Acquisition System based in Electroantennography to assess the Response of Grape Berry Moth to Volatile Molecules

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***Abstract*—In Southern Europe *Lobesia botrana*, commonly known as the grape berry moth, is a major pest of grapes. Traditionally the control of this pest is made using insecticides, however there is an increasing public concern about the negative side effects of these products. Also in Europe there has been an increase of the regulation for the use of pesticides. Therefore new, and alternative, less harmful pest control strategies are needed. One of these strategies is mating disruption (MD). This is an innovative and sustainable technique based on the saturation of the air with large quantities of pheromones produced by the female moths, interfering with the pheromone communication and therefore reduce the number of offspring. However in the Douro region, in Portugal, this technique has not always yield the best results because of factors such as the fragmented landscape and the fact that most vineyards are small and surrounded by other vegetation that can also house the grape moth. Therefore a project to study the impact of the Douro landscape in the distribution of the pheromone cloud is undergoing, to improve the use of this technique in Douro. To assess the distribution of the cloud and which surrounding vegetation is more attractive to the moth traditional techniques such as the use of traps can be used. However more sophisticated techniques, such as Electroantennography (EAG), can be employed. In this paper it is presented the development of the acquisition system for an EAG device, in the framework of the above mentioned project.**

***Index Terms*—Electroantenography, Grape Berry Moth, Acquisition System, Microcontroller**

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I. INTRODUCTION

GRape berry moth, Lobesia botrana Denis & Schiffermüller (Lepidoptera: Tortricidae) is a major insect pest of commercial vineyards, mainly in Southern Europe, including Portugal. It can cause damages to grapes, directly through the feeding activity of larvae and indirectly by promoting the infection of the grape by the grey mold fungus, *Botrytis cinerea* Persoon [1]. While, traditionally, the control of this important pest relied primarily on repeated insecticide treatments during the season, public concern about the negative side effects of these products as well as the increased regulation for pesticides use in Europe has generated a demand for less harmful pest control strategies, such as mating disruption (MD) [2].

MD is an innovative and sustainable technique based on the saturation of the air above and between grapevines with large quantities of synthetic copies of the sex pheromones produced by female moths to call males for mating [3]. This will interfere with pheromone communication and mate finding between males and females which results in reducing the number of offspring produced by the pest and thus the damage caused in treated areas.

According to Witzgall et al.[2], three main elements account for the fascination of insect sex pheromones and their feasibility for insect management:

- they are species specific;
- they are active in very small amounts;
- the vast majority are not known to be toxic to animals.

MD is registered for *L. botrana* in Portugal since 2002 (Isonet-L®, from Shin-Etsu Chemical Co.). Its use is considered of particular interest in Douro Demarcated Region, an important winegrowing area (43,670ha) located in the Northeast of Portugal, where “Port” D.O.C. wine and other remarkably high-quality table wines are produced, for the preservation of the clean reputation of wines, as well as compatibility with agro-tourism, which is a key economic activity in the region [4].

However, the implementation of MD used in the Douro Region has not always yielded the best results due to some constraints, among which the fragmented landscape of the region stands out. Also, most of the vineyards are of small size, often are bounded by other crops such as olive groves, and by unmanaged natural or abandoned crop habitats, where alternative plants hosts of *L. botrana* (e.g. the flax-leaved daphne, *Daphne gnidium* L.) are common.

Under the above conditions a project is in course with the objective to research the impact of the landscape of Douro Region in the distribution of the pheromone cloud, namely in what concerns to the role of the olive tree and the flax-leaved daphne in its distribution, in order to introduce improvements in the use of MD at the vineyard plot level through a more homogeneous distribution of this cloud. Therefore among the main objectives pursued, it is intended to set up a method to evaluate how the pheromone cloud is spread in both the treated and the untreated areas around, as well as to study the relationship between the amount of pheromone emission and the behavioural activity of the moth.

To evaluate how the pheromone cloud is spread in vineyards and to assess the attractiveness of the surrounding vegetation, which can be used as refuge, to the grape berry moth we can use classic techniques, such as the use of traps, or use more sophisticated methods such as the use of Electroantennography (EAG). The latter is the one that will be used, because besides easily providing, in laboratory, information about vegetation attractiveness to the grape berry moth, it will also allow in the future to develop an electronic method to assess the spreading of the pheromone cloud, to be used *in loco*.

In this paper it is therefore presented the development of the data acquisition system for an electroantennograph, and its test with insects to assess its correct operation. As above mentioned, the work presented in this paper is being developed in the framework of a bigger research project, and here it is presented only the development of the acquisition system for the electroantennograph, which will be used as a tool in the next phases of that project.

The next phase of the project is expected to run from April to October of 2019, when grape moths have reached adult stage, and it will consist in acquiring data using grape berry moths, and correlate these data to the attractiveness that vegetation has to *L. botrana*.

It will also be tested the reaction of the grape moth males to the pheromone and to vegetation that has been in contact with the pheromone, e.g., vine and olive tree leaves collected in the vineyards under treatment.

II. Data Acquisition System

In Electroantennography insect antennae are used as a sensor to detect volatile molecules to which insects react. Odors are perceived by insects by adapted sensillae on the antennae [5], and there will be a difference in the potential between the tip and the base of the antenna, when it is stimulated [6]. In our case, those stimulus consist in exposing the insect antenna to the pheromone (in the case of males) and volatile molecules of the vegetation under test. In a simple way, Electroantennography consists in detecting and measuring those differences in potential.

This is not a new technique as it was presented in 1957 by Schneider in [7], and since then it has been used in many scientific studies. Although some commercial solutions for Electroantennography exist, in this project, it was decided to build such a system from the scratch. This decision was taken because of some key factors:

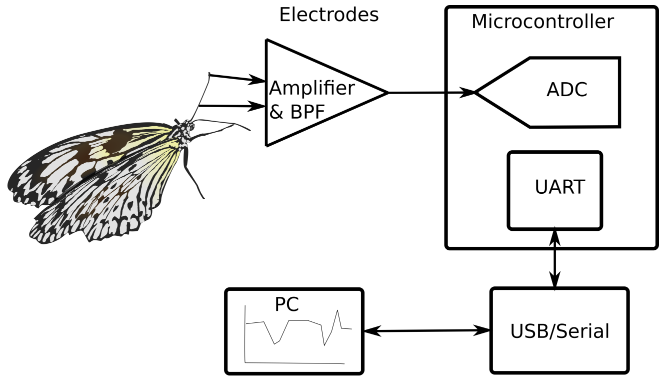

Fig. 1. Block diagram of the data acquisition system that was developed to detect and measure the electrical signals from the insects antennae.

- these commercial solutions can be very expensive, and therefore could make the main project economically unfeasible because lack of funding;
- to develop in-house know-how in the development such systems, that will be useful in other projects of the authors Research Centers, such as [8];
- by designing and building, from the scratch, such a system it is possible to develop custom hardware and software solutions that will fit the exact needs of the project. Also that hardware and software can be calibrated for the a specific insect that is going to use in the research work;
- in the future authors plan to adapt the data acquisition system presented in this paper into a battery powered portable device.

As above mentioned in Douro region vineyards, which are mountain vineyards, because of the shape of the terrain and because of the surrounding vegetation it can be very hard and technologically demanding, to predict the coverage area of the pheromone cloud. Therefore, from the above presented reasons for developing an Electroantennography based data acquisition system, the last above presented reason is very important for this project, because the developed system will be used to try to detect the coverage area of the pheromone cloud in vineyards.

A. Proposed System Architecture

In Fig. 1 are presented the main blocks of the data acquisition system:

- a pair of electrodes, to which the insect antenna will be connected to detect the difference in potential;
- an amplification and filtering stage used to amplify the small signals from the antenna, which is expected to range form a few μV to several mV [9];
- a microcontroller, which includes the Analog to Digital Conversion stage and that will send the data to a computer;
- a computer where the collected data are stored, visualized and analysed.

To acquire data in the PC, it was developed a simple data acquisition and visualization software, developed using Java technology. The objective of this software is to allow the user to visualize in real time the waveform of data collected by the acquisition system and save it into a file. Data is saved

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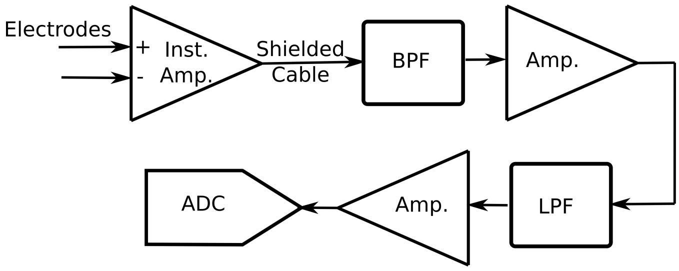

Fig. 2. Diagram block of the amplification and filtering stage.

in CSV format, which will allow an off-line analysis using the most common data analysis software applications.

Java was chosen to develop this applications but any other programming language could be used, provided that it has support to interface de serial port (for communications with the acquisition system) and it is supported by Linux. The latter will be important in the future evolution of the device into a portable device (based on an Linux embedded system). In [10] authors present some reasons for the use of Java in this type of systems.

B. Amplification and Filtering Stage

Because of the white noise and the 50Hz noise from the electrical power grid, that are picked up by the two electrodes, and because antennae signals are extremely small, the amplification stage was divided into two sections, as depicted in Fig. 2.

The first section corresponds to an Instrumentation Amplifier, with a high Common Mode Rejection Rate, to which the antenna is connected (through the electrodes). In this project it was used the AD8422BRZ Instrumentation Amplifier from Analog Devices.

This Instrumentation Amplification (IA) block is implemented in a single PCB that is placed as near as possible to the antennae to reduce, as much as possible, interference because of noise. After this first amplification the analog signal is sent to the data acquisition board using a shielded cable (Fig. 2 and Fig. 4). A previous prototype of this system had this amplification stage in the same printed circuit board as all other circuits, but electrodes had to be connected to the board using cables. Even though these cables were shielded, noise level was still too high for the circuit to be feasible. Higher noise immunity can be reached by housing this amplification stage inside an aluminum enclosure.

After the Instrumentation Amplifier, there is a Band Pass Filter block, with cut-off frequencies of 0.1Hz and 10Hz. This filter has two objectives:

- remove any offset voltage that could saturate the output of the next amplification stages;
- remove noise, such as the white noise and electrical grid noise that will be picked up by the electrodes.

The second amplification block uses a cascade of two low noise Operational Amplifiers (OPAMP), in this case OPA21 were used, but any low noise OPAMP with similar or better electrical characteristics can be used. By cascading two amplifiers, instead of a single amplifier, it will allow to obtain high overall gain without having a too high gain in a single amplifier that would result in a lower bandwidth, or even circuit instability.

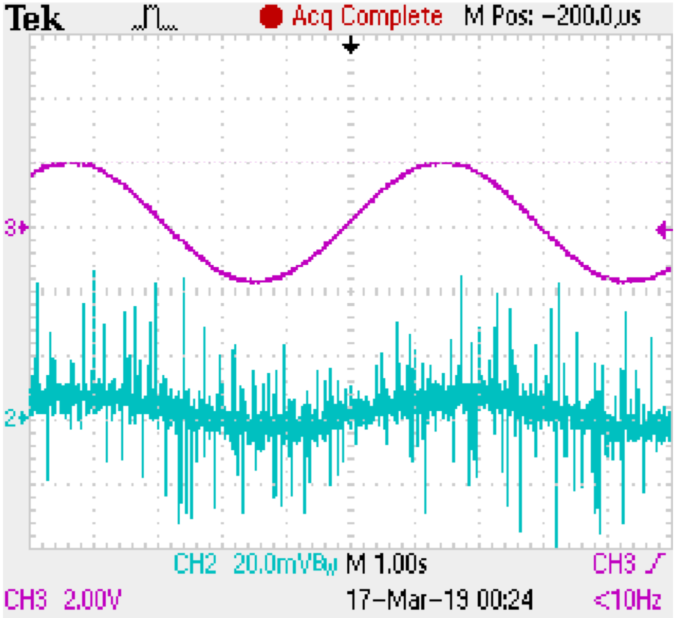

Fig. 3. Laboratory test made to amplification circuit using a low amplitude and frequency sine wave degraded by noise.

To test this block, the overall gain of this stage was set to $60dB$ and a low amplitude, approximately 4mV, and low frequency sine wave was injected in this circuit (Fig.3). As it can be seen in Fig. 3, even though the input signal is very degraded by noise, at the output of the circuit this noise is not perceptible.

C. Data Conversion and Transmission

After amplification and filtering data collected from the antenna must be converted into the digital domain. This process is made using the Analog-to-Digital (ADC) converter of a microcontroler. In this project a PIC18LF26K22 was used, which is a low power 8-bit microcontroller with a 10-bit ADC.

Besides the ADC this microncontroller also includes an asynchronous serial communication interface, which will be used to send data to the computer. Because most modern computers do not have such an interface, a serial to USB module was also used.

This device will be "seen" by the computer as a serial interface device, which will allow the use of standard communications libraries to interface the Java application [10]. However the use of the serial port of the microcontroller has disadvantage of limiting the maximum transmission speed of data and therefore limit the sampling frequency of the ADC. In the developed prototype a sampling frequency of 100Hz was used.

Before data is sent to the PC, it was implemented a FIR (Finite Impulse Response) digital low pass filter, with a cut-off frequency of 10Hz. The objective of this filter is provide an extra filtering to the signal, to remove any interference picked up by the microcontroller ADC.

This filter can be implemented both in the microcontroller or in the computer. In the future the decision where this filter will be computed will depend on the complexity of other tasks that might be needed to implement in the

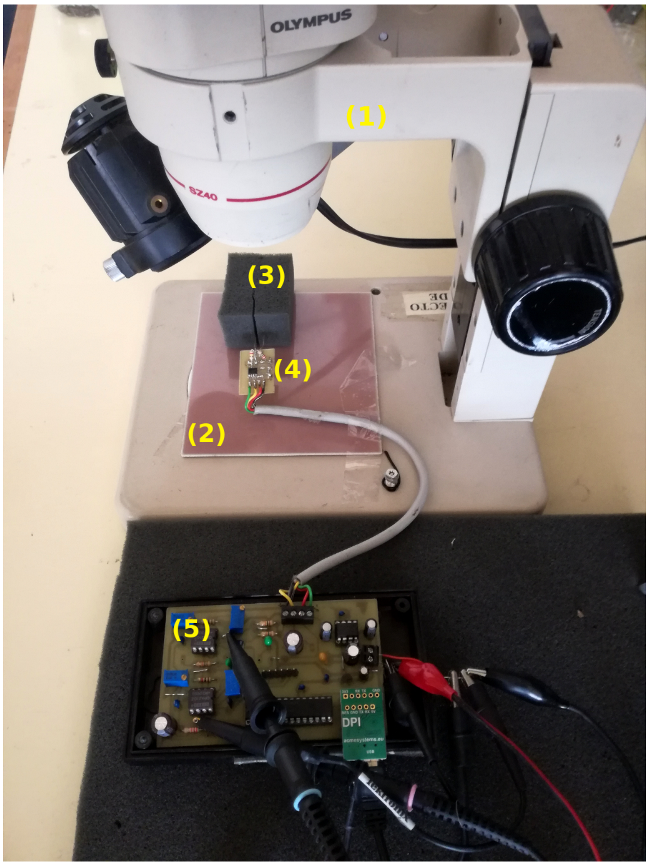

Fig. 4. Setup of the prototype used in laboratory to test the developed data acquisition system.

microcontroller (e.g. data compression) that might use too much CPU time.

III. Tests and Results

In this section are presented two tests made with two test subjects. These tests allowed authors to setup the gain of the amplification stage and verity the correct operation of all above mentioned blocks. These tests consisted in connecting test subjects antennae to the circuit using the electroded and expose the subjects to samples of air with and without volatile molecules.

To acquire data for tests, and in the future do acquire related to the grape moth response to vegetation and pheromone, the prototype presented on in Fig. 4 was used. This prototype includes:

- the magnifier (1), needed to help handle insects and correctly place the electrodes in the antenna;
- a grounded copper plate that is used to help reduce noise (2);
- (3) the insect foam holder, used to hold the insect still while signals are being recorded (more detail can be seen in Fig. 6);
- the instrumentation amplifier block, which as above explained must be placed as near as possible to the insect antenna (4);
- data acquisition circuit (5) .

Connected to the data acquisition circuit (5) there is also a personal computer as presented in Fig. 5.

Test subject are kept alive and mobilized in the foam, Fig. 6, and the antenna is connected to one electrode. Because after the conclusion of the first feasible prototype it was not possible to obtain grape berry moth test subjects, other types of lepidoptera were used to assess the system viability and to calibrate it. For all the tests made to the circuit, subjects were kept alive and after were released.

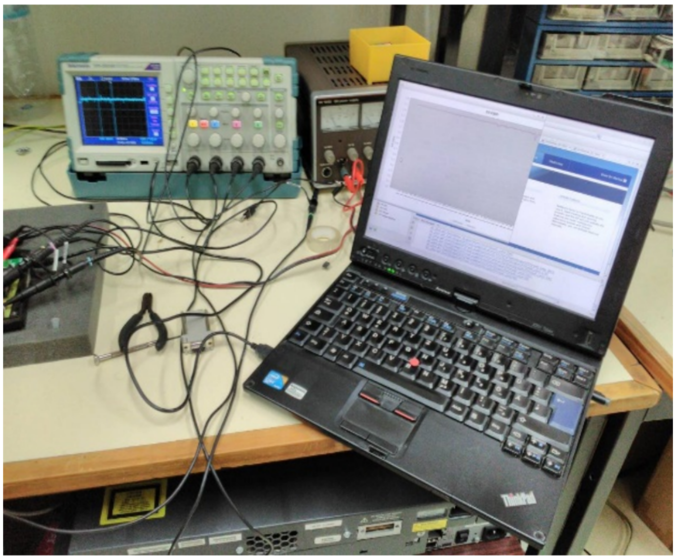

Fig. 5. PC used to acquired data.

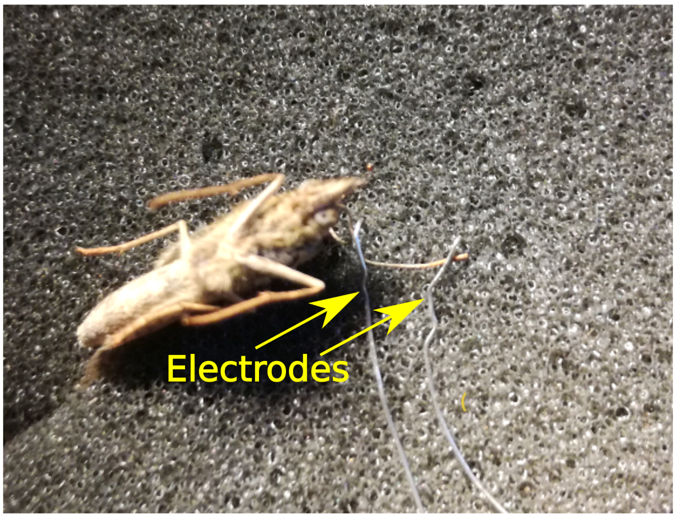

Fig. 6. Electrodes connected to a test subject (not a grape moth).

After being immobilized and connected to the electrodes, test subjects were exposed to samples of oregano and their response was recorded. In Fig. 7 and Fig. 8 are presented data recorded for two test subjects.

In both plots we can see a clear response of the test subjects when are exposed to air samples containing volatile molecules of the plant that is know to be attractive to these *lepidoptera*. Also there is no response when the test subjects were exposed to air samples without those volatile molecules.

IV. Conclusion

In the current stage of development, both the developed data acquisition circuit and software are ready for the next phase of the project, which is the acquisition of data using grape moth test subjects. The next generation of the grape berry moth is expected to reach the adult phase in the first

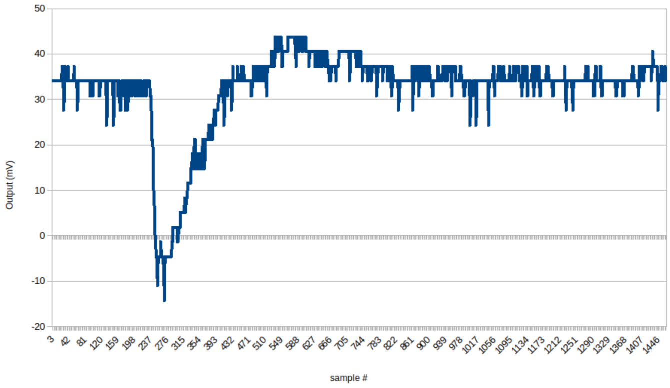

Fig. 7. Plot of data acquired using test subject 1, exposed to volatile molecules of oregano plant.

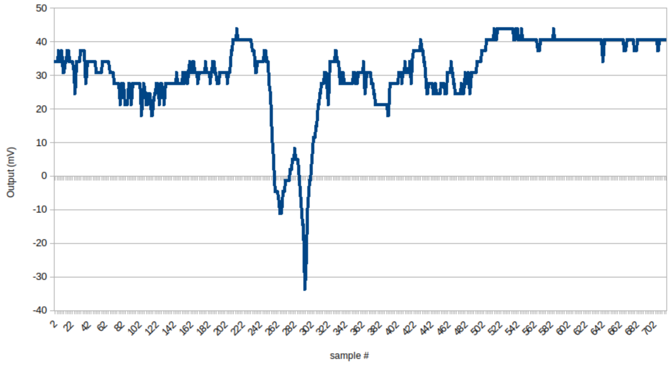

Fig. 8. Plot of data acquired using test subject 2, exposed to volatile molecules of oregano plant.

week of April, when they will be ready for the tests. In that phase data will be collected using both male and female grape berry moths, and it will be recorded their reaction to several samples of vegetation and to the pheromone. This last one will be only for grape moth males.

In parallel to these in-laboratory data acquisition, it will also be studied the possibility of evolving this system into a portable device, so it can be easily used in field work. At the moment these prototypes are using simple single sided printed circuit board (used only in the prototyping phase). Next, the data acquisition circuits will be converted into a daughter board to fit an embedded system, such as Raspberry Pi or a similar embedded device, with a small LCD screen and battery powered.

This embedded system will replace the computer that is currently used to store and display the data. Because the software for this solution is being developed using Java and standard libraries for serial communications [10], to interface the data acquisition module, no major integration problems are expected in this field.

One of the most challenging tasks will be the minimization of power consumption, to extend the battery life. This can be done either by using very low power devices in the data acquisition module, customizing the kernel and installed applications of the Operating System, removing features and applications that are not needed, disabling some peripherals or even modifying the system board.

In this stage of development, no data compression is being used in the serial port. Because effective data communications transmission rate, between the microcontroller and the computer, will limit the maximum ADC sampling frequency, if a higher ADC sampling frequency will be needed in the future, then fast compression algorithms (that ensure a low latency) will have to be considered. Other options might include the use of a microcontroller with integrated USB interface or even the use of a FLASH ADC and an FPGA (Field-Programmable Gate Array) as authors used in [12].

REFERENCES

[1] M. Fermaud and R. L. Menn, “Association of Botrytis cinerea with grape berry moth larvae. Phytopathology,” *Phytopathology*, no. 89, pp. 651–656, 1989.

[2] P. Witzgall, P. Kirsch, and A. Cork, “Sex pheromones and their impact in pest management,” *J. Chem. Ecol.*, no. 36, pp. 80–100, 2010.

[3] B. M. B.A. and M. Evenden, “When mating disruption does not disrupt mating: fitness consequences of delayed mating in moths,” *Entomologia Experimentalis et Applicata*, no. 140, pp. 50–65, 2012.

[4] C. Carlos, *Towards a sustainable control of arthropod pests in Douro Demarcated Region vineyards with emphasis on the grape berry moth, Lobesia botrana (Denis & Schifermüller)*. PhD Thesis, University of Trás-os-Montes and Alto Douro, Vila Real, Portugal, 2017.

[5] P. B. A. Cork, A. Gough, and D. Hall, *Gas Chromatography Linked to Electroantennography: A Versatile Technique for Identifying Insect Semiochemicals*. Springer, 1990.

[6] F. Schiestl and F. Marion-Poll, *Detection of Physiologically Active Flower Volatiles Using Gas Chromatography Coupled with Electroantennography*. Springer, Berlin, Heidelberg, 2002, vol. 21.

[7] D. Schneider, “Elektrophysiologische untersuchungen von chemo- und mechanorezeptoren der antenne des seidenspinners (bombyx mori l.),” *Z Vergl Physiol*, no. 40, pp. 8 – 41, 1957.

[8] P. Arnaldo, D. Gonzalez, I. Oliveira, F. Langevel, and I. Wynhoff, “Influence of host plant phenology and oviposition date on the oviposition pattern and offspring performance of the butterfly phengaris alcon,” *J Insect Conserv*, no. 18, p. 1115–1122, 2014.

[9] SYNTECH, *ELECTROANTENNOGRAPHY a practical introduction*. SYNTECH, Kirchzarten, Germany, 2015.

[10] P. Mestre, C. Serôdio, J. Matias, J. Monteiro, and C. Couto, *Java in the Loop of Data Acquisition Systems, Data Acquisition*. IntechOpen, 2010.

[11] P. Mestre, C. Serôdio, A. Azevedo, H. Correia, I. Bentes, and C. Couto, “Filling rate assessment of recycling containers using ultrasonic transducers,” *Measurement*, vol. 44, no. 6, pp. 1084 – 1095, 2011. [Online]. Available: http://www.sciencedirect.com/science/article/pii/S0263224111000923

[12] P. Mestre, A. Calçada, N. Carvalho, C. Serodio, P. Couto, J. Matias, P. Melo-Pinto, and J. Morais, “Low-cost Ultrasonic Probe to Assess Wood Defects and Parameters,” in *Lecture Notes in Engineering and Computer Science: Proceedings of The World Congress on Engineering 2013, WCE 2013, 3-5 July, 2013, London, U.K.*, 2013, pp. 993–998.