Early Detection of Stress in Greenhouse-Grown Industrial Hemp Plants by Hyperspectral Imaging

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Abstract

Industrial hemp (*Cannabis sativa*) is susceptible to nutrient deficiency of nitrogen, phosphorus, and potassium (NPK), leading to a decrease in yield and subsequently profits for growers. Therefore, in this study, we focused on monitoring the NPK fertilizing rate of industrial hemp plants in greenhouse conditions. Benchtop hyperspectral imaging was utilized to detect the NPK deficiency in three different cultivars (Trilogene Alpha, Atlas Wilhelmina, and UMN 5-4). A Resonon Pika L was used to scan the hemp plant canopy and obtain hyperspectral images in the greenhouse. Image scanning began 30 days after planting (DAP), and four days after nutrient stress application. Two additional rounds of image collection were carried out at four-day intervals. Four classifiers, multi-layer perceptron (MLP), random forest classifier (RFC), Stepwise discriminant analysis (STDA), and quadratics discriminant analysis (QDA), were used to classify stressed plants from control plants (fully fertilized). All varieties quickly responded to NPK deficiency and were recorded with high classification rates for all classes. Trilogene Alpha had the highest classification accuracy among the three hemp varieties using MLP and STDA - 94% and 97%, respectively, in DAP 30 and 98% and 97%, respectively in DAP 34. Feature selection was performed to select the most effective wavebands. The result of this study will lead to the development of an inexpensive sensor to detect hemp nutrient deficiencies before noticeable morphological changes in the greenhouse.

Keywords: Cannabis sativa, hemp, NPK, hyperspectral images, classification, fertilizer

1. Introduction

The main challenge in precision agriculture is detecting stress factors correctly before damage increases in the entire crop (Cruz et al., 2017; Ana I. de Castro et al., 2015; Luvisi et al., 2016; Roujean & Breon, 1995). Stress factors such as insects, nutrient deficiencies, pathogens, drought, and weed presence can decrease yield quality and quantity without proper management (Mahlein et al., 2010). Nutrient deficiency and plant disease economic threats could have disastrous consequences for host plants encountering novel pathogens. Plant disease diagnostic practice consists of visual inspection of suspect plants, collection of symptomatic leaves, and laboratory analyses, which are time-consuming, labor-intensive, expensive, and require experienced personnel. Therefore, a rapid technique to identify early-stage nutrient deficiency and the ability to discriminate between it and other diseases and abiotic stressors is highly desirable (Sankaran & Ehsani, 2012). Advancements in remote sensing technology offer opportunities for rapidly detecting plants by measuring plant spectral reflectance (spectral signature) and comparing the spectral reflectance of healthy and stressed plants (Lopez-Granados, 2011). The spectral reflectance of leaves can be affected by nutrient deficiency, creating differences in color, shape, cell wall degradation, or crop canopy morphology (Blackburn, 1998). Hyperspectral sensors are one of the most promising spectral-based sensors to monitor these changes, especially for nutrient deficiency, which creates chlorotic and necrotic symptoms (Mahlein et al., 2012). Innovative hyperspectral cameras in the visible and near-infrared range prove the capabilities of narrow bandwidth spectral data to detect early changes in plant physiology due to biotic and abiotic stresses(Lu et al., 2017; Mahlein et al., 2010). These remote sensing tools, in combination with powerful multivariate analysis tools, such as Neural Networks (NN), Decision Trees (DT), and Support Vector Machines (SVM), can be efficiently used to distinguish between more than one type of stressor and disease (Moshou et al., 2004; Zhang et al., 2007). G round-based spectral observations using a hyperspectral spectroradiometer have been conducted to characterize crop nutrient deficiencies, especially to evaluate the impact of nitrogen and environmental conditions on corn crops (Atkinson & Tatnall, 1997). In addition, using spectral vegetation indices (SVI) and band ratios can magnify differences in spectral signatures caused by stress factors, making separating infected plants from healthy plants easier (Mahlein et al., 2012). Monitoring nitrogen, iron, magnesium, and phosphorous can help farmers manage their fields by applying the optimum application rate, avoiding excessive fertilization, and reducing water use, which is costly and causes environmental pollution (Boroujerdnia et al., 2007; Gunkel et al., 2007; Strachan et al., 2002). Based on biochemical and biophysical plant changes due to nutrient deficiencies, ground-based spectral observations using hyperspectral spectroradiometers have been conducted to characterize these nutrient deficiency symptoms in crops. For instance, Tilling et al. (2007) applied best band selection to measure N variability in a wheat field; (Strachan et al., 2002) used hyperspectral information-based spectral to monitor different N application rates within area-grown corn; Osborne et al. (2002) determined combinations of wavelengths indicative of P and N deficiency in corn using spectral radiance measurements; Zhao et al. (2005) used ratios from leaf hyperspectral reflectance to evaluate the effects of N deficiency on sorghum growth. In addition, optical indices from hyperspectral remote sensing were employed to assess chlorophyll variability over crop plots with various nitrogen levels (Haboudane et al., 2004). Spectral measurement has been found to discriminate nutrient deficiencies and diseases in plants when the visual distinction is difficult (Ampatzidis et al., 2017; A. I. de Castro et al., 2015). Therefore, detecting and determining NPK deficiencies would be valuable when managing industrial hemp (Cannabis sativa) plants in greenhouses. This project studied the possibility of remotely detecting NPK deficiency. The main objective of the present study was to detect NPK nutritional deficiencies before obvious visual symptoms arose. Early detection of NPK can help growers develop management techniques to efficiently apply a successful NPK fertilization rate to industrial hemp in greenhouses. The objectives of this study are i) to select the optimal hyperspectral wavebands to detect NPK deficiency in hemp plants, ii) to evaluate and choose the best classification algorithm to accurately detect NPK deficiency in an early stage.

2. Materials and Methods

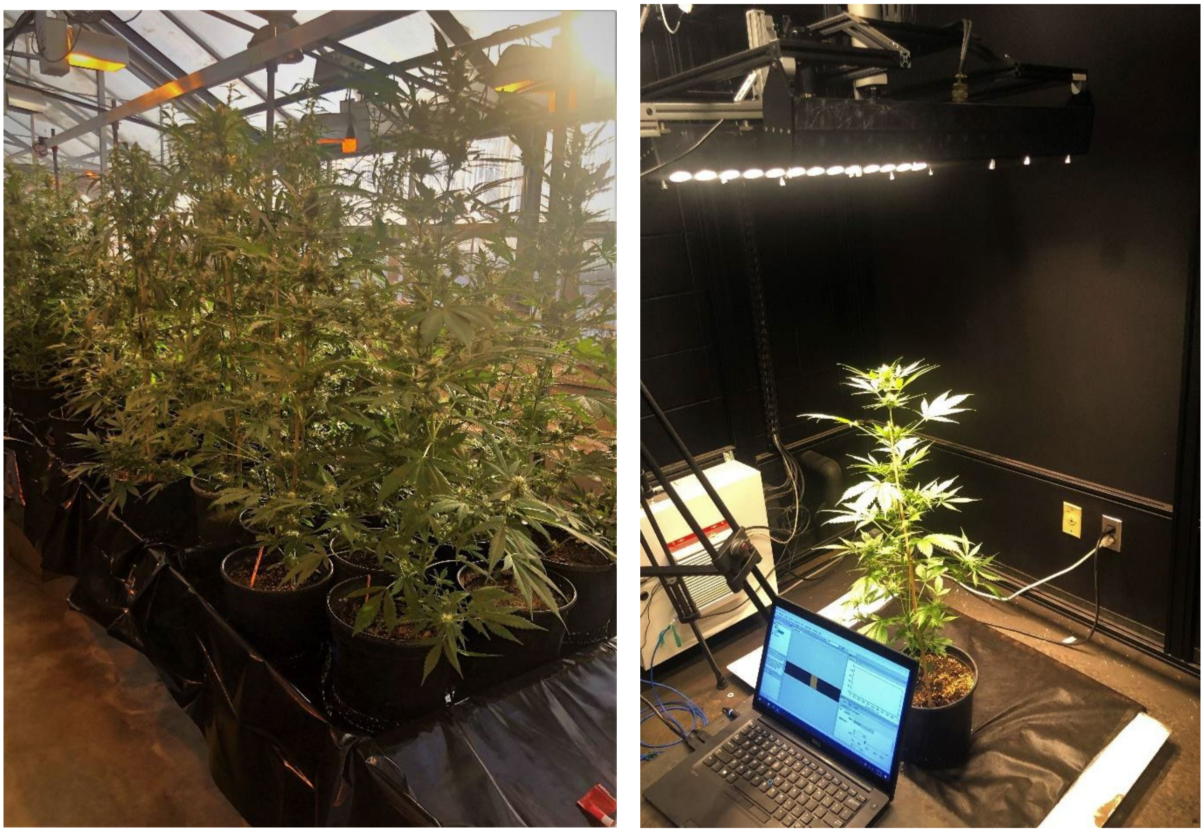
2.1. Plant and Samples Selection

The experiment was conducted in a greenhouse environment at the Plant Growth Facilities of the University of Minnesota - Twin Cities Saint Paul campus. The experiment included three hemp cultivars and breeding lines: Trilogene Alpha, Atlas Wilhelmina, and UMN 5-4. Trilogen Alpha and Atlas Wilhelmina are feminized auto-flowering cultivars bred for CBD production. UMN 5-4 is a vigorous breeding line selected from feral hemp collections and maintained by the University of Minnesota. Healthy seedlings were transplanted into 3-gallon pots at ~10 DAP, with one plant per pot. Pots were filled with Promix BRK growing media and fertilized with Osmocote Plus 15-9-12 (3-4 months) at a rate of 3g Osmocote per liter of growing media. This low fertilizer rate is insufficient for commercial hemp production and intended only to supply enough nutrients for plants to reach maturity. Two treatments were initiated at ~50 DAP. Healthy (H) plants received Jack's 20-10-20 Peat-Lite nutrient at a rate of 150 ppm via bottom water fertigation for the duration of the experiment and were used as the control. Nutrient stressed plots received no additional nutrients beyond the Osmocote. Plots were comprised of nine plants of a single cultivar/breeding line, each subjected to one of the two aforementioned nutrient regimes, for a total of six plots per repetition with two complete repetitions (Figure 1a).

2.2 Hyperspectral Data Collection

Spectral measurements were collected on Jan 3 (~60 DAP) & 7 (~64 DAP) (Fig. 1 a&b). The desired goal of the data collection was to detect the disease in a very early stage before showing any symptoms.

All collected samples were scanned using a benchtop hyperspectral imaging system (Fig.1 b), Pika L 2.4 (Resonon Inc., Bozeman MT, USA) equipped with a 23 mm lens which has a spectral range of 380–1020 nm, 281 spectral channels, 15.3º field of view, and a spectral resolution of 2.1 nm. Resonon Hyperspectral Imagers (RHI) are line-scan imagers (also called push-broom imagers). The system consists of a linear stage assembly that is moved by a stage motor. Regulated lights are placed above the linear stage to create optimal conditions for performing scans. The hyperspectral imaging system was set so the distance from the lens to the linear location was 0.5m. The lights were at the same level as the lens on a parallel plane. All scans were performed using the Spectronon Pro software (Resonon Inc., Bozeman MT, USA), which was connected to the camera system using a USB cable. Before performing scans of the leaves, dark current noise was removed using the Resonon cub software. Then the camera was calibrated by using a white tile (reflectance reference), provided by the manufacturer of the camera system, placed in the same conditions as where the scans were to be performed. The regions of interest (ROIs) were chosen manually by randomly selecting ten spectral scans from each canopy to avoid bias. The number of pixels for each RoI was about 2000, including representative leaf tissue (symptomatic and asymptomatic) to collect data from the whole leaf (Fig. 1b). The canopies of hemp plants were scanned in a separate room with stable lighting conditions. After each scan, the spectral data of the leaves were collected using post-processing data analysis software (Spectronon Pro, Resonon Inc., Bozeman MT, USA). Several areas on the leaves were selected using the selection tool, and the spectrum was generated. Several random spots on leaves were chosen for healthy and asymptomatic stages. Once the spectra were developed, the reflectance data were exported from the software in Microsoft Excel Sheets using the export option. White panels and dark lines with black covers were utilized for calibration.



(a) (b)

Figure 1: a) Industrial hemp plants in the greenhouse; b) Spectral measurement equipment setup with hemp plant with a hyperspectral camera and halogen light source shown.

2.3 Spectral Data Classification Methods

For the classification analysis, SPSS software and Python software were utilized to analyze and classify the spectral data of the four categories of plants: (i) healthy, (ii) asymptomatic, (iii) very early, and (iv) early stages. Four classification methods were utilized to analyze the collected data and classify the above categories: (I) the Neural Network Multilayer Perceptron (MLP); (II) Stepwise Discriminate Analysis (STDA); (III) Random Forest Classifier (RFC); (VI) Quadratic Discriminant Analysis (QDA). These methods were chosen because of their high classification accuracy in similar studies (Lu et al., 2017; Omrani et al., 2014).

2.3.1 Neural Network Multilayer Perceptron (MLP)

MLP is an artificial neural network that performs supervised machine learning. It is a more complicated process than a simple linear classifier and can analyze a substantial amount of data. MLP is considered an excellent function classifier for spectral reflectance data(Singh & Rao, 2005). Generally, the back-propagation technique in neural networks is utilized to adjust the network weights to improve classification accuracy (Palmer, 1987). This study randomly split the entire dataset into two datasets, including 70% training and 30% testing (Barros et al., 2008). The input layers used to train the MPL were: healthy, asymptomatic, very early, and early development stages. A cross-validation method was utilized to validate the results. Cross-validation classifies all variables.

2.3.2 Stepwise Discriminate Method (STDA)

STDA is commonly used in agricultural research to select valuable subjects of variables and to estimate the order of importance of each variable in the final prediction of a group membership. For example, the STDA method was applied in remote sensing applications to identify the dataset's patterns and determine the probability of a dataset (spectral data) belonging to a given group. This method utilizes backward elimination to remove features/factors that do not significantly affect the prediction while building a machine-learning model. Several parameters can be used to determine if a feature/factor substantially affects the forecast, including the Wilks Lambda, the Mahalanobis Distance, and the F value. If the F value of a variable is statistically significant in the discrimination group, then it means the variable contributes to the estimation of group participation. In this study, the input data contained the spectral reflectance of three hemp varieties to monitor the progress of NPK deficiency. The training dataset was 70%, and the testing dataset was 30%.

2.3.3 Quadratic Discriminant Analysis

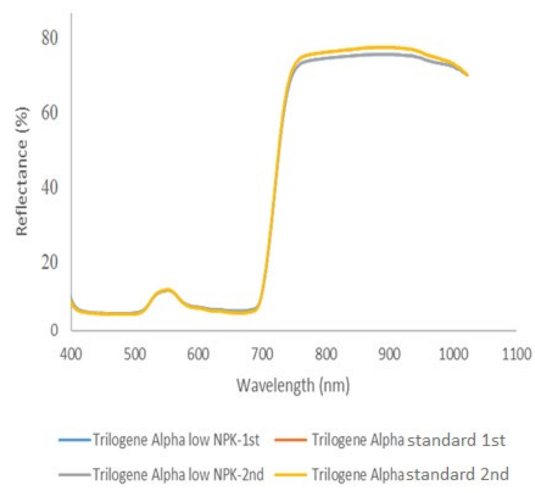
Discriminant analysis plays an essential role in biological and agricultural research. The most common discrimination methods in applied applications are parametric systems like the linear and quadratic discriminant analysis. However, there exist adjustments to these approaches, namely unbiased and predictive discriminant analysis, which lead to compact error rates in certain conditions

2.3.4 Random Forest Classifier

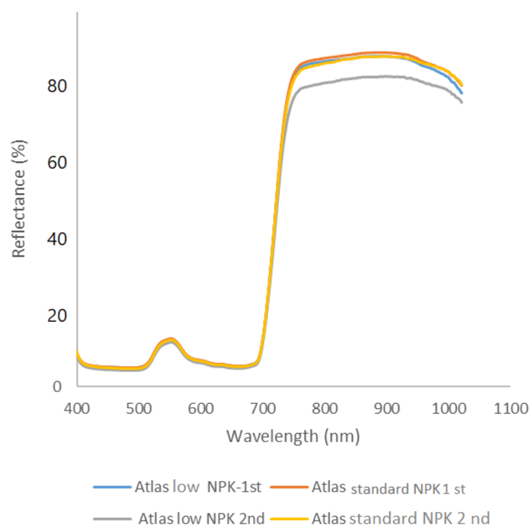
Random forests are, as a whole, a learning method for classification, regression, and other tasks that function by creating a forest of decision trees through the training time. Unlike decision trees, Random forest overcomes the weakness of overfitting its training data set and handles together numeric and categorical data. In other words, a random forest classifier contains a mixture of tree classifiers. Each classifier is produced using a random vector sampled autonomously from the input vector. Each tree companies a unit vote for the most common class to classify an input vector (Breiman & Friedman, 1988). The random forest classifier used for this study uses randomly selected features or a combination of features at each node to grow the tree.

4. Result and Discussion

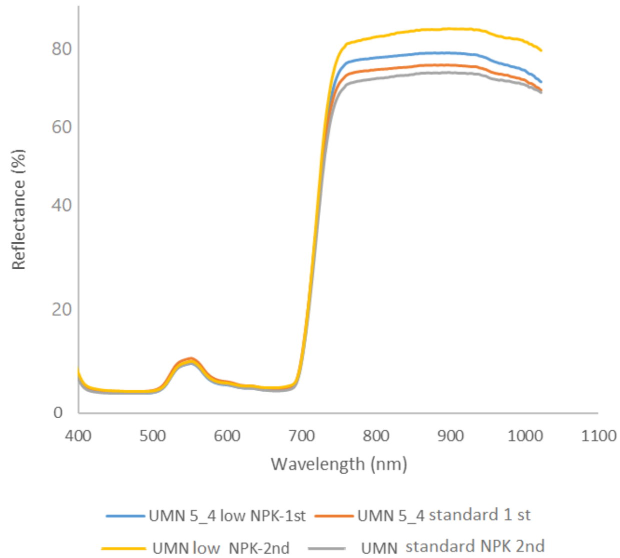
Mean hyperspectral curves (spectral signatures) for asymptomatic and advanced detection stages of NPK deficiency, as well as for standard NPK rate (which can be called a healthy (H) plant), are shown in Fig. 2 a, b, and c, for Alpha, Atlas, and UMN 5-4 respectively. In the early stage, there are no apparent differences to the naked eye in the spectral reflectance of the visible range (VIS; 400–700 nm) between the two varieties, Atlas and UMN 5-4. Fig. 2 shows two characteristic peaks, one in the green region of the spectrum and the highest reflectance in the near-infrared spectral region, which is typical for green vegetation. The expected peak in the green region of the spectrum is at 550 nm (Fig. 2) for any class. This stage may be due to the unchanging chlorophyll concentration, as chlorophyll tends to be more critically affected than other pigments when plants are under stress (Sims & Gamon, 2002). No stress occurred during this early stage, and the leaves appeared healthy in the visible range. Alpha was the only variety with higher reflectance in stressed plants than in the controlled plants. In the NIR region, all low rates of NPK had higher reflectance than standard rates. A standard rate of NPK had lower reflectance in the region (NIR) and a sharp shift to the NIR domain. Hence, early detection at asymptomatic stages is crucial and challenging because the plant is under stress when the symptoms develop. If it is not remediated in time, this will lead to early senescence or reduced crop production. In this state, before the appearance of visual symptoms, it is challenging to identify nutritional deficiencies using wavelengths in the visible region. Therefore, recourse to Proxy Gene Expression methods or remote sensing in the red-edge and NIR regions are good indicators to monitor nutrient deficiencies in the early stages (Heim et al., 2018; Nie et al., 2018). Hemp leaves kept the green color for two weeks, and then we could see some initial symptoms of NPK deficiency. The most relevant change concerning the early stage stress is observed in the NIR region. This will reduce the yield productivity, therefore identifying nutrient deficiency in the early stage, leading to improved field management and expecting the NPK deficiency in the very early stage before the situation of plants develops into complex damage.



(a)



(b)



(c)

Figure 2: The mean spectral reflectance of the industrial hemp plant for three varieties with low and standard NPK rates: a) Trilogen, b) Atlas, and c) UMN 5_4. 1st means the first-time spectral measurement, and 2nd means the second-time measurement.

Table 1: The classification results of industrial hemp plants: Atlas, Trilogene, and UMN 5_4 with four classification methods MLP, STDA, QDA, and RFC.

Category	Date	MLP (%)	STDA (%)	QDA (%)	RFC (%)
1st measurement					
Atlas standard NPK vs. low NPK	1-3-2022	86	86	85	84
Trilogene Alpha standard NPK vs. low NPK	1-3-2022	94	97	94	93
UMN 5_4 standard NPK vs. low NPK	1-3-2022	87	80	82	84
2nd measurement					
Atlas high standard NPK vs. low NPK	1-7-2022	92	94	92	93
Trilogene Alpha standard NPK vs. low NPK	1-7-2022	98	97	96	95
UMN 5_4 standard NPK vs. low NPK	1-7-2022	92	92	92	91

It was possible to differentiate between the three varieties at both asymptomatic and early stages of nutrient deficiency development (Table 1) shows the ideal spectral bands designated for early and late-stage detection of NPK deficiency using MLP classification methods. The results obtained with MLP were better than those achieved with QDA, RFC, and STDA, with classification percentages ranging from 92% to 98% in all datasets in the second trial. In contrast, the highest precision value achieved using STDA was 97%. Since the classification results in the second trial are not significant, they all have high classification rates ranging from 92-98. Therefore, the band selection was extracted as one set (six bands). Only one collection of feature selections was picked to ensure it is possible to build a sensor that can detect nutrient deficiency in greenhouses with different varieties. The sensor with six bands will not be limited to a specific variety. Each hemp plant has different plant morphology. For instance, Atlas is shorter than other varieties. Therefore, it needs an extra nutrient and water system based on the UMN 5-4 recommendations.

The best bands selected were: "**725.57, 732.05, 992.83, 854.39, 792.9, and 712.62 nm**". Most of the best bands resided between red-edge and NIR (700-900). As we mentioned, there were no significant differences between spectral curves in the visible range; the most differences were in the NIR range. Most of the low NPK rates showed higher NIR spectra than the standard NPK rate spectra. The curve record supports the result of band selection. The hemp plants did not show any symptoms during HS measurements. Therefore, there were no bands selected from the visible range. This could help growers make the right decision before nutrient deficiency increases in the greenhouse, making it challenging to remediate without loss. The concentration of chlorophyll did not change in this case. In general, chlorosis is the yellowing of leaf tissue due to a lack of chlorophyll. It may be confusing to the grower to determine the reason for the chlorosis since there are numerous possible causes: drainage, damaged roots, compacted roots, high alkalinity, and nutrient deficiency. However, it is difficult to differentiate between those of chlorosis quickly and accurately based on visual cues alone. Plant pathology and horticultural professionals depend on the apparent symptoms of symptoms and morphology patterns, which help determine the nature of the plant stress. In many cases, biotic stress symptoms are similar to abiotic stress symptoms. For instance, Abdulridha et al. (2019) could differentiate between N & Fe deficiency and Laurel Wilt Disease in avocados using the Hyperspectral technique.

5. Conclusion

Hyperspectral images with ideal feature selection can be utilized to differentiate nutrient deficiency in plants with substandard nutrient rates and develop an automated and low-cost stress detection system. The industrial hemp industry would benefit from an automatic and remote method for asymptomatic and early stress detection that would enable agronomists and growers to prevent the progression of nutrient deficiency, making timely management decisions easier and reducing degradation. Three hemp varieties were studied to ascertain their spectral reflectance of the nutrient deficiency by comparing low and standard fertilizer rates. The best classification methods were MLP and STDA, with classification rates between 91%-98% for the second round. This classification rate is considered a high rate value and can be an excellent indicator to differentiate between standard and low NPK rates. The best feature selection was chosen from MLP as follows: 725.57, 732.05, 992.83, 854.39, 792.9, and 712.62. All bands fall in the NIR and red edge, so it is possible to build a new sensor to detect nutrient deficiency in greenhouses with acceptable accuracy.

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