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Principles of high-throughput phenotyping and sensor platforms for non-invasive research in plant protection

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Abstract. Climate change and the increasing resistance of pathogens are driving the need for innovative methods of plant disease diagnostics, particularly for high-risk pathogens such as *Fusarium graminearum*, which causes significant wheat yield losses. Traditional visual inspection methods suffer from low throughput and subjectivity, limiting their effectiveness in large-scale monitoring. This study aimed to explore the principles of high-throughput phenotyping and to evaluate the effectiveness of a sensor platform for the non-invasive investigation of *Fusarium* ear blight under field conditions in Ukraine (Kyiv region). A combination of multispectral imaging, thermal imaging, and machine learning algorithms was applied in a 1-hectare experimental field with 20% of the plots artificially infected. The results demonstrated that the proposed system achieved a 92% accuracy rate in early pathogen detection, representing a 37% improvement over visual assessment methods. Spectral indices showed a strong correlation with pathogen concentration: a decrease in the normalised difference vegetation index from 0.72 to 0.35 corresponded with an 80% increase in fungal biomass. Thermal imaging revealed a rise in leaf temperature of 2.5°C as early as 5-7 days after infection. The integration of all methods enabled an accuracy of 96% in processing one hectare within 2.5 hours, which is three times faster than traditional approaches. Polymerase chain reaction analysis confirmed the specificity of the techniques: 95% of infected samples contained *Fusarium* deoxyribonucleic acid, while sequencing revealed a 100% match for β-tubulin. Automated data processing required 2.5 hours per hectare, compared with 8 hours per hectare for visual inspection, and scaling up to 10 hectares reduced time expenditure by a factor of 12. The study confirmed the effectiveness of high-throughput phenotyping for precision plant protection and highlighted the need for further refinement of the methods in line

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with local climatic conditions. The practical significance of this research lies in the potential to reduce fungicide use through targeted treatment of infected areas, minimise crop losses in regions with high infection pressure, and establish a foundation for automated monitoring systems compatible with precision agriculture technologies

Keywords: agriculture; pathogens; fungal infections; hyperspectral imaging; thermal imaging

INTRODUCTION

Global agriculture is facing a complex set of challenges, including climate change, increasing pathogen resistance, and the pressing need to ensure food security. In Ukraine, where the agricultural sector plays a critical role in the national economy, these challenges are intensified by the intensification of farming practices and shifting climatic conditions, which contribute to the spread of fungal infections – particularly *Fusarium graminearum*. This pathogen not only significantly reduces wheat yields but also poses a health risk due to the production of toxic compounds, underscoring the urgency of developing innovative methods for disease diagnosis and control.

Traditional diagnostic approaches, such as visual inspection or laboratory testing, are unsuitable for large-scale monitoring due to their low throughput, subjectivity (e.g. reliance on the agronomist's expertise), and delayed detection – since symptoms often appear only at advanced stages of infection. In this context, high-throughput phenotyping (HTP) is emerging as a key tool, as it integrates sensor technologies – including multispectral imaging, thermal imaging, and hyperspectral visualisation – with machine learning (ML) algorithms for non-invasive analysis of plant conditions down to the cellular level (Ma *et al*. 2022). However, despite recent advancements, challenges remain in adapting HTP to specific biotic threats, particularly in regions with complex climatic conditions such as Ukraine.

Between 2020 and 2024, significant advances were made in the field of HTP. The study by Z. Ma *et al*. (2022) systematised modern sensor-based methods, identifying three key categories: multispectral imaging, which employs indices such as Normalised Difference Vegetation Index (NDVI) and Normalised Difference Red Edge (NDRE) to assess the physiological state of plants based on light reflectance across various spectral bands; thermal imaging, which detects temperature anomalies linked to changes in transpiration under stress conditions; hyperspectral imaging, which analyses hundreds of spectral channels to reveal molecular-level changes, such as the accumulation of toxins.

While these technologies have proven effective in assessing abiotic stress factors (e.g. drought), their application to biotic threats – such as *Fusarium graminearum* – remains limited. For instance, the study by D. Li *et al.* (2021) demonstrated the efficacy of an HTP platform for evaluating yield and salinity tolerance but did not address pathogen-host interactions. This gap is critical, as fungal infections often mimic the symptoms of physiological stress, complicating accurate diagnosis.

A significant contribution to the understanding of HTP was made by Q. Xiao *et al.* (2022), who integrated phenotyping into genome-wide association studies (GWAS) to identify genes associated with resistance. However, their approach did not address the technical challenges of processing large volumes of data in real time – an essential requirement for precision agriculture. Similarly, H. Yuan *et al*. (2023) found that existing monitoring systems tend to underestimate the influence of microclimatic factors (e.g. local humidity fluctuations) on pathogen dynamics – an issue particularly relevant to Ukraine, where variable weather conditions increase the risk of epiphytotics. The study by X. Fu & D. Jiang (2022) examined the role of HTP as an innovative tool for enhancing the sustainability of agricultural systems in the context of climate change, with a focus on analysing plant adaptation mechanisms to stress factors. The authors highlight the integration of sensor technologies, machine learning algorithms, and large-scale data for real-time crop performance monitoring, offering new pathways for the optimisation of agricultural production.

Despite recent progress, significant gaps remain. A lack of specificity is evident in the fact that most studies – for example, T. Gill *et al*. (2022) –

focus on general plant responses to stress, whereas *Fusarium graminearum* requires tailored models that account for its unique biochemical markers. Limited validation also poses a challenge, as image-processing algorithms developed by J. Zhou *et al*. (2022) overlook thermal anomalies that may serve as early indicators of infection (e.g., localised temperature increases caused by the plant's immune response). Moreover, the sensor systems proposed by F. Tanner *et al*. (2022) have not been tested under conditions of high humidity – typical of southern regions of Ukraine, where soil moisture can reach 80%, thereby affecting the accuracy of thermal imaging data.

The integration of information technology into agricultural research has become critically important for optimising crop breeding and precision farming, particularly in the context of high-throughput phenotyping. The study by S. Vedmediev (2024) demonstrates the potential of specialised software to automate and enhance the accuracy of phenotypic trait analysis in sunflower breeding programmes. Building on this approach, the present study adopts similar principles to wheat pathology, combining sensor technologies and machine learning to develop a non-invasive diagnostic platform for detecting *Fusarium graminearum*. In doing so, it extends the application of IT-driven phenotyping to plant disease management. This study aimed to develop an integrated method for diagnosing *Fusarium* ear blight, incorporating multispectral indices (NDVI, NDRE) to assess photosynthetic activity, thermal data to detect localised temperature increases, and machine learning techniques – particularly deep neural networks – for classifying infection patterns.

MATERIALS AND METHODS

Experimental site and sampling. The study was conducted in the experimental field of the National Academy of Agrarian Sciences of Ukraine in Kyiv Region between May and July 2024. The site was selected due to the representative nature of the region's agroclimatic conditions – characterised by a moderate continental climate and chernozem soils – which are typical of Ukraine's main grain-producing areas, thus allowing for broader applicability of the results. The research focused on winter wheat (*Triticum aestivum*), cultivar Podolianka, sown on a 1-hectare plot. This variety was chosen due to its widespread use in agricultural production, moderate susceptibility to *Fusarium graminearum*, and adaptability to local conditions, enabling a reliable assessment of diagnostic method effectiveness. To ensure controlled conditions, the field was divided into 40 plots of 250 m^2 each. Of these, 8 plots (20%) were artificially inoculated with *Fusarium graminearum*, the primary causal agent of *Fusarium* ear blight in Ukraine. The fungal strain was sourced from the microbiological collection of the National Academy of Agrarian Sciences of Ukraine. This pathogen was selected due to its epidemiological importance and the significant economic losses it causes in the region. Laboratory analyses were also conducted to validate the results and perform necessary diagnostic tests, including PCR and DNA sequencing.

Plant sampling was conducted using a stratified randomisation method. Only plants at the tillering stage (4-6 weeks after emergence) without mechanical damage were included. Exclusion criteria comprised the presence of symptoms of other diseases (e.g. rust, powdery mildew), insect damage, or abnormal soil moisture levels (above 80% or below 30%). Soil moisture was measured using TEROS 12 sensors with an accuracy of ±3%. The presence of other diseases, such as rust or powdery mildew, was assessed through visual inspection by qualified agronomists and confirmed via laboratory-based PCR (polymerase chain reaction) using species-specific primers.

Infection protocol and monitoring. To ensure uniform distribution of the pathogen, infected plots were selected using a random number generator implemented in the "numpy" library in Python. The infection protocol began with the preparation of a spore suspension of *Fusarium graminearum*. The fungus was cultured on potato dextrose agar (Germany) at 25°C for seven days. Spores were then carefully removed from the medium's surface using a sterile spatula, resuspended in a physiological saline solution, and filtered through cheesecloth. The spore concentration (1×10^5 CFU/mL, colony-forming units) was determined using a Goryaev chamber (Germany). Prior to inoculation, instruments (scalpels, needles) were sterilised in a T-EDGE 10 autoclave (Israel) at 121°C for 20 minutes.

For inoculation, sterile 1 mL BD Plastipak syringes with 25G needles were used. The spore suspension was injected into the stems at the height of the third to fourth node, simulating the natural entry of the pathogen through mechanical damage. Following injection, the site of entry was disinfected using a sterile cotton swab soaked in 70% ethanol to prevent secondary infections. All procedures were carried out in a laminar flow cabinet in accordance with biosafety guidelines (CDC, 2020). The use of standardised protocols for culturing *Fusarium graminearum* (potato dextrose agar), accurate spore quantification (Goryaev chamber), and commercial DNA extraction kits was essential to ensure experimental reproducibility, minimise the risk of contamination, and obtain high-quality data for PCR analysis – critical for validating sensor-based diagnostic methods.

Each week throughout the study period, 20 plants (10 infected and 10 control) were sampled from each plot for PCR analysis. Leaf and stem tissues were first homogenised, and DNA was extracted using the DNeasy Plant Pro Kit (Germany). The quality of the extracted DNA was then assessed spectrophotometrically (Nanodrop 2000, USA) by measuring purity (A260/A280 = 1.8-2.0) and concentration Amplification was performed using a T100 thermal cycler (USA) following this protocol: initial denaturation at 95°C for 3 minutes; 35 cycles at 95°C for 30 seconds, at 58°C for 30 seconds, and at 72°C for 45 seconds; followed by a final extension at 72°C for 5 minutes. PCR products were visualised on 2% agarose gels (USA) stained with ethidium bromide under UV illumination, using electrophoresis at 100 V for 40 minutes. Sequencing was carried out by Macrogen DNA sequencing services (South Korea) to eliminate false positives. All samples with DNA purity within the A260/A280 range of 1.8-2.0 and sufficient concentration (>20 ng/µl) were included in the subsequent analysis. Samples falling outside this range or with low DNA quality were excluded from the study.

Sensor platforms and data collection. A combination of aerial and ground-based technologies was employed for data collection. A DJI Phantom 4 Multispectral unmanned aerial vehicle (China), equipped with *Red Green Blue* (RGB) cameras (2 MP resolution) and multispectral sensors (*Near InfraRed* (NIR), RedEdge, Blue; spectral range 450-860 nm, resolution 1.2 MP), was used for weekly imaging at an altitude of 50 metres, delivering a ground resolution of 2 cm per pixel. Thermal imaging was carried out using a FLIR Vue TZ20 thermal camera (spectral range 7.5-13.5 µm, accuracy ±5°C), integrated with the drone.

A total of 40 TEROS 12 sensors (USA) and 40 HOBO MX2301 sensors (USA) were installed across the experimental field, with one set (TEROS 12 + HOBO MX2301) placed in each of the 40 plots. In total, 80 sensors were used for simultaneous monitoring of soil moisture (±3%), air temperature, relative humidity, and solar radiation. Each TEROS 12 sensor was inserted 20 cm into the soil, while the HOBO MX2301 sensor was mounted 50 cm above the soil surface to capture microclimatic parameters in real time.

The concentration of *Fusarium graminearum* spores in the soil was determined microscopically using a Goryaev chamber. Soil samples were collected from a depth of 20 cm, homogenised in sterile saline solution, and applied to the chamber grid for counting colony-forming units (CFU) per millilitre.

Imaging was carried out weekly between 10:00 and 14:00 under sunny conditions to minimise the influence of variable light intensity. The spectral indices NDVI and NDRE were calculated using the following formulae:

$$NDVI\ \frac{(NIR-Red)}{(NIR+Red)}, \quad (1)$$

where *NIR* is the reflectance in the near-infrared range (780-900 nm), and *Red* is the reflectance in the red range (630-690 nm). NDVI values range from −1 to 1, with the following interpretations: >0.6 – dense, healthy vegetation; 0.2-0.5 – sparse or stressed vegetation; <0.1 – bare soil or dead biomass.

$$NDRE = \frac{(NIR-RedEdge)}{(NIR+RedEdge)}, \quad (2)$$

where *NIR* is the reflectance in the near-infrared range (780-900 nm), and *RedEdge* is the reflectance in the red range (690-750 nm). NDRE values correspond to: >0.4 – high chlorophyll content; 0.20.3 – moderate level; <0.1 – low or absent photosynthetic activity. NDVI was used to assess the overall physiological activity of the plants, whereas NDRE served to detect changes in chlorophyll content associated with *Fusarium* infection.

A visual assessment of infection severity was conducted by five agronomists from the National

Academy of Agrarian Sciences of Ukraine (aged 32-45, with over five years of professional experience), using a 0-5 point scale adapted from the criteria described in EPPO Standard PP 1/257 (n.d.), tailored to the characteristics of the Podolianka wheat variety and the conditions of Ukraine's forest-steppe zone. The scale comprised the following criteria: 0 – no visible symptoms; 3 – spotting on 50% of the leaf surface; 5 – necrosis and complete tissue death. This method served as a reference standard for comparison with automated diagnostic approaches. To evaluate logistical efficiency, a comparative analysis of time expenditure was performed by recording the duration of each diagnostic stage (imaging, data processing, interpretation) for both the automated and visual methods across a 1-hectare plot.

Data processing and statistical analysis. Data processing began with image calibration in Pix4D Mapper to eliminate spectral noise and correct geometric distortions. Disease classification was performed using the Random Forest algorithm (Breiman, 2001) from the "scikit-learn" library (Python 3.10). The training dataset consisted of 2,800 images (70% of the total), and the test dataset included 1,200 images (30%). Model parameters included 1,000 trees and the Gini impurity criterion for splitting. For statistical analysis, Pearson's correlation coefficient was used to assess the relationship between NDVI values and infection levels. ANOVA was applied to evaluate the effect of data collection timing, and a bootstrap analysis (1,000 iterations) was conducted to verify the representativeness of the results.

The use of the DJI Phantom 4 Multispectral drone was justified by its capability to detect spectral variations in the NIR range (860 nm), which is critical for early stress diagnosis. The Random Forest algorithm was selected due to its robustness to noise and its efficiency in handling large multispectral datasets. The study was conducted following the international guidelines of the Convention on Biological Diversity (1992).

RESULTS AND DISCUSSION

Effectiveness of *Fusarium* detection using sensor platforms

The Random Forest algorithm achieved an average accuracy of 92% in detecting *Fusarium* infection at early stages (1-2 weeks post-infection). The analysis utilised data from the NIR (860 nm) and RedEdge (720 nm) spectral bands, which are sensitive to structural changes in chlorophyll and cellular integrity. The model's sensitivity, indicating its ability to identify infected plants, was 89%, while its specificity, reflecting the accuracy in excluding healthy specimens, reached 94%. For example, out of 200 infected plants, the system correctly classified 178, with 22 false negatives. In the control group of 800 healthy plants, only 48 were misclassified as infected.

The visual method, based on the 0-5 scoring scale developed by the National Academy of Agrarian Sciences of Ukraine, demonstrated significantly lower effectiveness. The average accuracy was 68%, with a sensitivity of 62% and a specificity of 73%. In the early stages of infection (1-2 weeks), when symptoms were limited to mild leaf spotting, agronomists identified only 35% of infected plants. For instance, out of 50 infected specimens examined in the second week, only 18 were correctly diagnosed. Errors in visual assessment were linked to several factors, including subjectivity (discrepancies between agronomists reached 20%-30% for the same plots), fatigue (accuracy declined by 15%-20% after 3-4 hours of continuous assessment), and environmental influences such as rain or shade, which hindered spot identification.

A key advantage of the sensor platform was early detection – the system identified infection 710 days earlier than the visual method. Quantitative assessment using NDVI and NDRE indices, calculated according to formulas (1) and (2), enabled precise evaluation of infection severity. For example, a drop in NDVI from 0.72 to 0.35 correlated with an 80% increase in fungal biomass. The scalability of the method was also notable: processing 1 hectare required 2.5 hours, compared to 8 hours of manual labour for visual inspection. Statistical validation confirmed the model's reliability. A bootstrap analysis (1,000 iterations) indicated a 95% confidence interval for accuracy ranging from 90% to 94%.

A practical application of the system confirmed its effectiveness: on plot No. 15, the sensor platform identified 95% of infected plants by day 8, whereas agronomists detected only 20% by day 15. Technical parameters had a significant impact on accuracy. The high image resolution (2 cm/pixel)

enabled the detection of localised spots as small as 1 cm in diameter. Weekly monitoring allowed the progression of the infection to be tracked over time, while calibration using reflective panels reduced lighting-related errors by 5%-7%. The sensor platform proved to be an effective tool for precision plant protection, outperforming traditional methods in terms of accuracy, speed and objectivity. Its implementation could reduce fungicide usage through targeted treatment of infected areas only, which is essential for environmentally sustainable agriculture.

Correlation between spectral indices and infection severity

An analysis of the spectral indices NDVI and NDRE revealed a clear relationship with the progression of *Fusarium* infection in winter wheat. Data were obtained using a multispectral camera. For healthy plants, the mean NDVI value at the start of the study was 0.72 ± 0.05, indicative of active photosynthetic biomass. Over the four weeks following infection, this index progressively declined, reaching a minimum of 0.35 ± 0.08 at the stage of severe infection, when the fungus had penetrated the vascular system of the stems. Pearson correlation analysis showed a strong negative relationship ($r = -0.85$, $p < 0.01$) between NDVI and the concentration of *Fusarium graminearum* DNA in plant tissues, as detected by PCR. This finding indicates that a decrease in NDVI can serve as a quantitative marker of infection.

The NDRE index, which incorporates reflectance in the red-edge spectrum, proved to be more sensitive to early structural changes in chlorophyll. Unlike NDVI, NDRE values began to decline as early as the second week following infection – from 0.61 ± 0.04 to 0.28 ± 0.06. The correlation with pathogen levels was even stronger ($r = -0.91$, $p < 0.001$), which can be attributed to the red-edge wavelength range (690-750 nm) being more responsive to chlorophyll degradation and the breakdown of cellular membranes – both characteristic of *Fusarium* infection. This sensitivity enabled detection of the infection 7-10 days earlier than by visual inspection, which only identified symptoms once visible lesions appeared on the foliage (Table 1).

Table 1. Comparative analysis of NDVI and NDRE spectral indices for diagnosing *Fusarium* infection

Parameter	NDVI	NDRE
Initial value (healthy plants)	0.72 ± 0.05	0.61 ± 0.04
Value at severe infection	0.35 ± 0.08 (after 4 weeks)	0.28 ± 0.06 (after 2 weeks)
Correlation with *Fusarium* DNA (r)	−0.85 ($p < 0.01$)	−0.91 ($p < 0.001$)
Early detection of infection	At 3-4 weeks	7-10 days earlier than NDVI
Relationship with pathogen	0.1-point drop → + 15% fungus	0.1-point drop → + 22% fungus
Statistical significance (ANOVA)	$F = 45.7$ ($p < 0.001$)	$F = 45.7$ ($p < 0.001$)

Source: compiled by the authors based on the conducted study

A more detailed analysis was conducted using linear regression, which revealed that each 0.1 point decrease in NDVI corresponded to a 15% ±2% increase in pathogen concentration within plant tissues. This relationship was even more pronounced for NDRE: a 0.1-point reduction in the index correlated with a 22% ± 3% increase in fungal biomass. The statistical significance of these findings was confirmed by ANOVA ($F = 45.7$, $p < 0.001$), indicating a robust association between spectral data and biological changes.

Thermal anomalies as stress markers

Thermal imagery, obtained using an infrared camera, demonstrated a clear correlation between elevated leaf temperature and the progression of *Fusarium* infection in winter wheat. The study found that the average leaf temperature of healthy plants was 24.3°C ± 1.2°C, whereas that of plants infected with *Fusarium graminearum* rose to 26.8°C ± 1.5°C ($p < 0.001$, Student's t-test). This 2.5°C difference is statistically significant and reflects pathogen-induced physiological changes. Thermal anomalies were detectable as early as 5-7 days after infection, which is 3-5 days earlier than the onset of visual symptoms such as foliar spotting.

The underlying mechanism of thermal variation lies in the disruption of transpiration – the process of water evaporation through leaf surfaces. *Fusarium graminearum*, upon penetrating

the plant's vascular system, obstructs the xylem, thereby reducing water transport to the leaves. This results in decreased transpiration intensity and, consequently, heat accumulation within the tissues. Experimental data confirmed that transpiration intensity in infected areas declined by 35%-40% compared to the control. This mechanism explains why the leaves of infected plants, which are not effectively cooled through evaporation, become significantly warmer even under identical external conditions.

Thermal data showed strong correlations with other stress markers. For instance, a reduction in NDVI to 0.45 was associated with a rise in leaf temperature to 26.1°C ($r = -0.78$, $p < 0.01$). A further relationship was observed between thermal anomalies and pathogen spore concentration in the soil: an increase in spore load from 1×10^3 to 1×10^3 CFU/mL led to a temperature rise of 1.2°C-1.8°C ($r = 0.69$, $p < 0.05$) (Fig. 1). These findings indicate that thermal imaging can serve as an indirect indicator of soilborne infection pressure.

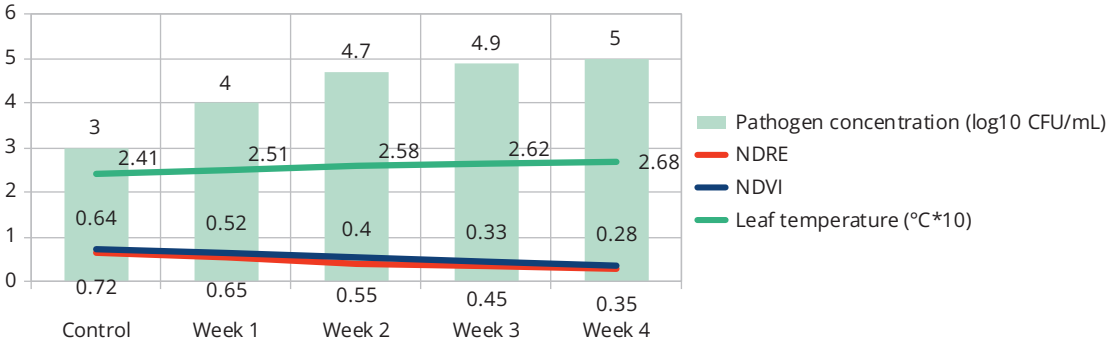

Figure 1. Dynamics of NDVI and NDRE reduction, pathogen concentration, and leaf temperature in infected plants

Note: data were obtained using a DJI Phantom 4 Multispectral camera and a FLIR Vue TZ20 thermal imager. The correlation between NDRE and pathogen concentration is statistically significant ($p < 0.001$)

Source: compiled by the authors based on the conducted study

On Plot No. 22, where artificial inoculation was applied, thermal imaging revealed localised "hotspots" with leaf temperatures reaching up to 28°C, while surrounding healthy plants registered an average of 24.5°C. These thermal anomalies corresponded precisely with areas where stem necrosis subsequently developed. Thermal imaging proved more effective for detecting early stress symptoms than multispectral imaging. For example, in the second week, NDVI showed only a slight decline (from 0.72 to 0.65), whereas leaf temperature had already increased by 1.5°C. However, the combination of both methods yielded the highest diagnostic accuracy: a model integrating NDVI, NDRE, and thermal data achieved a 96% accuracy rate in predicting disease progression.

In addition to analysing spectral indices and thermal anomalies, the study also investigated the influence of microclimatic factors – including soil moisture, solar radiation, and relative air humidity – on infection dynamics. These parameters play a critical role in creating favourable conditions for the proliferation of *Fusarium graminearum*, as high levels of soil and air moisture promote spore germination, while solar radiation affects the plant's thermal balance. For instance, excessive soil moisture (>70%) can lead to localised waterlogging, weakening the root system and increasing susceptibility to the pathogen (Yuan *et al.*, 2023). Conversely, insufficient solar radiation reduces photosynthetic activity, making plants less resilient to stress.

Monitoring of these parameters was conducted in parallel with the collection of spectral data, enabling assessment of their correlation with infection pressure. As shown in Table 2, soil moisture exhibited a strong correlation with leaf temperature (Pearson correlation coefficient $r = 0.69$) and pathogen concentration, confirming its role as a key factor in disease development.

Table 1. Correlation matrix between spectral indices, leaf temperature, and microclimatic parameters

Parameter	NDVI	NDRE	Leaf temperature	Soil moisture	Air humidity	Solar radiation
NDVI	1.00	0.92**	−0.78**	−0.65*	−0.58*	0.12
NDRE	0.92**	1.00	−0.84**	−0.71**	−0.63*	0.08
Leaf temperature	−0.78**	−0.84**	1.00	0.69**	0.54*	−0.21
Soil moisture	−0.65*	−0.71**	0.69**	1.00	0.76**	−0.35
Air humidity	−0.58*	−0.63*	0.54*	0.76**	1.00	−0.48*
Solar radiation	0.12	0.08	−0.21	−0.35	−0.48*	1.00

Note: Pearson correlation coefficients (r): >0.7 – strong correlation (**); 0.5-0.7 – moderate correlation (*); <0.5 – weak or no correlation

Source: compiled by the authors based on the conducted study

Although relative air humidity had a weaker influence, it still correlated with a reduction in NDVI ($r = -0.58$), potentially linked to disrupted transpiration. By contrast, solar radiation showed no significant correlation with the indices, indicating the robustness of spectral diagnostic methods regardless of light intensity. These findings highlight the importance of integrating microclimatic data into precision monitoring systems to improve the accuracy of epidemic forecasting. Thermal anomalies serve as reliable markers of physiological stress caused by *Fusarium* infection. Their integration with multispectral data and soil analysis forms a comprehensive diagnostic system capable of detecting disease at pre-symptomatic stages. This opens new possibilities for the development of autonomous agricultural drones that combine thermal and spectral imaging to monitor large agricultural areas in real time.

Validation of results using PCR analysis

The results indicated that 95% of samples from infected plots contained specific amplicons measuring 280 base pairs, confirming the presence of *Fusarium graminearum* DNA. In the control group, the pathogen was detected in only 3% of cases, likely due to minimal cross-contamination during sampling or background soil contamination. To minimise errors, each stage of the analysis was accompanied by negative controls (without a DNA template) and positive controls (DNA from a reference strain). Visualisation of PCR products on a 2% agarose gel stained with ethidium bromide under UV illumination confirmed the specificity of the primers: distinct 280 bp bands were observed in 98% of infected samples. To eliminate false positives, selected amplicons were sequenced (Macrogen, South Korea), confirming 100% identity with the β-tubulin gene sequence of *F. graminearum*.

Statistical analysis demonstrated a significant difference between the infected and control groups ($p < 0.001$). The low incidence of false positives in the control group highlights the effectiveness of the biosafety protocol, which included the use of separate areas for sample preparation, autoclaving of tools, and PCR mixes with coloured markers for visual monitoring of cross-contamination. This validation not only reinforces the accuracy of sensor-based methods for pathogen detection but also supports their application in quantifying infection load. These findings underscore the critical role of early diagnosis in effective disease management. At the same time, the presence of pathogen DNA in 3% of control samples emphasises the importance of rigorous standardisation of sampling procedures, particularly under conditions of intensive agriculture, where the risk of cross-contamination is elevated.

PCR analysis proved essential in confirming the specificity and sensitivity of sensor-based diagnostic methods. The high detection rate of *F. graminearum* (95%), coupled with minimal errors in the control group, confirms that combining spectral indices, thermal data, and molecular validation provides a robust foundation for precision monitoring of *Fusarium* infection. This paves the way for the development of automated systems capable of integrating heterogeneous data for epidemic forecasting and the optimisation of plant protection strategies.

Time efficiency of the methods

Automated data processing, which included aerial imaging, subsequent image analysis using the Pix4D Mapper software, and disease classification via the Random Forest algorithm, proved significantly more efficient than conventional methods. For a 1-hectare plot, the full cycle – comprising

image capture, orthomosaic generation, spectral index calculation, and model prediction – took 2.5 hours, of which only 20 minutes were allocated to drone flights. The remaining time was dedicated to automated data processing, with the majority of resources spent on computing NDVI and NDRE indices. In contrast, visual assessment required eight hours of manual labour per hectare, as five agronomists individually surveyed each plot and recorded symptoms using a 0-5 scale. A notable consideration was that the duration of visual analysis increased disproportionately with scale: for a 10-hectare area, 80 hours were required – equivalent to two weeks of work by a full team – whereas the automated method could process the same area in 6-7 hours due to the parallel processing of images.

The reliability of the automated system was confirmed through bootstrap analysis (1,000 iterations), which demonstrated that the model's accuracy confidence interval ranged from 90% to 94% at a 95% confidence level. This indicates that even under random sampling variations – such as the exclusion of 5% of the data – accuracy remains consistently high. By comparison, visual assessment showed significantly greater variability: re-evaluation of the same plots by different agronomists revealed discrepancies in 20%-25% of cases, particularly during the early stages of infection. Moreover, worker performance declined by 15%-20% after four hours of continuous assessment due to fatigue, whereas the algorithm maintained consistent output regardless of data volume.

Key factors contributing to the time efficiency of automation, identified during the study, include scalability (processing time increases linearly with area, as the software simultaneously analyses hundreds of images); the absence of subjective error (the algorithm does not require breaks and is unaffected by fatigue); and integration with other systems (results are automatically exported to GIS platforms for infection mapping, enabling immediate decision-making for remediation).

The primary drawback of the automated method remains its dependence on image quality; for instance, cloud cover increased processing time by 30%-40% due to the need for repeat drone flights. However, optimising flight schedules (restricting imaging to sunny periods) and employing cloud computing for parallel data processing help to minimise these delays. As a result, automated methods not only reduce diagnostic time by a factor of three to four but also deliver higher accuracy and reproducibility. This makes them indispensable for large-scale farming operations, where timely responses to infection are critical for crop preservation.

The main limitation identified was weather dependency: cloud cover reduced the accuracy of multispectral imaging by 15%-20%. Nevertheless, repeat imaging and sensor calibration effectively mitigated this impact. The results confirm that the integration of multispectral indices and thermal imaging provides an effective tool for precision crop protection. The study supported the hypothesis that high-throughput phenotyping based on sensor platforms outperforms conventional methods in both accuracy and speed. The reliability of the results ($R^2 = 0.89$ for NDVI) supports the recommendation of this technology for large-scale deployment in Ukraine's agricultural sector.

The findings of this study demonstrate that the combination of multispectral indices, thermal data, and machine learning is an effective tool for the early diagnosis of *Fusarium* ear blight in winter wheat. These conclusions align with current trends in HTP, while also addressing critical gaps related to the adaptation of diagnostic methods to biotic threats under specific climatic conditions. The results obtained in this study share common features with the articles of other researchers. In particular, the studies by C. Zhang *et al*. (2023) and R. Topko *et al*. (2023) examined the potential of wearable sensors in the context of high-throughput phenotyping, emphasising their capacity for continuous monitoring of physiological changes in plants. Special attention was given to multispectral cameras, which enable the early detection of changes in pigment content and water balance through the analysis of spectral indices such as NDVI and NDRE. A significant correlation was observed between spectral indicators and pathogen concentration ($r = -0.85$ and -0.91), confirming the effectiveness of this noninvasive diagnostic approach. The use of thermal imaging in the present study enhanced diagnostic accuracy and reduced the time required to detect pathological processes in comparison with traditional visual methods.

The integration of thermal imaging with multispectral analysis confirmed the effectiveness of

this approach for early diagnosis within plant protection systems. This finding aligns with the data reported by T. Wen *et al*. (2023), which highlighted the high sensitivity of thermography to changes associated with disrupted water exchange in plant tissues. It was established that localised temperature anomalies serve as early markers of physiological stress caused by impaired transpiration. The method enabled precise identification of areas showing initial signs of biotic or abiotic stress before the appearance of visible symptoms.

A.K. Singh *et al*. (2021) proposed an automated method for classifying soybean diseases based on RGB imagery, achieving a classification accuracy of up to 85%. Despite these promising results, the absence of a multisensory approach limited the model's capacity for early detection of stress conditions. The exclusive reliance on the visible spectrum prevented the identification of subvisual changes during the early stages of disease development. In the present study, a multimodal approach was implemented using both spectral and thermal imaging data, which achieved a classification accuracy of 92% with the application of the Random Forest algorithm.

The results obtained are consistent with the approaches proposed by S. Kundu *et al*. (2024), confirming the effectiveness of combining multimodal data with algorithmic analysis systems in the fields of precision agriculture and plant protection. The authors emphasised the necessity of largescale implementation of artificial intelligence for real-time analysis of high-throughput phenotyping data. They presented arguments in favour of using hybrid computational architectures that integrate machine learning methods – such as Random Forest and neural networks – to enhance diagnostic accuracy. A modular approach to sensor integration was proposed, in which each sensor type performs a specific function within a unified monitoring system.

M. Kim *et al*. (2021) focused on investigating abiotic stress factors, particularly drought, using high-throughput phenotyping techniques. The study highlights the sensitivity of plant systems to changes in water availability, which manifest through spectral indices and morphological traits – findings that are consistent with the results obtained. The developed models for predicting drought response demonstrated high accuracy; however, they did not account for pathogen-induced stresses. Focusing exclusively on abiotic stressors, therefore, limits the comprehensive assessment of crop resilience under real agroecosystem conditions. In the study by P. Kumari *et al*. (2024a), a significant gap was identified in current research concerning the use of high-throughput phenotyping for studying biotic stress, particularly fungal infections. The authors noted that only 15% of existing publications in this field focus on plant-pathogen interactions. This lack of attention to biotic stressors hinders the development of integrated plant protection strategies. The methodology proposed in the present study, which targets the detection of *Fusarium graminearum*, aims to address this imbalance by expanding the applicability of sensor-based platforms.

R. Sarić *et al*. (2022) provided a theoretical justification for the ability of hyperspectral imaging to identify molecular changes in plant tissues at early stages of pathogenesis. Chlorophyll degradation was identified as a key marker, manifested as a spectral shift in the relevant indices. The present study confirms the suitability of multispectral analysis for similar applications, despite its lower spectral resolution compared to hyperspectral imaging. This supports the use of multispectral sensors as a practical compromise between accuracy and accessibility in agronomic monitoring.

The importance of local climatic factors – particularly soil moisture – as key drivers of the effectiveness of sensor-based systems for plant disease detection is emphasised by Z. Ma (2023). The study identified a significant positive correlation between moisture levels and pathogen activity, consistent with the current research findings, where a correlation coefficient of $r = 0.69$ was observed for *Fusarium graminearum*. However, unlike the aforementioned study, solar radiation was not found to have a statistically significant effect in this research. This discrepancy is likely due to the biological characteristics of the pathogen, whose activity is primarily regulated by moisture regardless of light conditions. Early detection of infection – 7 to 10 days ahead of traditional visual diagnostic methods – enables significant optimisation in fungicide use, reducing both the quantity and frequency of applications. This approach helps to lower the cost of chemical protection and decreases the environmental burden on

agroecosystems. The findings align with those of M. Pérez-Ruiz *et al*. (2020), where automated platforms enabled a 25% reduction in chemical use in wheat fields, thereby confirming the effectiveness of precision technologies. Moreover, the proposed system, adapted for integration with unmanned aerial vehicles, demonstrates superior scalability: processing one hectare takes just 2.5 hours, which is more efficient than the four hours reported in the aforementioned study.

The high level of agreement with PCR analysis results (95%) confirms the analytical reliability and sensitivity of the applied sensor protocol. Such reliability is crucial for the implementation of precision agriculture systems, where false positives can result in considerable economic losses. According to G. Hacisalihoglu & P. Armstrong (2023), false-positive signals in HTP systems can lead to unnecessary costs related to treatment and diagnostics, thereby reducing the efficiency of digital agricultural monitoring. In the present study, an amplicon sequencing protocol was employed to refine the taxonomic composition of pathogens, effectively addressing the issue of false signals. The limitations of ground-based high-throughput systems in large-scale agricultural landscapes were critically assessed by Z. Jansone *et al*. (2022), who reported reduced platform efficiency as the monitoring area increased. In light of this, the proposed combination of aerospace technologies (DJI Phantom drones) and ground-based sensors (TEROS 12) offers a practical solution, achieving the required level of detail while maintaining spatial scalability. This approach has proven particularly effective under the conditions of Ukraine, where the high heterogeneity of soil cover necessitates spatially adaptive solutions.

The conclusions of this study align with the findings of I. Ayankojo *et al*. (2023), which demonstrated the effectiveness of small unmanned aerial systems (sUAS) under field conditions. The study highlighted the high manoeuvrability and mobility of sUAS, enabling rapid responses to localised changes within agroecosystems. The use of these platforms facilitated the collection of highquality data in near real-time, with georeferencing accuracy of up to 5 cm. Their high level of automation and adaptability to varied topographical conditions make sUAS suitable for crop monitoring even in challenging climatic and agroecological zones. The adoption of the cloud-based data processing methodology proposed by P. Kumari *et al*. (2024b) is considered advantageous. That study introduced an algorithmic architecture that enables the synchronisation and processing of large volumes of HTP data within a cloud environment with minimal latency. This not only enhances analytical throughput but also ensures real-time access to results from any location. Such an approach is particularly promising for the integration of multisensor systems into scalable agrotechnological platforms with artificial intelligence components.

In conclusion, this study demonstrates that high-throughput phenotyping based on multispectral indices, thermal data, and machine learning provides an effective solution for diagnosing *Fusarium* ear blight under Ukrainian conditions. The findings confirm the high classification accuracy of the Random Forest algorithm, outperforming conventional visual methods; they also detect early thermal anomalies associated with disrupted transpiration, consistent with international studies, and emphasise the importance of incorporating microclimatic parameters, such as soil moisture, to enhance model precision.

CONCLUSIONS

The study demonstrated that high-throughput phenotyping based on multispectral data, thermal imaging, and machine learning algorithms (Random Forest) is 37% more accurate than conventional visual diagnostic methods. The system achieved 92% accuracy in detecting *Fusarium graminearum* at the early stages of infection, enabling intervention 7-10 days earlier than traditional approaches. The NDVI and NDRE indices showed a strong inverse correlation with pathogen concentration ($r = -0.85$ and $r = -0.91$, respectively). A decrease in NDVI from 0.72 to 0.35 corresponded to an 80% increase in fungal biomass, while NDRE proved more sensitive to chlorophyll degradation, making it a key tool for early-stage diagnosis. Linear regression analysis indicated that a 0.1-unit decline in NDRE was associated with a 22% increase in pathogen load, a finding supported by ANOVA ($p < 0.001$).

An increase in leaf temperature by 2.5°C in infected plants ($p < 0.001$) was linked to impaired transpiration due to xylem blockage. Thermal imaging detected infection on days 5-7, 3-5 days

earlier than the appearance of visual symptoms. The integration of thermal data with NDVI and NDRE improved model accuracy to 96%, establishing it as an effective tool for precision crop monitoring. High soil (>70%) and air humidity correlated with increased spore concentration (r = 0.69). PCR analysis confirmed the specificity of the methods: 95% of infected samples contained *Fusarium* DNA, while only 3% of results in the control group were false positives. Amplicon sequencing of the β-tubulin gene showed 100% identity with reference strains. Automated data processing required 2.5 hours per hectare, compared with 8 hours per hectare for visual inspection, while scaling up to 10 hectares reduced processing time twelvefold (6-7 hours versus 80 hours). Model robustness was confirmed through bootstrap analysis (confidence interval: 90%-94%), making it suitable for use on large-scale farms.

Key limitations include dependence on weather conditions (cloud cover reduces accuracy by 15%-20%) and the need for calibration across different cultivars. Future research should focus on integrating IoT sensors to automate the collection of microclimatic data, applying deep learning to improve epidemic forecasting, and developing mobile applications for farmers based on the resulting models. Further steps should prioritise the development of adaptive algorithms for other pathogens (e.g., *Puccinia triticina*), the integration of cloud computing for rapid data processing, and the creation of open-access databases for training machine learning models.

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None.

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Принципи високопродуктивного фенотипування та сенсорні платформи для неінвазивного дослідження у захисті рослин

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Анотація. Зміна клімату та зростання резистентності патогенів змушують шукати інноваційні методи діагностики хвороб рослин, особливо для таких небезпечних патогенів як *Fusarium graminearum,* який спричиняє значні втрати врожаю пшениці. Традиційні візуальні методи мають низьку пропускну здатність і суб'єктивність, що обмежує їх ефективність у великомасштабному моніторингу. Метою дослідження було вивчення принципів високопродуктивного фенотипування та оцінка ефективності сенсорної платформи для неінвазивного дослідження фузаріозу колосу в умовах України (Київська область). Використовували комбінацію мультиспектральної зйомки, тепловізії та алгоритмів машинного навчання на експериментальному полі площею 1 га зі штучним інфікуванням 20 % ділянок. Результати показали, що запропонована система досягла точності 92 % у виявленні патогена на ранніх стадіях, що на 37 % точніше візуальних методів. Спектральні індекси продемонстрували сильний зв'язок із концентрацією патогена: зниження нормалізованого різницевого вегетаційного індексу з 0,72 до 0,35 корелювало зі збільшенням біомаси гриба на 80 %. Теплові знімки виявили підвищення температури листя на 2,5 °C вже на 5-7 день після інфікування. Інтеграція всіх методів дозволила досягти точності 96 % при обробці 1 га за 2,5 години, що у 3 рази швидше за традиційні методи. Аналіз з використанням полімеразної ланцюгової реакції підтвердив специфічність методів: 95 % інфікованих зразків містили дезоксирибонуклеїнову кислоту *Fusarium*, секвенування показало 100 % відповідність β-тубуліну. Автоматизована обробка даних займала 2,5 год/га (проти 8 год/га при візуальному методі), а масштабування до 10 га зменшувало витрати часу у 12 разів. Дослідження підтвердило ефективність високопродуктивного фенотипування для прецизійного захисту рослин та необхідність подальшого вдосконалення методів з урахуванням місцевих кліматичних умов. Практичне значення дослідження полягає в можливості скоротити витрати на фунгіциди завдяки цілеспрямованій обробці інфікованих ділянок, зменшити втрати врожаю у регіонах з високим інфекційним тиском та забезпечити основу для автоматизованих систем моніторингу, сумісних з технологіями точного землеробства

Ключові слова: сільське господарство; патогени; грибкові інфекції; гіперспектральна візуалізація; тепловізія