N O T E

Detection of Spotted-winged Drosophila (Diptera: Drosophilidae) Infestations in Blueberry Fruits[1]

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Spotted-winged drosophila, *Drosophila suzukii* Matsumura (Diptera: Drosophilidae), has become a significant pest of small and stone fruit. Unlike most *Drosophila* species, it tends to infest healthy, intact ripe fruit, as opposed to rotting or overripe fruit (Mitsui et al. 2006, Popul. Ecol. 48:233–237; Asplen et al. 2015, J. Pest Sci. 88:469–494). Spotted-winged drosophila adults are typically detected in the field using baited traps. This is useful in helping growers decide when to apply insecticides (Ebbenga et al. 2022, J. Entomol. Sci. 57: 516–529), but methods are also needed to estimate actual fruit infestation levels. Spectral imaging of fruit may provide a nondestructive alternative to extraction of larvae and could provide information on the infestation status of a single fruit. Such imaging has been tested for insect pests other than *D. suzukii*. For example, Peshlov et al. (2009, J. Near Infrared Spectrosc. 17:203–212) used near-infrared spectroscopy (NIRS) to detect infestation of wild blueberries (*Vaccinium*) by blueberry maggot, *Rhagoletis mendax* Curran (Diptera: Tephritidae). By measuring spectra of a live larva and subtracting it from an infested blueberry, they demonstrated that the NIR signal they recorded was from a larva and "associated chemical changes in the blueberries." Detectable differences between infested blueberry and larvae occurred between approximately 750 and 1300 nm, with a small differential signal at 600 nm. Tsuta et al. (2006, Food Sci. Technol. Res. 12:96–100) also used spectroscopy to discriminate between blueberry fruit and "foreign substances." They measured the spectra of various foreign substances, including worms, separately from the fruit. They detected a difference in the second derivative of absorbance between worms and berries between approximately 625 and 675 nm.

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The F-750 Produce Quality Meter (Felix Instruments, Camas, WA) is a handheld visible and near-infrared spectrometer that has been used to predict ripeness of various fruit correlated with fruit constituents, such as dry matter content of cherry, *Prunus avium* L. (Toivonen et al. 2017, Can. J. Plant Sci. 97:1030–1035). Light transmitted from the device's xenon tungsten lamp interacts with the scanned object and then is transmitted near the zone of illumination where the signal is recorded. The portability of this device allows its use in agricultural fields and may be useful beyond postharvest fruit quality determination. Infestation levels of *D. suzukii* eggs and first instars are undetectable by the human eye. If the F-750 meter is capable of distinguishing infested from uninfested fruit, the device would allow sorting or culling by small-acreage farmers who may not have storage facilities cold enough to arrest *D. suzukii* development (Aly et al. 2017, J. Econ. Entom. 110:87–93). This work was conducted to determine to what extent infestation can be detected using the F-750 spectrometer in blueberries and raspberries (*Rubus*).

To test the possibility that the F-750 spectrometer could be used to detect *D. suzukii*, in-field infestation assays and artificial infestation assays with store-bought blueberry fruit in the laboratory were initiated. For field infestation samples, 'Northblue' and 'Chippewa' blueberries (*V. corymbosum* L. × *V. angustifolium* Ait.) were harvested from the Sand Plain Research Farm of the University of Minnesota in Becker, MN (45°23′36.42″N, 93°52′36.3″W). Berries were collected weekly between 5 and 26 July 2017 (100 in week 1 and then 50 each in weeks 2, 3, and 4), scanned with the F-750 spectrometer in the laboratory, and then placed in 30-mL containers (Dart Container Corp., Mason, MI), with one fruit per container. The containers were incubated at room temperature (21–23°C), and fly emergence was recorded 1 wk later.

Artificial infestation assays were initiated with store-bought blueberry fruit in the laboratory. Blueberries were purchased from a grocery store, and berries with disease, previous insect infestation, or turgor loss were discarded prior to artificial infestation. Scans of fruit that were infested were compared with that of uninfested fruit and fruit that were uninfested but poked using an insect pin (BioQuip Insect Pins, Black, #0, Bioquip Products, Rancho Dominguez, CA) to simulate ovipositor injury without the presence of eggs or larvae. To infest fruit, individual berries were incubated with two mated *D. suzukii* females for 24 h in 30-mL clear plastic containers with a square of filter paper to absorb excess liquid. Adult flies were removed after 24 h, after which all containers were placed in a growth chamber at 23–25°C, with a 16-h light:8-h dark photoperiod. Individual fruits were scanned 4 or 7 d after infestation. Control treatments included (1) fruit not poked and not infested and (2) poked but not infested.

One-way blind tests were conducted in 2021. Intact fruit were rinsed three times with deionized water, air dried, dipped in 2% (v/v) propionic acid (Fisher Scientific, Hampton, NH) for 5 s to inhibit mold growth, air dried, and then poked with an insect pin to provide ovipositor entry sites. Individual fruits were placed in 30-mL containers and randomly assigned to an infested or uninfested category using R statistical software. Five female and five male (to insure mating) flies, approximately 3 d old, were added to each "infested" treatment container. No flies were added to containers serving as untreated controls. All containers were placed in a growth chamber kept at 23–25°C, with a 16-h light: 8-h dark photoperiod. After 36 h, flies were removed, and berries were viewed under a stereo microscope to check for the presence of egg breathing filaments. Containers of berries were then delivered to

the scanner who did not know which containers contained infested fruit. Fruits were scanned 48, 72, and 120 h after initial infestation and scored for larval emergence 7 d after introduction to *D. suzukii* adults.

Each berry was scanned once with the F-750 Produce Quality Meter. Blueberries were scanned with pedicel-ends down on the light-emitting surface. A ring was fabricated from Delrin acetal homopolymer (DuPont, Wilmington, DE, USA) to fit on top of the 11-mm reflector cone to improve the support of berries over the central ring of the small fruit adapter provided by the manufacturer. Only the machine-computed second derivatives of absorbance data were collected. The routine used by the F-750 meter can be explained as a nine-point second order Savitzky-Golay filter (Savitzky and Golay 1964, Anal. Chem. 36:1627–1639).

The accuracy rates of one-way blind predictive tests were conducted according to Hodgson et al. (2004, J. Econ. Entomol. 97:2127–2136). The probability of making a correct determination of infestation across all data sets $= \Sigma p_i$ (true positives$_i$ + true negatives$_i$), where p_i is the proportion of n data sets represented by set i; true positives$_i$ is the probability of correctly identifying infestation; and true negatives$_i$ is the probability of correctly identifying no infestation. Because there were seven data sets of similar size (50–99 berries), $p_i = 0.143$.

Field-harvested 'Chippewa' and 'Northblue' (Fig. 1A) berries showed similar patterns of spectral changes with harvest week. Mean second derivatives of absorbance in the 660- to 690-nm range increased, but decreased in the 692- to 715-nm range over time. None of the berries from harvests 1 and 2 were infested with *D. suzukii*. Twenty percent of harvest 3 and 96% of harvest 4 'Chippewa' berries and 42% of harvest 3 and 100% of the harvest 4 'Northblue' fruit were infested. Thus, mean second derivatives of absorbance of infested fruit were greater than that of uninfested fruit in the 660- to 690-nm range and less in the 692- to 715-nm range. Similarly, mean second derivatives of absorbance in the 660- to 690-nm range of artificially infested berries were greater, whereas those in the 692- to 712-nm range were less than those of berries that were uninfested or poked (Fig. 1B). Although scans of the field-grown and laboratory-infested berries suggested that spectral patterns could be used to discriminate between uninfested and infested fruit, accuracy of one-way blind assays varied from 53% to 71%, whereas error rates varied from 29% to 47% (data not shown), and the proportion of correct true positives was 0.13, whereas that of true negatives was 0.48 (Table 1). The method was better able to predict negatives (specificity) than positives (precision or recall).

The inability of the device used in this study to accurately predict positive infestations may be due to its decrease in signal-to noise ratio at about 980 nm. However, data obtained at wavelengths greater than 1000 nm may improve discrimination of infested and uninfested blueberries. Also, improved statistical analysis using partial least squares modeling and the use of machine learning algorithms could improve the predictive ability of spectrometers to detect *D. suzuki* infestation in berries.

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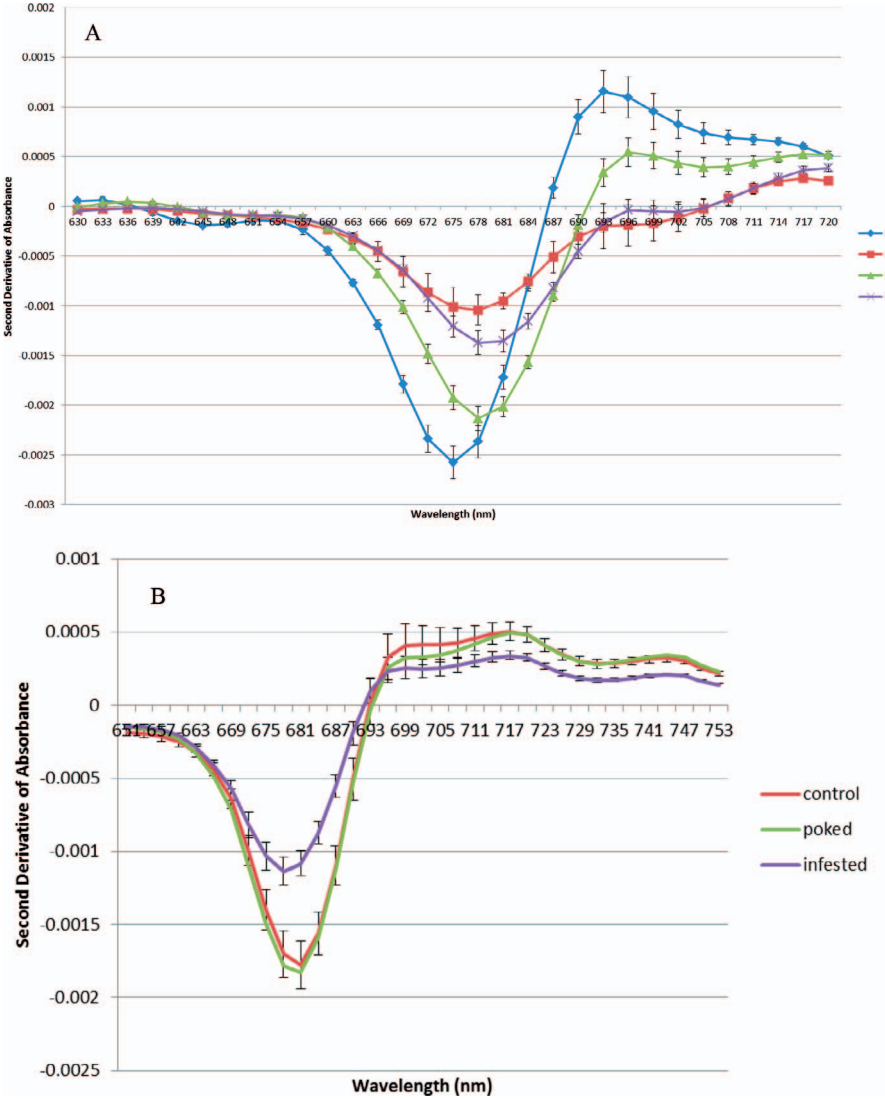

Fig. 1. Mean second derivatives of absorbance spectra for four weekly harvests (H1–H4) of 'Northblue' (A) blueberries and of store-bought blueberries left uninfested (control) (B), poked but not infested (control to simulate ovipositor injury without the presence of eggs or larvae), and artificially infested. Scans were taken 4 d after spotted wing drosophila were introduced to store-bought blueberries. Individual spectra were obtained from 50–100 berries for each experiment. Error bars indicate standard errors of means.

Table 1. Probability of correct and incorrect detections of eggs and/or larvae of spotted wing drosophila based on scans using the Felix F-750 spectrometer of infested blueberries.

		Proportion Correct		Proportion Incorrect	
Scanning Date	Insect Stage	True Positives	True Negatives	False Positives	False Negatives
10 February 2020	Larvae	0.07	0.63	0.23	0.07
10 March 2020	Eggs	0.12	0.44	0.08	0.36
4 January 2022	Eggs	0.20	0.46	0.17	0.17
6 January 2022	Eggs or larvae	0.04	0.61	0.19	0.17
19 January 2022	Eggs	0.15	0.38	0.22	0.25
21 January 2022	Eggs or larvae	0.14	0.47	0.13	0.26
23 January 2022	Larvae	0.16	0.4	0.2	0.24
	Sum	0.89	3.39	1.22	1.50
	Proportion correct or incorrect	0.13	0.48	0.17	0.22
	Correct total	0.61			
	Incorrect total	0.39			