The Effects of Biostimulants on Induced Plant Defense

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The search for innovative and alternative methods for chemical control to manage pests is an increasingly growing reality. The use of biostimulants such as plant growth promoting rhizobacteria (PGPR) and humic acids (HA) has been shown to improve many agronomic characteristics of plants while increasing yield. These biostimulants also alter the production of secondary metabolites with consequences for insect herbivores. Here we review the role of biostimulants such as PGPR and HA in promoting and eliciting plant defenses. The cascading effects of using these biostimulants on insect herbivores and their natural enemies are discussed in this context. Synergism between biostimulants are also discussed. The potential role of these products in augmenting agricultural productivity is highlighted as is further need for additional research. This review highlights the potential of this tool to enhance integrated pest management in agricultural production systems, reduce the use of pesticides, and increase the efficiency of fertilization while supporting healthier more pest-resistant plants.

Keywords: induced plant defense, plant stimulants, integrated pest management, plant growth promoting rhizobacteria, PGPR, humic acid, HA, natural enemies

1. INTRODUCTION

Plants are constantly under threat in the field, either by abiotic stresses or by the attack of diseases and pests, which can directly and often negatively influence their productivity (Al-Juthery et al., 2020; Vasseur-Coronado et al., 2021). Infestation by pests can result in damage ranging from leaf/root area reduction of the shoot/root by herbivory to the spread of pathogens, causing irreversible damage to the crop. However, in order to deal with herbivory and minimize the subsequent impacts caused by pests, plants have developed several adaptations that include constitutive and induced defense mechanisms of a physical and/or chemical nature (Dicke and van Loon, 2000; Moreira et al., 2014, 2018).

Constitutive chemical defenses comprise metabolites that are present in plant tissues without the need for the action of an herbivore for its expression, while induced defenses are activated following herbivory (Arimura et al., 2005). Chemical defenses can affect herbivores directly, through toxic or repellent secondary metabolites or anti-nutritional proteins (e.g., protease inhibitors, lectins) and indirectly by the release of volatile organic compounds (VOCs) attractive or repellant to herbivores and/or attractive to natural enemies (Dicke and van Loon, 2000; Halitschke et al., 2001; Chen, 2008; Rasmann et al., 2017; Aljbory and Chen, 2018). Synthesis of induced chemical defenses is triggered...
by elicitors derived from an herbivore’s oral secretions and damage-associated molecular patterns (DAMPs), and is modulated by changes in levels of the phytohormones salicylic acid (SA), jasmonic acid (JA), and ethylene (ET) (Hatcher et al., 2004; Kaplan et al., 2008; Schmelz et al., 2009; Erb et al., 2012; Lazebnik et al., 2014).

The protection conferred by these induced defenses can be exploited in pest management through application of substances that induce or strengthen plant defenses prior to herbivory. Induction through exogenous application can be accomplished by a range of products ranging from synthetic plant hormone mimics to soil amendments and microbes. Many of these lead to an increase in the resistance of plants to insects through induction of pre-existing mechanisms latent in the plant or defense priming (Stout et al., 2002; Walters and Heil, 2007; Bektas and Eulgem, 2015). Substances that act as priming agents promote defense activation more quickly after an attack which can be advantageous both to plants in terms of a lower energy cost approach to defense and to agricultural managers (Pieterse et al., 2001; Balmer et al., 2015; Conrath et al., 2015; Martínez-Medina et al., 2016). Generalizing response from application of substances designed to stimulate plant defense pathways is difficult, however. Each substance and compound is unique and can not only produce different and specific biochemical properties in the plant, but also result in different behavioral responses on the part of herbivores and natural enemies (Sobhy et al., 2012; Peñaflor and Bento, 2013; Filgueiras et al., 2019).

While some of these effects are relatively well-characterized, especially in terms of elicitors such as synthetic plant hormones that stimulate specific plant defense pathways (e.g., methyl salicylate), the effects of many biostimulants on induced plant defenses are only beginning to be quantified. This is in part due to the vast number of possible products encompassed in the definition of biostimulants (Brown and Saa, 2015; Du Jardin, 2015). Essentially any substance applied to plants with the express interest of enhancing positive effects on growth and productivity could be considered a biostimulant (Du Jardin, 2015; Al-Juthery et al., 2020). These products are obtained naturally from various economically and environmentally viable sources. Currently these products include extracts of seaweed, humic substances (humic acids and fulvic acids), chitin and chitosan derivatives, amino acids, protein hydrolysates, and microbes (Al-Juthery et al., 2020). Some have been showing promise for commercial agriculture, not only for enhancing plant growth above- and below-ground and/or crop productivity as it has been reported currently (Grover et al., 2020; Jiménez-Arias et al., 2021; Sakr et al., 2021), but also more specifically in enhancing plant defense against pests and pathogens (Brown and Saa, 2015).

Two of the more promising biostimulants used in this regard are microbial inoculants in the form of Plant Growth Promoting Rhizobacteria (PGPR) and the organic fertilizer Humic Acid (HA) (Figure 1). Each has an established history of efficacy from the standpoint of enhancing plant growth and each has produced mounting evidence in recent years that point to roles in stimulating plant defenses. While this is an emerging area and research is in its infancy, there is evidence of synergism between these biostimulants and clear paths forward in terms of expanding commercial use with benefits for large scale agriculture. Here we review the role and point out the potential of PGPR and HA in increasing plant resistance against insect herbivores.

2. PLANT GROWTH PROMOTING RHIZOBACTERIA (PGPR)

Plant growth promoting rhizobacteria (PGPR) are a common form of microbial inoculant already used as biofertilizers in agriculture for facilitating and improving nutrient absorption by plants while influencing plant morphogenesis and development (Bhattacharyya and Jha, 2012). PGPR has a long and storied history with the use of legumes to increase fertility documented before the common era (Bhattacharyya and Jha, 2012). These rhizobacteria are mostly gram-negative bacteria that can form associations with plant roots and increase plant growth through nitrogen fixation, phosphate solubilization, production of phytoheterophores, and modulation of phytohormones (Adesemoye et al., 2009). These changes in phytohormones influence plant responses to biotic and abiotic stress because they modulate defense signaling pathways that culminate in the production of secondary metabolites as well as activate defense priming (Pieterse et al., 2001, 2014; Bhattacharyya and Jha, 2012; Bulgarelli et al., 2013; Backer et al., 2018). The enhanced ability to defend itself in PGPR-colonized plants is often given by the activation of induced systemic resistance (ISR), but in some cases also by systemic acquired resistance (SAR) (Bhattacharyya and Jha, 2012; Annapurna et al., 2013; Pieterse et al., 2014; Conrath et al., 2015; Mhatre et al., 2019). Initially, both defense-priming processes were thought to act against only pathogens, however, they also play a role in enhancing plant defensive response against insect herbivores (Zamiodis and Pieterse, 2012; Conrath et al., 2015).

It has been well-documented that colonization by PGPRs can confer resistance to insect pests that feed on shoots or roots (Rasmann et al., 2017). These effects range from repellence and weight reduction effects on larvae (Disi et al., 2019), to an increase in nymph mortality (Dorta et al., 2020). Colonization by PGPRs can also reduce the feeding period and reproductive rate of some insects (Rashid et al., 2017). Other studies to a lesser extent have also documented that, depending on the system, PGPR colonization has either a neutral (Boutard-Hunt et al., 2009; Brock et al., 2018) or a positive effect on the insect herbivore population (Kempel et al., 2009; Table 1A). In addition, the effects of PGPR on plant-herbivore interactions can vary according to the level of specialization as well as the feeding habit of the herbivore (Van Oosten et al., 2008; Pineda et al., 2010). Generalist herbivores tend to suffer a greater impact than specialists. Experiments, with Arabidopsis thaliana plants inoculated with Pseudomonas fluorescens negatively affected the behavior of the generalist herbivore (Spodoptera exigua), but did not affect the specialist (Pieris rapae) (Van Oosten et al., 2008).

Other PGPR biostimulants have been well-studied in plants and have been shown to be responsible for modifying plant defenses and the profile of plant secondary metabolites.
increasing levels of hydroxycinnamates, benzoates, flavonoids, glycoalkaloids, phenols, stilbenoids, coumarins, lignins, monoterpens, and sesquiterpenes (Tomczyk, 2002; Salomon et al., 2014; Mhlongo et al., 2020; Jeon et al., 2021). Applications of PGPR can also induce systemic resistance in Arabidopsis plants by increasing the levels of glucosinolates and camalexin with negative impacts on herbivore performance (Pangesti et al., 2015). This phenomenon is not limited to Arabidopsis, however. PGPR can induce systemic resistance across plant families; in cotton (Gossypium hirsutum), for example, induced plants show higher levels of defense compounds such as gossypol that contribute to an increase in the plant’s resistance to pest attack (Zebelo et al., 2016).

PGPR can also increase plant resistance via recruitment of natural enemies in both above- and belowground environments. At the belowground level, for example, association with PGPR can change the emission of root volatiles in ways that increase the recruitment of entomopathogenic nematodes, which are important biological control agents of soil pests (Santos et al., 2014). At the aboveground level, PGPR can increase the diversity
TABLE 1A | Examples of wide use of beneficial bacteria in different cultures affecting the behavioral response of insects and other natural enemies of different orders.

| Bacteria                  | Plant          | Organism          | Effect                          | Site | References                      |
|---------------------------|----------------|-------------------|---------------------------------|------|---------------------------------|
| Serratia marcesens        | Cucumber       | Acalymma vitatum  | ↓ POPULATION                    | F    | Zehnder et al., 1997b           |
| Serratia marcesens        | Cucumber       | Diabrotica undecimpunctata (H) | ↓ POPULATION                  | F    | Zehnder et al., 1997a           |
| Pseudomonas putida        | Cucumber       | Acalymma vitatum  | ↓ POPULATION                    | F    | Zehnder et al., 1997b           |
| Pseudomonas putida        | Cucumber       | Diabrotica undecimpunctata (H) | ↓ POPULATION                  | F    | Zehnder et al., 1997a           |
| Pseudomonas spp.          | Rice           | Craphalocrocis medinalis (H) | ↓ LEAF CONSUMED, ↑ LARVAL MORTALITY, ↓ PUPAL WEIGHT | G    | Commare et al., 2002           |
| Bacillus spp.             | Bell Pepper    | Myzus persicae (H) | NO EFFECT NUMBER OF APHIDS PER LEAVES | F    | Herman et al., 2008            |
| Rhizobium leguminosarum   | White clover   | Spodoptera littoralis (H) | ↑ CATERPILLAR WEIGHT GAIN     | F    | Dempel et al., 2009            |
| Rhizobium leguminosarum   | White clover   | Myzus persicae (H) | ↑ POPULATION                    | F    | Dempel et al., 2009            |
| Bacillus spp.             | Green pepper   | Myzus persicae (H) | NO EFFECT ON THE PEST DENSITY AND NATURAL ENEMY | F    | Boutard-Hunt et al., 2009      |
| Bacillus subtilis         | Tomato         | Bemisia tabaci (H) | ↓ EMERGING ADULTS               | L    | Valenzuela-Soto et al., 2010    |
| Azospirillum brasilense    | Corn           | Diabrotica speciosa (H) | ↓ LARVAE WEIGHT GAIN            | L    | Santos et al., 2014            |
| Bacillus pumilus          | Corn           | Heterorhabditis bacteriophora (NE) | ↑ NATURAL ENEMY RECRUITMENT   | L    | Dusi et al., 2019              |
| Bacillus pumilus          | Corn           | Ostrinia nubilalis (H) | ↓ HOST CHOICE FOR OVIPosition   | L    | Dusi et al., 2019              |
| Bacillus pumilus          | Corn           | Diabrotica virgifera virgifera (H) | ↑ LARVAE REPELLENCe, ↓ WEIGHT GAIN OF LARVAE | L    | Dusi et al., 2019              |
| Kosakonia radiccinctans  | Arabidopsis    | Brevicoryne brassicae (H) | ↓ INSECT FEEDING               | L    | Brock et al., 2018             |
| Kosakonia radiccinctans  | Arabidopsis    | Myzus persicae (H) | ↓ INSECT FEEDING               | L    | Brock et al., 2018             |
| Kosakonia radiccinctans  | Arabidopsis    | Spodoptera exigua (H) | ↓ WEIGHT GAIN COMPARED TO CONTROL | L    | Brock et al., 2018             |
| Kosakonia radiccinctans  | Arabidopsis    | Pieris brassicae (H) | NO EFFECT ON THE PEST          | L    | Prabhuwarkrikeyan et al. 2014  |
| Bacillus subtilis         | Tomato         | Helicoverpa armigera (H) | ↓ RATE OF INFESTATION          | G    | Prabhuwarkrikeyan et al. 2014  |
| Bacillus velezensis       | Arabidopsis    | Myzus persicae (H) | ↓ SETTLING, FEEDING AND REPRODUCTION | L    | Rashid et al., 2017            |
| Bacillus thuringiensis    | Citrus         | Diaphorina citri (H) | ↑ NYMPHAL MORTALITY            | L    | Dorta et al., 2020             |

Insect designation refer to: herbivore (H) and natural enemy (NE). Effect designations refer to: increase (↑), decrease (↓), and neutral (NO EFFECT). Site designations refer to: Laboratory (L), Field (F) Greenhouse (G).

Furthermore, they can also modify the metabolic profile of plant defenses, which promotes greater resistance against insects (Schiavon et al., 2010; Razmjou et al., 2011). Similar to PGPRs, HAs have a storied history of use by humans. Vermicompost containing humic acids have been used extensively in small-scale agriculture. Soil amendment with vermicompost makes plants not only more vigorous, but also resistant to insect pests (Cardoza, 2011; Cardoza and Buhler, 2012; Sedaghatbaf et al., 2018). Despite the early evidence of vermicompost in enhancing plant resistance, just recently the role of HA has been investigated. The few studies available concern only aboveground insect pests and show that amendment with HA impacts development, survival, and fecundity of lepidopteran and aphid pests, resulting in reduced population growth (Mohamadi et al., 2017a,b; Nasab et al., 2019; Jafary-Jahed et al., 2020; Table 1B).

Little is known about the HA-induced changes in plant phenotype that affect insect herbivore performance nor the underlying mechanisms responsible for the increased resistance and abundance of predators and optimize host searching by parasitoids as host-infested plants emit a more attractive blend of herbivore-induced plant volatiles (HIPVs) (Pangesti et al., 2015).

3. HUMIC SUBSTANCES AND HUMIC ACIDS

Humic acids (HAs) are a subset of a broader category of hemic substances (HSs) and comprise a biologically active fraction characterized by being soluble in alkaline media and insoluble in acidic media (García et al., 2016). In addition to being able to modify the physical, chemical and biological properties of the soil, these compounds can alter plant hormonal metabolism resulting in the acceleration of plant growth and development, root elongation, increase in germination rates, mitigation of osmotic and heavy metal stress, and increase in biomass (Trevisan et al., 2011; Baldotto and Baldotto, 2014; Canellas et al., 2015; Nardi et al., 2017; Shah et al., 2018; Wong et al., 2020). Furthermore, they can also modify the metabolic profile of plant defenses, which promotes greater resistance against insects (Schiavon et al., 2010; Razmjou et al., 2011).
to insect pests. For example, the lower performance of the aphid *Brevicoryne brassicae* in HA-treated canola was not associated with the level of glucosinolates, phenols or flavonoids, secondary plant metabolites with putative defensive functions against insects (Nasab et al., 2019). Although not in the context of insect-plant interactions, it is well documented that HA or HS amendment increases the level of metabolites linked to the shikimic acid pathway, such as flavonoids and benzoic acids, because of the greater activity of the phenylalanine ammonia lyase and induction of the primary metabolism (Schiavon et al., 2010; Olivares et al., 2017; Aguiar et al., 2018). Despite the relevant role of phenolics in plant resistance against insect pests, the few suggestions in the literature to the protective function of HA against biotic stress only discuss phytopathogens (Aguiar et al., 2018; Jindo et al., 2020), while the potential to increase crop resistance to pests has been overlooked.

Recent evidence that HA (or HS in general in some cases) influences phytohormone signaling networks, transcription level of phytohormone-sensitive genes and transcription factors indicate their potential as a priming agent of anti-herbivore defenses. The role of HA as a priming agent has been highlighted as alleviating abiotic stress through regulation of several genes sensitive to jasmonates (jasmonic acid: JA), ethylene (ET) and salicylic acid (SA), which are important phytohormones in modulating herbivore-induced plant defenses (Canellas et al., 2020). More recent promising results have shown that either foliar or root HS treatment induces augmented levels of JA and JA-Isoleucine (the active form) in roots after 72 h (De Hita et al., 2020). Interestingly, their results coincide with the temporal dynamic of early alterations in abscisic acid (ABA) and indole-3-acetic acid (IAA) followed by JA, in similar fashion to the modulation of herbivore-induced plant defenses (Erb et al., 2012; Vos et al., 2013; Machado et al., 2016).

Another important aspect of signaling and modulating plant defense response to herbivores is the balance of oxidative metabolism (Erb and Reymond, 2019). Herbivory elicits the production of reactive oxygen species (ROS), which despite being important early signals for activating plant defenses, cause oxidative injury in cells and can interact antagonistically with the production of JA in ways that affect negatively plant resistance to some insects (Miller et al., 2009; Block et al., 2018). Treatment with HA often elevates the activity of antioxidative enzymes (e.g., superoxide dismutase, catalase, and peroxidase), which are important in ROS scavenging under stress conditions (Pizzeghello et al., 2001). The enhanced capacity in preventing oxidative injury in HA-treated plants might also alleviate the potential negative effects on herbivore-induced plant defenses (Aguiar et al., 2016; Hatami et al., 2018). However, further investigation is necessary to determine whether the greater antioxidative enzyme activity observed in HA-treated plants is due to the role of HA in modulating phytohormone signaling networks. Transcription level of phytohormone-sensitive genes and transcription factors indicate their potential as a priming agent of anti-herbivore defenses. The role of HA as a priming agent has been highlighted as alleviating abiotic stress through regulation of several genes sensitive to jasmonates (jasmonic acid: JA), ethylene (ET) and salicylic acid (SA), which are important phytohormones in modulating herbivore-induced plant defenses (Canellas et al., 2020). More recent promising results have shown that either foliar or root HS treatment induces augmented levels of JA and JA-Isoleucine (the active form) in roots after 72 h (De Hita et al., 2020). Interestingly, their results coincide with the temporal dynamic of early alterations in abscisic acid (ABA) and indole-3-acetic acid (IAA) followed by JA, in similar fashion to the modulation of herbivore-induced plant defenses (Erb et al., 2012; Vos et al., 2013; Machado et al., 2016).

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amendment influences the biology of the third trophic level via host/prey remains unknown. Moreover, future research is needed to address whether HA amendment changes the emission of constitutive and herbivore-induced plant volatiles with consequences for the recruitment of the third trophic level. Given the established influence of HA on the shikimic acid pathway, as mentioned before, and the ecological role of shikimic-acid derived volatiles to natural enemies (D’Alessandro et al., 2006; Ye et al., 2018), this question deserves attention as HA amendment likely interferes in the attraction of biological control agents. For example, if HA amendment elicits in intact plants the emission of attractive volatiles to natural enemies, it might have unintended consequences to pest biological control because of the enhanced attractiveness of plants with no host/prey (reward).

4. INTERACTIONS

Interference in pest biological control is a concern especially when multiple biostimulants are used in conjunction with each other. Plant defense pathways are known to exhibit cross-talk where activation of one pathway can have antagonistic effects on other pathways (Checker et al., 2018). Similar questions are eminently applicable to the combined use of different forms of biostimulants both for enhancing plant growth and for augmenting pest control: Can biostimulants be synergistic?

The short answer is yes. While studies are still somewhat scarce and synergistic effects are more often reported in terms of plant growth, some work has shown that the combined use of two biostimulants can have a synergistic effect on plants, making them more resistant than when treated with only a single biostimulant (Aguiar et al., 2018). Humic acid application can stimulate the production of root hair in the root system as well as the formation of adventitious roots (Olivares et al., 2017). This effect provides an increase of microorganisms adherence to these structures, making the inoculation potential for rhizobacteria greater and more efficient, since it is in this region that bacteria colonize and associate with plants (Olivares et al., 2017). Interestingly, synergism between biostimulants does not necessarily imply positive independent effects. In tomatoes and spinach, treatments of PGPR alone had no effect, while treatments of PGPR in conjunction with vermicompost containing humic substances had positive effects on yield, plant nutritional quality, and markers of soil quality (Song et al., 2015). Synergistic effects of combined biostimulant application are beginning to be validated across multiple systems and research groups have been able to reproduce results in the same crop types (Song et al., 2015; Rouphael and Colla, 2018).

Antagonist effects of combined biostimulant application are not unreported anecdotaly and should be considered in terms of effects on a plant’s ability to resist insect pests. While many of the synergistic effects described above may be excellent for plant growth, more nutritive tissue could also be highly attractive to pests, especially if such tissue is not defended. Studies on the effects of combined application of biostimulants on herbivores are limited and deserve further attention as efforts are made to implement use of these substances at larger agricultural scales.

5. CONCLUSIONS

Despite the nascency of understanding mechanistically the effects of biostimulant application in isolation and in combination, biostimulants continue to be used in agriculture. The means by which use of these substances can be optimized for enhanced agricultural productivity is of primary importance for continued development of their potential. With a multitude of effects—many that could potentially be synergistic—the use of PGPRs and HAs (or HSs) holds the promise to not only increase production of nutritive plants but also do so in such a manner that the plants themselves are well defended and require less use of pesticides. In implementing such techniques and fostering further research, holistic approaches to cascading effects of application resulting from induced plant defenses will go a long way.

AUTHOR CONTRIBUTIONS

RP, CF, JD, MP, and DW each contributed to the research and writing of this manuscript. All authors contributed to the article and approved the submitted version.

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**Conflict of Interest:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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