

## TRACES OF GLYPHOSATE IN CORN (*Zea mays* L.) AND AVOCADO (*Persea americana* Mill.) WEEDS SUPPORTED BY VANT AND RAMAN SPECTROSCOPY

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### ABSTRACT

Since the 1990s, radiometric and biophotonic sensor applications have provided reliable alternatives and rapid non-invasive diagnostics for the detection and delimitation of pests and diseases, as well as pesticide traces. Active sensors have been shown to be more accurate in detecting the heterogeneity of environmental factors based on thermal and infrared characteristics, chlorophyll fluorescence, and plant reflectance. Unmanned Aerial Vehicles (UAVs) equipped with infrared cameras locate and delimit weed abundance and diversity using Normalized Difference Vegetation Index (NDVI) algorithms, which highlight heterogeneous chlorophyll activity and herbicide-limited photosynthesis in crops. The resulting map serves as the foundation for collecting plant samples. In this study, the technology of these sensors was applied to determine glyphosate traces using Raman spectroscopy, which allowed a quick, low-cost, simple, and practical diagnosis with immediate results. These are essential characteristics for surveillance and monitoring activities, as well as the basis for a geo-referenced inventory of pesticides in production units. Of the 44 weed samples collected, eight were positive for glyphosate traces: two in corn and six in avocado. Weeds are identified as positive to glyphosate traces in Raman spectroscopy in the carotenoid and phenylpropanoid reduction ranges (1186 and 1213 cm<sup>-1</sup>) as a result of a decrease in the immune response. The vibrations presented by the amines in glyphosate and the C-OH of the carboxyl group are detected in the range of 1565 and 1567 cm<sup>-1</sup>.

**Keywords:** Pesticides, biochemical signatures, crops.

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## INTRODUCTION

Weeds (endemic and invasive) are a fierce competitor for cash and subsistence crops, causing significant losses due to a decrease in yield of up to 50 % in harvesting efficiency and approximately 20 % in productivity, as well as competition for light, water, and nutrients (Radosevich *et al.*, 2007). Some weeds can produce toxic substances that are transferred to the final agricultural product, resulting in the loss of certified seed, and they can also serve as hosts for pests and diseases (de Pardo *et al.*, 2010).

The crops most affected in yield and productivity worldwide by the presence of weeds are corn (*Zea mays* L.), soybean (*Glycine max* L. Merrill), and bean (*Phaseolus vulgaris* L.), with up to 50 % yield losses, followed by sunflower (*Helianthus annuus* L.) with up to 40 % losses; canola (*Brassica napus* subsp. *napus*), up to 25 %; and sugarcane (*Saccharum* spp.) and winter oats (*Avena sativa* L.), up to 10 % (Wall and Smith, 2000; Page *et al.*, 2017). In this regard, a typology known as “aggressive weeds” was created, which are weeds that have developed herbicide resistance, causing agrochemicals to be less effective, to be applied in greater volume, and to be used in combination with several herbicides.

Mexico has 36 % of the cases of herbicide resistant weeds, and 1.7 % of these are resistant to glyphosate (Heap, 2022). Since 1996, this country increased by 1500 % the preferential application of glyphosate for the planting of genetically modified corn, cotton, and soybeans (CONACyT, 2020). There is also evidence of the increase of this pesticide in Mexican agriculture, both conventional and subsistence, in corn production units (35 %), followed by citrus (14 %), pasture (12 %), sorghum (*Sorghum bicolor* (L.) Moench) (11 %), and avocado (*Persea amaricana* Mill.) (3 %) (Alcántara-de la Cruz *et al.*, 2021). The most concerning example is avocado, which has increased 251 % in the last 20 years (from 96 000 ha in 2000 to 241 000 ha in 2021) (SIAP, 2021), and whose production system includes the use of five herbicides: carfentrazone ethyl, diquat, glyphosate, oxyfluren, and simazine. Glyphosate is the most widely used of these as it has an extremely broad spectrum of action, combating 81 of the 86 species listed for avocado (Rodríguez-Campos *et al.*, 2017).

Glyphosate is an organophosphate herbicide with a simple chemical structure, but its four highly polar groups make it difficult to analyze using conventional methods. The so-called “gold standard” for trace detection techniques are gas chromatography (GC) and high-performance liquid chromatography (HPLC) (Dias *et al.*, 2019). Despite their remarkable advantages of high sensitivity and precision in quantitative detection, these techniques still require sample pretreatment and well-trained laboratory personnel, as well as hours or even days to complete the entire analysis process.

Since the 1990s, radiometric and biophotonic sensor applications have provided reliable alternatives and rapid non-invasive diagnostics for the detection and delimitation of pests and diseases, as well as pesticide traces (Mahlein *et al.*, 2012; Martinelli *et al.*, 2015). Precision agriculture, also known as digital agriculture or 4.0, involves the detection, quantification, and mapping of plant stress through monitoring and data collection, information processing, and decision support for the temporal and spatial

allocation of inputs for crop production (Pedersen and Lind, 2017; Bongiovanni and Lowenberg, 2017).

Active sensors are the most accurate at detecting the heterogeneity of environmental factors (Chaerle and van der Straeten, 2001). Symptoms (non-visible and early stage) of pests and diseases, as well as traces of pesticides, are determined using measurements based on thermal and infrared characteristics (Jones and Schofield, 2008), chlorophyll fluorescence (Chaerle and van der Straeten, 2001), and plant reflectance (Galieni *et al.*, 2021). Unmanned aerial vehicles (UAVs) equipped with infrared cameras are used to detect and delimit glyphosate impact on crops (Huang *et al.*, 2014; Daouk *et al.*, 2015). Multispectral images determine weed abundance and diversity using NDVI algorithms (Castaldi, 2017), and the resulting map serves as the basis for sample collection for the detection and impact of glyphosate and other herbicides (Esposito *et al.*, 2021). Once the plant sample is obtained, weed identification is carried out, and Raman spectroscopy (RS) is used for the determination of glyphosate traces and residues (Sato-Berrú *et al.*, 2004; Singh *et al.*, 2021, Farber *et al.*, 2019).

RS measures the inelastic scattering of light from a monochromatic source, providing information on the chemical composition by recording the molecular vibrations of the constituent components; the “biochemical signature” or “molecular fingerprint” is then obtained (Vallejo-Pérez *et al.*, 2016). Both infrared and Raman spectroscopy provide a characteristic spectrum of the specific vibrations of a molecule and are valuable in identifying a substance, as Raman spectra contain vibrational bands that can be assigned to carbohydrates, carotenoids, proteins, and phenylpropanoids affected by glyphosate (Singh *et al.*, 2021).

Based on this knowledge, we propose the hypothesis of using high spectroscopic and radiometric resolution sensors to detect glyphosate traces in weeds and map its distribution in agricultural lands in real time. This is a pioneer study in corn and avocado crops in Mexico, whose objective is to reveal the structural biochemical changes of weeds containing traces of glyphosate in the reduction of carotenoids and phenylpropanoids (1186 and 1213  $\text{cm}^{-1}$ ) resulting from a decrease in the defense response, when compared to the spectra obtained from the concentrations of applied pesticides specified positive to glyphosate traces in RS in the ranges of 1081 and 1082  $\text{cm}^{-1}$ , in the vibrations of phosphonate molecules in glyphosate. While mapping their distribution in agricultural premises, vibrations in the C-N bonds in the amino group part and those in the C-OH of the carboxyl group are detected between 1565 and 1567  $\text{cm}^{-1}$  (Singh *et al.*, 2021; Balafoutis *et al.*, 2017).

For this purpose, the precision agriculture (PA) methodology is utilized, which consists of a cyclical data collection system used for crop management and decision evaluation through smart farming technologies (SFT) such as infrared cameras mounted on UAVs, active biophotonic sensors to obtain geo-referenced spectral information of plant physiological parameters, as well as the characterization of environmental parameters of productive units processed in spatial information software such as Geographic Information Systems (GIS) and, later, through Principal Component Analysis (PCA).

## MATERIALS AND METHODS

### Study area

The experimental production units were determined by the consensus of both producers (who lent land) and researchers from the project “Evaluation and validation of agronomic practices for the agroecological management of weeds in corn and avocado crops” (UACH-CONACyT, 321134; 2022). In the municipality of Texcoco, State of Mexico, Mexico, four corn production units are rainfed, with only one property having auxiliary irrigation (two peri-urban and two in rural landscapes). It was also agreed to work on two avocado production units with an irrigated agroforestry landscape in Tetela del Volcán, Morelos, Mexico (Figure 1).

### Sampling

The multidisciplinary field work took place from June 13 to July 18, 2022, and consisted of sampling weeds in the early stages of growth (11 days after emergence) before weeding activities in the case of corn and in the ripening stage in the case of avocado, as well as the definition of treatment application and flight missions with the DJI Phantom 3 unmanned aerial vehicle (UAV) and onboard the Mapir Survey RGN camera (visible and near infrared-NIR) covering an area of 9 ha. A total of 44 samples collected throughout the study area were processed. Thirteen weeds were collected for corn and 31 for avocado (Table 1). The PA cyclic production process can be achieved through data acquisition, data processing, analysis and evaluation (decision making), and accurate application of operations (implementation,

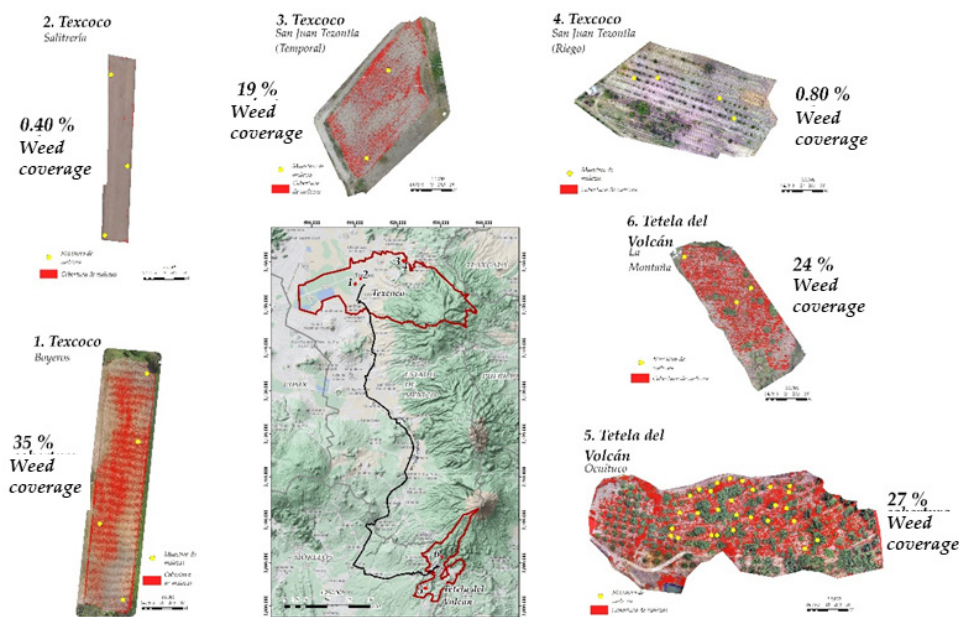


Figure 1. Location of the study area and identification of weed cover with the UAV.

**Table 1.** Characterization of the experimental and sampling production units of corn (*Zea mays* L.) and avocado (*Persea americana* Mill.).

State	Municipality	Plots	Lot	Variety	Area (ha)	Samples analyzed	Weed density (m <sup>2</sup> )
State of Mexico	Texcoco	Boyeros	Corn	Yellow	1	4	374
State of Mexico	Texcoco	Salitrería	Corn	Yellow	0.5	3	3
State of Mexico	Texcoco	San Juan Tezontla (Temporary)	Corn	Yellow and blue	1	2	6
State of Mexico	Texcoco	San Juan Tezontla (Irrigation)	Corn	Yellow and white	1.5	4	128
Morelos	Tetela del Volcán	Ocuituco	Avocado	Hass	4	28	550
Morelos	Tetela del Volcán	La Montaña	Avocado	Hass	1	3	498

classification, or typing). These three components define, in this case, risk management specific to weed density and the determination of glyphosate positives (Pedersen and Lind, 2017).

### Data acquisition

#### Spatialization

To determine the percentage of weed coverage, six photogrammetric missions were planned with Pix4D Capture, which delimited the UAV flight lines at 25 m, obtaining an 80 % overlap between aerial images with a GSD of 1 cm pixel. The aerial images were obtained using a 12-megapixel MAPIR Survey3 (550–850 nm) multispectral camera mounted to the UAV Phantom 3 Professional and processed in Agisoft Metashape Professional v1.5.2, resulting in six georeferenced orthomosaics. The NDVI algorithm (Castaldi *et al.*, 2017) was calculated to each orthomosaic and spectral information extraction was applied for the determination of percent weed coverage.

#### Weed collection

To measure weed population density in the field, the “W” method (SENASICA, 2013) was used, using a metallic quadrant (0.5 x 0.5 m), where the weeds remaining within the quadrant were counted and multiplied by four to obtain population per square meter (m<sup>2</sup>). The collected samples were stored in self-sealing plastic bags to maintain the cold chain but without freezing the samples, so they were placed in unicep coolers containing refrigerant gels.

### Raman diagnosis

For the determination of biochemical signatures of glyphosate traces in the collected weed samples, two high-purity pesticides (from eight commercial brands of glyphosate) were used and analyzed at the Center for the Application of Infrared Radiation, Alternative Energies and Materials (CARIEM) of CIACyT-UASLP. The Xplora Plus Raman Microscope was used, with the green laser at 100 % power and 785 nm excitation in a 20/2 second acquisition time that incorporates unique and powerful features in a reliable, high-performance system. These systems feature an enhanced range of options to measure with at least one other laser wavelength in the infrared, including EMCCD detection, combined Raman polarization, and Raman-AFM. Being a multisample Raman microscope, the observation technique of using microscope objectives with a typical spatial resolution of 2-4  $\mu\text{m}$  is improved. To obtain the reference spectra of glyphosate, a drop of both pesticides was taken with a glass pipette and placed on an aluminum slide for liquids.

The biochemical signature of the Raman Spectra of the two pesticides (Tackle 360 and Faena Fuerte) shows changes in the intensity level in comparison to the ranges 1081–1082 and 1565–1567  $\text{cm}^{-1}$ , where the vibrations of phosphonate molecules in glyphosate are found, as well as the vibration in the C-N bonds in the amino group and C-OH part of the carboxyl group (Table 2).

**Table 2.** Raman band assignment for glyphosate-positive weeds according to the literature.

Raman	Assignment	Interpretation
477–484	$\delta(\text{OH})+\rho(\text{CH}_2)+(\text{PO}_2)$	Vibration present in phosphonate molecules in glyphosate.
915	$\nu(\text{C-O-O})$ in plane, symmetrical	Cellulose, phenylpropanoids
870–895	$\rho(\text{CH}_2)+\delta(\text{NH})+\nu(\text{C-C})$	Vibration in bonded molecules of the phosphonate-carboxyl group bonding
989–992	$\rho(\text{CH}_2)+\delta(\text{OH})$	Methylene molecule balancing vibrations and bending of OH
1081–1082	$\nu(\text{C-N})+\nu(\text{C-OH})$	Vibration in the C-N bonds in the amino group part and C-OH of the carboxyl group.
1145–1157	$\beta$ -carotene	$\beta$ -carotene, which is a strong orange-red organic pigment.
1156	C-C stretching; $\nu(\text{C-O-C})$ , $\nu(\text{C-C})$ in glycosidic bonds, asymmetric ring respiration	Carotenoid, organic pigments present in plants
1186	$\nu(\text{C-O-H})$ next to the aromatic ring+ $\delta(\text{CH})$	Carotenoid, organic pigments present in plants
1213	$\delta(\text{C-C-H})$	Carotenoid, organic pigments present in plants
1325–1337	$\rho(\text{CH}_2)+\nu(\text{C-C})$	Stretch vibration of C-C bonds in glyphosate.
1425–1427	$\delta(\text{CH}_2)$	Flexural vibration of the methylene molecule
1525	-C=C-(in plane)	Carotenoid, organic pigments present in plants.
1565–1567	$\delta(\text{NH}_2)$ , $\delta(\text{NH}_2^+)$	Types of vibration exhibited by amines in glyphosate
1607	$\nu(\text{C-C})$ aromatic ring + $\delta(\text{CH})$	Phenylpropanoids

Source: Sato-Berrú *et al.* (2004), Farber *et al.* (2019).

### Glyphosate trace quantification

The Raman device was calibrated and configured for use. The laser power used on the Xplora Plus was 532 nm, with an acquisition time of 3 s in five accumulations and at 1 % of the laser power (mW). To avoid oxidative processes in the plant samples, they were analyzed no later than 48 hours after collection (Vallejo-Pérez *et al.*, 2016). Leaf samples were extracted from the corresponding bags and placed on a 76 mm x 26 mm glass slide. The leaves analyzed were carefully collected to avoid physical damage from improper handling, and there were no signs or symptoms of disease-causing agents or other residues. The selected samples were recorded in the logbook with label information and Raman device information to ensure traceability and trackability. Each leaf sample was placed on the sample holder at the focal point of the Xplora Plus, allowing the laser pass through the leaf beam for 3 s. The areas to be analyzed corresponded to the 0.5 and 0.75 portions of the leaf blade in longitudinal orientation to avoid hitting the central rib of the leaf. Once the portion of the plant tissue of interest was located, the sample was covered with cardboard material to prevent light from entering the sensor, and the Raman spectrum was obtained. The data obtained were analyzed using the Origin 2018 software, which removed the fluorescence from the Raman spectra.

After obtaining the biochemical signatures, a multivariate statistical method, such as Principal Component Analysis (PCA), can be used to reduce the number of variables or, in this case, group the main characteristics of the Raman spectra. This technique is widely used for one or more data sets without any prior knowledge about their nature. Component analysis reduces the dimension of the data set by finding an alternative set of coordinates known as principal components, which are orthogonal linear combinations of the original variables that explain a given and acceptable amount of variance. Considering that there is a sample with  $n$  individuals, each with  $p$  variables ( $X_1, X_2, \dots, X_p$ ), the sample space has  $p$  dimensions. PCA allows finding a number of underlying factors ( $Z < p$ ) that explain approximately the same as the original  $p$  variables. Whereas previously  $p$  values were required to characterize each individual, this analysis assumes that  $Z$  values, also known as principal components, are now sufficient.

Each principal component ( $Z_i$ ) is obtained by linear combination of the original variables. The first principal component of a group of variables ( $X_1, X_2, \dots, X_p$ ) is the normalized linear combination of these variables that has the highest variance:

$$Z_1 = \phi_{11} X_1 + \phi_{21} X_2 + \dots + \phi_{p1} X_p$$

The normalized linear combination implies that:

$$\sum_{j=1}^p \phi_{j1}^2 = 1$$

where the terms  $\phi_{11}$ , ...,  $\phi_{1p}$  define the component and can be interpreted as the contribution or weight/importance that each variable has in each component, therefore, they help to know what type of information each component collects (James *et al.*, 2021).

### RESULTS AND DISCUSSION

Eight of the 44 samples tested positive for glyphosate: two in corn and six in avocado (Table 3). The lot with the highest concentration of glyphosate traces was the Ocuítuco avocado lot in Tetela del Volcán, and the second was the Salitrería corn lot in the urban area of Texcoco. According to an interview with the farmers in charge of the production units, in the first case, they stated that they “had a health problem due to a weed that caused hives and skin rashes to the workers and that they applied glyphosate to eradicate it in the north area of their property three years ago”. In the second case, the producer stated that she just “rents the lot and does not know the type of management and application of poisons” (CONACyT project fieldwork logs, 321134/2022).

Although the percentage of glyphosate-positive weeds per plot is relatively low in avocado (0.3 %), leaves from trees adjacent and close to these weeds were also positive (five trees). In the case of positive corn plots, there is a distribution of 2 % in San Juan Tezontla, where the stubble leaf and corn also tested positive for glyphosate traces

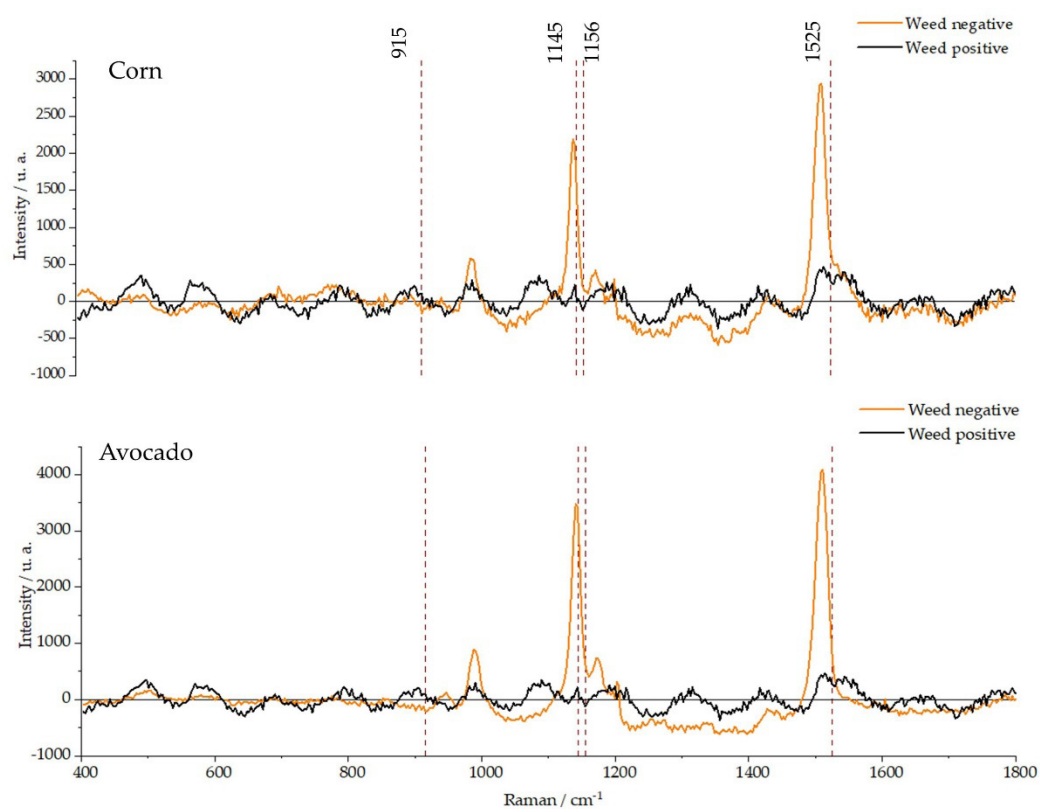
**Table 3.** Weed glyphosate trace identification and weed cover percentage in corn and avocado production units.

Plots	Weed Positive	Weed Negative	Total weed coverage (%)	Glyphosate-positive weed coverage (%)
Texcoco (Boyeros) Corn	0	<i>Ricinus communis</i> (4)	35	0
Texcoco (Salitrería) Corn	<i>Chenopodium album</i> (1)	<i>Rumex crispus</i> (2)	0.40	10
San Juan Tezontla (Temporary) Corn	<i>Baccharis linearis</i> (1)	<i>Baccharis linearis</i> (1)	19	15
San Juan Tezontla (Irrigation) Corn	0	<i>Brachiaria plantaginea</i> (4)	0.80	0
Tetela del Volcán (Ocuítuco) Avocado	<i>Amaranthus palmeri</i> (6)	<i>Solanum americanum</i> (8), <i>Malva sylvestris</i> (10), <i>Artemisia dracuncululus</i> (4)	27	20
Tetela del Volcán (La Montaña) Avocado	0	<i>Rumex crispus</i> (2), <i>Tithonia tubiformis</i> (1)	24	0

Source: Heap (2022), Syngenta (2022), CENGICANÑA (2013).

using Raman applications. The spectral analysis was carried out in two stages: (1) preprocessing of the spectra and (b) principal component analysis (PCA).

Trace glyphosate-positive weeds displayed bands at the positions of ranges 1081–1082 and 1565–1567  $\text{cm}^{-1}$  where the vibrations of phosphonate molecules in glyphosate (equivalent to the chemical fingerprint of the herbicides selected for this study) are found. The sample with the highest intensity in avocado was #32, while the sample with the highest intensity in corn was #25. Some herbicides interfere with chlorophyll biosynthesis and functional changes in chloroplasts. It can also affect the formation of other pigments, such as xanthophylls and carotenoids, causing interference in electron flow and a reduction in ATP production by inhibiting ATP-synthase activity in the photophosphorylation process (Corrêa *et al.*, 2018). In fact, glyphosate inhibits photosynthesis and stimulates stomatal closure. It also produces oxidative stress, inhibits aromatic amino acid synthesis, and decreases protein synthesis (Meloni *et al.*, 2019). The beta-carotene vibrations in the 1145-1156-1523  $\text{cm}^{-1}$  records of very low intensity in the glyphosate positives compared to the three highest peaks in the glyphosate-free weeds (Figure 2), where there is a low intensity portion of 3.31 % in comparison to the glyphosate-free ones.



**Figure 2.** Comparison of Raman spectra of glyphosate positive and negative weeds in experimental units of corn and avocado.

After obtaining the biochemical signatures, the PCA method was used to reduce the complexity of the Raman spectra and reduce their behavior to two variables, allowing for measuring, explaining, and predicting the degree of relationship and variation between the spectra obtained in positive-negative to glyphosate. Samples below 0 on the y-axis have positive values because they are close to the samples of the base herbicides of the study and show less variation (T, Ff). On the other hand, the samples that tested negative for glyphosate are above 0 on the same axis. Similarly, the positive samples show positive intensity values in both representative bands (1156 and 1525  $\text{cm}^{-1}$ ) (Table 4).

There were seven negative samples that shared some spectral signature characteristics with the positive samples. The PCA technique was used to distinguish any trace of glyphosate, resulting in a pattern of separation of negative and positive weeds (Figure 3). The PCA technique allowed the interpretation of complex data matrices, as well as the improvement of understanding of the state of the system under study and the identification of the factors influencing the system. All of this made it possible to differentiate the data according to positive and negative (Figure 3), to detect traces of glyphosate with a high degree of reliability, and to use this technique in later stages to classify new samples as "positive" or "free" (negative) of glyphosate (Vallejo-Pérez *et al.*, 2016). Therefore, this methodology proves to be reliable for sample identification. To carry out the following studies, it is necessary to consider the type of matrix or state of the sample to be evaluated, as well as the type of radiation or irradiation beam at which the experiments will be carried out. Radiometry and spectroscopy technologies are an accurate and cost-effective approach to early and geo-referenced search and detection of glyphosate traces in any agricultural production lot, in addition to an alternative for pre-diagnosis and sample selection for HPCL analysis in certified laboratories.

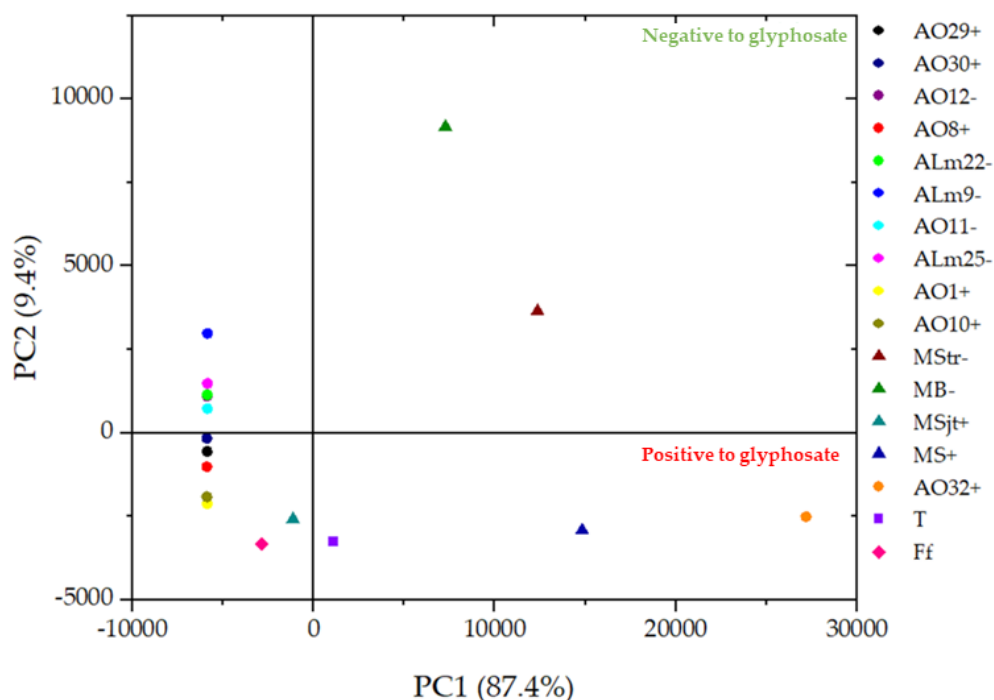
## CONCLUSIONS

Raman spectroscopy combined with Principal Component Analysis (PCA) demonstrated 86.9 % sensitivity and 89.2 % accuracy in distinguishing between glyphosate-positive weeds and healthy plants. The spatiality of plant sample collection in the field is determined by the 0.5 Normalized Difference Vegetation Index of the orthomosaics obtained by the Unmanned Aerial Vehicles and infrared photographs of weeds. Therefore, it is proposed that this protocol serve as the basis for a geo-referenced inventory of pesticides with the support of a catalog of spectral signatures and the use of Raman in real time (supported by intelligent mobile technologies), as well as part of the protocols for organic certification inventories. The intelligent algorithm is expected to make pesticide residue detection more instrumented and programmatic. Finally, the establishment of a Raman database of pesticides and the standardization of instruments should attract more attention in the field of rapid *in situ* detection in the future.

**Table 4.** Raman wave assignment values for weeds with glyphosate residues.

Sample number	(1156) cm <sup>-1</sup>	(1525) cm <sup>-1</sup>	Qualitative assignment	Lot
1	-100	409	Low	Avocado
2	334	894		Avocado
3	413	1067		Avocado
4	201	633		Avocado
5	143	492		Avocado
6	347	684		Avocado
7	130	388		Avocado
8	137	378	Low	Avocado
9	158	520		Avocado
10	53	265	Low	Avocado
11	164	460		Avocado
12	199	582		Avocado
13	180	541		Avocado
14	324	772		Avocado
15	187	518		Avocado
16	241	671		Avocado
17	234	626		Avocado
18	204	454		Avocado
19	277	727		Avocado
20	223	721		Avocado
21	184	422		Avocado
22	134	414		Avocado
23	271	685		Avocado
24	293	801		Avocado
25	232	532		Avocado
26	231	526		Avocado
27	285	711		Avocado
28	212	678		Avocado
29	19	91	Mean	Avocado
30	34	95	Mean	Avocado
31	568	1160		Corn
32	-97	402	Very high	Avocado
33	130	234	Mean	Corn
34	1089	1237		Corn
35	105	408	Very high	Corn
36	235	267		Corn
37	169	174		Corn
38	1074	1517		Corn
39	346	499		Corn
40	499	832		Corn
41	203	593		Corn
42	189	604		Corn
43	168	533		Corn
44	196	573		Corn

Source: Farber *et al.* (2019).



**Figure 3.** Principal component analysis of samples collected from corn and avocado lots. Lots: Corn (M), avocado (A); Plots: Boyeros (B), Salitrería (S), San Juan Tezontla-Temporal (Sjt), San Juan Tezontla-Riego (Str), Ocuituco (O), La Montaña (Lm); Pesticides: Takle 360 (T), Faena Fuerte (Ff); Positive (+) and Negative (-) result.

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