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Research Paper

A multispectral imaging system using solar illumination to distinguish faecal matter on leafy greens and soils

Colm D. Everard [a], Moon S. Kim [a,*], Mark C. Siemens [b], Hyunjeong Cho [c], Alan Lefcourt [a], Colm P. O'Donnell [d]

[a] Environmental Microbial and Food Safety Laboratory, Beltsville Agricultural Research Center, Agricultural Research Service, United States Department of Agriculture, MD, USA
[b] Department of Agricultural and Biosystems Engineering, University of Arizona, Tucson, AZ, USA
[c] Experiment and Research Institute, National Agricultural Products Quality Management Service, Republic of Korea
[d] School of Biosystems and Food Engineering, College of Engineering and Architecture, University College Dublin, Dublin 4, Ireland

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Faecal contaminated fruits and vegetables have been linked to several outbreaks of foodbourne diseases. In-field detection of faecal matter would allow the producer to take action to reduce faecal contaminated produce entering the post-harvest processing line and the human food supply. No viable systems to accomplish this task have been developed to date. To address this, a prototype imaging system was developed to detect faecal matter on leafy greens. The system principally comprised of two monochrome cameras that were used to simultaneously capture images of the same target at two separate wavelengths, 690 and 710 nm, by utilising a beam-splitter and bandpass filters. The 710 nm and 690 nm waveband images were used for pixel-by-pixel calculation of a ratio image of the target, to which a thresholding technique was applied to classify pixels as either faecal matter or non-faecal matter. The system was tested on spinach leaves (*Spinacia olerace*) and three types of soils in an outdoor environment. On all samples evaluated, the imaging system, coupled with this waveband ratio normalisation method, successfully distinguished faecal matter from spinach leaves and soil under varying atmospheric conditions. These findings are very encouraging and further study is needed to determine if such a technique would reliably detect faecal material in an agricultural field environment where leaf orientation and contamination concentration levels are highly variable.

1. Introduction

Leafy green crops are susceptible to contamination as a result of animal intrusion into outdoor production areas (USFDA, 2001; Xicohtencatl-Cort & Chacon, 2009). *Escherichia coli* O157:H7 from the gastrointestinal tracts of animals can be transferred onto leafy greens via direct contact with faecal matter; this can lead to potential foodborne illnesses and

* *Corresponding author*. Fax: +1 301 504 9466.
E-mail address: moon.kim@ars.usda.gov (M.S. Kim).
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death if the contaminated greens are consumed (Armstrong, Hollingsworth, & Morris, 1996; USFDA, 2001). Several outbreaks of *E. Coli* O157:H7 have been associated with leafy greens (Brandl, 2008). The Centers for Disease Control and Prevention reported that 19,507 infections, 4476 hospitalisations, and 75 deaths occurred due to foodborne illnesses during 2014 in the United States (CDC, 2015). The incident rates for *E. Coli* O157:H7 and *Salmonella* were 0.91 and 15.29 per 100,000 people (CDC, 2015). Preliminary data for 2015 show 20,107 infections, 4531 hospitalisations, and 77 deaths in the United States (CDC, 2016).

Early in-field detection of faecal matter can help producers avoid harvesting and mixing contaminated produce with uncontaminated produce, thereby reducing or preventing the introduction of human pathogens to the food supply. Further, large outbreaks would be less likely since there would be decreased opportunities for cross-contamination during the harvesting, processing, and packaging phases of production. Harvest and process equipment downtime required for cleaning and sanitising can also be reduced by not harvesting faecal contaminated produce.

Everard, Kim, and Lee (2014) reported that hyperspectral fluorescence imaging with violet light-emitting diode (LED) excitation performed better than both reflectance (halogen light source) and ultraviolet LED excitation for detection of a range of diluted faecal contaminations on leafy greens. Everard, Kim, Cho, and O'Donnell (2015) subsequently developed a 4-waveband algorithm, i.e. 680:688 nm × 703:723 nm, which identified 100% of faecal contaminants on both fresh and non-fresh spinach leaves using hyperspectral fluorescence imaging. These wavelengths were selected to distinguish the spectral differences between faecal matter and intact green leaves in the red and far-red regions of the spectrum created by chlorophyll *a* molecules.

Recently, Everard, Kim, and O'Donnell (2016) successfully used single point field-spectroscopy with normalisation techniques to distinguishing faecal contaminants on spinach leaves from both soil on spinach leaves and uncontaminated spinach leaf portions. In that study, a ratio of 710:688 nm coupled with thresholding was used to identify faecal matter. The algorithm was developed to remove the need for calibration using a reference panel, which is unreliable as atmospheric/environmental changes can occur during the lag time between reference panel and target scans. The normalisation technique assumed that atmospheric/environmental changes result in equal magnitude response changes at 688 and 710 nm.

The sun emits both ultraviolet and visible light which interacts with faecal matter, leaf, or soil sample, and the response can be captured using spectroscopic techniques (Kim, Chappelle, Corp, & McMurtrey, 1993). The red and far red regions of green vegetation's spectral response to solar illumination are reported to be affected by both ultraviolet and visible light (Chappelle et al., 1999; Lichtenthaler, Buschmann, Rinderle, & Schmuck, 1986). There is relatively low reflectance between 680 and 725 nm for green vegetation due to high chlorophyll *a* light absorption (Campbell, Middleton, Corp, & Kim, 2008).

The objectives of this research were to develop and test a dual monochromatic camera imaging system to detect faecal matter in an outdoor environment on 1) spinach leaves and 2) various types of soils. An image algorithm was developed to distinguish faecal matter on horizontal samples over several days with differing atmospheric conditions. Using spectral filters with lower-cost monochromatic cameras allows for simultaneous two-band imaging of sample materials. An in-field imaging device to detect faecal contamination on both the crop and the surrounding soils could be part of a food safety risk reduction strategy.

2. Materials and methods

2.1. Sample preparation

Pre-washed "ready-to-eat" spinach leaves (*Spinacia oleracea*) were purchased from a local food store. Faecal matter from Holstein cows was obtained at the Dairy Operations Unit, Beltsville Agricultural Research Center, MD, USA. After 16 h drying of the faecal matter in a forced-air oven at 80 °C, a grinder (Sample Grinder, C.W. Brabender Instruments Inc., South Hackensack, NJ, USA) was used to reduce the faecal particle size to less than 2 mm. Next, reconstitution with distilled water restored the ground samples to their original moisture level. This procedure ensured that content of originally large particulates were not excluded during subsequent contaminant application to leafy greens that was performed by pipette. Soils selected for analysis were representative of the three main soil types found in the vegetable producing region around Yuma, AZ, USA. These included: Soil 1, Holtville clay (clayey over loamy, smectitic over mixed, superactive, calcareous, hyperthermic Typic Torrifluvents); Soil 2, Indio silt loam (coarse-silty, mixed, superactive, calcareous, hyperthermic Typic Torrifluvents); and Soil 3, Gadsden clay (fine, smectitic, calcareous, hyperthermic Vertic Torrifluvents). Soils were collected near Yuma, AZ from sites identified using the US Department of Agriculture – Natural Resources Conservation Service (USDA-NRCS) Web Soil Survey (USDA-NRCS, 2016) tool. At each site, approximately 1 kg of the soil type was collected from the top 300 mm of the soil surface, thoroughly mixed and placed in a bag.

Compositional analyses for moisture content and organic matter content of the faecal matters and soils were carried out in triplicate. Forced-air drying for 24 h at 105 °C was used for moisture determinations. For organic matter content, a Paragon Furnace (Paragon Industries, Mesquite, Texas, USA) was used at 550 °C for faecal matter while the Walkley-Black Colorimetric method (Walkley & Black, 1934) was used for soils.

In this study, two sets of experiments were conducted. In the first, spinach leaf samples were contaminated by pipetting 0.25 ml volumes of faecal matter in 12–15 mm diameter spots onto the adaxial leaf surfaces. Using the same volumes and spot sizes, soil was also pipetted onto each leaf at different adaxial locations. The soil sample was prepared by drying the soil in a forced air oven at 80 °C for 16 h and then diluting the dry sample with distilled water to 1:2. Fifteen leaf samples were analysed on each of seven different days ($n_L = 15 \times 7 = 105$). For each batch of fifteen leaf samples, faecal matter was applied on all of the leaves and the three