Innovative Technologies in Plant Protection and Nutrition: Current Status and Global Trends

Technologies innovantes dans le domaine de la protection et de la nutrition des végétaux : situation actuelle et tendances mondiales

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ABSTRACT. Plants in the environment face constant stresses either biotic or abiotic. These stresses can significantly reduce the productivity of important crops worldwide, with annual crop yield losses ranging from 25% to 50% of the total production. Biotic stress includes herbivores, pests and pathogens. Thus, plants developed a multilayer defence system to prevent the problem of biotic stress which includes the constitutive (SAR) and induced defence system (ISR). The excessive use of synthetic chemicals has detrimental effects on the environment and human health, which discourages pesticide application in the agriculture sector. As a result, researchers worldwide have shifted their focus towards alternative eco-friendly strategies to prevent plant diseases. A variety of biological control agents are available for use. Currently, researchers are exploring the use of beneficial microorganisms as an eco-friendly strategy to control crop diseases. A range of bacterial genera and fungi have demonstrated great potential as biocontrol agents for various plant diseases.
Apart from these, to date, researchers in biotechnology exploring the plant Systemic Acquired Resistance (SAR) and Induced Systemic Resistance (ISR) the role and mode of action against phytopathogens and plant stress.
In this paper an effort has been made to describe the new biostimulants and natural elicitors made by biotechnology and nano technology in the last years, as a new insight to the increase of SAR and ISR in plant defence system.
RÉSUMÉ. Les plantes présentes dans l'environnement sont confrontées à des stress constants, qu'ils soient biotiques ou abiotiques. Ces stress peuvent réduire considérablement la productivité de cultures importantes dans le monde entier, avec des pertes annuelles de rendement allant de 25 % à 50 % de la production totale. Les stress biotiques comprennent les herbivores, les ravageurs et les agents pathogènes. Les plantes ont donc développé un système de défense multicouche pour prévenir le problème du stress biotique, qui comprend le système de défense constitutif (SAR) et le système de défense induit (ISR). L'utilisation excessive de produits chimiques synthétiques a des effets néfastes sur l'environnement et la santé humaine, ce qui décourage l'application de pesticides dans le secteur agricole. En conséquence, les chercheurs du monde entier se sont tournés vers des stratégies alternatives respectueuses de l'environnement pour prévenir les maladies des plantes. Il existe toute une gamme d'agents de lutte biologique. Actuellement, les chercheurs explorent l'utilisation de micro-organismes bénéfiques comme stratégie écologique pour lutter contre les maladies des cultures. Divers genres de bactéries et de champignons ont démontré un grand potentiel en tant qu'agents de lutte biologique contre diverses maladies des plantes.
En outre, à ce jour, les chercheurs en biotechnologie explorent la résistance systémique acquise (SAR) et la résistance systémique induite (ISR) des plantes, leur rôle et leur mode d'action contre les phytopathogènes et le stress des plantes.
Cet article s'efforce de décrire les nouveaux biostimulants et éliciteurs naturels créés par la biotechnologie et la nanotechnologie au cours des dernières années, afin d'apporter un nouvel éclairage sur l'augmentation de la SAR et de l'ISR dans le système de défense des plantes.
MOTS-CLÉS. Produits bioactifs, biotechnologie, système de défense induit, nanotechnologie, pathogénie végétale, résistance systémique acquise.
KEYWORDS. Bioactive Products, Biotechnology, Induced Defence System, Nanotechnology, Plant pathogenesis, systemic acquired resistance.

1. INTRODUCTION

Globally, one third of greenhouse gas emissions come from food systems, according to the Intergovernmental Panel on Climate Change (IPCC). Within the EU, agriculture is responsible for almost 11% of EU greenhouse gas emissions, which mainly come from methane emissions from livestock farming and nitrous oxide from soils. It should also be emphasized that agriculture is constantly linked to the environment, both positively and negatively. In Europe, the intensification and industrialization of the agricultural sector are continually ongoing; this is primarily demonstrated in the use of pesticides and artificial fertilizers [CRI 21]. Therefore, some agricultural systems should undergo extensification. As emphasized by [KEE 18] the current degradation of soil and land presents a severe challenge to society because it contributes to climate change, loss of biodiversity, and consequently, a decrease in agricultural production. The conversion of forests, meadows, or wetlands into arable land will result in the loss of valuable products (wood, fibers, energy), on the one hand, and the loss of ecosystem functions (carbon sequestration, carbon storage, water cycle regulation, and biodiversity) on the other. Hence, expanding agricultural land at the expense of other valuable ecosystems is not perceived as an ecological and feasible option. A possible scenario is to allocate part of the land used for feed purposes to production intended directly for human consumption, while reducing food waste. However, this would require a change in diet and a reduction in the consumption of animal products, which in turn, according to some studies, would make it possible to feed the world's population in 2050 using the principles of sustainable agriculture; this will be possible while implementing the EGD's assumptions [PAW 24].

The European Green Deal, adopted in 2019, is a package of environmental and climate policy initiatives. It aims to make Europe climate neutral by 2050. A key aspect of this is transitioning to a more sustainable and resource-efficient food system. This includes reducing the use of synthetic fertilizers and plant protection products, while ensuring no deterioration in soil fertility. The Green Deal also promotes organic farming and the development of a circular economy for fertilizers, encouraging the use of bio-based and recycled nutrient sources. The individual objectives span many different sectors, such as environment and biodiversity, agricultural production and food, energy, transport, industry, construction.

Agriculture is a key part of this transition. The excessive use of pesticides and antimicrobials in agriculture constitutes another challenge, with serious environmental and health impacts, resulting in water pollution, soil degradation and the development of antimicrobial resistance. The common agricultural policy (CAP) has been reformed to support environmental and climate objectives more effectively. For 2023-2027, 40% of the CAP budget is allocated to climate action. According to the European Environment Agency 60-70% of EU soils are in an unhealthy state. Depleted soil and degraded agricultural ecosystems have a lower capacity to produce food [ALM 23], [BAZ 23].

The Farm to Fork program includes the following targets:

25% of EU agriculture to become organic by 2030.

50% reduction in pesticide use by 2030.

20% reduction in fertilizer use by 2030.

30% reduction in nutrient losses by at least 50%.

40% reduction in antimicrobial/antibiotic use in agriculture and aquaculture by 50% by 2030.

50% reduction in sustainable food labelling.

50% reduction in food waste by 2030.

10 billion € dedicated to related R&D.

However, agriculture and forestry can make a crucial contribution to combat climate change by absorbing carbon from the atmosphere.

In this paper we have tried to describe the new eco-friendly biostimulants and natural elicitors produced by biotechnology and nano technology in recent years.

2. THE CONTRIBUTION OF NEW TECHNOLOGIES TO THE ACHIEVEMENT OF GOALS

Can the new technologies being developed today cope with the demands of modern agriculture?

"A world without animals is possible, a world without plants is unthinkable..."

It is known from ancient texts that plants are affected by diseases that concerned humans even before 4000 years BC. At that time and many years later, humans could offer little help to the plants that were part of their diet. In antiquity, and especially in ancient Greece, Egypt and in the Mediterranean countries, there are important references concerning the cultivation method and the usefulness of many plant species. The monoculture of plant species domesticated by humans began to present, over time, problems that wild species did not have and were due to various diseases, parasites or competitive plants that we today call weeds. The problems that crop faced by external enemies were significant and even affected the survival of a portion of the population. At that time, the possibilities of solving these problems were almost non-existent, with the only possibility being weeding the weeds by hand. But over time, research and science began to show up and try to provide solutions to many problems that existed in crops. A unique gap in the progress of science and research existed in the dark years of the Middle Ages, when science was under persecution and people placed their hopes for the protection of their crops in nature and divine forces. But after the Middle Ages research and science began to try again to provide solutions to many problems that existed in the cultures and to escape from the old concepts that were imposed during this dark age [CHE 24].

Researchers who succeeded in solving Phyto pathological problems received awards, such as the one Tillet received from the French Royal Academy in 1755 for finding a way to prevent grain blast. In 1882 in Bordeaux, France, Professor Alexis Millardet, in research he conducted on a disease in the vineyard, discovered the fungicidal action of copper and prepared the Bordigal slurry, which is still widely used today to combat many fungal diseases [MAY 98].

The phenomenal growth of the pesticide industry has its origins in the war industry. During the interwar period 1925-1940, the synthesis of new chemical compounds for war purposes showed that some of these substances also had insecticidal activity. The first organic pesticide applied in agriculture was DDT, which was manufactured in 1874 by a German chemist and its insecticidal activity was discovered in 1939 by the Swiss T. Müller, who received the Nobel Prize for this [FRE 08], [WHO 07].

There are over 100,000 species of fungi and other microorganisms on earth, but only about 200 species cause serious damage to agriculture. In order to develop and survive on the planet, humans have altered many of the balances of ecosystems. Agriculture is an example that has caused serious disruption to the balance between plants, but also between plants and animals. The cultivation of a single plant species in large areas causes a great imbalance in the environment and favors the development of harmful microorganisms for that crop, thus making the use of chemical pesticides necessary [COD 06].

In the 1990s, the World Health Organization (WHO) estimated that over 1.5-2 million people are poisoned each year using pesticides and over 300,000 dies. Systemic pesticides cause greater damage, as they could penetrate all plant and animal tissues, thus killing the organisms that feed on them, such as bees. Due to monocultures, the reckless use of pesticides and herbicides, agriculture over the last 40 years has been responsible for a 52% reduction in biodiversity, the irreversible loss of species of agricultural interest by 75%, increased vulnerability to diseases, soil degradation and the outbreak of pandemics (which are directly related to ecosystems and biodiversity) [AGR 05].

Pesticides are distributed by large multinational companies, which above all put their profits before the health of users, consumers and the environment. Pesticides that were used freely for a long time were found to be very dangerous for humans and the environment and the producing companies were forced to withdraw them. Bees and other pollinating insects are essential for the pollination of 84% of plant species. The use of a group of systemic insecticides (nicotinoids) was found to be responsible for the mass death of pollinators and the services of various countries demanded the immediate withdrawal of nicotinoids from the pesticide market [GRA 08].

The pressure exerted worldwide by various ecological and environmental organizations has resulted in the banning of many dangerous chemical active substances, and the creation of a large gap in the way farmers act to protect their crops from the destructive pests and weeds that threaten them.

3. THE RISK OF LOSING THE AGRICULTURAL PROFESSION AND THE FOOD CRISIS

The question that arises after the withdrawal of dangerous chemical pesticides is how professional farmers will continue their production without financial burden and reduction in profits and without a food crisis?

The answer to this question is given first by the plants themselves and then by researchers and science. Plants are not only natural organisms, but they are also organisms that are subject to the laws of nature. The natural laws that plants are subject to are the maintenance of three basic balances:

a. the balance of energy,

b. the balance of water and

c. the balance of assimilation (nutrition).

The vegetation and development of plants is analyzed through these three balances. Biological processes through plant mechanisms and organs aim to maintain these balances and should not be treated as individual phenomena, moreover research has shown that a one-sided approach never works properly. Plants, unlike animal organisms, cannot move from their positions and any treatment we do must aim to maintain their physical activity and the basic balances mentioned above and not to strengthen them in a different direction.

To date, the interventions we make on plants are mainly unilateral (apart from modern hydroponic greenhouses) and the principles of energy, nutrition, and water balance are almost never respected. When an entomological, mycological, or bacteriological attack occurs, we intervene with the aim of solving the specific problem.

Plants, because they grow under different conditions, most of the time have to react to many different influences at the same time. This has led producers to simultaneously use many different chemicals in order to maintain commercial production at high levels of quantity and quality. The frequent and continuous use of plant protection and nutrients has caused many fungi, bacteria and insects to become addicted to specific active substances, resulting in the reduction or elimination of their action and at the same time the increase in their residues in food. While the reckless use of chemical fertilizers has created problems in the structure of soils and significant contamination of the water table in many areas of the world [LEW 16].

Multinational companies were forced to withdraw toxic formulations including nicotinoids and thus created a gap in crop protection and crop nutrition. In recent decades, significant changes have been observed in the way of plant protection and nutrition, and farmers are led to use products that are friendly to the environment, the user and the consumer, as well as products that have a multifaceted and not unilateral approach to the protection and development of plants [COD 06].

It is necessary that Agronomists - consultants and producers, have a full understanding of the complex mechanisms and interrelationships that plants use to cope with external challenges on their own. The great challenge for the agricultural consultant is to help the plant in real time respond and maximize its performance and of course its survival time while maintaining its balance in the best possible way.

3.1 The natural defense of plants

Plants are among the oldest organisms that have evolved on earth and one of the main reasons for their evolution is their ability to defend themselves against biotic and abiotic factors that threaten them. The metabolic activity for the synthesis of the necessary components, which constitute the fundamental structure and shape the way plant cells function, is characterized as primary metabolism. The biochemical mechanisms involved in primary metabolism do not show significant differences between cells, tissues, organs or organisms. However, a very large part of metabolic products is produced only in individual tissues and at specific stages of development. These biomolecules originate from intermediate compounds of primary metabolism and are synthesized through biochemical pathways that together constitute secondary metabolism. The products of secondary metabolism were previously considered waste or by-products of primary metabolism and their value for the functioning of fundamental defense mechanisms necessary for the survival of plant species was not appreciated. But in recent years, and especially after the decoding of DNA, research has made great strides in better understanding the mechanisms related to plant defense, as well as the defense strategies that plants develop to deal with biotic and abiotic stresses on their own [KIN 00].

In general, plants defend themselves with physical and chemical mechanisms that they possess. Physical mechanisms include thicker leaves, thorns, needle-like leaves with oxalic acid crystals, milky sap, gum, glue, etc. Chemical defense is the most diverse and sophisticated strategy they use to deal with pests. This strategy includes various groups of chemical compounds of secondary metabolism, called phytoalexins. Such are the group of phenolic compounds, terpenes and nitrogenous compounds. The phytoalexin group includes substances such as terpenes, flavonoids, nicotine, cocaine, caffeine, anthocyanins and many others. Phytoalexins play an important role in the self-defense of plants that produce them to deal with specific external insults [CHE 24], [FRE 08].

Plant defense is divided into two categories: fundamental and induced defense.

Plant organisms give priority to the defensive shielding of the surfaces exposed to the external environment. The epidermis and its appendages, the cutin and waxes of the epidermal layer of aboveground organs, and the suberin that mainly covers underground organs constitute the main part of the fundamental defense. Many secondary metabolites, such as phenolic compounds, contribute to the fundamental defense. Lignin strengthens cell walls, tannins indiscriminately precipitate proteins and make them indigestible, while a large number of phenolic substances such as terpenes and essential oils play an active role in the plant's defense against pathogens and insects.

Nitrogenous compounds also include alkaloids (morphine, caffeine, cocaine, nicotine, etc.) and constitute the most widespread group of defense molecules. Some pathogens have mechanisms to neutralize or bypass the pre-existing fundamental defense. In order to overcome these mechanisms of pathogens, plants also have their own mechanisms that we call induced defense. The process of pathogen attack, colonization and reproduction is called pathogenesis. The strains of pathogens that successfully complete the process of pathogenesis are called infectious strains. The invasion of pathogens inside the plant tissues signals a series of reactions, the speed of which also determines the success of neutralizing the invader. This neutralization is not limited to the area of infection but characterizes the entire body of the plant and is called Systemic Acquired Resistance (SAR). SAR is based on the production by the plant itself of a group of chemical substances, such as some special polypeptides called defense proteins (Defense proteins DP), or Pathogenesis Related proteins (Pathogenesis Related PR). These proteins include Chitinase, Chitosan, Gluconase, Peroxidase, Proteinase, Systemin, etc.

Most plants produce defense proteins themselves and their defense response is kept at a low level until they are attacked by a pathogen or pest. Then the defense mechanism is mobilized and the plant inhibits other functions and uses a large percentage of its energy to produce defense proteins to deal with the attack. Specifically, immediately after an attack, the first step the plant takes to initiate its defense response is to identify the type of threat (e.g. whether it is a bacterium, fungus, virus, wind, drought, temperature, frost, etc.).

After detecting the attack, the cells closest to the point of attack begin to self-destruct sequentially and die. This is called the hypersensitivity response (HR) and is visible by the creation of a yellow ring around the point of attack (Fig. 1).

Figure 1 Hypersensitivity response (yellow halo) after Xanthomonas Citri attack on young citrus leaf. Brown halo are colonies of Xanthomonas Citri.

The initiation of HR (hypersensitive response) is the first step in the overall defense response of plants referred to as Systemic Acquired Resistance (SAR) and Induced Systemic Resistance (ISR). Immediately after the local hypersensitivity response, the cells closest to the site of attack produce messenger molecules called elicitors. These spread throughout the plant and alert the rest of the cells to the attack. This induces the overall initiation of the defense system in all healthy cells of the plant. There are two classes of elicitor molecules, salicylic acid, which induces SAR, and jasmonic acid or ethylene, which induces ISR [VAL 04].

All the above briefly explains the way that plants have been used for thousands of years to deal with various attacks from microorganisms, insects and abiotic factors. However, in commercial crops, pathogens manage to destroy a large part of the crop before the plants fully mobilize their defense mechanisms [NEP 20].

The science of biotechnology, after thorough study and experimentation, has managed to identify with great precision the defense proteins and the molecules that trigger (elicitors) of plant defense. Today, after the bans and restrictions on the use of chemical plant protection products and fertilizers, there is great interest in the use of molecules that stimulate defense in plants (elicitors) in combination, many times, with integrated pest management (IPM) programs [PAN 09].

In order for plants to strengthen their defenses and achieve maximum production, they must have all the necessary nutrients available for their uninterrupted growth. Photosynthesis is the mechanism by which plants, through sunlight and the uptake of carbon dioxide (CO_2) from the atmosphere, produce sugars that are used to produce energy and other chemical compounds needed for their growth. Therefore, increased photosynthesis is a key point for plant health. Another important factor for the healthy growth of plants is the soil in which their root system develops and the beneficial microorganisms that inhabit it. The health and good development of the root system directly depends on the composition of the soil and its ability to adequately provide the necessary nutrients required for rational plant growth. Photosynthesis and soil quality are key elements that must be considered when implementing programs to enhance the natural defenses of plants [VAR 04].

3.2 The promises of the innovative achievements of biotechnology and nanotechnology.

The way in which plants defend themselves against external enemies was presented in detail, in order to sufficiently understand the mechanisms of plant function. However, modern science can today have a decisive intervention in plant protection and plant nutrition using the achievements of biotechnology and nanotechnology. In the USA, India, Australia and recently in Europe, many companies involved in biotechnology, nanotechnology and biostimulants have turned their interest to finding solutions that relate to strengthening the natural defense of plants and protecting them from external enemies by avoiding the use of chemical pesticides.

A reliable example of producing novel environmentally friendly products is the company KeyPlex. Since 1998, KeyPlex (USA) in collaboration with the United States Department of Agriculture (USDA) after many investigations in the laboratory and in the field identified the molecular structure of defense proteins as well as the molecules that initiate (elicit) the natural defense SAR and demonstrated that the company's products, KeyPlex 350 and KeyPlex 350 DP, fertilizers containing peptides, stimulate the initiation of defense mechanisms (SAR) of plants [MAY 98].

The Environmental Protection Agency (EPA) has granted permission for the use of these products in agricultural crops without any restrictions.

Today, KeyPlex manufactures and markets products that contain ready-made defense proteins and enzymes (elicitors) that are responsible for transmitting the initiating signal to healthy cells of the mechanisms of Systemic Acquired Resistance (SAR) of plants as well as Induced Systemic Resistance (ISR). These products are special biostimulants of plant origin, which, in addition to the special biotechnologically produced molecules, are also equipped with all the necessary nutrients as well as highly reactive alpha ketonic acids, in order to maintain the plant's energy at a high level during the attack. The mode of action of defensive peptides is complex. For example, fungi during the attack produce pectinase, defensive peptides inhibit the production of pectinase, resulting in the reduction or elimination of the attack [YI 13].

Many of the new biotechnology products contain special yeasts or saccharomyces, which when sprayed during flowering, have time to colonize on the stamens before the fungus or bacteria, such as Erwinia in pome fruits and prevent the attack. These peptides can destroy the membranes of bacteria or fungi.

KeyPlex's biotechnology products also cause the plant itself to create special peptides that reach the roots in a few minutes and neutralize the bacteria while at the same time they wrap around the viruses, they encounter within the plant tissues and encapsulate them, thus preventing their reproduction.

In the US, a product from the company Green Light Bio was recently granted a license by the Environmental Protection Agency (EPA), which is based on the use of innovative dsRNAi (double stranded RNA) technology, which has the ability to inhibit the production of specific proteins, which are essential for the continuation of the life of insects or pathogens that attack plants. This product, in a minimal dose corresponding to an amount equal to a teaspoon and covering an area the size of a large football field, can effectively combat an insect that is one of the biggest enemies of potatoes, a major pest *Leptinotarsa decemlineata* (Colorado potato beetle) [ROD 10].

Figure 2 Leptinotarsa decemlineata (Colorado potato beetle).

The breakdown of this product occurs within three days, without leaving residues on the fruits or the soil and does not require the use of any type of chemical for its effective control. This product only combats specific insects and does not affect beneficial insects or bees. Also, with the same technology, products are produced that target mites in the varroa mite of bees, a very destructive mite that is one of the most serious enemies of bee hives.

Research is expanding to produce new herbicides, fungicides and insecticides. In Australia, the company AUSSAN, which deals with plant nutrition, produces products from active flavonoids that increase the rate of photosynthesis and plants bind more carbon dioxide from the atmosphere to produce carbohydrates. The excess carbohydrates are channeled into the soil through root exudations and in this way the carbon footprint in the atmosphere is reduced while at the same time the soil is regenerated due to the increased activity of beneficial microorganisms. These products are not chemicals, but products derived from the plants themselves and even contribute to the fight against nematodes due to flavonoid secretions from the roots. For its contribution to soil regeneration and the reduction of the carbon footprint, the company received an award from the UN [PIE 09].

Nanotechnology has brought to the market silicon nanoparticles, such as Nano Pro, which load the active substances of systemic insecticides, fungicides, herbicides or fertilizers into their pores, thus increasing their action and reducing the quantities or number of repetitions of chemicals used to combat plant pathogens or enhance their nutrition. Many products, such as copper, zinc, silver, defense peptides, special enzymes, fertilizers, etc. are now produced using biotechnology or nanotechnology methods and in this way a significant reduction in the quantities used until now is achieved without any reduction in effectiveness, resulting in a reduction in the negative impacts the that the old-style formulations had on the environment, the soil, the user, the consumer and on food [CUT 10].

In our previous research, [GIA 24] we investigated the effectiveness of specific organic biostimulants (1) Magnablue + Keyplex 350 (Mg + Kp350); (2) Cropbiolife + Keyplex 120 (Cpl + Kpl120); (3) Keyplex 120 (Kpl120); and (4) Magnablue + Cropbiolife + Keyplex 120 (Mgl + Cpl + Kpl120) on the nutrition and physiology in black chokeberry (Aronia) plants, as well as the quality of their berries.

Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) revealed that the Magnablue + Cropbiolife + Keyplex treatment significantly increased concentrations of P, Ca, and K. Additionally, it enhanced photochemical efficiency (Fv/Fm), water-splitting efficiency (Fv/Fo) in PSII, and the performance index (PI) of both PSI and PSII in chokeberry leaves (Fig 3). Improvements in photosynthesis, such as CO2 assimilation (A), transpiration (E), and water-use efficiency (A/E), were also noted under biostimulant applications compared to the control. Upon harvesting the ripe fruits, they were examined for their quality characteristics and post-harvest physiology. Precisely, parameters such as weight, ascorbic acid, flesh consistency, total antioxidant capacity, anthocyanin concentration, and total phenols of the berries were notably higher under cultivation with biostimulants compared to the control. These data suggest that selecting appropriate biostimulants can enhance plant yield and fruit quality by potentially activating secondary metabolite pathways.

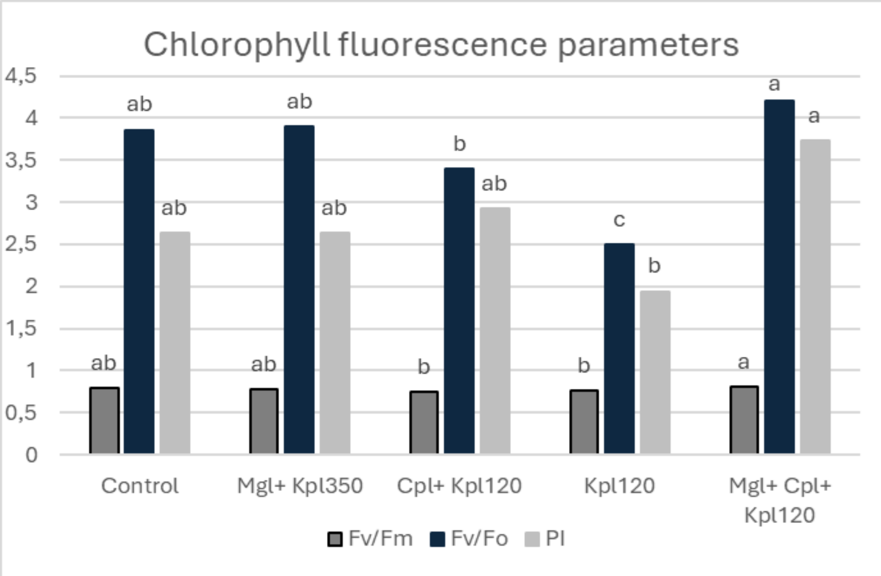

Figure 3 Differential response of chlorophyll fluorescence indices of Aronia leaves under different biostimulants (mean value, n=4).

In addition, since one of the most important problems faced by vegetables in the greenhouse is attacks from nematodes [COL 06], we examined two new biological products Key Eco oil and CROPBIOLIFE (extract of vegetable oils and active flavonoids) in greenhouse cucumber (*Cucumis sativus* L.) cultivation in terms of their effectiveness in the soil–plant relationship, measuring how plant and fruit physiology, nutrition, and structure are affected by nematodes, as well as their fertility and mobility [OUZ 25a]. The chemicals currently used to address this issue have little effectiveness and, in many cases, are toxic to the plants.

Based on our results, the application of bioproducts altered the nematode population, reducing the presence of plant-parasitic species. Treated plants showed fewer root knots, with microscopic analysis revealing a scarcity of giant cells, which are the nematodes' essential feeding sites (Fig 4). The higher efficiency of the oxygen-evolving complex (OEC, Fv/Fo) of cucumber leaves under bioproducts was significantly correlated with the maximum efficiency of PSII photochemistry (Fv/Fm).

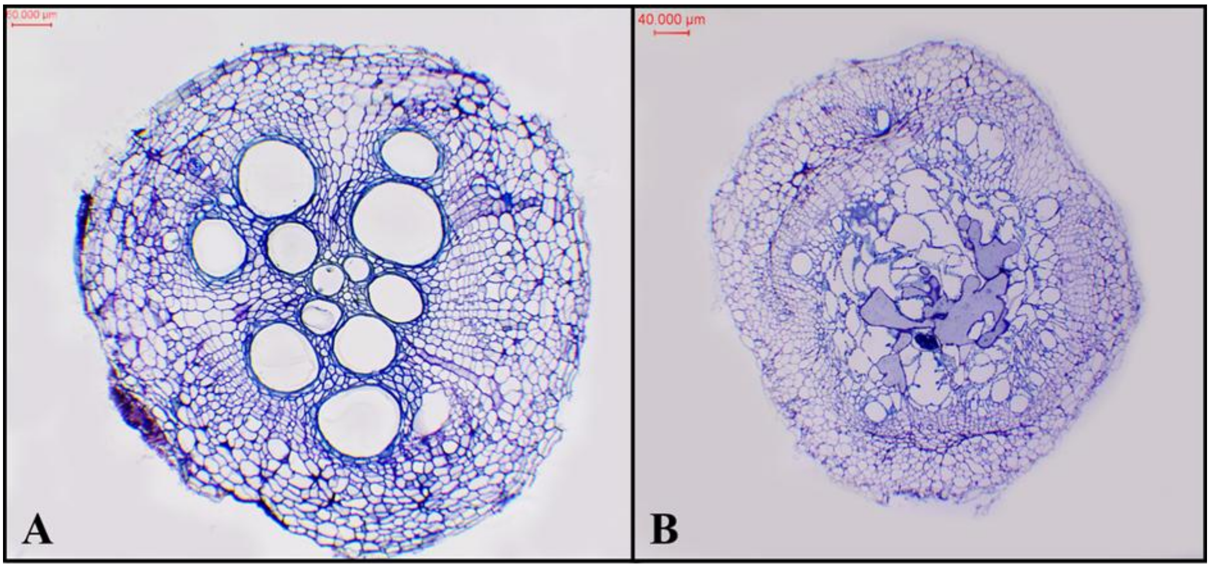

Figure 4 Transverse root sections show (A) a root section without nematode infection and (B) a root section from a gall formed due to nematode infestation.

The decreased non-photochemical quenching (NPQ) in control leaves correlated well with the lower chlorophyll concentration, offering a smaller light-harvesting antennae compared to treated leaves. The increased quantum yield of PSII photochemistry (PHPSII) and electron transport rate (ETR) under bioproducts can explain the higher net photosynthesis (Pn) and transpiration rate (E). Similarly, chlorophyll fluorescence measurements in fresh mature cucumber fruits under bioproducts revealed a more efficient PSII system compared to the control. Cultivation with these substances had beneficial effects on fruit quality as fresh weight, maintenance of flesh consistency and color, ascorbic acid concentration (which was two times higher than that of control) and sugars.

Biostimulants applied to plants strengthen nutritional efficiency, abiotic stress tolerance, and fruit biochemical traits [NEP 20]. Our recent research aimed to elucidate the effects on the growth, physiology, and quality of Chardonnay grapevine plants and fruits under the conventional cultivation and biostimulant (nano-products) formulation [OUZ 25b]. The applied products contain special defense proteins, alpha ketonic acids, especially humic and fulvic acids, oligopeptides, produced from hydrolyzed proteins, copper nanoparticles in the form of ions as well as flavonoids. Plants were subjected to different treatments during the whole cultivation period of the grapes (April to October) in Northern Greece (Drama-Adriani, Lazaridis Domain) under natural environmental conditions. Measurements such as mass, color, texture, and consistency (fruits) were performed on the fruits that had ripened on the grapevine, while chlorophyll fluorescence measurements were performed on the foliage at harvest. Chlorophyll fluorescence indices measured by IRGA and Imaging PAM revealed the highest photosynthetic efficiency of plants treated with biostimulants. Beneficial effects were also observed under the application of biostimulants in the maintenance of flesh consistency, ascorbic acid concentration, and phenols (Fig 5). In conclusion, the application of innovative nano-products serving as biostimulants is a sustainable tool to improve productivity, which aligns well with the strategy of the European Green Deal.

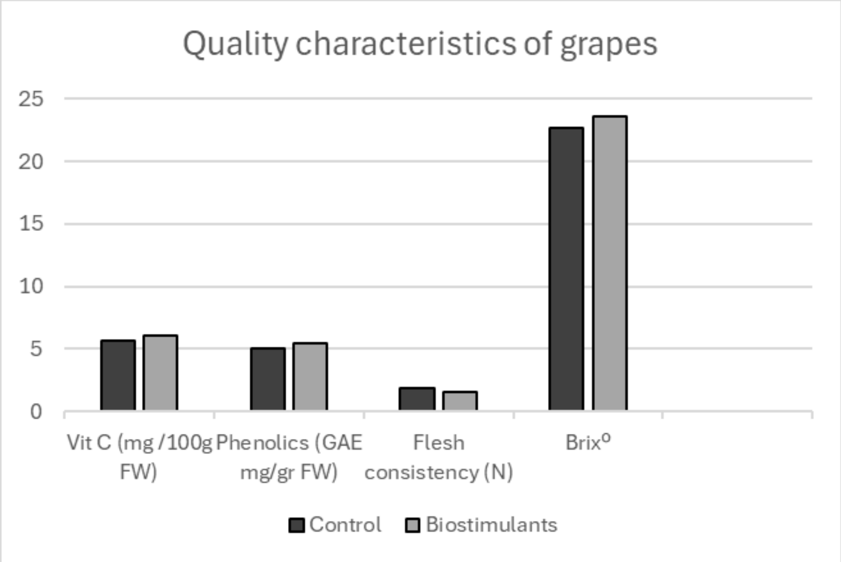

Figure 5 Texture and biochemical analysis of grapes after conventional and biostimulant cultivation (mean value, n=30).

Erwinia amylovora is the type species of the genus Erwinia that belongs to the family Enterobacteriaceae. *Erwnia amylovora* causes fire blight, a devastating plant disease affecting a wide range of host species within the Rosaceae subfamily Spiraeoideae and is a major global threat to commercial apple and pear production [PIQ 15]. Moreover, strains infecting plants in the genus Rubus belonging to the subfamily Rosoideae, including blackberry and raspberry, have also been reported. In the last two centuries this pathogen has spread worldwide and, in consequence, *E. amylovora* has been cataloged as a quarantine organism in the European Union, where it is subject to phytosanitary legislation, and, recently, has been included in the top 10 plant pathogenic bacteria. The resistance of those bacteria to common coppers and antibiotics can now be eliminated if, in the Integrated Pest Management program that we apply alternately with traditional plant protection products and biotechnology biostimulants, coppers with ion formulations, such as, for example, ionic copper, produced by the company Geogreen Agro Solutions, which contains a significantly reduced amount of metallic copper, has great effectiveness, biostimulant and systemic action and bacteria or fungi cannot develop resistance.

Alternating the spraying program with products that, as we mentioned earlier, contain special defensive peptides with alpha ketonic acids, e.g. KeyPlex 350 DP and KeyPlex 120, saccharomyces, yeasts, bacteriophage viruses such as AgriPhage, dsRNAi, such as Calantha by Green Light Bio, now provide a solution to attacks by *Erwnia* by interrupting the functioning of the resistance mechanisms it develops [MAY 98].

4. CONCLUSIONS

The progress in the field of plant protection and nutrition are rapid and the gap left by the withdrawal of many chemicals that farmers used until now has already been filled. The goals that have been set for achieving the new w5. 5ay of plant protection and nutrition are:

• the activation of plant defense with products that contain or cause the creation of special defense proteins,

• the production of special enzyme initiators that activate the self-defense of plants,

• the use of bioactive products (biostimulants), which can function by maintaining the energy balance of plants at the highest possible level during the attack.

The extension of plant protection remains and is the major issue. Farmers must understand that in a few years they will leave forever the old plant protection system in which a chemical product indiscriminately killed many species of insects or fungi, destroying harmful and beneficial organisms at the same time, with catastrophic effects on biodiversity, the environment and the health of consumers. Nevertheless, and because crops have enormous economic, social and environmental value, a safe risk that farmers can take is to alternate the application of new and innovative products with the traditional conventional products they used.

Biotechnology, nanotechnology, the biostimulant industry, the decoding of DNA, the decoding of plant defense mechanisms and the possibilities of interventions and use of RNA, provides important answers and solutions to professional agriculture, and help to avoid chemical and organic products by replacing them with substances that are friendly to the environment and humans.

Young farmers are now familiar with electronic technology and information gathering so adopting changes and transitioning to Green Agriculture will be quite easy for them. However, the development of techniques for inducing resistance using biotic and abiotic agents has a short history. Commercial products are few and more research is needed. One policy option would be to increase investment in fundamental and applied research. It is expected that successful practical implementation will require further development to find the most effective application methods. Another policy option would therefore be to invest in the education and training of extension workers and farmers in environmentally plant propagation and cultivation.

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