analysis. Also, NIR measurements have to be adequately transformed. Chemically, as the most important reason, selected wavelengths in the equations were not related directly to the injury. Although the NIR region is composed of radiation with wavelengths of 700-3000 nm (Hruschka, 2001), the priority feature of radiation used in the prevalent device is permeability of NIR spectra to samples which are relatively large and contain more than 80% water.

NIR spectra in the range from 650 to 1000 nm are related to overtones of C-H, O-H and N-H stretching, but not related to complex chemical bonds of certain proteins, lipids and carbohydrates (Iwamoto et al, 1994). Although the NIR region used in the device was fixed, mechanical and statistical improvement of the prevalent device may enhance the probability for detecting infested fruits.

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近赤外線分光法を利用したモモシンクイガに加害されたリンゴの識別の試み

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摘　　要

モモシンクイガに加害されたリンゴ‘ふじ’を近赤外線分光法で識別できるか，現行の近赤外線非破壊選果機を使って評価した．被害果の近赤外スペクトルデータと被害度を分析して検量線を作成したところ，検量線による予測値と実測の被害度との間に相関係数0.8868±0.3688の相関関係があることがわかった．被害果の約90％を除去する被害度の予測値0.37において，90.15％の被害果と同時に39.24％の健全果も除去されるので，十分な識別率とは言えないが，現行の近赤外線選果機でモモシンクイガ被害果の識別の可能性が示唆された．