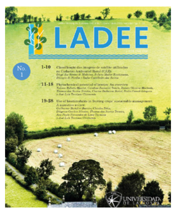

Use of biostimulants in fruiting crops' sustainable management: A narrative review

Uso de bioestimulantes en el manejo sustentable de cultivos frutales: una revisión narrativa

Uso de bioestimulantes no manejo sustentável de plantas frutíferas: uma revisão narrativa

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Keywords: Agroecosystems, fruticulture, orchards, sustainability, bioinputs

Abstract

Introduction: On a global scale, the obstacles to fruticulture correspond to the lack of skilled labor, the limited amount of available arable land, and the high costs of acquiring fertilizers and pesticides. These inconveniences, linked to environmental impacts and ecotoxicological damage, indicate that scientists, industries, and fruit growers have shown interest in the development of biotools for fruiting crops' management aiming at orchards' optimal production, such as biostimulants. This bioinput stimulates plant nutrition processes independently of the product's nutrient content, aiming to improve efficiency in the use of nutrients, tolerance to abiotic stress, and the quality and availability characteristics of nutrients available in the growth medium. **Objective**: Thus, this narrative review aims to analyze the state-of-the-art regarding the use of biostimulants in fruticulture, compile information on the proper application of these bioinputs and present alternatives to the diffusion of biostimulants in fruit agroecosystems. The totality of bioestimulants' action mechanisms still needs to be better understood. **Results**: The applicability of biostimulants in the management of fruiting crops proved to be a relevant possibility to grant sustainability to production systems in fruticulture and reduce costs, increasing productivity, shelf life, and reducing damage caused by climatic adversities in crops, mainly hydric stress. **Conclusions**: The development of specific legislation for biostimulants should contribute substantially to generating credibility with farmers in order to differentiate, for example, foliar fertilizers and microbial agents.

Resumen

Introducción: A escala mundial, los obstáculos para la fruticultura corresponden a la falta de mano de obra calificada, la limitada cantidad de tierra cultivable disponible y los altos costos de adquisición de fertilizantes y pesticidas. Estos inconvenientes, ligados a impactos ambientales y daños ecotoxicológicos, indican que científicos, industrias y fruticultores han mostrado interés en el desarrollo de bioherramientas para el manejo de plantas frutales con el objetivo de la producción óptima de los huertos, como los bioestimulantes. Este bioinsumo estimula los procesos de nutrición de las plantas independientemente del contenido de nutrientes del producto, con el objetivo de mejorar la eficiencia en el uso de los nutrientes, la tolerancia al estrés abiótico y las características de calidad y disponibilidad de los nutrientes disponibles en el medio de cultivo. **Objetivo:** Así, esta revisión narrativa tiene como objetivo analizar el estado del arte en cuanto al uso de bioestimulantes en la fruticultura, recopilar información sobre la correcta aplicación de estos bioinsumos y presentar alternativas a la difusión de bioestimulantes en agroecosistemas frutícolas. La totalidad de los mecanismos de acción de los bioestimulantes aún debe comprenderse mejor. **Resultados:** La aplicabilidad de bioestimulantes en el manejo de cultivos frutales demostró ser una posibilidad relevante para otorgar sostenibilidad a los sistemas productivos en fruticultura y reducir costos, aumentando la productividad, la vida útil y reduciendo los daños causados por las adversidades climáticas en los cultivos, principalmente el estrés hídrico. **Conclusiones**: El desarrollo de una legislación específica para bioestimulantes debe contribuir sustancialmente a generar credibilidad con los agricultores para diferenciar, por ejemplo, fertilizantes foliares y agentes microbianos.

Palabras clave: Agroecosistemas, fruticultura, huertos, sostenibilidad, bioinsumos

Resumo

Introdução: Em escala global os entraves da fruticultura correspondem à falta de mão-de-obra especializada, à quantidade limitada de terra arável disponível e aos elevados custos para a aquisição de fertilizantes e pesticidas. Esses inconvenientes, atrelado aos impactos ambientais e aos danos ecotoxicológicos, apontam que cientistas, indústrias e fruticultores têm demonstrado interesse no desenvolvimento de bioferramentas para o manejo de plantas frutíferas visando a ótima produção dos pomares, a exemplo dos bioestimulantes. Esse bioinsumo estimula processos de nutrição das plantas de forma independente ao teor de nutrientes do produto, visando melhorar a eficiência no uso de nutrientes, a tolerância ao estresse abiótico e as características de qualidade e disponibilidade de nutrientes disponíveis no meio de crescimento. **Objetivo**: Assim, essa revisão narrativa tem como objetivo analisar o estado da arte referente ao uso de bioestimulantes na fruticultura, compilar informações sobre a aplicação adequada desses bioinsumos e apresentar alternativas à difusão dos bioestimulantes nos agroecossistemas frutícolas. A totalidade dos mecanismos de ação dos bioestimulantes ainda precisa ser melhor compreendida. **Resultados**: A aplicabilidade de bioestimulantes no manejo de culturas frutíferas revelou-se uma relevante possibilidade de conceder sustentabilidade aos sistemas de produção em fruticultura e redução de custos, incrementando a produtividade, a vida de prateleira e a redução de danos causados por adversidades climáticas nas culturas, principalmente estresse hídrico. **Conclusões**: O desenvolvimento de legislações específicas para bioestimulantes deverá contribuir substancialmente para gerar credibilidade aos agricultores de modo a diferenciar, por exemplo, fertilizantes foliares e agentes microbianos.

Palavras-chave: Agroecossistema, fruticultura, pomares, sustentabilidade, bioinsumos

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1. Introduction

In Brazilian agribusiness, fruticulture represents one of the segments that stood out in recent decades in the fruit production both for fresh consumption and for industrialization. Brazil occupies the third position in the ranking of the largest fruit producers in the world, behind only China and India. The annual Brazilian fruit production exceeded 40 million tons and this contributed to employing about 6 million people. In addition to job creation, fruticulture features the diversification of small and medium-sized farmers' properties, keeping producers in rural areas, stimulating family succession and greater productivity in relation to grain crops, which translates into better profitability for fruit growers.

However, in recent years, fruticulture has faced challenges regarding the adequate production of food for the constantly growing world population (Zulfiqar et al., 2020), mainly due to the lack of specialized labor, by the limited amount of available arable land and by the high costs of acquiring traditional chemical inputs (fertilizers and pesticides). These inconveniences, linked to environmental impacts and ecotoxicological damage, indicate that scientists, industries, and fruit growers have shown interest in the development of tools for the management of fruiting crops aimed at optimal production in orchards.

The use of inputs with lower financial investments and that grant the sustainability of agroecosystems has been intensified due to the high prices of those conventional ones, which generally show variation linked to foreign currencies and international stock exchanges. This action contributes to the establishment of sustainable agrifood systems, which represents one of the requirements to enable the development of fruit farming that is ecologically correct, socially fair, technologically adequate, economically viable, and culturally accepted. Among the agricultural practices that contribute to the sustainability of fruit systems are the use of biostimulants (Kisvarga et al., 2022). These bioinputs represent a sustainable and effective solution that complements their synthetic counterparts, bringing benefits to agrobiodiversity, the environment, human health, and the economy (Basile et al., 2020).

In general, biostimulants are used in crops with high added value, such as fruit, vegetables, and flowers, grown in greenhouses or outdoors, to increase yield and quality in a sustainable way (Colla & Rouphael, 2015). This drives horticultural companies to develop new biostimulant products and more effective bioactive molecules (Bulgari et al., 2019). The literature shows that biostimulants do not have negative effects on ecosystems or human health due to the low biological toxicity of their components, their rapid degradation in the environment, their low mobility in food and their low application rate (Kisvarga et al., 2022).

Understanding the concepts, mechanisms, and action modes, applicability in fruticulture and the effects on plants will help fruit growers to leverage the use of biostimulants as a strategic tool to improve fruit products' production and quality. Therefore, this narrative review aims to analyze the state-of-the-art regarding the use of biostimulants in fruticulture, compile information on the proper application of these bioinputs and present alternatives to the diffusion of biostimulants in fruit agroecosystems.

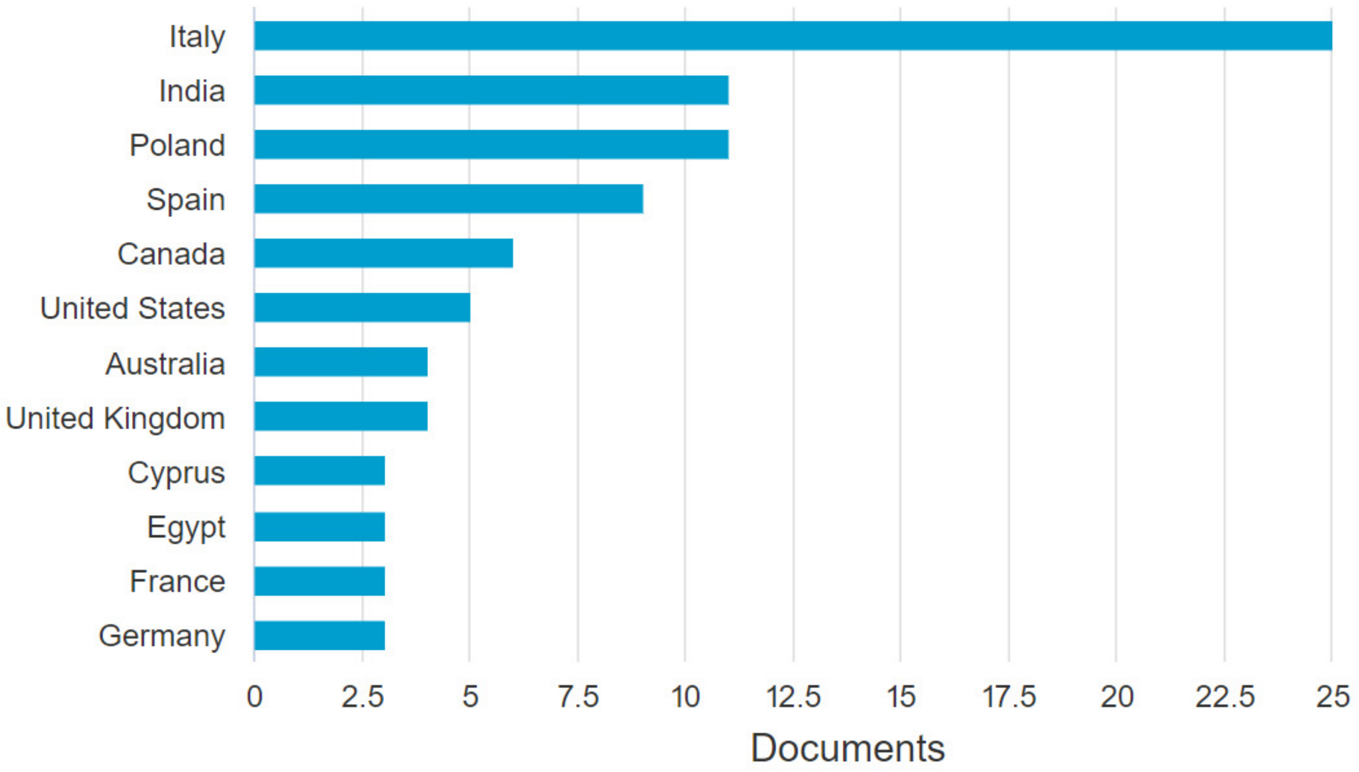
2. Definitions and categories of biostimulants

The term "plant biostimulant" was first used by Zhang and Schmidt (1997). This word was initially used by specialists in horticulture to describe substances that promote plant growth, ignoring nutrients, soil amendments, and pesticides from the concept (Du Jardin, 2015).

Conceptually, the same author defined plant biostimulant as any substance or microorganism applied to plants with the aim of increasing nutritional efficiency, tolerance to abiotic stress and/or crop's quality characteristics, regardless of its nutrient content; by extension, plant biostimulants also designate commercial products containing mixtures of these substances and/or microorganisms (Du Jardin, 2015).

The topic involving plant biostimulants has been debated in recent years in relation to their regulatory framework (Basile et al., 2020). From a search in the Scopus database (https://www.scopus.com), using 'biostimulants' and 'horticulture' as keywords and 'and' as Boolean logic of the search, we found 87 documents and verified that Europe, especially Italy, dominates research on these bioinputs (Fig. 1).

Fig. 1. Number of documents, by country or territory, on biostimulants in horticulture. Mostly, Italy dominates research on the subject and contributes significantly to the advancement of science to understand the mechanisms of action of these bioinputs.



Source: Scopus database.

The European Parliament defines a plant biostimulant as a fertilizer product that stimulates plant nutrition processes independently of the product's nutrient content, aiming to improve one or more of the following plant or rhizosphere characteristics: nutrient use efficiency, abiotic stress tolerance, quality characteristics, and availability of nutrients confined in the soil or rhizosphere (European Union [EU], 2019). Based on this concept, biostimulants were categorized, according to the agricultural functions performed, into: 1) non-microbial biostimulants, which include natural bioactive substances (humic and fulvic acids, hydrolyzed animal and vegetable proteins, extracts of marine macroalgae and silicon); 2) microbial biostimulants, which include beneficial microorganisms [for example, Arbuscular Mycorrhizal Fungi (AMF) and nitrogen-fixing bacteria (N) of the *Rhizobium, Azotobacter* and *Azospirillum* genera] (Rouphael & Colla, 2020).

Brazilian legislation does not grant specific registration to biostimulants. Products not classified as pesticides or nutrient sources, with bioactive components that stimulate plant growth and development, that improve crop's productivity and quality, and that increase tolerance to abiotic stresses, are covered by Decree 4.954 (Presidency of the Federative Republic of Brasil [RFB], 2004) and are classified as biofertilizers.

It should be noted that the legal definition of biofertilizers in Brazil (Decree 4.954, RFB, 2004) disregards resistance inducers in plants and beneficial fungi/bacteria. These microorganisms, according to Brazilian legislation, are classified as inoculants. However, the national legislation regarding organic production, defined in Normative Instruction 64 (Minister of State for Agriculture, Livestock and Food Supply of Brazil [MAPA], 2008), links the term biofertilizer to the "product that contains active components or biological agents, capable of acting directly or indirectly on all or part of the cultivated plants [...]" (Art. 2, num. I). This definition, which includes biological agents, allows us to consider beneficial fungi and bacteria as biofertilizers for organic production.

According to Normative Instruction 25 (MAPA, 2009), fertilizer products with nutrients concentrations, registered as organomineral fertilizers for foliar application and which must guarantee 6% of carbon (C) as an organic fraction, can be formulated with humic/fulvic acids, amino acids, seaweed extracts and plant extracts with chelating agents, complexing agents or additives.

Non-microbial biostimulants can be obtained from organic materials and include humic substances, complex organic materials, beneficial chemical elements, inorganic salts, seaweed extracts, chitin and chitosan derivatives, peptides, amino acids, and other N-containing substances. In addition to phytoregulators and phytohormones, biostimulants are indirect growth promoters, such as carbohydrates, which, when added to root exudates, positively influence the ion-growth medium interaction, increasing root development and plant survival (Araujo et al., 2019).

Microbial biostimulants that act as biofertilizers are biological products containing live microorganisms that, when applied to seeds, plant surfaces or soil/substrate, promote growth by various mechanisms. Biofertilizers can be used as complements to mineral fertilizers. Microbial inoculants are represented mainly by free-living bacteria and fungi that have been isolated from a variety of environments, including soils, plants, plant residues, water and composted manure. Among the biofertilizers that have been studied are Plant Growth Promoting Rhizobacteria (PGPR) (Calvo et al., 2014).

The use of these bioinputs in the world's agroecosystems is an important strategy in the search for sustainable agriculture. Since they are less aggressive to the environment and can improve plant growth by activating their defense system. Biostimulants based on inorganic salts were able to reduce the severity of 49 fungal diseases in different tissues (roots, stems, leaves and fruits) in 35 plant species, including cereals, fruits, vegetables, ornamentals and native plants (Calvo et al., 2014; Nardi et al., 2016). Also, biostimulants positively influenced the expression of genes and the activity of operational enzymes in the metabolism of the apple tree (*Malus domestica* Borkh.). The red coloring of apples was incremented with a product based on plant extracts containing ethylene and anthocyanin biosynthesis amino acids precursors, chlorophyllase, potassium oxide, and monosaccharide regulators (Fenili et al., 2019).

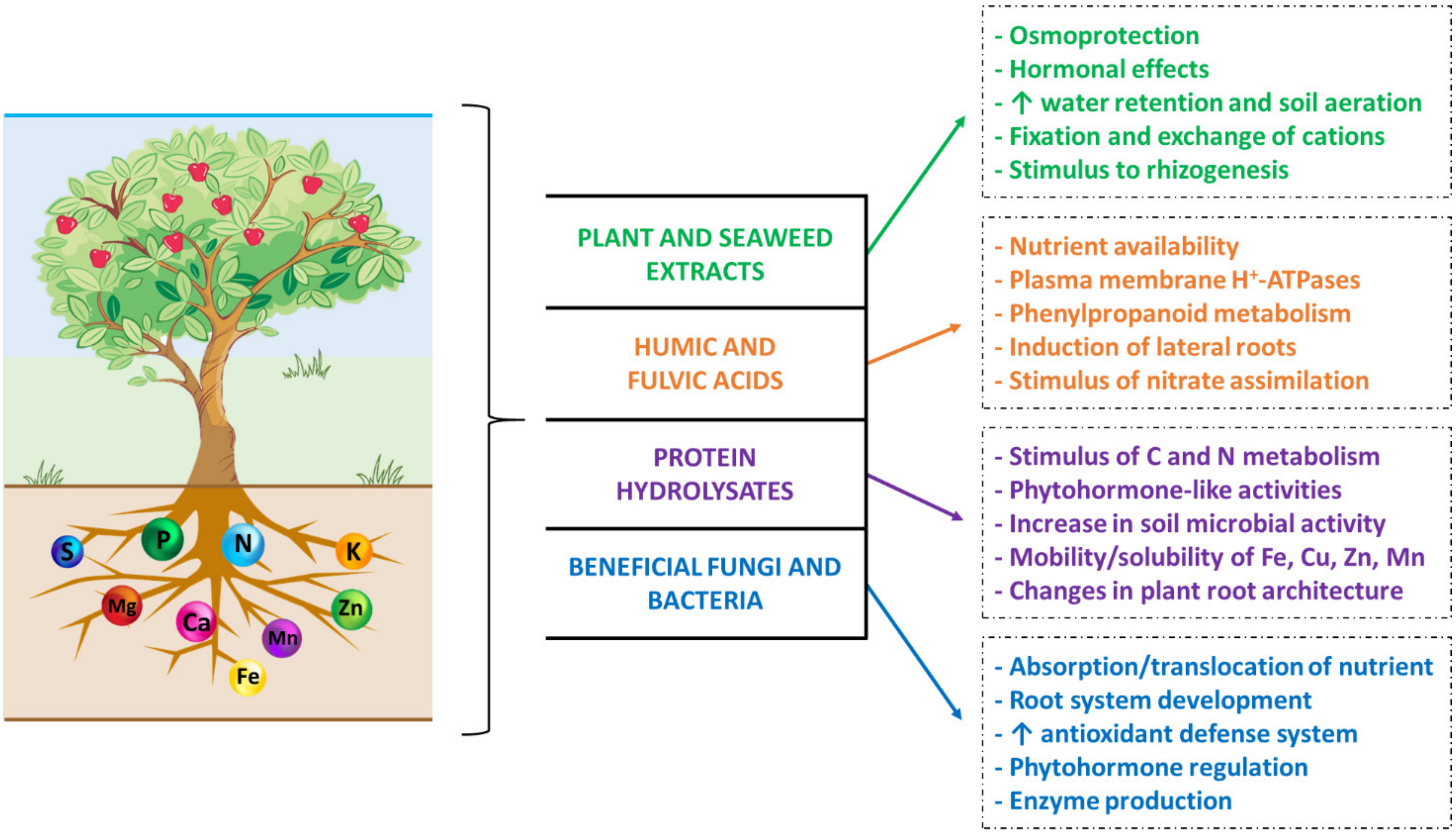
The use of biostimulants accelerated the growth of passion fruit seedlings (*Passiflora* spp.), making them taller in a shorter period of time. In this case, the phytoregulators involved in plant growth and development stimulated cell division, differentiation, and elongation, and also increased nutrient uptake and utilization. However, when used in excess, biostimulants can cause physiological disorder in the plant (Gonçalves et al., 2018).

Therefore, to enhance only the beneficial effects of biostimulants on plants, it is important to know and understand their action mechanisms. This will allow fruit growers to establish appropriate management practices that are consistent with the sustainability of crop agroecosystems.

3. *Biostimulants' action mechanisms*

The positive effects of biostimulants, which can be single or multicomponent, are attributed to bioactive compounds that stimulate plant growth, such as phytohormones, amino acids, and nutrients (Zulfiqar et al., 2020). These compounds help in the development of plants in several ways (Kocira et al., 2018). However, the biostimulants' action mechanisms (Fig. 2), still need to be better understood. Here, we present the latest findings reported in the literature from joint efforts by scientists, industry, and producers.

Fig. 2. Synthesis of the main mechanisms of biostimulants based on plant and seaweed extracts, humic and fulvic acids, protein hydrolysates and beneficial microbes.



Source: Own authorship.

At low concentrations, biostimulants are capable of inducing biochemical, physiological, and molecular responses in plants, such as improved flowering, plant growth, yield, nutritional, and functional quality of the product and its shelf life. Physiological and molecular aspects are capable of stimulating C and N metabolism, triggering key enzymes, regulating key genes involved in the detoxification of reactive oxygen species, increasing the antioxidant defense system (increases superoxide dismutase and catalase and decreases oxidative stressors by hydrogen peroxide and malondialdehyde) and the production of secondary metabolites, improve photosynthetic activity and water relations, increase the accumulation of osmolytes (glycine-betaine, proline, and sorbitol), improve the characteristics of the growth medium, trigger phytohormone-like activities, improve epiphytic and rhizosphere microbial populations and modulate the root system in terms of the volume of soil/substrate explored (Rouphael et al., 2017; 2018; Ertani et al., 2018; Basile et al., 2020).

3.1. *Plant and seaweed extracts*

Du Jardin (2015) reported that little was known about the biostimulant activities of plant extracts and that attention to these bioinputs was focused on their pesticidal properties. With the advancement of molecular biotechnology, scientists have proven that the use of plant extracts provides physiological benefits to cultivated plants. Among these effects, we highlight the characteristics of hormonal balance and osmoprotection, which act inside plant cells and protect them against dehydration. Thus, their metabolic activities are maintained at adequate levels, even in stressful situations (Cavalcante et al., 2020).

For example, on grapevine (*Vitis vinifera* L.) the use of edible coatings of *Aloe vera* (L.) Burm.f. maintained the chemical quality of the berries because these biostimulants extended the storage period, reduced moisture loss and respiration rate, and reduced the proliferation of microorganisms (Pessenti et al., 2022). It is also important to highlight that the dynamism of plants in agroecosystems can be mediated by allelochemicals, from allelopathy, which has been receiving more and more attention in the context of sustainable crop management (Du Jardin, 2015).

In a review on biostimulants from organic plants and fruit quality, using the VOSView bibliographic analysis software, Rodrigues et al. (2020) showed that one of the most researched areas corresponded to seaweed extracts. Among the constituents of seaweed extracts that can promote plant growth are polysaccharides (laminarin, alginates, and carrageenans) and their degradation products, micro and macronutrients, sterols, nitrogenous compounds (betaines) and hormones (Craigie, 2011). The main brown algae (phylum Ochrophyta, class Phaeophyceae) used as biostimulants belong to the *Ascophyllum*, *Fucus*, and *Laminaria* genera. Carrageenans, in turn, originate from red algae (phylum Rhodophyta) (Du Jardin, 2015).

When added to the growth medium, seaweed polysaccharides act in gel formation and improve the physical characteristics of the soil or substrate (improve water retention and aeration). By promoting the fixation and exchange of cations, the polyanionic compounds of algae function in soil remediation (Calvo et al., 2014). Furthermore, these biostimulants can activate PGPR present in the rhizosphere and suppress phytopathogens that inhabit the growth medium. In plants, seaweed extracts generating nutritional effects by providing macro and micronutrients to plants and also improve plant development due to hormonal effects (Du Jardin, 2015).

A. nodosum based extracts have been used as a tool to improve tolerance to abiotic stresses and increase fruit quality in grapevines, cultivar 'Cabernet Sauvignon'. The application of improved ecophysiological characteristics and increased the total sugar and polyphenol content of berries in dry and hot years (Pessenti et al., 2022). The literature indicates that the hormonal effects caused by *A. nodosum* are explained by the negative and positive regulation of hormone biosynthetic genes in plant tissues and by the hormonal content of the extracts used (Wally et al., 2013).

Spirulina platensis is one of the main species of algae used in agriculture and in the pharmaceutical industry. In its commercial form, *S. platensis* has a large number of nutrients that are absorbed by the plant. Microalgae of the genus *Scenedesmus* are also used as biostimulants and contain chlorophylls a and b, xanthophylls (lutein and prasinoxanthin) and carotenoids α, β and γ as constituents. These microalgae can stimulate rhizogenesis to increase the percentage of rooting of pomegranate cuttings (*Punica granatum* L.) due to the nutritional content associated with brassinosteroids (Gomes et al., 2019).

3.2. *Humic and fulvic acids*

Chemically, Humic Substances (HS) are the product of a saponification reaction by alkaline extraction of soils and sediments (Canellas et al., 2015), which are classified, according to their molecular weights and solubility, in humines, Humic Acids (HA) and Fulvic Acids (FA) (Du Jardin, 2015). HA are soluble in alkaline Hydrogen potential (pH) and insoluble in acidic pH, while FA are soluble in alkaline and acidic pH (Olk et al., 2019). The application of HS in agriculture has been recognized as a product since the 1980s (Calvo et al., 2014), because HA and FA combine to convert minerals into organic compounds that can be easily utilized by plants (Kumar & Aloke, 2020).

These substances, which represent the largest reservoir of organic-C on the earth's surface, originate from animal, plant, and microbial decomposition (Canellas et al., 2015), and constitute key components of the growth medium's fertility and structure because they act on the chemical, physical, physical-chemical, and biological properties of soils/substrates (Rouphael & Colla, 2018). Especially, HS can to retain water and nutrients, improve Cation Exchange Capacity (CEC), increase nutrient availability and generating an aerated structure of the growth medium (Lehmann & Kleber, 2015).

The benefits of HS to plants may result from its phytohormone-like activity, since the literature reports that the structure of HS includes hormones (Pizzeghello et al., 2001), such as gibberellic acid (Pizzeghello et al., 2002), isopentenyladenosine (Pizzeghello et al., 2013), and indoleacetic acid (Jindo et al., 2012). In the latter case, it was possible to confirm the hypothesis proposed by Concheri et al. (1996) that auxin-like HS activity could induce root development in plants.

Another effect of HS on plant growth is the enhancement of nutrient uptake and the elongation of lateral root growth by inducing Adenosine Triphosphatase (ATPase) activity in the plasma membrane (Jindo et al., 2020; Popa et al., 2022), and this enhances plant development, including yield and fruit quality. The use of fertilizers containing HA and FA improved juice production, vitamin C content and total acid, total sugar and soluble solid contents in lemon [*Citrus limon* (L.) Burm. f.] (He et al., 2022). In grapevine, different concentrations of HA potentiated leaf development, physiological activity, production and berry quality (Popescu & Popescu, 2018). The foliar application of organic acids in olive tree (*Olea europaea* L.) intensified the biochemical properties of olives (Nargesi et al., 2022).

The mechanisms that explain the biostimulant action of HS involve: 1) increased absorption of nutrients linked to the higher CEC of the growth medium with polyanionic HS; 2) increased phosphorus (P) solubility; 3) stimulation of the plasma membrane H^+-ATPases which, when Hydrolyzing Adenosine Triphosphate (ATP), convert the released free energy into transmembrane electrochemical potential; 4) protection against stress by phenylpropanoid metabolism, which originates phenolic compounds involved in plant defense responses to stressors; 5) improvement in the induction of lateral roots and stimulation of nitrate assimilation through the upregulation of nitrate reductase and glutamate dehydrogenase enzymes (Du Jardin, 2015; De Pascale et al., 2017).

Piccolo (2002) showed that HS are supramolecular aggregates and their stability and reactivity depend on the pH of the surrounding environment and the ionic strength of the solution. Therefore, low molecular weight organic acids, such as root exudates, break the structure of macroaggregates and generating subunits of biologically active molecules, responsible for the effects on plants (Nardi et al., 2016). This and other mechanistic studies contributed to the development of humeomics (Nebbioso et al., 2015), aiming to understand the effects of HS on specific plant metabolic processes; this accelerates the development of HS-based biostimulants for use in agriculture (Canellas et al., 2015).

3.3. *Protein hydrolysates*

Protein Hydrolysates (PH) are mixtures of amino acids, polypeptides, and oligopeptides, which act as signaling molecules, derived from protein sources and obtained by enzymatic, chemical (with strong acids or alkalis) or thermal hydrolysis of agricultural by-products of animal origin (leather, viscera, feathers, blood) and/or vegetable (leguminous seeds, alfalfa hay, corn wet milling) (Schaafsma, 2009).

These biostimulants are available as liquid extracts or in soluble powder and in granular form and are applied by foliar spraying and also via irrigation of the growth medium and as a seed treatment (Colla et al., 2015). More than 90% of the PH market applied to horticulture comes from animal protein subjected to chemical hydrolysis, since PH obtained from plant biomass and enzymatically produced are less common because they were recently introduced in the biostimulants market (Colla et al., 2017).

PH modulate molecular and physiological processes in plants, which trigger growth (mainly in root and leaf biomass), increase yield and mitigate abiotic stresses in crops (Yakhin et al., 2017). PH's action mechanisms that benefit plants include: 1) activation of the enzymes Fe(III)-chelate reductase [iron (Fe) metabolism], nitrate reductase, nitrite reductase, glutamine synthetase, glutamate synthase and aspartate aminotransferase (reduction and assimilation of N) and citrate synthase, isocitrate dehydrogenase and malate dehydrogenase (tricarboxylic acid cycle); 2) phytohormone-like activities (auxin and gibberellin); 3) greater action of antioxidant enzymes, pigment biosynthesis and production of secondary metabolites; 4) increase in soil microbial activity; 5) improved mobility and solubility of micronutrients, in particular Fe, zinc (Zn), manganese (Mn) and copper (Cu); 6) changes in plant root architecture (Ertani et al., 2017; RouphaeL et al., 2018; Sestili et al., 2018).

The beneficial effects caused by PH result from the use of very low doses (Ertani et al., 2014), and depend on the species/cultivar, ecophysiological characteristics, time and mode of application and leaf permeability (Kunicki et al., 2010; Ertani et al., 2014). Animal PH spraying improved papaya (*Carica papaya* L.) (Morales-Payan & Stall, 2003), and banana (*Musa* spp.) (Gurav & Jadhav, 2013), productivity. However, the literature also reports that the use of animal origin PH can cause phytotoxicity and growth depression of fruiting crops (Lisiecka et al., 2011).

3.4. *Beneficial fungi and bacteria*

The use of microbial biostimulants, represented by PGPR (*Azospirillum, Azotobacter* and *Rhizobium*, for example) and endophytic fungi (AMF, Sebacinales and *Trichoderma* spp., for example), helps to maintain the crop yield stability in line with the agroecosystem cultivation sustainability and represents a biotool to mitigate stressful conditions for plants (Rouphael & Colla, 2018).

Several studies show the potential of microbial consortiums as plant biostimulants, rhizobacteria and rhizo fungi, which function as agricultural probiotics (Woo & Pepe, 2018), and can quantitatively and qualitatively modulate the rhizosphere microbial population, with a beneficial effect on the soil ecosystem (Rouphael & Colla, 2018). These microorganisms are applied to the soil/substrate to increase crop productivity through metabolic activities (Bulgari et al., 2019). Among the agricultural crops impacted by microbial biostimulants are fruiting crops (Table 1).

TABLE 1. EFFECT OF MICROBIAL BIOSTIMULANTS ON FRUITING CROPS.

Fruiting crops	Microbe	Effects	Reference
Apple [*Malus hupehensis* (Pamp.) Rehder]	*Trichoderma asperellum*	Increased seedlings growth and young branch elongation.	Wang et al. (2022)
Blackberry (*Rubus glaucus* Benth)	*T. asperellum*	Increased crop yield and fruit weight.	Viera et al. (2019)
Blueberry (*Vaccinium* spp.)	*Bacillus amyloliquefaciens*, *B. subtilis* and *B. licheniforims*	Increased growth, fruit quality and production.	Yu et al. (2020)
Citrus (*Citrus reticulata* Blanco)	*Funneliformis mosseae*, *Diversispora versiformis* and *Rhizophagus intraradices*	Increased fruit quality and root physiological activity.	Cao et al. (2021)
Goldenberry (*Physalis peruviana* L.)	*Mycorrhizal community*, *R. intraradices* and *Rhizophagus clarus*	More profuse root system and better chemical quality fruits.	Chiomento et al. (2022)
Grape (*Vitis berlandieri* Planch.)	*Pseudomonas putida* and *B. simplex*	Increased graft callusing, scion shoot growth, cane hardening and nursery survival rate.	Sabir (2013)
Kiwifruit [*Actinidia deliciosa* (A. Chev.) C. F. Liang & A. R. Ferguson.]	*Bacillus* sp., *Paenibacillus polymyxaa* and *Comamonas acidovorans*	Rooting stimulation and root growth.	Erturk et al. (2010)
Melon (*Cucumis melo* L.)	*Azospirillum brasilense*	Increased yield and economic returns.	Vendruscolo et al. (2020)
Strawberry (*Fragaria X ananassa* Duch.)	*Azotobacter*	Increased growth, runner production, yield and fruit quality.	Mishra & Tripathi (2011)
	T. harzianum	Increased relative yield, growth, productivity and anthocyanins accumulation in fruits.	Lombardi et al. (2020)
	Claroideoglomus etunicatum	Increased shoot and root biomass and total anthocyanins, flavonoids and polyphenols contents.	Chiomento et al. (2021)
Walnut (*Juglans regia* L.)	*Pseudomonas chlororaphis* and *Arthrobacter pascens*	Increased plant height, shoot and root dry weight, phosphorus and nitrogen uptake.	Yu et al. (2012)

Source: Authors.

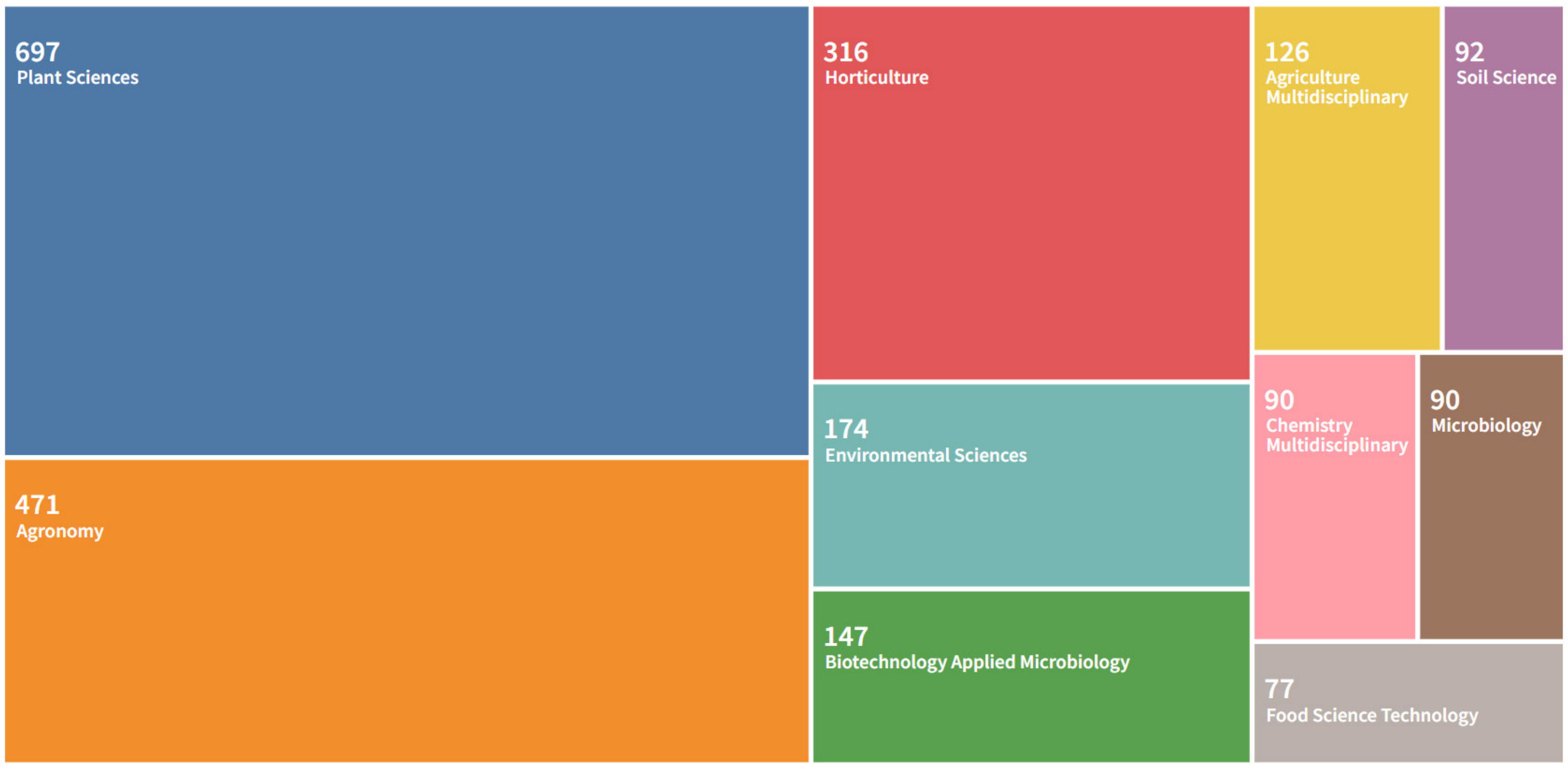
The direct and indirect action mechanisms of phytostimulation of rhizobacteria and rhizofungi include: 1) improvement in the efficiency of nutrient use, mainly N, P, Fe, Zn and Mn; 2) water use efficiency and photosynthetic capacity; 3) better development of the root system; 4) balance of the cellular oxidative state and better antioxidant defense system; 5) hormonal regulation (abscisic acid, indole-3-acetic acid, jasmonic acid, salicylic acid); 6) higher enzymatic activity and production in the rhizosphere (De Pascale et al., 2017; Bitterlich et al., 2018; Castiglione et al., 2021).

New biotechnologies have enabled an exponential increase in the number of researches and experiments linked to the benefits of microbial biostimulants (Castiglione et al., 2021). However, the effectiveness of microbial biostimulants needs to be better validated in field experiments, since these bioinputs are often used as supplementary therapies instead of being used to their full potential in crop management (Fadiji et al., 2022).

4. Biostimulants application in fruiting crops

A search in the Web of Science database (https://www.webofscience.com), using 'biostimulants' as a keyword, resulted in 2 262 publications and indicated that 14% of the documents deal with biostimulants in the horticulture field (Fig. 3), which includes fruiting crops. Thus, we grouped the literature according to fruiting crops (berries, citrus, apple, papaya, passion fruit and grapevine) to report some results on the effects of plant biostimulants under normal or stressful conditions, in the field or greenhouse, aiming at the growth and development of plants, fruit production and quality, and effects on soil ecosystems.

Fig. 3. Based on searches in the Web of Science database, research linked to the use of plant biostimulants departs from plant sciences to agronomy and focuses on horticulture.



Source: Web of Science database.

4.1. *Berries*

The use of the rhizofungus *T. asperellum* significantly improved the yield and weight of blackberry (*R. glaucus*) fruits, and the organic matter content was correlated with the fungal population in the soil (Viera et al., 2019). Garcia-Seco et al. (2015) demonstrated that the use of the rhizobacterium *Pseudomonas fluorescens* in blackberry (*Rubus* sp.), 'Loch Ness' cultivar, increased the expression of some flavonoid biosynthesis genes and the concentration of selected flavonoids in fruits.

In blueberry (*Vaccinium corymbosum* L.), 'Legacy' cultivar, the combined application of a microbial consortium formed by *B. subtilis*, *B. licheniformis*, *B. megaterium*, *B. polymyxa*, *B. macerans*, *P. fluorescens*, *P. putida*, *Nocardia corallina*, *Saccharomyces cerevisiae*, and *T. viride* (OIKO-BAC® 174; Oikos Chile Ltda, Chile), and humic acid derived from leonardite (Biosolve® 174; Oikos Chile Ltda, Chile) increased shoot dry weight (50%) and root dry weight (43%) of seedlings and improved N and potassium (K) uptake from the soil (Schoebitz et al., 2016).

In raspberry (*Rubus idaeus* L.), 'Tulameen' cultivar, the use of the monospecific mycorrhizal inoculant based on *R. intraradices* (MYKOS®; Xtreme Gardening, Canada) increased the number of flowers per plant (33%) and the number of fruits (35%). Furthermore, the weight of a single berry increased more strongly with rates of fertilizer application in plants inoculated with AMF than in non-inoculated plants. As a consequence, AMF inoculation increased raspberry production by 43% compared to non-mycorrhizal plants (Chen et al., 2022).

In strawberry, Soppelsa et al. (2019) reported that the application of *A. nodosum* extract and PH based on alfalfa improved the content of phenolic compounds in the fruits, while the use of chitosan increased the firmness of the strawberry pulp. Ismail and Ganzour (2021), when performing foliar applications of white wattle extract (*Moringa oleifera* L.), observed increases in fresh and dry biomass, in carbohydrate, vitamin C and anthocyanin contents and in total yield.

4.2. *Citrus*

The application of seaweed extract based on *A. nodosum* (Stimplex® Crop Biostimulant; Acadian Seaplants, Nova Scotia, Canada), via foliar spraying or soil soaking, attenuated the effects of water stress on young 'Hamlin' sweet orange [*Citrus sinensis* (L.) Osbeck], grafted onto 'Carrizo' citrange [*Poncirus trifoliata* (L.) Raf. × *C. sinensis*] and 'Swingle' citrumelo (*P. trifoliata* × *C.* × *paradisi* Macfad.). Plants grown in pots and treated with the seaweed extract had greater vegetative growth (longer shoots, greater leaf area and greater dry mass of shoots and leaves), regardless of the rootstock used. Water use efficiency was higher in plants treated with *A. nodosum* under water stress and grafted on 'Swingle' (Spann & Little, 2011). Koo (1988) reported that the use of an extract with this same brown seaweed increased the production of 'Sunburst' (*C. reticulata*) and 'Valence' (*C. sinensis*) mandarin oranges by 11%.

When investigating the use of microbial biostimulants, we verified that the use of AMF (*Funneliformis mosseae* and *Paraglomus occultum*) in citrus (*P. trifoliata*) increased the dry weight of the root, the length of the main root, the number of lateral roots, the concentrations of fructose and glucose and caused less accumulation of proline in the roots (Zhang et al., 2018). Previously, Wu et al. (2017) demonstrated that these same plant fungal species also induced osmotic adjustments in *P. trifoliata* under controlled substrate conditions. The concentration of inorganic (K^+ and Ca^{2+}) and organic (sucrose, glucose, and fructose) osmotically active substances was higher in leaves of plants inoculated with AMF under water stress, while proline (involved in osmotic adjustment) was reduced. AMF also increased the structure and stability of aggregates in the rhizosphere and this improved water availability and plant growth. In citrus, the role of aquaporins in the cell membrane, involved in water transport, was to ensure growth and water availability to plants subjected to water stress and inoculated with AMF. Aquaporin genes regulate the responses of its complex mechanisms to mycorrhization and water stress (Basile et al., 2020).

4.3. *Apple*

The isolated application of *A. nodosum* extract, vitamin B, and PH based on alfalfa in apple tree, 'Red Jonathan' cultivar, potentiated the levels of phenolic compounds and antioxidant activity (Soppelsa et al., 2018). Two years later, this same research group verified that the use of seaweed extract based on *A. nodosum* (Algavis®; SERBIOS, Polesine, Italy) increased the reddish coloration of fruits and the final concentration of anthocyanin in the skin, in addition to reduce the physiological disorder 'Jonathan's spot' after 160 days of storage. The increase in the concentration of Ca, Zn, and Mn in the skin of apples after *A. nodosum* applications, together with alterations in the phenolic profile during storage, were identified as the possible causes of the lower susceptibility of the fruits to post-harvest disorders (Soppelsa et al., 2020).

Przybyłko et al. (2021), when studying the 'Topaz', 'Odra', and 'Chopin' cultivars and an improved clone U 8869, showed that the use of a microbial biostimulant (Micosat® F; CCS Aosta, Quart, Italy) formed by AMF (*Glomus mosseae*, *G. viscosum*, and *G. intraradices*) and PGPR (*B. subtilis* and *Streptomyces* spp.) improved N nutritional status, which promoted vigorous tree growth and more efficient uptake of magnesium (Mg) from the soil.

In the national market, some multinational companies are betting on the use of biostimulants to improve the redness of the apple epidermis. Many of these products are composed of calcium (Ca), molybdenum (Mo) and K, in addition to minimal concentrations of ethylene and jasmonates.

4.4. Papaya

Concentrations of 0.4% to 1.6% of biostimulant based on the seaweed *S. platensis* improved the agronomic performance of papaya seedlings (*Carica papaya* L.), 'Formosa' and 'Solo' groups, because they increased number of leaves, plant height, stem diameter, leaf area, root length, biomass (fresh and dry) and Dickson quality index (Guedes et al., 2018).

The application, via foliar spraying, of humic and fulvic acids (Humitec®; Tradecorp, Hortolândia, Brazil) in papaya tree, 'Formosa' group, improved the phytometric morphology of the shoot and root system and the papaya seedlings quality (Cavalcante et al., 2011).

Prasad et al. (2022) reported that the application of turmeric leaf extract (*Curcuma longa* L.) on papaya, 'Red Lady' cultivar, prolonged maintenance of fruit quality because it increased nutritional (total carotenoids, ascorbic acid) and biochemical attributes (total soluble solids, titratable acidity and total phenolics) throughout storage and provided greater consumer preference and sensory score.

4.5. *Passion fruit*

Ferreira et al. (2007) studied the effects of a biostimulant based on 4-indole-3-ylbutyric acid, gibberellic acid, and kinetin (Stimulate®; Stoller do Brasil, Campinas, Brazil) on passion fruit (*Passiflora edulis* f. *flavicarpa* Degener). The biostimulant applied to the seeds promoted increases in the percentage of emergence and seedling development.

The consortium between *T. asperellum* and *T. harzianum* increased the number and size of chloroplasts, plant physiology characteristics, yield and quality of passion fruit (*P. caerulea* L.) (Şesan et al., 2020).

4.6. *Grapevine*

In an experiment conducted in pots and in a greenhouse, foliar spraying of seaweed extract (IPA®; BiotechMarine, Pontrieux, France) induced a significant increase in the leaf water potential of grapevine (V. vinifera), 'Sangiovese' cultivar. This improvement in the water status of the plants submitted to the extract was translated into increased values of stomatal conductance and carbon dioxide (CO_2) exchange rates (Mancuso et al., 2006).

Nikolaou et al. (2003) showed that the addition of *Glomus mosseae* in the soil under grapevine (*V. vinifera*) cultivation, 'Cabernet Sauvignon' cultivar improved leaf water potential, stomatal conductance and CO_2 exchange rates compared to non-mycorrhized vines.

When studying different classes of biostimulants, Pessenti et al. (2022) found that abscisic acid and *A. nodosum* extracts increased the total anthocyanin and polyphenol contents in 'Cabernet Sauvignon' berries and wine.

Soil K silicate application stimulated leaf area expansion rates and plant height of 1-year-old cuttings of 'Cabernet Sauvignon' exposed to saline stress. These effects were associated with the mitigation of the negative influence of salinity on leaf photosynthesis, probably because silicon plays a significant role in protecting the photosynthetic apparatus. This effect was also suggested by the increase in maximum yield and potential photochemical efficiency of photochemical reactions in photosystem II (Qin et al., 2016).

In national viticulture, many companies are betting on the commercialization of products that enhance the purple hue of the skin of the grape, especially in cultivars that tend to have a pink skin, such as 'Rubi'. These products, which activate the anthocyanins of the epidermis, have Ca, Mg, K, ethylene and abscisic acid in their compositions.

5. Final considerations and future prospects

The use of biostimulants in agriculture has increased a lot in the last 15 years, mainly due to their multifaceted properties (Rouphael & Colla, 2018). The diffusion of biostimulants application on a world scale can be seen by an exponential growth in their commercialization, whose market in 2018 exceeded US$ 2.3 billion. It is estimated that between 2019 and 2025 the growth of this market will be 1.4% per year, since biostimulants have proven to provide improvements to biogeocenosis, in addition to contributing to optimal and sustainable production, mainly because they improve the efficiency of water and nutrients use to crops and increase plant tolerance against abiotic stressors.

The totality of biostimulants' action mechanisms still needs to be better understood. In this case, scientific research is an excellent tool for generating and disseminating information on the use of these bioinputs in the management of fruiting crops. Knowledge to increase crop productivity and quality, fruit conservation and food security is built in education, research, development, and innovation institutions. The findings arising from these surveys must, therefore, be disseminated in the scientific community and articulated with the productive, commercialization and export sectors. This characterizes the dynamism that must occur between scientists, private industries, legislators and producers.

The generation of new technical-scientific information on the effects and potential use of biostimulants provides farmers with information about the real potential and impacts of these bioinputs on agricultural production. Furthermore, the State provision of technical assistance and rural extension, to build ecologically sustainable and socially inclusive agricultural and agrifood systems, will underlie the dissemination of the use of inputs that are less harmful to agroecosystems (Caporal, 2020).

The applicability of biostimulants in the fruiting crops' management proved to be a relevant possibility to grant sustainability to production systems in fruticulture and reduce costs, increasing productivity, shelf life and reducing damage caused by climatic adversities in crops, mainly hydrical stress. There are numerous biostimulant alternatives that incite physiological mechanisms in fruiting crops, which can replace or reduce the use of exogenous inputs in agricultural production units.

Plant biostimulants are a new generation of products available on the market, which can be useful to achieve agricultural sustainability policies (Castiglione et al., 2021). However, it is important to highlight the urgent need for further studies with biostimulants in perennial crops (Rodrigues et al., 2020). Revealing more about the complex signaling network for alleviating environmental disturbances between biostimulants and fruiting crops, under circumstances that approximate natural conditions as closely as possible (commercial orchards), will allow scientists, private industries, policymakers and interested parties to develop and/or improve molecular tools to improve the effectiveness and consistency of the positive effects of these bioinputs.

After reaching a good understanding of the action mechanisms and modes of plant biostimulants, we will be able to advance to the next generation of these products (biostimulants 2.0), where synergies and complementary mechanisms can be functionally designed, through the application of microbial agents (rhizobacteria and rhizofungi) and not microbial agents (algae and plant extracts, humic substances and protein hydrolysates) to make agriculture more sustainable and resilient (Rouphael & Colla, 2020).

Finally, the development of specific legislation for biostimulants should contribute substantially to generating credibility with farmers in order to differentiate, for example, foliar fertilizers and microbial agents. This is due to the fact that the biostimulants' action modes, their way of penetrating plants and the syntheses capable of forming new compounds, are different from any other class of products for agriculture.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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