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Bioprospecting bacterial and fungal volatiles for sustainable agriculture

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Keywords

Abstract
Current agricultural practice depends upon a wide use of pesticides, bactericides and fungicides. Increased demand for organic products indicates consumer preference for reduced chemical use. Therefore, there is a need to develop novel sustainable strategies for crop protection and
enhancement that do not rely on harmful chemicals and/or genetic modification. Microbial (bacterial and fungal) volatile organic compounds (MVOCs) are intriguingly complex and dynamic, and can modulate the physiology of plants and microorganisms by regulating metabolomics, genomics and proteomics status. Hence, MVOCs can be exploited to use as an ecofriendly, cost effective and sustainable strategy for agricultural practices. An increasing body of evidence indicates that MVOCs might become alternative to harmful pesticides, fungicides and bactericides as well as genetic modification.

Introduction

Bacteria and fungi are the major inhabitants of soil rhizosphere, the narrow zone of soil that surrounds and is influenced by plant roots and which is considered to be one of the most dynamic interfaces on Earth. In agro-ecosystems, the rhizosphere microbiota have been shown to have a profound influence on plant growth, nutrition and health [1, 2]. Numerous organisms are responsible for these processes, partaking in innumerable interactions between plants, antagonists and mutualistic symbionts, both below and above ground [3-5]. To help plants to defend against attack from multiple pathogens, sophisticated alternative interactions involving plant growth promoting rhizobacteria (PGPR) and fungi (PGPF) occur, through the activation of induced systemic resistance (ISR) [6].

Many of the current insights into the above mentioned interactions and processes have originated from direct physical contact between interacting partners. However, in the last decade considerable progress is also being made in understanding the role microbial signals and microbial volatile organic compounds (MVOCs) in below- and above-ground multitrophic interactions and their roles in modulating growth, nutrition and health of interacting partners [7-13].

Microorganisms produce a plethora of intriguingly complex and dynamic MVOCs, which are defined as compounds that have high enough vapor pressures under normal conditions to significantly vaporize and enter the atmosphere [1]. Despite increasing attention on the importance of MVOCs in both atmospheric (“above-ground”) and soil (“below-ground”) ecosystems [7, 14-17], their functional role remains elusive. Only recently, a few studies have
shown the wealth of MVOCs for the modulation of crop growth, development, defense, inter- and intra-specific communication [2]. Recent literature reports the documentation of MVOCs produced by just 400 microorganisms of the 10,000 described microbial species and millions of species existing on earth [10].

At the plant-microbe community level, substantial progress has been made in studying various strains of PGPR, PGPF and phytopathogen MVOCs multifaceted role in agro-ecosystems. Chemical ecologists consider MVOCs as potential semiochemicals that function as attractants and repellants to insects and other invertebrates. For agriculture scientists, MVOCs are seen as bio-control agents to control various phytopathogens and as bio-fertilizers for plant growth promotion. In the food industries, the MVOCs bio-control properties are used to prevent post-harvest plant diseases. Most recently, MVOCs have been considered as a potential source of biofuel.

Because many recent reviews have considered the multifaceted importance of MVOCs, including the regulation of VOC emissions, the role of VOC in plant rhizosphere processes (i.e. competence, pathogenesis, symbiosis) and their potential functions as quorum sensing signals both for microbial growth and regulation of root development [8-10, 18], we will not repeat this in detail. Instead, this article will focus on the role of MVOCs in plant growth, nutrients and health perspectives and possible exploitation of MVOCs significance role from lab conditions to the open field conditions. Here, we review recent progress in MVOCs research for crop welfare and suggest that a conceptual framework is needed to stimulate adoption of MVOCs at open field condition as a possible substitute for the hazardous chemical pesticides and fertilizer.

**MVOCs in the field for crop welfare**

Under highly competitive but symbiotic conditions, MVOCs are particularly important for antibiosis and signaling, and may serve as regulators of plant growth and development. The ecological functions of microbial volatiles are not understood in detail, but several functions such as inter and intra species communication, defense and plant growth-promotion/priming have been suggested. Research over the last 10 years has led to an increasingly clear conceptual understanding of the role MVOCs for the crop welfare. These studies demonstrated the
modulation of metabolomics, genomics and proteomics of crop plants upon MVOCs treatment. MVOCs influence on modulation of phytohormones, induction of systemic acquired resistance, defense and priming response, multiple pathogen resistance, and change in plant biomass, growth and development have been extensively studied and reviewed elsewhere [7, 9, 14, 15, 17, 19-23]. Here, we emphasize selected examples of how microbial MVOCs modulate above mentioned multifaceted interactions.

Exposure of Arabidopsis plants to MOVOCs from rhizosphere strains of Bacillus subtilis and B. amyloliquefaciens resulted in significant growth promotion. Further investigation on the volatile profile revealed that 2,3-butanediol is the major volatile compound contributing to this phenotypic effect [13, 24]. Similarly, exposure of tobacco plants to Pseudomonas chlororaphis MVOCs promoted growth via GacS kinase-dependent production of 2,3-butanediol [25]. These GacS kinases also regulate the synthesis of signal molecules such as acyl-homoserine lactones (AHL), suggesting that 2,3-butanediol and other MVOCs may belongs to a novel class of chemical signals that bacteria utilize to communicate with neighboring organisms [25]. B. subtilis emitted 2,3-butanediol contributes to salt tolerance and ISR in Arabidopsis, whereas the same compound produced by P. chlororaphis resulted in Arabidopsis drought tolerance and enhanced disease resistance against Erwinia caratovora but not against P. syringae pv. tabaci [12, 25-27]. Many other bacterial volatiles from species which are present in the plant rhizosphere, such as Burkholderia cepaci and Staphylococcus, show growth promoting features although their chemical structures are yet to be determined [21]. There are certain bacterial genera including Burkholderia, Chromobacterium, Pseudomonas, Serratia and Stenotrophomans, whose volatile profiles have shown to have adverse effects on plant growth and development [19, 22]. Transcriptional and molecular analysis of Arabidopsis exposed to growth inhibiting volatile profiles of Serratia plymuthica and Stenotrophomnas maltophilia suggest an important role of the WRKY18 transcription factor in volatile-mediated plant growth inhibition [28]. Growth modulation, ISR and drought tolerance observed in plants after microbial volatile exposure depend on genomic, metabolomic and proteomic changes, which are largely attributed to alterations on phytohormone levels. The influence of 2,3-butanediol from B. subtilis on plant growth and ISR is due to modulation of ethylene and auxin homeostasis. Similarly, drought tolerance induced by 2,3-butanediol from P. chlororaphis depends on jasmonic and salicylic acid, although the involvement other phytohormones and their cross talk could not be
ruled out [12, 13, 27, 29]. Transcriptomic, proteomic and metabolomic analyses of Arabidopsis exposed to \textit{B. subtilis} suggests the involvement of different signaling pathways for enhanced growth, involving activity of cell wall modification, stress responses, hormone regulation, antioxidant enzymes activity and photosynthesis [29-31].

Similar studies were conducted to understand the role fungal volatile profile on plant growth, nutrients and health. \textit{Trichoderma viride} volatiles induce significant changes in Arabidopsis, including increased lateral roots, taller, bigger and early flowering phenotypic changes [32]. 1-octen-3-ol is commonly produced by many fungi and contributes to enhance plant resistance to the necrotrophic fungal pathogen \textit{Botrytis cinerea} by inducing defense signaling cascades [33, 34]. \textit{Alternaria alternata}, \textit{Penicillum charlesii} and \textit{P. aurantiogriseum} volatile profile promote growth and starch accumulation in several plant species [35]. Interestingly, volatiles from a non-pathogenic strain of \textit{Fusarium oxysporum}, MSA35, associated with a group of ectosymbiotic bacteria promotes lettuce growth [36, 37]. Further studies on this strain revealed that sequiterpenes such as β-caryophyllene produced by the ectosymbiotic bacterial species are the major volatile compounds responsible for the enhanced growth [37]. Ectomycorrhizal truffles such as \textit{Tuber borchii}, \textit{T. indicum} and \textit{T.melanopsorum} produce volatiles that mediated inhibition of leaf growth and root development in Arabidopsis [38].

Collectively, these studies demonstrate that MVOCs have profound effects on plant metabolism, growth and health. However, many of the current insights into the role of MVOCs in modulating plant growth and defense are obtained from either laboratory or greenhouse experiments. Quite recently, a study has been conducted at the field level to induce crop defense against multiple pathogens and to attract natural enemies of aphids. This study provided useful insights of possible implementation of MVOCs as crop protection and biocontrol agents in open field conditions [39]. We now have the means to begin a new era of MVOCs that might potentially replace costly and unsustainable chemical pesticides and fertilizers and limit the use of genetically modified crops. Table 1 lists some bioactive MVOCs and their effects on plants.

**Deployment of MVOCs in the open field**

The search for novel molecules with biotechnological applications is termed “bioprospecting”. For most of the 20th century, fungal and bacterial bioprospecting has focused on the search for
traditional secondary metabolites with drug value (e.g. penicillin, lovastatin) or for enzymes with new applications (e.g. biomass degrading enzymes from thermophiles). A concerted search for new biotechnological products among MVOCs will require a paradigm shift in the scientific community’s thinking [15]. MVOCs represent a new frontier in bioprospecting. However, although considerable progress has been made in our understanding of MVOCs for crop welfare at lab conditions, we are still far from implementing them under field conditions. Relatively recent studies conducted on volatile application at open field condition suggest that MVOCs can be applied to trigger defense against both pathogens and herbivores [39]. This is just the beginning but we still need to optimize proper conditions for the effective implementation of MVOCs at the field level.

There are many limitations of MVOCs for field applications: a) identification of bioactive MVOCs; b) optimization of concentration of specific volatiles or blend of volatile compounds; and c) application at the field level. The latter, by considering MVOCs physical and chemical properties, is the most difficult and challenging task. For instance, 2,3-butanediol field treatment on tobacco led to significant reduction in disease symptoms, whereas no significant results were observed when cucumber plants were treated to fight the biotrophic pathogen *Pseudomonas syringae* [39-41]. However, an artificial VOC mixture prepared on the basis of the composition of the VOCs (mainly alcohols and esters) mimicked the inhibitory effects of the natural MVOCs released by *Saccharomyces cerevisiae* on citrus black spot, caused by the fungus *Guignardia citricarpa* at postharvest. Thus, MVOCs produced by the yeast or the artificial mixtures might be a promising control method for citrus black spot or others postharvest diseases [42, 43]. So far, MVOCs are successfully applied at field level as a foliar spray and soil dumping [39-41] but there are no comparative studies using different methods of field application to provide a better understanding of effective and optimized methods.

**Conclusions and future perspectives**

Studies of MVOCs application at the field level are still in their infancy. More experiments and field trials are needed to prove their worth and provide sustained industry pipelines leading to a commercial production that meets farmers’ needs. Consumers are well aware of the hazardous effect caused to the environment and human health by pesticides and chemical fertilizers. Alternative to this, genetically modified crop plants and recently proposed genetically edited
crops [44] could provide a solution, but most countries have lengthy, cumbersome and expensive regulatory frameworks, which slow down the use of genetically modified crop plants. Now it is time to adopt emerging MVOCs, a new sustainable approach that can be available in a cheaper, efficient, effective and ecofriendly manner. MVOCs are equivalent to biopesticides or biofertilizers. The market breadth and demand for these naturally derived compounds increased considerably in the recent years around the world but their use is still only 4% of the global pesticide market [45, 46]. Researchers realized the importance of MVOCs for the crop welfare under lab conditions and recently extended their studies to field level with certain success. We are now beginning to understand the multi-facet interaction of MVOCs with microorganisms and crop plants and further studies should be done by field level testing different crop species and obtaining reproducible results which could satisfy farmers’ and consumers’ needs. However, several questions remain unsolved (see Box 1).

In our opinion, MVOCs possess a high potential impact for crop welfare and sustainable agriculture but we are just beginning to understand their role and still far from agricultural applications. In the coming years we assume MVOCs will outperform chemical pesticides and fertilizers and will become novel candidate for sustainable agriculture.

References


Table 1. List of bioactive MVOCs and their effects on plants

<table>
<thead>
<tr>
<th>Bacterial or Fungal species and strain</th>
<th>Identified volatile compounds</th>
<th>Effects on interacting organisms</th>
<th>Ref.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Arthobacter agilis UMCV2</strong></td>
<td>N,N-dimethyl-hexadecanamine</td>
<td>Growth promotion</td>
<td>[47]</td>
</tr>
<tr>
<td><strong>Bacillus amyloliquefaciens IN937a</strong></td>
<td>2,3-Butanediol, Acetoin</td>
<td>Growth promotion and induced systemic resistance (ISR)</td>
<td>[12, 13]</td>
</tr>
<tr>
<td><strong>Bacillus megaterium XTBG34</strong></td>
<td>2-pentylfuran</td>
<td>Growth promotion</td>
<td>[48]</td>
</tr>
<tr>
<td><strong>Bacillus subtilis GBO3</strong></td>
<td>2,3-Butanediol, Acetoin</td>
<td>Growth promotion and ISR</td>
<td>[12, 13]</td>
</tr>
<tr>
<td>Bacterial or Fungal species and strain</td>
<td>Identified volatile compounds</td>
<td>Effects on interacting organisms</td>
<td>Ref.</td>
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<tr>
<td><em>Fusarium oxysporum</em> MSA 35</td>
<td>β-caryophyllene</td>
<td>Induced shoot length, root length and fresh weight of lettuce seedlings</td>
<td>[37]</td>
</tr>
<tr>
<td>Many species of bacteria, fungi and plants</td>
<td>2-butanone</td>
<td>ISR, emission of green leaf volatiles to attract natural enemies of Aphid</td>
<td>[24, 39, 53, 54]</td>
</tr>
<tr>
<td>Many species of bacteria, fungi and plants</td>
<td>3-pentanol</td>
<td>ISR, pheromone, in response to herbivore produced by plant to attract natural enemies</td>
<td>[39, 55-58]</td>
</tr>
<tr>
<td>Mold fungi</td>
<td>1-octen-3-ol</td>
<td>Induced defense and protection against <em>Botrytis cinerea</em></td>
<td>[33]</td>
</tr>
<tr>
<td><em>Muscodor albus</em></td>
<td>Isoamyl acetate</td>
<td>Collectively they acted synergistically to kill a broad range of plant- and human-pathogenic fungi and bacteria</td>
<td>[59]</td>
</tr>
<tr>
<td>Bacterial or Fungal species and strain</td>
<td>Identified volatile compounds</td>
<td>Effects on interacting organisms</td>
<td>Ref.</td>
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</tr>
<tr>
<td>Muscodor albus</td>
<td>2-methyl butanol, isobutyric acid</td>
<td>Volatile mixture were effectively used to control postharvest plant diseases</td>
<td>[64]</td>
</tr>
<tr>
<td>Muscodor crispans</td>
<td>Mixture of volatile compounds</td>
<td>Effective against a wide range of plant pathogens, including the fungi <em>Pythium ultimum</em>, <em>Phytophthora cinnamomi</em>, <em>Sclerotinia sclerotiorum</em> and <em>Mycosphaerella fijiensis</em> (the black sigatoka pathogen of bananas), and the serious bacterial pathogen of citrus, <em>Xanthomonas axonopodis</em> pv. citri. In addition, the VOCs of <em>M. crispans</em> killed several human pathogens, including <em>Yersinia pestis</em>, <em>Mycobacterium tuberculosis</em> and <em>Staphylococcus aureus</em>.</td>
<td>[60]</td>
</tr>
<tr>
<td>Muscodor yucatanensis</td>
<td>Mixture of volatile organic compounds</td>
<td>Mixture of volatile organic compounds produced by <em>M. yucatanensis</em> have allelochemical effects against other endophytic fungi, phytopathogenic fungi and plants.</td>
<td>[63]</td>
</tr>
<tr>
<td>Phoma sp</td>
<td>Unique mixture of volatile organic compounds, including a series of sesquiterpenoids, some alcohols and several reduced naphthalene derivatives.</td>
<td>The volatiles of <em>Phoma sp.</em> possess antifungal and fuel properties Some of the test organisms with the greatest sensitivity to the <em>Phoma sp.</em> Volatiles were <em>Verticillium</em>, <em>Ceratocystis</em>, <em>Cercospora</em> and <em>Sclerotinia</em>.</td>
<td>[62]</td>
</tr>
<tr>
<td>Bacterial or Fungal species and strain</td>
<td>Identified volatile compounds</td>
<td>Effects on interacting organisms</td>
<td>Ref.</td>
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</tr>
<tr>
<td>Phomopsis sp</td>
<td><img src="image" alt="sabinene, isoamyl alcohol, 2-methyl propanol" /></td>
<td>Volatile mixture of Phomopsis sp. possess antifungal properties and an artificial mixture of the VOCs mimicked the antibiotic effects of this organism with the greatest bioactivity against a wide range of plant pathogenic test fungi including: <em>Pythium, Phytophthora, Sclerotinia, Rhizoctonia, Fusarium, Botrytis, Verticillium, and Colletotrichum</em>.</td>
<td>[61]</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa PAO1, PAO14, Tb, TBCF10839 and PUPa3</td>
<td>HCN</td>
<td>Growth inhibition</td>
<td>[49]</td>
</tr>
<tr>
<td>Pseudomonas chlororaphis O6</td>
<td>2,3-Butanediol</td>
<td>Growth promotion, ISR and drought stress tolerant</td>
<td>[25, 27]</td>
</tr>
<tr>
<td>Pseudomonas fluorescens A112</td>
<td>Not determined</td>
<td>Growth inhibition (shoot and root)</td>
<td>[50]</td>
</tr>
<tr>
<td>Pseudomonas</td>
<td>Not determined</td>
<td>Growth inhibition</td>
<td>[21]</td>
</tr>
<tr>
<td>Bacterial or Fungal species and strain</td>
<td>Identified volatile compounds</td>
<td>Effects on interacting organisms</td>
<td>Ref.</td>
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<tr>
<td><em>Bacillus trivialis</em> 3Re2-7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rhizosphere strains (isolated from rhizosphere of lemon plants) L263, L266, L272a, L254, L265a and L265b</td>
<td>Volatile mixture</td>
<td>Growth promoting and modulation of root architecture</td>
<td>[52]</td>
</tr>
<tr>
<td>Rhizosphere strains (more than 42 strains predominantly from <em>Burkholderia</em> genus)</td>
<td>Not determined</td>
<td>Growth inhibition or promotion (dose dependent)</td>
<td>[51]</td>
</tr>
<tr>
<td><em>Serratia marcescens</em> MG-1</td>
<td>Not determined</td>
<td>Growth inhibition</td>
<td>[21]</td>
</tr>
<tr>
<td><em>Serratia plymuthica</em> 3Re4-18, HRO-C48, IC14</td>
<td>Not determined</td>
<td>Growth inhibition</td>
<td>[21, 49]</td>
</tr>
<tr>
<td>Bacterial or Fungal species and strain</td>
<td>Identified volatile compounds</td>
<td>Effects on interacting organisms</td>
<td>Ref.</td>
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<tr>
<td><em>Stenotrophomonas maltophilia</em></td>
<td>Not determined</td>
<td>Growth inhibition</td>
<td>[21]</td>
</tr>
<tr>
<td>R3089</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Stenotrophomonas rhizophila</em></td>
<td>Not determined</td>
<td>Growth inhibition</td>
<td>[21]</td>
</tr>
<tr>
<td>P69</td>
<td></td>
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<td></td>
</tr>
<tr>
<td><em>Trametes gibbosa</em></td>
<td><img src="" alt="1-octen-3-ol" /></td>
<td>Serves as attractant for fungus eating beetles</td>
<td>[65]</td>
</tr>
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<tr>
<td><em>Trametes versicolor</em></td>
<td><img src="" alt="γ-patchoulen" /> <img src="" alt="δ-cadinene" /> <img src="" alt="Isolatedene" /> <img src="" alt="β-guaiene" /></td>
<td>Serves as attractant for fungus eating beetles</td>
<td>[66]</td>
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<tr>
<td>Bacterial or Fungal species and strain</td>
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<td>Ref.</td>
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</tr>
<tr>
<td><em>Trichoderma virens</em></td>
<td><img src="image1" alt="β-caryophyllene" /> <img src="image2" alt="β-elemene" /> <img src="image3" alt="Germacrene D" /> <img src="image4" alt="δ-cadinene" /></td>
<td>Growth promotion and induction of defense responses of <em>Arabidopsis thaliana</em> against <em>Botrytis cinerea</em></td>
<td>[34]</td>
</tr>
<tr>
<td><em>Tuber melanosporum</em>, <em>Tuber indicum</em> and <em>Tuber borchii</em> (truffles)</td>
<td><img src="image5" alt="2-octenal" /></td>
<td>Growth inhibition</td>
<td>[38]</td>
</tr>
</tbody>
</table>
Outstanding Questions Box 1

VOCs play important signaling roles for bacteria and fungi but also for other organisms in their natural environments. Many ecological interactions are mediated by VOCs, including those between fungi and plant, bacteria and plants, plant-plants, arthropods-plants, ect. The diverse functions of MVOCs can be exploited in biotechnological applications for biofuel, biocontrol, and mycofumigation. MVOCs represent a new frontier in bioprospecting, and the study of these gas-phase compounds promises the discovery of new products for human exploitation (medical, agricultural and industrial arenas) and will generate new hypotheses in fundamental biology. However, the mechanisms through which MVOC respond to their surrounding must be better understood in order to be more predictive about which role and effect on their surrounding. Some key questions remain to be answered:

- What is the advantage of the plant to perceive (M)VOCs?
- Which plant proteins participate in the perception of MVOCs?
- What is the identity of MVOCs responsible for induction of plant growth/defense?
- Are plants able to perceive MVOCs from their bacterial and fungal pathogens, and are they able to induce defense mechanism?
- Are plants able make the difference between MVOCs produce by host or non host pathogens?
- Can MVOCs be used as biopesticides?
Glossary

Above-ground: a position measured with respect to the underlying ground surface.

Agrochemicals: a generic term for the various chemical products used in agriculture. In most cases, agrichemical refers to the broad range of pesticides, including insecticides, herbicides, and fungicides. It may also include synthetic fertilizers, hormones and other chemical growth agents, and concentrated stores of raw animal manure.

Below-ground: a position measured with respect to the upper ground surface.

Biofertilizer: a substance containing living microorganisms which, when applied to seed, plant surfaces, or soil, colonizes the rhizosphere or the interior of the plant and promotes growth by increasing the supply or availability of primary nutrients to the host plant.

Biofilm: any group of microorganisms in which cells stick to each other on a surface.

Biopesticides: include several types of pest management intervention: through predatory, parasitic, or chemical relationships. The term has been associated historically with biological control and the manipulation of living organisms.

Bioprospecting: the search for new natural and sustainable molecules in the hope of finding novel biotechnological applications.

Crop welfare: is the provision of a minimal level of well-being and social support for all crops.

Info-chemical: information-conveying chemicals including kairomones, allelochemicals or pheromones that play a crucial role in food web interactions.

Microorganism: a very diverse kingdom that includes all the bacteria and archaea and almost all the protozoa. They also include some members of the fungi, algae, and animals such as rotifers.

Multitrophic interactions: incorporation of species from different trophic or nutritional levels interacting in the same system.

MVOCs: microbial volatile organic compounds that have high enough vapor pressures under normal conditions to significantly vaporize and enter the atmosphere.

Mycofumigation: the use of gas-producing fungi to kill other microorganisms via production of MVOCs.

Plant growth inhibition: reduction of plant growth determined by environmental factors, such as temperature, available water, available light, carbon dioxide and available nutrients in the soil or by the actions of pathogenic and saprophytic organisms and herbivores.

Priming: exposure to conditions by which the processing of a target stimulus is aided or altered by the presentation of a previously presented stimulus.

Rhizobacteria: root-colonizing bacteria that form symbiotic relationships with many plants. Though parasitic varieties of rhizobacteria exist, the term usually refers to bacteria that form a relationship beneficial for both parties (mutualism).
**Rhizosphere**: a narrow region of soil that is directly influenced by root secretions and associated soil microorganisms. It contains many bacteria that feed on sloughed-off plant cells, termed rhizodeposition, and the proteins and sugars released by roots.

**Sustainable agriculture**: an integrated system of plant and animal production practices having a site-specific application that will last over the long term.