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SENSORS AND BIOSENSORS FOR PATHOGEN AND PEST DETECTION IN AGRICULTURAL SYSTEMS: RECENT TRENDS AND OPORTUNITIES

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Dissertação de Mestrado, apresentada ao Programa de Pós-Graduação em Agronegócios, da Universidade Federal do Rio Grande do Sul, como requisito parcial para a obtenção do título de Mestre em Agronegócios.

Orientador: Prof. Dr. Homero Dewes

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I dedicate this work to all agricultural science professionals that, despite many adversities, restlessly continue to pursue the endeavor of food production through technological advances. I hope that the discussions and results presented here may contribute for technical enhancements in agricultural systems, making it more sustainable for future generations to come.

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ABSTRACT

Pathogen and pest-linked diseases across agriculture and ecosystems are a major issue towards enhancing current thresholds in terms of farming yields and food security. Recent developments in nanotechnology allowed the designing of new generation sensors and biosensors in order to detect and mitigate these biological hazards. However, there are still important challenges concerning its respective applications in agricultural systems, typically related to point-of-care testing, cost reduction and real-time analysis. Thus, an important question arises: what are the current state-of-the-art trends and relationships among sensors and biosensors for pathogen and pest detection in agricultural systems? Targeted to meet this gap, a comparative study is performed by a literature review of the past decade and further data mining analysis. With the majority of the results coming from recent studies, leading trends towards new technologies were reviewed and identified, along with its respective agricultural application and target pathogens, such as bacteria, viruses, fungi, as well as pests like insects and parasites. Results have indicated lateral flow assay, lab-on-a-chip technologies and infrared thermography (both fixed and aerial) as the most promising categories related to sensors and biosensors driven to the detection of several different pathogenic varieties. The main existing interrelations between the results are especially associated to cereals, fruits and nuts, meat and dairy along with vegetables and legumes, mostly caused by bacterial and fungal infections. Additional results also presented and discussed, providing a fertile groundwork for decision-making and further developments in modern smart farming and IoT-based agriculture.

Keywords: agribusiness, plague control, pest management, plant infection, animal infection, innovation, food safety, food industry, semiconductor, big data, IoT, IRT, LFA, LOC, UAV.

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LIST OF ABBREVIATIONS

AFI - Aerial Fluorescence Imaging
AIRT - Aerial Infrared Thermography
BL - Bioluminescence
CL - Chemiluminescence
CMOS - Complementary Metal-Oxide-Semiconductor
EIS - Electrochemical Impedance Spectroscopy
EL - Electro-induced Luminescence
ELISA - Enzyme-linked Immunosorbent Assay
EMF - Electromotive Force
FCM - Flow Cytometry
FISH - Fluorescence In-Situ Hybridization
FOBS - Fiber-Optic Biosensor
FRET - Förster Resonance Energy Transfer
GC - Gas Chromatography
HH - Handheld
HPLC - High-Performance Liquid Chromatography
IF - Immunofluorescence
IoT - Internet of Things
IRT - Infrared Thermography
LC - Liquid Chromatography
LFA - Lateral Flow Assay
LIBS - Laser-Induced Breakdown Spectroscopy
LOC - Lab-On-A-Chip
MEMS - Microelectromechanical Systems
NDVI - Normalized Difference Vegetation Index
PCR - Polymerase Chain Reaction
POCT - Point-of-Care Testing
QCM - Quartz Crystal Microbalance
SAM - Self-Assembled Monolayer
SAW - Surface Acoustic Wave
SEM - Scanning Electron Microscopy
SPR - Surface Plasmon Resonance
UAV - Unmanned Aerial Vehicles
VOC - Volatile Organic Compounds

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CHAPTER 1: GENERAL INTRODUCTION

1.1 Introduction

The continuum evolution of technology in the agriculture, as we know, have been allowing increasing yield growth results in crop production, in a global basis. Meanwhile, global demand for agricultural goods is increasing, and may continue to do so for decades, propelled by a 2.3 billion person projected increase in population and greater per capita incomes, expected until 2050 (TILMAN et al., 2011). This phenomenon stress the capacity of agriculture to meet food needs without further sacrificing the environmental integrity of local landscapes and the global environment. Agriculture's main challenge for the coming decades will be to produce sufficient food and fiber for a growing global population at an acceptable environmental cost (ROBERTSON; SWINTON, 2005). Hence, advances in technology are particularly important in the quest to close yield gaps worldwide, as it shape directly the ability to mitigate possible losses throughout the agricultural production chains.

Nevertheless, current trends show that human managed ecosystems, as well as the services they provide, are more likely to be vulnerable to diseases (FOLEY et al., 2005). This tendency indicates how the world's food security is also increasingly endangered by these factors. Thus, the maintenance of increasing productivity levels relies deeply on continued innovation to control weeds, diseases, insects, and other pests as they evolve resistance to different control measures, or as new species emerge or are dispersed to new regions (GODFRAY et al., 2010). Additionally, among all scientific fields in which pathogens represent real threats, the agriculture and food production systems steps up as the leading area of interest. More can be seen in Fig. 1.

Pathogens and pests proliferation and contamination are considered historically linked with disease-causing problems in agriculture. As these contamination outbreaks may often cause crop losses and nourishment decrease in human population, it is reasonable to relate it also as a key element for food security. Most pests and pathogens are kept in check not by pesticides but by natural enemies, immunities, and various ecological and physiological plant defense strategies (HAJEK, 2004). Still, for high-demand cropping systems, the regulation of important pathogen ans pest populations relies not only on natural predation, but also on management improvements and technology applications (ROBERTSON; SWINTON, 2005).

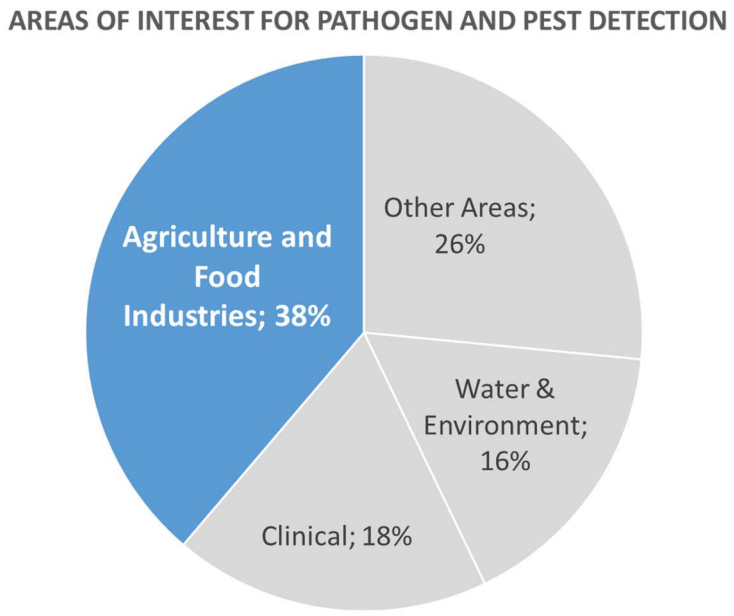

Figure 1: Areas of interest for pathogen detection applications (adapted from LAZCKA; CAMPO; MUÑOZ, 2007)

Taking a closer look in the global major crops, it is possible to identify the real impact (estimated losses) due to pathogens proliferation, through Fig. 2. Moreover, Fig. 3 also relates these losses in terms of geographical localization (per continent). In this case, the author (ROSENZWEIG et al., 2001), also states that as his study uses percentage to indicate losses, it is unclear (from the scientific point of view) how much of it is been driven also by other factors – i.e., economic and technology – especially in the case of the African and Asian continent.

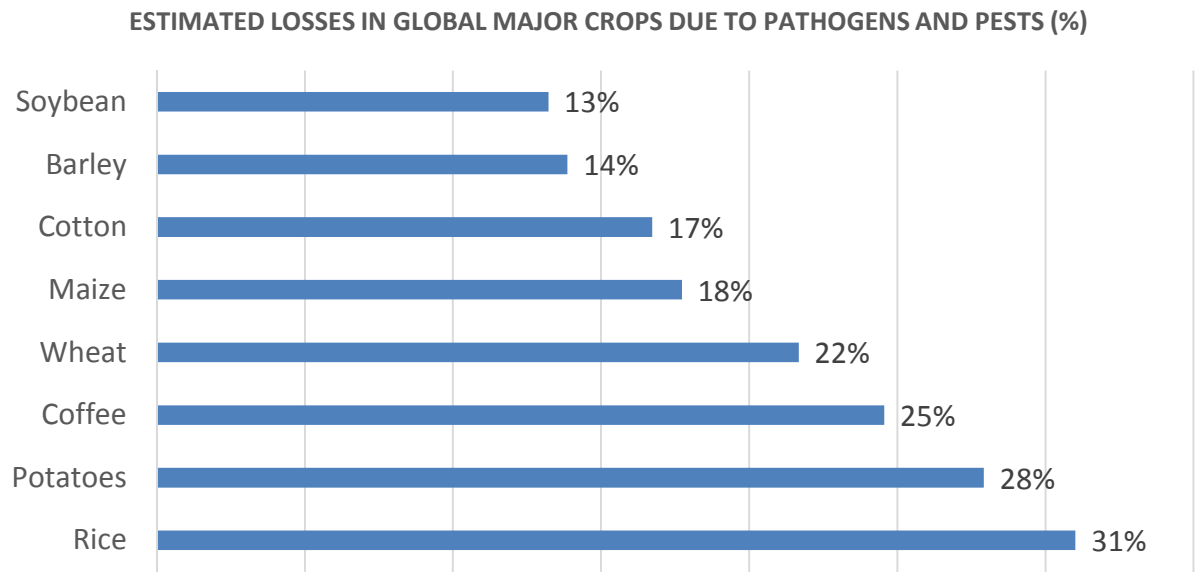

Figure 2: Estimated losses in global major crops due to pathogens (adapted from ROSENZWEIG et al., 2001)

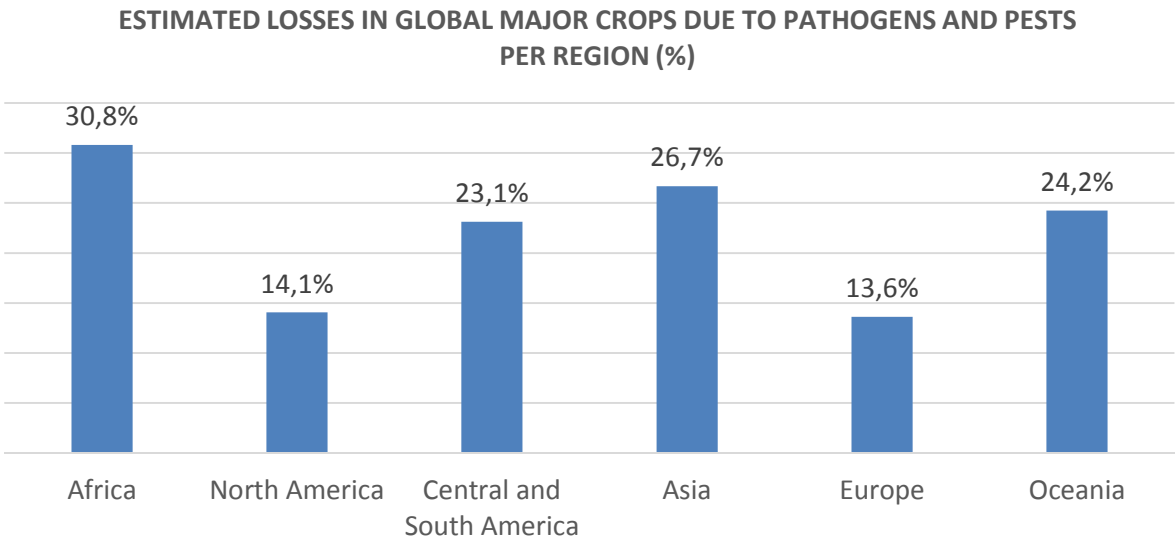

Figure 3: Estimated losses in global major crops due to pathogens, per continent (adapted from ROSENZWEIG et al., 2001)

Indirectly, human nutrition represent the most important driver for agricultural industry. Unfortunately, this same production chain creates a potential ground for pathogen organisms, such as virus and bacteria, resulting in many foodborne illnesses. Recent research by the United States Department of Agriculture estimated that the 15 most common foodborne pathogens alone have caused approximately 8.9 million cases of foodborne illness in the United States in 2013, with US$15.6 billion in overall medical costs, including productivity losses, costs of premature deaths and associated diseases (USDA, 2014). Further data can be found in Table 1.

Table 1: Pathogens - Cost of Foodborne Illnesses and Number of Cases (adapted from USDA, 2014)

Pathogen	Estimate Cost of Foodborne Illness (US$)	%	Number of Cases	%
Salmonella (non-typhoidal)	3.666.600.031	23,5%	1.027.561	11,5%
Toxoplasma gondii	3.303.984.478	21,2%	86.686	1,0%
Listeria monocytogenes	2.834.444.202	18,2%	1.591	0,0%
Norovirus	2.255.827.318	14,5%	5.461.731	61,3%
Campylobacter (all species)	1.928.787.166	12,4%	845.024	9,5%
Clostridium perfringens	342.668.498	2,2%	965.958	10,8%
Vibrio vulnificus	319.850.293	2,0%	96	0,0%
Yersinia enterocolitica	278.111.168	1,8%	97.656	1,1%
Escherichia coli O157	271.418.690	1,7%	63.153	0,7%
Vibrio (all other non-cholera species)	142.086.209	0,9%	17.564	0,2%
Shigella (all species)	137.965.962	0,9%	131.254	1,5%
Cryptosporidium parvum	51.813.652	0,3%	57.616	0,6%
Vibrio parahaemolyticus	40.682.312	0,3%	34.664	0,4%
non-O157 Shiga toxin-producing Escherichia coli	27.364.561	0,2%	112.752	1,3%
Cyclospora cayetanensis	2.301.423	0,0%	11.407	0,1%
	15.603.905.962		8.914.713	

As most of United States' crop types and agriculture best practices have been spread into many other countries and continents, we can expect to find similar pathogen-crop patterns elsewhere in world. This factor steps up to the challenge of dealing efficiently towards this issue. Therefore, new practices must be deployed in order to increase the resilience of the food system, particularly in the disease related issues. (FOLEY et al., 2011).

Current pathogen detection methods require great laboratorial infrastructure, as well as high cost and long analysis gaps (periods). The standard main methods are Conventional Culturing and Colony Counting, Enzyme-linked Immunosorbent Assay (ELISA) and Polymerase Chain Reaction (PCR). These methodologies reflect differences in its necessary equipment, reliability and timing, but none of them offers solutions for application issues, such as high cost, lack of portability and time-consuming techniques for analysis.

In order to narrow this gap, further recent developments in nanotechnology and micro-engineering allowed the designing of compact and long standing sensors and biosensors, which consists in analytical devices with physicochemical detectors, used for the detection of a specific chemical substance or physical parameter (BANICA, 2012). Sensors themselves have different types of classification, usually based upon its sensing elements (*i.e.* receptors) and its transduction platforms (*i.e.* type of detector). Particularly in the case of biosensors, biological components are combined to the sensor structure through the use of biological recognition elements (also named bioreceptors) as well as its electronic device for signal conversion (known as transducer). Above all, this breakthrough represented a major milestone for laboratory based real-time analysis.

However, there are still important challenges concerning sensor and biosensor application, especially related to its widespread employment in agricultural systems, not to mention also problems in terms of field usage (outside lab infrastructures). Portability-oriented design is a key element for current biosensors. New portable technologies have been researched, developed and are now embedded into many types of sensors – such as CMOS technology, UAV mounted systems and wireless connections – which are already spread out through several supply and production chains, playing a promising role in the agricultural systems management. Yet greater advances in this field are still expected in the forthcoming years (JUNG et al., 2014).

Although various methods for plant and animal disease identification have already been developed and some have been implemented, their application is limited due to multiple reasons: they are either time consuming, destructive, demand a skilled technician, require laboratory set-

up, do not provide real-time monitoring (e.g., FISH, ELISA, IF, FCM, GC) and/or display low specificity (e.g., imaging techniques). Growers are interested in a solution that could help them identify pathogen infections in crops in a rapid, real-time and non-destructive fashion so that timely intervention and preventative treatments can be performed to contain the infection and minimize the crop losses. This would allow the growers to save millions of dollars in fungicide costs, by allowing them to localize sprayings and timely applications rather than preemptive spray massive regions of crop field (FANG; RAMASAMY, 2015).

Although many sensors and biosensors are still in the development and testing phases, some have already reached the consumer market as handheld devices – portable units used for field measurements – or are routinely used in a laboratory setting. New discoveries in nanotechnology and material science as well as being able to custom engineer the analytical device will further push the development of useful and reliable biosensors. In addition, recent research show that all breakthroughs in biosensors are now been driven by low-cost, real-time and portable/disposable technologies (RONKAINEN; HALSALL; HEINEMAN, 2010).

By taking a further look at the agricultural industry, it is possible to list some of its important singularities, in the application point-of-view. For instance, such characteristics, along with its typical landscape scenario – based on countryside areas with limited access to modern (and usually "urban") lab facilities – represent a major challenge in the diffusion of new techniques, as pathogen detection within nanotechnology-applied biosensors with real time analysis. Equally, the lack of infrastructure from the average farm estate favors the application of biosensors only if all necessary robustness criteria are considered among its design, such as power supply and handling. A broader view of all interrelations between these factors can be seen in Fig.4.

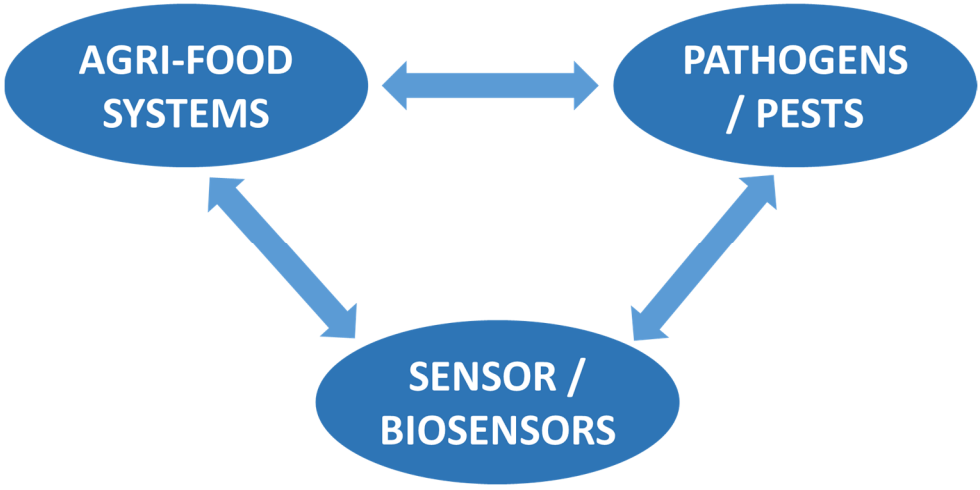

Figure 4: Bidirectional interrelations between Agricultural Systems, Pathogens and Sensors/Biosensors

Several works have been dedicated to evaluate possible trends on sensors and biosensors with pathogen detection methods embedded (FANG; RAMASAMY, 2015; FARRIS; HABTESELASSIE; PERRY, 2008; GRACIAS; MCKILLIP, 2004; KUCKENBERG; TARTACHNYK; NOGA, 2009; LAZCKA; CAMPO; MUÑOZ, 2007; POLTRONIERI et al., 2014; TOMBELLI et al., 2000; VELUSAMY et al., 2010; ZHAO et al., 2014), especially upon the technical point-of-view, with the design and engineering bias. Similarly, to solve problems and to improve yields in the agricultural systems also represent a substantial topic of current academic research (BRESTIC; ZIVCAK, 2013; CHAERLE et al., 2004; JANSEN et al., 2015; LENTHE; OERKE; DEHNE, 2007; MAHLEIN et al., 2012; PERERA; MARRIOTT; GALBALLY, 2002; SOZER; KOKINI, 2009; STOLL et al., 2008).

Yet, from the scientific research angle, it is clear the lack of academic papers among journals and publications, driven to explore all the agricultural applications within sensors and biosensors for pathogen and pest detection. Furthermore, these new technologies represent an important tool for food security, with potential to create positive impacts in yield concerning all agricultural production chains.

The approach of is type of study – which is based on meta-analysis and lay emphasis on all trends for sensor and biosensor-based pathogen detection systems in the agricultural production chains – has an significant strategic relevance in the aspect of enhancing food security and minimizing losses throughout crop harvesting and food processing. Thereafter, given the strategic role of agribusiness for the world economy, it is important to promote studies driven to support improvements in management and decision-making throughout the farming sectors.

Multidisciplinary study is increasingly becoming a key element for analysis and discussion of all present topics in agricultural sciences. Thus, throughout in this field of study, whenever the analysis is made through only one discipline, it might became insufficient for the fully comprehension of certain phenomena, with its inherent complexities.

Thereby, we present here the groundwork of this research project, which is developed under an extensive literature review on pathogen detection sensors and biosensor systems, as well as its direct application in the agricultural production chain. We divided this preliminary literature review into the following two main topics and chapters: Introduction To Sensors And Biosensors; Pathogen Detection Techniques (For Sensor and Biosensors Applications);

1.2 Problem Statement

The main subject that this study intends to address is:

What are the current state-of-the-art trends in technology of sensors and biosensors for pathogens and pests detection in agricultural systems?

Food security has been a major concern topic since the dawn of mankind. Over the course of the last century, great progress was achieved with the widespread of new technologies and best practices concerning yield increase and microbiology for the detection of several types of pathogens in crops. Several are the studies already carried out the area of pathogenicity, particularly with the agricultural industry bias (BATZ et al., 2005; MEAD et al., 1999; NYACHUBA, 2010; PIRES et al., 2012). Recent developments in nanotechnology also allowed the designing of new generation sensors and biosensors, which represented a milestone for laboratory based real-time analysis.

Nevertheless, new studies have shown challenges ahead in the forthcoming years, facing cost, efficiency and portability among upcoming technologies and its applications in the agricultural and food sectors (YOON; KIM, 2012). Technical improvements such as microfuidics, point-of-care testing (POCT), spectroscopy and internet of things (IoT) integration represents the most promising advances that are been held in this field of study, in order to meet these new barriers.

However, still many technical issues remain as holdbacks for the spreading of nanotechnology based portable sensors and biosensors in the agribusiness systems. Hence, the importance of fostering new guidelines and trends in this field, as well as its technical advantages and applications, strikes as a thriving subject for academic research.

1.3 Objectives

The objectives guiding the execution of this work, which gives focus at technological improvements in pathogen and pest control for agricultural systems, were:

1.3.1 General

Present a comparative study of technological trends among sensors and biosensors for pathogens and pests detection in agricultural systems, with groundwork structured out of literature review (from papers published worldwide over the past decade) and further data mining analysis.

1.3.2 Specific

1) Review relevant scientific papers on the subjects of sensor, biosensor and pathogen and pest detection, as well as its applications in agricultural systems;
2) Identify possible existing relationships between sensors, biosensors, pathogens, pests and agricultural systems;
3) Provide significant information in order to help decision-making process by the agricultural producer and policy makers;

CHAPTER 2: INTRODUCTION TO SENSORS AND BIOSENSORS

Sensors and biosensors are basically devices driven to process chemical substances or physical parameters into electrical signals. Regardless of its functionality or composition, they all require a specific analyte (defined as the component of interest within a given sample) prior to its operation. Technically it is possible to divide sensors and biosensors into two basic items: a sensing element and a transduction component. Sensing elements divided into receptors (sensors) and bioreceptors (biosensors) and are responsible for the recognition of specific targeted elements (i.e. analyte). Transduction components (popularly known as transducers) are electronical devices that converts signals from one form of energy into another, enabling the sensor and biosensor to value its necessary measureable property among the analyte. Figures 5 and 6 allow a more detailed understanding of its composition.

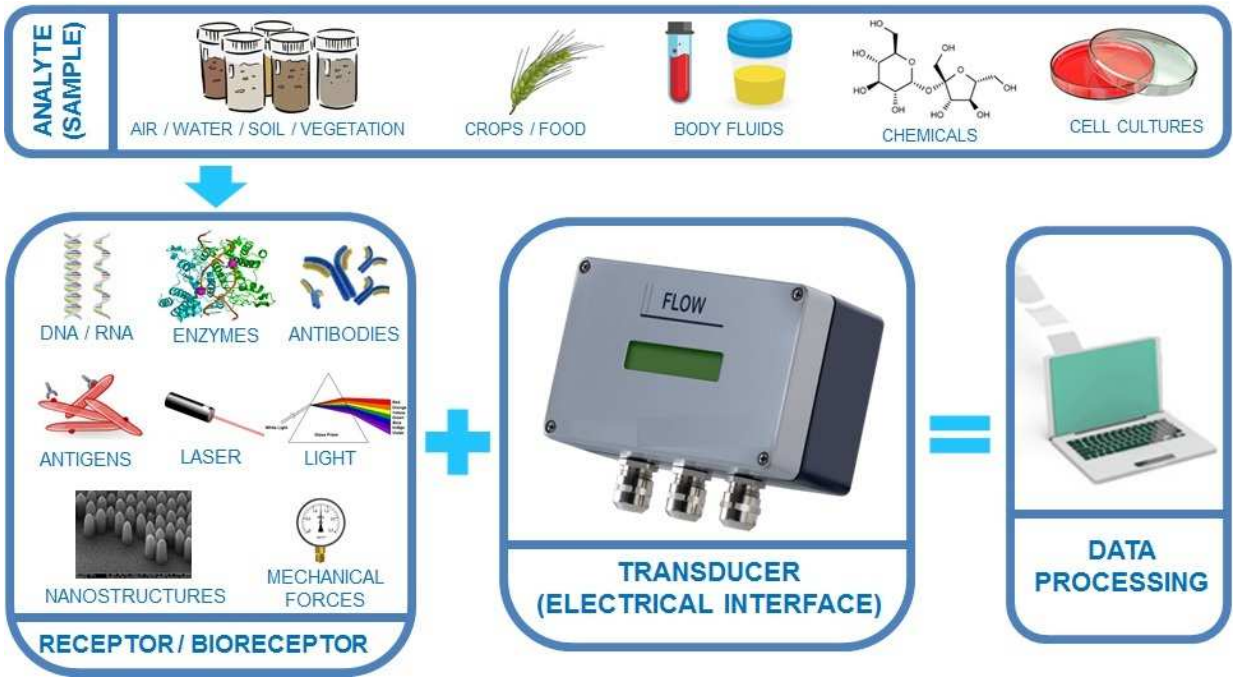

Figure 5: Sensor and biosensor components and its necessary elements for analysis

Conventional assays for analyte measurement always require the use of some sort of reagents, which are used to treat samples in many steps. This process increases timing and reduces portability, creating an extra obstacle from the analysis point of view. A typical example would be the glucose (analyte) concentration measurement in a biological fluids (sample). By using conventional methods, samples require preprossessing with reagents in order to provide the proper assessment of the analyte. Besides been considered a laboratory-based assay, this process does not provide an instant result.

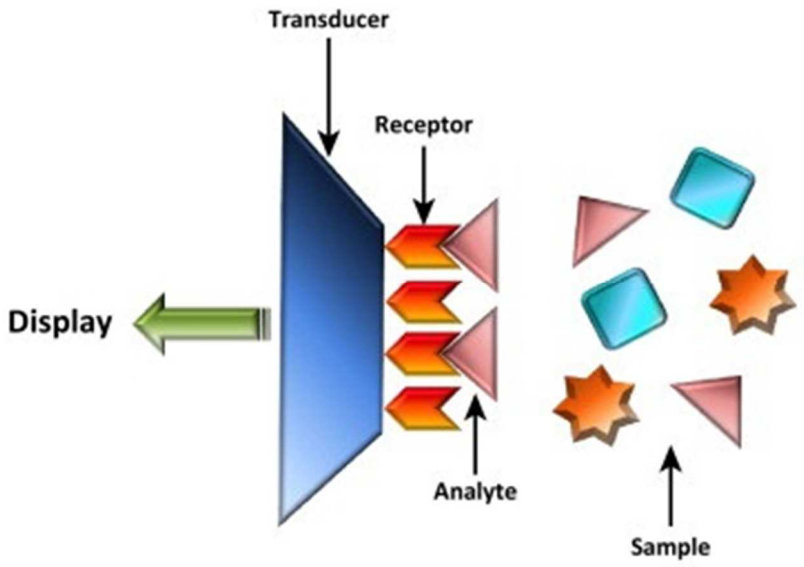

Figure 6: Typical sensor components and its interconnections

Whereas by measuring it directly with a biosensor (designed specifically for glucose concentration), it would be necessary simply dipping the sensor into the sample. In this case, the concentration measure would be provided through different reactions among both bioreceptor and transducer. Therefore, in contrast to the conventional assay method (reagent based), biosensor's simplicity and measurement speed are considered its main advantages.

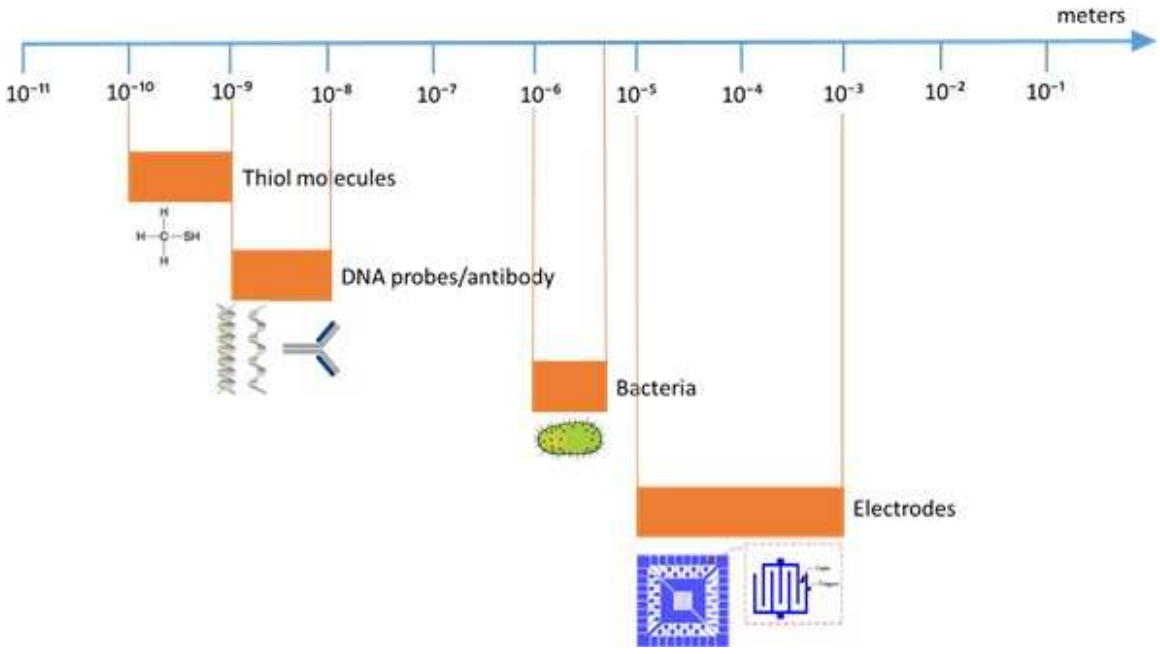

Figure 7: Diagram representing the comparative sizes of sensor and biosensor components (adapted from LAZCKA; CAMPO; MUÑOZ, 2007)

2.1 Analytes and Samples

Prior to establishing any sensor or biosensor system, it is vital to settle the composition of the sample itself, needed for analysis, as well as its target analyte. Analytes are the component of interest within a given sample, such as specific identities, concentrations or properties. Samples, on the contrary, represent a larger combination of environmental elements (air, water, soil and vegetation) and crops, along with biological fluids (blood, urine, saliva, etc), chemicals and cellular component. Upon the analyte's definition, it is possible to determinate all further requirements for the sensing elements and transducing components.

2.2 Sensing Elements

All elements that are able to react with the targeted analyte and detect its necessary physical parameter or chemical substance are designated as sensing elements. They can be classified into receptors (in the case of typical sensors) and bioreceptors (for biosensors).

2.2.1 Receptors

The receptor is a sensing component that recognizes specific targeted elements (technically referenced as analyte). This element recognition is essential for the designing of sensor technologies. They are divided according to (HULANICKI; GLAB; INGMAN, 1991).

2.2.1.1 Optical Receptors

Sensing energy in the form of light (i.e. photons) and its wave (amplitude, phase, polarization and spectrum), light spectrum, wave velocity, refractive index, emissivity, reflectivity and absorption.

2.2.1.2 Electrochemical Receptors

These receptors are the ones that respond directly to the presence of electrochemical interaction within an analyte. Such chemical reactions may be electrically stimulated or may resulted from a spontaneous interaction without electric current interference.

2.2.1.3 Electrical Receptors

All structures that detect electrical stimuli, as charge, current, potential, voltage, conductivity, electric and magnetic fields (amplitude, phase, polarization and spectrum) caused

by the interaction with a specific analyte. In this type of receptor, no chemical or eletrochemical reaction occurs.

2.2.1.4 Mass-Sensitivity Receptors

Receptors transforming mass changes (caused by accumulation of the analyte at specially modified surfaces) into property changes in a support material.

2.2.1.5 Magnetic Receptors

These receptors are based on the sensing of changes in magnetic properties.

2.2.1.6 Mechanical Receptors

Detecting mechanical forces, such as linear or angular positions, acceleration, force, stress, pressure, strain, mass, density, moment, torque, shape, roughness and orientation.

2.2.1.7 Thermometric Receptors

All structures capable to perceive changes in temperature, flux, specific heat and thermal conductivity.

2.2.2 Bioreceptors

Bioreceptors are molecular structures that use a biochemical mechanism for signal recognition. They are responsible for connecting the sample (analyte) to the sensor (transducer) for proper measurement. VELUSAMY et al. (2010) classifies bioreceptors into five different major categories, including antibody/antigen, enzymes, nucleic acids/DNA, cellular structures/cells, biomimetic and bacteriophage (phage). The enzymes, antibodies and nucleic acids are considered the main classes of bioreceptors, which are widely used in biosensor applications. Although enzymes figures among the biorecognition elements, they are mostly employed to function as labels than actual bioreceptors.

2.2.2.1 Antibody Bioreceptors

Antibodies are common bioreceptors used in biosensors. Antibodies may be polyclonal, monoclonal or recombinant, depending on their selective properties and the way they are synthesized. In any case, they are generally immobilized on a substrate, which can be the detector surface, its vicinity, or a carrier (LAZCKA; CAMPO; MUÑOZ, 2007).

The way in which an antigen and an antigen-specific antibody interact is similar to a lock-and-key fit (VO-DINH, 2008). An antigen-specific antibody fits its unique antigen in a highly specific manner, so that the three-dimensional structures of antigen and antibody molecules are matching. Due to this three-dimensional shape fitting, and the diversity inherent in individual antibody make-up, it is possible to find an antibody that can recognize and bind to any one of a large variety of molecular shapes.

This unique property of antibodies is the key that makes the immunosensors a powerful analytical tool and their ability to recognize molecular structures allows one to develop antibodies that bind specifically to chemicals, biomolecules, microorganisms, etc. One can then use such antibodies as specific probes to recognize and bind to an analyte of interest that is present, even in extremely small amounts, within a large number of other chemical substances (VELUSAMY et al., 2010).

2.2.2.2 Enzyme Bioreceptors

Enzymes are also largely employed for biorecognition, due to its robustness. They are chosen based on its specific binding capability and catalytic activity. The used enzyme (with a suitable substrate) shall provide enough electron transfer to the electrode or transducer (VO-DINH, 2008). Enzymes offer the advantages of high sensitivity, possibility of direct visualization and are stable for years. But there are some disadvantages found when using enzymes as labels, which include multiple assay steps and the possibility of interference from endogenous enzymes. Using enzymes as labels offers several advantages over fluorescently labeled and radiolabeled substances (VELUSAMY et al., 2010).

2.2.2.3 Nucleic Acid Bioreceptors

Recent advances in nucleic acid recognition have enhanced the power of DNA (deoxyribonucleic acid) biosensors and biochips. In the case of nucleic acid bioreceptors for pathogen detection, the identification of a target analyte's nucleic acid is achieved by matching the complementary base pairs that are often the genetic components of an organism. Since each organism has unique DNA sequences, any self-replicating microorganism can be easily identified (WONG; LEWIS, 2017).

Biosensors based on nucleic acid as biorecognition element are simple, rapid, and inexpensive and hence it is widely used in pathogen detection. In contrast to enzyme or antibodies bioreceptors, nucleic acid recognition layers can be readily synthesized and regenerated. DNA

damage is one of the most important factors to be considered when nucleic acid bioreceptor are used. Hundreds of compounds bind and interact with DNA. Detection of chemicals may cause irreversible damage to DNA by changing the structure of DNA and the base sequence, which in turn disturbs the DNA replication (WONG; LEWIS, 2017).

2.2.2.4 Cellular Bioreceptors

In cellular structures/cells based bioreceptors biorecognition is either based on whole cell/microorganism or a specific cellular component that is capable of specific binding to certain species (WONG; LEWIS, 2017).

2.2.2.5 Aptamer Receptors

Aptamers are molecules (consisting usually from short strands of oligonucleotides or peptides) that are able bind to a specific target molecule. Compared to traditional antibodies, aptamers present many technical advantages as a type of sensing element. Besides being small, chemically stable and present low cost, they offer exceptional flexibility concerning its structural design, which represent higher sensitivity and selectivity. Moreover, the aptamer combination alongside with nanomaterials has push forward its overall performance.

2.2.2.6 Biomimetic Receptors

A receptor that is fabricated and designed to mimic a bioreceptor (antibody, enzyme, cell or nucleic acids) is often termed a biomimetic receptor. Though there are several methods, such as genetically engineered molecules and artificial membrane fabrication, the molecular imprinting technique has emerged as an attractive and highly accepted tool for the development of artificial recognition agents (VELUSAMY et al., 2010).

2.2.2.7 Bacteriophages

Bacteriophages have been employed as biorecognition elements for the identification of various pathogenic microorganisms. These powerful bacteriophages (phages) are viruses that bind to specific receptors on the bacterial surface in order to inject their genetic material inside the bacteria (WONG; LEWIS, 2017). These entities are typically of 20–200 nm in size (SINGH et al., 2009). Phages recognize the bacterial receptors through its tail spike proteins. Since the recognition is highly specific, it can be used for the typing of bacteria and hence opened the path for the development of specific pathogen detection technologies (VELUSAMY et al., 2010).

2.2.3 Immobilization Strategies (Biosensors Only)

Specifically considering applications in biosensors, it is usually necessary to add intermediary steps in order to enhance bioreceptors operations. In other words, an immobilization strategy must be defined for the proper performance of the biological recognition element. It is possible to list three main classes of bioreceptors that are likely to be used along a immobilization strategy: nucleic acids, enzymes and antibodies.

Enzymes can be used to label either antibodies or DNA probes much in the same fashion as in an ELISA assay. In the detection of pathogenic bacteria, however, enzymes tend to function as labels rather than actual bacterial recognition elements. Rather than DNA probe-based biosensors, antibody-operating devices have currently been used more often. Antibodies may be polyclonal, monoclonal or recombinant, depending on their selective properties and the way they are synthesized (LAZCKA; CAMPO; MUÑOZ, 2007).

This section addresses the three major immobilization strategies, all using gold substrates due to its importance in the area of immunosensors and DNA probes (which is considered a key element of most bacterial biosensors).

2.2.3.1 The Advin-Biotin System

This method relies on the anchoring of biomolecules to a surface coated with a specific biotin-binding protein: avidin. It is considered a simple but very effective system. One of the most advantageous features of this system is that, although the affinity constant between avidin and biotin is rather high (ca. 10−15 mol^−1 L), the bonding allows multiple washing and re-use of the same sensing device (TOMBELLI et al., 2000). However, the high cost of all reagents involved in this technique has to be taken into consideration.

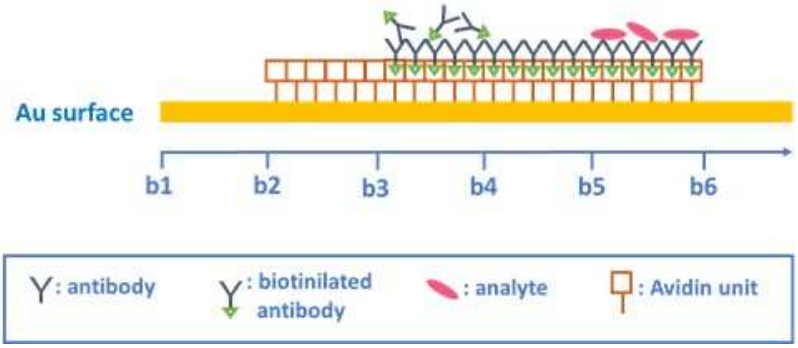

Figure 8: Schematic representation (cross-section) of the advin-biotin system (adapted from LAZCKA; CAMPO; MUÑOZ, 2007). b1) clean surface b2) avidin coating; b3) addition of biotinylated antibody; b4) wash step; b5) sample addition and b6) sample detection

2.2.3.2 Adsorption on Gold

Adsorption on gold consists of in the attachment of the antibodies on a specific gold substrate. As these attachments are randomly created, the exact orientation of the binding sites cannot be defined or controlled. It is considered the simplest and quickest method (among biological recognition elements and immobilization strategies), although having the least reliability index. Fig. 8 outlines the basic steps of this technique.

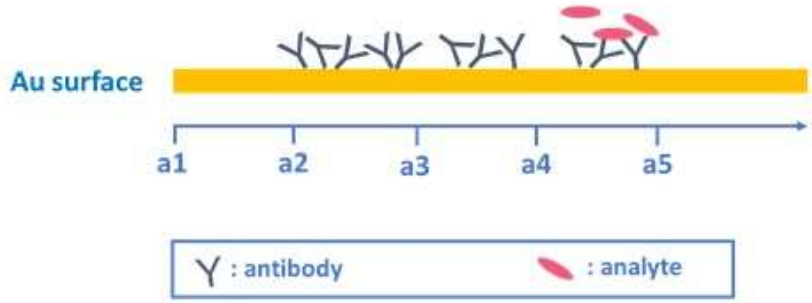

Figure 9: Schematic representation (cross-section) of adsorption on gold (adapted from LAZCKA; CAMPO; MUÑOZ, 2007). a1) clean surface a2) immersion in antibody solution; a3) wash step; a4) sample addition and a5) sample detection

2.2.3.3 SAMs

Self-Assembled Monolayer processes (or simply SAMs) consists in the immersion of gold plates into specific solutions (normally formed by a suitable surfactant in a high purity solvent. The immersion of gold in an ethanol solution (containing disulphides or thiols) is the most common SAM type. Dictating the dimensions of the recent formed monolayer, is the radical attached to the sulphide atom(s). Alkanethiols form the most important group of SAM-formation compounds. Immediately hereafter, a pre-determined bio-molecule is linked to the other end of the thiol. Familiarity with the biomolecule is needed in order to achieve the optimum orientation and enhance biosensor performance. Depending on this, different forms of chemical modification and activation are required (LAZCKA; CAMPO; MUÑOZ, 2007).

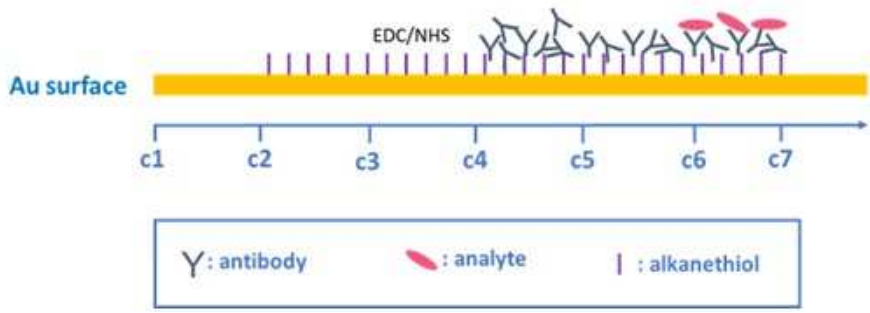

Figure 10: Schematic representation (cross-section) of SAM system (adapted from LAZCKA; CAMPO; MUÑOZ, 2007)

Considering the robustness of SAMs-based immunosensing devices, this technique has a wide range of different sorts of applications.

2.3 Transduction Platforms

Transduction platforms are constituted basically of electronic transducers, which can be technically described as electronical devices that converts signals from one form of energy into another. This process is known as transduction. That is to say, such components may apply different sorts of signals in order to convert them into specific ones, for experimental purposes. This typical behavior, combined with receptors and bioreceptors, enables the measurement of technical properties within analytes (samples), as well as the conversion of its signals into proper analysis forms.

The discovery of the first transducing materials and properties goes back to the 1800's, although it was only in the past century (particularly after 1948, with the advent of the transistor technology) that this technology really thrived. Considered key elements in all sensor and biosensor detection systems, new transducer systems and methods continues to be developed in present days.

Transducers are classified according to many variables, such as their application, method of energy conversion, nature of the output signal, etc. DE MARCELLIS; FERRI, (2011) categorize transducer can be classified as, Primary, Secondary, Analog, Digital, Electrical, Mechanical, Active and Passive, as described in Fig. 11.

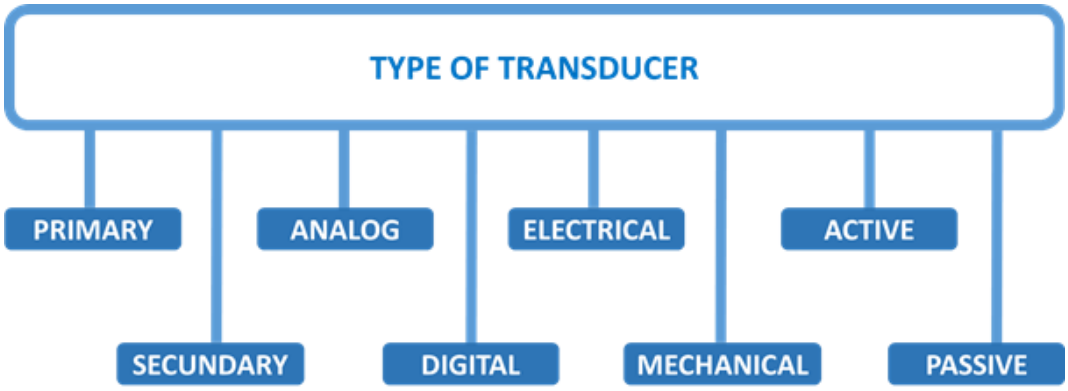

Figure 11: Transducers classification based upon its transducing method

The primary transducer is also known as detector or sensor. It senses a physical parameter such as pressure, humidity, temperature, etc and converts it into suitable physical parameter which is readable. The secondary transducer converts the output of primary transducer into electrical signal. The analog transducer converts the input signal into an analog output which is continuous function of time. The digital transducer converts the input signal into an electrical output which is in the form of pulses. Electrical transducers are the ones which sense the physical parameter, converting it into electrical signals. The mechanical transducer is based on the

conversion of one form of physical quantity into another. Active transducers are also called as self-generating transducers. It is that type of transducer which does not require any external (auxiliary) power supply to produce output. Passive transducer is also known as externally powered transducer. It is the type of transducer that requires an auxiliary power supply to produce output.

Based on these functionalities mentioned above, it is possible to divide all transduction platforms into 4 main categories: Chemical, Electrochemical, Optical and Mass-Sensitivity.

2.3.1 Chemical

This transduction technique relies on the transformation of chemical information (like concentration of a specific component within a sample) into an analytic useful signal. All chemical transducing elements are based on an analyzer that responds to a particular analyte in a selective and reversible way and transforms input chemical quantity, ranging from the concentration of a specific sample component to a total composition analysis, into an analytically electrical signal. Chemical transduction is just the primary link of the measuring chain, i.e. an interface between the chemical analyte and the electronics needed for signal processing.

Some typical properties associated with chemical transduction platforms are:

- Chemical contact between sensitive layer and the analyte;
- The sensitive layer is on a platform that allows transduction of the change to electric signals;
- After exposure to the analyte, the sensitive layer suffers a change in its chemistry (reaction);

2.3.1.1 Gas Detection

Among most known techniques, it is possible to emphasize Gas Chromatography (GC) as an analytical method based on the vaporization and decomposition of different compounds. In plant disease or pathogen detection, it typically involves the separation and profiling of volatile chemical substances from infected plants. The pathogen infections of plants can result in the release of specific volatile organic compounds (VOCs) which represent highly indicative signals of the type of stress experienced by plants. As VOCs are produced when green leaf plants are damaged pathogenically and even mechanically, they can be analyzed using GC technique to detect the presence of the specific VOC as an indicative of a particular disease. Thus, after identification, such substances can be later cross-related with its respective pathogen or disease. (JANSEN et al., 2015)

To enhance the performance of compound separation and analysis, the gas chromatography is often combined with mass spectrometry (GC-MS) to identify unknown compounds in the volatile sample (PERERA; MARRIOTT; GALBALLY, 2002). The GC can provide accurate information about plant diseases due to its wide range of data collected from the VOC sample. However, unlike the imaging system (which can directly obtain the data on-field), GC requires sampling of pre-collected VOC for a longer time before data analysis, which severely limits its on-field application(FANG; RAMASAMY, 2015).

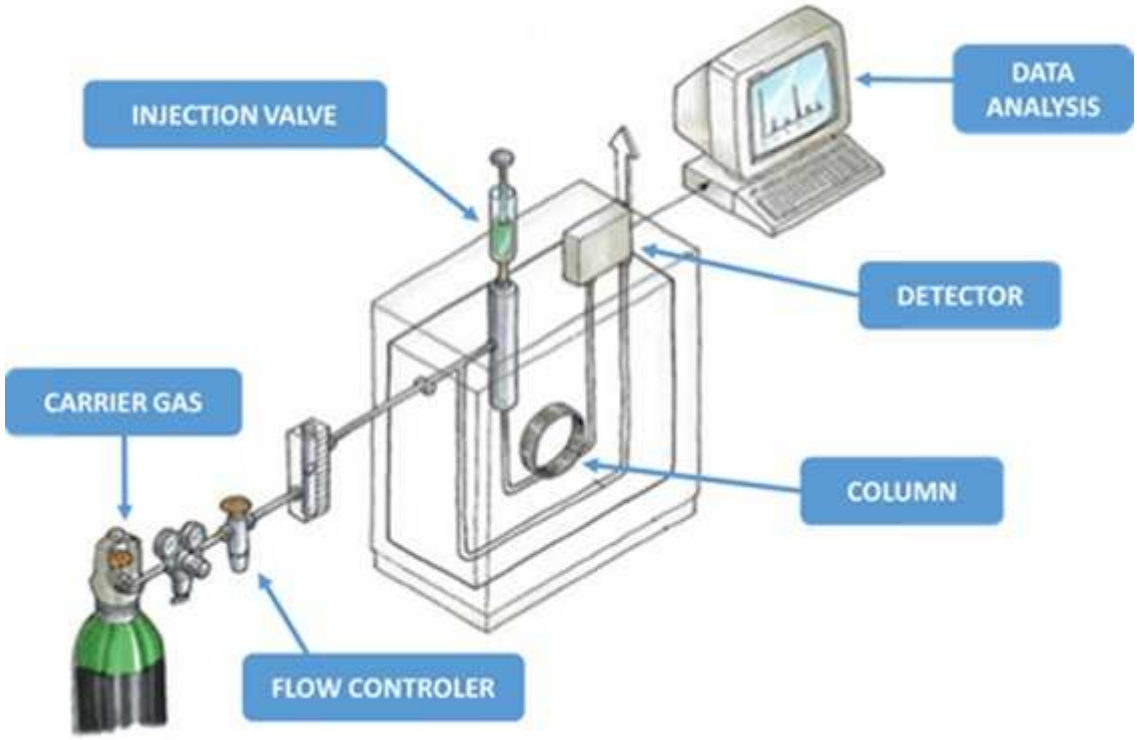

Figure 12: Gas Chromatography (GC) scheme showing each individual component used in the technique and the standard operational setup

2.3.1.2 Liquid Detection

Analogous to GC, Liquid Chromatography (LC) is a technique used to separate, identify, and quantify each component within a sample. The separation occurs based on the interactions of the sample with the mobile and stationary phases. Typically, in this method the sample mixture is dissolved among a pressurized liquid solvent, before been pumped and passed through a column filled with a solid adsorbent material. Components within the mixture are separated in the column based on each's affinity for the mobile phase. Also, each component in the sample interacts slightly differently with the adsorbent material, causing different flow rates for each component and leading to the separation of the components as they flow out the column.

Operating in a significantly higher pressures (between 50–350 bar), High-Performance Liquid Chromatography (HPLC) has also higher sensitivity for distinguishing compounds throughout the mixture separation, in comparison to standard LC techniques, which relies on on the force of gravity.

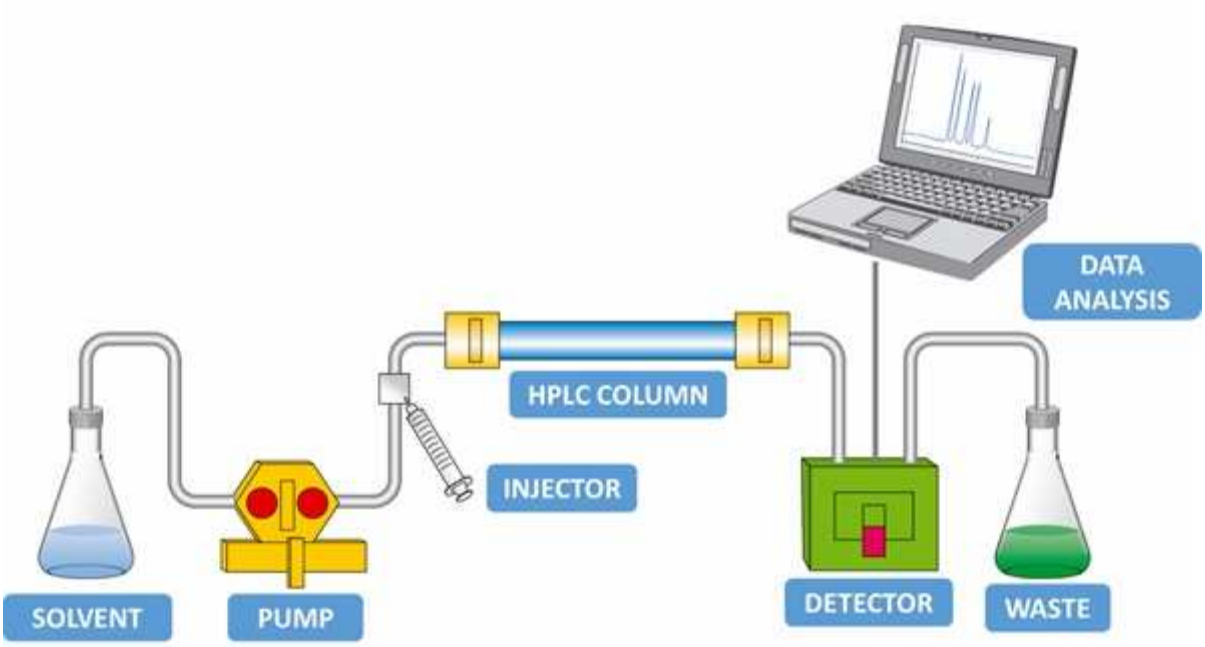

Figure 13: High-Performance Liquid Chromatography (HPLC) scheme showing each individual component used in the technique and the standard operational setup

2.3.2 Electrochemical

Electrochemical based detection methods are another possible mean of transduction that has been commonly used for identification and quantification of foodborne pathogens. Electrochemical biosensors can be classified into amperometric, potentiometric, impedimetric and conductometric, based on the observed parameters such as current, potential, impedance and conductance respectively. Although the electrochemical detection has several advantages like low cost, ability to work with turbid samples and easy miniaturization, their sensitivity and selectivity are slightly limited when compared to optical detection (VELUSAMY et al., 2010).

Some typical properties associated with electrochemical transduction platforms are:

- Its physically small size;
- Its real time operation;
- Its associated low cost (typically less expensive and more convenient than an equivalent instrument for the same electrochemical measurements).

2.3.2.1 Amperometric Methods

Besides been the most common electrochemical detection method used in sensors and biosensors, the amperometric technique works on the grounds of an existing linear relationship between analyte concentration and current. It has been used for pathogen detection, having a superior sensitivity than potentiometic method. In amperometric-based detection the sensor potential is set at a value where the analyte produces current. Thus, the applied potential serves as the driving force for the electron transfer reaction, and the current produced is a direct measure of the rate of electron transfer (VELUSAMY et al., 2010).

Technically it relies on the production of an electrical current when a potential is applied between two electrodes. The sensor potential is set at a value where the analyte, directly or indirectly, produces a current at the electrode. In the case of biosensors, where direct electron exchange between the electrode and either the analyte or the biomolecule is not permitted, redox mediators are required (LAZCKA; CAMPO; MUÑOZ, 2007).

Considered as an amperometric subclass, voltammetric techniques are based on the measurement of current as the potential is varied, in order to obtain information about a specific analyte. The analytical data for a voltammetric experiment comes in the form of a graphic which plots the current produced by the analyte versus the potential of the working electrode.

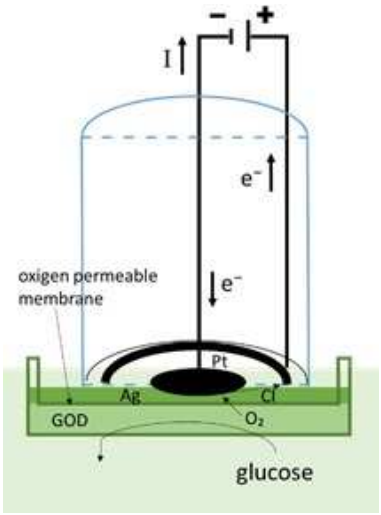

Figure 14: Schematic representation of an amperometric detection system

As an example of amperometric-based sensor, the metal oxide gas sensing system is considered one of the most important electrochemical applications. With similarities to the chemical GC (gas chromatography) methods, it is based on the measurement of conductivity changes of VOCs within a gas-sensing material. They have attracted much attention due to their low cost and flexibility in production; simplicity of their use; large number of detectable gases/possible application fields (WANG et al., 2010)

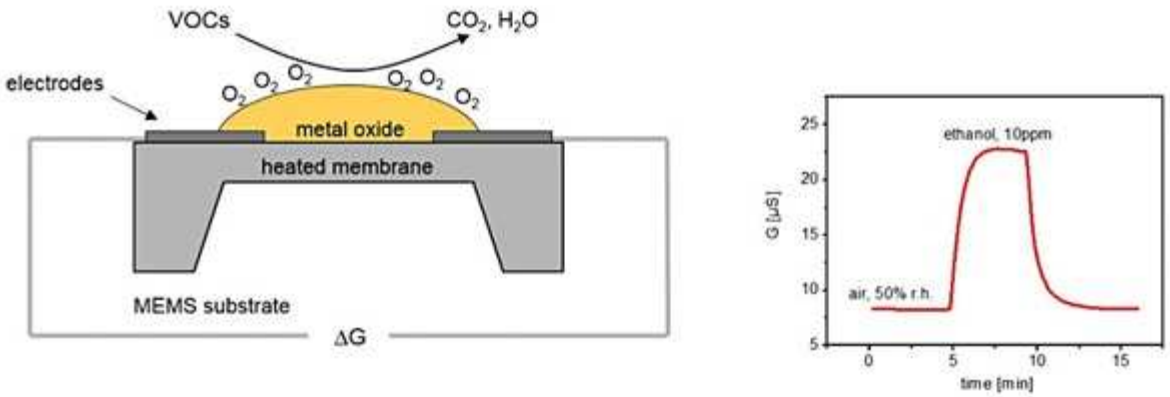

Figure 15: Schematic representation of a standard metal oxide transduction system (adapted from WANG et al., 2010)

2.3.2.2 Potentiometric Methods

In potentiometric-based detection the bio-recognition process is converted into a potential signal. Usually a high impedance voltmeter is used to measure the electrical potential difference (voltage) or electromotive force (EMF) between two electrodes at near zero current. Since potentiometry generates a logarithmic concentration response, the technique allows the detection of extremely small concentration changes. Not many potentiometric biosensors were found for the detection of pathogens (VELUSAMY et al., 2010). As an example of potentiometric detection, it is possible to list electrolyte gas sensors, which are used mainly for CO and other hydrocarbons detection.

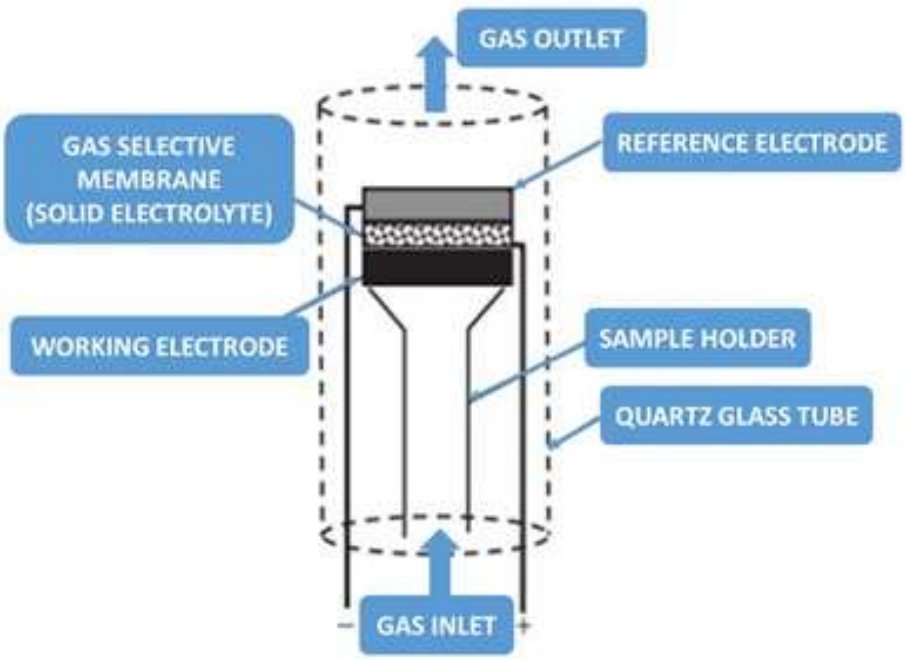

Figure 16: Schematic representation of an electrolyte gas transduction system

2.3.2.3 Impedimetric Methods

Impedimetric transduction techniques have been used to detect and quantify a vast variety of foodborne pathogens. Basically, it relies on the integration of electrical impedance with biological recognition technology. Within this method, Electrochemical Impedance Spectroscopy (EIS) represents a powerful tool for the study of conducting materials and interfaces. EIS is playing an important role in the biosensor development as it has high sensitivity and easy setup. This technique measures the electrochemical impedance response of an electrochemical system (cell) to an applied potential, and the frequency dependence of this impedance can reveal underlying chemical processes.

In EIS measurements, a controlled AC electrical stimulus of between 5 and 10 mV is applied over a range of frequencies, and this causes a current to flow through the biosensor, depending on different processes. EIS is a widely used technique for probing bioaffinity interactions at the surfaces of electrically conducting polymers and can be employed to investigate 'labelfree' detection of analytes via impedimetric transduction. Though, EIS offers label-free detection compared to amperometry or potentiometry (VELUSAMY et al., 2010).

Since the 1990s, impedance methods have been used for bacterial identification. These methods record the changes in the sensor electrical impedance induced by bacterial metabolism and cell growth as a result of the release of ionic metabolites from the living cells (carbon dioxide and organic acids produced by catabolism and ion exchange through the cell membrane) (POLTRONIERI et al., 2014).

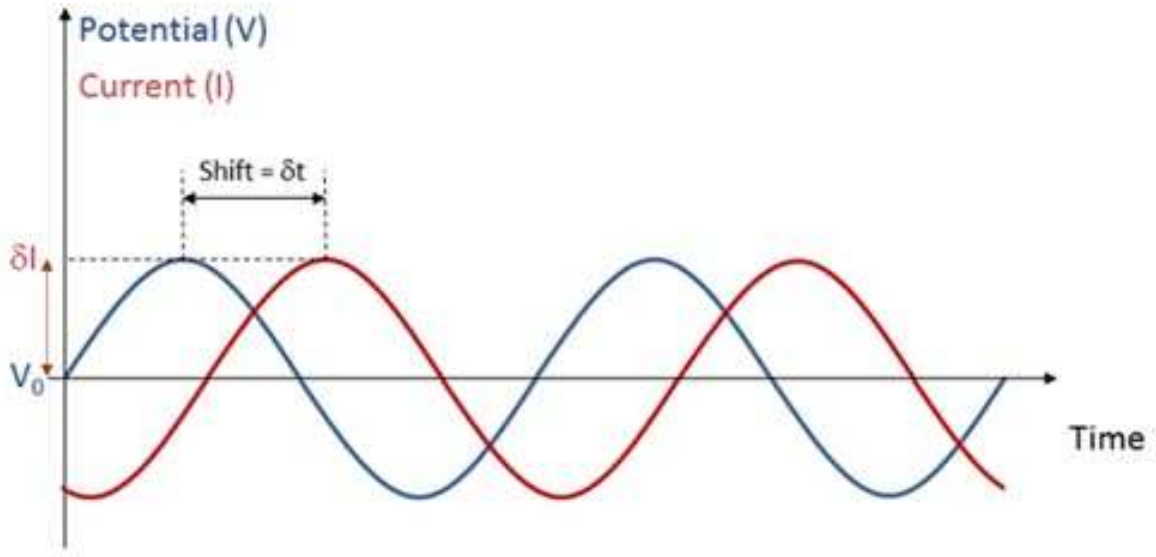

Figure 17: Example of oscillating perturbation in cell voltage, providing an oscillating current response (adapted from CHEUNG et al., 2010)

2.3.2.4 Conductometric Methods

Conductometric-based systems bond the relationship between conductance and a bio-recognition technique. Most reactions involve a change in the ionic species concentration, which leads to a change in electrical conductivity or current flow. Normally, a conductometric biosensor consists of two metal electrodes separated by a certain distance and an AC voltage applied across the electrodes causes a current flow. During a bio-recognition event the ionic composition changes and the change in conductance between the metal electrodes are measured (VELUSAMY et al., 2010).

2.3.3 Optical

Optical transducers in sensor and biosensors are applied to measure the responses to illumination or to light emission. Optical biosensors offer advantages in terms of miniaturization, low cost, disposability and no electrical interference. Because fiber sensors are made of glass, they are environmentally rugged, and can tolerate high temperatures, vibrations, shock, and other harsh conditions. They are also seen as relatively safe and biocompatible for use within the human body (MEHRVAR, 2010).

Been one of the most popular technologies, optical-based sensors and biosensors are currently considered a key technology for pathogen detection (in comparison to conventional analytical methods). This is especially due to the advantages of the optical transducer, in high measurement selectivity and sensitivity, as well as its small size and low-cost relation (typically with biodegradable electrodes). This class is described as a compact analytical device containing a sensing element integrated (or connected) to an optical transducer system.

Biosensor detection typically relies on an enzyme system, which catalytically converts analytes into products that can be oxidized or reduced at a working electrode and maintained at a specific potential. Optical sensors and biosensor techniques are categorized into many types, usually based on absorption, reflection, refraction, infrared, Raman, chemiluminescence, dispersion, fluorescence, and phosphorescence (ZHAO et al., 2014). However, all the above subclasses require a suitable spectrometer to record the spectrochemical properties of the analyte. The most commonly employed techniques of optical detection are surface plasmon resonance and fluorescence due to their sensitivity. Optical techniques using fiber optics, laser, prism and waveguides are also employed for pathogen detection (VELUSAMY et al., 2010).

2.3.3.1 Luminescence Detection

Luminescence-based techniques for the detection of microbial pathogens have been extensively applied in industrial operations, where the continuous monitoring of pathogen (bacterial or virus) contamination is essential for food safety. The primary advantage of all luminescence-based assays is its sensitivity and low time-consuming. In this section, we describe three main sub-classes of luminescence detection systems that have been already adapted for commercial use: Bioluminescence (BL); Chemiluminescence (CL) and Electro-induced Luminescence (EL)

Bioluminescence (BL) is a naturally occurring process by which living organisms convert chemical energy into light. Light-emitting pathways have been identified in bacteria, insects, and other eukaryotic organisms. Chemiluminescence (CL) is generally defined as the production of light by chemicals during an exothermic reaction, and CL differs from BL in that light production is not catalyzed by biological reactions. Although not as widely used in industrial applications, CL is sometimes preferred to BL-based detection systems due to the relative simplicity of the reaction and the elimination of certain steps sometimes required for the optimization of BL. CL has been used mainly for the detection of foodborne pathogens in combination with immunoassays (FARRIS; HABTESELASSIE; PERRY, 2008). Electro-induced Luminescence (EL) is an optical and electrical phenomenon in which a substance emits light in response to the passage of a strong electric field or an electric current, artificially induced.

2.3.3.2 Fluorescence Detection

Similarly to the luminescence imaging concepts described in the previous section, fluorescence detection in sensors and biosensors occurs when a valence electron is excited from its ground state to an excited singlet state. The excitation is produced by the absorption of light of sufficient energy. When the electron returns to its original ground state it emits a photon at lower energy. Another important feature of fluorescence is the little thermal loss and rapid light emission taking place after absorption.

Specifically in phytopathology, changes in the chlorophyll fluorescence (among leaf cells) can be related to pathogen disease and infections. Based on this, the Fluorescence Imaging technique uses optical microscopes to apply specific incident lights in the sample. The changes observed in the fluorescence parameters can be used to analyze possible pathogen infections. This phenomenon is due to the cell's photosynthetic apparatus and photosynthetic electron transport reactions (KUCKENBERG; TARTACHNYK; NOGA, 2009).

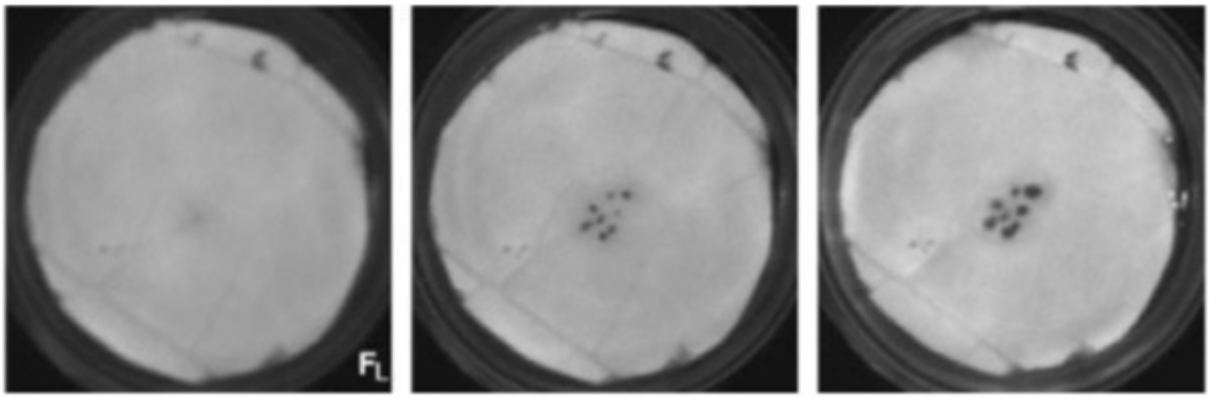

Figure 18: Tobacco mosaic virus (TMV) lesion development in tobacco leaf disc floating on water through chlorophyll fluorescence time-lapse imaging. Inoculation images at three time-points (30 h, 2 and 3 d) after TMV infection. (adapted from CHAERLE et al., 2004)

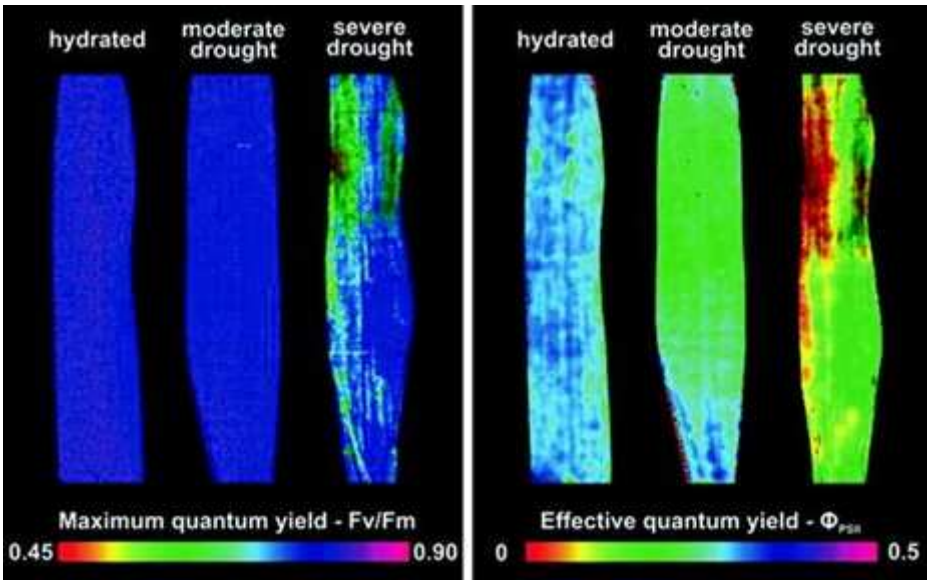

Figure 19: The chlorophyll fluorescence imaging screens of well-hydrated, moderately and severely drought-stressed wheat leaves. The figures shows a two-dimensional distribution (with recorded values) of two different light intensities (adapted from BRESTIC; ZIVCAK, 2013)

Förster resonance energy transfer (or simply FRET) sensors are based on the transfer of energy from a donor fluorophore to an acceptor fluorophore. It is able to report whether a food sample contains salmonella down to a detection limit of 2 gmL−1. Fluorescence detection, in contrast to SPR, is also used in combination with established techniques such as PCR and ELISA (LAZCKA; CAMPO; MUÑOZ, 2007).

Although fluorescence measurement provides sensitive detection of abnormalities in photosynthesis, the practical application of this technique in a field setting is limited due to portability and cost issues.

2.3.3.3 Colorimetric Thermography

Colorimetric sensors and biosensors make use of the changes in the color of a special compound to determine the concentration of the target analytes. This technique consists in measuring (through colorimetric method) a chromophoric product called p-nitrophenol (PNP), which is hydrolyzed by bacterium using methyl parathion. Based on this mechanism, colorimetric transducers have been widely used in developing microbial biosensors for the detection of methyl parathion.

Thermography is a technique based on imaging the differences in surface temperature. In the case of pathogens detection, it allows the comparative analysis from the color spectrum differences of plant leaves and canopies. This is made possible through the use of infrared radiation, which is emitted and later captured by thermographic cameras. Previous reports have demonstrated that the loss of water in plants (regulated by stomata) would be affected by phytopathogens, as changes in plants temperature are a direct consequence from pathogen infection. Therefore, the resulting disease can be monitored through thermographic imaging and the amount of water transpired can be determined to assess potential pathogen related diseases among plants, without the external temperature influences (LENTHE; OERKE; DEHNE, 2007).

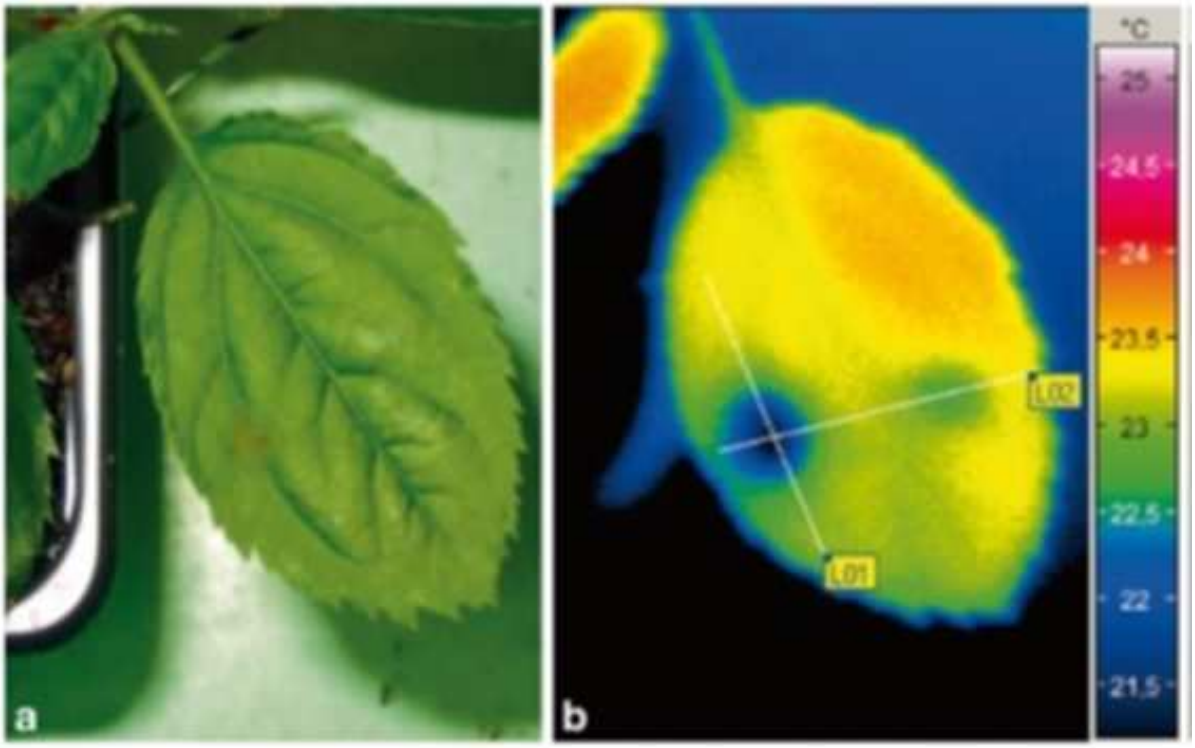

Figure 20: Effect of developing scab lesions on spatial heterogeneity in leaf temperature of apple leaves caused by Venturia inaequalis. First thermal effects became detectable 6 days after inoculation: A) Leaf overview and B) Thermogram with transects (MAHLEIN et al., 2012)

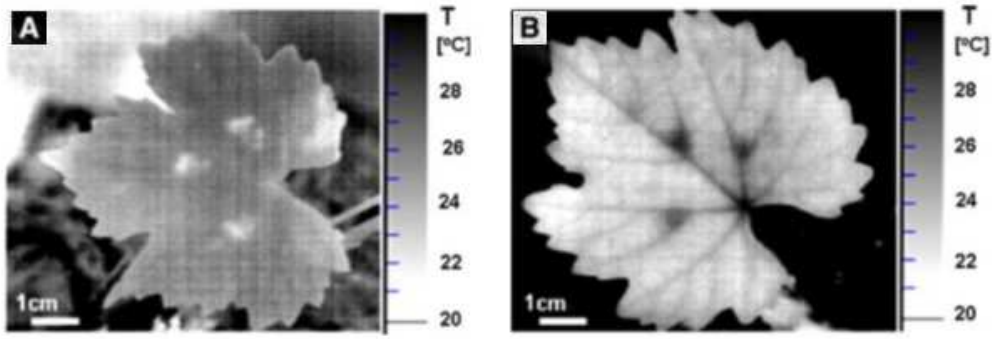

Figure 21: Thermal image comparison from Vitis vinifera L. cv. Riesling leaf presented in grey scale. A) Non- inoculated leaf and B) Inoculated leaf (on day 4 after inoculation with three drops of Plasmopara viticola) (adapted from STOLL et al., 2008)

Thermography is also a promising tool to monitor the heterogeneity in the infection of soilborne pathogens. However, the practical applicability of thermography for disease monitoring is limited due to its high sensitivity to the change of environmental conditions during measurements. Additionally, thermographic detection lacks the specificity towards diseases, and therefore cannot be used to identify the type of infection or distinguish between diseases that produce similar thermographic patterns (STOLL et al., 2008).

2.3.3.4 Hyperspectral Techniques

Hyperspectral techniques are an image-based method that can be used to obtain useful information about the plant health over a wide range of spectrum between 350 and 2500 nm. Hyperspectral imaging is increasingly being used for plant phenotyping and crop disease identification in large scale agriculture. The technique is highly robust and it provides a rapid analysis of the imaging data. Furthermore, hyperspectral imaging cameras facilitate the data collection in three dimension, with X- and Y- axes for spatial and Z- for spectral, which contributes to more detailed and accurate information about plant health across a large geographic area (MAHLEIN et al., 2012).

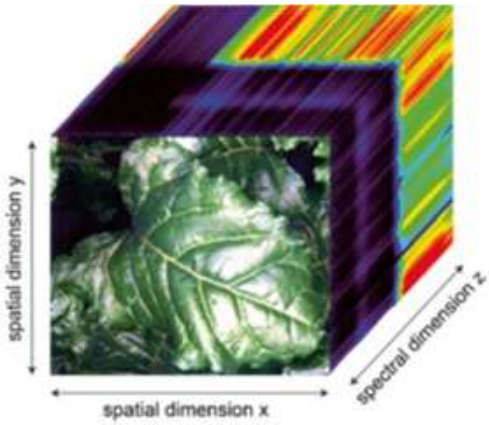

Figure 22: Structure of hyperspectral image data cube of sugar beet leaf with spatial dimensions X and Y and spectral dimension Z displaying the continuous color spectrum for each pixel of the image (MAHLEIN et al., 2012).

Hyperspectral imaging have been widely used for plant disease detection by measuring the changes in reflectance resulting from the biophysical and biochemical characteristic changes upon infection. Magnaporthe grisea infection of rice, Phytophthora infestans infection of tomato and Venturia inaequalis infection of apple trees have been already identified and reported using hyperspectral imaging techniques (FANG; RAMASAMY, 2015).

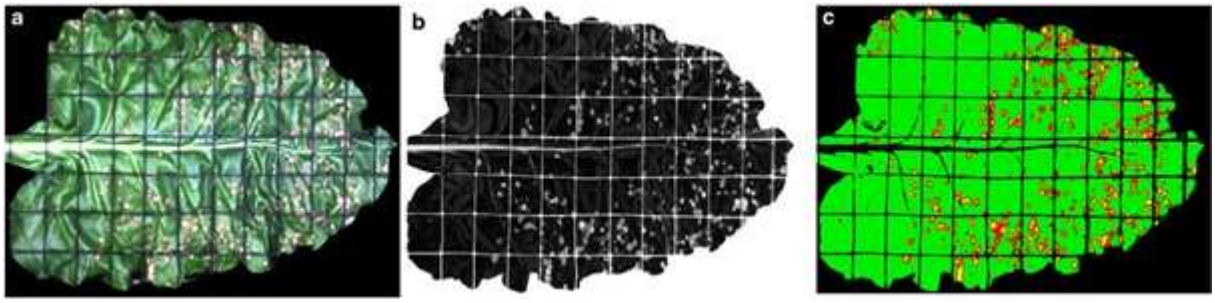

Figure 23: Hyperspectral classification of a sugar beet leaf after 14 days after inoculation with fungus Cercospora beticola Sacc.: A) Overview of sugar beet leaf, B) grey-scale rule image for healthy tissue (where dark pixel belong to the class 'healthy'), and C) colour image classification for 'healthy' (green), 'margin' (yellow), and 'necrotic' (red) (adapted from MAHLEIN et al., 2012)

2.3.3.5 Surface Plasmon Resonance (SPR)

Surface Plasmon Resonance (SPR) sensors and biosensors have been used in the direct and indirect detection of pathogenic microorganisms. This technique was largely studied in label-free immunosensors for the detection of bacteria. SPR can be used for the setup of immunosensors applied to the detection of food pathogens in enrichment broth, in liquids or in food dilutions. The SPR technique for biosensing allows real-time monitoring of chemical and bio-chemical interactions occurring at the interface between a thin gold film and a dielectric interface or transparent material, such as the liquid analyte (POLTRONIERI et al., 2014).

By using the evanescent wave phenomenon to measure changes in refractive index (very close to the sensor's surface), SPR is considered an optical technique. The evanescent wave produced by an incident, monochromatic light beam is able to interact with free electrons (plasmons) in the metal film at a special angle (α) of incident light (SPR angle). SPR applications are designed to measure changes in refractive index caused by structural alterations in the vicinity of a thin film metal surface. Its operating consists in a glass plate covered by a gold thin film is irradiated from the backside by p-polarised light (from a laser) via a hemispherical prism, and the reflectivity is measured as a function of the angle of incidence, θ. The resulting plot is a curve showing a narrow dip. This peak is known as the SPR minimum. (LAZCKA; CAMPO; MUÑOZ, 2007).

This technique's main drawbacks are its high investment (equipment cost) and large instrument size, as well as its complexity (as specialized staff is required). Fig. 24 schematically shows its operational system.

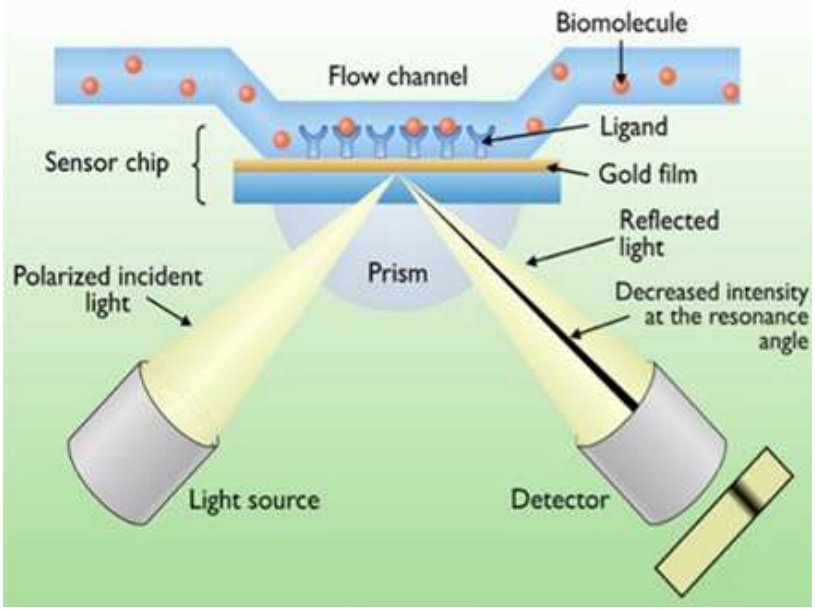

Figure 24: Schematic representation of a SPR biosensor operating system (adapted from POLTRONIERI et al., 2014)

2.3.3.6 Fiber Optic

Fiber optic transduction platforms basically correspond to fiber-based devices that use optical fibers to detect certain parameter such as temperature or mechanical strain, as well as concentrations of chemical substances, acceleration, rotations, pressure, vibrations and displacements. This technique is mainly used in remote sensing applications.

The fiber optic cable consists of a glass or plastic core surrounded by a layer made of cladding material. The difference in densities between the core and the layer enables the cables to act based on the total internal reflection principle, which states that the light striking a boundary between two components will be totally reflected without any loss in light energy. The reflected light is then transmitted to the sensor, which converts the light energy into electrical signals. Most of the fiber optic sensors are multiplexed along the length of a fiber by using light wavelength shift for each sensor or by determining the time delay as light passes along the fiber. A typical fiber optic transduction system consists of a fiber optic cable connected to an amplifier or a remote sensor.

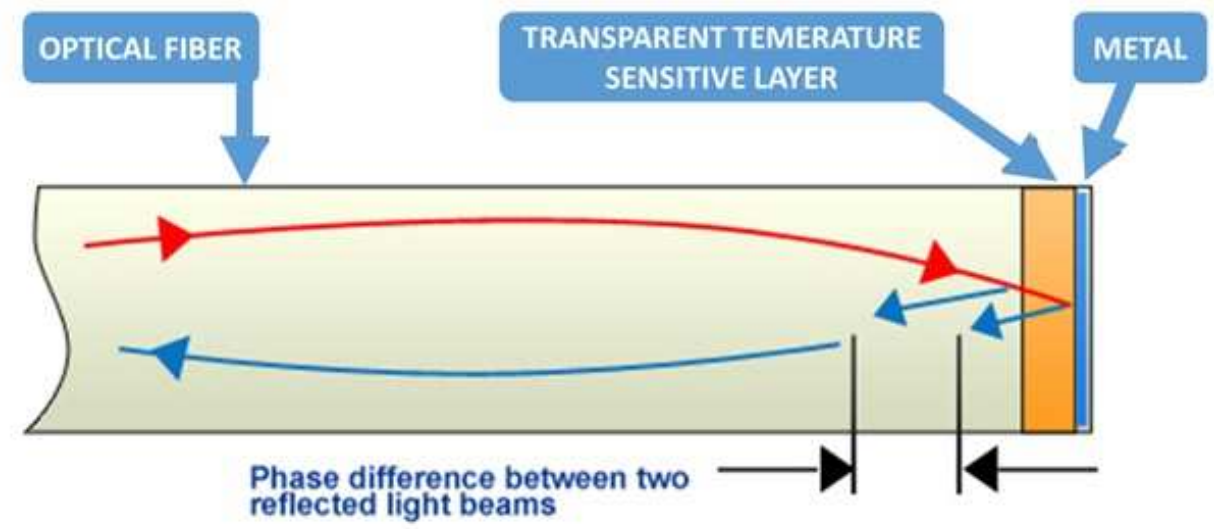

Figure 25: Schematic representation of a typical fiber optic temperature transduction system using phase interference

Fiber optic-based transduction systems are very light in weight and small in size. They can also endure in explosive environments and resist to high temperature. This is due to the existence of an electrically insulating material which also allows them for high voltages applications, without risks of electrical sparks. Additionally, fiber optic sensors are significantly resistant to electromagnetic frequency interference. They present a high sensitivity, with excellent range and resolution.

Propagation of light through a fiber or waveguide can be very sensitive to the surroundings, which makes the optical fibers excellent detectors for a variety of applications in foods such as identification and detection of pathogens. Also, it was highlighted that the detection limits of fiber optical sensors and biosensors are comparable to the sophisticated large bench-top instruments (VELUSAMY et al., 2010). With the further development of optical transducers, better electronics, and improved immobilization methods, fiber-optic biosensors will be increasingly applied to industrial processes, environmental monitoring, food processing, and clinical applications (MEHRVAR, 2010).

2.3.4 Mass-Sensitivity

Mass-sensitive transduction platforms are based on measurement of small changes in the analyte's mass. Thus, its sensors and biosensors are suitable for very sensitive detection.

2.3.4.1 Piezoelectric

Besides been an effective alternative technique to other type of sensors (such as surface plasmon resonance - SPR), piezoelectric biosensors operates by measuring resonance frequency changes on a quartz crystal microbalance (QCM), following mass changes on the probe/transducer surface. Typically, as piezo-electric crystals vibrate under the influence of an electric field, this type of biosensor is capable of sensitive detecting minimum amounts of analytes according to a linear relationship between deposited mass and its frequency response.

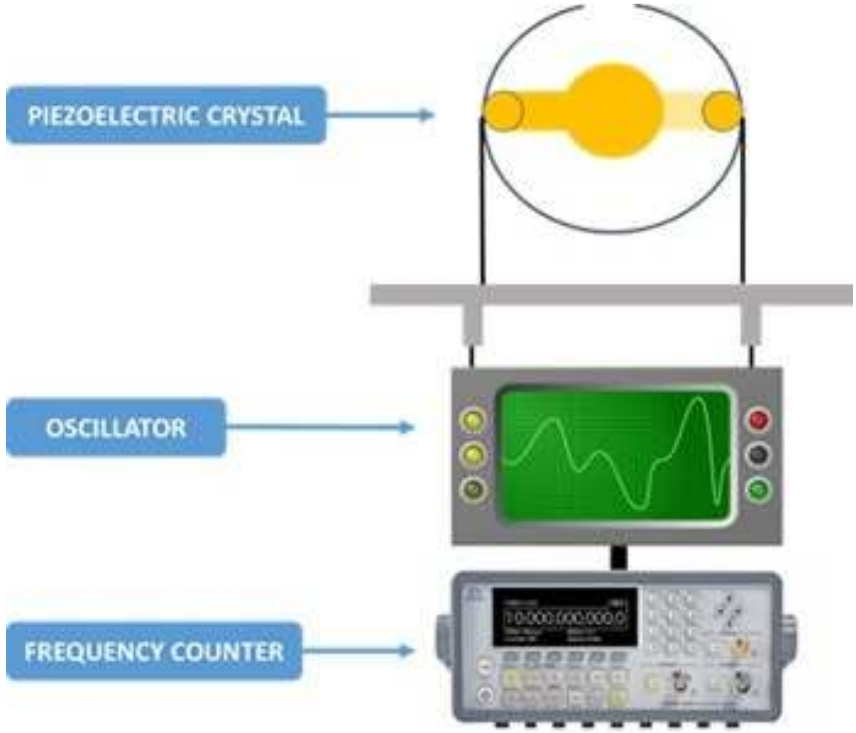

Figure 26: Schematic representation of piezoelectric biosensor system

The oscillation frequency depends on the crystal's thickness and cut, each one having a characteristic resonant frequency. This resonant frequency changes as molecules adsorb or desorb from the surface of the crystal. This frequency change is easily detected by relatively unsophisticated electronic circuits. The major drawback of these devices is the interference from atmospheric humidity and the difficulty in using them for the determination of material in solution. They are, however, inexpensive, small and capable of giving a rapid response.

2.3.4.2 Surface Acoustic Wave (SAW)

Surface Acoustic Wave (SAW) transduction systems are a class of microelectromechanical systems (MEMS), which senses physical phenomena through the modulation of surface acoustic waves. The sensor transduces an input electrical signal into a mechanical wave, which can be easily influenced by physical phenomena (unlike electrical signals). This wave is then transduced back into an electrical signal. Changes in frequency, amplitude, phase, or time-delay between the input and output electrical signals can be used to measure the presence of the desired phenomenon.

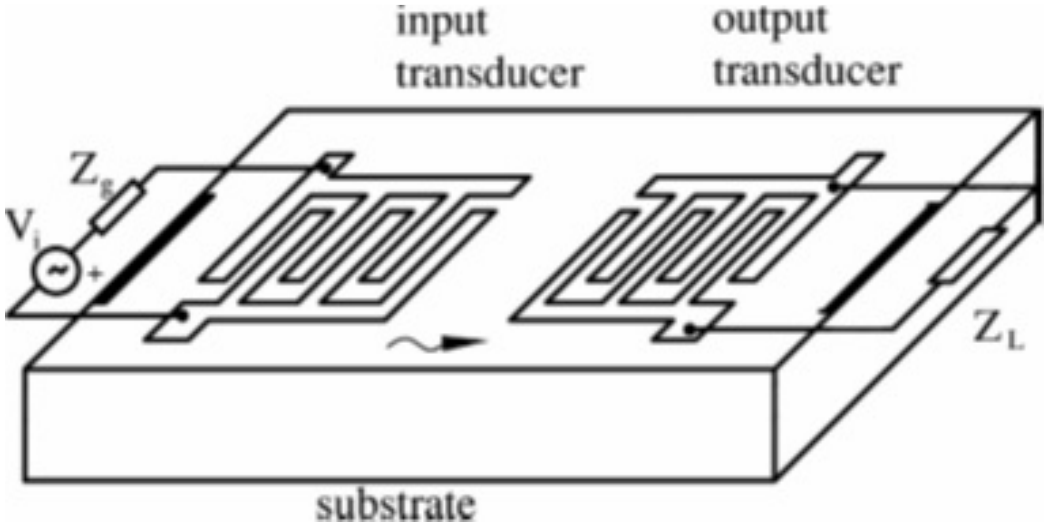

Figure 27: The basic structure of a SAW transduction device (HRIBŠEK; TOŠIĆ; RADOSAVLJEVIĆ, 2010).

Surface acoustic wave (SAW) devices have been widely used in different fields and will continue to be of great importance in the foreseeable future. These devices are compact, cost efficient, easy to fabricate, and have a high performance, among other advantages. SAW devices can work as filters, signal processing units, sensors and actuators. They can even work without batteries and operate under harsh environments. Its operating principles include temperature sensors, pressure sensors, humidity sensors and biosensors (LIU et al., 2016).

2.3.4.3 Micro-Cantilever

Micro-cantilever sensors have attracted substantial attention as a highly sensitive platform for chemical and biological detection due to their simplicity, sensitivity and their ability for label-free and real-time in situ monitoring. In the last two decades, a number of versatile sensors based on micro-cantilevers have been developed for the detection of microorganisms and biomolecules such as proteins, DNA, RNA, etc. The sensing mechanism in the micro-cantilever is based on adsorption of the target molecules on immobilized receptors on the cantilever surface which changes the mechanical properties of the cantilever. Molecular adsorption results in the cantilever bending due to adsorption-induced forces while the resonance frequency changes due to mass loading. Selectivity in detection depends on the selectivity of the immobilized receptors. Despite the many advances in the development of cantilever sensors, multiple drawbacks exist that limit their translation to clinical applications (ETAYASH et al., 2016).

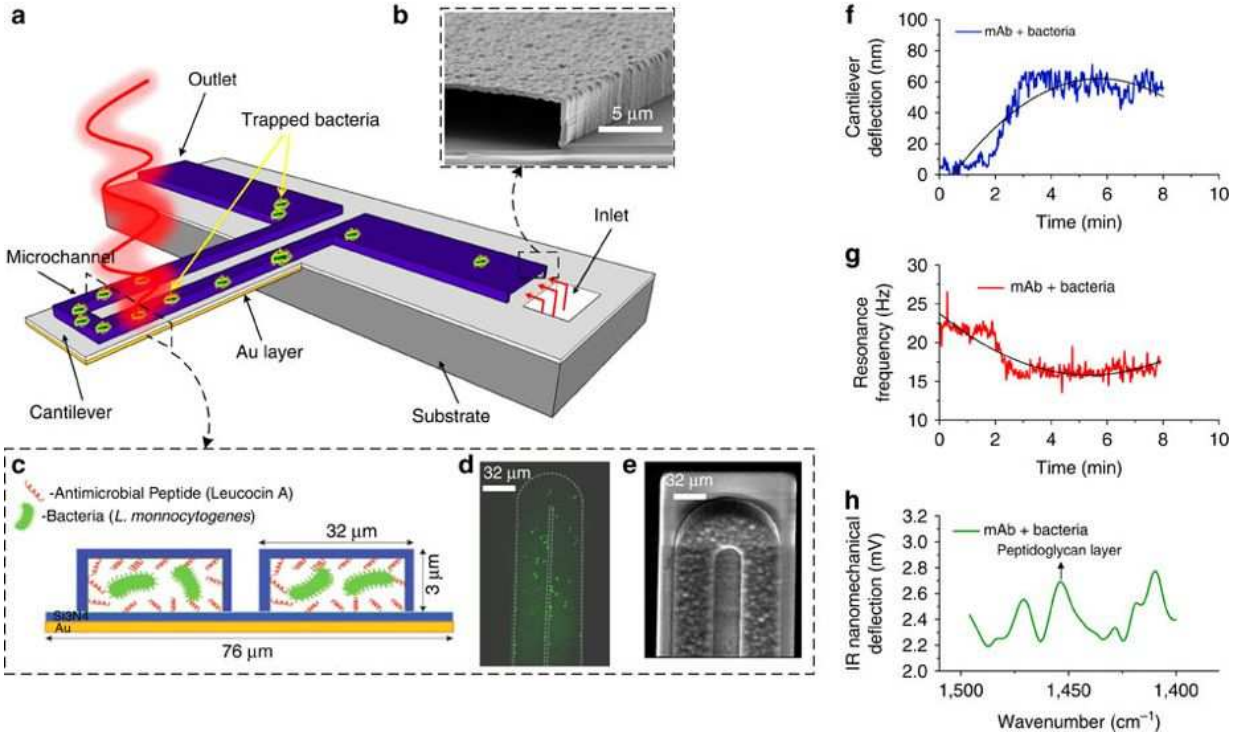

Figure 28: Overview of the micro-cantilever transduction process.
(a) Micro-cantilever filled with bacteria supported on a silicon substrate. (b) Scanning electron microscopy (SEM) image of the cross-section of an inlet, located on bottom side of the chip (c) Cross-section of the 32 µm wide microchannel of the cantilever. (d) Fluorescent image from the top side of the Micro-cantilever, filled with bacteria. (e) SEM image of the tip of the Micro-cantilever. (f) When the bacteria inside the Micro-cantilever absorbs infrared light, local heat is generated that results in the nanomechanical deflection. (g) The resonance frequency is sensitive to the increased mass caused by the adsorption of bacteria inside the Micro-cantilever. (h) When the Micro-cantilever is illuminated with a certain range of infrared light, a plot of the nanomechanical deflection of the Micro-cantilever shows the wavelength where the bacteria absorb infrared light. This can provide excellent selectivity in a complex mixture (ETAYASH et al., 2016).

2.4 Signal and Data Processing

2.4.1 Signal Conditioning

Consisting in a number of techniques required to make the sensor's output signal suitable for processing, conditioning processes represents a key feature for any transducer-based system. Typically, it may comprehend the signal's amplification, filtering, converting, range matching and isolation. The overall goal is to improve the transducer's output signal. Aimed at boosting the signal strength, amplification processes - which are based on the increase of its amplitude - are considered the most common and important ones. However, there are also other important characteristics (defined as secondary) that need to be considered, such as signal's filtering and isolation.

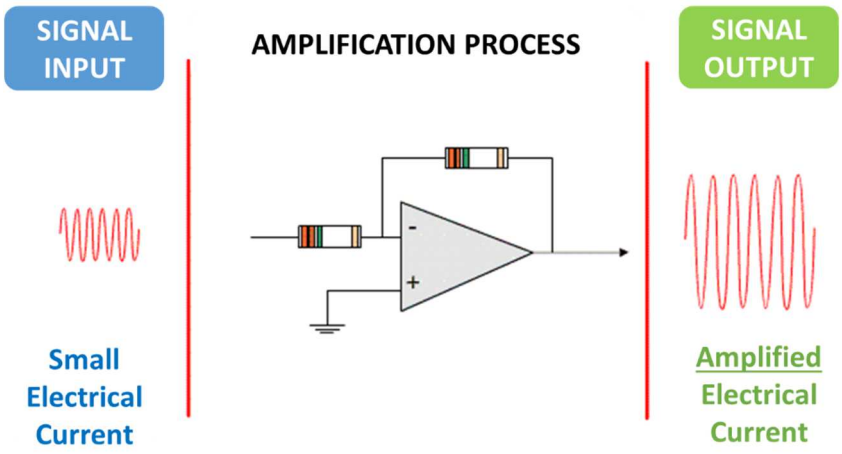

Figure 29: Representation of a standard signal amplification process applied to electrical current: an input current is amplified while passing through a series of electrical components, driven to enhance its amplitude.

2.4.2 Electronic Data Processing

After amplification, filtering and noise cancellation, all analog signals must be converted to digital numeric values, allowing them to be recognized as measurable by standard computer systems. This processing is performed through different platforms and operating systems, most commonly Windows, Linux, Mac, Android and iOS.

CHAPTER 3: PATHOGEN AND PEST DETECTION TECHNIQUES (FOR SENSOR AND BIOSENSOR APPLICATIONS)

Currently used methods for microbial and viral pathogenic agent's detection (and identification) have been developed in different periods throughout the 20th century. They depend almost entirely on specific laboratorial equipment and tools for microbiological and biochemical targeting and analysis.

Detection and identification of diseases could be realized both direct and indirectly, through several methods. Direct detection of diseases includes molecular and serological methods that could be used for high-throughput analysis when large numbers of samples need to be analyzed. In these methods, the disease causing pathogens such as bacteria, fungi and viruses are directly detected to provide accurate identification of the disease/pathogen. On the other hand, indirect methods identify the plant diseases through various parameters such as morphological change, temperature change, transpiration rate change and volatile organic compounds released by infected (FANG; RAMASAMY, 2015).

Moreover, some techniques are time-consuming, detaining all attempts on real-time analysis. Others have low detection limits, creating an extra disadvantage. Regardless of its environmental constraints, nearly all methods have reached some level of robustness, enabling some techniques to be consolidated among the scientific community. Considering the real-time and portable requirements described earlier in this study, we now narrow the current techniques into seven main methods, between sensors and biosensors - used for the direct and indirect detection of pathogens and pests - as described in Fig. 30.

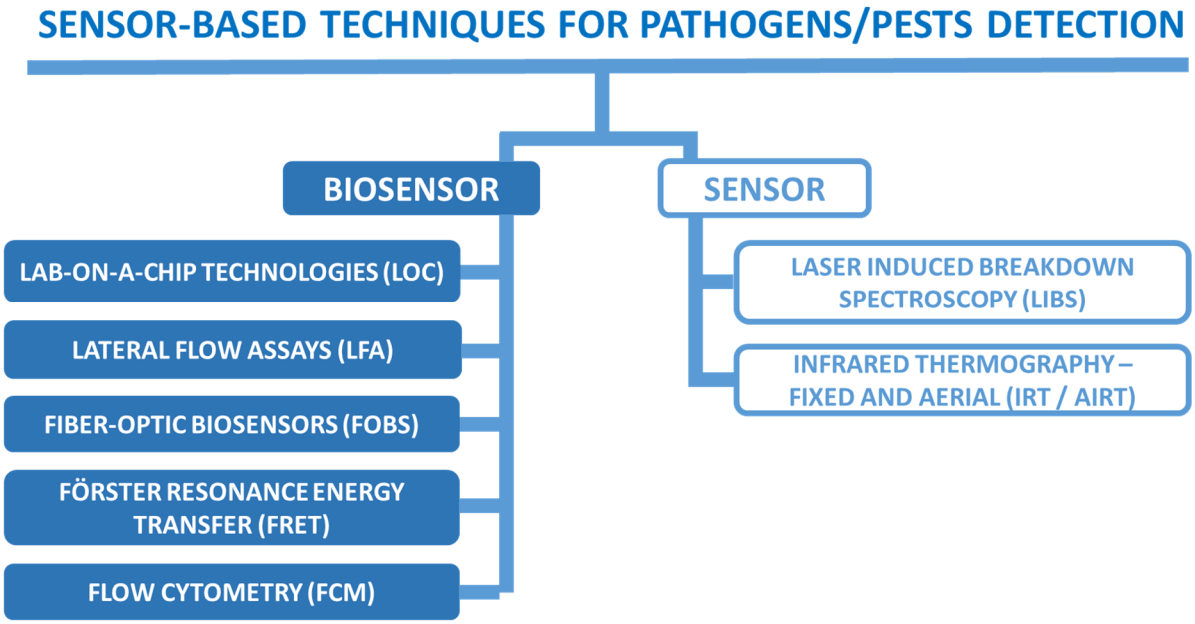

Figure 30: Sensor and Biosensor main techniques for pathogens ans pests detection

3.1 Biosensor-Based Methods

Each sensor-based technique for pathogens detection has application particularities. The techniques described in this section were selected among the ones based on biosensor technologies, considering both direct and indirect analytical methods. In practical terms, only techniques that are able to identify pathogen organisms (virus or bacteria, for instance) upon prior reactions with bioreceptors (with or without any technical correlation) are listed below.

3.1.1 Lab-on-a-Chip Technologies

Rather than a specific type of sensor, lab-on-a-chip (LOC) technologies represent a larger class of sensors, based on a scaled down microfluidic devices for basically carrying out laboratory operations (such as PCR, hybridization and DNA sequencing) outside the scope of standard laboratories. In other words, these techniques typify the most recent developments in the field of science and technology for the whole laboratory standard operations, as it has been reduced to a small chip capable of carrying out various functions.

Keeping to its word, micro, miniaturized volume samples and reagents have lowered the time taken to analyze a reaction apart from the distinctive behavior of liquids at the nano scale which has permitted substantial control of molecular interactions and concentrations. Also, amount of chemical waste and the cost of reagents has been minimized very drastically (PATEL; MAHESHWARI; CHANDRA, 2016).

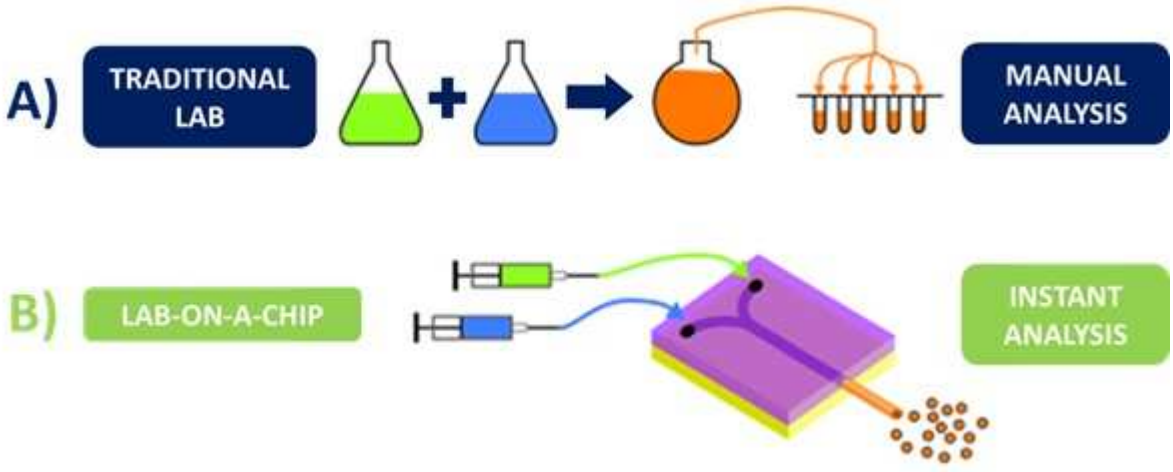

Figure 31: Schematic comparison between traditional lab analysis procedures (a) and lab-on-a-chip sensor technique (b)

The development of such sensors intended to enable controlled conditions for scientific measurements, without the need of a formal laboratory in order to address challenges faced during standard bioanalysis. Therefore, further development was carried out and the new field of microfluidics was established as the basis of lab-on-a-chip technologies. Basically, microfluidic can be described as the techniques responsible for preparation, handling and processing of extremely small sample volumes (prior to its signal transduction) in a sensor or biosensor.

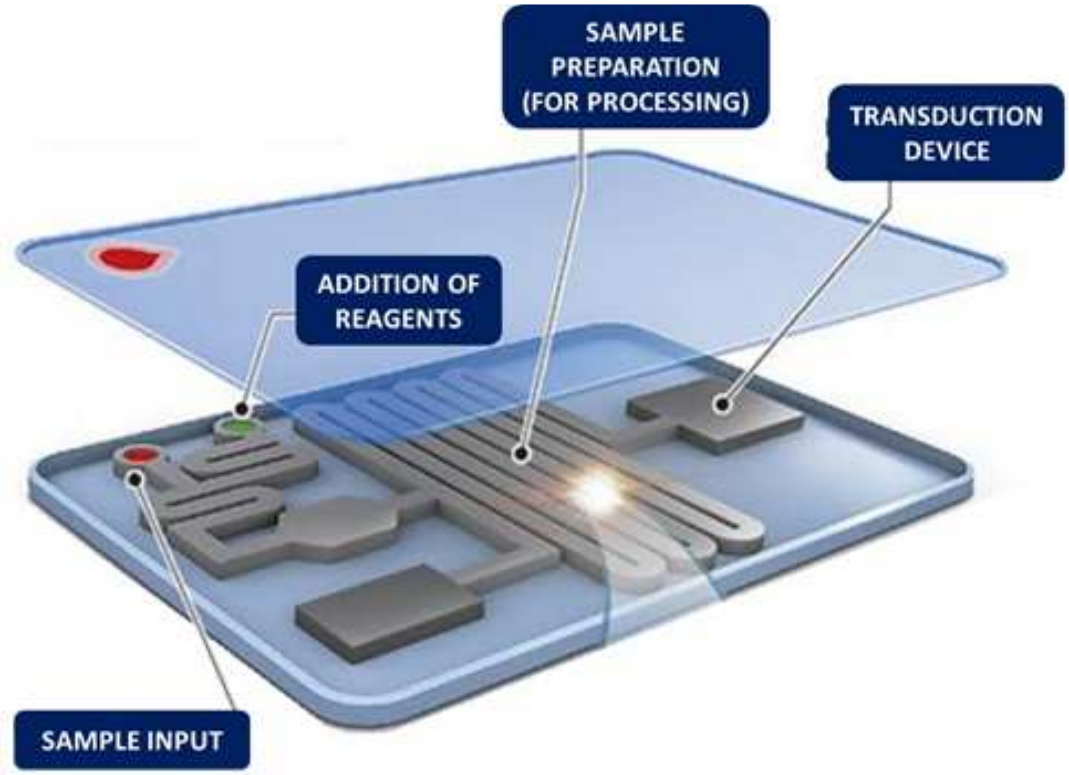

Figure 32: Standard microfluidic-based lab-on-a-chip sensor/biosensor

The polymerase chain reaction (PCR) technique applied in LOC biosensors consists in the amplification of a single copy (or a few copies) of nucleic acid (DNA or RNA) segment across several orders of magnitude, generating from thousands to millions of copies of a particular sequence. It is based on the isolation, amplification and quantification of a short nucleic acid sequence including the targeted bacteria (or virus) genetic material. PCR was developed in the mid 80's and it is widely used in pathogen detection (LAZCKA; CAMPO; MUÑOZ, 2007). Although having many advantages in comparison with other techniques, PCR still has limitations in the technical point-of-view, such as the impossibility to discriminate cells among viable and non-viable ones (due to the constant presence of DNA, regardless of its dead or alive status).

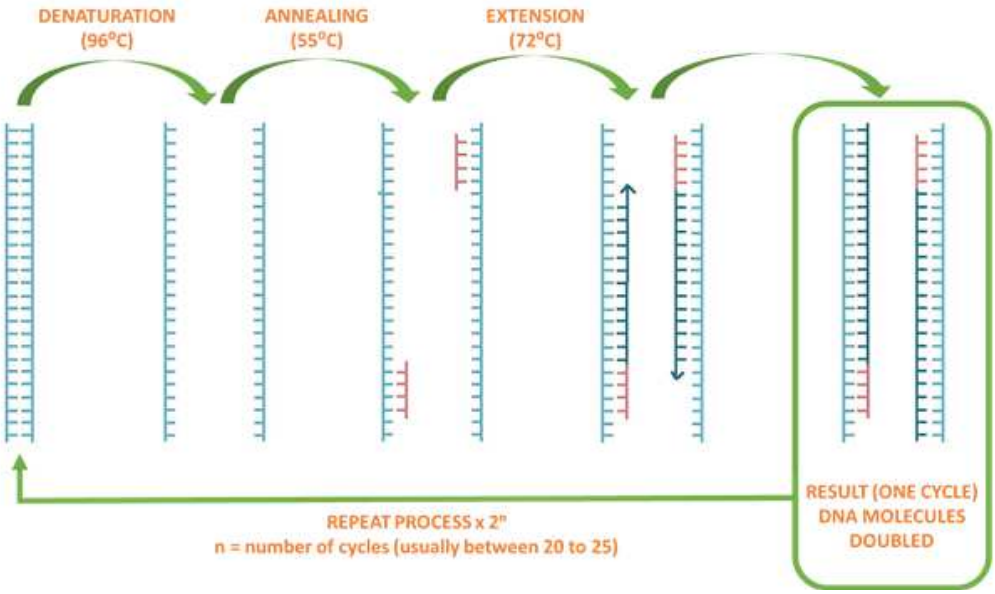

Figure 33: Polymerase chain reaction (PCR) description for one cycle results

Hybridization – another molecular technique used among lab-on-a-chip biosensors – is a method for measuring genetic similarities between different organisms, based on pools of nucleic acids sequences (DNA or RNA). Typically, this phenomenon occurs when single-stranded deoxyribonucleic acid (DNA) or ribonucleic acid (RNA) molecules anneal to a complementary DNA or RNA. Specifically in the case of DNA (double-stranded sequence), this process take place after molecule separation into single strands (generally by raising the surrounding temperature). Additionally, complementary sequences (also from single stranded nucleic acids) are placed within the sample and the surrounding temperature is lowered. This causes both separated single-stranded molecules (original one and complementary) to anneal to each other, in a process called hybridization. Two main sub techniques for hybridization are currently disseminated: DNA-DNA hybridization and Fluorescence *in situ* hybridisation (FISH).

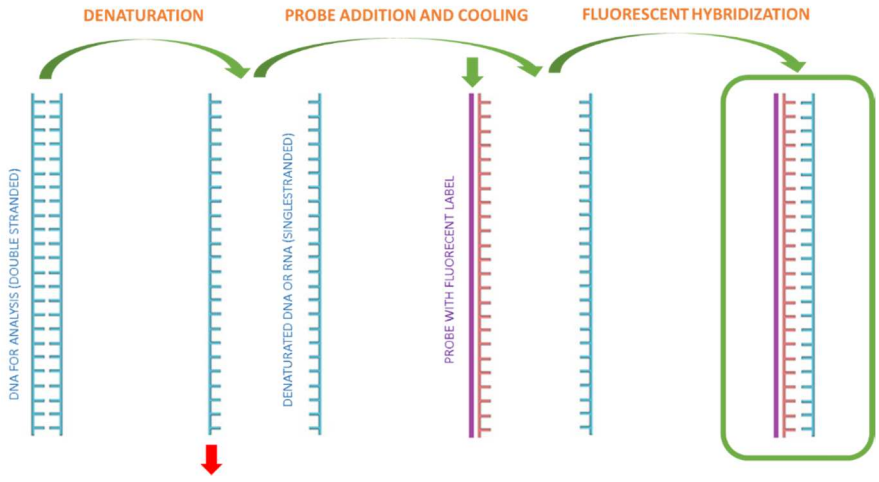

Figure 34: Fluorescence in situ hybridisation (FISH) technique scheme

The rapid emerging of lab-on-a-chip technologies indicates the potential to revolutionize food, agriculture and biosystems industries. Examples of potential applications of microfluidics in food industry include monitoring pathogens and toxins in food and water supplies and detection of antibiotics in dairy food products. In addition, microfluidics enables applications in agriculture and animal sciences such as nutrients monitoring and plant cells sorting for improving crop quality and production, effective delivery of biopesticides, simplified in-vitro fertilization for animal breeding, animal health monitoring, vaccination and therapeutics. (NEETHIRAJAN et al., 2011).

However, there are still several issues to be resolved before applying lab-on-a-chip sensors to field applications, including the pre-treatment of a sample, proper storage of reagents, full integration into a battery-powered system and demonstration of very high sensitivity (YOON; KIM, 2012). Taking into consideration that LOC is a comparatively newer technology, these types of sensors are not yet full-proof in the majority of its possible applications. Also, even considering its great precision in microfabrication, they're tolerances may often be higher when compared to precision engineering. They can undergo certain physical and chemical effects such as surface roughness or chemical interactions of construction materials on reaction processes, but LOC sensors still faces a long journey towards becoming a commercially available and widespread technology.

3.1.2 Lateral Flow Assay (LFA)

Lateral flow assay (LFA) sensors are simple-to-use diagnostic biosensors driven to detect the presence (or absence) of a target analyte within a liquid sample (matrix), through the use of external forces (microfluidics and capillary action) along various zones of polymeric strips, on which specific molecules that can interact with the analyte. Considering the target analyte as pathogens in animals, or contaminants in crops, water supplies or animal feeds, this type of sensor can be used for medical diagnostics either for home testing, point of care testing, or laboratory use.

LFA sensors typically contains a control line (to confirm the test is working properly), along with one or more target or test lines. Due to its operation characteristics, LFA's design is normally intuition-driven, in order to incorporate user's protocols, requiring minimal training prior to its operation. The sensor's results are usually qualitative (visual reading only) or quantitative (visual reading combined with processing technology).

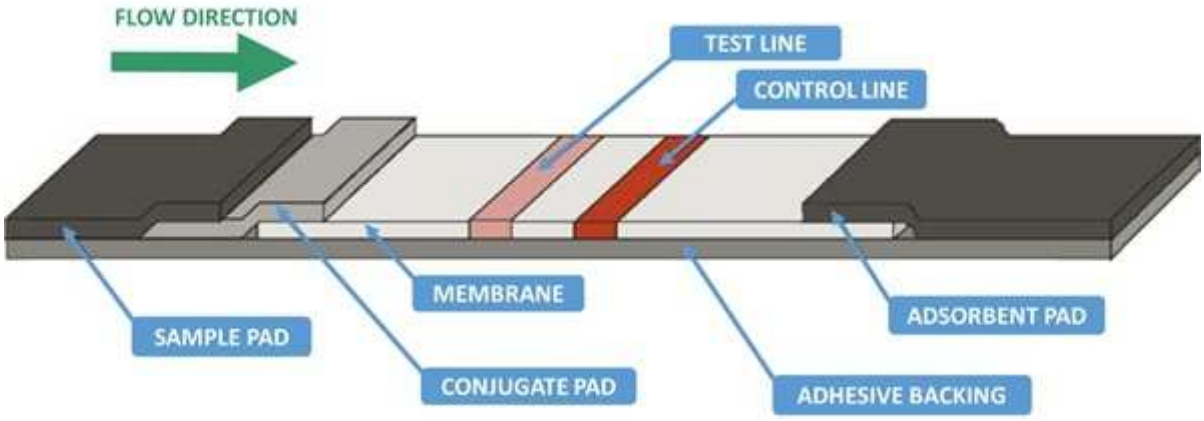

Figure 35: Lateral flow assay-based sensor components and operation scheme

Most lateral flow biosensors are based on immunoassays, which consists of a biochemical test to measure the presence (or concentration) of small or macro molecules in a solution through the use of an antibody or an antigen. After the molecule is detected through the immunoassay, it is often designated as analyte. In many cases, the analyte consists of a protein, albeit it may hold other types of molecules (within different sizes and formats) as long as the proper antibodies that have the adequate properties for the assay are executed. Among this field, the most known sub techniques are known as enzyme-linked immunosorbent assay (ELISA) and immunofluorescence (IF), as described in the figure below. Specifically in the case of immunofluorescence, light microscopy is applied in order to identify fluorescent dyes within the cell's biomolecule targets.

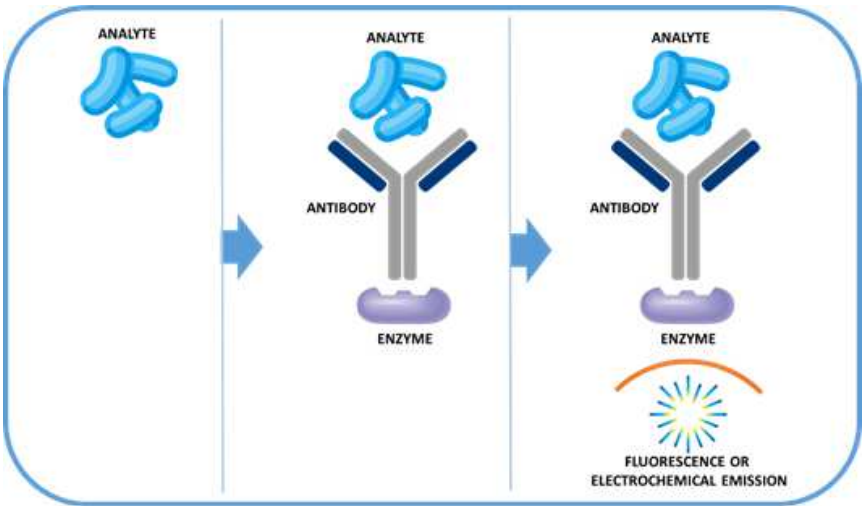

Figure 36: Antibody attached to enzyme interacts with analyte, emitting fluorescence or electrochemical signals

Despite having a characteristic of low sensitivity, lateral flow assay-based biosensors have the capability to play a major role across a number of industry sectors, including agriculture, veterinary and food industry, due to its versatile nature, low-cost, simple operation and portability.

3.1.3 Fiber-Optic Biosensors (FOBS)

Fiber-optic biosensors (FOBS) are optical fiber-derived devices which use optical field to measure biological species such as cells, proteins, and DNA. Thus, it is considered a modified version of the standard fiber-optic sensor. Because of their efficiency, accuracy, low cost, and convenience, FOBS are promising alternatives to traditional immunological methods for biomolecule measurements. Tapered fiber-optic biosensors (TFOBS) are a type of FOBS which rely on special geometries to expose the evanescent field to interact with samples. In order to amplify sensitivity and selectivity, TFOBS are often used with various optical transduction mechanisms such as changes in refractive index, absorption, fluorescence, and Surface Plasmon Resonance (LEUNG; SHANKAR; MUTHARASAN, 2007).

This technique is frequently used along with standard immunoassays, in order to detect specific molecules within an analytes through the use of a bioreceptor (an antibody or an antigen). In this case, the transmission properties of the light are modulated by changes in the refractive index (RI) of the solution in the region surrounding the fiber, due to the presence of an evanescent wave outside the fiber, penetrating within the external medium for distances of the order of hundreds of nanometers. The implementation of a sensing biolayer on the fiber surface containing a bioreceptors selective to a well-defined target, gives the opportunity to detect surface RI changes associated to the biochemical interaction between the target and the biolayer (CHIAVAIOLI et al., 2017).

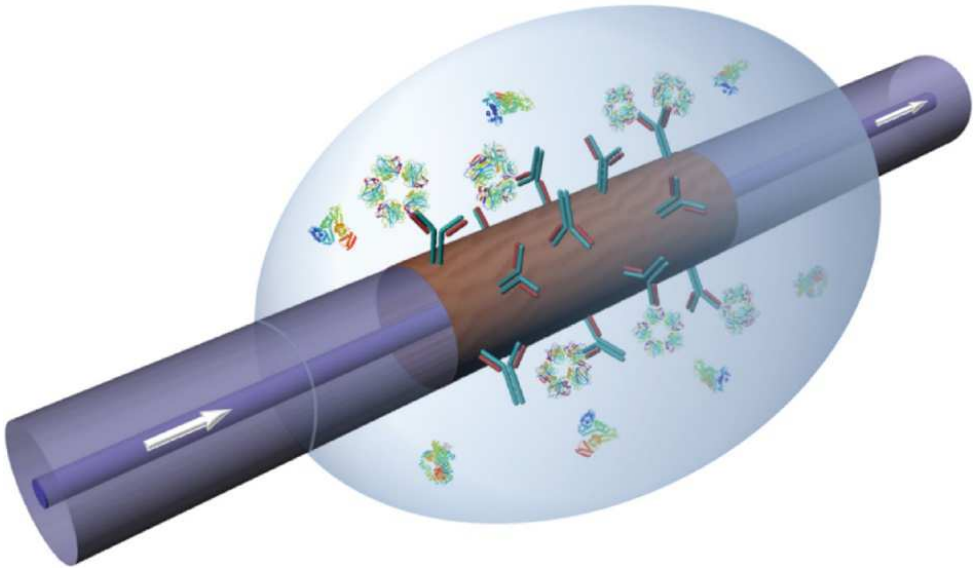

Figure 37: Schematic illustration of the biosensing capability of a standard FOBS. The biological recognition element is covalently bound onto the functionalized surface of the overlay (wrinkled brown) leading to the formation of a sensing biolayer, and the specific target (ring-shaped pentameric antigen) will specifically interact with the recognition element, generating a change in the optical signal traveling in the fiber (white arrows). Any other non-specific biomolecules present in the complex matrix will not bind to the receptor on the sensing layer, thus, not generating any change in the optical signal traveling in the fiber (CHIAVAIOLI et al., 2017).

Apart from the limit of detection and selectivity, it is important to recognize the advantages of fiber-optic biosensors including chemical-inertness, their compatibility to a wide range of surface modification, the potential for remote sensing, lowcost, and the ready availability of inexpensive lasers and photodetectors. Given its promising advantages, it is likely that FOBS will remain a popular choice among researchers and practitioners for detection of biological agents (LEUNG; SHANKAR; MUTHARASAN, 2007).

3.1.4 Förster Resonance Energy Transfer (FRET)

Förster Resonance Energy Transfer, known also as Fluorescence Resonance Energy Transfer - or simply FRET - is a physical phenomenon regarding energy transfer between two light-sensitive molecules (named fluorophore or chromophore). These molecules are, in fact, fluorescent chemical compounds that can re-emit light upon light excitation. Therefore, in this process initially a donor fluorophore absorbs energy due to the excitation of incident light, transferring the excitation energy to a nearby chromophore, the acceptor. FRET microscopy relies on the ability to capture fluorescent signals from the interactions of labeled molecules in single living or fixed cells. If FRET occurs, the donor channel signal will be quenched and the acceptor channel signal will be sensitized or increased (SEKAR; PERIASAMY, 2003).

FRET has been widely employed as a spectroscopic technique in all fluorescence-based applications, including pathogens diagnostics, among various others sensing properties.

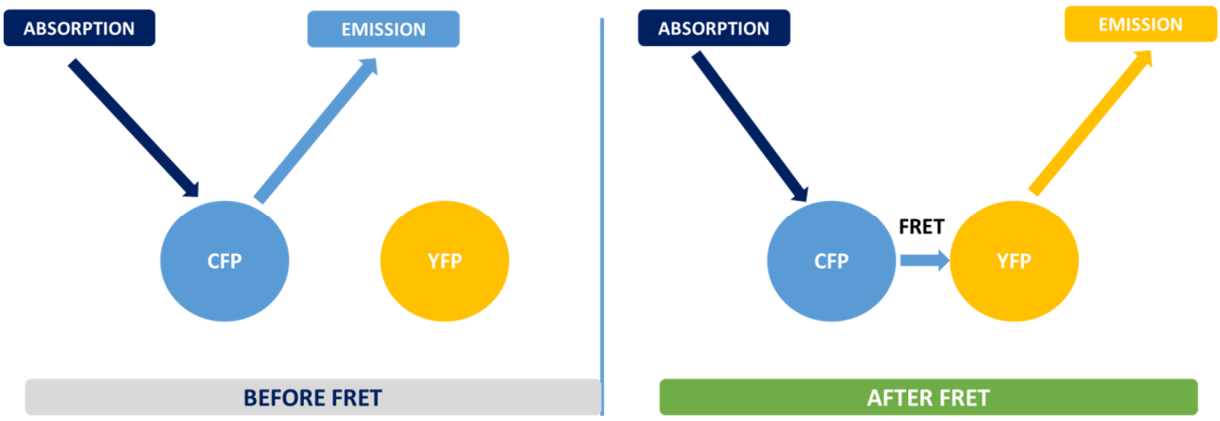

Figure 38: Representation of FRET phenomenon. CFP (donor) and YFP (acceptor) absorption and emission spectra. The overlap of the cyan fluorescent protein (CFP) emission spectrum and the yellow fluorescent protein (YFP) absorption spectrum.

Additionally, Aerial Fluorescence Imaging (AFI) may be consider as a complementary method to standard FRET, as it combines fluorescence imaging and UAVs (unmanned aerial vehicles) applications. With improvements in spatial, spectral and temporal resolution of aerial remote sensing, UAVs will enable near real-time visual assessment for crop monitoring in the field yield predictions, crop status mapping, weed detection, and disease and nutrient deficiency detection. Moreover the development of these miniaturized, affordable light-weight unmanned aerial vehicles have enabled the acquisition of high resolution images for various remote sensing applications (ZAMAN-ALLAH et al., 2015).

3.1.5 Flow Cytometry (FCM)

FCM is an optical technique based on laser or impedance, which is widely used for cell counting and sorting, as well as biomarker detection and protein engineering. Applied for quick identification of cells while passing within an electronic detection device among a liquid stream, this technique has the advantage of been capable to measure several different parameters simultaneously. Its technical operation relies on an incident laser beam, enabling the measurement of the scattering and fluorescence reflected from the sample. Although FCM has been primarily applied to study cell cycle kinetics and antibiotic susceptibility, to enumerate bacteria, to differentiate viable from non-viable bacteria, and to characterize bacterial DNA and fungal spores, it is still a relatively new technique for plant disease detection application. FCM in combination with fluorescent probes has been applied for rapid detection of foodborne bacterial pathogens. FCM has also been proven to be efficient for detection of soil borne bacteria (PIYASENA; GRAVES, 2014).

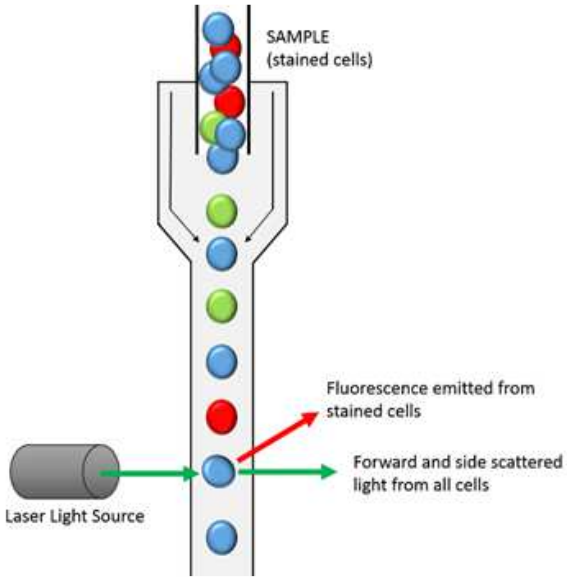

Figure 39: Flow Cytometry operational system scheme (adapted from CHEUNG et al., 2010).

3.2 Sensor-Based Methods

The techniques described in this section are considered to be based on sensor analytical methods. That is to say, it is listed below only techniques with capacity to identify pathogen organisms and pests indirectly, through the usage of electromagnetic, optical, mechanical, thermal, chemical receptors.

3.2.1 Laser Induced Breakdown Spectroscopy (LIBS)

Laser Induced Breakdown Spectroscopy - or simply LIBS - is a type of analytical sensor based on chemical spectroscopy. Basically it uses a highly energetic laser pulse in order to form a plasma, which atomizes and thermally excites the samples (with temperatures that exceed 100,000 K). This plasma formation begins only when the focused laser reaches a certain temperature level, favorable for the optical breakdown of the sample's molecules. This threshold depends generally on environmental conditions and, more importantly, the analyte's material.

Theoretically, LIBS sensors can analyze any matter regardless of its physical state (solid, liquid or gas). This analytical flexibility is due to the fact that all elements emit light in specific frequencies (among a spectrum) when excited to properly high temperatures. Hence the capacity of LIBS sensors to detect (in principle) all elements within a sample. Its performance is limited only by the applied laser power, as well as the sensitivity and wavelength range of its spectrometer and detector. As long as the chemical elements of the sample needed for analysis are known among science, LIBS sensors may be employed to assess its relative composition, or even to monitor the presence of impurities, for example.

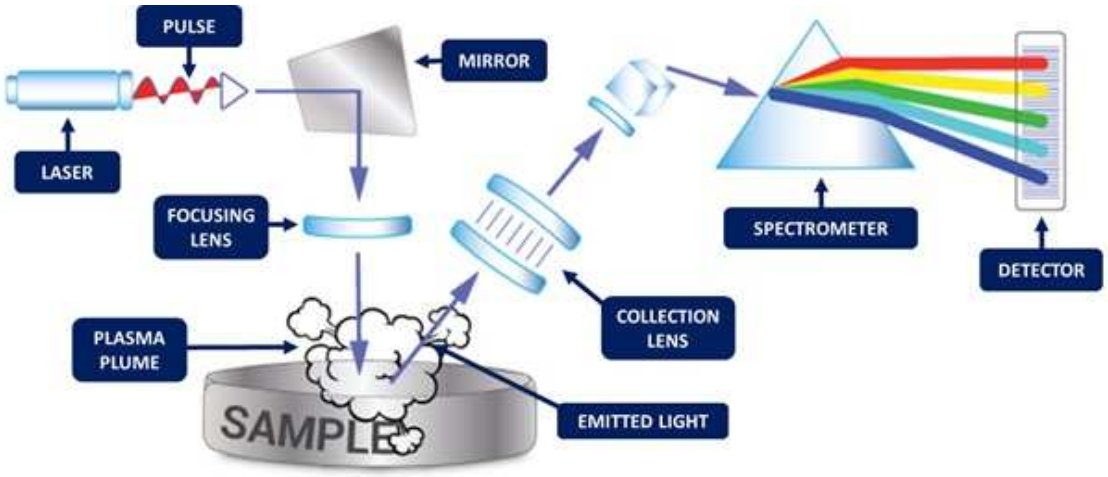

Figure 40: Standard LIBS sensor operation technique: A focused laser is first fired at a sample with sufficient pulse energy as to create a plasma around the area struck. Bound atomic electrons are striped from the atoms comprising the material. As the plasma cools, atoms recombine with electrons and in the process emit light in the UV, optical and IR regime

LIBS technique is considered essentially a non-destructive or minimally-destructive process. This is due to the small amount of material consumed during the analysis, as well as the mild power density radiated (less than one watt) onto the sample. Handheld operation devices for LIBS applications, also called HH-LIBS, represent the state-of-the-art technology, in terms of portability and field usage. Essential for almost all agricultural purposes, HH-LIBS are especially interesting for analytical measurements within soil, crops, livestock and vegetation.

Figure 41: Typical handheld operation LIBS sensor (adapted from RANULFI et al., 2018)

One of its major advantages is the capacity to profile a sample in terms of depth, by repeatedly discharging the laser in the same position. This effectively makes the laser pulse go deeper into the analyte after each shot. It can also be applied to surface contamination removal, where the laser is discharged a number of times prior to the analysing shot. LIBS is also not time consuming, enabling results within seconds, making it particularly useful for high volume analyses or on-line industrial monitoring. Portable LIBS (such as HH-LIBS) systems are more sensitive, faster and may detect a wider range of elements (particularly from light) than other similar techniques such as portable x-ray fluorescence. And LIBS does not use ionizing radiation to excite the sample, which is both penetrating and potentially carcinogenic.

Nevertheless, this technique presents some limitations, such as analytical reproducibility, driven by inherent variations in the laser spark and its resultant plasma. Also, the accuracy of LIBS measurements stays within 10% and precision is often around 5%. Its detection limits can range from >100 ppm to <1 ppm, but may vary according to the type of component measured and the experimental device used.

3.2.2 Fixed and Aerial Infrared Thermography

Integrating the colorimetric thermography concepts described previously, with fixed stations or unmanned aerial vehicles (UAV), Infrared Thermography (IRT) technique represents one of the moist promising tools for analytical assays, especially in agricultural systems. IRT and AIRT are innovative diagnostic tools driven to detect thermal anomalies on the external surface. It is considered a non-invasive technique, which registers the temperature distribution through the usage of an UAV (or fixed station) embedded with thermal cameras, receiving and processing the infrared radiation emitted from the target surface.

AIRT applications also enables the possibility to correlate infrared images into Normalized Difference Vegetation Index (NDVI) images, which are considered an alternative, in terms of further data processing in agricultural systems.

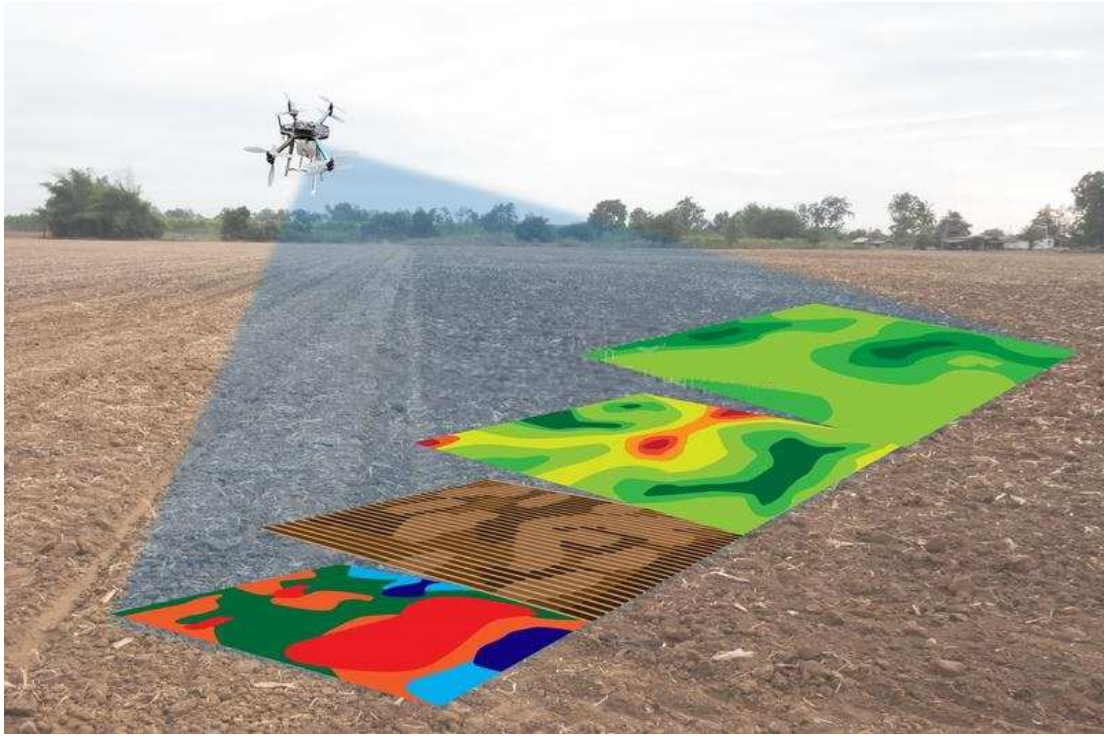

Figure 42: Example of AIRT application for the detection of thermal anomalies within a specific crop . In this case, multispectral images (with NDVI) are also represented (adapted from KHANAL; FULTON; SHEARER, 2017).

Unmanned aerial vehicle platforms (UAVs) equipped with sensors are emerging as a promising and low cost tool for precision agriculture and crop management. The application of unmanned aerial remote sensing has shown advantages in terms of large scale crop condition monitoring, such as yield forecasting, quality control and disease identification due to the high spectral and spatial resolution (on its mounted sensors).

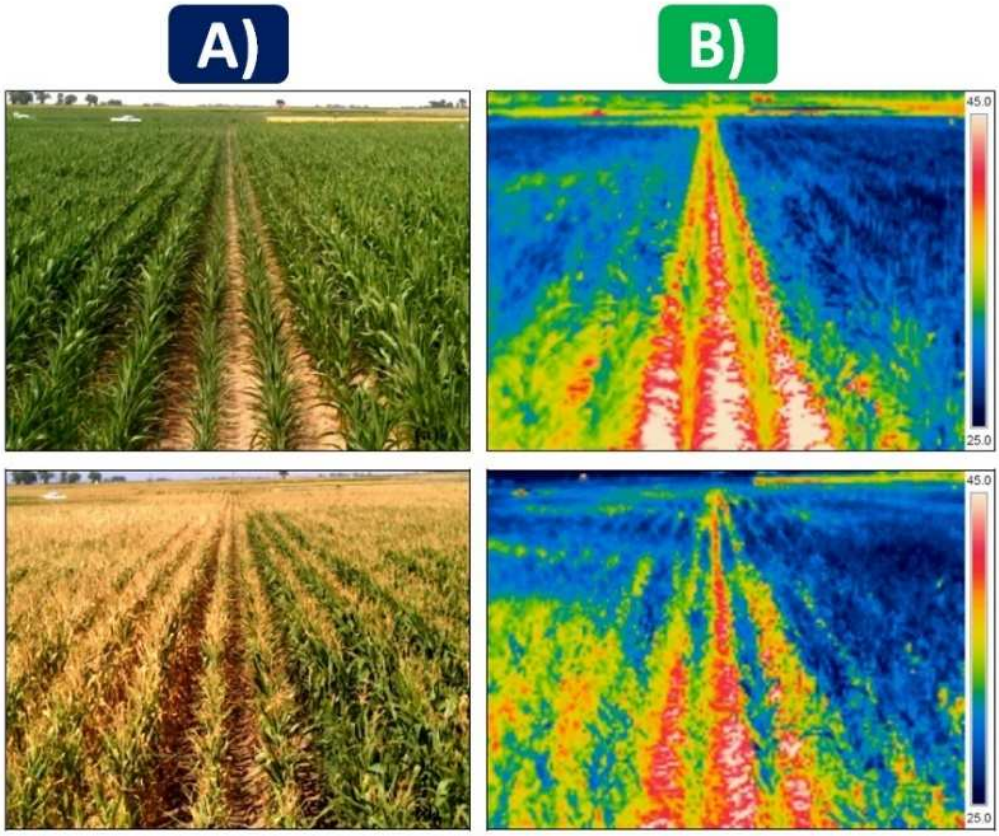

Figure 43: Demonstration of IRT application with UAV: a) visible images; b) thermal images. The legends on the right side of the thermal images represent temperature color ramp in °C. (adapted from TAGHVAEIAN; CHÁVEZ; ALTENHOFEN, 2013).

CHAPTER 4: MATERIALS AND METHODS

The correct definition of all necessary steps for methodology implementation is a key element concerning all academy research studies. Hence the importance of the methodological approach for this study, which is presented below.

Figure 44: Methodology implementation flow:
Step 1 – Application sector definition; Step 2 – Database and keywords definition; Step 3 – Data collection; Step 4 – Selection criteria and result filtering; Step 5 – Pathogen, pest, sensor and application identification and Step 6 – Trend and relationship analysis

4.1 Step 1: Application Sector Definition

The first step is this study's methodology relies heavily on the definition of the specific economical sector in which it will be applied. In the case of this study, all attention is going to be given to the agricultural systems and its opportunities regarding new technical applications for pathogen ans pest detection. In this field, the main areas in terms of applicability and relevance that have direct and indirect impact in its performance are described below.

a) Plant pathogen and pest detection in crops (direct impact)

b) Animal pathogen detection in livestock (direct impact)

c) Plant pathogen detection in soil (indirect impact)

d) Plant pathogen detection in pastures (indirect impact)

4.2 Step 2: Database and Specific Keywords Definition

All pathogen and pest detection techniques for sensor and biosensor applications (already arranged within Chapter 3) were used as a basis for this step. In order to acquire a large range of scientific papers and patents for analysis, several keywords were chosen. Some of them were introduced with boolean operator “AND”, and others with operator “OR”. The complete list of selected ones are shown on Table 2 below.

Table 2: Keywords used for data base analysis

Method	Keyword 1	Keyword 2	Keyword 3	Keyword 4
Biosensor	"Lab-on-a-Chip"	agric*	Pathogen*	
Biosensor	"Lateral Flow"	agric*	Pathogen*	
Biosensor	Fiber Optic	agric*	Pathogen*	
Biosensor	FRET	agric*	Pathogen*	
Biosensor	Cytometr*	agric*	Pathogen*	Food
Sensor	Laser Induced Breakdown Spectroscopy	agric*	Pathogen*	"LIBS"
Sensor	Thermograph*	agric*	Pathogen*	Infrared*
	Topic			
	AND			

All keywords were used along with the search field “TOPIC”, which, in this case, gathers results from different data base fields, such as Title, Abstract, Author Keywords and Keywords Plus. No geographical restrictions were applied, and the search was limited to papers published from 2000 to 2018.

4.3 Step 3: Data Colletion (Scientific Papers and Patents)

The research was conducted into the most important databases of scientific journals, such as Web of Science, Scopus, Cilea and SciDirect. Furthermore, several patent databases were also included in this study, e.g. Derwent World Patents Index (DWPI), Patentscope (WIPO) and European Patent Office (EPO).

Titles, abstracts, author keywords and keywords plus from more than 800 publications, journals and patents were screened and examined. Thus, all relevant results were gathered in two different software basis: Mendeley (reference management software) and Microsoft Excel (spreadsheet developer software, for data preparation, calculation, graphing tools, pivot tables).

4.4 Step 4: Selection Criteria Definition and Result Filtering

The selection criteria chosen to identify and filter all relevant articles and patents (related to the objectives of this review) is based on the topic analysis (title, keyword and abstract) within the complete data collection. The complete steps are described below:

(1) addressing only results related to the sensors and biosensors for pathogen and pest detection listed on Chapter 3;

(2) focusing on results for all agriculture application categories, among cereals, fruits, vegetables, meat and derivatives, as well as the dairy industry and the crop-related renewable energy sector;

(3) targeting only results concerning techniques applied outside laboratorial infrastructure;

By expressly defining the selection criteria, it enabled the filtering and narrowing of all 832 results into exactly 185. More details can be seen in the figure below.

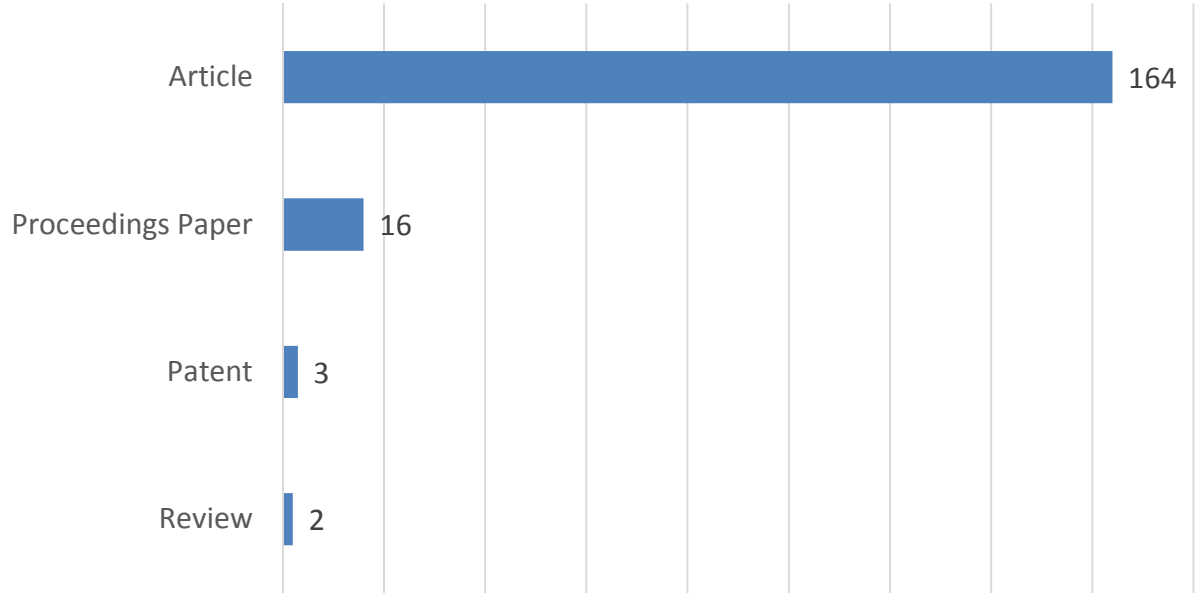

Figure 45: Total number of results (found after data filtering) divided per type: featuring 186 scientific papers, 16 proceedings papers, 3 patents and 2 reviews, which fitted the selection criteria previously defined

Results coming from 111 different scientific journals and conference proceedings were finally selected, plus 3 patent registers, for a total number of 164 papers reviewed (Table 3). The journals mainly belong to fields such as precision agriculture, food research, sensors and biotechnology, phytopathology and virological methods.

Table 3: List of the journals selected and related articles reviewed

JOURNAL	NUMBER OF RESULTS
PRECISION AGRICULTURE	12
PHYTOPATHOLOGY	8
PLOS ONE	7
BIOSENSORS & BIOELECTRONICS	6
PLANT DISEASE	5
JOURNAL OF VIROLOGICAL METHODS	5
JOURNAL OF AOAC INTERNATIONAL	5
SENSORS	4
EUROPEAN JOURNAL OF PLANT PATHOLOGY	4
FRONTIERS IN PLANT SCIENCE	3
FUNCTIONAL PLANT BIOLOGY	3
COMPUTERS AND ELECTRONICS IN AGRICULTURE	2
CANADIAN JOURNAL OF ANIMAL SCIENCE	2
FRONTIERS IN MICROBIOLOGY	2
FRONTIERS IN PATHOGEN DETECTION: FROM NANOSENSORS TO SYSTEMS	2
PLANT PHYSIOLOGY	2
APPLIED AND ENVIRONMENTAL MICROBIOLOGY	2
SCIENTIFIC REPORTS	2
TALANTA	2
BMC MICROBIOLOGY	2
TOXINS	2
PLANT PATHOLOGY	2
ANALYST	2
PLANT PHYSIOLOGY AND BIOCHEMISTRY	2
JOURNAL OF FOOD PROTECTION	2
ANALYTICA CHIMICA ACTA	2
JOURNAL OF PLANT PATHOLOGY	2
FOOD AND BIOPROCESS TECHNOLOGY	2
JOURNAL OF THE AMERICAN SOCIETY FOR HORTICULTURAL SCIENCE	2
BIOSYSTEMS ENGINEERING	2
GESUNDE PFLANZEN	2
JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY	2
Proceedings of the 9th International Symposium on Precision Agriculture.	1
MSPHERE	1
JOURNAL OF THE SCIENCE OF FOOD AND AGRICULTURE	1
ENVIRONMENTAL CHEMISTRY LETTERS	1
BULLETIN OF MATHEMATICAL BIOLOGY	1
ANALYTICAL BIOCHEMISTRY	1
CROP SCIENCE	1
FOOD ANALYTICAL METHODS	1
MEDYCYNA WETERYNARYJNA-VETERINARY MEDICINE-SCIENCE AND PRACTICE	1
ANALYTICAL CHEMISTRY	1
PHYSIOLOGIA PLANTARUM	1
FOOD BIOPHYSICS	1
CEREAL RESEARCH COMMUNICATIONS	1
FOOD CONTROL	1
SENSING AND INSTRUMENTATION FOR FOOD QUALITY AND SAFETY	1
FOODBORNE PATHOGENS AND DISEASE	1
WATER RESEARCH	1
FRONTIERS IN BIOLOGICAL DETECTION: FROM NANOSENSORS TO SYSTEMS VII	1
AGRONOMY JOURNAL	1
AGRICULTURAL WATER MANAGEMENT	1
MOLECULAR BREEDING	1
APPLIED BIOCHEMISTRY AND MICROBIOLOGY	1
NANOSCIENCE, NANOTECHNOLOGY AND NANOENGINEERING	1
APPLIED MICROBIOLOGY AND BIOTECHNOLOGY	1

JOURNAL	NUMBER OF RESULTS
PLANT CELL TISSUE AND ORGAN CULTURE	1
FULLERENES NANOTUBES AND CARBON NANOSTRUCTURES	1
CENTRAL EUROPEAN JOURNAL OF BIOLOGY, 1	1
APPLIED OPTICS	1
ANALYTICAL AND BIOANALYTICAL CHEMISTRY	1
APPLIED SPECTROSCOPY	1
REMOTE SENSING	1
IEEE SENSORS JOURNAL	1
SPECTROCHIMICA ACTA PART A-MOLECULAR AND BIOMOLECULAR SPECTROSCOPY	1
IEEE TRANSACTIONS ON NANOBIOSCIENCE	1
VETERINARY MICROBIOLOGY	1
INDIAN JOURNAL OF ANIMAL SCIENCES	1
ZOONOSES AND PUBLIC HEALTH	1
INTERNATIONAL Agrophysics	1
JOURNAL OF VETERINARY DIAGNOSTIC INVESTIGATION	1
INTERNATIONAL JOURNAL OF APPLIED EARTH OBSERVATION AND GEOINFORMATION	1
LETTERS IN APPLIED MICROBIOLOGY	1
ISHS ACTA HORTICULTURAE	1
MICROCHEMICAL JOURNAL	1
ISPRS GEOSPATIAL WEEK 2015	1
MOLECULAR PLANT PATHOLOGY	1
ZUCHTUNGSKUNDE	1
MYCOLOGICAL RESEARCH	1
2009 ASABE ANNUAL INTERNATIONAL MEETING	1
NATIONAL SCIENCE COUNCIL	1
JOURNAL OF ANIMAL SCIENCE	1
BMC GENOMICS	1
ARCHIVES OF VIROLOGY	1
6TH IEEE INTERNATIONAL CONFERENCE ON NANO/MOLECULAR MEDICINE AND ENGINEERING	1
JOURNAL OF APPLIED MICROBIOLOGY	1
ACTA HORTICULTURAE	1
JOURNAL OF DAIRY RESEARCH	1
PLASMA SCIENCE & TECHNOLOGY	1
JOURNAL OF DAIRY SCIENCE	1
POULTRY SCIENCE	1
JOURNAL OF FIELD ROBOTICS	1
PROCEEDINGS OF SPIE	1
JOURNAL OF FOOD ENGINEERING	1
REAL-TIME IMAGING	1
AGRICULTURE ECOSYSTEMS & ENVIRONMENT	1
CRITICAL REVIEWS IN IMMUNOLOGY	1
JOURNAL OF HAZARDOUS MATERIALS	1
CRITICAL REVIEWS IN MICROBIOLOGY	1
JOURNAL OF MICROBIOLOGICAL METHODS	1
CROP PROTECTION	1
JOURNAL OF PHYTOPATHOLOGY	1
TRANSACTIONS OF THE ASABE	1
JOURNAL OF PLANT DISEASES AND PROTECTION	1
VITIS	1
BIOSENSORS AND BIOELECTRONICS	1
ZEITSCHRIFT FUR NATURFORSCHUNG SECTION C-A JOURNAL OF BIOSCIENCES	1
BIOSENSORS-BASEL	1
CURRENT RESEARCH TOPICS IN APPLIED MICROBIOLOGY AND MICROBIAL BIOTECHNOLOGY	1
JOURNAL OF THE BRAZILIAN CHEMICAL SOCIETY	1
JOURNAL OF AGRICULTURAL SCIENCE AND TECHNOLOGY	1

4.5 Step 5: Pathogen/Pest, Sensor and Application Identification

Been one of the most work-demanding steps, in this phase all 185 selected results were analyzed individually in order to identify its respective pathogen or pest, sensor/biosensor as well as application field within the agricultural systems. Analysis of the full content of every result was used so that all necessary information could be recognized. Additionally, all selected results were classified into the following categories described in Table 4.

Table 4: Categories used for classification of pathogen, pest, sensor and application analysis

Pathogen/Pest Categories	Sensor Categories
Algal	Infrared Thermography
Bacterial	Flow Cytometry
Fungal	Fiber-Optic Biosensor
Insect	FRET
Parasites	Lateral Flow Assay
Synthetic	LIBS
Viral	Lab-On-A-Chip

Most of articles and patents analyzed in this work were related to more than one category within agricultural application as well as pathogen/pest. Hence, the number of results considered for both these categories reached higher levels than the total of articles and patents results (185).

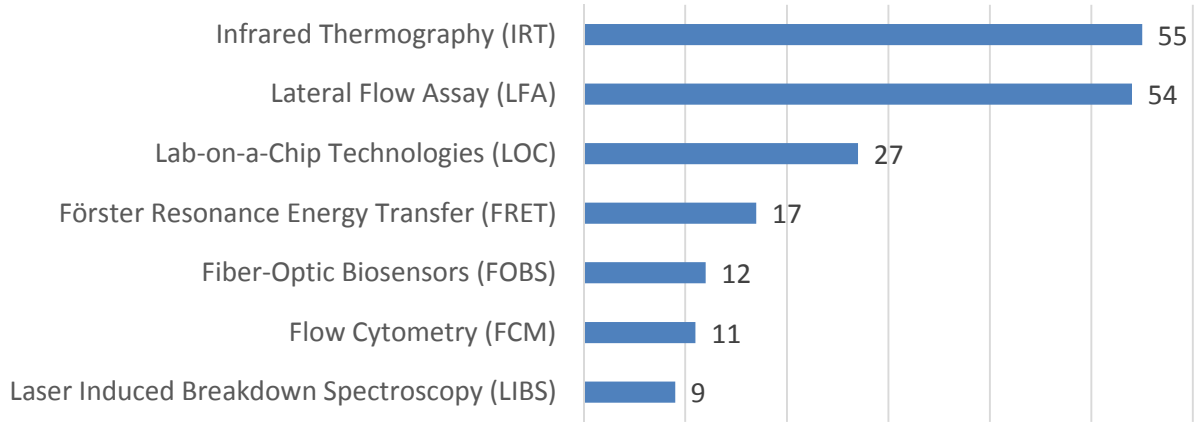

Figure 46: Results per category of sensor and biosensor

From this step on, a specific software for data mining and visualization (Orange) was used for all qualitative text analysis. The result of this step can be seen in the bar charts and word clouds below.

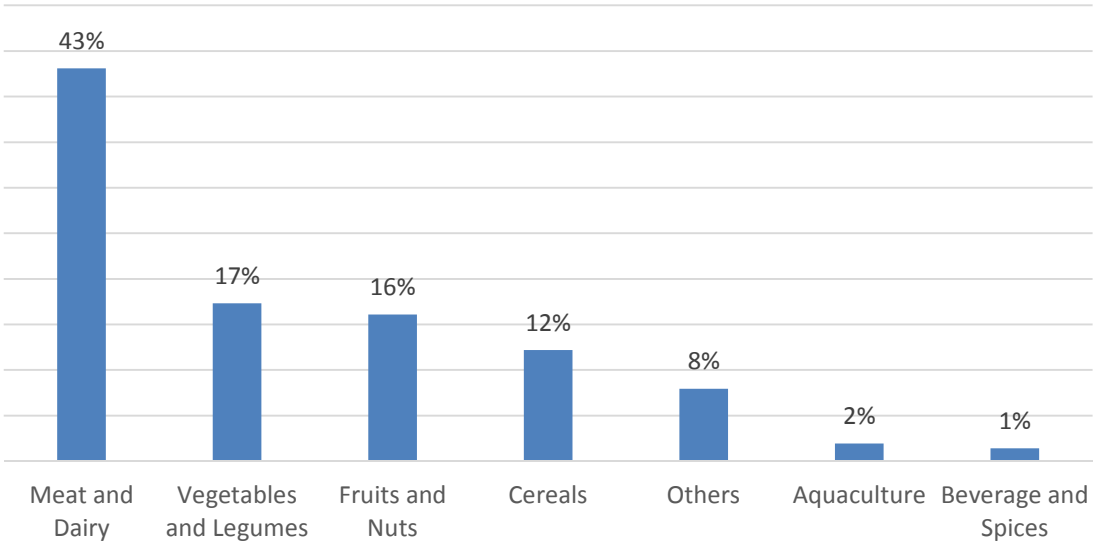

Figure 47: Bar chart generated by the results per agricultural application category among 185 articles and patents, using Orange software for data mining, visualization and qualitative text analysis.

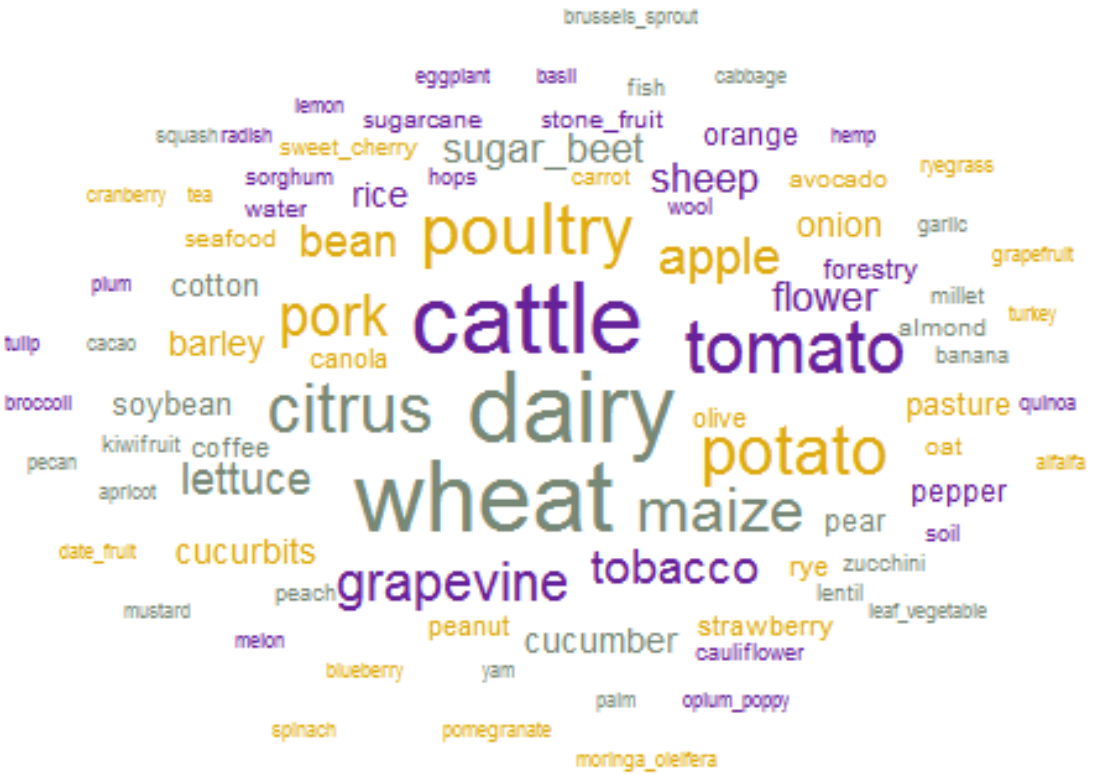

Figure 48: Word cloud generated by the specific results per agricultural application among 185 articles and patents, using Orange software for data mining, visualization and qualitative text analysis.

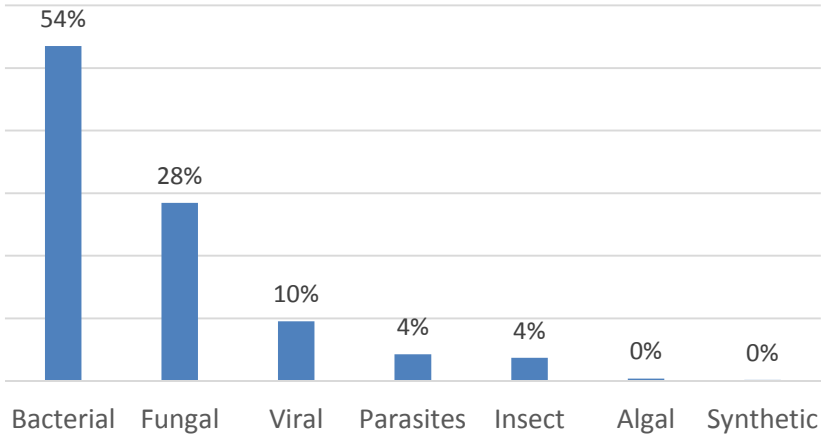

Figure 49: Bar chart generated by the results per pathogen/pest category among 185 articles and patents, using Orange software for data mining, visualization and qualitative text analysis.

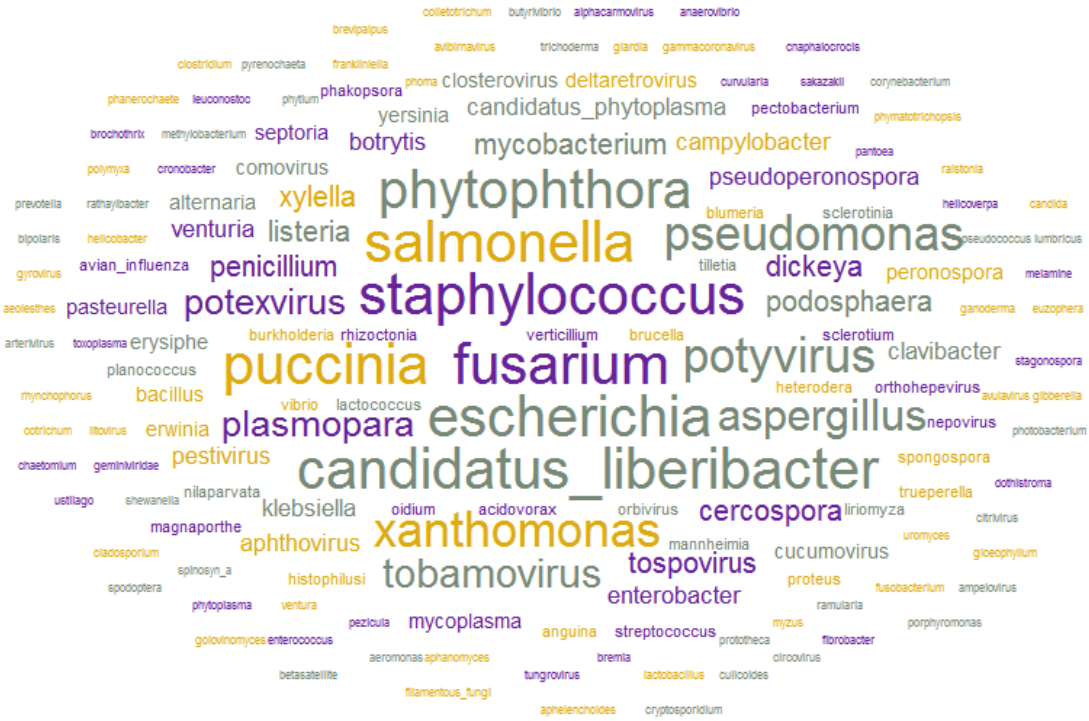

Figure 50: Word cloud generated by the specific results per pathogen/pest among 185 articles and patents, using Orange software for data mining, visualization and qualitative text analysis.

4.6 Step 6: Trends and Relationships Analysis

At the start of this phase, all necessary articles and patents for the study were already set for further research. Big data tools were used to perform specific analysis among this selection, in order to identify possible trends within agricultural systems, pathogens, pests and sensors/biosensors. This step sought to deliver the majority of potential links between sensors and biosensor technologies, pathogenic organisms and its correlated applications in agricultural systems.

CHAPTER 5: RESULTS

Throughout this chapter, we present all relationships found between sensors, biosensors, pathogens, pests and the agricultural systems in which they are applied. Based on the outcome from the big data analysis, this study unfolds its final results using relations diagrams (also known as interrelationship diagrams). This is particularly due to the immensely large number of data needed for exhibition, as well as the practical and easy-to-understand characteristics that this sort of tool is able to portrait, in terms of relationships between two or more factors.

Using the categories defined previously in Table 4 for agricultural applications, the results were divided according to (FAO, 2017): aquaculture; beverages and spices; cereals; fruits and nuts; meat and dairy; vegetables and legumes and others.

5.1 Aquaculture

This category combines the production and cultivation of aquatic animals or plants, all driven for the food industry. Been the result of 5 articles from different authors, in which lateral flow assays overbalances the sensor category as the main technique.

Table 5: Processed results for aquaculture production systems (ranked by publication date), showing associations between applications, sensor categories and pathogens/pests, specifically for each author

Application	Sensor Category	Detection Mode	Pathogen / Pest		Authors
Crab	Lateral Flow Assay	Direct	Staphylococcus aureus		(BANERJEE; JAISWAL, 2018)
Fish	Lateral Flow Assay	Direct	Aphanomyces invadans		(QI et al., 2016)
Seafood	Lateral Flow Assay	Direct	Salmonella spp		(HOERNER et al., 2011)
Seafood	Flow Cytometry	Direct	Toxoplasma gondii		(SHAPIRO et al., 2010)
Fish Seafood	Lab-On-A-Chip	Direct	Aeromonas hydrophila Vibrio cholerae	Vibrio parahaemolyticus Vibrio vulnificus	(RASOOLY; HEROLD, 2008)

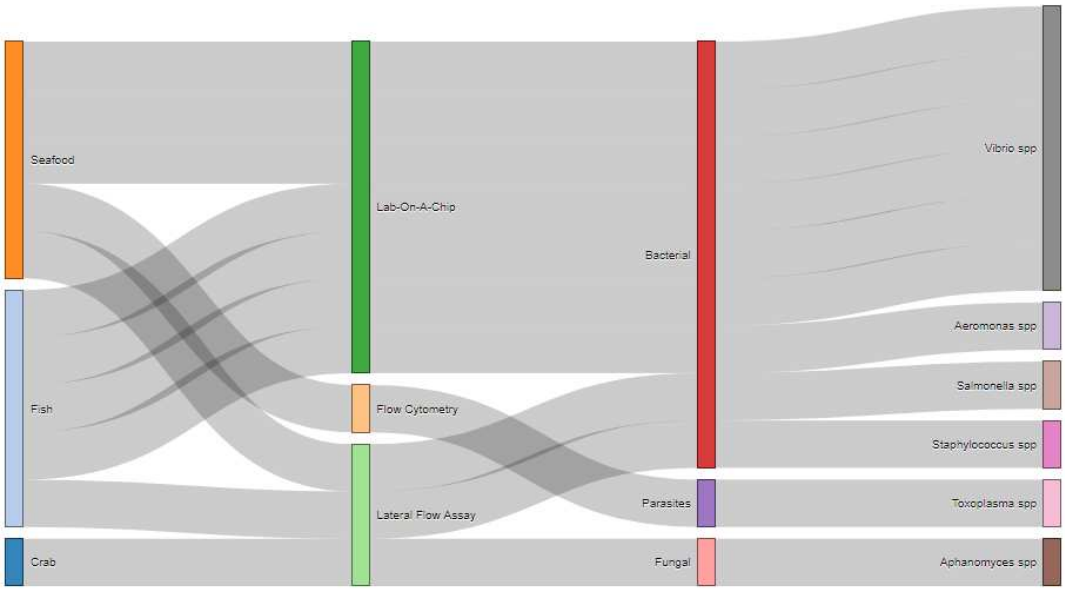

Figure 51: Relations diagram between aquaculture applications and its respective sensor categories, with linkage to the pathogen/pest's specific category and genera

5.2 Beverages and Spices

Outcoming from 7 articles and one patent, the results mention different sorts of agricultural crops used in the beverage and spice areas, along with its identified pathogen/pest and detection method. Lab-on-a-chip technologies and lateral flow assays appear as the main type of sensors, driven to detect 8 genera of pathogens, between bacteria, fungi and viruses, thoughtout mostly coffee and pepper cultivation systems.

Table 6: Processed results for beverages and spices production systems (ranked by publication date), showing associations between applications, sensor categories and pathogens/pests, specifically for each author

Application	Sensor Category	Detection Mode	Pathogen / Pest	Authors
Pepper	Lab-On-A-Chip	Direct	Chili_leaf_curl_betasatellite	(TAHIR et al., 2018)
Coffee	FRET	Direct	Aspergillus_spp Penicillium_spp	(QIAN et al., 2015)
Coffee	Lab-On-A-Chip	Direct	Xylella_fastidiosa	(WHIDDEN et al., 2015)
Tea	Laser Induced Breakdown Spectroscopy	Indirect	Brevipalpus_phoenicis	(LIU et al., [s.d.])
Ginger	Lab-On-A-Chip	Direct	Enterobacter_cloacae	(RASOOLY; HEROLD, 2008)
Pepper	Lateral Flow Assay	Direct	Tomato_spotted_wilt_virus_TSWV	(MARGARIA; CIUFFO; TURINA, 2004)
Pepper	Lateral Flow Assay	Direct	Pepino_mosaic_virus_PepMV	(SALOMONE; ROGGERO, 2002)

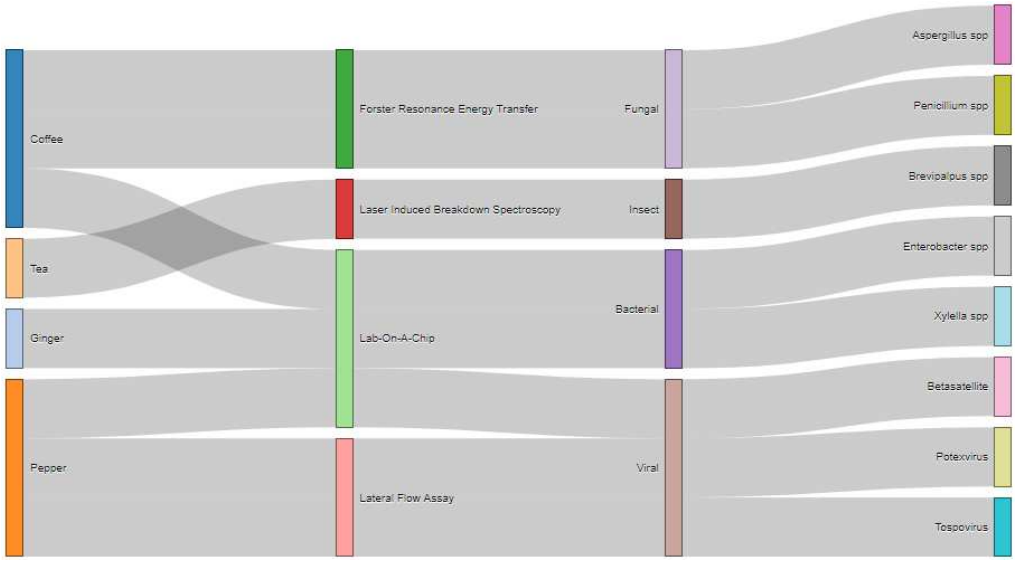

Figure 52: Relations diagram between beverage and spices applications and its respective sensor categories, with linkage to the pathogen/pest's specific category and genera

5.3 Cereals

Been globaly one of the main categories of agricultural crops cultivated for the food industry, cereals represent a wider range of possibilities, in terms of applications, pathogens and sensing techniques. Mentioned by 29 articles, 5 proceedings paper and one review, it's possible to highlight fungi as the main pathogen category, with 88% of the applications been driven to detect it. Wheat, maize and rice appear as the leaders in terms of crop application for all categories, between pathogens and sensors. In terms of of sensor and biosensor, results were concentrated in infrared thermography (25%), förster resonance energy transfer (22%), lab-on-a-chip technologies (18%) and lateral flow assay (18%).

Table 7: Processed results for cereal production systems (ranked by publication date), showing associations between applications, sensor categories and pathogens/pests, specifically for each author

Application		Sensor Category	Detection Mode	Pathogen / Pest		Authors
Maize		Lateral Flow Assay	Direct	Ustilago_maydis	Magnaporthe_oryzae	(BURNHAM-MARUSICH et al., 2018)
Maize Millet	Rice Wheat	Lab-On-A-Chip	Direct	Curvularia_lunatus Fusarium_graminearum	Bipolaris_spp Magnaporthe_grisea	(KIM et al., 2018)
Wheat		Infrared Thermography	Indirect	Fusarium_culmorum	Fusarium_graminearum	(MASRI et al., 2017)
Maize		Flow Cytometry	Direct	Fusarium_verticillioides Fusarium_sporotrichoides	Fusarium_proliferatum_conidia Fusarium_graminearum	(BANATI et al., 2017)
Maize		FRET	Indirect	Aspergillus_flavus		(DE SAEGER; LOGRIECO, 2017)
Maize		Lateral Flow Assay	Direct	Aspergillus_flavus		(KACHAPULULA; AKELLO; BANDYOPADHYAY, 2017)
Wheat		FRET	Direct	Tilletia_indica		(KASHYAP; KUMAR; SRIVASTAVA, 2017)
Wheat		Infrared Thermography	Indirect	Puccinia_triticina		(KHANAL; FULTON; SHEARER, 2017)
Barley	Wheat	FRET	Indirect	Puccinia_triticina	Blumeria_graminis	(MAHLEIN, 2016)
Barley Maize	Rye Wheat	Lab-On-A-Chip	Direct	Puccinia_striiformis Fusarium_culmorum		(FANG; RAMASAMY, 2015)
Barley Maize Oat	Sorghum Wheat	FRET	Direct	Aspergillus_spp Penicillium_spp		(QIAN et al., 2015)
Barley		FRET	Indirect	Blumeria_graminis		(ELLINGER; VOIGT, 2014)
Rice		Lateral Flow Assay	Direct	Rice_tungro_bacilliform_virus_RTBV		(UDA et al., 2014)
Wheat		Infrared Thermography	Indirect	Stagonospora_nodorum		(ANTONUCCI et al., 2013)
Maize	Wheat	Lateral Flow Assay	Direct	Pantoea_stewartii		(CHEN et al., 2013)
Wheat		Infrared Thermography	Indirect	Fusarium_spp		(MAHLEIN et al., 2012)
Maize		Lateral Flow Assay	Direct	Cladosporium_herbarum Fusarium_spp	Gibberella_spp	(ROBERTSON et al., 2011)
Wheat		Infrared Thermography	Indirect	Blumeria_graminis		(VADIVAMBAL; JAYAS, 2011)
Wheat		Lateral Flow Assay	Direct	Fusarium_graminearum		(CHRPOVA et al., 2008)
Oat	Wheat	Lateral Flow Assay	Direct	Fusarium_spp		(MOLINELLI et al., 2008)
Barley Maize	Wheat	Infrared Thermography	Indirect	Fusarium_spp		(SCHMALE; DINGUS; REINHOLTZ, 2008)
Wheat		Infrared Thermography	Indirect	Puccinia_striiformis		(HUANG et al., 2007)
Rice		Fiber-Optic Biosensor	Indirect	Cnaphalocrocis_medinalis	Nilaparvata_lugens	(YANG; CHENG; CHEN, 2007)
Wheat		Infrared Thermography	Indirect	Puccinia_triticina	Septoria_tritici	(LENTHE; OERKE; DEHNE, 2007)
Wheat		Infrared Thermography	Direct	Puccinia_striiformis		(MOSHOU et al., 2006)
Wheat		Infrared Thermography	Indirect	Blumeria_graminis Puccinia_striiformis		(HELLEBRAND et al., 2006)
Wheat		Fiber-Optic Biosensor	Indirect	Podosphaera_fusca		(GRAEFF; LINK; CLAUPEIN, 2006)
Wheat		Laser Induced Breakdown Spectroscopy	Indirect	Erysiphe_graminis Puccinia_recondita	Septoria_tritici	(TARTACHNYK; RADEMACHER; KUEHBAUCH, 2006)
Wheat		FRET	Direct	Tilletia_indica		(TAN; MURRAY, 2005)
Wheat		Infrared Thermography	Indirect	Puccinia_striiformis		(MOSHOU et al., 2005)
Wheat		Infrared Thermography	Indirect	Septoria_tritici		(NICOLAS, 2004)
Rice		Fiber-Optic Biosensor	Indirect	Nilaparvata_lugens		(YANG; CHENG, 2001)
Cereal Irrigation		Fiber-Optic Biosensor	Direct	Spinosyn_A		(LEE; WALT; NUGENT, 2001)
Wheat		Infrared Thermography	Indirect	Fusarium_graminearum		(DELWICHE; KIM, 2000)

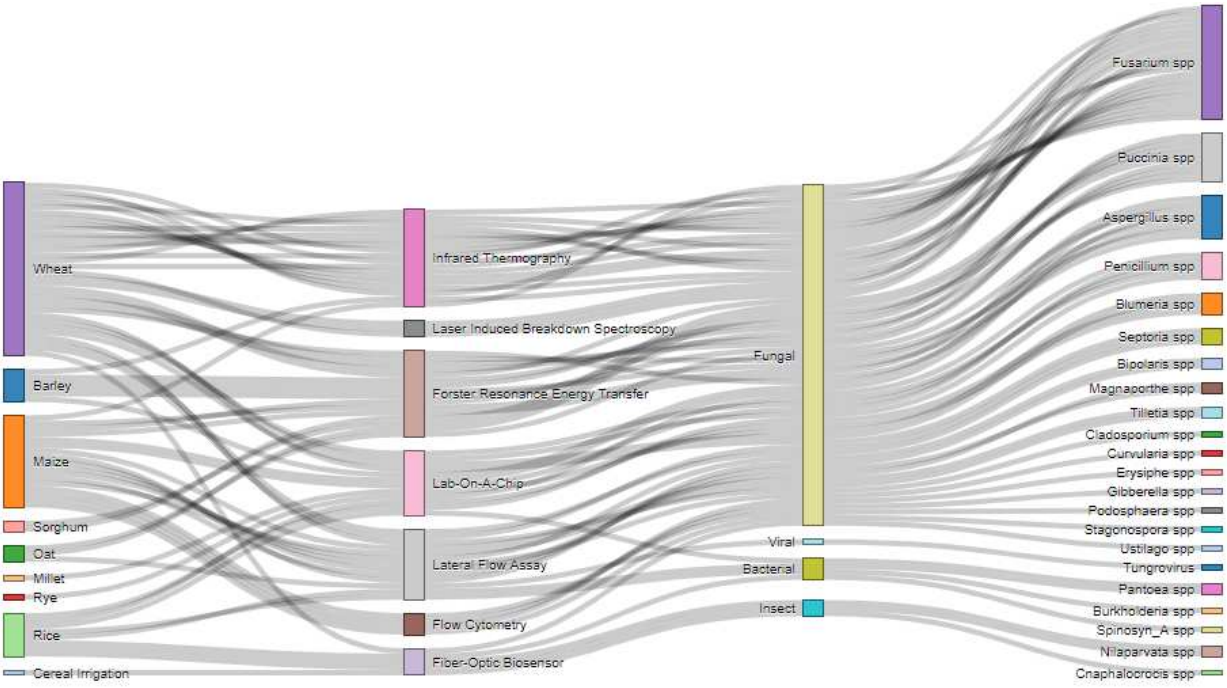

Figure 53: Relations diagram between cereals applications and its respective sensor categories, with linkage to the pathogen/pest's specific category and genera

5.4 Fruits And Nuts

Summarizing a large number of species, in terms of application, fruits and nuts results were concentrated in 50 articles and 2 reviews. Its most representatives sensing techniques are infrared thermography (32%), lateral flow assay (27%) and lab-on-a-chip (15%), mostly divided into citrus (28%), grapevine (18%) and apple (13%) as major varieties. Among bacterial and fungi, the leading genera for detection were candidatus_liberibacter spp, xanthomonas spp, plasmopara spp, xylella spp, aspergillus spp and venturia spp.

Table 8: Processed results for fruits and nuts production systems (ranked by publication date), showing associations between applications, sensor categories and pathogens/pests, specifically for each author

Application	Sensor Category	Detection Mode	Pathogen / Pest	Authors
Citrus	Lateral Flow Assay	Direct	Botrytis_cinerea	(BURNHAM-MARUSICH et al., 2018)
Apple Pear Pomegranate Strawberry	Lab-On-A-Chip	Direct	Penicillium_expansum Xanthomonas_axonopodis Botrytis_cinerea	(KIM et al., 2018)
Banana	Lateral Flow Assay	Direct	Xanthomonas_campestris	(NAKATO; MAHUKU; COUTINHO, 2018)
Apple	FRET	Indirect	Pezicula_malicorticis	(PIECZYWEK et al., 2018).
Melon	Infrared Thermography	Indirect	Dickeya_dadantii	(PINEDA et al., 2018)
Peanut	FRET	Indirect	Aspergillus_parasiticus	(DE SAEGER; LOGRIECO, 2017)
Peanut	Lateral Flow Assay	Direct	Aspergillus_parasiticus	(KACHAPULULA; AKELLO; BANDYOPADHYAY, 2017)
Citrus Peanut Grapevine	FRET	Direct	Candidatus_Phytoplasma_aurantifolia Aspergillus_niger	(KASHYAP; KUMAR; SRIVASTAVA, 2017)
Apple Grapevine	Infrared Thermography	Indirect	Venturia_inaequalis Plasmopara_viticola	(KHANAL; FULTON; SHEARER, 2017)
Almond Stone_Fruit	Lateral Flow Assay	Direct	Xanthomonas_arboricola	(LÓPES-SORIANO et al., 2017)
Citrus	Laser Induced Breakdown Spectroscopy Lateral Flow Assay	Direct	Candidatus_Liberibacter_africanus Candidatus_Liberibacter_americanus Candidatus_Liberibacter_asiaticus	(RANULFI et al., 2017) (DANDEKAR et al., 2010)
Apple Pear Stone_Fruit	Lateral Flow Assay	Direct	Candidatus_Phytoplasma_mali	(VALASEVICH, 2017)
Peach	Flow Cytometry	Indirect	Penicillium_expansum	(ZHANG et al., 2017)
Avocado	Infrared Thermography	Indirect	Pepper_mild_mottle_virus_PMMV	(BARÓN; PINEDA; PÉREZ-BUENO, 2016)
Grapevine	Lab-On-A-Chip	Direct	Plasmopara_viticola	(DUFOUR et al., 2016)
Grapevine	Lab-On-A-Chip	Direct	Xylella_fastidiosa	(HAO et al., 2016)
Citrus	FRET	Direct	Citrus_tristeza_virus	(SHOJAEI et al., 2016)
Date_fruit	Laser Induced Breakdown Spectroscopy	Indirect	Rhynchophorus_ferrugineus	(FAROOQ et al., 2015)
Orange Citrus Strawberry	Infrared Thermography	Indirect	Candidatus_Liberibacter_africanus Candidatus_Liberibacter_americanus Candidatus_Liberibacter_asiaticus	(GAGO et al., 2015) (LI et al., 2014) (SANKARAN et al., 2013)
Banana	Lateral Flow Assay	Direct	Xanthomonas_campestris	(HODGETTS et al., 2015)
Grapevine	FRET	Direct	Aspergillus_spp Penicillium_spp	(QIAN et al., 2015)
Grapevine	Lab-On-A-Chip	Direct	Grapevine_fanleaf_virus_GFLV	(RETTCHER et al., 2015)
Blueberry Citrus Grapevine Pecan	Lab-On-A-Chip	Direct	Xylella_fastidiosa	(WHIDDEN et al., 2015)
Apple	Laser Induced Breakdown Spectroscopy	Indirect	Aeolesthes_holosericea Euzophera_semifuneralis Planococcus_citri	(MA; DONG, 2014)
Kiwifruit	Infrared Thermography	Indirect	Pseudomonas_syringae	(MAES et al., 2014)
Sweet_Cherry	Lateral Flow Assay	Direct	Little_cherry_disease_LCD	(MEKURIA; ZHANG; EASTWELL, 2014)
Citrus	Lateral Flow Assay	Direct	Candidatus_Liberibacter_asiaticus	(RIGANO et al., 2014)
Stone_Fruit	Lateral Flow Assay	Direct	Plum_pox_virus_PPV	(ZHANG et al., 2014)
Apple Grapevine	Infrared Thermography	Indirect	Venturia_inaequalis Plasmopara_viticola	(MAHLEIN et al., 2012)
Citrus	FRET	Direct	Xylella_fastidiosa	(BRADY; FASKE; MITCHELL, 2011)
Apple Pear	Lateral Flow Assay	Direct	Erwinia_amylovora	(BRAUN-KIEWNICK et al., 2011a) (BRAUN-KIEWNICK et al., 2011b)
Apple	Infrared Thermography	Indirect	Venturia_inaequalis	(OERKE; FROEHLING; STEINER, 2011)
Grapevine	Infrared Thermography	Indirect	Plasmopara_viticola	(VADIVAMBAL; JAYAS, 2011) (STOLL et al., 2008) and (STOLL; SCHULTZ; BERKELMANN-LOEHNERTZ, 2008)
Citrus	Lateral Flow Assay	Direct	Xanthomonas_citri Xanthomonas_fuscans	(RIGANO et al., 2010)
Citrus	Laser Induced Breakdown Spectroscopy	Indirect	Candidatus_Liberibacter_asiaticus	(VERBI PEREIRA et al., 2010)
Orange	Infrared Thermography	Indirect	Penicillium_italicum Phytophthora_citrophthora	(SIGHICELLI et al., 2009)
Grapevine	Fiber-Optic Biosensor	Direct	Planococcus_spp Pseudococcus_spp	(NAIDU et al., 2009)
Grapefruit	Infrared Thermography	Indirect	Xanthomonas_axonopodis	(QIN et al., 2008)
Citrus	Fiber-Optic Biosensor	Indirect	Xanthomonas_axonopodis	(BELASQUE; GASPAROTO; MARCASSA, 2008)
Papaya	Lab-On-A-Chip	Direct	Enterobacter_cloacae	(RASOOLY; HEROLD, 2008)
Apple	Infrared Thermography	Indirect	Venturia_inaequalis	(STEINER; BUERLING; OERKE, 2008)
Kiwifruit	Fiber-Optic Biosensor	Indirect	Botrytis_cinerea Sclerotinia_sclerotiorum	(COSTA et al., 2007)
Apple	Infrared Thermography	Indirect	Venturia_inaequalis	(OERKE et al., 2005)
Strawberry	Lateral Flow Assay	Direct	Escherichia_coli	(MUHAMMAD-TAHIR; ALOCILJA, 2004)
Cranberry	Infrared Thermography	Indirect	Pseudomonas_syringae	(WORKMASTER; PALTA; WISNIEWSKI, 2000)

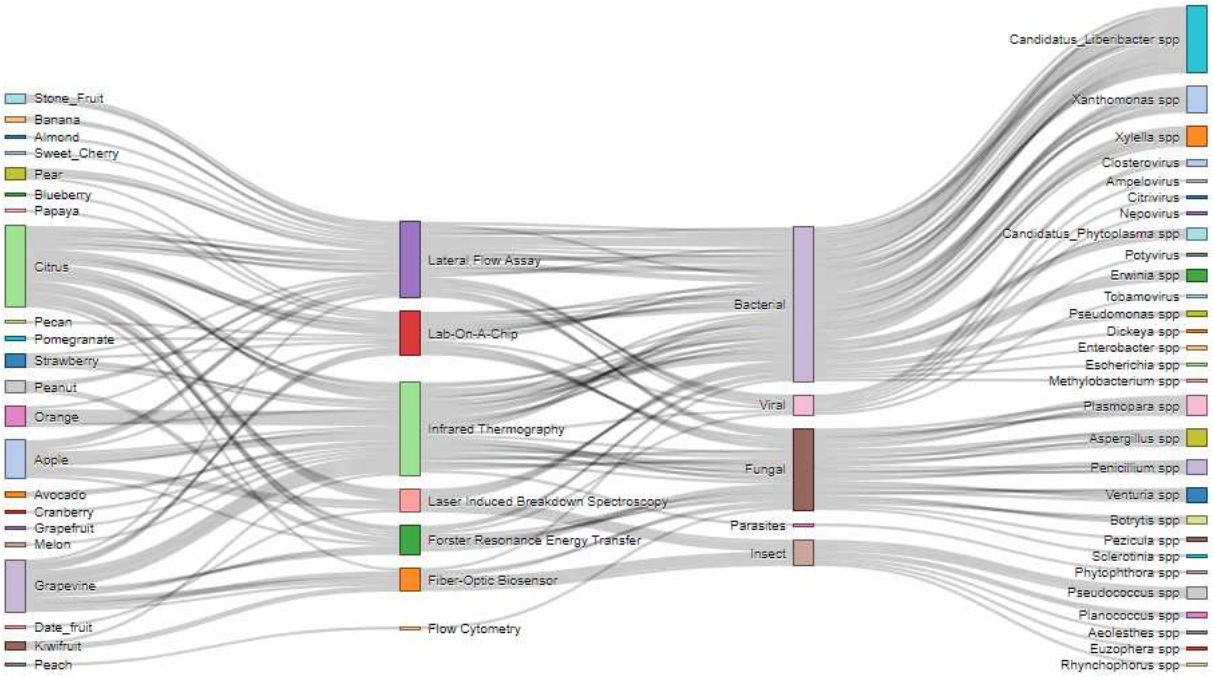

Figure 54: Relations diagram between fruits and nuts applications and its respective sensor categories, with linkage to the pathogen/pest's specific category and genera

5.5 Meat And Dairy

Playing a major role in terms of protein production throughout the agricultural systems, meat and dairy applications were indicated as results in 45 articles, 3 proceedings papers and 2 patent. Among all sensors, lab-on-a-chip technologies and lateral flow assays respresented 80% of all results. In terms of application, the main subcategories were dairy (32%), cattle (23%), poultry (16%) and pork (16%). Compiling results shows approximately 80% of the sensors and biosensors driven to detect pathogens originating from bacteria and fungi, represented in its majority by listeria spp; staphylococcus spp; fusarium spp; campylobacter spp; salmonella spp; candidatus_liberibacter spp; escherichia spp and aspergillus spp.

Table 9: Processed results for meat and dairy production systems (ranked by publication date), showing associations between applications, sensor categories and pathogens/pests, specifically for each author

Application		Sensor Category	Detection Mode	Pathogen / Pest		Authors
Cattle Dairy	Pork Poultry	Lateral Flow Assay	Direct	Escherichia_coli Staphylococcus_aureus	Bacillus_anthracis Salmonella_typhimurium	(BANERJEE; JAISWAL, 2018)
Cattle	Dairy	Lateral Flow Assay	Direct	Aspergillus_fumigatus		(BURNHAM-MARUSICH et al., 2018)
Cattle Poultry Pasture	Pork Dairy	Lab-On-A-Chip	Direct	Candida_albicans Fusarium_semitectum		(KIM et al., 2018)
Dairy		Infrared Thermography	Indirect	Staphylococcus_aureus		(ZANINELLI et al., 2018)
Cattle Dairy Lamb	Pork Poultry	Lab-On-A-Chip	Direct	Campylobacter_jejuni Escherichia_coli Listeria_monocytogenes	Salmonella_typhimurium Staphylococcus_aureus	(ALAHI; MUKHOPADHYAY, 2017)
Cattle Dairy	Lamb Pork	Lab-On-A-Chip	Direct	Staphylococcus_aureus		(SHI et al., 2015) (YANG et al., 2009)
Poultry		Lab-On-A-Chip	Direct	Avian_Influenza		(MOULICK et al., 2017)
Pork		Infrared Thermography Lateral Flow Assay	Indirect Direct	Classical_swine_fever_virus_CSFV		(PETRY et al., 2017) (SAMBANDAM et al., 2017)
Cattle Dairy		Lab-On-A-Chip	Direct	Enterobacter_spp Klebsiella_pneumoniae	Pseudomonas_aeruginosa Staphylococcus_aureus	(RENNER et al., 2017)

Cattle	Dairy	Flow Cytometry	Direct	Bovine_leukemia_virus_BLV	Mycobacterium_avium	(VENEGAS-VARGAS et al., 2017)
Dairy		Fiber-Optic Biosensor	Indirect	Staphylococcus_spp		(ABDELGAWAD et al., 2016)
Ryegrass		Lateral Flow Assay	Direct	Rathayibacter_toxicus		(ARIF et al., 2016)
Poultry		Lateral Flow Assay	Direct	Salmonella_Enteritidis		(MOZOLA et al., 2016)
Cattle	Dairy	Infrared Thermography Lateral Flow Assay FRET	Indirect Direct	foot_and_mouth_disease_FMD		(NIEDBALSKI, 2016) (BISWAL et al., 2012) (JAULENT et al., 2007)
Poultry		Lateral Flow Assay	Direct	Campylobacter_coli	Campylobacter_jejuni	(SCHALLEGGER et al., 2016)
Cattle	Dairy	FRET	Direct	Bovine_leukemia_virus_BLV		(YANG et al., 2016a) / (YANG et al., 2016b)
Pasture		Infrared Thermography	Indirect	Alternaria_spp		(BARANOWSKI; JEDRYCZKA; MAZUREK, 2015)
Poultry		Lab-On-A-Chip	Direct	Campylobacter_spp		(MORANT-MIÑANA; ELIZALDE, 2015)
Poultry		Lab-On-A-Chip	Direct	Avian_Influenza		(JIANG et al., 2015)
Cattle		Lab-On-A-Chip	Indirect	Escherichia_coli		(LAMOUREUX et al., 2015) (TERAO et al., 2015)
Pork		Lab-On-A-Chip	Indirect	hepatitis_E_virus_HEV	Yersinia_spp	(MEYER et al., 2015)
Cattle	Dairy	Lateral Flow Assay	Direct	Filamentous_fungi		(THORNTON; WILLS, 2015)
Dairy		Forster Resonance Energy Transfer	Direct	Bacillus_thuringiensis Listeria_monocytogenes	Salmonella_Typhimurium	(BURRIS et al., 2012)
Pasture		Lateral Flow Assay	Direct	Pantoea_stewartii		(CHEN et al., 2013)
Cattle Dairy	Lamb Pork	Lateral Flow Assay	Direct	Listeria_spp		(ALLES et al., 2012)
Dairy		Infrared Thermography	Indirect	Staphylococcus_aureus		(FRANZE et al., 2012) (BERRY et al., 2003)
Pork		Lateral Flow Assay	Direct	Porcine_circovirus_2		(JIN et al., 2012)
Dairy		Lateral Flow Assay	Direct	Staphylococcus_aureus		(MULDOON et al., 2012)
Cattle Poultry	Turkey	Lateral Flow Assay	Direct	Salmonella_spp		(HOERNER et al., 2011) (JAGADEESAN et al., 2011)
Poultry		Lab-On-A-Chip	Direct	Salmonella_enterica		(JENKINS et al., 2011)
Dairy		Fiber-Optic Biosensor	Direct	Melamine		(QIN; CHAO; KIM, 2010)
Cattle Dairy Lamb	Pork Poultry	Flow Cytometry	Direct	Toxoplasma_gondii		(SHAPIRO et al., 2010)
Cattle	Dairy	Lateral Flow Assay	Direct	Brucella_melitensis		(BRONSVOORT et al., 2009)
Dairy		Lab-On-A-Chip	Direct	Escherichia_coli		(MULVANEY et al., 2009)
Cattle	Dairy	FRET	Direct	Mycobacterium_avium		(SPANGLER; SPANGLER; TARTER, [s.d.])
Pork		Flow Cytometry	Direct	Salmonella_Typhimurium		(BOYEN et al., 2007)
Dairy		Flow Cytometry	Direct	Brucella_melitensis Corynebacterium_bovis Enterobacter_aerogenes Escherichia_coli Klebsiella_spp Mycoplasma_spp Pasteurella_spp	Proteus_spp Prototheca_spp Pseudomonas_aeruginosa Staphylococcus_spp Streptococcus_spp Trueperella_pyogenes	(KOESS; HAMANN, 2008)
Cattle Dairy Lamb Pork Poultry		Lab-On-A-Chip	Direct	Bacillus_spp Campylobacter_spp Clostridium_spp Enterococcus_spp Escherichia_coli Helicobacter_pylori	Klebsiella_pneumoniae Lactococcus_lactis Listeria_spp Salmonella_spp Staphylococcus_aureus	(RASOOLY; HEROLD, 2008)
Poultry		Flow Cytometry	Indirect	Infectious_bronchitis_virus	Newcastle_disease_ND	(QIU et al., 2007)
Pasture		Fiber-Optic Biosensor	Direct	Cucumber_mosaic_virus	Oilseed_rape_mosaic_virus	(HUANG et al., 2005)
Cattle	Dairy	Lateral Flow Assay	Direct	Mycobacterium_paratuberculosis		(MUHAMMAD-TAHIR; ALOCILJA; GROOMS, 2005)
Cattle		Infrared Thermography	Indirect	bovine_viral_diarrhoea_BVD_virus		(SCHAEFER et al., 2004)
Cattle		Lab-On-A-Chip	Direct	bovine_viral_diarrhoea_BVD_virus		(DITCHAM et al., 2001)

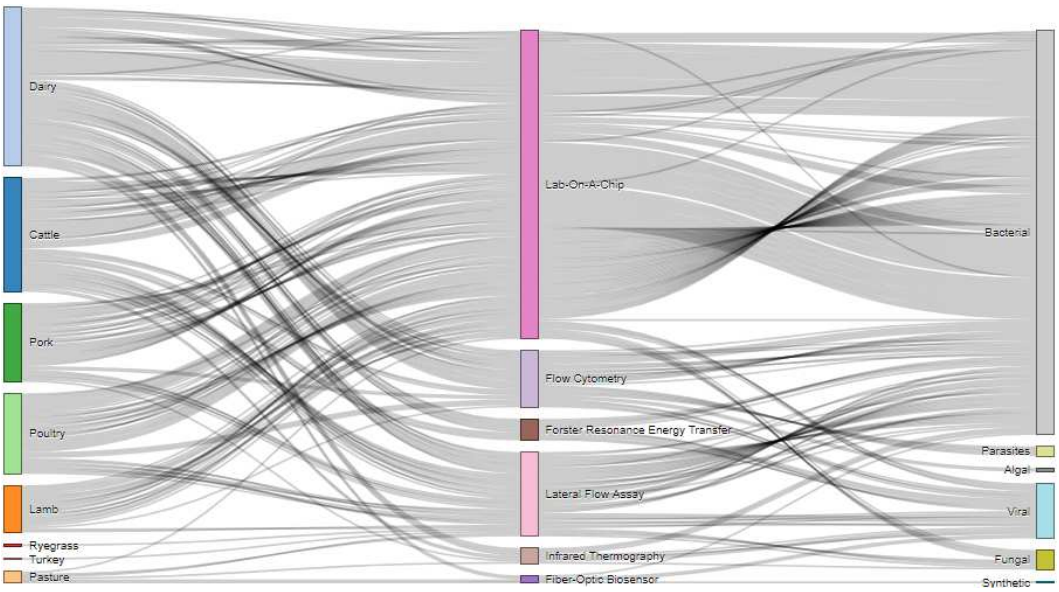

Figure 55: Relations diagram between meat and dairy applications and its respective sensor categories, with linkage to the pathogen/pest's specific category

5.6 Vegetables and Legumes

The vegetables and legumes category indicated results in 47 articles, 4 proceedings papers and one review. In terms of sensors and biosensors used, the leading methods are based in lateral flow assay (31%), infrared thermography (26%), lab-on-a-chip (19%) and förster resonance energy transfer (12%). Among all 19 varieties mentioned in the results, the largest number of sensor techniques are related to tomato, potato, sugar beet, cucurbits, bean and lettuce. Considering the pathogens which these techniques intend to detect, results indicated them typically diffused among fungi (37%) and bacteria (31%), divided almost equally into 47 genera of pathogens.

Table 10: Processed results for vegetables and legumes production systems (ranked by publication date), showing associations between applications, sensor categories and pathogens/pests, specifically for each author

Application	Sensor Category	Detection Mode	Pathogen / Pest	Authors
Lettuce	Lateral Flow Assay	Direct	Salmonella_typhimurium	(BANERJEE; JAISWAL, 2018)
Bean Carrot Garlic Onion Tomato	Lateral Flow Assay	Direct	Stromatinia_cepivora	(BURNHAM-MARUSICH et al., 2018)
Tomato	Flow Cytometry	Direct	Clavibacter_michiganensis	(HAN et al., 2018)
Sugar_Beet	Infrared Thermography	Indirect	Heterodera_schachtii	(JOALLAND et al., 2018)
Avocado Bean Cucurbits Onion Potato Tomato	Lab-On-A-Chip	Direct	Cotrichum_gloeosporioides Fusarium_oxysporum Botrytis_cinerea Alternaria_alternate Phoma_destructiva Pseudomonas_syringae	(KIM et al., 2018)
Potato	Lateral Flow Assay	Direct	Potato_virus_X_PVX	(RAZO et al., 2018)
Cucurbits	Lateral Flow Assay	Direct	Acidovorax_citrulli	(ZENG et al., 2017)
Tomato	Flow Cytometry	Direct	Pectobacterium_carotovorum	(AHMED; ARIF; ALVAREZ, 2017)
Potato	Infrared Thermography	Indirect	Frankliniella_tuberosi Liriomyza_huidobrensis Myzus_persicae Phytophthora_infestans	(FAYE et al., 2017)
Onion Potato Sugar_Beet Tomato	FRET	Direct	Aspergillus_niger Phytophthora_spp Polymyxa_betae Phytophthora_spp Xanthomonas_axonopodis	(KASHYAP; KUMAR; SRIVASTAVA, 2017)
Cucurbits Sugar_Beet	Infrared Thermography	Indirect	Pseudoperonospora_cubensis Cercospora_beticola	(KHANAL; FULTON; SHEARER, 2017)
Sugar_Beet	Infrared Thermography	Indirect	Phythium_aphanidermatum	(MARTINEZ et al., 2017)
Zucchini	Infrared Thermography	Indirect	Dickeya_dadantii Podosphaera_fusca	(PINEDA et al., 2017)
Potato	Lateral Flow Assay	Direct	Dickeya_dianthicola Dickeya_solani	(SAFENKOVA et al., 2017)
Cucumber	Infrared Thermography	Indirect	Pseudoperonospora_cubensis	(SIMKO; JIMÉNEZ-BERNI; SIRAULT, 2017)
Bean Lettuce Sugar_Beet	FRET	Indirect	Xanthomonas_fuscans Bremia_lactucae Cercospora_beticola	(MAHLEIN, 2016)
Potato	Lateral Flow Assay	Direct	Ralstonia_solanacearum	(PANFEROV et al., 2016)
Zucchini	FRET	Indirect	Dickeya_dadantii	(PÉREZ-BUENO et al., 2016)
Tomato	Lateral Flow Assay	Direct	Tomato_spotted_wilt_virus_TSWV	(SZOSTEK et al., 2016)
Brussels_sprout	Lateral Flow Assay	Direct	Alternaria_brassicae	(WAKEHAM, ALISON J.; KEANE, GARY; KENNEDY, 2016)
Bean Cucurbits Lettuce Potato Tomato	Lab-On-A-Chip	Direct	Cowpea_mosaic_virus_CPMV Cucumber_mosaic_virus_CMV Lettuce_mosaic_virus_LMV Phytophthora_infestans Potato_virus_Y_PVY	(FANG; RAMASAMY, 2015)
Lettuce	Lab-On-A-Chip	Direct	Listeria_monocytogenes	(HUANG et al., 2015)
Bean	Infrared Thermography	Indirect	Pseudomonas_syringae	(PÉREZ-BUENO et al., 2015)
Bean	FRET	Direct	Aspergillus_spp Penicillium_spp	(QIAN et al., 2015)
Tomato	Infrared Thermography	Indirect	Oidium_neolycopersici	(RAZA et al., 2015)
Radish Tomato	Lateral Flow Assay	Direct	Escherichia_coli	(TERAO et al., 2015)
Potato	Lateral Flow Assay	Direct	Clavibacter_michiganensis	(SAFENKOVA et al., 2015)
Tomato	FRET	Indirect	Oidium_neolycopersici	(ELLINGER; VOIGT, 2014)
Tomato	Lab-On-A-Chip	Direct	Fusarium_oxysporum	(MALAPI-WIGHT et al., 2014)
Cucumber	Infrared Thermography	Indirect	Fusarium_oxysporum	(WANG et al., 2013) /(WANG et al., 2012)
Carrot Potato	Flow Cytometry	Direct	Pectobacterium_carotovorum	(FRÖHLING et al., 2012)
Cucumber	Infrared Thermography	Indirect	Pseudoperonospora_cubensis	(MAHLEIN et al., 2012)
Potato	Lateral Flow Assay	Direct	Spongospora_subterranea	(BOUCHEK-MECHICHE; MONTFORT; MERZ, 2011)
Moringa oleifera	Laser Induced Breakdown Spectroscopy	Indirect	Escherichia_coli Klebsiella_pneumonia Pseudomonas_aeruginosa Staphylococcus_aureus	(MEHTA et al., 2011)
Cucumber	Infrared Thermography	Indirect	Pseudoperonospora_cubensis	(VADIVAMBAL; JAYAS, 2011)
Potato	Lateral Flow Assay	Direct	Clavibacter_michiganensis	(EL-BADRY; EL-HADDAD; ELPHINSTONE, 2009)
Onion	Infrared Thermography	Indirect	Burkholderia_cepacia	(WANG et al., 2009)
Sugar_Beet	Infrared Thermography	Indirect	Cercospora_beticola Erysiphe_betae Heterodera_schachtii Ramularia_beticola Rhizoctonia_solani Uromyces_betae	(HILLNHUETTER; MAHLEIN, 2008)

Onion		Lab-On-A-Chip	Direct	Enterobacter_cloacae		(RASOOLY; HEROLD, 2008)
Sugar_Beet		Infrared Thermography	Indirect	Cercospora_beticola		(CHAERLE et al., 2004)
Tomato		Fiber-Optic Biosensor	Indirect	Liriomyza_spp		(XU et al., 2007)
Spinach	Tomato	Lab-On-A-Chip	Direct	Salmonella_spp	Escherichia_coli	(SAGE, 2007)
Potato		Lateral Flow Assay	Direct	Spongospora_subterranea		(PORTA-PUGLIA; MIFSUD, 2006)
Tomato		Infrared Thermography	Indirect	Phytophthora_infestans		(ZHANG; QIN; LIU, 2005)
Lettuce		FRET	Direct	Pyrenochaeta_lycopersici		(DUFRESNE; JENNI; FORTIN, 2004)
Alfalfa	Lettuce	Lateral Flow Assay	Direct	Escherichia_coli		(MUHAMMAD-TAHIR; ALOCILJA, 2004)
Soil		Lateral Flow Assay	Direct	Rhizoctonia_solani		(THORNTON et al., 2004)
Tomato		Infrared Thermography	Indirect	Phytophthora_infestans		(ZHANG et al., 2003)
Eggplant Potato	Tomato	Lateral Flow Assay	Direct	Pepino_mosaic_virus_PepMV		(SALOMONE; ROGGERO, 2002)
Tomato		Infrared Thermography	Indirect	Pseudomonas_syringae		(WISNIEWSKI; GLENN; FULLER, 2002)

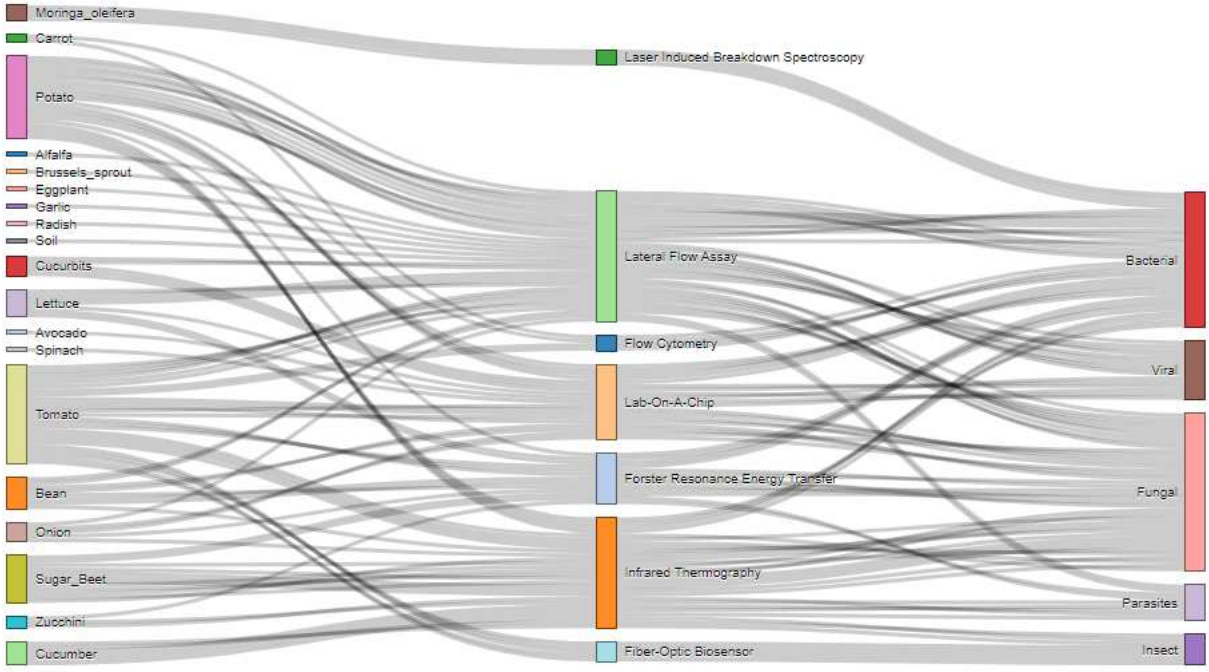

Figure 56: Relations diagram between vegetables and legumes applications and its respective sensor categories, with linkage to the pathogen/pest's specific category

5.7 Others

With the largest variety of different possible crops gathered into this category, results revealed 25 articles, 4 proceedings papers and one review. The respective sensors and biosensors are typical based in lab-on-a-chip technologies (38%), infrared thermography (27%) and lateral flow assay (20%), all designed for pathogen detection among mostly fungi and viruses, which sums up more than 70% of the results. The main genera are: tobamovirus, xylella spp, potyvirus, fusarium spp, potexvirus, gloeophyllum spp, spodoptera spp, peronospora spp, verticillium spp, phoma spp, phytophthora spp, phakopsora spp. As to the agricultural applications, tobacco, flowers, cotton, forestry and soybean emerge as the leading varieties.

Table 11: Processed results for other applications in agricultural systems (ranked by publication date), showing associations between applications, sensor categories and pathogens/pests, specifically for each author

Application	Sensor Category	Detection Mode	Pathogen / Pest		Authors
Olive	Lab-On-A-Chip	Direct	Xylella_fastidiosa		(CHIRIACÒ et al., 2018)
Cotton Forestry Hemp Hops Soybean Sugarcane	Lab-On-A-Chip	Direct	Helicoverpa_armigera Spodoptera_litura Chaetomium_globosum Gloeophyllum_abietinum Gloeophyllum_trabeum	Phanerochaete_sordida Phoma_herbarum Fusarium_semitectum Spodoptera_litura	(KIM et al., 2018)
Flower	Infrared Thermography	Indirect	Podosphaera_fusca		(MINAEI; JAFARI; SAFAIE, 2018)
Soybean	Laser Induced Breakdown Spectroscopy	Indirect	Aphelenchoides_besseyi		(RANULFI et al., 2018)
Cotton	Lab-On-A-Chip	Direct	Geminiviridae_spp		(TAHIR et al., 2018)
Canola Palm	FRET	Direct	Sclerotinia_sclerotiorum Ganoderma_boninense		(KASHYAP; KUMAR; SRIVASTAVA, 2017)
Olive Rose	Infrared Thermography	Indirect	Verticillium_dahliae	Peronospora_sparsa	(KHANAL; FULTON; SHEARER, 2017)
Sugarcane	Lateral Flow Assay	Direct	Candidatus_Phytoplasma_mali		(NAIDOO et al., 2017)
Tobacco	Laser Induced Breakdown Spectroscopy	Indirect	Tobacco_mosaic_virus_TMV		(PENG et al., 2017)
Tobacco	Infrared Thermography	Indirect	Dickeya_dadantii		(BARÓN; PINEDA; PÉREZ-BUENO, 2016)
Soybean	Lateral Flow Assay	Direct	Soybean_mosaic_virus		(ZHU et al., 2016)
Canola	Infrared Thermography	Indirect	Alternaria_spp		(BARANOWSKI; JEDRYCZKA; MAZUREK, 2015)
Tobacco	Lab-On-A-Chip	Direct	Tobacco_mosaic_virus_TMV Tobacco_rattle_virus_TRV		(FANG; RAMASAMY, 2015)
Flower	Lateral Flow Assay	Direct	Xanthomonas_axonopodis		(JUN-HAI et al., 2015)
Forestry	Infrared Thermography	Indirect	Dothistroma_septosporum		(SMIGAJ et al., 2015)
Tobacco	Lab-On-A-Chip	Direct	Xylella_fastidiosa		(WHIDDEN et al., 2015)
Opium_Poppy	Infrared Thermography	Indirect	Peronospora_arborescens		(CALDERÓN et al., 2014)
Cotton	Flow Cytometry	Direct	Fusarium_oxysporum	Verticillium_dahliae	(NI et al., 2012)
Flower	Lab-On-A-Chip	Direct	Cymbidium_mosaic_virus_CymMV Tomato_spotted_wilt_virus_TSWV		(CHANG et al., 2012)
Sugar_beet Tobacco	Infrared Thermography	Indirect	Cercospora_beticola Tobacco_mosaic_virus_TMV		(MAHLEIN et al., 2012)
Tobacco	Infrared Thermography	Indirect	Tobacco_mosaic_virus_TMV		(VADIVAMBAL; JAYAS, 2011)
Flower	Lateral Flow Assay	Direct	Phytophthora_ramorum		(BULAJI et al., 2010)
Tulip	Infrared Thermography	Indirect	Tulip_breaking_virus_TBV		(POLDER et al., 2010)
Soybean	Lab-On-A-Chip	Direct	Phakopsora_pachyrhizi		(MENDES et al., 2009a) / (MENDES et al., 2009b)
Cotton	Infrared Thermography	Indirect	Phymatotrichopsis_omnivora		(HUANG et al., 2008)
Forestry	Lateral Flow Assay	Direct	Phytophthora_ramorum		(KOX et al., 2007)
Flower	Fiber-Optic Biosensor	Indirect	Phytoplasma_spp		(CHOI et al., 2004)
Flower	Lateral Flow Assay	Direct	Calibrachoa_mottle_virus		(LIU et al., 2003)
Tobacco	Lateral Flow Assay	Direct	Pepino_mosaic_virus_PepMV		(SALOMONE; ROGGERO, 2002)

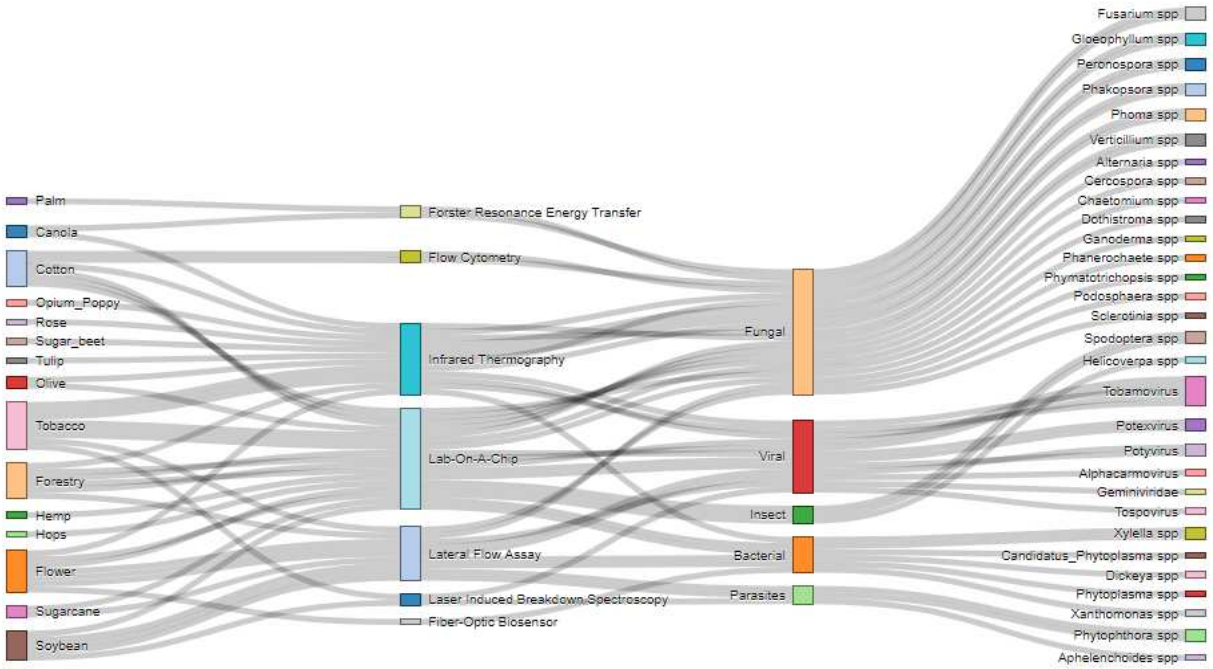

Figure 57: Relations diagram between other applications in agricultural systems and its respective sensor categories, with linkage to the pathogen/pest's specific category and genera

5.8 Combined Results

In order to compare each sensor and biosensor technique along with its agricultural and pathogen/pest applications, it is vital to consider not only the respective occurrence and importance (through academic records), but also its versatility, which is measured by the number of different relationships between these previous factors. The relations diagram between all categories (application, sensors/biosensors and pathogens/pests) can be seen in below.

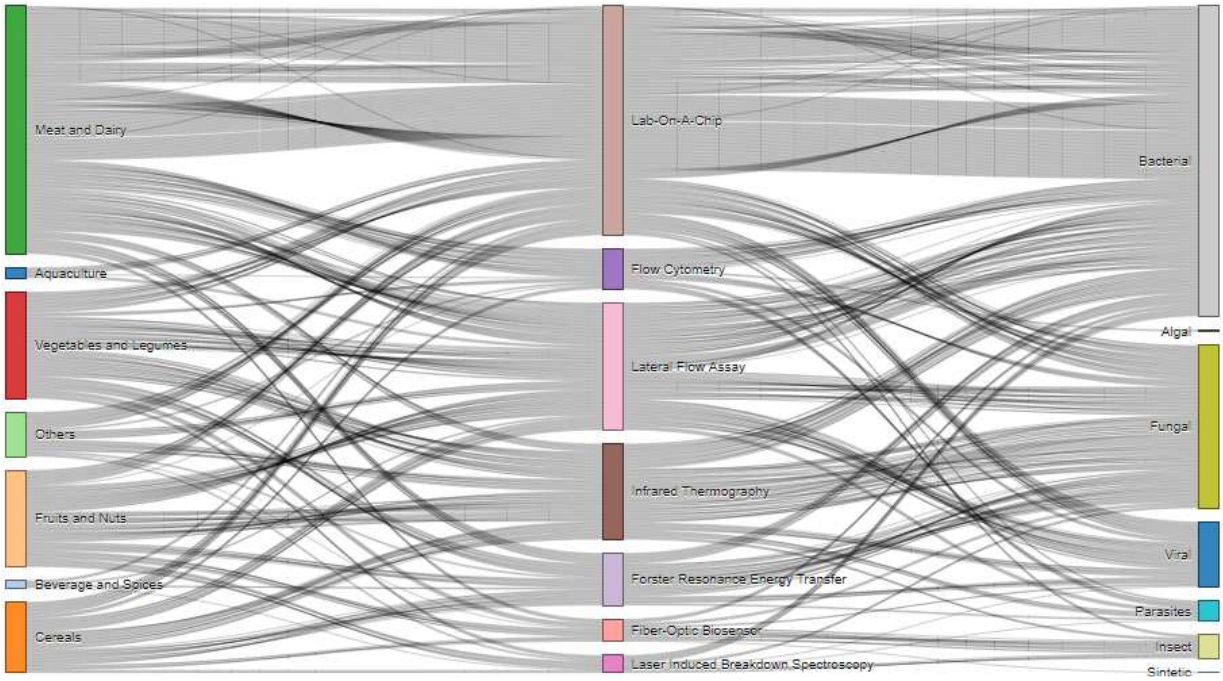

Figure 58: Combined results considering all category relations between agricultural applications and pathogens/pests among all 185 articles and patents, using Orange software for data mining, visualization and qualitative text analysis.

Concerning all agricultural applications considered in this work, the meat and dairy category stands in the forefront, with 42% of all relationship results between sensors/biosensors and pathogens/pests, followed by vegetables and legumes (18%), fruits and nuts (17%), cereals (12%), others (8%), aquaculture (2%) and beverages and spices (1%). Similarly, in relation to the leading technologies in sensors and biosensors, the relationships indicates lab-on-a-chip technologies as the most versatile technique with 39% of all results. Subsequently, comes lateral flow assays (22%), infrared thermography (16%), förster resonance energy transfer (9%), flow cytometry (7%), fiber-optic biosensor (4%) and laser induced breakdown spectroscopy (3%)

Furthermore, with reference to the main pathogen and pest categories considered in this study, it is possible to point out bacterial as the major one with 53% of the relationships found. Immediately followed by fungal (28%), viral (11%), insects (4%) and parasites (4%). Algal and

synthetic, with limited number of ocurrences and relations, appear at zero percentage among all results. Going further into pathogens and pests genera, the most common ones are shown below.

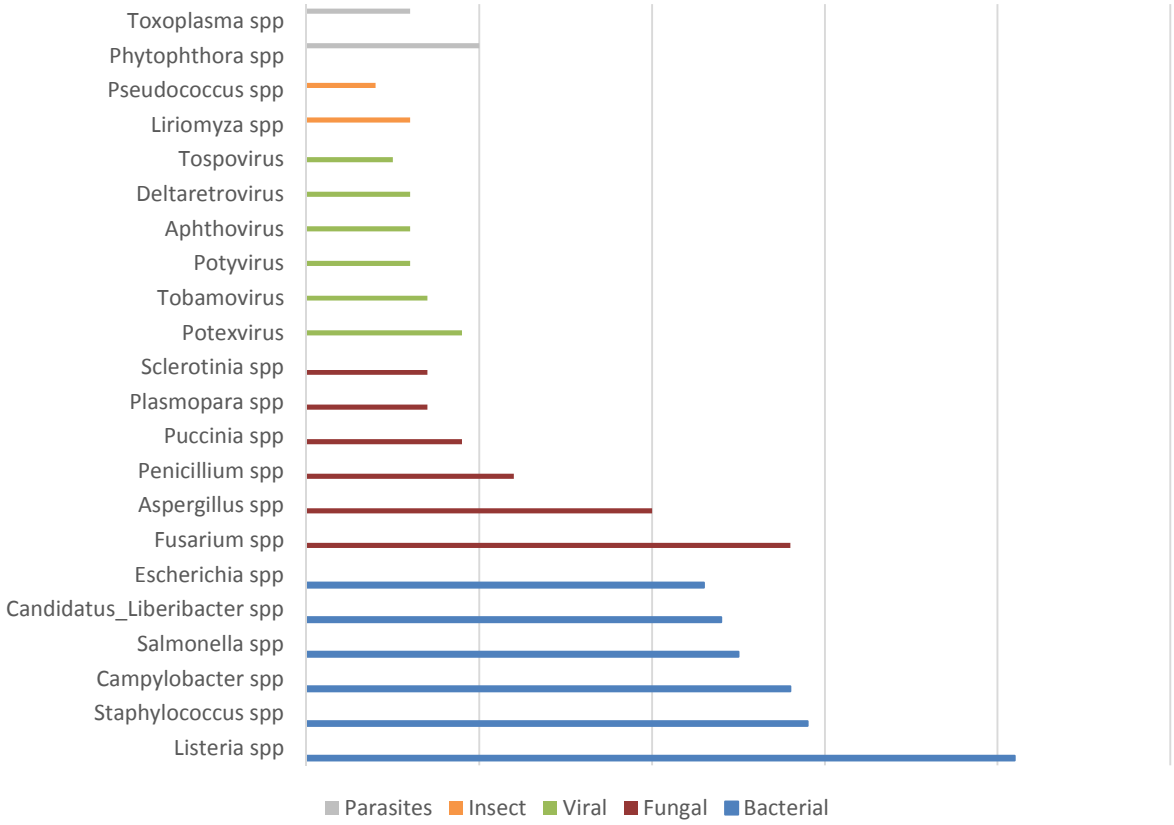

Figure 59: Distribution of most common genera of pathogens and pests detected among sensors and biosensors considered in this work

In spite of having the largest number of results in articles, some types of sensors and biosensors performed differently in terms of relationships between pathogens, pests and agricultural applications. Thus, *i.e.* some technologies were considered more or less versatile, considering practical necessities in agriculture. Figure 60 below shows the comparison of these factors.

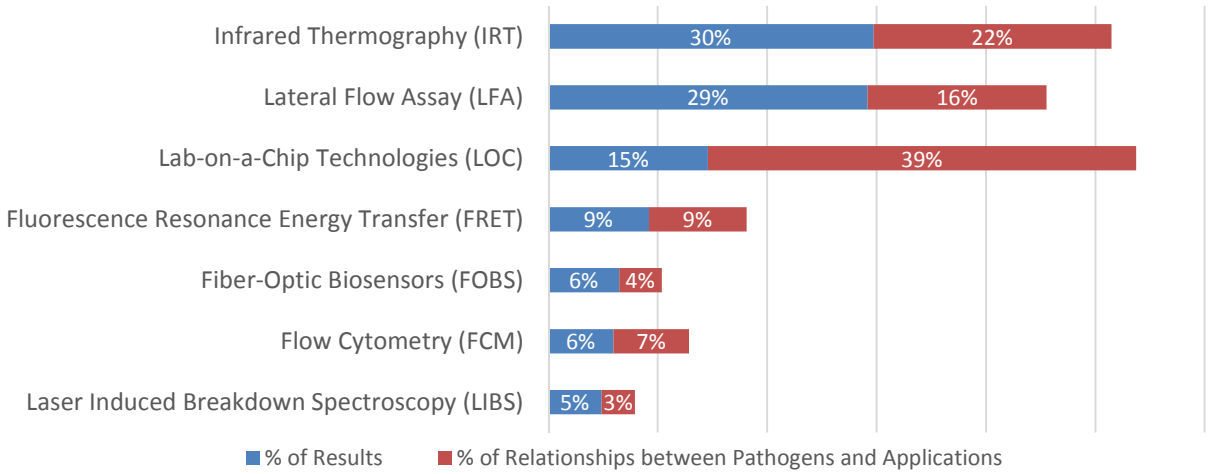

Figure 60: Comparison between number of results (blue) and number of relations of pathogens, pests and agricultural applications

In terms of detection mode, all results were also classified between direct and indirect, with reference to the way in which the sensors and biosensors recognition systems are driven to. Thus, we found that 56% of all results indicated direct detection modes, in opposition to indirect recognition, which designated 44% of all sensors and biosensors operations found throughout this study. We also observe a clear association between some of the categories and its respective detection mode. This phenomenon is typically important in regard to lateral flow assays and lab-on-a-chip technologies, which are found to be mainly direct detection techniques, in contrast to infrared thermography, defined distinctly as indirect. Figure 61 and 62 below shows these splitted results.

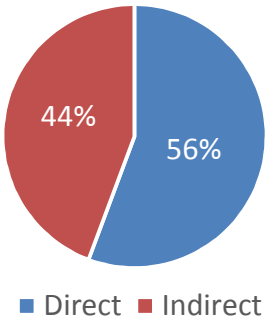

Figure 61: Detection mode classification (direct and indirect) shown as percentage index among all results

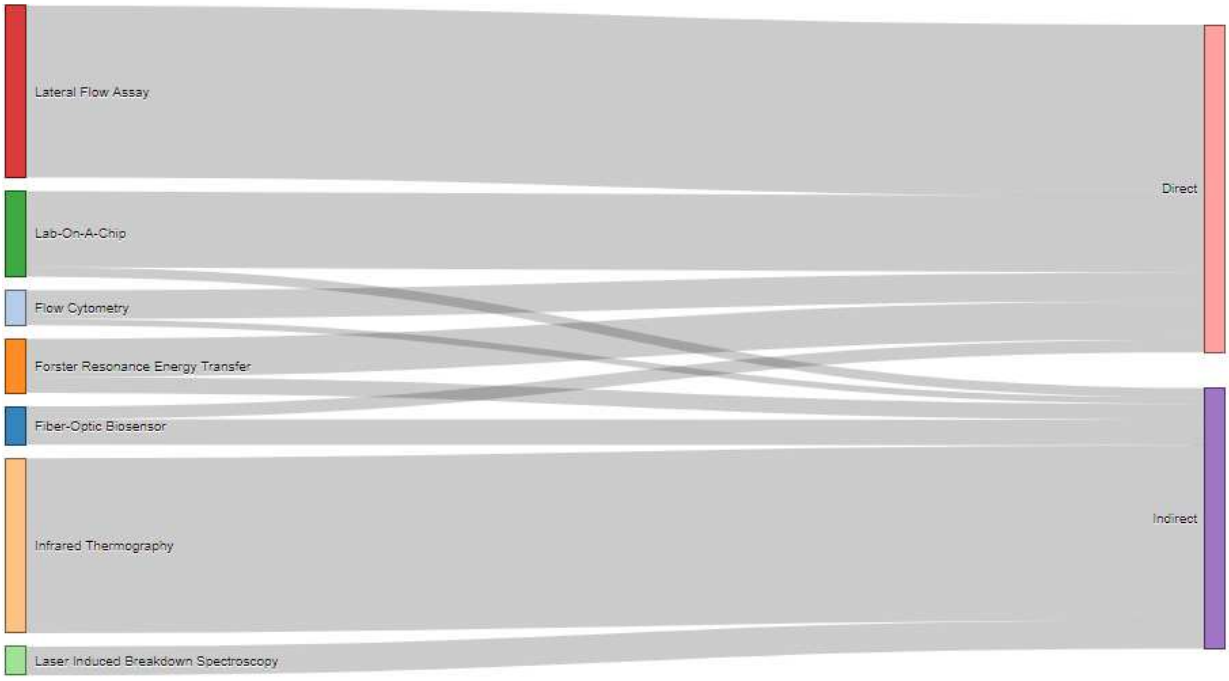

Figure 62: Relations diagram compiling all 185 results, relating all sensor/biosensor categories and its respective detection mode (direct and indirect)

CHAPTER 6: FINAL CONSIDERATIONS

This review of the literature about sensors and biosensors driven to the detection of pathogens ans pests throughout the agricultural systems showed that the majority of the results comes from recent studies. These records typically focus on specific examples of normally one type of sensor/biosensor applied to a low variety of crop or animal production systems, aiming to detect an average of one or two genera of pathogen and pest, simultaneously or not. In general, there is a lack of specific studies combining agricultural application, pathogen and pest hazards and the best sensor or biosensor technique to detect it, and thus meet these requirements, particularly in terms of low cost, portability and real-time analysis.

Mature technologies such as lateral flow assays – developed in the 1980's – still tends to be leader in some agricultural areas like vegetables and legumes as well as meat and dairy, due to its technical simplicity associated with low time-consuming and cost. However, this method also implies some intrinsic aspects equally regarding the manner in which the analysis needs to be performed, like the necessity of reaching the analyte or sample in a near range, and sometimes also fulfill mechanical operations prior to the analysis. Likewise, this study indicates that large equipment dependent techniques as förster resonance energy transfer and flow cytometry are overall still minor technical options, applied only for specific fungal and bacterial detection in dairy and cereals, albeit having major advantages in terms of sensibility and time consuming.

Leading trends towards new technologies in terms of sensor techniques were identified, such as lab-on-a-chip, acting as a major option in aquaculture, beverages and spices, meat and dairy among others agricultural systems. This family of biosensors combines several different types of detection technologies, alongside modern methods like microfluidics, nanostructures and semiconductor devices. Nevertheless, LOC still faces the same limitations as the LFA explained previously. Equally acknowledged as a future trend among sensor-based techniques, infrared thermography and its aerial variation using UAV's (AIRT), have been considered as a promising and low cost method for the detection of pathogens ans pests throughout several different types of crops and animals production systems, particularly in this study, cereals, fruits and nuts, vegetables and legumes, among other categories.

Notwithstanding, laser induced breakdown spectroscopy also arise as a favorable option when it comes to portability and real-time analysis of certain types of crops, like cereals and fruits and nuts, especially for the detection of bacterial infections. It suffers, however, from its relative high cost equipment, despite its excellent portability. Additionally, the same results indicated that fiber-optic biosensors have narrow application possibilities, been only considered for the identification of some specific genera of insects, besides its quite novel technology.

Overall, in this study we reviewed some of the current and promising types of sensors and biosensors developed for the detection of plant and animal diseases, all caused by pathogens such as bacteria, viruses, fungi, as well as pests like insects and parasites. It was detected that although more established sensor techniques such as flow cytometry and förster resonance energy Transfer are already widely available for plant and animal disease detection, they are also relatively difficult to operate, requiring large equipments, limiting its applicability, in terms of point-of-care. Fiber-optic biosensors and laser induced breakdown spectroscopy are alternatively modern, yet both still face issues concerning the spreading of its usage throughout agriculture systems for biological hazards and plague control. Finally, results have indicated lateral flow assay, lab-on-a-chip technologies and infrared thermography (both fixed and aerial) as the most promising categories related to sensors and biosensors driven to the detection of several different pathogenic and pest varieties in agricultural systems.

For future studies, we recommend that not only pathogens ans pests, but also different sorts of agricultural threats may be taken into consideration in terms of academic research. This is particularly important regarding drought issues and heavy metals contamination, which represent major hazards, menacing worldwide agricultural systems. Thus, justifying it as a important scientific subject, enabling the application and review of an even wider range of sensor and biosensors. In addition, collaborative studies in this field could be extended, identifying patterns in agricultural systems over time and analyzing the evolution and declining of the technologies mentioned previously would also be scientifically relevant.

One of the limitations of this study lies upon the lack of data obtained from patents and private research organizations. This is particularly complex to gather due to the way in which these results are commonly described by copyrighters, not been specific enough for academic purposes. Another restriction was towards the use of the data mining and analysis software for further statistical studies, combining regression and correlation, for instance. Despite not been mentioned initially in the objectives, such processed statistical data would provide an even deeper

support for the results presented previously in this work. In addition, another important limitation is the shortage of results – and therefore scientific data – regarding applications in certain specific agricultural categories, despite its relevance, like aquaculture, beverage and spices among others.

The potential of several types of sensors and biosensors for plant and animal disease detection across agricultural systems has been comprehensively reviewed in this study. The advent of new techniques based on nanotechnology, as well as the fusion of different methods – such as UAV and hyperspectral imaging or PCR and microfluidics – resulted in new breakthroughs in terms of agricultural management. Likewise, many of the advances mentioned throughout this study laid the foundation for modern agriculture-based IoT and smart farming.

In this study, we assessed a wide range of relationships within different modern technologies and several disease-causing pathogens and pests, all of them applied to agricultural production systems based on crops as well as animals. The results identified a strong dissemination component, in terms of information and technique application, which indicates the social role and relevance of academical research in agricultural sciences.

Aimed at identifying the recent technological trends and relationships among sensors and biosensors for pathogens and pests detection in the agricultural systems, this study is the result of a multidisciplinary endeavor between different fields of science, typifying what is considered to be the most essencial and perennial characteristic of agricultural sciences. Thereby, contributing positively to the technology innovation approach as a fundamental tool in order to meet even higher yield gaps, mitigating both animal and plant diseases, as well as food security worldwide.

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