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Short Report

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Posted Date: September 23rd, 2025

DOI: https://doi.org/10.21203/rs.3.rs-7543685/v1

Version of Record: A version of this preprint was published at Journal of Plant Diseases and Protection on January 4th, 2026. See the published version at https://doi.org/10.1007/s41348-025-01210-1.

Preliminary assessment of hyperspectral imaging for detecting *Halyomorpha halys* and *Nezara viridula* feeding sites on bean pods: A proof-of-concept study

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Abstract

This proof-of-concept study explores the potential of hyperspectral imaging (HSI) for non-destructive characterization of feeding damage by two invasive stink bug species on bean pods under controlled laboratory conditions. We compared spectral signatures of feeding sites caused by *Halyomorpha halys* and *Nezara viridula* on *Phaseolus vulgaris* seven days post-infestation. Using a limited dataset of 45 observations from 20 pods (12 individual plants), we identified distinct spectral modifications induced by both species. *H. halys* feeding increased visible spectrum reflectance (450-700 nm) with reduced near-infrared (NIR) reflectance, while *N. viridula* caused more severe spectral changes with substantial NIR decreases (up to 31% at 800 nm). Difference spectra revealed species-specific sensitivity patterns with maximum responses at 540 nm (green) and 740 nm (NIR) for both species, though *N. viridula* showed consistently larger deviations from healthy tissue. Key wavelengths for damage characterization were identified at 660-680 nm and 730-780 nm for both species. While these initial findings suggest potential spectral discrimination between stink bug feeding damage and healthy tissue, we emphasize that this preliminary study requires extensive validation with larger sample sizes, multiple cultivars, comparison with other herbivores, and field conditions before any practical application. The results provide first insights into spectral responses to stink bug feeding that warrant comprehensive follow-up investigation practical hyperspectral-based pest symptom monitoring tools that could enable more targeted management strategies against these economically significant pests.

Keywords: Stink bugs, invasive species, symptom detection, hyperspectral imaging, spectral signature, machine learning, Pentatomidae

Introduction

The invasive stink bugs *Halyomorpha halys* (Stål) and *Nezara viridula* (Linnaeus) represent significant economic threats to agricultural production worldwide. In Europe, both species have established populations causing substantial crop losses. For instance, in North Rhine-Westphalia, Germany, a single bell pepper producer suffered losses of € 300.000 due to stink bug infestations in 2021. These pentatomid species feed through piercing-sucking mouthparts, injecting digestive enzymes that cause mechanical and biochemical damage to plant tissues (Liu & Bonning, 2019; Serteyn et al., 2020). Typical symptoms of infestation include localized wilting, malformations, necrosis, fruit abscission, morphological deformities, and tissue malformation (Panizzi, 1997; Corrêa-Ferreira & De Azevedo, 2002; Wiman et al., 2015; Haye et al., 2015) and are often visually similar between species, making differentiation challenging (Haag & Dieckhoff, 2024). Current detection relies on visual scouting, which often identifies damage too late for effective intervention.

HSI has proven valuable for detecting various plant stresses by capturing subtle changes in tissue optical properties across narrow spectral bands (Kuska & Mahlein, 2018). While HSI applications exist for detecting stink bug individuals in field monitoring (Sorbelli et al., 2023), spectral characterization of species-specific feeding damage remains unexplored. This study aimed to: (1) characterize spectral signatures of stink bug feeding damage, (2) identify species-specific patterns, and (3) determine key detection wavelengths.

Materials and Methods

Plant cultivation

Bean plants (*Phaseolus vulgaris* L. cv. 'Cerdon') were grown under controlled conditions (22±1°C, 65±5% RH, 16:8 h light:dark photoperiod) until pod development stage BBCH 75. Laboratory colonies of *H. halys* and *N. viridula* were kept at 21±1°C, 65±5% RH in in ventilated, transparent BugDorms breeding cages (L 30 x W 30 x H 30 cm) (Veldshop.nl B.V, Groningen, Netherlands) and fed with hazelnuts and fresh beans. For controlled infestation, individual adult stink bugs (both sexes) were starved for 48 hours, then caged on single pods using transparent organza bags for two days. Control treatments included non-infested pods and mechanical injury (5 µm needle puncture). We examined 20 pods total: 5 non-infested, 5 mechanically injured, 5 exposed to *H. halys*, and 5 to *N. viridula* (Fig. 1). All measurements were performed seven days post-infestation, a timepoint determined through preliminary trials.

HSI data processing

Spectral reflectance (400-1000 nm, 2.73 nm resolution) was measured using a line scanner (ImSpector V10E line scanner, Specim, Finland) mounted on a microscope mounted on a stereo microscope foreoptic (Z6 APO, Leica, Wetzlar, Germany) with a magnification up to 7.3x. Bean pods were scanned using an XY-moving stage in a darkened room with standardized dual linear illumination (Schott, Germany). Reflectance was calculated from white reference and dark current measurements using ENVI 5.1 software and smoothed with a Savitzky-Golay filter (Savitzky and Golay, 1964).

Regions of interest (ROIs) were selected visually by a single observer based on symptom appearance, without independent validation. This subjective approach introduces observer bias and limits reproducibility. Equal-sized rectangular ROIs were extracted for: healthy tissue (n=15), mechanical injury (n=5), marginal symptoms (n=10), and severe symptoms (n=10) from both species. The final dataset comprised 45 observations (Fig. 1).

Statistical analyses were performed in R (R Core Team, 2023) included difference spectra ($Diff_\lambda = \mu_{symptomatic} - \mu_{healthy}$) and spectral ratios ($\text{Spectral ratio}_\lambda = \frac{\mu_{healthy}}{\mu_{symptomatic}}$) at each wavelength λ, where λ = 450 to 850 nm, in order to identify the wavelength or wavelengths appropriate for distinguishing between infestation and symptom severities. The data was prepared for ML as a dichotomous classification was performed to distinguish between "healthy" and "stink bug feeding sites". The feature importance was calculated according to Kuhn (2020) based on the trained random forest model (Breiman et al., 2022).

Results

This study examined the potential of HSI sensors for the detection, identification, and differentiation of the feeding sites of two invasive stink bug species – *Halyomorpha halys* and *Nezara viridula* – on common bean pods under controlled conditions.

Hyperspectral signatures of feeding sites of *H. halys* and *N. viridula*

Control ROIs from non-infested pods showed typical vegetation reflectance: low visible reflectance, green peak (550 nm), red edge transition (700 nm), and high NIR plateau (Fig. 2A). Mechanical injury caused minimal deviation (4-10% NIR reduction) while maintaining the overall spectral pattern (Fig. 2B).

H. halys feeding modified spectra differentially by damage severity (Fig. 2C). Marginal symptoms showed minor changes, while severe damage increased visual reflectance (500-700 nm) and reduced NIR by up to 15% at 800 nm. *N. viridula* produced stronger modifications (Fig. 2D): marginal symptoms already showed measurable changes, and severe damage caused 31% NIR reduction at 800 nm with consistently elevated visible reflectance.

Both species maintained characteristic vegetation features (green peak, red edge) but with altered intensities. Standard deviations were notably larger for biological damage than mechanical injury, suggesting heterogeneous tissue responses.

Despite controlled conditions and starvation pretreatment, only 5 of 20 exposed pods (25%) developed visible symptoms suitable for analysis—highlighting the unpredictability of stink bug feeding behaviour.

Difference spectra and spectral ratios for stink bug feeding sites

Difference spectra revealed wavelength-specific responses. *H. halys* marginal damage showed minimal deviation (±0.01%), while severe damage increased visible reflectance up to 0.08% (450-700 nm) with maximum sensitivity at 540 nm and 740 nm. *N. viridula* severe symptoms produced dramatic NIR decreases (-0.16% at 750-800 nm).

Spectral ratios confirmed stronger *N. viridula* impacts, reaching 1.44 (540 nm) and 1.59 (740 nm) for severe symptoms versus maximum 1.35 and 1.40 for *H. halys*. Both species showed severity-dependent changes, but absolute magnitudes differed substantially.

Feature importance identifies relevant wavelengths

Random Forest analysis (n=35) identified two diagnostic regions: primary importance at 660-680 nm (importance value ~100%) and secondary at 730-780 nm (45-52%). Visible spectrum (450-650 nm) and far-NIR (>800 nm) showed consistently lower importance (~20%).

Discussion

This proof-of-concept study demonstrates that HSI can detect feeding damage by *H. halys* and *N. viridula* on bean pods, revealing distinct spectral modifications between species under controlled conditions. However, substantial methodological challenges limit immediate practical application.

The more pronounced spectral alterations from *N. viridula* feeding—particularly the 31% NIR reduction versus 15% for *H. halys* at 800 nm—align with known salivary composition differences. The higher protein count in *N. viridula* saliva (305 versus 238 proteins; Serteyn & Francis, 2019) could likely explain the more severe tissue damage and stronger spectral responses. Increased visible reflectance (450-700 nm) coupled with decreased NIR reflectance indicates chlorophyll degradation and cellular disruption, consistent with enzymatic digestion patterns (Peiffer & Felton, 2014). The sensitivity peak at 540 nm corresponds to anthocyanin accumulation, a typical stress response (Merzlyak et al., 2008). Anthocyanins function as a plant defence mechanism in response to various stressors including defence against herbivores and pathogens (Lee & Gould, 2002). According to sensitivity analyses for a spectral range from 400 to 2500 nm, elevated reflectance around 700 nm is a widespread indicator of stress situations (Cibula & Carter, 1991).

Currently, HSI is applied for the detection of *H. halys* and *N. viridula* individuals in field monitoring for integrated pest management, not for the differentiation of the feeding site of the stink bug species (Sorbelli et al., 2023; Kargar et al., 2024; Tetila et al., 2024). Gadalla & Öztekin (2023) have dealt with image processing and the classification of hazelnuts damaged by *H. halys* using machine learning (ML), while Almstedt et al. (2024) dealt with the detection of *H. halys* punctures on apple fruits. Unlike these previous studies we uniquely characterized feeding site spectral properties. The wavelength regions identified parallel those for other arthropod damage; Herrmann et al. (2012) documented similar but lower-amplitude changes from spider mite feeding on beans. The greater spectral modifications from stink bugs likely reflect their more destructive piercing-sucking mechanism versus cellular content removal by mites. The high within-class variability corroborates Zeilinger et al. (2015), who found no correlation between salivary sheath counts and tissue damage. This variability reflects stink bugs complex feeding behaviour—probing multiple sites, injecting variable saliva amounts, and feeding for different durations—creating a mosaic of damage states that challenges classification schemes. The 25% success rate for visible damage despite controlled conditions and starvation pretreatment highlights unpredictable feeding behaviour, consistent with Schweiger et al. (2024). This fundamental challenge suggests that generating standardized spectral libraries for field application will be exponentially more difficult than anticipated.

The subjective ROI selection based on visual symptoms introduces observer bias and limits reproducibility. Future studies must incorporate objective validation through chlorophyll fluorescence, biochemical markers, or histological analysis. Our limited dataset (45 observations, single cultivar) severely restricts generalizability. Bean cultivars vary in thickness, pigmentation, and defensive chemistry—all influencing spectral properties. Without comparing other herbivores or abiotic stresses, claims of species-specificity remain premature.

The identified wavelengths (660-680 nm, 730-780 nm) suggest potential for simplified multispectral systems, reducing costs versus full hyperspectral sensors. However, substantial damage class overlap indicates that species discrimination remains problematic. Developing operational spectral libraries requires standardized protocols and collaborative efforts similar to plant disease libraries (Bohnenkamp et al., 2021). Advanced ML, particularly deep learning incorporating spatial patterns (Chakhvashvili et al., 2024), shows promise but requires substantially larger datasets than currently available.

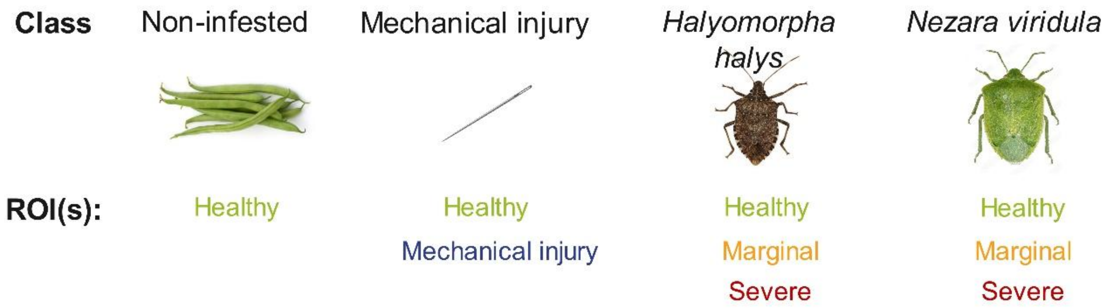

Figure 1: Classification scheme for the assessment of damage to bean pods (Phaseolus vulgaris). Reference images are shown for non-infested beans, mechanical injury and feeding damage caused by the invasive marmorated stink bug (Halyomorpha halys) and the green stink bug (Nezara viridula). Damage is classified in the categories "Healthy", "Mechanical injury", "Marginal" and "Severe" based on the respective appearance of the region of interest (ROI).

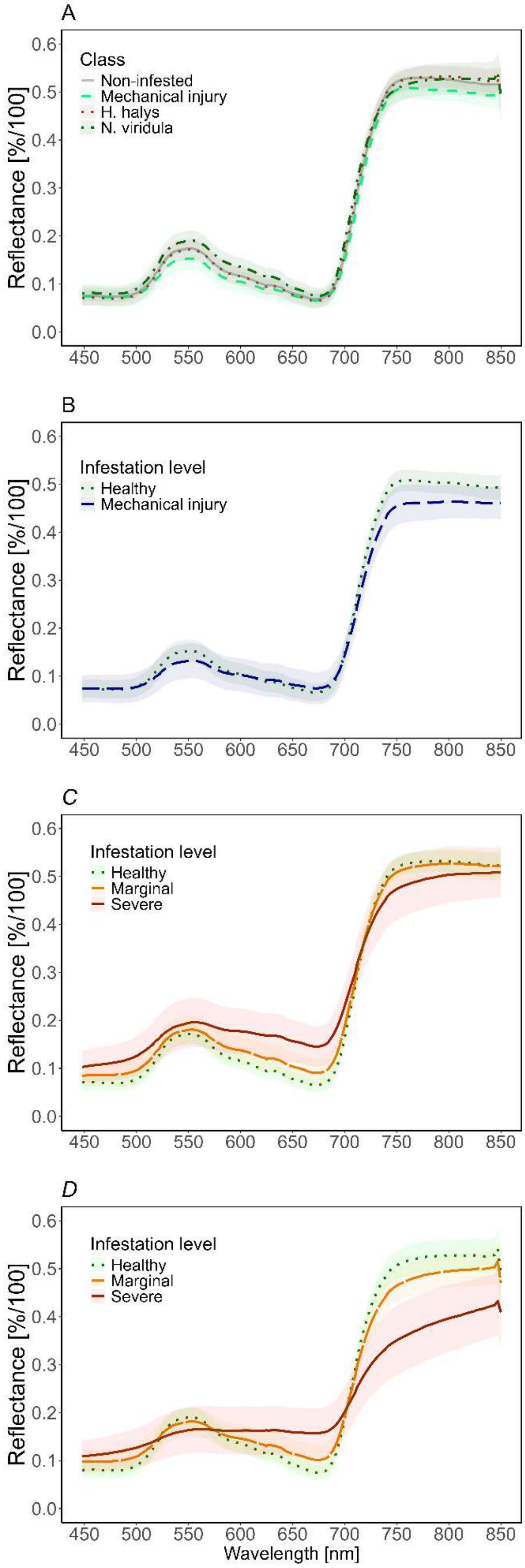

Figure 2: Spectral signatures of different tissue areas of bean pods in the wavelength range of 450-850 nm; (B) influence of mechanical puncture compared to healthy tissue; (C) influence of infestation with H. halys; (D) influence of infestation with N. viridula. The spectra represent mean values (lines) and standard deviation (hatched areas) of reflection as a function of wavelength 7 days after colonization (n=5).

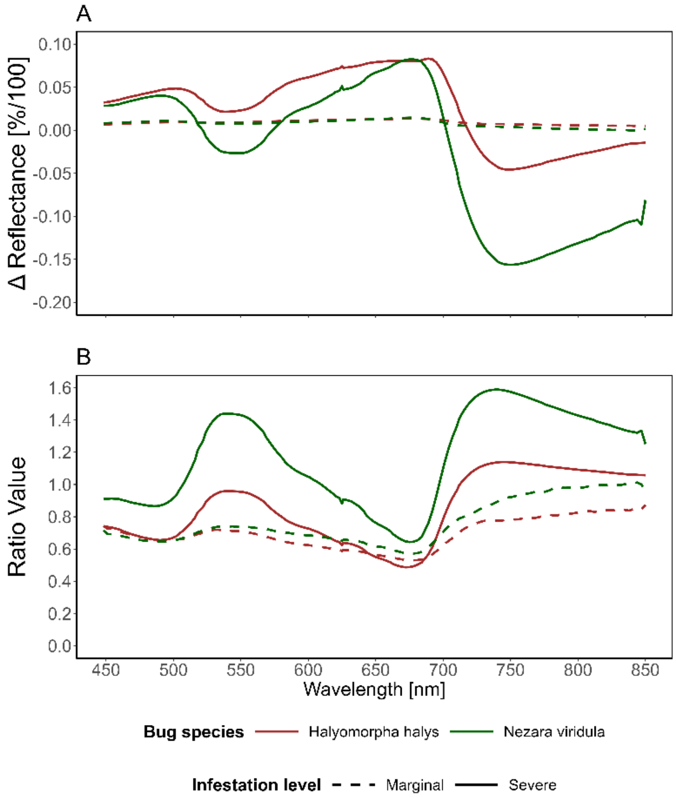

Figure 3: Differential spectra of feeding sites (A) of the stink bug species H. halys (brown) and N. viridula (green) on bean pods depending on symptom severity. The dimensionless spectral ratios (B) were calculated by subtracting the reflection of healthy beans from the reflection of symptomatic bean pods. Ratio value of beans infested by H. halys and N. viridula at different symptom severity levels. The reflection for all measurements was measured 7 days after colonization (n=5).

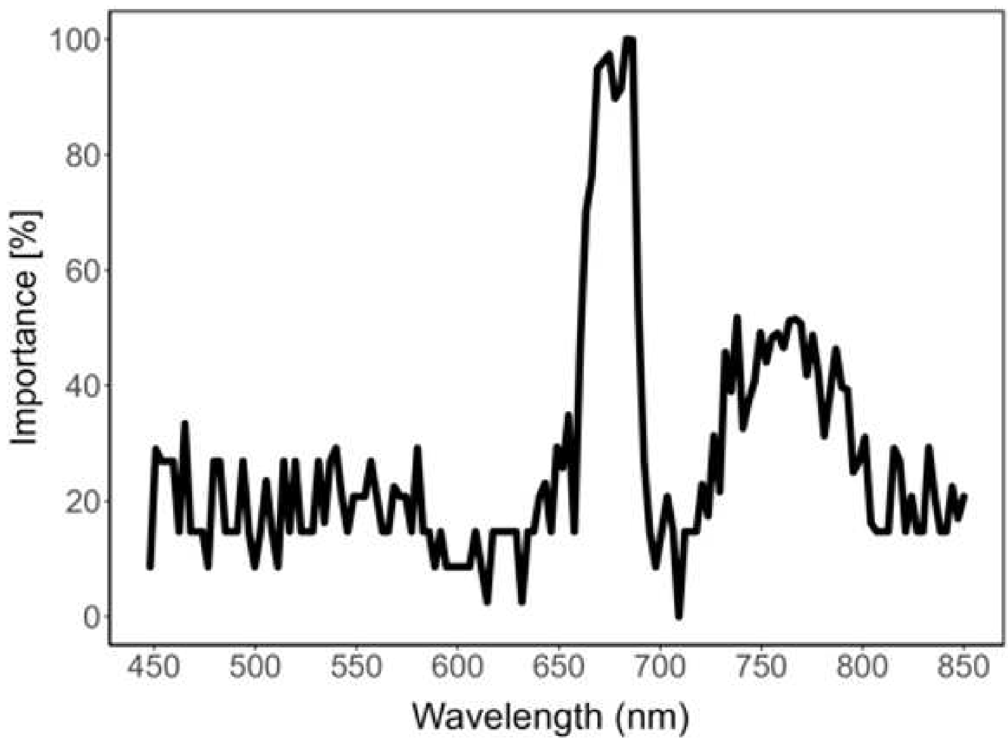

Figure 4: Feature importance spectra used for machine learning classification. Spectral feature importance for distinguishing between healthy and symptomatic bean pod samples, with a prominent peak at approximately 680 nm and elevated importance in the 720-800 nm region.

Data availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Acknowledgements
This study was kindly sponsored by the Plant Protection Service, Landwirtschaftskammer North Rhine-Westphal, Köln and by the Plant Pathology, Faculty of Agricultural, University of Bonn, Bonn, Germany.

Funding
The authors received no financial support for the research, authorship, and/or publication of this article.

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Ethics declarations
Conflict of interest
The authors declare that they have no conflicts of interest.

Consent for publication
All authors of the manuscript have read and agreed to the publication. All authors have agreed to the submission to the journal.