Spectral variability analysis Lupinus Mutabilis Sweet under nanofertilizers and chelates application through spectroscopy and Unmanned Aerial Vehicles (UAV) multispectral images.

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Abstract

Lupine is an Andean legume that has gained importance in Ecuador due to its protein content in the grain. However, in recent times the production of this crop has been affected by inadequate nutritional management. In order to avoid such circumstances, the current study spectrally analyzed lupine cultivation by the effect of the application of nanofertilizers and Fe and Zn chelates, within two controlled trials, using a radiometer spectrum, an active crop sensor and a multispectral sensor mounted on a UAV. Vegetation indices were generated and statistically analyzed using an ANOVA and Tukey tests. In the field trial, the treatments lacked to indicate significant improvements, while in the greenhouse trial, the nanofertilizer treatments indicated better results compared to the control ones. Furthermore, the chelate treatment presented a certain degree of toxicity for the plant.

1. Introduction

Precision agriculture is understood as the set of techniques aimed at optimizing agricultural inputs according to the spatial and temporal variability of production, which is achieved with the correct allocation of inputs depending on the needs and potential of each management area (Chartuni et al., 2007; Hedley, 2015; Köksal & Tekinerdogan, 2019; Bhakta et al., 2019). In order to study, evaluate and understand spatial and temporal variations, it is necessary to use technologies such as Global Positioning System (GPS), satellites, remote sensors and images of the area, linked to Geographic Information Systems (GIS) (Croner et al., 1996; Xue et al., 2002; Seelan et al., 2003; Sonti, 2015; Banu, 2015; Thakur et al., 2017). In this sense precision agriculture provides georeferenced information through maps of soil fertility or yield, which using remote sensors is capable to generate immediate information, as well as distributing agricultural inputs in an optimal way, in order to satisfy food needs (Roberson, 2000; Fulton et al., 2018; Lund et al., 1999; Abdullahi et al., 2015).

Hereby, nanotechnology focuses on the development, characterization and use of materials with very small dimensions such as < 100 nm (Weiss et al., 2006; Pal et al., 2011; Sawhney et al., 2008). In this group of materials are nanoparticles, which have great potential in agriculture since they are able to be used as nanofertilizers (Raliya et al., 2017; Arguello, 2016; Zulfiqar et al., 2019; Liu and Lal, 2015). Nanofertilizers focuses on the synthesis of macro and micro nutritive elements for crops, but mainly on micronutrients (Zn, Cu, Mn and Fe) (Manjunatha et al., 2016; Dimkpa & Bindraban, 2017; Guha et al., 2020). Plants need mineral nutrients to complete their life cycle, since they are involved in metabolic functions and are part of the organic structure. These nutrients are classified according to their concentration, being macronutrients which are required in large quantities (N, P, K) and micronutrients in small amounts (Ca, Mg, S, Fe, Mn, B, Zn, Co, Mo), while they have the same importance as macronutrients (Sinfield et al., 2010; Ejaz et al., 2011; Singh and Mishra, 2012; Toor et al., 2021). Iron plays an important role as components of the enzymes involved in the transfer of electrons in the process of photosynthesis (Rout & Sahoo, 2015; Imsande, 1998). The lack of this micronutrient is observed in young leaves as it presents interveinal chlorosis, which reduces the synthesis of complexes protein-chlorophyll in chloroplasts, while the lack of zinc causes the reduction of the growth of internodes (Das, 2014; Magri et al., 2020). Therefore, the leaves may be small, deformed and present chlorosis between the nerves of the old leaves (Zeiger & Taiz, 2006; Rajendran et al., 2009; Zhao et al., 2016).

Lupine or chocho (*Lupinus mutabilis Sweet*) is an Andean legume, which has a high protein content (approximately 46% in dry grain), compared to the rest of legumes and other Andean cereals (Muñoz et al., 2018; Mikić et al., 2013; Jacobsen & Mujica, 2008; Miano et al., 2015; Mora Villacís et al., 2020; Murgueitio-Herrera et al., 2022). This legume provides phosphorus, iron and zinc, which are the main minerals in the human body, therefore, it is considered a product that contributes to the food sovereignty of many countries. The lupine is part of the variety of agricultural products that have an important nutritional content, because it has a high percentage of protein, and other nutrients that are essential for human health and for food sovereignty in Ecuador (Villacrés et al., 2006; Giunta, 2014; Peña, 2016).

In Ecuador, lupine cultivation is located in the highland region, where it is capable of adapting to different types of soil, especially in dry and sandy agro-ecological zones between 2,600 and 3,400 meters above sea level (Falconí et al., 2013; Carlos et al., 2018; Struelens et al., 2021). It develops in environments where rainfall fluctuates between 300 to 600 mm per year and with a temperature between 7 and 14 ° C (Blackmore et al., 2021; Caicedo & Peralta, 2000).

Anthracnose is a disease that affects stems, leaves, pods and seeds, in all stages of lupine plant development, therefore, this problem alters the spectral response and therefore the value of the NDVI vegetation index, which allows evaluating the health of the vegetation (Falconí, 2012; Horning, 2008).

Remote sensing is an useful technology for precision agriculture, as with the use of satellite platforms for agricultural management they are very important to monitor agricultural production (Brisco et al., 1998; Segarra et al., 2020; Khanal et al., 2017; Torres et al., 2022). However, within the agricultural-scale applications they have not been adopted as expected due to a variety of challenges such as pixel resolutions, infrequent coverage, clouds and slow delivery of information to users (Saiz-Rubio & Rovira-Más, 2020). Nonetheless, unmanned aerial vehicles (UAV) or drones provide a remote sensing platform with the ideal characteristics for data acquisition, such as high spatial resolution, on-demand coverage, and fast information delivery (Nebiker et al., 2008; Feng et al., 2015; Hunt & Daughtry, 2017)

Remote sensing, being a discipline that obtains information from an element by detecting and analyzing its radiated energy, lays its foundations in the study of spectroscopy (Gholizadeh & Kopačková, 2019). This refers to the observation and study of the electromagnetic spectrum, and is based on the interaction of radiant energy with matter, that is, it measures the intensity of radiation as a function of the wavelength of an object (Pu, 2017). That implies the measurement of energy in many parts of the electromagnetic spectrum, which allows monitoring the vegetation in a spatial and temporal way, make predictions of crop production, monitoring of agricultural land use, as well as early detection and control of diseases of the crop (Weiss & Jacob, 2020; Yang, 2020; Viera-Torres et al., 2020).

There are several remote sensing studies focused on analyzing the spectral response across the phenological stages of crops, such as those by Psomas et al. (2005) on grasslands and Manevski et al. (2011) on Mediterranean plants. They are also notable a variety of research developed in the cultivation of lupine, where the spectral behavior of the crop was analyzed, after a seed disinfection application, implemented in greenhouse and field (Tapia-Silva et al., 2011; Kosewska et al., 2016; Sinde-González et al., 2021).

Based on the aforementioned, the present study aims predominantly to determine the spectral variability in the phenological states of the lupine crop in two controlled trials (greenhouse and field). This shall be reached in order to analyze the effects of addition of chelates and nanofertilizers, for purposes of control and monitoring of this focused crop.

2. Materials and Methods

2.1 STUDY AREA

The study area is located in the central part of the Inter-Andean Valley, inside the highlands of continental Ecuador, a country characterized by a complex geodynamic setting associated by a variety of natural hazards (Toulkeridis et al., 2017; Toulkeridis and Zach, 2017; Macías et al., 2024). Within the Campus IASA I, in the farm Prado of the University of the Armed Forces ESPE (Fig. 1), two tests were conducted, being in a greenhouse with a translucent plastic cover and two in the field, with an area of 60 m^2 and 240 m^2 respectively. This zone has a humid mesothermic equatorial climate, with an average annual temperature of 14 ° C. The average annual rainfall is of about 1500mm being located at an altitude of 2748 meters above sea level. Additionally, there is a type of soil of the molisol order, which is characterized by having a high content of organic matter. See Fig. 1.

2.2 MATERIAL

The obtaining of spectral data was conducted using a Spectral Evolution PRS-1100 spectroradiometer, which records spectral information in a range of 320–1100 nm. In addition, a GreenSekeer crop sensor was used, which emits bursts of red and infrared light and measures the light reflected by the leaves of the crop, with which it is possible to obtain Normalized Difference Vegetation Index (NDVI) values. In order to obtain spectral information of the plant canopy, a DJI MAVIC PRO UAV was used, where a multispectral sensor MAPIR Survey 3W-Red + Green + NIR (RGN, NDVI) was mounted. With that sensor we were able to obtain information in near infrared light of 850nm, red from 660nm and 550nm green. Control points were obtained using a precision Trimble R8 GPS. Qgis software was used for geospatial data management.

The methodology and material usage are summarized in Fig. 2.

3.2 Establishment of trials

Two trials were established in which the first was in a plastic greenhouse, with an approximate area of 60 m^2, and the second was performed in the field where there was an approximate area of 240 m^2. A Completely Random Design (CRD) was applied with five treatments and three repetitions (R1, R2, R3), with a total of 15 experimental units per trial. In the greenhouse, each experimental unit has an approximate area of 4m^2, while in the field each experimental unit has an area of approximately 15m^2. The project had five treatments, of which the first represents the control (T1), the second and third treatment (T2 and T3) are the application of chelates of (Fe, Zn) at concentrations of 2gr / l and 4gr / l respectively, while the fourth and fifth treatment (T4 and T5) are the application of nanofertilizers of (Fe, Zn) at concentrations of 540ppm and 270ppm respectively. The application was conducted by foliar spraying with the use of an atomizer, until the plant reaches the point of runoff. In planning the sampling times, the phenology of the lupine was considered as a basis. Then, four samplings were conducted in the phenological stages of vegetative development, flowering, reproductive and sheathing. It should be noted that the samplings were performed between two or three days after the application of the treatments. The distribution of the trials in greenhouse and in field can be seen in Fig. 3.

3.3 Data Capture

The spectroradiometer (See Fig. 4) was used to capture spectral data in the two trials. In each experimental unit ten plants were selected and from each one three leaves were chosen, located in the upper third of the plant. Therefore, thirty spectral data were obtained per experimental unit, while this process was repeated in the different samplings. The spectrum radiometer generates a file with a .sed extension, which is compatible with Microsoft Excel software. In it, databases were set up according to the repetitions, treatments and phenological states, of the three spectral values of each plant. A mean was performed in order to obtain an average value of each one, resulting in ten values for each experimental unit.

Using the crop sensor (See Fig. 4), NDVI values were automatically obtained both in the field and in the greenhouse. In order to ensure the accuracy of the readings, the sensor was placed at a distance between 60–120 cm above the crop, parallel to the ground. In this way, in each experimental unit a value was taken per plant, resulting in 10 NDVI data for each unit in the different samplings.

3.4 Aerial image capture with UAV

With the use of the UAV in this study, the aim has been to explore the possibility of its future application as a tool for analyzing the effect of nanofertilizers on larger-scale crops. Therefore in the field trial, a total of five flights were performed with the DJI Mavic Pro UAV and a Survey 3W-Red + Green + NIR camera, taking multispectral images on the same days of spectral data collection. For the execution of the flight, the Pix4D Capture application was used, in which the flight parameters such as longitudinal and transverse overlap, height and terrain area were established. In order to georeference the multispectral images obtained, four control points were established, and subsequently materialized in cylindrical landmarks 40 cm high and 10 cm in diameter. Of the same it was tracked for two and a half hours each in static mode, while later it was post-processed with the PPP method.

In the processing of the images obtained, the Pix4Dmapper software was used, with the 3D Maps template, capable of generating ortho mosaics. The process is divided into three steps, being Initial Processing, Mesh and Point Cloud Generation, as well as MDS and Orthomosaic Generation. Once the orthomosaics had been generated, the radiometric calibration was performed using the Mapir Camera Control application and the MAPIR Calibration Ground Target PackageV2 card (See Fig. 5). This process was repeated for the different flights, in order to obtain orthomosaics in different phenological stages.

3.5 Generation of vegetation indices

The vegetation indexes, Normalized Difference Vegetation Index (NDVI), Transformed Normalized Difference Vegetation Index (TNDVI), green band (GNDVI), Grassland Curing Index (GCI), Renormalized difference vegetation index (RDVI), Relative Vigor Index (RVI) and Difference Vegetation Index (DVI) were calculated from the spectral data (Tucker, 1979; Pettorelli, 2013; Naji, 2018; Melilos et al., 2020). Additionally, the DVI index was used to discriminate between soil and plant values, with the aim of avoiding values that could contaminate subsequent extractions (See Fig. 6). In the multispectral images obtained, the NDVI index was calculated and to obtain the value of the vegetation index per plant, they were georeferenced using the RTK method, with which 150 points were obtained (See Fig. 7). A buffer was generated at these points and the Zonal Statistics tool was applied.

3.6 Statistic analysis

Prior to the ANOVA analysis of the vegetation index values obtained by different methods, an exploratory analysis of the data was conducted. This was generated in order to organize and prepare the data, detect failures in the data collection and identify outliers. The statistical analysis begins with the hypothesis tests approach between the different treatments applied to the cultivation of lupine. Several hypotheses were then raised for both trials. Regarding the treatments, it is confirmed that H_0, are the vegetation indices that are not efficient to identify characteristics associated with the treatment of nanofertilizers and Fe and Zn chelates in lupine cultivation. The H_1 are the vegetation indices that are efficient to identify characteristics associated with the treatment of nanofertilizers and Fe and Zn chelates in the lupine crop. Regarding the phenological states, the H_0 are the vegetation indices which are not efficient to spectrally characterize the lupine crop during its phenological development, and, finally, the H_1 are the vegetation indices which are efficient to spectrally characterize the lupine crop during its phenological development.

4 Results

4.1 Data capture

A total of 1782 spectral signatures were obtained with the espectroradiometer in the trial, associated with the four samplings conducted, during the phenological development of the lupine. The values of the NDVI index, by using the crop sensor, a total of 600 values and 594 values were obtained for field and greenhouse trials respectively. In the field trial, four orthomosaics were obtained in RGN, one for each phenological state during the development of the crop. The flight parameters were the same for all the flights (Table 1).

Table 1
Flight parameters

Flight time	6 min 59 s
Longitudinal overlap	90%
Transverse overlap	80%
Flight height	30 m
Total surface	37 m x 82 m
GSD	1 cm
Speed	3 m/s
Flight lines	5

4.2 Statistical analysis of the vegetation index

Vegetation indices were calculated, such as $NDVI_E$, GNDVI, TNDVI, DVI, RVU, RDVI, GCI by means of the spectral response and $NDVI_S$ of the crop sensor, for each treatment, during the four phenological stages. From the images taken with UAV in the field trial, a map of $NDVI_{UAV}$ in each phenological stage resulted. From this information, descriptive statistics such as mean and standard deviation, data distribution graphs and box plot were obtained. The analysis was performed in order to verify the existence of a normal distribution among the data and to obtain outliers.

4.3 Analysis of variance (ANOVA)

In the field and greenhouse test, the analysis of variance was applied, to the vegetation indices obtained from the radiometric data, $NDVI_S$ values from the crop sensor, and $NDVI_{UAV}$ values acquired through the UAV. This occurred in order to determine the hypotheses raised, and to identify differences between the treatments and phenological states in the lupine crop, starting from the VI. Table 2 lists the ρ - treatment values and phenological state resulting from the trial.

Table 2 indicates that there is statistical significance in the vegetation indices when analyzing the treatments and phenological states of the lupine crop, therefore, the alternative hypothesis is accepted. For the NDVI obtained with the crop sensor, there is no statistical difference in the treatment of chelates and nanofertilizers of Fe and Zn, therefore, the null hypothesis is accepted. However, for the case of phenological states, there is statistical significance, thus that, the alternative hypothesis previously exposed is accepted.

Table 2. ρ - value for Vegetation Indices, $NDVI_S$ - greenhouse test.

Index		**ρ – value**	
		Treatment	**Phenological state**
Spectral	$NDVI_E$	< 0,0001*	< 0,0001*
	RVI	< 0,0001*	< 0,0001*
	GNVI	< 0,0009*	< 0,0001*
	TNDVI	< 0,0001*	< 0,0001*
	RDVI	< 0,0001*	< 0,0001*
	DVI	< 0,0001*	< 0,0001*
	GCI	< 0,0032*	< 0,0001*
Crop sensor	$NDVI_S$	0,5149	< 0,0001*
** Statistical significance ρ-value < 0,05*			

Table 3. ρ - value for Vegetation indices, $NDVI_{UAV,}$ $NDVI_S$ – field trial.

		ρ - value	
	Index	Treatment	Phenological state
Spectrals	$NDVI_E$	0,1835	< 0,0001*
	TNDVI	0,2001	< 0,0001*
	GNDVI	0,0831	< 0,0001*
	RVI	0,7576	< 0,0001*
	RDVI	< 0,0001*	< 0,0001*
	DVI	< 0,0001*	< 0,0001*
	GCI	< 0,0141*	< 0,0001*
UAV	$NDVI_{UAV}$	< 0,0331*	< 0,0001*
Crop sensor	$NDVI_S$	< 0,0035*	< 0,0001*

** Statistical significance ρ-value < 0,05.*

The ρ - values presented in Table 3 indicate statistical significance only in the RDVI, DVI and GCI indices through the application of nanofertilizers and chelates. Therefore, the alternative hypothesis is accepted, in the case of phenological state the p - values indicate statistical significance in all the indices, then the alternative hypothesis previously exposed is accepted. For the $NDVI_{UAV}$ and $NDVI_{S}$ statistical significance was found in treatments and phenological status. Once the indices were established with ρ - value < 0.05, the statistical test of Tukey was applied at 5%, in order to find differences in means between the treatments and phenological states.

4.4 Tukey's statistical test

Greenhouse treatments

The vegetation indices $NDVI_{E}$ and TNDVI behave in a similar way, therefore, the results of the Tukey test indicate that treatments 2, 4 and 5 (T2, T4 and T5), which occupy the same range (A), that is, statistically they are equivalent, while treatment 1 (control) occupies a different rank (B). This demonstrates that spectrally it is possible to differentiate between a lupine plant with and without fertilizers (chelates and nanofertilizers). However, a difference cannot be established between nano-fertilizer and chelate treatments, since they are statistically equivalent.

The results of the Tukey test of the GNDVI and GCI vegetation indices indicate that treatment T5 occupies the range (A), while treatments 2 and 3 (T2 and T3) occupy a different range (B), that is, statistically equivalent. However, treatments 1 and 4 (T1 and T4) can be located in A or B. Vegetation indices DVI and RDVI behave in a similar way. The results of the Tukey test demonstrates that treatment 4 (T4) occupies the range (A), and treatments 1, 3, and 5 (T1, T3 and T5), occupy a different range (B), that is, statistically they are equivalent, while treatment 2 (T2) can be located in A or B.

Greenhouse phenological states

The results of the Tukey test of the vegetation indices $NDVI_{E}$ and TNDVI indicate that phenological stages 3 (vegetative development), 4 (flowering) and 6 (sheathing) were classified into three different categories (A, B, and C), while phenological state 5 (reproductive) can be located in B or C, which allows us to deduce that the lupine crop can be differentiated spectrally by phenological state. Tukey's test of the GNDVI and GCI vegetation indices demonstrate that phenological stage 6 (sheathing) presents a range (B), while phenological stages 3, 4 and 5 occupy a different range (A). This indicates that spectrally it is possible to differentiate between the phenological states of the lupine crop. However, a difference cannot be established between states 3, 4, and 5, because they are statistically equal. In the case of the DVI and RDVI vegetation indices, it is evident that the four phenological states analyzed occupy a different range, which allows us to deduce that, spectrally, the lupine crop can be differentiated by phenological state, as listed in Table 4.

Table 4
Tukey test at 5% of the DVI and RDVI indices for the phenological stages in the greenhouse *trial.*

Phenological State	Description	DVI	RDVI	Range			
		Mean	Mean				
5	Reproductive	85,46	8,62	A			
3	Vegetative development	82,72	8,49		B		
4	Flowering	77,41	8,23			C	
6	Sheathing	76,02	8,11				D
Means with a common letter are not significantly different (ρ > 0.05)							

Field Treatments

During the application of the Tukey test on the RDVI values, the means of each treatment are demonstrated where treatment T1 belongs to range A, followed by T5, while T4 is included in range B. Therefore, there is no significant difference between their means, as the T2 treatment is in the BC ranges, while the T3 treatment appears in the C range. The DVI index describes a grouping of treatments similar to the RDVI, because it was proposed to improve the DVI and NDVI indices. In this case, the T5 treatment is in the A-B ranges, resulting that there is no significant difference between the control and Fe and Zn nanofertilizers at 270 ppm concentration.

Tukey's test in the GCI index indicates that treatment T2 presented the highest mean, while treatment T5 and T1 are in the ranges A - B coinciding with the DVI index. Hereby, there is no significant difference, while on the other hand, there is a difference between treatment T2 and T4 with respect to T3. For the NDVI obtained from the crop sensor, it presents two ranges, being for range A the treatments T4 and T1, ranges A - B the treatments T5 and T2, while range B includes the treatment T3. In other words, there is only a significant difference between the treatment T4, T1 with respect to T3, this being the lowest mean and it coincides with the rest of the indices in this aspect. The results of the Tukey test for the $NDVI_{UAV}$ index indicates that there is a significant difference only between treatments T3 and T1, with T3 being the maximum mean of all treatments, thus, this result differs from the rest of the indices analyzed.

Phenological states- field

Tukey's test by phenological state, applied to the NDVIE and TNDVI indices, yields equal ranges for the means. Three ranges are identified, in which in range A is state 5, range B in states 4 and 6, and finally, state 3 in range C. This confirms that the NDVIE and TNDVI indices identify spectral characteristics that differentiate between phenological stages of the lupine crop. Tukey's test of the RDVI and DVI indices indicates a distribution of ranges similar to the NDVIE index, with three ranges where states 4 and 3 are significantly distinguished, while states 5 and 6 are in the same range. The behavior of the RDVI values indicate that state 4 presents the highest mean compared to the NDVI index, which was state 5. Additionally, there is no significant difference between the last two states 5 and 6. The GNDVI and GCI indices presented a behavior similar to the previously described $NDVI_E$ index (See Table 5), and additionally coincides with the $NDVI_{UAV}$, since state 5 is the highest mean. Thus, the hypothesis raised is corroborated, that the indices are efficient to spectrally characterize the lupine crop during its phenological development.

Table 5
Tukey's test for GNDVI, GCI indices by phenological state.

Phenological State	**Description**	**GNDVI mean**	**GCI mean**	**Range**			
5	Reproductive	0,73	5,44	A			
4	Flowering	0,70	4,64		B		
3	Vegetative development	0,68	4,20			C	
6	Sheathing	0,66	3,98				D

Means with one letter in common are not significantly different (p < 0,05)

The Tukey test for the NDVI values obtained from the GreenSeeker crop sensor, demonstrates the means of the different phenological states and they are classified into three ranges. There is a significant difference between states 3 and 6, while states 5 and 4 are not distinguished. Tukey's test for the NDVI values obtained by UAV indicates four ranges, one for each phenological state, see Table 6. In other words, the stages during the phenological development of the lupine crop are significantly distinguished, unlike the $NDVI_E$, which did not present a significant difference between stages 4 and 6.

Table 6
Tukey's test for $NDVI_{UAV}$ index by phenological state.

Phenological State	**Description**	**NDVI mean**	**Range**			
5	Reproductive	0,63	A			
3	Vegetative development	0,59		B		
6	Sheathing	0,53			C	
4	Flowering	0,35				D

Means with one letter in common are not significantly different (p < 0,05)

4.5 Comparison of $NDVI_S$ values between trials

From the data obtained from the crop sensor, graphs (See Fig. 8) were realized that illustrate the behavior of each treatment in the different phenological states for each trial, in order to perform a comparative analysis between trials. From the first application of nanofertilizers and chelates, an improvement was identified in the T4 and T5 treatments with respect to the initial state in the two trials. On the other hand, the T3 treatment indicates a decrease in the NDVI value for the field test, while it is maintained for the greenhouse. The phenological state of flowering demonstrates a decrease in the NDVI value in all treatments, this due to the susceptibility to diseases that this state presents. For the two trials a recovery of treatment T4 is given compared to the rest. Finally, in the sheathed state, the treatments with nanofertilizers indicate higher averages than the control. Treatment T3 presented the lowest means throughout the vegetative development of the lupine crop.

5 Discussion

5.1 Greenhouse trials

Treatments

The information was generated from a spectroradiometer, a crop sensor and an UAV with modified lens, in order to generate vegetation indices (NDVI, TNDVI, SR-RE, NDRE, $NDVI_E$ and CCI). Within the studies previously mentioned, the NDVI and TNDVI indices were not efficient to identify significant differences between treatments, while in the current investigation these indices indicated that there is a significant difference between treatments T2, T3, T4 and T5, with T1 (control).

In the case of the GNDVI and GCI indices, it is observed that treatment 2 (T2) presents the lowest value, while (T5) the highest. The GCI and GNDVI indices present a higher value in both treatment 5 and 4, because these indices have shown greater sensitivity to variations in chlorophyll content. The RDVI and DVI vegetation indices demonstrate that treatment 1 has the lowest value, while (T4) the highest. These indices, by making use of the red and near-infrared portions of the electromagnetic spectrum, are able to establish reflectance combinations that are sensitive to chlorophyll concentration, foliage agglomeration, and canopy leaf area. The effect of nanofertilizers on lupine cultivation can contribute to decreases in NIR wavelengths and increase in red wavelengths, thus causing an increase in the values of these vegetation indices (Agapiou et al., 2012).

In several studies conducted by applying the vegetation indices NDVI and TNDVI, significant differences were established between the phenological states of the crop, but with little precision, that is, one phenological state cannot be differentiated from the other (Sinde-González et al., 2021; Simbaña & Tello, 2020). Similarly, in such studies the NDVI and TNDVI indices presented the same results, but unlike previous studies, when applying the DVI and RDVI vegetation indices, it was possible to establish significant differences in the four phenological stages of the crop.

5.2 Field trial

Treatments

In the study of Simbaña & Tello (2020), significant differences were encountered between seed fertilization treatments applied to lupine cultivation through the NDVI and TNDVI indices obtained from spectral data. However, in the present project, these indices did not indicate significant differences between the treatments applied for the field trial, and, on the other hand, the DVI, RDVI and GCI indices were able to significantly differentiate the applied treatments. According to the DVI and RDVI indices, both, nanofertilizers and chelates did not present improvements with respect to the control, which differs from another study on the foliar application of NPK nanofertilizer in potatoes, where significant effects on the yield and quality of the crop were found (El-Azeim et al., 2020).

According to the Tukey test for the GCI index, the T4 treatment indicates a higher mean than the control treatment, as previously confirmed, where ZnO nanofertilizers were sprayed on bean plants generating important improvements in plant biomass, root length and chlorophyll content (Tarafdar & Raliya, 2014). From the results obtained in the field, from the Tukey test for the $NDVI_S$ and $NDVI_{UAV}$ indices, both indices significantly distinguished between applied treatments. Additionally, they presented a strong contrast, since in the index $NDVI_S$, the treatment with the lowest mean was T3, the opposite of the $NDVI_{UAV}$ index, in the case of the crop sensor. This difference may be related to the data collection height, since the crop sensor performs readings of the upper part of the canopy of the studied plant, that is, the reflected energy detected, belongs to the cover closest to the sensor, while the radiation or obtained from the images by UAV comes from the entire crop canopy (Klaas, 2014).

In the field trial, the NDVI and TNDVI indices obtained from radiometric data significantly distinguished three of the four phenological states studied, indicating greater precision than in the study by Simbaña & Tello (2020), where only two phenological states with the same indices were differentiated. It also presents a precision similar to that found by Sinde-González et al., (2021) in their study on lupine using the NDVI and TNDVI indices obtained from radiometric data in a field trial. On the other hand, the RDVI and DVI indices significantly differentiated three states, as did the NDVI. The reproductive state presented the highest NDVI value, while the flowering state was the highest in DVI and RDVI. This contrast may be due to the fact that the NDVI index is more sensitive to low and medium concentrations of chlorophyll than RDVI, and the presence of chlorophyll in the leaves is closely related to plant health (Haboudane & Miller, 2004; Matamoros & Falconí, 2018). Finally, the GNDVI and GCI indices indicated greater precision compared to the rest of the indices obtained from radiometric data, since they significantly distinguished each phenological state. This coincides with the study by Sinde-González et al. (2021) where they analyzed the CCI index to distinguish between phenological states in lupine cultivation.

The $NDVI_{UAV}$ index significantly differentiated each phenological state, unlike the study by Sinde-González et al. (2021), where they found significant differences only in one phenological state. This may be related to the type of camera used, while in the present study, the UAV camera was used, being different to a Parrot Sequoia. Regarding the behavior of the $NDVI_{UAV}$ value throughout the vegetative development, it is observed that the flowering state presented the lowest average, which according to De la Casa & Ovando (2007), in their study on corn. The value of the NDVI index presented greater variation when it blooms, likewise, this phenological state presents a high correlation with the crop yield.

Comparison between trials

According to the preliminary sampling (development 1) the NDVI values varied from 0.78–0.81 for the field trial, while for the greenhouse they were of about 0.73–0.79. This is because factors such as leaf thickness, leaf age and leaf area index can influence the spectral response of the leaf (Katsoulas & Elvanidi, 2016). Regarding the treatments T4 and T5 (nanofertilizers) they yielded an increase in the value of the index after the first application for both tests, since the application of ZnO nanofertilizers increases the chlorophyll content (Tarafdar & Raliya, 2014). On the other hand, treatment 3 (chelates) in the field trial presented the lowest value of NDVIS in all phenological states, because it contains a concentration higher than the recommended one, causing a possible phytotoxicity in the plant (Molina & Meléndez, 2002).

In the flowering state, the different treatments determined a significant decrease in the index value in the two trials, as a product of the sensitivity to diseases that this state presents (Falconí & Bracho, 2019). Additionally, this agrees with studies on lupine and corn, in which the NDVI value decreased and varied significantly in this phenological state (Sinde-González et al., 2021; De la Casa & Ovando, 2007).

In the reproductive state, the T5 in the field and T4 in the greenhouse indicated an increase in the value of NDVIS, surpassing the control treatment since nanofertilizers are useful for disease management (Elumalai et al., 2014). Finally, in the sheathed state, a decrease in the value of NDVIS was identified, which is observed when the crop is close to physiological maturity (Guamán, 2018) On the other hand, in another study, a decay in the relation of rice grain yield with the values of the crop sensor was found due to possible effects related to the decrease of the canopy in front of the sensor's field of view (Ali & Thind, 2014).

Conclusions

The use of geospatial tools in the cultivation of lupine, allows the spectral characterization of theeffect of the application of nanofertilizers and Fe and Zn chelates in which the use of these instruments it was possible to generate indices that allowed to identify significant differences between treatments, as well as between phenological states.

From the field trial, four RGN orthomosaics were obtained, one for each phenological state. These were used to generate NDVI maps and obtain a total of 600 weighted NDVI values.

From the vegetation indices NDVI, TNDVI, GNDVI, GCI, DVI and RDVI, it can be concluded that they are efficient to spectrally characterize the lupine crop due to the application of nanofertilizers and Fe and Zn chelates in controlled trials. In each of the analyzes treatments T4 (Nanofertilizers Fe and Zn 540 ppm) and T5 (Nanofertilizers Fe and Zn 270 ppm), allow to obtain higher values with respect to the control. In the case of the vegetation indices NDVI and TNDVI, the treatments with nanofertilizers (T4 and T5) present higher values, meaning that they improved the health status of the crop. Likewise, The GNDVI and GCI indices indicate higher values in the treatments with nanofertilizers. Therefore, it is concluded that chlorophyll levels are higher, because these indices are sensitive to this parameter. Similarly, the vegetation indices DVI and RDVI presented higher values in treatments T4 and T5, compared to the control (T1). That is, the application with nanofertilizers improved the cell structure of the culture.

Through the use of the spectroradiometer, it was possible to generate vegetation indices that allowed to significantly differentiate between treatments applied to the cultivation of lupine in the field. The DVI and RDVI indices that use the NIR band establish that the nanofertilizers (T5) did not alter the cell structure of the plant, while the GCI index that uses the green band, identified a higher concentration of chlorophyll in the T4 treatment, therefore, increased pigmentation in the leaves. In terms of precision, the DVI and RDVI indices stood out compared to the GCI index since they differentiated three of the five treatments applied. On the other hand, the NDVI index obtained from the crop sensor indicated better pigmentation in the T4 treatment leaves, while the $NDVI_{UAV}$ presented a precision similar to the GCI index.

All the indices obtained from the spectral signatures in the greenhouse and field tests proved to be efficient to distinguish between the different phenological states of the lupine crop. The $NDVI_{E}$, TNDVI, RDVI and DVI indices only managed to significantly differentiate three states of the four analyzed, while the GNDVI and GCI indices presented greater sensitivity when distinguishing each phenological state. This established a decrease in pigmentation during the sheathed state, due to the proximity of senescence of the plant.

The indices calculated using spectral data were efficient to distinguish between phenological states. In the greenhouse test, the DVI and RDVI indices presented greater precision to significantly differentiate the four phenological states, while in the field test the GNDVI and GCI indices indicated greater precision because they are more sensitive to chlorophyll content. Similarly, the NDVI index obtained using the multispectral camera (RGN, NDVI) significantly differentiated each state, which establishes the importance of spectral resolution when conducting studies on different crops.

Through the use of the spectroradiometer spectrum it was possible to demonstrate that after the application of Fe and Zn nanofertilizers implied an improvement in the structure and chlorophyll content of the leaf and therefore in the development of the lupine plant in the greenhouse test. Contrarily, in the field trial the applied treatments did not yield an improvement in relation to treatment 1 (control), this related to environmental factors such as temperature, light, relative humidity and application time that influence the success of foliar fertilization. Additional treatment T3 caused a deterioration in chlorophyll, as well as in the cellular structure of the plant in both tests. On the other hand, according to the indices obtained from spectral data, $NDVI_{S}$ and $NDVI_{UAV}$, a decrease in vigor and health of the plant is established in the flowering state due to the susceptibility to diseases that this state presents.

Declarations

Conflict of interest:

The authors declare no conflict of interest.

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Author Contribution

Conceptualization ISG, EM, CF, Methodology ISG, CF, Software ISG, Formal Analysis ISG, EM, CF, Investigation ISG, EM, CF, Data Curation ISG, Writing original Draft ISG, Writing Review ISG, EM, CF, MGD, TT, Editing Supervision ISG, EM, CF, MGD, TT

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Data Availability

Data of this research will be freely available at https://drive.google.com/drive/folders/1BUV3-knOJ4Tj-IXFQYoMdqBAq0KhGPFN?usp=sharing

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Figures

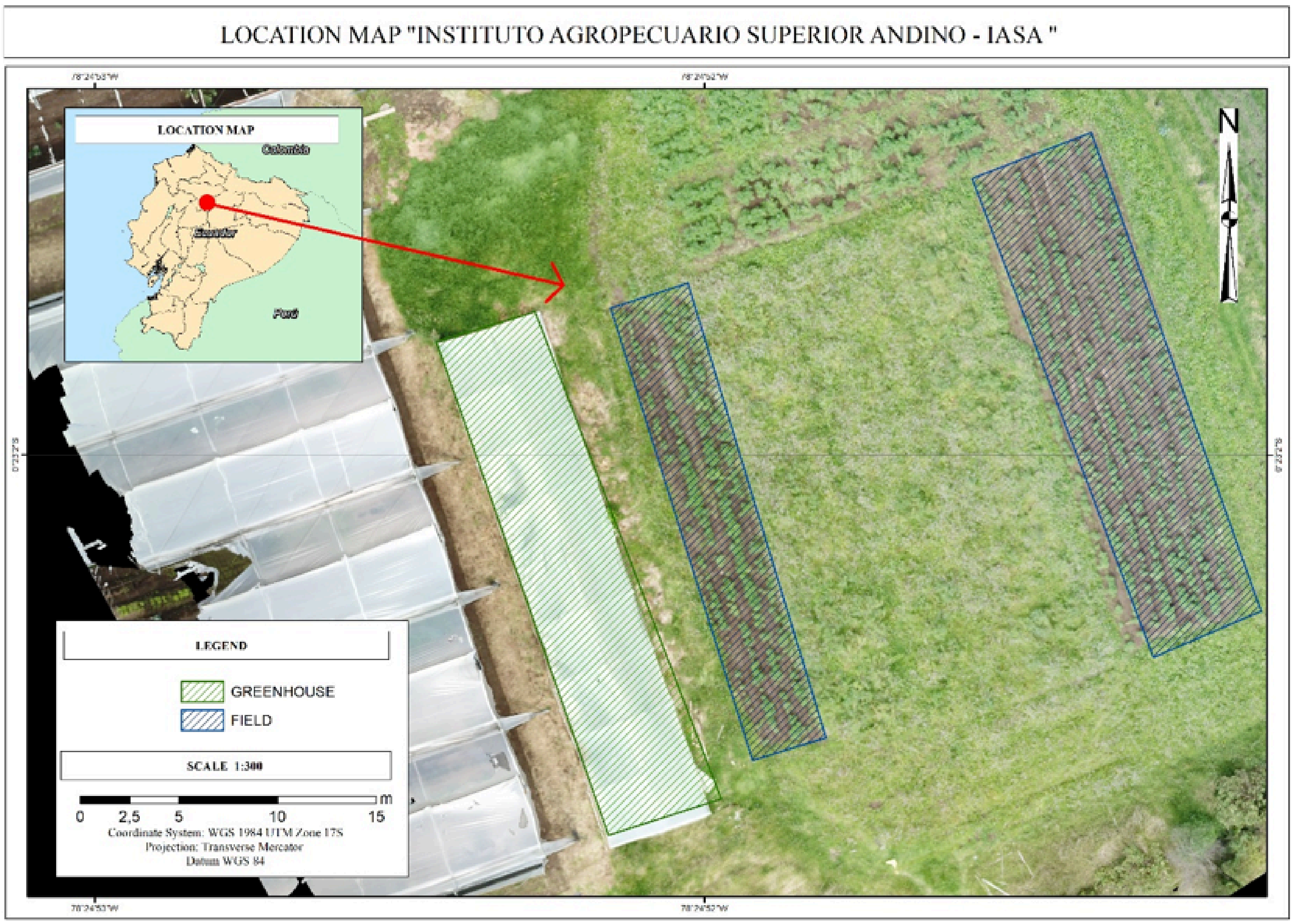


Figure 1

Location map of controlled trials.

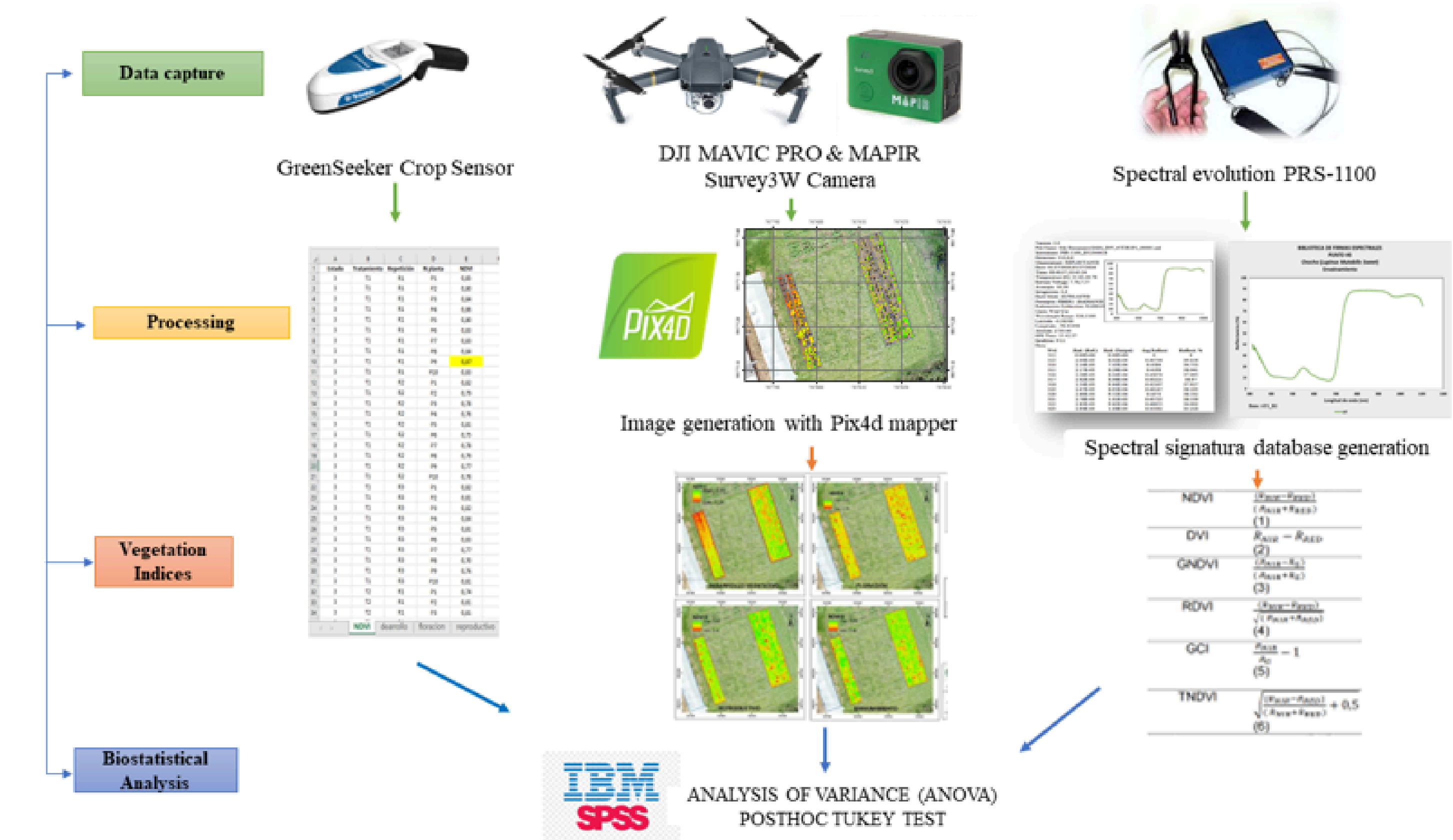


Figure 2

Methodology flow diagram

Bloque 1	Bloque 2	Bloque 2		Bloque 1	Bloque 2	Bloque 3
T2_R3	*T2_R2*	*T3_R3*		*T2_R3*	*T2_R1*	*T1_R2*
T2_R1	*T5_R3*	*T1_R3*		*T4_R2*	*T3_R3*	*T4_R3*
T4_R3	*T5_R2*	*T4_R2*		*T1_R1*	*T5_R3*	*T2_R2*
T3_R2	*T1_R2*	*T4_R1*		*T5_R1*	*T5_R2*	*T4_R1*
T5_R1	*T3_R1*	*T1_R1*		*T1_R3*	*T3_R2*	*T3_R1*

Figure 3

Distribution of treatments per experimental unit in a) greenhouse b) field

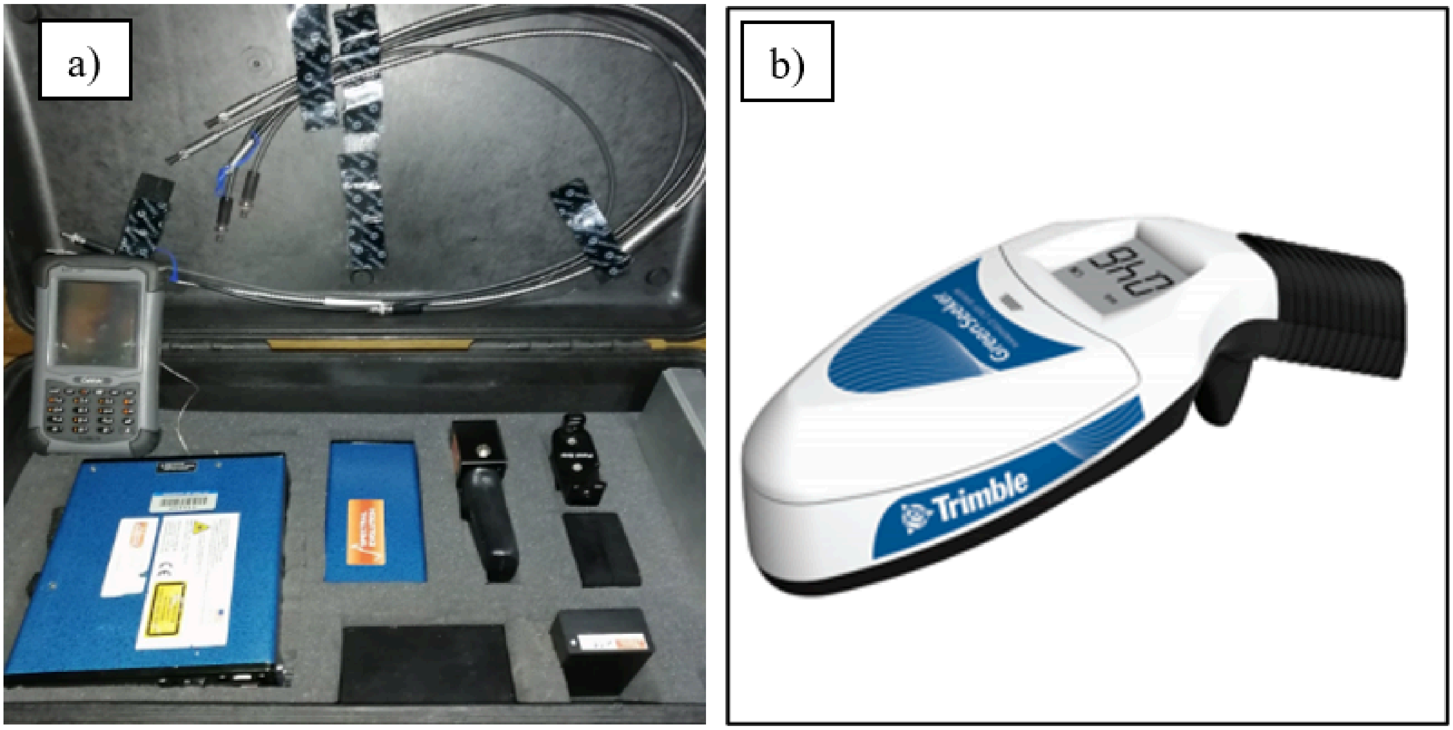


Figure 4

Equipment for data acquisition a) PRS-1100 spectrum radiometer b) GreenSeeker sensor

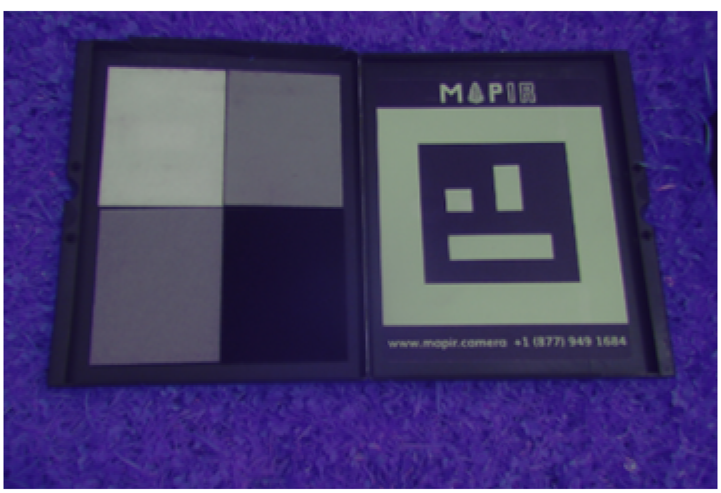


Figure 5

Calibration Target V2 (MAPIR)

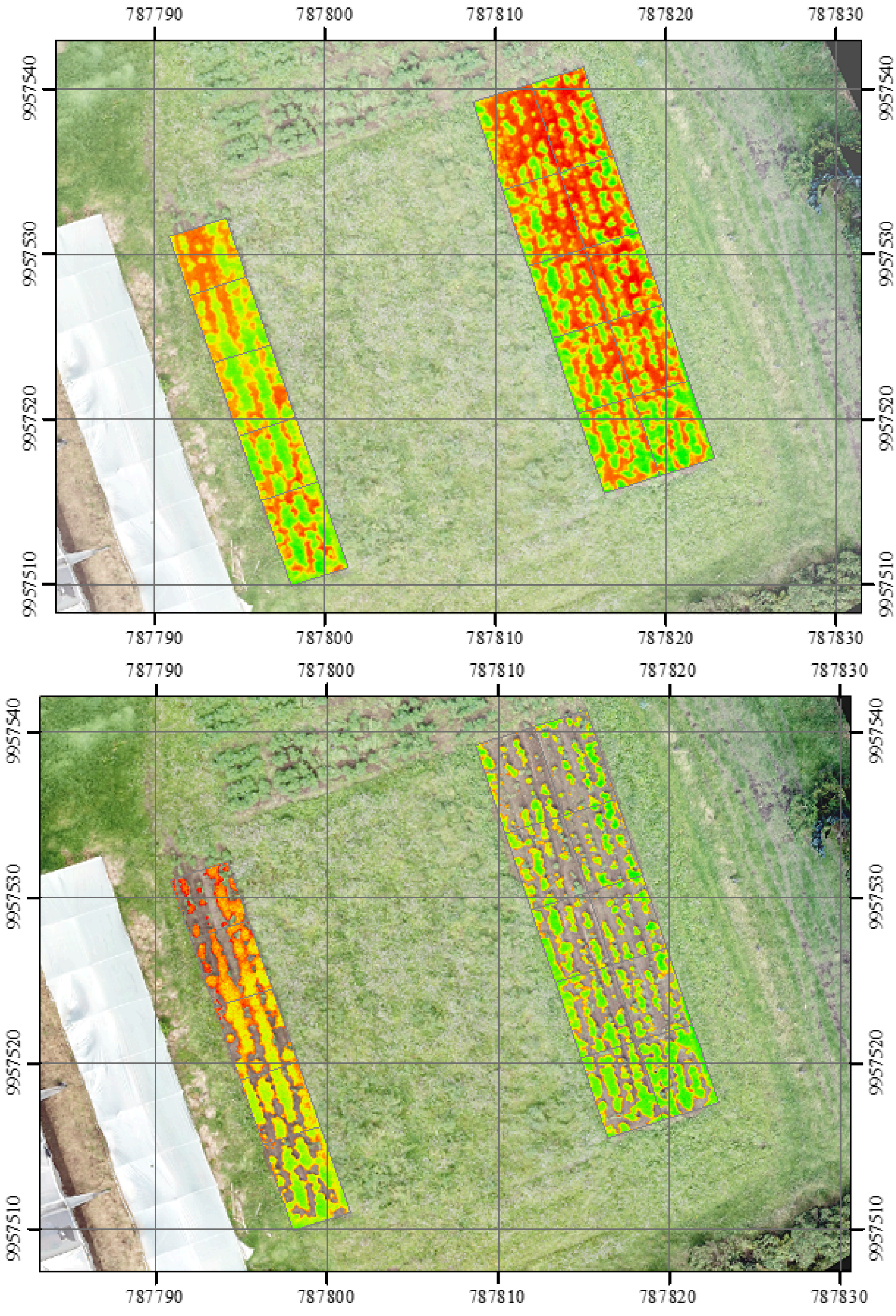


Figure 6

Discrimination between plant and soil pixels

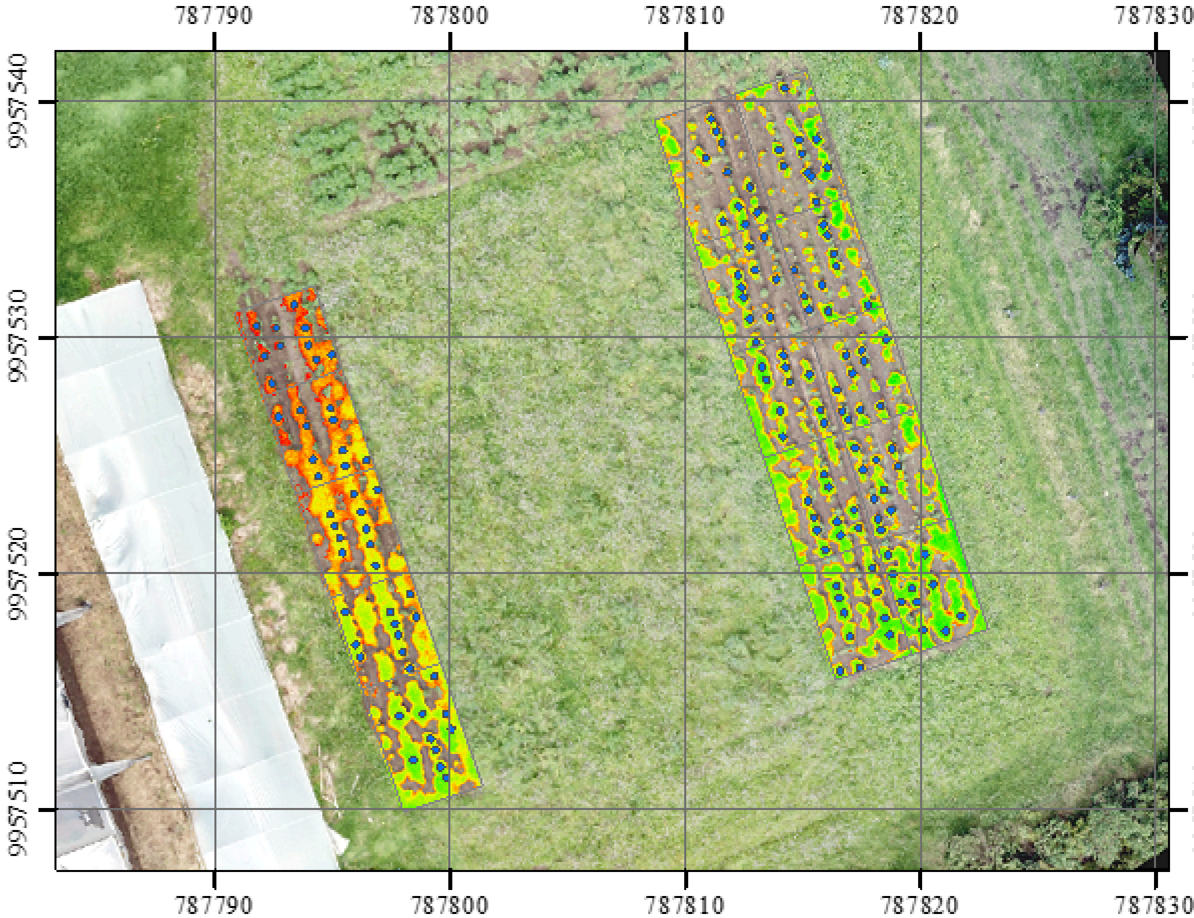
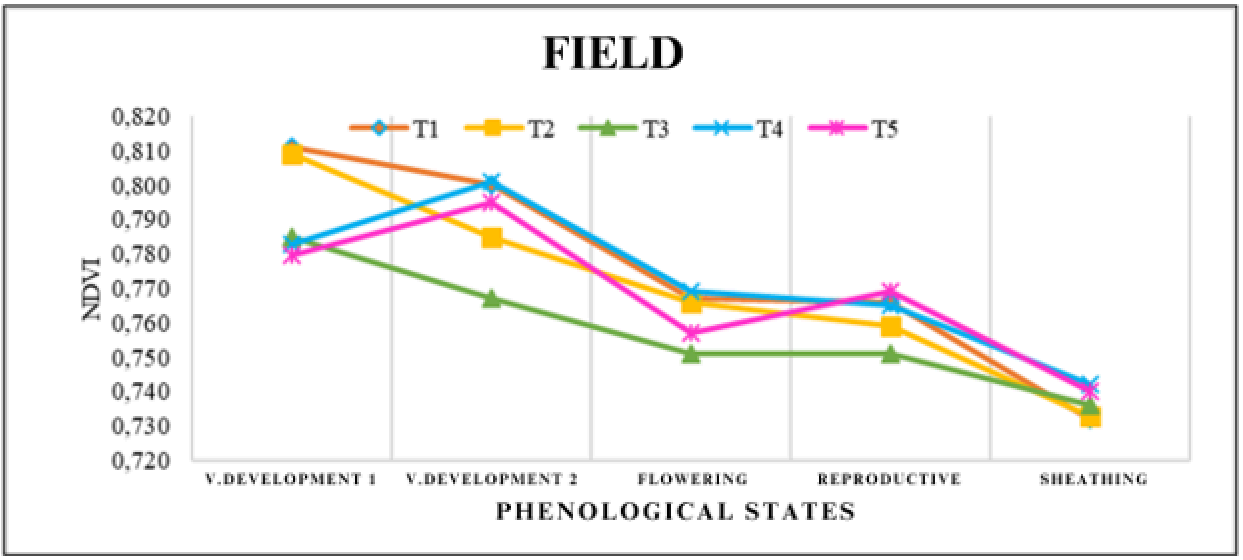


Figure 7

RTK plant positioning and NDVI index with mask.



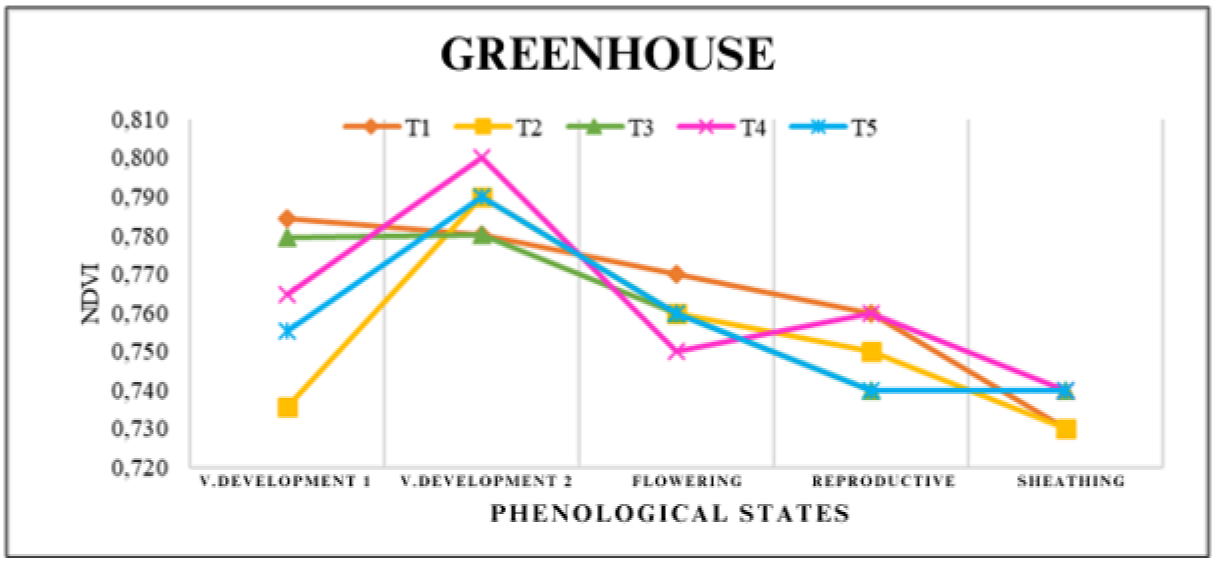


Figure 8

Treatment behavior for phenological stages in field and greenhouse.