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原著論文

The Ability of a Double-Sensored NIR Device to Detect Apples ('Fuji' Cultivar) Injured by the Peach Fruit Moth, *Carposina sasakii* Matsumura (Lepidoptera: Carposinidae)[†1]

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Abstract

We evaluated the ability of a near infrared (NIR) spectroscopy device to detect apples ("Fuji" cultivar) injured by the peach fruit moth, *Carposina sasakii*. The scanned NIR spectra of whole fruit were collected using two detectors located at the top and base of the inspection box in this device. Further, several calibration equations were developed using injured apples harvested in 2004 and 2005. The calibration developed with the apples harvested in 2004 predicted injured apples at 0.87±1.95 (r±SEP). When validated, an accuracy of this calibration was calculated at 92.4%. The calibration developed with the apples harvested in 2005 predicted injured apples at 0.85±2.79. When validated, the accuracy of this calibration was calculated at 94.5% for the apples harvested in 2004 and 93.7% for apples in 2005, respectively.

Key words: apple, *Carposina sasakii*, near-infrared spectroscopy, nondestructive inspection, calibration

Introduction

The peach fruit moth, *Carposina sasakii* Matsumura, is the most serious pest of pome fruits in Japan, Korea, China, and Russia (Narita, 1986). The first-instar larvae bore into fruits with a microscopic sign on the surface, eat the pulp of fruits, and develop in them. After growing in the fruits, the fifth-instar larvae make a hole to escape from the fruits onto the ground for pupation. Since the hole made by these larvae is visible, the fruits with the hole are usually recognized as injured fruits during the fruiting season and after harvesting. On the other hand, it is difficult to detect the microscopic signs by the first-instar larvae on the surface of the fruits even after harvesting. Infested fruits may be packed and sold if the larvae remain in them. To avoid the packing and selling of injured fruits, growers must spray insecticides at two-week intervals to prevent the invasion of first-instar larvae of *C. sasakii* into fruits.

As an alternative way to avoid the distribution of injured fruits in the market, we focused on nondestructive detection by near infrared (NIR) spectroscopy. In Japan, NIR spectroscopy is used to predict fruit quality, mainly sugar content, for quality sorting of fresh fruits (Kawano et al., 1992). NIR spectroscopy applied to agricultural products is based on the permeability of NIR and multivariate analysis of NIR absorbance (Iwamoto et al., 1994). This may also be applied to detect injured fruits. In the United States, NIR spectroscopy has been used successfully to detect

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insects hidden in individual wheat kernels (Dowell et al., 1998), parasitized weevils (Baker et al., 1999), and parasitized fly puparia (Dowell et al., 1999). In Japan, apples injured by *C. sasakii* were investigated using a commercial NIR device on a trial basis (Toyoshima et al., 2006). The base of the inspection box of this device has a detector for NIR spectra (Fig. 1a), which analyzes the NIR spectra passed through the lower part of the fruits. Injured areas may not be biased at the lower part; rather, they may be distributed in the whole fruit. With the help of this device it was detected that 40% of the uninjured fruits was missed as injured when 90% of the injured fruits was estimated as injured fruits.

In this study, another commercial NIR device was used to detect injured apples. The device has two detectors located at the top and base in the inspection box (Fig. 1b), and it analyzes NIR spectra at 2-nm intervals. The dual detectors may detect the injured areas at the upper as well as lower part of fruits. The analysis of 2-nm intervals may be more sensitive to detect the change of NIR absorbance than the device used in the previous paper that analyzes NIR spectra at 5-nm intervals (Toyoshima et al., 2006). In this study, we evaluate the calibration equations developed with apples harvested in 2004 and 2005 and discuss the possibility of excluding injured fruits with an NIR device.

Materials and Methods

Collection of injured fruits

Apple fruits ("Fuji" cultivar) naturally infested by *C. sasakii* were harvested on November 15, 2004, from an unsprayed apple orchard in our experimental field.

In 2005, a strain of *C. sasakii* (Ishiguri and Shirai, 2004) was used to make injured apples in a sprayed orchard in our experimental field. *C. sasakii* eggs collected from a stock culture were attached on a weekly basis to the surface of 100 apple fruits ("Fuji" cultivar) from June 29 to August 29, 2005. The infested fruits were wrapped with paper bags during the experimental period to prevent additional oviposition by the *C. sasakii* inhabiting the orchards. Conventional management was conducted in this orchard during the fruiting season. As samples for NIR practice, fruits (100–120 mm in diameter) were harvested on November 7, 2005.

Collection of NIR spectra and determination of injury level of fruits

Fruits were randomly grouped into two sets, namely, a calibration set and a validation set. A calibration equation was developed from the multiple regression analyses of the NIR spectral data and the degree of injury ("injury level" hereafter) of fruits in the

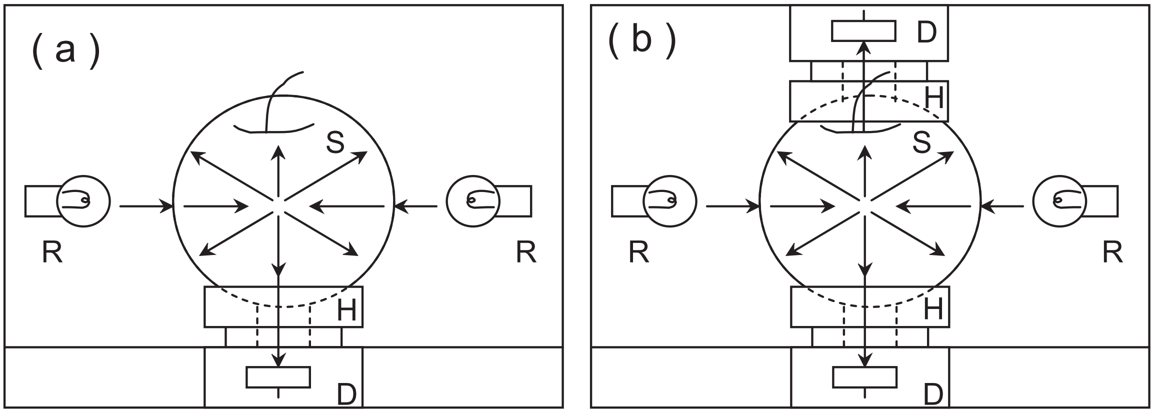

Fig. 1. Schematic illustration of transmittance geometry in the inspection box of the single-sensored (a) and double-sensored (b) NIR devices. The fruits were placed on a conveyor belt and passed between the halogen lamps positioned to illuminate the fruit on the belt from both sides (five lamps on each side). The transmittance of light passing through the fruit was measured by a detector positioned toward the lower portion of the fruit (a) and by two detectors positioned toward the upper and lower portions of the fruit (b). The arrows indicate the light image. D: Detector. H: Holder on the conveyor belt (a and b), and movable holder to cover the top of the fruits (b); the latter was attached to the roof of the inspection box (b). R: Radiation source (halogen lamps). S: Sample.

calibration set. It was validated against the spectral data and injury level of fruits in the validation set. Prior to the determination of the injury level, the fruits were scanned by the NIR device ("FQA-21®", FANTEC Research Institute, Kosai, Shizuoka, Japan) to obtain NIR spectra. In this device, the fruits were placed on a conveyor belt and passed between halogen lamps that illuminate them from both sides. Two detectors that are located at the top and base of the inspection box (Fig. 1b) received NIR transmitted through fruits, and were measured at 2-nm intervals of wavelength ranged from 650 nm to 1000 nm.

Detection should be conducted binomially as "injury" or "no injury"; however, the analysis of multiple regressions is usually used in the NIR spectroscopy for intact fruits (Iwamoto et al., 1994). Therefore, the injury level of fruits must be determined as continuous variables for regression analysis. Ideally, the cubic volume of the injured region in an injured fruit was measured as the injury level of the fruit. In this study, however, while the actual volume of the injured region was not measured, the injury level was mimetically determined by scoring three injury indices on the surface of and inside the fruits.

Initially, the injury index on the surface of the fruits was visibly scored as 0 (without hole) or 1 (with hole). As the second injury index, the amount of feces accumulated in the core of the fruits was estimated because NIR is absorbed efficiently into the black materials as the feces produced mainly by fifth-instar larvae of *C. sasakii*. The fruits were cut into two pieces along their vertical axes, and both faces of the section were digitally photographed. Based on the digital pictures, the amount of feces in the core of fruits was scored into five ranks: 0: no feces; 1: less than a quarter of core filled with feces; 2: less than half of the core filled with feces; 3: less than three quarters of the core was filled with feces; and 4: more than three quarters of the core was filled with feces.

As the third injury index, the degree of the injured pulp of fruits was estimated. The fruits were cut into 12 pieces along their vertical axes, and the number of pieces injured was counted to obtain the injury index. In addition, both faces of each piece were digitally photographed for further estimation of injury level. Based on the digital pictures, the injury index of the face of each piece was scored into the five ranks mentioned above. The injury and feces in the core of fruits was not estimated in this scoring process. The scores of the 12 pieces were averaged after scoring each piece.

At last, the injury level of each fruit was obtained by summing three injury indices of the surface, core, and face of 12 pieces (counted (Calibration 1) or averaged scores (Calibration 2)).

In the treatment in 2005, injured apples were obtained by artificial infestation in which a few eggs were attached on the surface of apples. Therefore, among the samples, there were many apples with 0 injury because *C. sasakii* larvae do not develop well in intact apples (Kim and Lee, 2002; Ishiguri and Toyoshima, 2006). As a result, the frequency distribution of the injury level was skewed in the 2005 samples. Another quantity index for uninjured fruits must be determined by the other criteria to improve the skew distribution. The commercially available calibration for detecting browning apples ("F-DAS®", FANTEC Research Institute) efficiently detects apples that will become brown in the near future. In the calibration using the samples harvested in 2005, therefore, the degree of browning of uninjured fruits was determined by the calibration. The degree of browning was scored between 0 and 3 at an interval of 0.5 (Calibration 3). Then, the index "3" was added to each injury level of injured fruits.

Data analysis

The transmitted NIR spectrum at a wavelength was obtained as the relative absorbance of a band, which was calculated by the equation $As(\lambda) = \log(Ir(\lambda)/Is(\lambda))$, where λ is the wavelength (nm); $As(\lambda)$, the relative absorbance of the samples at λ nm; $Ir(\lambda)$, the intensity of light transmitted through the reference at λ nm; and $Is(\lambda)$, the intensity of light transmitted through the samples at λ nm. The relative absorbance of the spectra was applied to second derivatives in order to separate overlapping bands and to remove baseline shifts (Iwamoto et al., 1994). Next, the values of injury level and second derivatives were used to develop equations for calibration. The analytical procedure was based on a modified stepwise multiple linear regression using the software "F-DAS®" incorporated in FQA-21. In the analytical procedure, five wavelengths were selected as variables to minimize both the standard error of prediction (SEP) and standard error of calibration (SEC) (Iwamoto et al., 1994). The following equation was developed to predict the injury level:

$$\text{Injury level} = K + K_1 d^2\text{As}(\lambda_1) + K_2 d^2\text{As}(\lambda_2) + K_3 d^2\text{As}(\lambda_3) + K_4 d^2\text{As}(\lambda_4) + K_5 d^2\text{As}(\lambda_5)$$

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

Two equations were developed by the NIR spectra collected from two detectors located at the top and base in the inspection box, and these equations were validated using the fruits in the validation set. An equation derived from the NIR spectrum measured by the top detector was used to predict the injury level of the upper portion of the fruits, while another equation derived from the NIR spectrum measured by the base detector was used to predict the injury level of the lower portion of the fruits. During the prediction procedure, the two injury levels predicted by these two equations were compared and the higher value was adopted as the injury level of a fruit; this is because the higher value represents the injury level in the upper or lower portion of the fruits. Finally, the detection threshold was determined to minimize the error of prediction. Apples are usually predicted into four categories. If the injury level of injured apples was higher than the detection threshold, the injured apples could be predicted as injured apples by the calibration (A). The injured apples with a lower injury level than the detection threshold could be missed as uninjured apples by the calibration equations (B). Uninjured apples were also checked for whether their injury level were lower or higher than the detection threshold. If the uninjured level of uninjured apples was lower than the detection threshold, the uninjured apples could be predicted as uninjured apples by the calibration (C). The uninjured apples with a higher injury level than the detection threshold could be missed as injured apples by the calibration (D). The error of prediction was described as B+D. The accuracy of the calibration equations was calculated as following equation: Accuracy (%) = 100*(the number of A+ the number of C)/total number of apples investigated.

Results and Discussion

A simple correlation coefficient (*r*) with SEP and the accuracy of two calibrations, which have two equations derived from NIR spectra collected at the top and base detectors, are listed in Table 1. Calibration 1 was developed with the injury level, which were obtained by summing the three injury indices of the surface, core, and face of the 12 pieces (counted) of the apples harvested in 2004. Calibration 2 was developed with the injury levels, which were obtained by summing the injury indices of the surface, core, and face of the 12 pieces (averaged) of the apples harvested in 2004. When the equation developed by the top detector's spectra was used for detection, the simple correlations between the actual and predicted injury levels by the equation were 0.85±2.20 in Calibration 1 and 0.84±2.11 in Calibration 2. When two equations developed by the top and base detectors' spectra were used for detection, the simple correlations were 0.88±2.06 in Calibration 1 and 0.87±1.96 in Calibration 2. In both calibrations, the accuracy of detection with two equations (top and base) was higher than that with a single equation (top or base). These results indicate that the dual detection of NIR with top and base detectors was useful to increase the accuracy of detecting injured apples, compared with the current NIR device with the base detector. The accuracy of detection in Calibration 2 was relatively higher than that in Calibration 1 in each position of the detectors. Therefore, the calibration development of NIR from two detectors by averaging visibly determined injury areas was effective and useful to obtain calibration equations with high accuracy for the inspection of injured fruits.

Details of Calibrations 2 and 3 are shown in Table 2. Calibration 2 in Table 2 is the same as that in Table 1. The multiple correlation coefficient (*R*) and standard error of correlation (SEC) in Calibration 2 were 0.87 and 1.98 for the top detector and 0.85 and 2.18 for the base detector, respectively. When validated with apples harvested in 2004, the correlation between actual and predicted injury levels is shown in Fig. 2. In the validation samples (n = 236), the injury levels

Table 1. Comparison of accuracy to detect injured fruits by single and dual detectors

Position of Detector(s)	Calibration 1		Calibration 2	
	r ± SEP	Accuracy	r ± SEP	Accuracy
Top	0.85 ± 2.20	84.3%	0.84 ± 2.11	86.4%
Base	0.85 ± 2.07	85.6%	0.84 ± 2.08	88.1%
Top & Base	0.88 ± 2.06	90.3%	0.87 ± 1.96	92.4%

Calibration and validation were conducted by the injured apples harvested in 2004.

Table 2. Two sets of calibration equations for the NIR device with dual detectors to estimate the apple fruits ("Fuji" cultivar) injured by *C. sasakii* larvae

Name	Position [1]	Equation	R ± SEC
Calibration 2	Top	y= 9.54 -22.96 d^2As(680) +38.71 d^2As(700) -266.23 d^2As(720) +215.31 d^2As(736) + 93.29 d^2As(808)	0.87 ± 1.98
	Base	y= 10.48 -25.36 d^2As(680) +50.50 d^2As(696) -236.55 d^2As(716) +220.83 d^2As(744) + 99.50 d^2As(804)	0.85 ± 2.18
Calibaration 3	Top	y= 22.52 -43.36 d^2As(680) +72.08 d^2As(696) -288.84 d^2As(716) +356.91 d^2As(748) + -65.35 d^2As(916)	0.86 ± 1.55
	Base	y= 23.62 -36.96 d^2As(680) +81.92 d^2As(696) -444.71 d^2As(716) +422.43 d^2As(732) +-679.42 d^2As(840)	0.82 ± 1.78

[1] Location of detectors in the NIR device.

Calibration 2 and 3 were developed by injured apples harvested in 2004 and 2005, respectively.

ranged from –0.5411 to 3.4570 in 42 uninjured apples (white circles and triangles in Fig. 2) and from 0.8254 to 13.5392 in 194 injured apples (black circles and triangles in Fig. 2). At the predicted injury level of 2.50 as the detection threshold for this calibration, 6.78% of the injured apples (black triangles in Fig. 2) passed through the inspection procedure as uninjured apples, while 0.85% of the uninjured apples (white triangles in Fig. 2) were excluded as injured apples.

Calibration 3 was developed with the injury levels, which was obtained by summing the scores of the surface, core, and face of the 12 pieces (averaged) of the apples harvested in 2005. The injury levels of uninjured fruits were also determined by the calibration for browning in apples. Then, multiple correlations were obtained as 0.86±1.55 for top and0.82±1.78 for base detectors. When validated by apples in 2005, the simple correlation was 0.89±1.39 (n = 284), shown in Fig. 3a. In the validation samples, the predicted injury level ranged from –0.65 to 3.90 in 177 uninjured apples (white circles and triangles in Fig. 3a) and from 0.98 to 13.32 in 107 injured apples (black circles and triangles in Fig. 3a). At the predicted injury level of 4.00 as the detection threshold for this calibration, 3.17% of the injured apples (black triangles in Fig. 3a) passed through the inspection procedure as uninjured apples, while 3.17% of the uninjured apples (white triangles in Fig. 3a) were excluded.

When Calibration 3 was also validated by the validation samples harvested in 2004, the correlation between actual and predicted injury levels was 0.85± 2.79 (Fig. 3b). In the validation samples, the predicted injury levels ranged from –1.36 to 5.48 in 42 uninjured apples (white circles and triangles in Fig. 3b) and from 1.39 to 14.16 in 194 injured apples (black circles and triangles in Fig. 3b). At the predicted injury level of 4.00 as the detection threshold for this calibration, 4.66% of the injured apples (black triangles in Fig. 3b) passed through the inspection procedure as uninjured apples, while 0.85% of the uninjured apples (white triangles in Fig. 3b) were excluded.

The accuracy of detecting injured apples was calculated at 92.4% in Calibration 2, 94.5% in Calibration 3 when validated by apples harvested in 2004, and 93.7% in Calibration 3 when validated by apples harvested in 2005. In the previously cited study, it was shown that wheat kernels containing third- and fourth-instar rice weevil larvae could be detected by NIR with greater than 95% confidence (Dowell et al., 1998). Almost all studies on this technology for

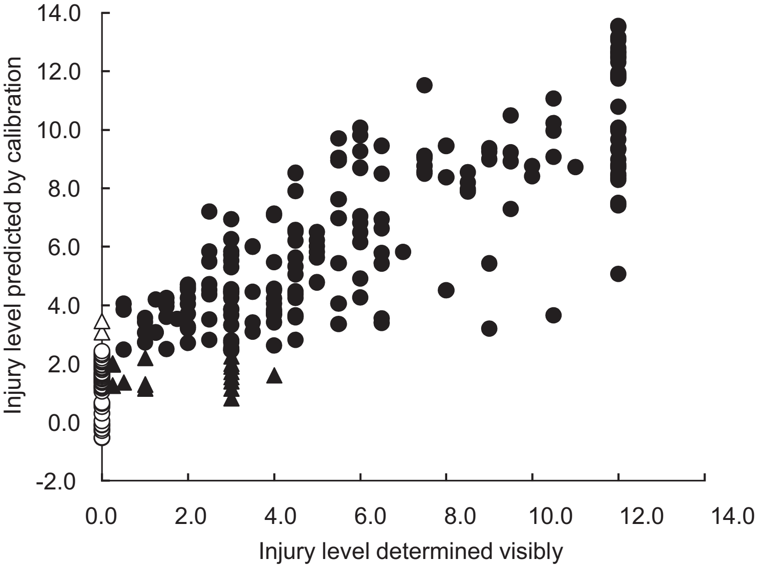

Fig. 2. Relationship between the injury levels determined visibly and those predicted by Calibration 2 after validating the apples harvested in 2004. The simple correlation coefficient and SEP were 0.87 and 1.96, respectively (*n* = 236) and the accuracy was 92.4% . Abbr.: ○ – Uninjured apples; △ – Uninjured apples with higher injury level than 2.50; ▲ – Injured apples with lower injury level than 2.50; ● – Injured apples.

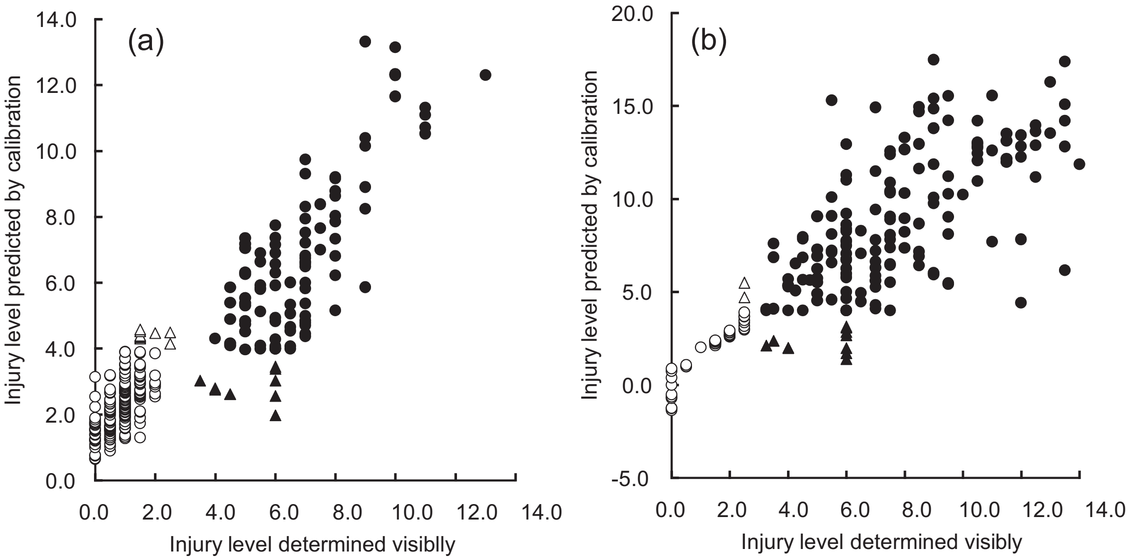

Fig. 3. Relationship between the injury levels determined visibly and those predicted by Calibration 3, in which the injury level of uninjured apples was determined by the commercially available calibration for browning. (a) The injury levels obtained by the Calibration 3 after validating the apples harvested in 2005. The simple correlation coefficient and SEP were 0.89 and 1.39, respectively (*n* = 284), and the accuracy was 93.7% . (b) The injury levels obtained by Calibration 3 after validating the apples harvested in 2004. Simple correlation coefficient and standard error of prediction were 0.85 and 2.79, respectively (*n* = 236), and the accuracy was 94.5% . Abbr.: ○ – Uninjured apples; △ – Uninjured apples with higher injury level than 4.00; ▲ – Injured apples with lower injury levels than 4.00; ● – Injured apples.

detecting insects involved the use of small-sized fruits as samples (Dowell et al., 1998, 1999; Baker et al., 1999). On the other hand, the apple fruits are big and juicy samples; this condition is not optimal for nondestructive detection with NIR. Therefore, it is a major accomplishment that the NIR technology has a potential to detect apples injured by *C. sasakii*.

For further improvement, effective wavelengths should be used to detect injured apples. In the present study, we used a wavelength ranging from 680 to 916 nm (Table 2); this wavelength range does not match with the wavelength region that corresponds to the physical and biochemical characteristics of the insect larvae. A relatively longer wavelength region, i.e., more than 1000 nm, corresponds to the C-H overtone and combination regions. As previously reported, the C-H overtone regions correspond to the chitin absorption regions (Dowell et al., 1998). However, this wavelength region is strongly absorbed in juicy fruits. Therefore, optic characteristics and detector sensitivities must be improved to use the effective wavelength region for detecting insect larvae in apple fruits. NIR spectroscopy is now expected to detect injured and/or rotten fruits as well as to predict the fruit quality. Since the NIR device used in this study is now commercially available, NIR spectroscopy would provide an opportunity to use this technology at the market place if the accuracy to detect *C. sasakii*-injured fruits is further improved.

Acknowledgments

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ダブルセンサー近赤外装置のモモシンクイガ加害リンゴの検出精度

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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，上下で透過スペクトルを検出する近赤外線非破壊選果機を使って評価した．2004年および2005年に収穫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，2004年のサンプルで作成したCalibration 2による予測値と実測の被害度との間に0.87 ± 1.95（相関係数±予測誤差）の相関関係があり，判別精度は92.4%であった．2005年のサンプルで作成したCalibration 3を2004年のサンプルで評価すると，予測値と実測の被害度との間に0.85 ± 2.79の相関関係があり，判別精度は94.5%であった．Calibration 3を2005年のサンプルで評価すると，予測値と実測の被害度との間に0.89 ± 1.39の相関関係があり，判別精度は93.7%であった．