Plant and Crop protection / Sanidad Vegetal y Protección de Cultivos

doi: http:// dx.doi.org/10.15446/acag.v64n3.44729 e-ISSN 2323-0118

Detection of *Eurhizococcus colombianus* (Hemiptera: Margarodidae) in blackberry plants by near–infrared spectroscopy

Detección de *Eurhizococcus colombianus* (Hemiptera: Margarodidae) en mora por espectroscopía del infrarrojo cercano

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Rec.: 15.05.2014 Acep.:03.12.2014

Abstract

A key aspect in the study of underground pests is the selection of appropriate methods for sampling and analysis. Knowledge of the population parameters of such insects depends on the use of tools sensitive enough for their detection in a complex environment as the one of the soil and the roots. Near infrared (NIR) spectroscopy have been suggested as a suitable, non–destructive sampling tool, which takes advantage of specific optical signatures in different groups of plants and organisms. The aim of this study was to assess the use of NIR spectroscopy in leaves and rhizosphere soil samples as an analytical technique to define the presence of the underground insect *Eurhizococcus colombianus* in blackberry crops, in the Eastern of Antioquia. The information obtained in seven farms distributed in five municipalities indicates that it is possible to classify plants with presence or absence of the insect through the spectral patterns of leaves and rhizosphere soil within each farm. However, it was not possible to establish a general model involving the data gathered from all farms. These results allow us to glimpse a promising non–destructive tool to understand the conditions accounting for the presence of the insect in the crop. It also would help to build management strategies of such insects based on ecological knowledge, which in turn will help farmers to make sound and timely pest control decisions.

Key words: Detection of infestation, Colombian ground pearl, NIR, non–destructive sampling, *Rubus glaucus* Benth.

Resumen

La selección de métodos apropiados para muestreo y análisis es un aspecto importante en el estudio de plagas subterráneas. El conocimiento de su biología, depende del uso de herramientas sensibles para su detección en el ambiente complejo del suelo y las raíces. Recientemente se ha propuesto el uso de espectroscopia infrarroja cercana (conocida como NIR, por su sigla en inglés) para el diagnóstico fitosanitario no destructivo en cultivos, aprovechando la manifestación de propiedades ópticas únicas para cada grupo de plantas y organismos. El objetivo de este trabajo fue evaluar el uso de espectroscopía NIR en muestras de hojas y suelo rizosférico, para detectar la presencia del insecto subterráneo *Eurhizococcus colombianus* en cultivos de mora (*Rubus glaucus* Benth.), en la región del Oriente antioqueño. La información obtenida en siete fincas distribuidas en cinco municipios indica que a través de los patrones espectrales de las hojas y el suelo rizosférico es posible clasificar las plantas con presencia o ausencia del insecto dentro de cada finca. Sin embargo, no fue posible establecer un modelo general para todas las fincas. Los resultados obtenidos permiten vislumbrar una herramienta no destructiva muy promisoria para detectar el insecto y entender las condiciones asociadas con su presencia en el cultivo, lo que favorecería el diseño de estrategias de manejo de este tipo de plaga, con base en el conocimiento de su ecología, ayudando así a la toma de decisiones ambientalmente amigables, razonables y oportunas por parte de los agricultores.

Palabras clave: Detección de infestación, muestreos no destructivos, NIR, perla de tierra colombiana, *Rubus glaucus* Benth.

Introduction

Blackberry (*Rubus glaucus* Benth.) is one of the most important crops for rural economy in high Andean parts of Colombia; however, its development is limited because the damage caused by causa *Eurhizococcus colombianus* (Jakubski, 1965) (Hemiptera: Margarodidae), an insect with underground habits, known as Colombian ground pearl that was registered as a plague of economic importance more than 30 years ago (Posada *et al.*, 1978). Although the symptoms of damage in the aerial parts caused by this insect have not been define precisely, authors like Castaño (2000), Carvajal (2002) and Osorio (2005) reported that blackberry plants infested with ground pearl have nodules in the roots that block respiration and nutrition. A consequence, some plants show chlorosis, defoliation, rachitis, stunting, lower emission of stems, poor flowering or decline in production, small and dry fruits, that eventually die. An important methodological aspect the study of this and other soil pests is field sampling or under controlled conditions, since the method and tools used are essential for inferring population parameters of pests or quantify their economic importance (Rodríguez del Bosque *et al.*, 2010). In recent years, several studies worldwide have focused on the development or adaptation of methods for detecting insects in their natural environment. Some of them include the use of acoustic sensors, microtomography by X-rays, harmonic radar, computed tomography, magnetic resonance, ultrasound and near-infrared and visible spectroscopy, (Mankin *et al.*, 2000; Reynolds and Riley, 2002; O´Neal *et al.*, 2004; Johnson *et al.*, 2007; Kotwaliwale *et al.*, 2011). Most of the equipment use for that can only be used at the lab scale, so that it is required to validate them in order to develop portable systems that can be used directly in the field

The near-infrared spectroscopy (NIR) is a non-destructive and accurate rapid analytical technique, which is widely used in industry and scientific research. This technique allows detection of molecular structures of a wide range of organic compounds, based on the principle that each molecule has a characteristic spectrum, similar to a fingerprint, which is generated by the interaction of infrared light with specific links of the molecules present in these compounds (Roberts *et al.*, 2004; Susurluk *et al.*, 2007). Furthermore, by multivariate statistical techniques and appropriate calibration, the spectra can be used to develop predictive models or qualifiers of unknown samples based on its NIR spectrum (Acuna and Murphy, 2007). In entomology, from the premise that each species and stage of development of an insect has a unique chemical composition, this technique has been used mainly for taxonomic purposes and for non-destructive insect screening inside stored fruits and grains (Dowell *et al.*, 1999; Maghirang and Dowell, 2003; Paliwal *et al.*, 2004; Karunakaran *et al.*, 2003). However, its use in the field to detect insects in the soil is still emerging (Foley *et al.*, 1998; Lui *et al.*, 2009). With the aim of evaluating a potential system to detect de *E. colombianus* that allows the development of other studies in insect ecology and management, this research used infra-red spectroscopy measurements of rhizospheric soil and leaves from blackberry crops with and without *E. colombianus*. With few exceptions, for each evaluated farm the construction of a qualitative model from spectroscopy study was possible, allowing the classification of infected plants with or without *E. colombianus*, suggesting the possibility of using this technology to diagnose the insect in blackberry plants on a fast and non-destructive basis.

Materials and methods

Area of study

This research was performed at the east region of Antioquia, which is the main producing area of blackberry in the Department of Antioquia (Colombia) and one of the most affected by *E. colombianus* according to the most recent biophysical and socioeconomic characterization done for this crop by Ríos *et al.* (2010).

Selection of farms

For the selection of experimental farms areas with at least two plots cultivated with blackberry of the same age and variety, where one of them recorded the presence of the insect in the roots of the plants and the other not, were searched. This selection strategy of matched batches allows better control of potential confounding factors. Accordingly, during the second half of 2012 weekly visits to the farms were conducted in the municipalities of La Ceja, El Retiro, Guarne, Envigado, San Vicente, Rionegro, La Unión, Santa Elena and Granada. Initially seven farms were selected in five of these municipalities whose characteristics are summarized in Table 1. Once the farms identi-

Table 1. Information of the selected farms for the study, plants age, blackberry variety and incidence of the pest.

Municipality	Village	Farm	Geographical location	Variety	Plants/ plot (no.)	Age (Years)	Incidence of *E. colombianus**
Guarne	Guapante Abajo	La Frijolera	Latitude: 06º 17´39.7" N Longitude: 75º 24´17.4" W Altitude: 2453 masl	Castilla	460 260	7 years 7 years	10/10 0/10
Rionegro	Llanogrande	La Selva 1	Latitude: 06º 08´04.3" N Longitude: 75º 25´02.7" W Altitude: 2143 masl	Castilla	248 380	5 years 5 years	0/10 10/10
Rionegro	Llanogrande	La Selva 2	Latitude: 06º 08´04.3" N Longitude: 75º 25´02.7" W Altitude: 2143 masl	Castilla	248 380	5 years 5 years	0/10 10/10
La Ceja	San Rafael	El Encanto	Latitude: 05º 57´33.7" N Longitude: 75º 27´28.4" W Altitude: 2304 masl	San Antonio	750 750	7 years 7 years	8/10 0/10
El Retiro	La Amapola	La Torre	Latitude: 06º 00´22.1" N Longitude: 75º 29´01.1" W Altitude: 2382 masl	San Antonio	700 700	5 years 5 years	0/10 10/10
Envigado	Pantanillo	El Reposo	Latitude: 06º 11´06.8" N Longitude: 75º 29´41.6" W Altitude: 2364 masl	Castilla	1200 1500	7 years 7 years	0/10 8/10
El Retiro	Pantalio	Campo Alegre	Latitude: 05º 58´53.1" N Longitude: 75º 30´14.9" W Altitude: 2133 masl	San Antonio	200 400	4 years 4 years	8/10 0/10

***** Number of plants with presence of *E. colombianus* in the roots/number of evaluated plants.

fied, the incidence of *E. colombianus* in blackberry crop was evaluated using the methodology proposed by Osorio (2005).

Collection and processing of samples

From each plot 10 plants were selected randomly following a zig-zag path, in total were 20 samples per farm and 140 in total. In each plant, leaves and rhizosphere soil was collected separately. The soil collected was the one adhered to the plants roots; for that the samples were strongly shaken till soil was removed and the roots were separated. Then, in the soil Microbiology Lab of the Universidad Nacional de Colombia – Medellin, they were dried out at room temperature in plastic trays for 5 days. Finally, they were crushed with a wooden rolling pin and passed through a sieve of 500µ before storing them in paper bags.

Leaf samples were collected in different strata of the plant using trimming scissors; to move them to the lab they were wrapped in absorbent paper and placed on plastic bags. Then they were dried out in the oven at 40 °C for 5 days on metal trays. Finally, they were grounded in an electrical grinding machine Thomas-Wiley with sieve 40µ and stored in paper bags.

NIR spectra gathering

The 280 samples processed (140 for rhizosphere soil and 140 from leaves) were analyzed in the Soil Lab of the Universidad Nacional de Colombia – Medellin by near-infrared spectroscopy-NIR in order to obtain the spectra patterns of each one of them. The samples were placed on a glass container covering all the bottom and placing them one by one on the detector window of the spectroscope (FTNIR, Buchi NIRFlex 500). The reads were done by reflectance at wavelengths between 1000 and 2500 nm with 2 nm intervals. In total 280 spectra were obtained -140 from leaves and

140 from soil- that covered the two categories of *E. colombianus* incidence. Each spectrum was composed of 1501 reflectance values, one per each wavelength measured.

Building of a classifying model of insect incidence

To find the classification model for presence or absence of *E. colombianus* from the spectra data of leaves and rhizosphere soil of each farm it was used the qualitative analysis of the chemometric software NIRcal that was incorporated in the spectroscope. The first step to create the model consisted on feeding the system with data from two categories of incidence measured in the field (absence - presence), the spectra data found per farm and type of sample and the wavelengths used (1000 to 2500 nm). Then, to reduce the contribution of the physical effects of the samples (particle size) on the NIR spectra and to improve the model, automatic adjustments of the software on parameters like normalization, correction of multiplicative dispersion, normal standard deviation, smoothing and derivate, were used. For data classification in one of the two categories of incidence based on the NIR spectra, cluster analysis was used with principal components analysis (PCA). Finally, a selection of a classifying model based on the 'Q' value or quality attribute obtained by the model, which should be close to 1.

Selection of possible variables associated with the insect presence

Both, in leaves and rhizosphere soil, the principal component was selected as the one grouping the most variability of data. Then, the variables were identified (wavelength) that composed such component and finally, based on the charge of the variables the wavelengths that were more associated to the presence of the insect were established.

Results and discussion

Getting NIR spectra

In Figure 1 the NIR spectra from the leaves and rhizosphere soil are shown. When comparing visually the spectra from plants with and without affectation by *E. colombianus* it is observed that, both, in leaves and rhizosphere soil, the general spectra pattern in both categories of incidence is the same.

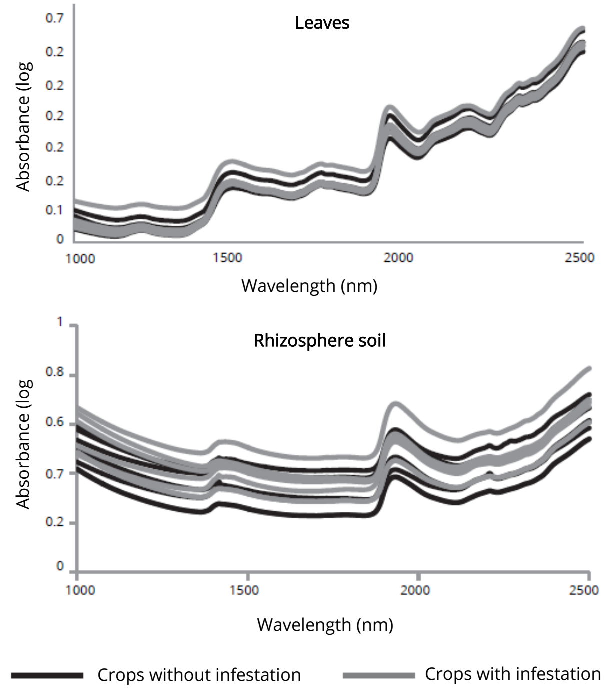

Figure 1. Characteristics of infrared spectra of leaves and rhizosphere soil samples coming from blackberry crops with infestations of *E. colombianus*, in the East of Antioquia (Colombia).

Model to classify incidence

Cluster analysis with PCA for the obtained spectra in samples from each farm showed 91 and 94% of certainty for the samples of leaves and rhizosphere soil among the respective categories (with or without insect). The 'Q' values or quality attributes obtained were higher than 0.8 in 50% of the analysis. For each farm and sample type were selected the two first principal components (PC1 and PC2) which explained between 54 and 100% of the behavior of the original variables. The figures of each farm for the PC1 and PC2 scores for the leaf samples (Figure 2) and rhizosphere soil (Figure 3) showed, in general, a clear difference between those coming from plants with or without the insect presence. When analyzing the spectra as group in the seven farms it was not possible to obtain a general classifying method for the incidence of *E. colombianus.*

Selection of the possible variables associated with the presence of the insect

Considering that for the farms, except for Campo Alegre, a good discrimination on the axis of the first principal component was observed, the NIR spectra regions with higher load on this component were identified for leaves (Figure 4) and rhizosphere soil (Figure 5). Possibly, these regions are associated with the presence of the insect.

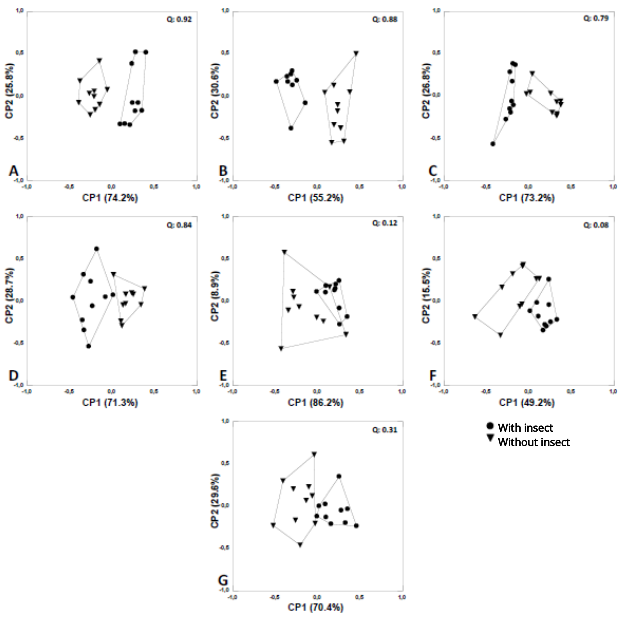

Figure 2. Classification of leaf samples after the analysis Cluster-PCA. A. Farm La Selva (Rionegro); B. Farm La Selva (Rionegro); C. Farm La Frijolera (Guarne); D. Farm El Encanto (La Ceja); E. Farm La Torre (El Retiro); F. Farm El Reposo (Envigado); G. Farm Campo Alegre (El Retiro). West of Antioquia (Colombia).

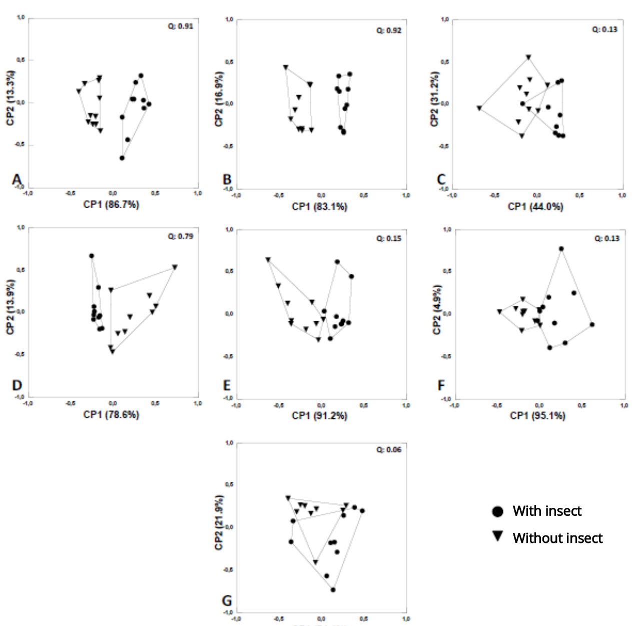

Figure 3. Clasification of rhizosphere soil after the analysis Cluster-PCA. A. Farm La Selva (Rionegro); B. Farm La Selva (Rionegro); C. Farm La Frijolera (Guarne); D. Farm El Encanto (La Ceja); E. Farm La Torre (El Retiro); F. Farm El Reposo (Envigado); G. Farm Campo Alegre (El Retiro). West of Antioquia (Colombia).

In Figures 4 and 5 is possible to observe that, in general, in all the farms the samples of rhizosphere soil have a very similar pattern related to the regions conforming the PC1 and, therefore, of the group differentiation. Such regions appeared to be represented as those points that are further away from the center on the horizontal axis, meaning, 1400 – 1499 nm, 1800 – 1899 nm and 1900 – 1999 nm. For the leaves, this patterns is changing between the farms. In this study the use of NIR spectroscopy was evaluated as a tool to detect the presence of the underground insect *E. colombianus* (Hemiptera: Margorodidae) in blackberry crops (*R. glaucus*) in the east of Antioquia. The results indicate that the NIR spectra analysis of the leaf or rhizosphere soil samples from each farm allow a clear differentiation among plants coming from crops with or without presence of the insect. Nonetheless, when analyzing the spectra obtained in the seven farms as a unique data set, it was not possible to get a classifying model for incidence of *E. colombianus*. These results suggest that the patterns coming from fractions of the near-infrared spectra can be considered as promising parameters for *E. colombianus* detection in blackberry crops and also to understand the general conditions associated to the presence of the insect in the crop. Also it was evident the requirement for calibration of this tool in each farm.

The NIR spectroscopy use has been widely accepted for its advantages over other conventional analytic techniques (Blanco and Villarroya, 2002). In agreement with these authors, a clearly visible advantage on this study is the capability of getting, without the use of chemicals, large amounts of data (spectra) from minimally processed solid samples (leaves and rhizosphere soil), reducing the costs and time for analysis.

According to Lavine (1998), a possible disadvantage is that the complex matrices of spectrum data from NIR require the knowledge and application of complex chemometric methods (mathematical and statistical procedures) to extract useful information. But this difficulty can be avoided thank to computational advances and development of new algorithms that are incorporated in the spectroscopy equipment. The PCA is a relevant multivariate analysis to cluster spectrum data of similar characteristics and stablish classification methods for unknown samples (Pali-

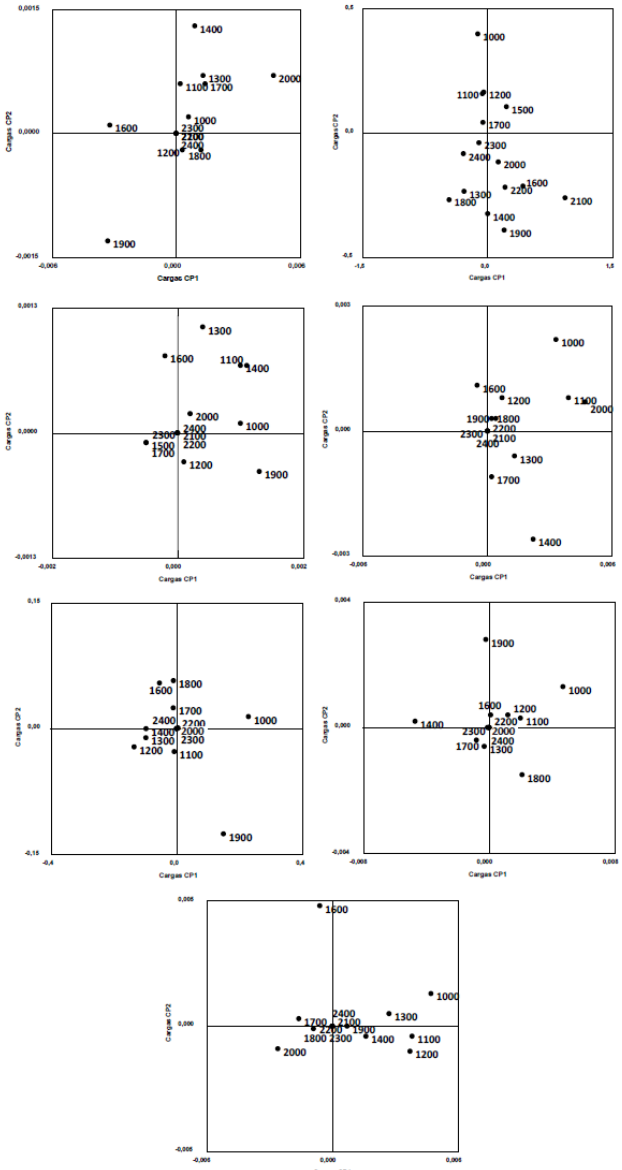

Figure 4. NIR regions comprising the principal components PC1 and PC2 for the leaf samples of each farm. NIR regions horizontally and vertically separated contribute more to the differentiation of samples with or without presence of insects. From left to right and top to bottom: Farm La Selva (Rionegro); Farm La Selva (Rionegro); Farm La Frijolera (Guarne); Farm El Encanto (La Ceja); Farm La Torre (El Retiro); Farm El Reposo (Envigado); Farm Campo Alegre (El Retiro). West of Antioquia (Colombia).

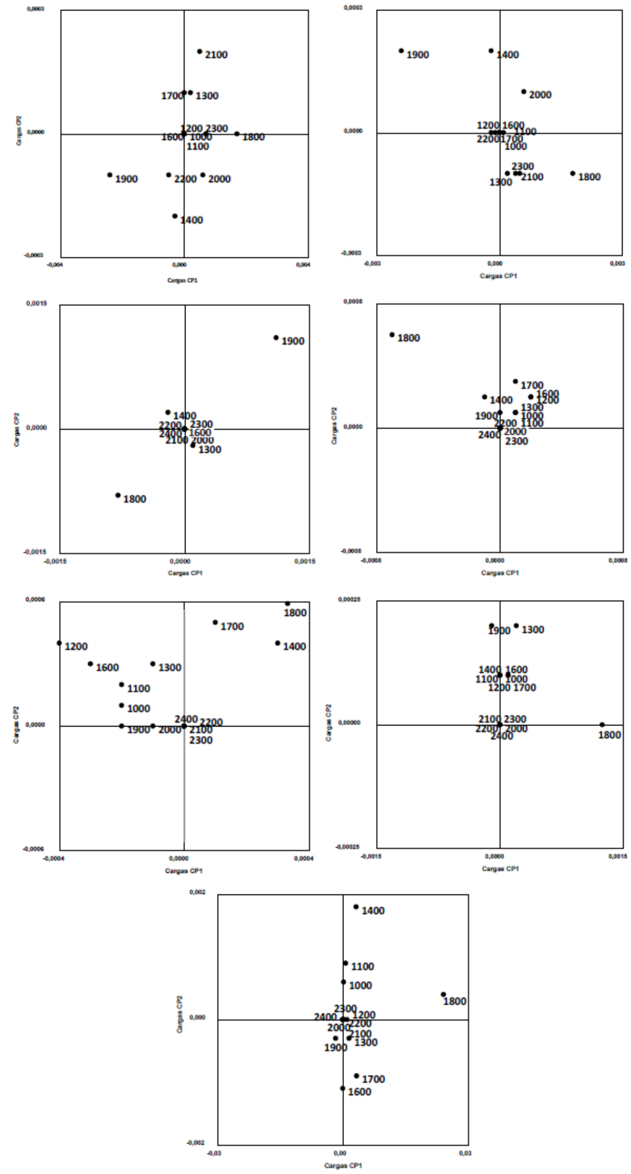

Figure 5. NIR regions comprising the principal components PC1 and PC2 for the rhizosphere soil samples of each farm. NIR regions horizontally and vertically separated contribute more to the differentiation of samples with or without presence of insects. From left to right and top to bottom: Farm La Selva (Rionegro); Farm La Selva (Rionegro); Farm La Frijolera (Guarne); Farm El Encanto (La Ceja); Farm La Torre (El Retiro); Farm El Reposo (Envigado); Farm Campo Alegre (El Retiro). West of Antioquia (Colombia).

wal *et al.*, 2004; Xing and Guyer, 2008; Liu *et al.*, 2010 and Singh *et al.*, 2010). A clear example of such is the work done by Maghirang and Dowell (2003) on insects in stored rains, where using NIR could differentiate ($P < 0.05$) healthy wheat grains from those containing *Sitophilus oryzae* L. insect.

While it is true that the cluster analysis with PCA showed a clear differentiation among the samples coming from crops with or without affection of *E. colombianus*, it is not possible to establish a general classifying model for the incidence based on the spectra data from the seven farms. This suggests that the NIR patterns obtained per farm depend on the chemical and physical characteristic of the analyzed samples in this research, such as size particle and presence of chemical bonds C–H, N–H, O–H y S–H (Blanco and Villarroya, 2002), properties that can be specific for each place (Blanco *et al.*, 1998) because they are influenced by the natural conditions and by the induced conditions due to soil use (Jaramillo, 2009) and agronomical management of plants. As they are complex samples (leaves and rhizosphere soil) obtained under non-controlled conditions, it is possible that their properties were not suitable for adjustment to a single model only by pretreating the data (Candolfi *et al.*, 1999). Therefore, for later studies it may be convenient to count with a larger matrix of spectra data that includes the physico-chemical variations expected in the samples and, with a more rigorous sampling methodology on the kind of leaves and rhizosphere soil that should be collected, which can be included as support variables on a discriminant analysis.

Some researchers like Smith (1999) declare that the infrared spectroscopy works better on pure samples, since all the bands can be assigned to a simple molecular structure; however, Downey and Boussion (1996) tested that the NIR spectroscopy can be used a discriminatory tool of complex organic materials when differentiating coffee mixes with 95% of assertiveness based on the caffeine concentration. Although in this research particular compounds associated with the plant-insect interaction were not detected, the methodology that represents in a general manner spectra bands of the compounds released by the plant, soil conditions, insect or interaction among them was constructed. To identify the key components of these interactions it is necessary to use pure compounds as standards. From this point and for a more specific detection of some compounds, other analytical techniques can be used, like middle infrared spectroscopy, which represents more defined spectra bands although the sample preparation is very elaborate.

According to Foley *et al.* (1998) the NIR spectroscopy has a high potential as a holistic tool to investigate complex attributes in natural systems. A good example of this focus is the study from Rutherford and Van Staden (1996) on the resistance of different sugarcane cultivars to stem borers attack. These researchers developed a model on NIR data to predict the resistance of sugarcane cultivars to the lepidoptera *Eldana saccharina* Walker, finding that the wavelengths that contributed the most to the resistance inn sugarcane belonged to wax components (alcohols and carbonyl components) in the stem surface. This suggests that when samples or complex processes are studied they are not associated to only one variable but to a considerable set of them (Wold, 1991). Under the evaluated conditions in this study and after analyzing the wide information contained in the PC1 of each farm (Figures 4 and 5), it can be said that there is more than one spectra band associated to the presence of the insect in the leaf samples (1000 – 2099 nm) and in the rhizosphere soil (1400 – 1499 nm; 1800 – 1899 nm; 1900 – 1999 nm).

A possible explanation of why the NIR regions conforming he PC1 in the rhizosphere soil are similar for the seven farms (Figure 5), independently of the pretreatment used, can be found in the type of soil. All of them are samples from Andisol, which showed unique and distinctive characteristics of these soils, like high water retention capacity (Jaramillo, 2009). According to Cambule *et al.* (2012) the humidity id detected by the NIR spectra with peaks at 1400 and 1900 nm, that belong to the OH groups in the soil moisture. However, these interpretations should be taken cautiously since the cited authors referred to samples obtained from different environmental conditions.

As for the variables responsible of the spectra differentiation in leaves, it is possible to say the lack of a similar PC1 for all the farms is due to variations in the chemical components, both primary and secondary in the leaves (Edwards *et al.*, 1993), that although not able to identify precisely through this technique, allowed to develop models to explain a high percentage of the variability in each farm. This result agrees with a study cited by Foley *et al.* (1998), in which through the NIR a model that explained 88% of the variation in resistance to defoliation of eucalyptus trees was developed, based solely on spectral characteristics of the foliage and not in identifying the most important bands in the spectrum.

Given the above, it is conceivable that by refining spectroscopic analysis in leaves the insect *E. colombianus* can be monitored without destroying the plants. This will allow the farmer to take appropriate, effective and economic decisions on time for the insect management and therefore, it will generate a lower negative impact in the environment by the control measurements taken. In addition, it encourages to think of the application of fundamentals of NIR spectroscopy to develop portable equipment to quickly identify the presence or absence of the insect in the field, saving time and money to the farmer.

Conclusions

Infrared spectroscopy used as comparative analytical technique allowed the classification of plants or soil of blackberry crop by the presence or absence of underground insect *E. colombianus*. If calibrated previously, this tool could be incorporated as a new method of nondestructive phytosanitary diagnosis that would improve the knowledge and sanitary monitoring of the blackberry crop in Colombia. Additionally, it is advisable to include complementary analytical techniques when you want to go from these exploratory studies to test hypotheses or to the detection of specific compounds involved in plant-insect interactions.

Acknowledgements

To the Research Direction of the Universidad Nacional de Colombia - Medellin –DIME, to the Colombian Corporation of Agricultural Research –Corpoica- La Selva, to the Soil Lab of the Universidad Nacional de Colombia - Medellin, to the research group on symbiotic Systems of the Universidad Nacional de Colombia - Medellin, to the reviewers of this article and specially to the blackberry producers in the east of Antioquia for allowing to carry out this work in their farms.

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