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Published in:
Agronomy

Link to article, DOI:
[10.3390/agronomy12030659](https://doi.org/10.3390/agronomy12030659)

Publication date:
2022

Document Version
Publisher's PDF, also known as Version of record

[Link back to DTU Orbit](#)

Citation (APA):
Afonso, S., Oliveira, I., Meyer, A. S., & Goncalves, B. (2022). Biostimulants to Improved Tree Physiology and Fruit Quality: A Review with Special Focus on Sweet Cherry. *Agronomy*, 12(3), Article 659.
<https://doi.org/10.3390/agronomy12030659>

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Review

Biostimulants to Improved Tree Physiology and Fruit Quality: A Review with Special Focus on Sweet Cherry

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Abstract: Due to the increasing global population and the continued need to sustainably increase agricultural production, the agricultural sector requires innovative strategies to increase productivity and efficiency in the use of resources. Biostimulants have emerged as new, promising, and environmentally friendly products to promote the overall sustainability of production systems. Humic and fulvic acids, protein hydrolysates, seaweed extracts, chitosan and other biopolymers, inorganic compounds, beneficial fungi, and bacteria are widely accepted categories of biostimulants, with proven potential in improving plant growth, increasing crop production, and quality of the final product. Some of them also have the capacity to enhance nutrient uptake and improve stress tolerance of the crop. Sweet cherry is a highly appreciated fruit, with a significant economic value, linked to production yield and quality attributes influencing consumer acceptability. However, this fruit presents several undesirable characteristics, such as physiological disorders (e.g., fruit cracking) and a short shelf-life. Several approaches are used to enhance not only sweet cherry production, but also cherry quality, with the latest efforts being placed in biostimulants. The present review focuses on the most recent findings on the use of biostimulants in sweet cherry production.

Keywords: *Prunus avium* L.; biostimulants; humic and fulvic acids; protein hydrolysates; chitosan; seaweed extracts; inorganic compounds; microorganisms



Citation: Afonso, S.; Oliveira, I.; Meyer, A.S.; Gonçalves, B. Biostimulants to Improved Tree Physiology and Fruit Quality: A Review with Special Focus on Sweet Cherry. *Agronomy* **2022**, *12*, 659. <https://doi.org/10.3390/agronomy12030659>

Received: 20 February 2022

Accepted: 8 March 2022

Published: 9 March 2022

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1. Introduction

The growing pressure on crop productivity, including plants and fruits, caused by the increasing world population, is a demanding challenge, as reduction of the use of agrochemicals with negative impacts on humans and ecosystems is compulsory. Hence, new strategies must be found, including those from the bio-based industry, using circular economy principles [1,2]. Fruit production, yield, and quality are influenced by several factors, including both biotic and abiotic. Stresses caused by such factors can reduce production yield, sometimes significantly, but also negatively influence the quality characteristics of any fruit [3]. The ability to regulate plant growth and, at the same time, to reduce the impact of these biotic and abiotic stresses, can provide tools to achieve maximum productivity and quality in plants. Even though abiotic stresses can be prevented to some extent by providing plants their optimal growth conditions (including fertilization and watering) and using plant growth regulators (PGRs) (auxins, cytokinins, gibberellins, strigolactones, brassinosteroids), the use of biostimulants is becoming a common practice in production systems [4,5]. Due to their ability to change physiological processes in plants, linked to growth, production, fruit set, and stress mitigation, biostimulants have recently received

considerable interest from science and enterprises [6,7]. Furthermore, being bio-based products, their impact on biodiversity, environment, human health, and economy is considerably less when compared to inorganic and organo-mineral fertilizers [8]. Biostimulants exert their function by regulation of several physiological and molecular mechanisms, including stimulation of the carbon (C) and nitrogen (N) metabolism, enhancing of antioxidant defenses and production of secondary metabolites, increasing photosynthetic activity and improving water relations, enhancing soil characteristics, both chemical and physical, activating hormone-like activities (particularly auxins, cytokinins, and gibberellins), improving epiphytic and rhizosphere microbial populations and modulating root system apparatus, regarding biomass, branching, density, diameter, length, and volume of soil/substrate exploited [7]. Even so, the beneficial results of the use of a given biostimulant in one crop cannot be generalized for other types of crops, especially in what concerns open-field fruit production, rather than those grown under greenhouse conditions. This difference is probably linked to the effects of application frequency and the favorable climatic conditions in controlled environments [9,10]. Although some limitations can be found for the use of biostimulants, they are being increasingly applied in fruit production, including sweet cherry. To the best of our knowledge, this is the first review aiming to provide an updated overview of the known and potential benefits of plant biostimulants on the growth, yield potential, nutritional and functional quality of sweet cherries. This fruit—sweet cherry—is among the most appreciated in the temperate areas of Europe [11], preference owing by its organoleptic features (color, brightness, flavor, aroma, and texture), but also by its recognized benefits for human health that influence consumer acceptability [12,13]. Even though sweet cherry is a fruit that allows producers to obtain significant economic profits, it has several undesirable characteristics. Agronomic features such as fruit set, flowering, and fertilization can be problematic, with climatic conditions having a major effect on them, either by delaying or causing irregular flowering, reducing bud opening, and hindering pollination [14,15], which can significantly influence the obtained yield. Other issues with sweet cherries are linked to physiological disorders of fruits, namely cracking, pitting, wrinkling, and darkening of stalk color, which can negatively influence the market price of this crop [16]. Sweet cherry also has a shelf-life comparably shorter than other fruits, being highly susceptible to quality losses under postharvest conditions [17,18]. Considering all these challenges that sweet cherry production presents, all strategies that can maximize yield, without compromising quality and sustainability must be addressed, and this review aims to provide a synopsis of the applicability of biostimulants to this specific fruit tree.

2. Definition and Main Categories of Biostimulants

Although the available research about biostimulants has been growing fast in the last years, a clear definition of “biostimulants” is still to be coined. The use of the term “biostimulant” includes products with various descriptions, namely biogenic stimulants, metabolic enhancers, plant strengtheners, positive plant growth regulators, elicitors, allelopathic preparation, plant conditioners, phytostimulators, biofertilizers, or biofertilizer/biostimulant [4]. Definitions for what can be ultimately considered biostimulants have been evolving since the 1950s, starting from “Every living tissue (human, animal, and plants), when exposed to unfavourable, but non-lethal conditions, undergoes biochemical restructuring with the formation in it of special substances which are biogenic stimulators of non-specific nature, stimulating the life reactions of the organism, in which they introduced in, one way or another”, by Filatov [19]. The industry also has its own proposed definition, adopted by the European Biostimulant Industry Council (EBIC), which, together with the Biostimulant Coalitions in the United States, is one of the world’s largest organizations of the manufactures of such products [20]. This council suggests “Plant biostimulant means a material which contains substance(s) and/or microorganisms whose function when applied to plants or the rhizosphere is to stimulate natural processes to benefit nutrient uptake, nutrient efficiency, tolerance to abiotic stress, and/or crop quality, independently of its nutrient content” [21]. The most recent definition, in the context of the revision of the EU

fertilizer legislation (2019) [22], and still under discussion, stands as “A plant biostimulant is any microorganism or substance based on natural resources, in the form in which it is supplied to the user, applied to plants, seeds or soil and any other substrate with the intention to stimulate natural processes of plants to benefit their nutrient use efficiency and/or their tolerance to stress, regardless of its nutrients content, or any combination of such substances and/or microorganisms intended for this use”. Although the clear definition of what a biostimulant is, from the point of view of regulatory legislation is of great importance, it should also be defined from a scientific point of view. Their classification may be performed using the mode of action and the origin of the active ingredient [23] or based on their action in the plants or on the physiological plant responses [24]. Another suggestion has been put forward by du Jardin [25], stating that “any definition of biostimulants should focus on the agricultural functions of biostimulants, not on the nature of their constituents nor on their modes of actions”. In fact, it was this author’s analysis and categorization of biostimulants that was influential in informing the development of subsequent legislation and regulation in the European Union [4]. As the definition of biostimulants has evolved, the categorization of these products has also been changing through the years. Again, Filatov [19] was the first to create categories of biostimulants, with four groups of biogenic stimulants suggested. Later, nine categories, based on raw materials used to derive biostimulants were suggested by Ikrina and Kolbin [26], and, to date, the most widely accepted classification, with seven categories, was put in place by du Jardin [4,25]. These categories are humic and fulvic acids, protein hydrolysates and other N-containing compounds, seaweed extracts and botanicals, chitosan and other biopolymers, inorganic compounds, beneficial fungi, and beneficial bacteria, and will be briefly addressed here.

2.1. Humic and Fulvic Acids as Biostimulants

Humic and fulvic acids are part of the humic compounds in the soil, with the addition of humins [25] and derive from soil organic matter decomposed by plants, animals, and microorganisms, as well as from the activity of soil fauna [27,28]. They are separated into three categories, based on their behavior in inorganic acidic and alkaline solvents: humic acids are soluble in basic media, fulvic acids are soluble in both alkali and acid media, and humins are not extractable from soil [29,30]. Their role in plant and soil functions has been reviewed by Berbara and García [30] and includes the control of nutrient availability, soil/atmosphere carbon and oxygen exchange, and the transformation and transport of toxic chemicals. Furthermore, plant physiology and the composition and function of rhizosphere microorganisms are affected by humic substances in soils [31].

2.2. Protein Hydrolysates and Other N-Containing Compounds

Another category of biostimulants is protein-based products, which can be divided into protein hydrolysates (PHs) (a mixture of peptides and amino acids of animal or plant origin) individual amino acids (glutamate, glutamine, proline) and glycine betaine [32,33]. The former is prepared by several approaches, namely enzymatic, chemical, or thermal hydrolysis from animal and plant residues [28,34]. The latter include the known structural twenty amino acids that take part in protein synthesis, but also those non-protein amino acids found abundantly in some plants [32,35]. PHs’ application is mainly foliar, although they are also applied to substrate and seeds [36]. PHs have demonstrated, both in open field and in greenhouses, the ability to increase nutrient absorption, density, length, and number of lateral roots [33,37], as well as improved enzymatic activity, increased photosynthetic rate and stomatal conductance, plant growth, and productivity, especially under environmental stress conditions [5,38–40].

2.3. Seaweed Extracts and Botanicals

Seaweeds have been used in coastal regions as soil fertilizers or amendments for centuries [41]. Liquid extracts from seaweeds have been available since the 1950s, their commercially focused products are now common in agricultural and horticultural crops [41,42]. Indeed, the use of seaweeds as plant growth and vigor promoters was put in place for several decades, until synthetic fertilizers took their place as the prime option [43]. Seaweed extract (SWE) contains a wide variety of components linked to the specific source of the seaweed, the time of collection, and the extraction process [42,44,45]. In their composition, both organic and mineral compounds are present, such as unique and complex polysaccharides, only found in seaweeds, plant hormones [42,46,47], namely cytokinins, auxins, abscisic acid, gibberellins, and other classes of hormone-like compounds, such as sterols and polyamines [41,48]. Polysaccharides are major components of brown seaweeds, and two of the most studied brown macroscopic algae are *Ascophyllum nodosum* and *Ecklonia maxima* [33]. There are numerous reports of beneficial effects of the application of these seaweed extracts linked to increases in plant growth and yield parameters as well as an increase of protein and nutrient content [49,50]. High concentration of phenolic compounds, antioxidant activity, and positive effects of these SWE on stress mitigation have also been recently reported after their application [51–53].

Another recent and novel seaweed liquid extract prepared from *Ulva lactuca* (Chlorophyta), *Pterocladia capillacea*, and *Jania rubens* (Rhodophyta) has been applied as plant biostimulants. The foliar application of this seaweed mixture enhanced the morpho-agronomic (higher significant plant height, branches, chlorophyll, dry matter, leaf-P, and leaf-K, increased total yield, fruit length, and fruit diameter) and bioactive properties (higher content in ascorbic acid, flavonoids, and phenolics and improved antioxidant capacity) of hot pepper *Capsicum annuum* [54].

Botanicals are defined as substances extracted from plants that are to be used in pharmaceutical or cosmetic products, but also as food ingredients or plant protection products [55]. Although much is still to be studied regarding the use of botanical extracts as biostimulants, they are known to be used as pesticides, although several pieces of evidence point out their applicability as biostimulants [25]. Indeed, recent works show how plant-derived extracts help to improve stress resistance or plant growth and fruit setting [56].

2.4. Chitosan and Other Biopolymers

Chitosan is formed when over 80% of the acetyl groups of the N-acetyl-d-glucosamine residues present in chitin, a co-polymer of N-acetyl-d-glucosamine and d-glucosamine, are removed. Depending on the sizes of the molecules, chitosan has the potential to be used in several industries, such as food, cosmetics, or medical ones [25,57]. It can also be used in agriculture, as a biostimulant, due to its ability to stimulate plant growth, tolerance to abiotic stress, and improved crop resilience to pathogen attack [58,59]. Preharvest application of chitosan shows an increase in fruit yield [57] and many chitosan-treated plants obtained a large production of hydrogen peroxide (H₂O₂) which led to the induction of plant defense enzymes, including phenylalanine ammonia-lyase (PAL), an important enzyme in the biosynthesis of phenolic compounds. The induction of PAL through the application of chitosan correlated well with the accumulation of phenolic compounds reported in several plants [60]. Chitosan postharvest application prevents decay during storage, delays microbial infection, and extends fruits storability [59]. Chitosan proteome and transcriptome changes caused after application of chitosan to plants [60] show its effect in the development regulation [25], and evidence shows that it may also directly control gene expression particularly those linked to the activation of plant defense signaling pathways [61,62].

2.5. Inorganic Compounds

Essential elements are those considered to be required by an organism to complete its life cycle. There are 17 elements essential to all plants, and they can be divided into macronutrients and micronutrients. The former includes C, H, O, Ca, K, Mg, N, S, and P, of which C, H and O account for approximately 95% of plant dry matter, while the others are typically present at >1000 mg/kg dry weight. Microelements, also called trace elements, comprise Cl, B, Cu, Fe, Mn, Mo, Ni, and Zn, which are usually present at <100 mg/kg dry weight [63]. Chemical elements, such as Al, Co, Na, Se, and Si, that promote plant growth, but are not required by all plant species, are entitled beneficial elements [63]. They can be found in soils and plants in different insoluble forms or inorganic salts, ionic compounds composed of negatively charged anions and positively charged cations [64], including phosphites, phosphonates, bicarbonates, silicates, sulfates, and nitrates [25,28]. Although their mode of action is not completely understood yet, these compounds are able to promote plant growth, quality of plant products, and tolerance to abiotic stress by several mechanisms, including the rigidification of cell wall, osmoregulation, reduction of transpiration, radiation reflection leading to thermal regulation, enzyme activity, plant nutrition, antioxidant protection, interactions with symbionts, responses to pathogens and herbivores, heavy metals toxicity protection or synthesis, and signaling of plant hormone [62,63]. These compounds—inorganic salts—have been used against fungal diseases, as they have some important advantages, including their low cost, reduced impact on the environment, and low mammalian toxicity [64,65]. This fungicidal activity appears to be derived from their ability to change osmotic, pH, and redox balance, as well as influence the activity of hormones and enzymes involved in stress responses [28].

2.6. Beneficial Fungi and Bacteria

In addition to the diversity of compounds/substances that are considered biostimulants, the definition of the European Biostimulant Industry Council [20] also includes microorganisms. In this group, there are beneficial fungi and bacteria. Fungi- and bacteria-based biostimulants are of particular importance, as they positively influence soil biodiversity, playing an important role in mitigating the impacts of agricultural activity on the environment [33,66] and benefiting plants, regarding their nutrition, productivity, and stress resistance, both abiotic and biotic [67].

Among the beneficial fungi, the most known are the arbuscular mycorrhizal fungi (AMF). These root fungal symbionts are associated with over 90% of all plant species [25], and can modify plant–soil interactions, enhancing plant growth when under stressful edaphic conditions [68]. Of the known effects related to the inoculation of plants with AMF is an increase in water adsorption, improving tolerance to drought [69], and nutrient adsorption (N, P, and K) [70–73]. They are also able to reduce the negative effects of salinity and alkalinity conditions [74,75]. These effects are attributed to the fact that arbuscular mycorrhizal fungi increase the root area, the penetration of those in the substrate, but also by activating and excreting various enzymes [68]. However, some drawbacks have been pointed out to these fungi, namely the difficulty to propagate them on a large scale, but also the need to better understand the host specificities and dynamics of mycorrhizal communities within agroecosystems [25]. More recently, attention has been given to other fungal endophytes, such as *Trichoderma* spp., as they are easier to multiply in vitro, and can be used as a model for understanding the mechanisms of nutrient transfer between fungal endosymbionts and their hosts [25,76,77]. These saprotrophic fungi can promote plant growth, by producing antimicrobial compounds, by parasitizing other fungal pathogens, but also by increasing solubility of some soil micronutrients or by producing indole-3-acetic acid or auxin analogs [6,78–81]. However, plants can present interaction with a wide variety of soil fungi, from various taxa, belonging to arbuscular mycorrhizal fungi, orchid mycorrhizal fungi, ericoid mycorrhizal fungi, ectomycorrhizal fungi, *Piriformospora*, and other root endophytes fungi such as *Fusarium* spp., *Penicillium* spp., and *Aspergillus* spp., all recognized as being plant growth-promoting fungi [82]. Arbuscular mycorrhizal fungi

(AMF) are known to have a major role in plant nutrition, as they can improve mineral uptake by plants [83] and are widely used in horticulture, namely *Rhizophagus* (formerly known as *Glomus*) *intraradices* and *Funneliformis* (formerly known as *Glomus*) *mosseae* [84]. The use of AMF in several horticultural crops has proven to result in beneficial effects, including higher mineral content, marketable fresh yield, increased growth, and improved chemical characteristics, in parallel to higher resistance to diverse stresses, such as drought and saline conditions, nutrient deficiency, heavy metal pollutants, and adverse soil pH conditions [85].

Other microbial-based biostimulants are those containing bacteria, either plant growth-promoting rhizobacteria (PGPRs) and plant growth-promoting bacteria (PGPBs), both comprising free-living bacteria mainly isolated from the rhizosphere [86]. Regarding beneficial bacteria, du Jardin [25] refers to two different types, namely the mutualistic endosymbionts of the type *Rhizobium* and the mutualistic rhizospheric PGPRs. Of those, PGPRs are the most common and are currently considered as effective for biostimulation of plant growth [32]. PGPRs are described as being able to influence plant performance in several ways, namely increased yield, growth, micro- and macronutrient uptake, higher chlorophyll and mineral content, as well as being able to respond to biotic and abiotic stresses, thoroughly reviewed by Ruzzi and Aroca [87] and Mehmood et al. [88]. These effects are due to the ability of PGPRs to alter the hormonal profile of a given plant, the emission by PGPRs of volatile organic compounds that stimulate plant growth, the increase of availability and intake of nutrients by plants, or by reducing the susceptibility of plants to abiotic stress factors [87–89].

3. Use of Biostimulants in Sweet Cherry and Other Fruit Crops

3.1. Humic and Fulvic Acids

There are several works regarding the use of biostimulants in sweet cherry, although the number of references available is quite dissimilar when comparing all the groups of biostimulants referred before. For humic and fulvic acids, there are some works regarding their effects on plant growth and fruit quality [90]. The use of humic acids combined with the application of Fe or Zn led to an apparent increase in the uptake of these two minerals, as their amount in treated leaves is significantly higher than in those where only Fe or Zn was used [90]. The use of humic acid combined with grape seed extracts resulted in a reduction of stress indicators in cherry leaves [91], namely the reduction of membrane degradation and an increase of peroxidase activity, to counteract the oxidative stress. The application of humic acid to sweet cherry trees resulted in an increase in the mineral content (nitrogen (N), phosphorus (P), potassium (K), and calcium (Ca)) of leaves, improving the health status of those plants. However, increasing levels of humic acid does not result in the same increase in mineral content of leaves [92]. The use of fulvic acids combined in an integrated nutrition regime [93] resulted in increased root density and fruit yield. Furthermore, changes in some fruit parameters, namely higher dry matter and K content, were also detected. The application of leonardite (an oxidized form of lignite with a brown and coal-like appearance, with high content of humic acid) contributed significantly to a positive effect in leaf nutrient contents and fruit chemical parameters (pH, total soluble solids, total phenolic content, and antioxidant activity), with the exception of Mg in leaves [94]. These results appear to be related to the ability of humic substances to improve availability and uptake of micronutrients, linked to the increase of root cell membrane permeability. The use of humic acid to correct iron chlorosis in cherry leaves has also been proven. Kalınbacak and Köksal [95] report that humic acid alone or used combined with FeSO_4 can help cherry trees to recover from iron chlorosis. Another potentiality that may be linked to the use of humic acids in sweet cherries is its ability to reduce cracking. Although nothing is known regarding the effect of humic acid on this phenomenon in cherries, its use on pomegranate has proven to significantly reduce cracking [96] and could be a novel strategy to reduce losses caused by this physiological disorder.

3.2. Protein Hydrolysates

The use of protein-based products, protein hydrolysates, and individual amino acids in sweet cherries has been tested sometimes. Available data show an intricate effect of some of these compounds on plant physiology and fruit characteristics. The use of glycine betaine in cultivar (cv.) Staccato resulted in bigger fruits, with increased content in soluble solids, polyphenols, vitamin C, and antioxidant activity, but lower acidity [97]. The same treatment resulted in changes in the visual aspect of fruits. Other works show a decrease in plant characteristics, such as photosynthetic parameters, namely stomatal conductance (gs), after spraying with glycine betaine plant of cv. Skeena and resulted in an increase of relative water content [98]. The same authors showed an increase in leaf pigment content, soluble sugars, soluble proteins, and starch. In fruits of cv. Skeena, the use of glycine betaine increased fruit weight, color at ripening [99], and anthocyanin content, but decreased carotenoid content and ascorbic acid [100]. Cracking incidence was reduced of cv. Sweetheart fruits when using glycine betaine, with higher wax content, cuticle, and epidermal thickness [101]. The use of BLUPRINS, a bud breaking agent containing amino acids, as well as polysaccharides, nitrogen, and calcium showed that it can be used to advance and uniform sprouting and flowering in cherry [102].

3.3. Seaweeds as Biostimulants

As referred to above, seaweed products contain a wide variety of substances, which are related to the specific source of the seaweed, time of collection, and extraction process [42,44,103,104]. Early reports indicate that the use of an *Ascophyllum nodosum* seaweed-based product in sweet cherry cv. Simone trees resulted in increased fruit quality, predominantly an increase in fruit weight and a higher number of fruits having larger size [105]; furthermore, fruit cracking was reduced by up to 10% [106]. Further reports indicate that the use of seaweed biostimulants, namely *Ascophyllum nodosum*, is able to reduce cherry cracking in cvs. Skeena and Sweetheart, with an associated increase in weight, diameter, pH, and fruit waxes [107]. Positive effects are also found in leaves of cherry trees, with reduced damages to cell membranes, improved water status, and increases in the content of soluble sugars, organic acid, and carotenoids, but also with higher production yields [98–100]. The fruit of cv. Staccato is also positively affected by the use of *Ascophyllum nodosum*, with results pointing out an increase in fruit size, soluble solids content, with higher pH and lower acidity. Additionally, the content of bioactive compounds, namely polyphenols and vitamin C, was also increased [97]. Using other important brown macro-alga, *Ecklonia maxima*, as a base for biostimulants for use in sweet cherry resulted in an increased fruit set and yield of cv. Bing [108–110].

3.4. Chitosan and Other Biopolymers

The use of chitosan and other biopolymers in sweet cherry production has also been studied, but it is most commonly used as a postharvest strategy to enhance shelf life. Chitosan is a deacetylated form of chitin that can be produced both naturally and industrially, with several sizes and used in numerous industries, including the food, cosmetics, medical, and agricultural sectors [25]. Its use regulates numerous plant defense signaling pathways in plants [111] and it is considered a less toxic and economical compound, biodegradable and environmentally friendly, edible, and safe for both animals and plants [112]. Its use as postharvest treatment in cherry is described as being able to reduce weight loss, delaying fruit ripening, as they present lower acidity, and a very important characteristic, it can lower the rate of softening of fruits [113]. Even if limited use of chitosan for sweet cherry is known, in preharvest treatments, some effects could be expected when used for this fruit. In raspberries, yield and fruit firmness were improved by the preharvest application of chitosan [114]. For strawberries, the use of chitosan results in improved external quality traits, namely fruit average size, firmness, and color, but also in the bioactive concentration recorded [115,116] and yield [58]. Positive effects concerning the use of chitosan were also recorded for grapevine, with increased contents of anthocyanin and phenolic acids [117].

For nectarines and peach, chitosan has also proven to be an interesting strategy to enhance the quality of fruits: nectarines treated with chitosan presented firmer pulp and higher soluble solids content [118], while, for peach, in addition to increased fruit dimensions and weight, higher yield, fruit firmness, acidity, and ascorbic acid were also found [119]. Yield, fruit dimension, and plant growth of mango were also improved by the application of chitosan [120]. Other crops can also have increased yields with field application of chitosan, including tomato, sweet pepper, cowpea, Chinese garlic, cucumber, mung bean, potato, maize, and rice [121–126]. In addition to changes in yield and chemical and physical parameters of fruits, chitosan also has an additional important trait: antifungal activity. Indeed, sweet cherry is a very perishable fruit and can undergo postharvest decay, mainly caused by filamentous fungi, such as the well-known plant pathogens *Monilinia* spp. and *Botrytis cinerea*, and occasionally by *Rhizopus stolonifer*, *Alternaria alternata*, *Penicillium expansum*, and *Cladosporium* spp. [127]. When used in preharvest applications, chitosan was effective in significantly reducing the infection index of postharvest diseased in sweet cherry [128], but also in table grapes and oranges, either against artificially inoculated or uninoculated pathogens [127]. Other biopolymers are also considered biostimulants and have proven to be useful in plant and fruit production. Laminarin, a storage glucan of brown algae, as well as carrageenans, major cellular constituents of red algae, are known to function as an elicitor of plant defense [129,130]. The use of biopolymers of polysaccharides (polyglucosamine) in apricot resulted in increased fruit set, fruit size, and the ratio of soluble solids [131]. In plum tomatoes, the use of a biopolymer combination of carboxymethyl and Pluronic F127 resulted in higher protein content, GABA (γ -aminobutyric acid), and MEA (monoethanolamine) [132].

3.5. Inorganic Compounds

In sweet cherries, the use of potassium silicate resulted in increased fruit flesh firmness [133]. More recently, phosphite has been increasingly used as pesticide, fertilizer, and biostimulant. As a biostimulant, its use resulted in improved nutrient uptake and assimilation, increased product quality and nutritional value, tolerance to abiotic stress, while being able to improve root growth and yield. In addition, phosphite is largely used as a tool for pathogen control, being registered as a fungicide and bactericide in several countries [134]. The effects of the use of phosphite in fruit crops have been widely studied, with interesting results. Early works showed that the use of potassium phosphite resulted in similar biochemical responses to the ones achieved with calcium phosphate [135] with the use of the same compound was able to increase the yield of large size fruit, total soluble solids, and the ratio of soluble solids to acid in orange were improved [136,137], as were flower number, fruit set, and yield [138]. An initial review of the effects of phosphites was performed by Rickard [139]. This review highlights the positive effects of this compound in several fruit crops, in addition to the already mentioned oranges. For peaches, sugar content and soluble sugars increased, while for raspberries, higher fruit firmness was recorded. For strawberries, changes in fruit were recorded regarding the content of ascorbic acid, anthocyanin, and sugar content [140,141], but also in free amino acid and protein in leaves [141,142]. Although information about the use of phosphite in sweet cherry cultivation is missing, a positive influence might be expected, considering the results from the referred-to studies.

3.6. Bacterial- and Fungal-Based Biostimulants

Although the use of fungi in sweet cherry is not very common, their benefits have already been briefly recorded: application of *Trichoderma harzianum* in two *Prunus* rootstocks during the rooting phase resulted in increased shoot growth and plant development [143], mainly due to changes in phytohormone levels [144], but also resulting from the acidification of the medium, as this could favor the diffusion of cations from the medium [145]. Other results indicate that the use of a mixture of AMF (*Rhizophagus intraradices*, *Funneliformis mosseae*, *Claroideoglossum etunicatum*, *Rhizophagus clarus*) increases root diameter, root

volume, and root fresh and dry weight of one-year-old sweet cherry trees (cv. *Vanda*) grafted onto the “Gisela 5” rootstock [146]. Positive results were also observed for cherry rootstocks, “Edabriz” and “Gisela 5” inoculated with several *Glomus* species. Results pointed out that the mycorrhizal cherry rootstocks were healthier and had higher Zn and P contents [147]. Similar results were also observed by [148] when inoculating *Glomus mosseae* and *G. fasciculatum* during acclimatization of micropropagated sweet cherry rootstocks. Higher content of Fe, Cu, Zn, and P was found in inoculated plants, although with different responses caused by the fungal species or the rootstock (“Gisela 5”, “Edabriz”, or “Damil”). The inoculation of *Glomus intraradices* in sweet cherry rootstock “Colt” also improves the survival rate of inoculated plants [149]. Even though no data are referring to the effect of the use of fungal biostimulants in plant performance or fruit quality, in sweet cherry, results available in other fruits indicate the possible beneficial effects. Indeed, in a thorough review of the effects of the use of plant growth-promoting fungi, positive impacts were recorded for photosynthetic efficiency, flowering, yield, and photosynthetic and bioactive compounds in a large variety of crops [150].

The available data regarding the use of bacteria in sweet cherry cultivation also highlight the positive effects of this type of biostimulants. Foliar application of *Pseudomonas* BA-8 and *Bacillus* OSU-142 in cv. Ziraat resulted in increased yield per trunk cross-sectional area, fruit weight, and shoot length [151], but also fruit length, diameter, seed weight, and soluble solids content [86]. Furthermore, the mineral content of leaves was increased namely for N, P, K, but also Fe, Zn, and Mn [151–153]. The use of bacteria strains had positive effects on reducing the effects of salt stress in a sweet cherry sapling, namely in what concerns plant growth, root weight, chlorophyll content, leaf water content, photosynthetic activity, water use efficiency, and root vitality [154,155].

4. Conclusions and Future Perspectives

Finding innovative but sustainable solutions to increase crop production while reducing the negative impacts on the environment of agricultural inputs is a priority in modern intensive agriculture. Biostimulants are in the frontline as novel strategies to achieve that goal, but knowledge about their use in the fruit production industry have to be increased, aiming to close the gap on information about efficacy and repeatability of their application. In addition to aiming the studies on biostimulants to their characterization, concerning the description of active ingredients and mode of action, how they are influenced by climatic conditions, possible negative carry-over effects of repeated applications, and the cost-benefit of their use have to be clearly addressed. In cherry crops, the beneficial effects of biostimulants are evident (Table 1), as their application has the ability to improve plant growth and tree performance and increase nutrient uptake and production yields while improving the nutritional value and quality of fruits by reducing fruit cracking (Figure 1). For sweet cherry, there is still a long way to go, as studies on the beneficial effects of several categories of biostimulants are lacking, concerning tree status as well as fruit quality. This research must focus also on finding the best cultivar/environment/management practices balance in order to select the best combination possible, ensuring sustainability and profitability to producers and quality for consumers.

Table 1. Effects of the application of biostimulants on sweet cherry trees.

Biostimulant	Application Method	Trail Setup	Recorded Effects	References
Humic acids	Soil application	Field	Increased zinc and iron leaf concentrations	[90]
	Soil application and foliar fertilization	Field	Reduced membrane degradation and increased peroxidase activity in leaves	[91]
	Foliar spray	Field	Increased mineral content of leaves	[92]
	Soil application	Field	Positive effects in leaf and fruit chemical parameters	[94]
	Foliar spray	Field	Leaf recovery from iron chlorosis	
Fulvic acids	Soil application	Field	Increased root and fruit dry matter and yield	[93]
Glycine betaine	Foliar spray	Field	Fruit increased in size and content in soluble solids, polyphenols, vitamin C, and antioxidant activity	[97]
	Foliar spray	Field	Decreased stomatal conductance (gs) and increased of relative water content	[98]
	Foliar spray	Field	Increased fruit weight and color at ripening	[99]
	Foliar spray	Field	Increased anthocyanin content, but decreased carotenoid and ascorbic acid content	[100]
	Foliar spray	Field	Reduced fruit cracking incidence and increased wax content and cuticle and epidermal thickness	[101]
Amino acids	Foliar spray	Field	Advanced and uniformed sprouting and flowering	[102]
Seaweeds	Foliar spray	Field	Increased fruit weight	[105]
	Foliar spray	Field	Reduced fruit cracking	[106]
	Foliar spray	Field	Increased yield and fruit firmness	[108]
	Foliar spray	Field	Increased fruit set and yield	[109]
	Foliar spray	Field	Increased fruit set and yield	[110]
	Foliar spray	Field	Reduced fruit cracking and increased fruit dimension	[107]
	Foliar spray	Field	Higher production, yields, and improved status of leaves	[98–100]
	Foliar spray	Field	Increased fruit size, soluble solids, polyphenols, and vitamin C content	[97]
Chitosan	Foliar spray	Field	Reduced storage decay	[128]
Silicate	Soil application	Field	Increased fruit flesh firmness	[133]
Fungal-based biostimulants	Root inoculation	Growth chamber	Increased shoot growth and plant development of rootstocks	[143–145]
	Soil application	Field	increased root diameter, volume, fresh and dry weight	[146]
	Root inoculation	Glasshouse	Higher Zn and P contents in rootstocks	[147]
	Root inoculation	Growth chamber	Increased Zn and P uptake in rootstocks	[148]
	Root inoculation	Glasshouse	Improved survival rate of rootstocks	[149]
Bacteria-based biostimulants	Foliar spray	Field	Increased yield, fruit weight, and shoot length, increased leaf mineral content	[151]
	Foliar spray	Field	Increased fruit length, diameter, seed weight, and soluble solids content	[152]
	Foliar spray	Net house	Increased N, P, K, Fe, Zn, and Mn leaf content of seedlings	[153]
	Soil application	Glasshouse	Reduced effects of salt stress in saplings	[154]
	Soil application	Field	Increased net photosynthetic rate, water use efficiency, and root vitality	[155]

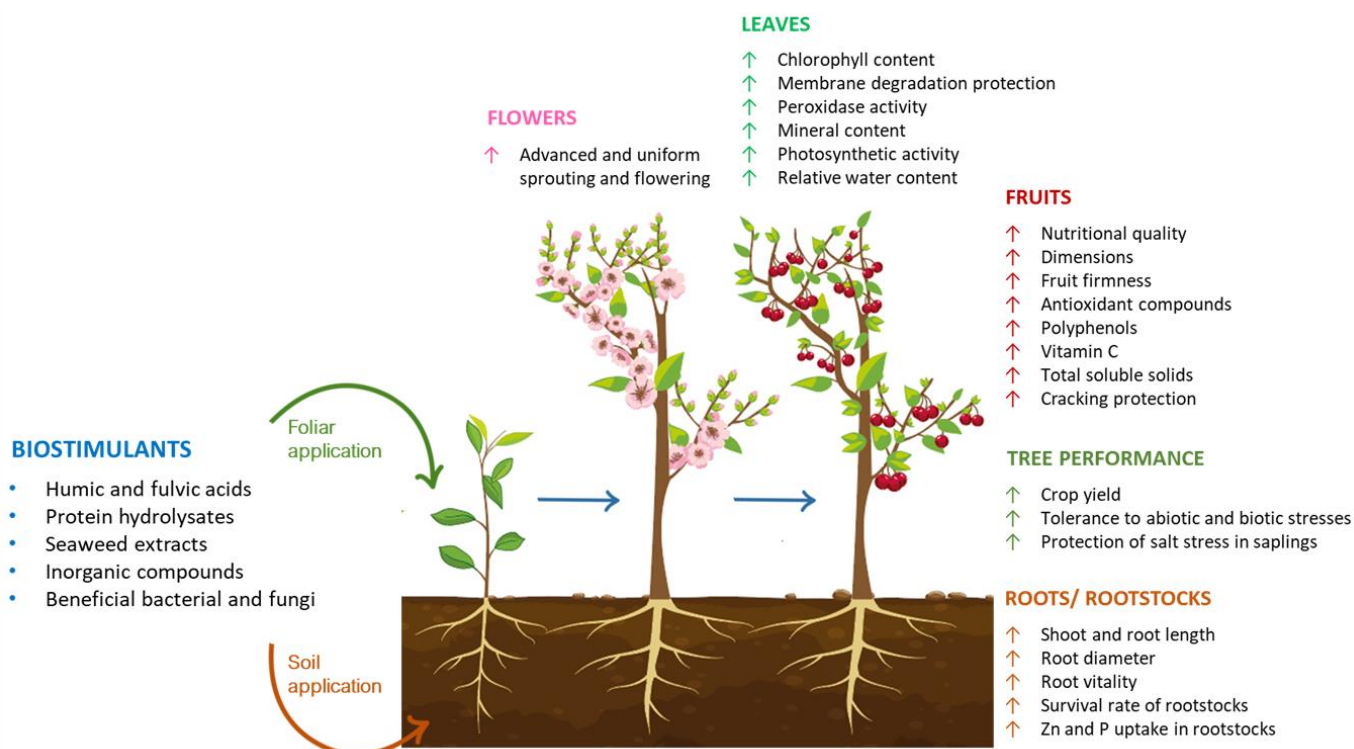


Figure 1. Schematic representation of biostimulants use on sweet cherry tree.

Author Contributions: Conceptualization, S.A., I.O., A.S.M. and B.G.; writing—original draft preparation, S.A.; writing—review and editing, S.A., I.O., A.S.M. and B.G.; supervision, A.S.M. and B.G. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by “Fundo Europeu Agrícola de Desenvolvimento Rural (FEADER)” and by “Estado Português” in the context of “Ação 1.1 «Grupos Operacionais»”, integrated in “Medida 1. «Inovação» do PDR 2020—Programa de Desenvolvimento Rural do Continente—Grupo Operacional para a valorização da produção da Cereja de Resende e posicionamento da subfileira nos mercados (iniciativa n.º. 362)”. This work is supported by National Funds by FCT—Portuguese Foundation for Science and Technology, under the project UIDB/04033/2020.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: Sílvia Afonso is grateful to FCT, MCTES, and FSE for the PhD Fellowship SFRH/BD/139922/2018.

Conflicts of Interest: The authors declare no conflict of interest.

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