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5 **Chapter 15: Considerations with biostimulants in turfgrass**

6 *Michael Fidanza, Pennsylvania State University, USA;*

7 *Cale Bigelow, Purdue University, USA;*

8 *Stanley Kostka, Pennsylvania State University, USA;*

9 *Erik Ervin, University of Delaware, USA;*

10 *Roch Gaussoin, University of Nebraska-Lincoln, USA;*

11 *Frank Rossi, Cornell University, USA;*

12 *John Cisar, Cisar Turfgrass Research Services, USA;*

13 *F. Dan Dinelli, North Shore Country Club, USA;*

14 *John Pope, Pope Soils Consulting and Counseling Services, USA;*

15 *and James Steffel, Lehigh Agricultural and Biological Services, USA*

16

17 1 Introduction

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22

23 1 Introduction

24

25 The term “biostimulants” and biostimulant products have been misunderstood and often misused
26 as potential “miracle cures” in the turfgrass industry, and these products were often dismissed as
27 “snake oil”, “foo-foo juice” or “foo-foo dust” (e.g., sarcastic reference to the mythical foo-foo
28 tree). Some biostimulant products make performance claims substantiated with scientific
29 research conducted in turfgrass ecosystems, while other products lack direct evidence of their
30 benefit within a turfgrass management program (Fidanza et al., 2019).

31

32 The Merriam-Webster Dictionary (Springfield, MA, USA) lists “biostimulant” as derived from
33 “bio” (~life) and “stimulus” (~a thing that evokes a specific functional reaction in an organ or
34 tissue, or a thing that arouses activity or energy in something, or an interesting or exciting
35 quality). Dr. Richard Schmidt (Emeritus Professor; Virginia Polytechnic Institute and State
36 University, Blacksburg, VA, USA) is considered the pioneer of biostimulant research in turfgrass
37 science. Dr. Schmidt along with Dr. Xunzhong Zhang (Virginia Polytechnic Institute and State
38 University, Blacksburg, VA, USA) studied and evaluated various substances that promoted plant
39 growth without the plant’s response attributed to fertilizers, nutrients, or pesticides. Drs. Schmidt
40 and Zhang’s concise definition is “*Biostimulants are organic materials that when applied in
41 small or minute quantities enhance plant growth and development*” (Bigelow et al., 2021;
42 Fidanza et al., 2015; Schmidt and Chalmers, 1993). The use of the word “minute” in this
43 definition was important and intended to differentiate the fact that these substances, compared to
44 traditional nutrients and/or soil amendments, elicited a measurable and beneficial response at
45 much lower application rates (Bigelow et al., 2021). Drs. Schmidt and Zhang explained the plant
46 biostimulant effect as attributed to a hormonal response and the plant protection effect against
47 abiotic stress as attributed to antioxidant production, and both of those effects made possible
48 from low concentrations of exogenous applications. Later, the term “metabolic enhancers” was
49 used, but the important distinction was that something positive was happening to the plant
50 beyond what mineral nutrition supplied (Zhang and Schmidt, 1997).

51

52 More recently, du Jardin (2015) defined a plant biostimulant as “any substance or microorganism
53 applied to plants with the aim to enhance nutrition efficiency, abiotic stress tolerance, and/or

54 crop quality traits, regardless of nutrient content”. Plant biostimulants also refer to commercial
55 products containing mixtures of those aforementioned substances, compounds, and
56 microorganisms (du Jardin, 2015). The term “plant biostimulant” often is used to describe the
57 various categories of compounds and substances used in these products: plant growth hormones
58 (e.g., abscisic acid, auxins, cytokinins, gibberellic acid, etc.), microorganisms (e.g., *Bacillus* spp.,
59 *Trichoderma* spp., mycorrhizae, etc.), amino acids, humic and fulvic acids, plant defense-
60 activating substances, plant growth-promoting compounds, vitamins, pigments and oils, soil
61 amendments and soil conditioners, composts and compost teas, and more (Bigelow et al., 2021;
62 Crouch and Van Staden, 1993; Ervin, 2013a, 2013b; Fidanza et al., 2015; Kostka and Fidanza,
63 2017).

64
65 The European Biostimulant Industry Council (EBIC; <https://biostimulants.eu>) defines
66 biostimulants as: “Agricultural biostimulants include diverse formulations of compounds,
67 substances, and other products that are applied to plants or soils to regulate and enhance the
68 crop’s physiological processes, thus making them more efficient; biostimulants act on plant
69 physiology through different pathways than nutrients to improve crop vigor, yields, quality and
70 post harvest shelf life/conservation.” The EBIC also has a functional definition of plant
71 biostimulants as follows: “A material which contains substance(s) and/or microorganisms whose
72 function, when applied to plants or the rhizosphere, is to stimulate natural processes to benefit
73 nutrient uptake, nutrient efficiency, tolerance to abiotic stress, and/or crop quality, independently
74 of its nutrient content.” Of note, the EBIC’s functional definition expands beyond the ‘plant’ to
75 also include the ‘soil’ (e.g., rhizosphere) (Fidanza et al., 2019). The Association of American
76 Plant Food Control Officials (AAPFCO; <https://aapfco.org>) defines biostimulants as: “...any
77 substance or compound other than primary (e.g., N, P, and K), secondary (e.g., Ca, Mg, S), and
78 microplant nutrients (e.g., Fe, Cu, etc.), that can be demonstrated by scientific research to be
79 beneficial to one or more plant species when applied exogenously; ...a substance or material,
80 with the exception of nutrients or pesticides, which has the capacity to beneficially modify plant
81 growth.” Of note, the ASPFCO definition of biostimulants refers to the term “beneficial
82 substance”.

83
84 Biostimulants are often categorized by “what they are” (e.g., how are these substances or
85 compounds or component materials described chemically or physically?) and “what they do”
86 (e.g., how do these substances or compounds benefit the turfgrass plant or the turfgrass soil/root
87 zone?) (Fidanza et al., 2019). Therefore, a classification strategy is needed to organize and
88 clarify helpful information for biostimulants utilized in the turfgrass industry. Also, the turfgrass
89 practitioner and stakeholder would benefit from knowing not only what a biostimulant is
90 composed of, but how those commercially available biostimulant products may benefit managed
91 turfgrass ecosystems.

92

93 **2 Classification of biostimulants for turfgrass**

94

95 A proposed classification method or strategy for listing biostimulants in turfgrass is presented in
96 Table 1. Overall, biostimulants are listed as primarily targeting the plant or soil/rhizosphere, then
97 further organized by category to describe their composition, followed-by active or functional
98 ingredients (e.g., compounds, substances, other descriptive terms) listed within each category.
99 Examples of common names for biostimulant products are listed for each category.

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Table 1. Proposed classification of biostimulants for turfgrass science and industry.

----- PLANT -----		
Category ¹	Examples of active or functional ingredients	Examples of biostimulant products ²
I Phytohormones	Abscisic acid Auxins Cytokinins Ethylene Gibberellic acid Others	algae, indoleacetic acid, benzyl-adenine, gibberellins, kelp, seaplant, seaweed extracts, and more
II Biopolymers, protein hydrolysates, and other N-containing compounds	Amino acids (e.g., proline, etc.) Antioxidants Betaines Chitin Enzymes Fatty acids Non-protein amino acids Peptides Polyamines Polysaccharides Vitamins Others	amino acids, chitosan, glycine betaine, and more
III Other botanical or synthetic bioactive compounds	Elicitor compounds Induced systemic resistance compounds Plant defense activator compounds Others	acibenzolar- <i>S</i> -methyl, jasmonic acid, salicylic acid, and more

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¹Category based on chemical and/or physical composition.

²Broad or general name of a biostimulant product listed; no product trade name provided. Should an additional column list the specific biostimulant function of those biostimulant products, and/or should the manufacturer include that information on their product label?

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109

Table 1. Proposed classification of biostimulants for turfgrass science and industry (continued).

----- SOIL/RHIZOSPHERE -----		
Category ¹	Examples of active or functional ingredients	Examples of biostimulant products ²
IV Humic substances	Fulvic acid Humic acid	leondardite, and more
V Organics	Biochar Bio-extracts Bio-fertilizers Composts/compost extracts Soil amendments/supplements Others	biochar, composts, compost teas, kelp, seaplant, seaweed extracts, vermi/worm extracts, and more
VI Inorganics/minerals	Al, Co, Na, Mo, Se, Si, etc. Phosphites Others	phosphite salts, and more
VII Biologicals/microbials	Beneficial fungi - Arbuscular mycorrhizal fungi <i>Trichoderma</i> spp. Others Beneficial bacteria - <i>Bacillus</i> spp. and other species Plant growth promoting rhizobacteria Others Other beneficial organisms	Many
VIII Soil surfactants ³	--?--	--?--
IX Other naturally derived or synthetic bioactive compounds	Elicitor compounds Induced systemic resistance compounds Plant defense activator compounds Others	--?--

110 ¹Category based on chemical and/or physical composition.

111 ²Broad or general name of a biostimulant product listed; no product trade name provided. Should an additional
112 column list the specific biostimulant function of those biostimulant products, and/or should the manufacturer include
113 that information on their product label?

114 ³Proposed location of soil surfactants as a category if some of those soil surfactants are to be considered or included
115 as biostimulants.

116 2.1 Phytohormones

117
118 Salisbury and Ross (1991) define a plant hormone as: “*an organic compound synthesized in one*
119 *part of a plant and translocated to another part, where in very low concentrations it causes a*
120 *physiological response.*” Plant hormones or phytohormones are considered chemical messengers
121 in plants. Phytohormones are often referred to as ‘signal molecules’ that occur in very low
122 concentrations, and are vital to plant growth and development, and regulation and function of
123 many physiological processes (Mauseth, 2019). Of note, each plant cell is capable of producing
124 phytohormones, unlike animals which require specialized hormone-synthesizing glands. The
125 most common phytohormones utilized as plant biostimulants are abscisic acid, auxins,
126 cytokinins, ethylene, and gibberellic acid.

127 128 2.1.1 Abscisic acid

129 Abscisic acid (ABA) is associated with water regulation in plants as indicated by an increase in
130 ABA concentration in leaves under drought stress (Swamy and Smith, 1999). ABA involvement
131 with plant adaptation to drought stress includes regulation of stomatal aperture, transpiration, leaf
132 senescence, and cellular osmotic conditions (Chen et al., 2018). ABA also is associated with the
133 plant’s ability to mitigate abiotic stress from salinity and temperature, and possible biotic stress
134 tolerance by signaling plant pathogen defense mechanisms (Mauch-Mani and Mauch, 2005;
135 Wilkinson and Davies, 2002). Some examples of ABA research in turfgrasses include: foliar
136 application of ABA improved Kentucky bluegrass (*Poa pratensis* L.) growth under drought
137 stress (Wang et al., 2003), and ABA’s role in heat tolerance (Li et al., 2014), cold tolerance
138 (Zhang et al., 2013a), salinity stress (Huang et al., 2021b), and photosynthesis (Chen et al.,
139 2018).

140 141 2.1.2 Auxins

142 Auxins are produced in shoot and root tips and promote cell division and elongation and many
143 aspects of plant growth and development (Salisbury and Ross, 1991). Auxin is responsible for
144 phototropism (e.g., shoots growing upward, toward the light) and gravitropism (e.g., roots
145 growing downward into the soil) (Mauseth, 2019). Indole-3-acetic acid (IAA) is the most
146 common naturally occurring auxin and included as the auxin component of many biostimulant
147 products (Sanderson et al., 1987). An example of auxin research in turfgrass is: foliar
148 applications of IAA with the shoot absorbed plant growth regulator trinexapac-ethyl to creeping
149 bentgrass (*Agrostis stolonifera* L.) maintained under water-deficit environmental conditions may
150 promote root viability and drought tolerance resulting in improved turfgrass quality (Zhang et al.,
151 2017).

152 153 2.1.3 Cytokinins

154 Cytokinins are involved with plant growth and development and stress-response processes, and
155 in particular with cell division and delaying of leaf senescence (e.g., plant senescence is the
156 process of aging in plants; plants have both stress-induced and age-related developmental aging)
157 (Ervin, 2013b, Haberer and Kieber, 2002). This delay of leaf senescence or “stay green” effect is
158 a plant stress response in which cytokinins inhibit the action of senescence-inducing enzymes,
159 slowing the degradation of chlorophyll, and maintaining photosynthetic rates and root viability
160 (Ervin, 2013a; Haberer and Kieber, 2002). In plants, cytokinins are produced in healthy, actively
161 growing root tips and translocated to shoots via the transpiration stream (Ervin, 2013a; Haberer

162 and Keiber, 2002). Consequently, as summer root decline proceeds in cool-season turfgrasses,
163 leaf tissue cytokinin levels also decline (Ervin, 2013a).

164
165 An example of a commonly used biostimulant product in this category is seaweed extract
166 (SWE), also referred to as seaplant or kelp. SWE is derived from brown, green, or red
167 macroalgae and has been utilized in the turfgrass industry since the 1950s (du Jardin, 2015; Khan
168 et al., 2009; Sanderson and Jameson, 1986; Sangha et al., 2014). SWE is widely used in turfgrass
169 culture and management due to their positive effects on turfgrass growth and abiotic stress
170 mitigation from drought, nutrient deficiency, soil salinity, and extreme temperatures (Bigelow et
171 al., 2010; Fidanza et al., 2015; Kostka and Fidanza, 2017; Wang et al., 2012, 2013, 2014; Zhang
172 and Ervin, 2008). SWE from the brown seaweed (*Ascophyllum nodosum* [L.] Le Jolis) has been
173 shown to contain biologically active concentrations of natural cytokinins of trans-zeatin riboside
174 and isopentenyl-adenine (Zhang and Schmidt, 1999). Although SWE is best known for
175 containing cytokinins and cytokinin-like compounds, SWE potentially can contain many other
176 beneficial and potentially biologically active compounds (e.g., polysaccharides, fatty acids,
177 vitamins, mineral nutrients, and more) (Craigie, 2011; Khan et al., 2009). Some examples of
178 cytokinin research in turfgrass are: benzyl-adenine increased gross CO₂ exchange rate,
179 chlorophyll and nonstructural carbohydrate contents of Kentucky bluegrass, indicating
180 antisenescence activity (Goatley and Schmidt, 1990; Kane and Smiley, 1983); foliar application
181 of SWE has been shown to increase leaf cytokinin content and antioxidant activity while
182 decreasing lipid peroxidation and delaying senescence in creeping bentgrass drought stress
183 (Zhang and Schmidt, 1999, 2000), increase turfgrass tolerance to salinity stress (Nabati et al.,
184 1994), to ultraviolet B irradiation (Ervin et al., 2004), and to heat stress (Liu et al., 2002; Zhang
185 and Ervin, 2008).

186 187 **2.1.4 Ethylene**

188 While abscisic acid, auxin, cytokinin, and gibberellic acid exist in the plant in liquid form,
189 ethylene is a gaseous phytohormone that regulates plant growth (e.g., the development of leaves,
190 flowers, and fruits), senescence, response to environmental stresses (e.g., heat and freezing
191 stresses), and often interacts with other phytohormones (Iqbal et al., 2017). Heat stress is a
192 major contributor to leaf senescence and other physiological damage in cool-season grass
193 species, and ethylene and cytokinins are two phytohormones associated with leaf senescence (Xu
194 and Huang, 2009). Some examples of ethylene research in turfgrass are: foliar application of an
195 ethylene synthesis inhibitor or a synthetic cytokinin was associated with suppressed leaf
196 senescence and improved heat tolerance of creeping bentgrass (Xu and Huang, 2009), and the
197 inhibition of ethylene production in annual bluegrass (*Poa annua* L.) may improve winter
198 survival during environmental conditions associated with ice encasement (Laskowski and
199 Merewitz, 2020). Foliar application of ethephon, which is converted to ethylene in leaf tissue,
200 has been shown to reduce rooting and hasten chlorosis in cool and warm-season turfgrass species
201 (Marcum and Jiang, 1997; McCullough et al., 2005a, 2005b). Ethephon, however, has been
202 shown to be a very effective annual bluegrass seedhead inhibitor, given proper application timing
203 (Patton et al., 2018; Peppers et al., 2021).

204 205 206 **2.1.5 Gibberellic acid**

207 Gibberellic acid (GA) controls important plant growth functions such as cell elongation and stem
208 growth, seed germination, flower development, and flowering time (Schwechheimer, 2008). The
209 number of known gibberellins exceeds 100 and they are classified as GA₁, GA₂, GA₃, etc.
210 (Salisbury and Ross, 1991). GA₃ was the first commercially available gibberellin (De Casto et
211 al., 2020). Use of GA products is currently recommended to reverse the effects of an over-
212 application of anti-GA plant growth regulators (Ervin, 2013b). In low light or shade conditions,
213 GA can accumulate in turfgrass thus causing shoot elongation and leaf etiolation (Tan and Qian,
214 2003).

215
216 Some examples of gibberellic acid research in turfgrass are: annual bluegrass seed germination is
217 accelerated with scarification plus exogenous application of GA (De Castro et al., 2020),
218 increased stolon growth up to a certain concentration in bermudagrass (*Cynodon* sp.) and
219 creeping bentgrass (Juska, 1958), and under shade, GA₃ reduced ultradwarf bermudagrass
220 (*Cynodon dactylon* [L.] Pers. × *C. transvaalensis* Burt-Davy) visual quality and chlorophyll
221 levels, but did not affect lateral regrowth of stolons (Bunnell et al., 2005). In addition, there are
222 examples of research which explored the use of plant growth regulators in turfgrasses and their
223 relationship to gibberellic acid, other phytohormones, and plant physiological responses
224 (Gaussoin and Branham, 1987; Gaussoin et al., 1997; Heckman et al., 2001a, 2001b, 2001c,
225 2001d; Li et al., 2020). Of note, commercially available PGRs for turfgrasses (e.g., flurprimidol,
226 paclobutrazol, prohexadione calcium, trinexapac-ethyl, etc.) are synthetic chemicals that interact
227 with the gibberellin biosynthesis pathway thus “regulating” a phytohormone response versus
228 “eliciting” a biostimulant-induced phytohormonal response.

229 230 **2.2 Biopolymers, protein hydrolysates, and other N-containing compounds**

231
232 Examples of compounds in this category include amino acids (e.g., betaine and proline) and
233 chitin.

234 235 **2.2.1 Amino acids**

236 Amino acids are the “building blocks” for proteins, enzymes, nucleic acids, antioxidants, and
237 other secondary compounds (Guo et al., 2021; Salisbury and Ross, 1991). The L-form of amino
238 acids are assimilated by plants, and these L-amino acids and short-chain peptides are reported to
239 increase plant N uptake, increase root mass, activate natural defense mechanisms, and enhance
240 photosynthesis (Salisbury and Ross, 1991). For example, L-proline has been shown to improve
241 water stress tolerance in plants (Ashraf and Fooland, 2007), and lysine metabolism is related to
242 abiotic and biotic stress response in plants (Yang et al., 2020). These amino acids and short-chain
243 peptide compounds are derived by chemical or enzymatic hydrolysis of animal or plant by-
244 products, fermentation metabolites by microorganisms, or chemical modification and synthesis
245 (du Jardin, 2015). Glycine betaine (GB) is the amino acid betaine derived from glycine, and is
246 synthesized in the chloroplast, and is associated with mitigation of abiotic stress from drought,
247 salinity, extreme temperatures, ultraviolet radiation, and heavy metals (Ashraf and Fooland,
248 2007). Commercial production of glycine betaine is from sugar beet (*Beta vulgaris* L.), which is
249 purified from molasses during the sugar extraction process (Ashraf and Fooland, 2007).

250
251 Other responses from amino acids applications, for example phenylalanine, include increased
252 production of antioxidants, the key components in dealing with abiotic stress (Kauffman et al.,

253 2007). Although primarily plant-directed, when applied to soil, microflora (e.g., bacteria and
254 fungi) can readily access and assimilate amino acids, resulting in increased soil microbial
255 biomass, which could be an important component of soil health (Moody et al., 2007). Amino
256 acids can be a readily available N source for rapid root and foliar uptake in turfgrass, suggesting
257 that they also have potential as fertility management options or to maximize plant use of existing
258 soil nutrients (McCoy et al., 2020; Radkowski et al., 2020).

259
260 Some examples of documented amino acid research in turfgrasses include: increase in creeping
261 bentgrass visual quality, chlorophyll content, and superoxide dismutase (antioxidant) content
262 from amino acid applications thus indicating a potential role for foliar amino acids in a creeping
263 bentgrass fertilization program (Zhang et al., 2013b), foliar applications of branched-chain amino
264 acids to creeping bentgrass increased shoot density and root weight (Mertz et al., 2019), GB
265 applications to creeping bentgrass was shown to ameliorate drought stress and this effect was
266 related to maintenance of the antioxidant enzyme system (Gan et al., 2018), perennial ryegrass
267 under high temperature stress and treated with hydrolyzed amino acids resulted in improved
268 photosynthetic efficiency (Botta, 2013), foliar application of GB to perennial ryegrass under low
269 temperature stress resulted in enhanced growth via osmotic adjustment (Mickelbart and Boine,
270 2020), and creeping bentgrass treated with tryptophan containing a fermentation byproduct or
271 purified tryptophan plus urea (46N-0P₂O₅-0K₂O) resulted in improved leaf and root IAA content,
272 root biomass, and overall turfgrass quality compared to applications of urea alone (Mertz et al.,
273 2017).

274 275 **2.2.2 Chitin**

276 Chitin is the second most abundant natural biopolymer in the world, after cellulose (Shamshina
277 et al., 2019). The main source of chitin is from shells of marine crustaceans, particularly crab and
278 shrimp, but other living organisms biosynthesize chitin including algae, insects, and fungi (Yan
279 and Chen, 2015). The chemical structure of chitin is a polysaccharide, and is reacted with an
280 alkaline substance (e.g., sodium hydroxide) to produce chitosan. Therefore, chitosan is a linear
281 polysaccharide composed of randomly distributed components of $\beta(1\rightarrow4)$ -linked D-glucosamine
282 and N-acetyl-D-glucosamine (Shamshina et al., 2019).

283
284 Chitosan is used to stimulate plant growth and to induce a biological response to abiotic and
285 biotic stresses (Hou et al., 2021). The plant's response to chitin is determined by species and
286 growth stage of development, and by chitin structure and concentration (Hou et al., 2021).
287 Research on chitosan has focused on its use as an "elicitor" (e.g., an elicitor is a molecule that
288 triggers a hypersensitivity response in plants) of stress response signaling (e.g., stomatal closure
289 to manage transpiration and water use) (du Jardin, 2015). Chitosan interacts with many cellular
290 components (e.g., DNA, plasma membranes, etc.) to bind to specific receptor sites that activate
291 defensive genes which results in increased protection from plant pathogens and insect pests, but
292 more broadly to enhance tolerance to abiotic stress (e.g., drought, salinity, nutrient deficiency or
293 toxicity) (Hou et al., 2021).

294
295 Current research is exploring the metabolic mode of action of chitin and chitosan
296 polysaccharides in plant abiotic stress tolerance, and also the suppression of plant pathogens (du
297 Jardin, 2015). Of note, complex polysaccharides in SWE may have similar effects (Sun et al.,
298 1997). An example of chitin research in turfgrass is: chitosan may be helpful to alleviate heat-

299 induced leaf senescence in creeping bentgrass by regulating chlorophyll metabolism and
300 influencing the production of antioxidants and heat shock proteins (Huang et al., 2021a).

301

302 **2.2.3 Polysaccharides**

303 Algae (e.g., seaweed) produce a diversity of biologically active compounds that can enhance
304 plant growth and resistance to abiotic and biotic stresses (Stadnik and Freitas, 2014). These
305 compounds include polysaccharides which are essentially branched or unbranched chains of
306 monosaccharides (~carbohydrate molecules). Some examples of polysaccharides are cellulose,
307 glycogen, and starch. Similarly, microorganisms (e.g., bacteria, fungi, actinomycetes) also
308 produce a diversity of polysaccharides that can influence how a microbe adsorbs onto surfaces, is
309 protected from desiccation, and interacts with plants to trigger of plant's response to abiotic or
310 biotic stresses. An example of a polysaccharide are ulvans. These water-soluble polysaccharides
311 are obtained from cell walls of green macroalgae (*Ulva* spp.) commonly known as "sea lettuce"
312 (Sangha et al., 2014). In plants, ulvans have been shown to signal a defense response to
313 pathogen infection (Jaulneau et al., 2010). No specific research with ulvans or other seaweed
314 polysaccharides and turfgrasses has been published, although this represents an opportunity to
315 further explore biotic stress mitigation in amenity turfgrasses.

316

317 **2.3 Other botanical or synthetic bioactive compounds**

318

319 This is a "place-holder" category for plant-directed compounds not yet described or fully
320 understood, or for compounds that do not fit the description of the other categories. Other
321 naturally-derived plant compounds or synthetic materials may be listed in this biostimulant
322 category (Table 1). An example of an organic compound in his category is acibenzolar-*S*-methyl
323 (ASM), which is a synthetic analog of salicylic acid (Kessman et al., 1996). ASM is referred to
324 as a "plant defense activator", because it is not directly toxic to fungal pathogens but produces an
325 induced systemic resistance response and thus activates a plant's natural defense system (Cole,
326 1999). Of note, salicylic acid is involved in both abiotic and biotic stress defense due to its
327 ability to signal for the activation of plant immune systems (Durner et al., 1997; Schmidt and
328 Zhang, 2001). ASM is a component of commercial turfgrass fungicides that contain the active
329 ingredients chlorothalonil and fluazinam (Clarke et al., 2020). Examples of ASM research in
330 turfgrass are: ASM associated with improved turfgrass disease management (Hsiang et al., 2011;
331 Lee et al., 2003), and also ASM associated with the ability to mitigate abiotic heat and drought
332 stress in turfgrass (Jespersen et al., 2017).

333

334 **2.4 Humic substances**

335

336 Humic substances (e.g., humic and fulvic acids) are natural decomposition constituents of soil
337 organic matter, typically derived from leonardite (a natural form of humates), associated with the
338 surface layers of "brown coal" deposits. Leonardite describes the base material used in
339 production of humic acids, and was named after A.G. Leonard, the first director of the North
340 Dakota Geological Survey in recognition of his contributions in this field (Fidanza et al., 2015).
341 Humic substances derived from leonardite are considered brown-black polymeric acids that
342 exhibit both hydrophobic and hydrophilic characteristics and bind to soil mineral surfaces (Adani
343 et al., 2006). Humic acid can be considered as a natural soil conditioner because it contains high

344 concentrations of trace minerals and has a greater effect on soil with alkaline pH and lower
345 cation exchange capacity (Chen, 1996; Wang et al., 1995).

346
347 Benefits of three active humic compounds (e.g., fulvic acid, humic acid, and humins) in humic
348 substances include increased soil nutrient and water holding capacity (e.g., increased cation
349 exchange capacity), prevention and reduction in leaching of soil nutrients, chelators of organic
350 molecules and minerals facilitating increased plant root absorption, enhanced soil enzyme and
351 metabolic activity, and more (Barrett, 2015; Liu et al., 1998; Schmidt and Zhang, 1998). Some
352 examples of humics/humic acid research in turfgrasses include: applications of SWE plus humic
353 acid to tall fescue (*Festuca arundinacea* Schreb.; syn. *Schedonorus arundinaceus* [Schreb.]
354 Dumort., nom. cons.) sod increased canopy photosynthesis efficiency and recovery from post-
355 harvest heat injury (Zhang et al., 2003), applications of humic acid were beneficial to creeping
356 bentgrass shoot and root growth (Hunter and Anders, 2004), foliar application of leonardite
357 humic acid prior to dry-down resulted in increased quality, photochemical efficiency, root mass,
358 and leaf α -tocopherol levels during drought (Zhang and Erivn, 2004), and sequential foliar
359 applications of leonardite- or peat-based humic acid during Kentucky bluegrass sod
360 establishment resulted in greater root mass and root strength than the fertilizer control (Ervin and
361 Roberts, 2007).

362 363 2.5 Organics

364
365 Many organic compounds, materials, and products can be placed into this category (Table 1).
366 Bigelow and Soldat (2013) and Waddington (1992) provided recent reviews and considerations
367 with chemical and physical soil amendments for turfgrass ecosystems. Traditionally, organic
368 amendments such as peat moss, manures, biosolids, composts, and other materials have been
369 added to sand-based turfgrass root zones to increase water and plant nutrient retention and
370 availability, but these benefits can decline within a few years as those amendments decompose
371 (Zhang et al., 2012; Waddington, 1992). Ideally, organic materials and substances applied to
372 turfgrass soils should be sufficiently decayed and biologically stable and decompose very slowly
373 so their benefits or positive impact can be expressed over a long time (Bigelow and Soldat,
374 2013). Thus, due to their stability and low cost, the peat mosses are the most frequently used
375 organic amendment for synthetic sand-based root zones.

376
377 Another recent example of a stabilized organic material being utilized for turfgrass root zones is
378 biochar (Brockhoff et al., 2010). Biochar represents a high carbon content, highly porous, and
379 tremendously stable (e.g., extremely resistance to microbial degradation) substance produced
380 from the pyrolysis (e.g., heating organic material > 500 °C in the absence of oxygen) of various
381 biomass feedstocks (e.g., wood chips, plant residues, animal manures, or other agricultural waste
382 products) (Spokas et al., 2012). Some examples of organic materials/products research in
383 turfgrasses include: beneficial use of biochar for water and nutrient retention for sand-based root
384 zones and turfgrass establishment (Vaughn et al., 2015), beneficial inclusion of compost and
385 biochar on soil biology of turfgrass subjected to deficit irrigation (Hale et al., 2021), benefits of
386 compost-amended soil for nutrient availability of turfgrass (Wright et al., 2007), and benefits of
387 compost and compost tea for turfgrass (Bero and Soldat, 2021). Another example of an organic
388 biostimulant product applied to turfgrass is Worm Power[®] (AquaAid Solutions; Rocky Mount,

389 NC; USA), which is vermicompost extract utilized to improve biological and physical health of
390 the turfgrass root zone (for more information: <https://tinyurl.com/2ch8ekmr>).

391

392 **2.6 Inorganics/minerals**

393

394 Many inorganic/mineral compounds and products can be placed into this category (Table 1).
395 Phosphite (PO_3^{3-}) of has become the most common inorganic compound incorporated into many
396 turfgrass management programs, particularly with disease management and suppression (Havlin
397 and Schlegel, 2021). In most products, phosphite is delivered as potassium phosphite in the form
398 of either potassium dihydrogen phosphite (KH_2PO_3) or dipotassium hydrogen phosphite
399 (K_2HPO_3) (Landschoot and Cook, 2005). Silicon in the form of silica (e.g., silicon dioxide
400 [SiO_2]) is example of an inorganic mineral with potential benefits for abiotic and biotic stress
401 reduction of turfgrasses (Guertal and Datnoff, 2021; Schmidt et al., 1999; Zeller et al., 2021).
402 Some examples of inorganic/mineral products research in turfgrasses include: use of potassium
403 phosphite to suppress snow mould disease (*Microdochium nivale* [Fr.] Samuels and Hallett) in
404 cool-season turfgrass (Dempsey et al., 2012), the use of silica to enhance disease suppression in
405 warm-season turfgrass (Datnoff and Rutherford, 2003), and foliar applications of potassium
406 silicate to bermudagrass, tall fescue, and perennial ryegrass irrigated with saline water was
407 helpful to alleviate sodium-induced stress (Esmaeili et al., 2015).

408

409 **2.7 Biologicals/microbials**

410

411 Numerous biological/microbial organisms can be placed into this category (Table 1). Arbuscular
412 mycorrhizal fungi form a mutually symbiotic relationship with plant roots, in which roots
413 provide carbohydrates for the fungi and the fungi aid in access and transfer of nutrients and water
414 to the plant roots, and also water balance, and abiotic and biotic stress tolerance or protection (du
415 Jardin, 2015). *Trichoderma* spp. is another example of a fungal organism that has been studied
416 for biopesticide (e.g., fungicide-like properties) and biocontrol (e.g., inducer of disease
417 resistance) benefits and facilitating abiotic stress tolerance in plants (du Jardin, 2015). *Bacillus*
418 spp. is the most common example of a bacterial organism utilized for biological control of plant
419 pathogens, and this is achieved via direct suppression by the release of antipathogen compounds,
420 or via indirect mechanism such as outcompeting the pathogen for space or food, or activating or
421 inducing plant defense systems (Lugtenberg and Kamilova, 2009; Schlatter et al., 2017). Current
422 research is exploring plant growth promoting rhizobacteria (PGPR) and their ability to confer
423 beneficial effects on plant growth and development by increased nutrient uptake (e.g., nitrogen
424 and phosphorus), synthesizing plant growth promoting compounds, activating abiotic and biotic
425 stress tolerance mechanisms, and possibly more (Lugtenberg and Kamilova, 2009; Rosier et al.,
426 2018).

427

428 Zuberer (2012) summarized 35 years of research in this area to conclude: (i) microbial function
429 and growth in turfgrass soils are similar to agricultural soils or native grassland soils; (ii) soil
430 microbial populations are resilient and changes to the community are most likely achieved
431 through altering the soil environment towards a more favorable habitat; and (iii) best practices
432 for managing the microbiology in the turfgrass root zone are those that adhere to well established
433 agronomic principles and practices (e.g., healthy turfgrass will provide the necessary resources
434 for soil microbes to thrive and function). Some examples of research on biologicals in turfgrasses

435 include: synthetic fungicides applied at reduced label rates in combination with biological
436 control agents (e.g., *Bacillus* spp.) provided an opportunity for dollar spot disease management
437 in creeping bentgrass (Marvin et al., 2020), environmental factors and an actively growing
438 turfgrass root system may have a greater influence and impact on soil microbial activity
439 (Bigelow et al., 2002), and SWE can impart positive effects on soil microbial populations,
440 especially PGPR that facilitate nutrient uptake or provide a source of biopesticidal metabolites or
441 plant defense activators such as ulvans (Mueller and Kussow, 2005; Sangha et al., 2014).

442

443 **2.8 Soil surfactants**

444

445 Agriculture, horticulture, and turfgrass industry practitioners commonly refer to soil surfactant
446 products as “wetting agents” (Fidanza et al., 2019; Kostka and Fidanza, 2019). The term “soil
447 surfactant” is a more appropriate and descriptive label from a soil science perspective (Kostka
448 and Fidanza 2018). Soil surfactant chemistries used in turfgrass management have been reviewed
449 in Zontek and Kostka (2012). With over 300 soil surfactants commercially available globally,
450 Fidanza et al. (2020) proposed a classification strategy for soil surfactants in the turfgrass
451 industry, the majority of which in the global marketplace are of non-ionic chemical composition.
452 Worthy of note, the first patent of a non-ionic soil surfactant was U.S. Patent #2,867,944,
453 “Method of treating soil by non-ionic surface active agents” by L.W. Fletcher (Havertown, PA,
454 USA) was first published in 1959 (<https://patents.google.com/patent/US2867944>). Should all or
455 some specific soil surfactants be listed as a biostimulant? Can soil surfactants “behave as
456 biostimulants”, or “facilitate a biostimulant effect” when applied to turfgrass root zones?
457 Scientists and stakeholders in academia, government, and industry have begun to explore this
458 question further (Fidanza et al., 2019; Kostka and Fidanza, 2019).

459

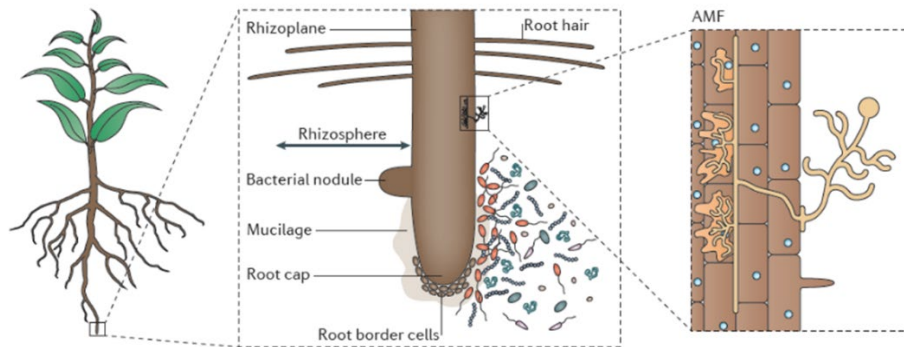
460 In managed amenity turfgrass ecosystems, soil surfactants are primarily and traditionally used
461 for water conservation, improving irrigation use efficiency, and ameliorating soil water
462 repellency (Kostka et al., 2007; Soldat et al., 2010). The utilization of soil surfactants is
463 considered the number one water conservation strategy among golf course superintendents in the
464 USA (Gelernter et al., 2015). Some examples of research on soil surfactants in turfgrasses
465 include: use of soil surfactants to alleviate soil water repellency in sand-based turfgrass root
466 zones (Cisar et al., 2000; Kostka, 2000; Dekker et al., 2019), beneficial use of soil surfactants
467 with irrigation programs (Mitra et al., 2006; Moore et al., 2010), use of soil surfactants to
468 improve delivery and efficacy of soil-directed fungicides (Fidanza, 2015; Fidanza et al., 2007)
469 and nematicides (Webb, 2022), and use of soil surfactants to increase nitrogen uptake of
470 bermudagrass and reduce nitrate leaching (Abagandura et al., 2021a, 2021b).

471

472 **2.8.1 Soil surfactants and the turfgrass rhizosphere**

473 In 1904, German agronomist and plant physiologist Lorenz Hiltner (1862-1923) first
474 documented the term “rhizosphere” to describe the plant-root interface (Hiltner, 1904). “Rhiza”
475 is a word of Greek origin that means “root” or “root-like”, and Hiltner (1904) first described the
476 rhizosphere as that specific area surrounding a plant root that is inhabited by microorganisms,
477 and these microorganisms are influenced by chemicals released from the roots. The rhizosphere
478 is a concept that pertains to the soil-root interface, and recently has been described as the zone of
479 biological, chemical, and physical influence generated by root growth and activity (Philippot et
480 al., 2013; Pinton et al., 2007). The rhizosphere also has been defined as that zone of soil

481 surrounding a plant root where the biology, chemistry, and physical properties and activities of
 482 the soil are influenced by the root and compounds exuded by the root (e.g., mucilage), and these
 483 influences change both radially and longitudinally along the root (Figure 1), and the rhizosphere
 484 also facilitates communication between the plant and the microbiome (Bias et al., 2004; McNear,
 485 2013).
 486



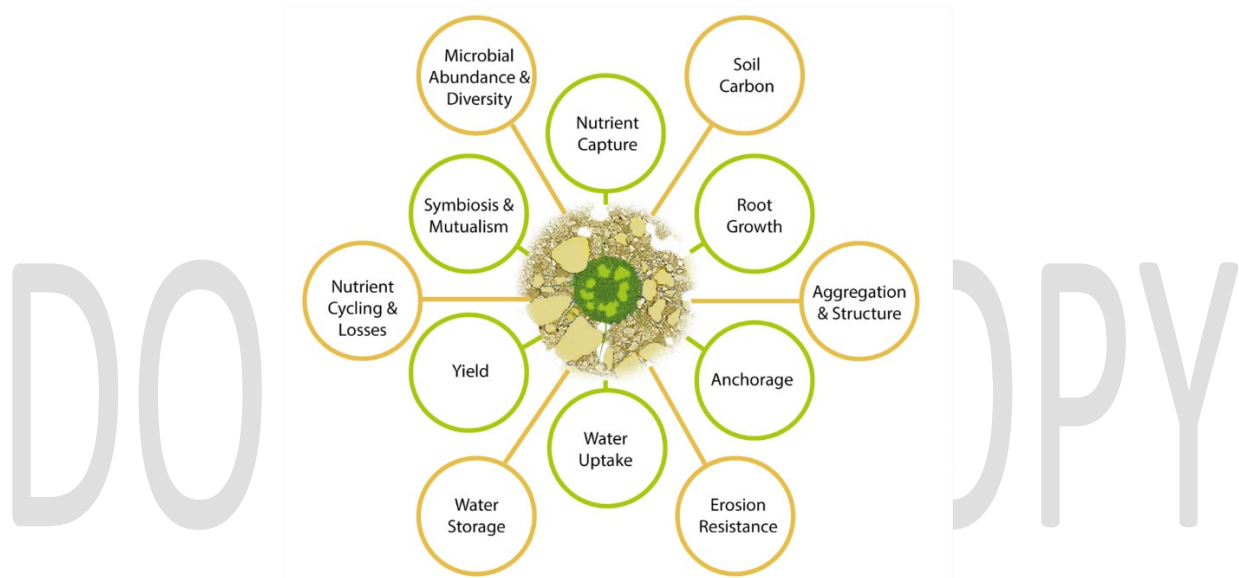
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 488
 489 **Figure 1.** An example of a root section illustrating the rhizosphere (e.g., interface between plant
 490 roots and soil); AMF = arbuscular mycorrhizal fungi. Source: Philippot, L., Raaijmakers, J.M.,
 491 Lemanceau, P., and van der Putten, W.H. 2013. Going back to the roots: the microbial ecology
 492 of the rhizosphere. *Nature Reviews Microbiology* 11(11):789-799.

493
 494 Hallett et al. (2022) provided a review of plant roots and their capacity to improve soil, and in
 495 particular the importance of root hairs: "...just as dabbing paint with a brush allows it to penetrate
 496 into nooks and crannies on surfaces, root hair can aid the influence of plant roots by penetrating
 497 into soil pores too small for roots and distributing rhizodeposits into a greater soil volume". Root
 498 hairs are single cell outgrowths from the root epidermis that increase root surface area and
 499 explore the bulk soil. Rhizodeposits are materials exchanged from plant to soil and dominated by
 500 root exudates, mucilage, and sloughed cells. Root exudates are substances excreted by roots and
 501 composed of sugars, amino acids, organic acids, and other carbon compounds. Mucilage
 502 typically composed of viscous polysaccharide (e.g., sugar) compounds secreted at the root tip,
 503 and is needed to lubricate soil particles as the root pushes through that abrasive soil environment.
 504 Mucilage also represents a significant food source for soil microorganisms and can influence
 505 soil-water relations (Carminati et al., 2016). The rhizosheath is defined as the soil that adheres
 506 strongly to the root through action of root hairs and rhizodeposits. Overall, root hairs not only
 507 help the plant access localized resources (e.g., air, nutrients, and water), but they also sequester
 508 carbon (e.g., deposits of exudates, mucilage, rhizosheaths) and this ultimately improves the soil
 509 and root zone environment (e.g., binding soil particles, improving soil structure, etc.). (Bigelow
 510 et al., 2022; Hallett et al., 2022).

511
 512 Plants invest in improving their root zone environment at the root-soil interface (e.g., the
 513 rhizosphere) (Gregory et al., 2022). By exuding materials such as mucilage, a high molecular
 514 weight biopolymer that absorbs and retains water, plants can remain hydrated for longer periods.
 515 Once dry-down cycles occur, however, water retention and rewetting are compromised due the
 516 presence of small quantities of amphiphilic compounds that render the mucilage hydrophobic
 517 thus impeding rehydration and access to water and nutrients in a diversity of species (Hallett et
 518 al., 2003; Carminati et al., 2010; Ahmed et al., 2016). Their work indicates that soil treated with

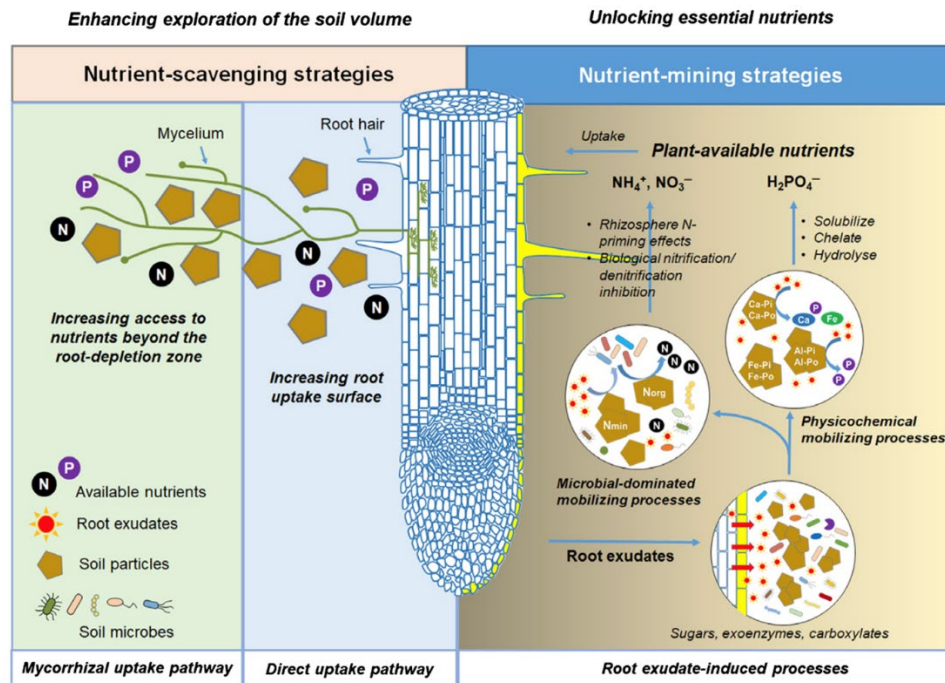
519 a surfactant modifies mucilage behavior (e.g., reducing swelling, increasing mucilage viscosity,
 520 and increasing the speed of wetting while resulting in a reduced rate of plant transpiration).
 521 Their work also suggests that in surfactant treated soil, applied irrigation results in faster root
 522 rehydration and a larger proportion of that applied water is plant available. While the rate of
 523 plant transpiration was slowed, turgidity duration was markedly increased with a concomitant
 524 maintenance of plant performance and tolerance to water stress. Soil surfactants may play an
 525 important role with modifying the turfgrass rhizosphere and thus influence the diversity of
 526 hydraulic processes and functions at the rhizosphere level (Figures 2 and 3) (Bigelow, 2022;
 527 Carminati and Vetterlein, 2013; Carminati et al., 2011, 2016; Fidanza et al., 2019, 2020; Kostka
 528 and Fidanza, 2018, 2019; Singh et al., 2018).

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Figure 2. Illustration indicating processes and functions of the rhizosphere, and that *everything from the soil which passes through the plant passes through the rhizosphere*. Of note, “Yield” pertains to crop production, but could be substituted for “Quality and Resilience” when pertaining to amenity turfgrass. Source: Hallett, P.D., Marin, M., Bending, G.D., George, T.S., Collins, C.D., and Otten, W. 2022. Building soil sustainability from root-soil interface traits. Trends in Plant Science 27:688-698.



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Figure 3. Illustration of root exudates functioning to enhance or influence nutrient acquisition with the rhizosphere. Soil surfactant applications in turfgrass root zones may play a beneficial role in this process. Source: Wen, Z., White, P.J., Shen, J., and Lambers, H. 2022. Linking root exudation to belowground economic traits for resource acquisition. *New Phytologist* 233:1620-1635.

550 In recent studies, Ahmadi et al. (2017) built on these earlier observations noting that not only did
551 surfactant treatment increase and maintain access to water, but that surfactant modification of
552 mucilage increased microbial activity in the rhizosphere, increased nutrient uptake efficiency,
553 and resulted in increased rooting and development of rhizosheaths. Moreover, the production
554 and distribution of a range of plant and microbial enzymes in the rhizosphere were associated
555 with soil health and suppression of soil-borne pests and pathogens (Ahmadi et al., 2018). As
556 Hallett et al. (2022) so obviously state: *everything from the soil which passes through the plant*
557 *passes through the rhizosphere* (Figures 2 and 3). It is becoming apparent that strategies to
558 “engineer” the rhizosphere may have profound effects normally associated with biostimulants.
559

560 Roots are essential for the acquisition of water and nutrients/minerals from soils (Salisbury and
561 Ross, 1991). Root exudates are important for the belowground, interspecies communication
562 between plants and other plants, and/or microorganisms, and thus modifications to roots and the
563 rhizosphere can result in improved plant performance and increased stress tolerance (Ahmed et
564 al., 2016; Bias et al., 2004; Rosier et al., 2018). Therefore, strategies to manipulate the
565 rhizosphere to maintain or enhance connectivity of roots and root hairs to soil particle surfaces
566 can be expected to stimulate the rhizosphere microbiome to influence soil and plant health
567 (Duddek et al., 2020, 2022). If root and root hair connectivity can be maintained, plant resilience
568 should be optimized. Enhanced rhizosheath development has been documented in turfgrass
569 grown in surfactant treated soils (Ahmadi, personal communication). Preliminary, unpublished
570 studies show that certain surfactants increase rooting in cool- and warm-season turfgrasses under

571 greenhouse and field conditions (personal communications: T. Malehorn [iGin Research,
572 Westminster, MD, USA], X. Zhang [Virginia Polytechnic Institute and State University,
573 Blacksburg, VA, USA], S.B. Martin [S.B. Martin Turf Research, Florence, SC, USA], P. Kirby
574 [Indigo Specialty Products, Rocklea, Australia]). Further, turfgrass remains turgid even as soil
575 water deficits increase suggesting that better root/root hair connectivity is being maintained
576 (Figure 4). A body of evidence is building that certain diverse root zone processes can be
577 “engineered” by surfactants to optimize rhizosphere and soil biophysical, microbiological, and
578 chemical properties (Figure 4) (Ahmadi et al., 2017, 2018; Bias et al., 2006; Duddek et al., 2020,
579 2022).
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581



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583

584 **Figure 4.** An example of a beneficial application of a soil surfactant to improve the volumetric
585 water content status of the root zone and possibly optimize the function of the rhizosphere of this
586 creeping bentgrass fairway and putting green. A: left side of fairway was untreated. D: right side
587 of fairway and putting green treated with soil surfactant (ProWet Evolve[®]; TurfCare; Kildare,
588 Ireland). B: drought stress visible in the turfgrass sward, indicating root with compromised
589 physiological function. C: illustration depicting poor root-to-soil contact. E: healthy and dense
590 turfgrass visible on right side, indicating that roots in the soil surfactant-treated turfgrass root
591 zones maintain root to soil contact and access to water and nutrients. F: illustration depicting
592 good or optimum root-to-soil contact. Photo by D. Jones. Images in C and F from: Weil and
593 Brady, 2022. Images in B and E from: Duddek et al., 2020, 2022.

594

595 **2.9 Other naturally derived or synthetic bioactive compounds**

596

597 This category is a “place-holder” for soil-directed compounds not yet described or fully
598 understood, or for compounds that do not fit the description of the other categories. To date, no
599 compounds are placed into this category.

600

601 **3 Considerations with the use of biostimulants for sustainable turfgrass management**

602

603 When asked to consider using a biostimulant product, a prudent and responsible turfgrass
604 manager would ask “*What is in it?*” and “*What does it do?*”, among other questions (Fidanza et
605 al., 2019). Carrow (1993) published an inciteful list of eight questions to ask when evaluating a
606 soil amendment or other product for turfgrass use and management (Table 2).

607

608 **Table 2.** Should I use this product on my turf? Eight questions to ask when evaluating a soil
609 amendment or other turf product to help you make sound, fact-guided agronomic decisions.¹

610

- 611 1. Is this product needed in my situation?
- 612 2. Are independent test results available?
- 613 3. What is the magnitude of response?
- 614 4. Does this product provide consistent results?
- 615 5. What is the duration of response?
- 616 6. Are there better alternatives?
- 617 7. Do benefits justify the costs?
- 618 8. Should I try this product on a trial area?

619

620 ¹Source: Carrow, R.N. 1993. Eight questions to ask: Evaluating soil and turf conditioners. *Golf Course Management*
621 61(10):56, 58, 60, 64, 70.

622

623 The purpose of asking those questions is to help guide the turfgrass manager towards making the
624 best fact-based agronomic decision. Fidanza et al. (2019) further expanded on those important
625 questions to ask when a turfgrass manager is considering a biostimulant product (Table 3). These
626 questions further explore a biostimulant’s intended use (e.g., abiotic or biotic stress, plant
627 nutrient efficiency, etc.) and ability to produce or facilitate the desired turfgrass response.

628

629

630 **Table 3.** What are key questions golf course superintendents, greenkeepers, course care
631 managers, sports field managers, and lawn and landscape professionals should ask when
632 considering a biostimulant product?¹

- 634 • What's in it? What is the product's composition or active ingredient(s) or component(s)?
- 635 • What is its' function? How does the product claim to benefit turfgrass?
- 636 • Will the product function in all climates, soil types, turfgrass species, and turfgrass
637 cultural practices and management programs?
- 638 • Does the product function best to help with abiotic (e.g., drought, heat, salt) or biotic
639 (e.g., insects, pathogens, traffic) stresses?
- 640 • Where's the data? What does the research-based data show that the product does when
641 the product is applied to turfgrass? Were the effects both qualitative and quantitative in
642 replicated field and/or controlled greenhouse research?
- 643 • Does the manufacturer have clear research-based data showing that all or most of the
644 product's active ingredients are essential to its function? Does the data show how
645 individual ingredients, when tested against the formulated product, no longer provide the
646 functional benefit?

648 ¹Adapted from: Fidanza, M., Kostka, S., Ervin, E., and Bigelow, C. 2019. The European Union's view on
649 biostimulants: What may be coming our way. *Golf Course Management* 87(9):58-62.

650
651 Products that contain a biostimulant or combinations of biostimulants may provide turfgrass
652 management options to maintain or improve turfgrass quality and function during abiotic and/or
653 biotic stress conditions. Moderating and mitigating these stresses are an important strategy to
654 establishing and maintaining a healthy, resilient, and sustainable turfgrass sward. As these
655 stresses increase, a number of plant physiological processes may be compromised: decline in
656 efficiency of light capture (photosynthesis) and carbon utilization (respiration); increase in
657 amount of destructive reactive oxygen species (free radicals) in leaves, shoots, and roots; leaves
658 and shoots stop allocating energy to roots and draw energy and resources from the roots; and root
659 decline which precedes shoot decline and is often coupled with root infection from secondary
660 plant pathogens (Yakhin et al., 2017). Therefore, should a biostimulant product or program
661 become a valuable part of sustainable turfgrass management? The answer to that question may
662 depend on what exactly the turfgrass practitioner wants to accomplish (e.g., better rooting, better
663 tolerance of heat or drought stress, improved recovery from heat or drought stress, traffic
664 tolerance, turf recovery, disease prevention, turf recovery from disease, better color or visual
665 quality, better playability, etc.).

666
667 Many biostimulant products are formulated from multiple components and/or are of undefined or
668 vaguely-defined composition (e.g., as components in fertilizer formulations or as soil
669 amendments). While biostimulant activity may be measured from such formulations, is the
670 observed plant or soil response a consequence of a single biostimulant component or from
671 multiple components in the formulation or product? In studies conducted in growth chambers
672 and in the greenhouse, effects of single components can be measured. However, measuring such
673 effects in the field is often more challenging. One or more complex formulations may need to be
674 applied to observe a visual plant response. The challenge then becomes explaining which
675 components were responsible for the observed effects.

676

677 Turfgrass practitioners should critically evaluate turf for the response desired. If a turf manager
678 decides to include a biostimulant product as a component of their turf management program, it
679 would be important to make many observations and measurements throughout the year and
680 document what you are seeing so that you can make informed decisions going forward. Also be
681 sure to review all available information and consider that the use of a biostimulant or any product
682 should be predicated on results from independent and replicated third-party research.

683

684 **5 Summary and future trends**

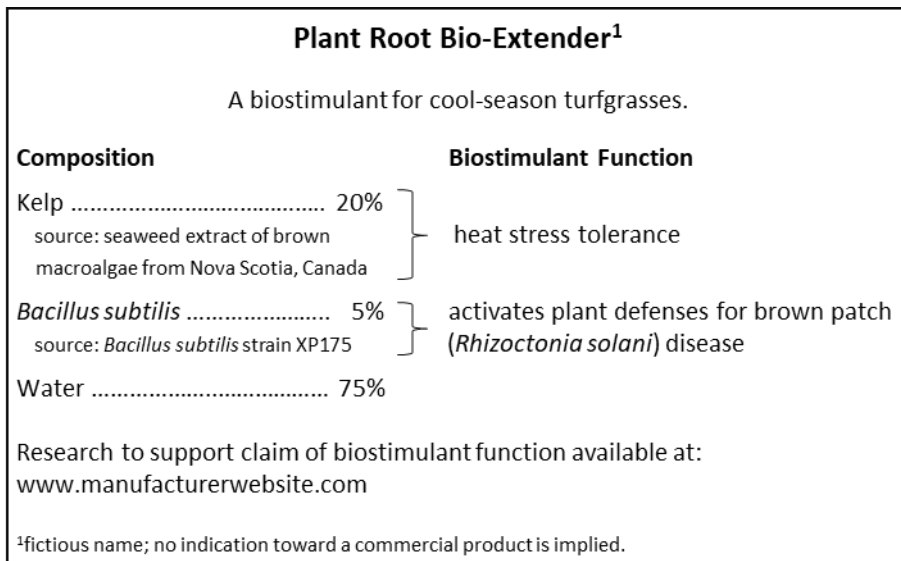
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686 Why should there be a functional definition of plant biostimulants? The European Union's view
687 is that biostimulants should support ecology-based agricultural and food production systems. The
688 EBIC's last part of their biostimulant definition: "...to stimulate natural processes ...
689 independently of its nutrient content" indicates that although a biostimulant product may contain
690 a fertilizer component (e.g., N or Fe), the observed demonstrated plant response is due to the
691 biostimulant and not the fertilizer effect. Therefore, the European Union has proposed
692 regulations to support and enforce a claims-based definition of plant biostimulants (e.g.,
693 biostimulant products should be defined by their function, not by the ingredients inside the
694 product container) (Fidanza et al., 2019). The European Union also proposed that biostimulant
695 products should have credible demonstrated effects to support the product claims (e.g.,
696 documented research to support product label statements for the crops and markets targeted). For
697 example, if a biostimulant product contains a microorganism that induces a plant defense
698 response to a plant pathogen, then the manufacturer must provide data supporting that claim for
699 that particular crop and use. Therefore, the manufacturer would have to prove the biostimulant
700 inside the product performs the function for that particular crop or use as stated on the label
701 (Fidanza et al., 2019; Ricci et al., 2019; Yakin et al., 2017).

702

703 Currently the EBIC is collaborating with government, academia, and industry to develop
704 guidelines for compiling data and information obtained from consistent and reliable research
705 results. The guidelines contain two main points: (i) data must be generated to support the
706 biostimulant claim (e.g., biostimulant function), and (ii) data must be used to place biostimulant
707 product into the European Union market and/or support commercial product claims. Of note, the
708 data required will depend on the biostimulant's claim or declared function (Fidanza et al., 2019).
709 Would biostimulant product manufacturers be required to include the specific biostimulant
710 function or claim on the product label (Figure 5)?

711



712
713

Figure 5. Example of an ideal biostimulant product label that would include information on both composition (e.g., active ingredients and sources) and biostimulant functions or claims.

716

717 Regarding the required research-based data needed to support biostimulant claims, the European
718 Union has proposed guidelines and criteria for laboratory, greenhouse, and growth chamber
719 studies that include proper data analysis and interpretation (e.g., the use of a 90% probability
720 statistic) and field trials conducted under real-world conditions and under various environmental
721 and geographic conditions. Of note, peer-reviewed scientific journal publications can be used to
722 support a biostimulant claim if it is of acceptable quality under the European Union guidelines
723 (Fidanza et al., 2019).

724

725 Should the U.S. Department of Agriculture (USDA; Washington, DC, USA) adopt the European
726 Union’s claims-based definition of biostimulants for use in the United States? Recently, the U.S.
727 Environmental Protection Agency (USEPA; Washington, DC, USA) has assembled a draft
728 document of guidelines for clarifying which biostimulants, biological substances, mixtures, and
729 associated product label claims should be considered as plant growth regulators (source:
730 [https://www.epa.gov/pesticides/draft-guidance-plant-regulators-and-claims-including-plant-](https://www.epa.gov/pesticides/draft-guidance-plant-regulators-and-claims-including-plant-biostimulants)
731 [biostimulants](https://www.epa.gov/pesticides/draft-guidance-plant-regulators-and-claims-including-plant-biostimulants)). Those compounds identified as plant growth regulators will be subject to
732 regulation under the Federal Insecticide, Fungicide and Rodenticide Act as pesticides. Of note,
733 the USDA’s proposed definition of biostimulant is: “...*plant biostimulant is a substance(s),*
734 *microorganism(s), or mixtures thereof, that, when applied to seeds, plants, the rhizosphere, soil*
735 *or other growth media, act to support a plant’s natural nutrition processes independently of the*
736 *biostimulant’s nutrient content. The plant biostimulant thereby improves nutrient availability,*
737 *uptake or use efficiency, tolerance to abiotic stress, and consequent growth, development, quality*
738 *or yield.”* (source: [https://www.epa.gov/pesticides/draft-guidance-plant-regulators-and-claims-](https://www.epa.gov/pesticides/draft-guidance-plant-regulators-and-claims-including-plant-biostimulants)
739 [including-plant-biostimulants](https://www.epa.gov/pesticides/draft-guidance-plant-regulators-and-claims-including-plant-biostimulants)).

740

741 In the USA, however, a structured regulatory path to register biostimulants has not been
742 established, thus precluding validation afforded by regulatory oversight and its value to
743 developers and users of the technology. Therefore, the Biostimulant Industry Workgroup (BIW)

744 was established in 2020 as a collaboration of the Biological Products Industry Alliance (BPIA;
745 Oakton, VA, USA) and The Fertilizer Institute (TFI; Arlington, VA, USA). In response to U.S.
746 Department of Agriculture (USDA; Washington, DC, USA) and U.S. Environmental Protection
747 Agency (USEPA; Washington, DC, USA) reports on biostimulants submitted to congress in
748 2019, BWI prepared and released a draft document: “United States Biostimulant Industry
749 Recommended Guidelines to Support Efficacy, Composition, and Safety of Plant Biostimulant
750 Products” (www.bpia.org). This draft document was developed by BIW representatives with
751 input from a wide range of stakeholders and reviewers and released in 2022. The document
752 offers clarification of terminology and a suggested framework for a regulatory process requiring
753 scientifically sound data in support of product label claims and safety of biostimulant products.
754 The biostimulant industry awaits the outcome of those interactions among the USDA, USEPA,
755 BPIA, and TFI.

756
757 Regardless of the biostimulant product or strategy it is important to note that biostimulants are
758 not a substitute for essential mineral nutrients and a sound agronomic-based turfgrass
759 management program. If the goal is to include or incorporate biostimulants as part of an overall
760 plant and soil health program, then the research in turfgrass ecosystems has demonstrated that
761 they must be applied in advance of those abiotic and biotic stresses to optimize their benefits.
762 There are many exciting innovations on the nearby horizon and evidence-based efforts will lead
763 the way toward a better understanding of how biostimulants will help maintain and improve
764 plant and soil health (Yakhin et al., 2017). Today, much more scientific research is focused on
765 the development, evaluation, use, function, and benefits of biostimulants for sustainable
766 agronomic practices in intensively managed amenity turfgrass ecosystems.

767 **6 References**

768 Abagandura, G.A., Park, D., and Bridges, Jr., W.C. 2021a. Surfactant and irrigation impacts on
769 soil water content leachate of soils and greenhouse substrates. *Agroecosystems, Geosciences and
770 Environment* 4:220153.

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