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MICROBIAL DYNAMICS AND INTERACTIONS IN THE SPERMOSPHERE

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■ **Abstract** The spermosphere represents a short-lived, rapidly changing, and microbiologically dynamic zone of soil surrounding a germinating seed. It is analogous to the rhizosphere, being established largely by the carbon compounds released into the soil once the seed begins to hydrate. These seed exudations drive the microbial activities that take place in the spermosphere, many of which can have long-lasting impacts on plant growth and development as well as on plant health. In this review, I discuss the nature of the spermosphere habitat and the factors that give rise to its character, with emphasis on the types of microbial activities in the spermosphere that have important implications for disease development and biological disease control. This review, which represents the first comprehensive synthesis of the literature on spermosphere biology, is meant to illustrate the unique nature of the spermosphere and how studies of interactions in this habitat may serve as useful experimental models for testing hypotheses about plant-microbe associations and microbial ecology.

INTRODUCTION

Seeds represent a remarkable stage of plant development that enables them to persist for decades in a state of suspended animation and, under the appropriate set of conditions, awaken to rapidly give rise to a new developing plant. Over the millennia, plants and the seeds they produce have evolved in association with a diversity of microorganisms. These associations may occur as the seed develops and matures (8, 51, 56, 89) or during dormancy and germination in soil. In some cases (e.g., nodulating bacteria with legumes), these associations are rather specific and may account for the presence of particular microorganisms with certain plant species or genotypes (28). In other cases, they may be casual and nonspecific. Associations developing on and around seeds germinating in soil are among the most significant, largely because such interactions mark the first point of contact between plants, pathogens, and soil microorganisms, with either beneficial or harmful results for plant growth, development, and health.

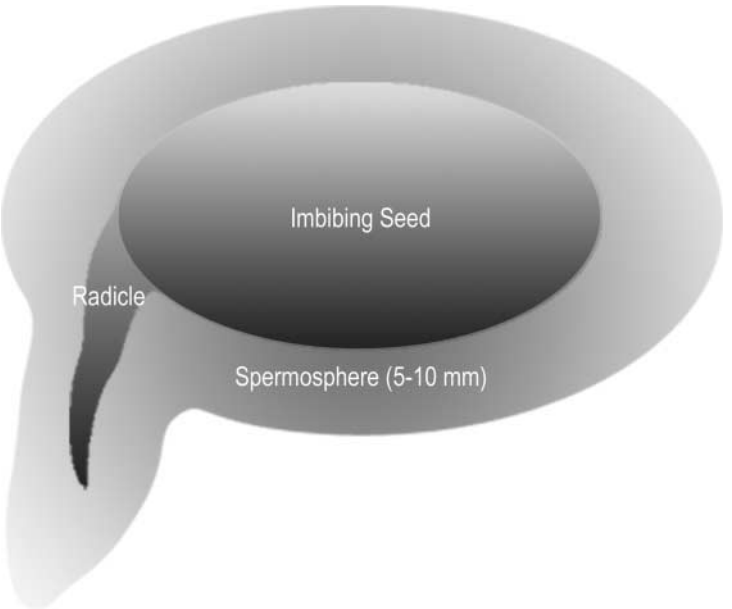

Figure 1 Schematic representation of the spermosphere.

The germinating seed and surrounding soil represents a rich habitat for microbial development and interaction. The main energy source for microorganisms in this habitat is the carbon released by the seed into the surrounding soil. The habitat known as the spermosphere is the zone of microbial interaction around the seed that is under the influence of seed carbon deposition (Figure 1). This review focuses on the nature of this habitat, its microbial dynamics, and interactions and aims to stimulate further work in spermosphere biology.

Definition and Some Key Historical Observations

Our understanding of the spermosphere has developed rather recently, particularly in comparison with the evolution of our knowledge of the rhizosphere. The concept of the rhizosphere, the zone of microbial stimulation around a plant root, was developed and described around the turn of the twentieth century (74). However, the role that seeds played in promoting and establishing those microbial interactions was largely ignored until the 1940s and 1950s, when the concept of the spermosphere emerged. The spermosphere was first mentioned in a study of seedling pathogens of forage grasses, in which Slykhuis (208) noted that "the development [of *Fusarium culmorum*] in the environment in the immediate vicinity of germinating seeds was different from that in the surrounding soil." He defined this region as the "spermatosphere," being aware of the microbiological uniqueness of this region and speculating that the spermatosphere was of particular importance in regulating the activities of seed and seedling pathogens.

It was not until the late 1950s and early 1960s that the concept of the spermosphere was fully developed by Onorato Verona, who defined the spermosphere as the zone of elevated microbial activity around a germinating seed (234). In his 1958 paper, he described the spermosphere in some detail, including the role of seed exudates and mucilages in regulating the microbial stimulation he observed. He also provided microscopic evidence for the stimulation of soil microorganisms by the seed. He developed this concept more completely in a 1963 paper (235) in which he provided more examples of the microbial stimulation occurring in soil around seeds. As with Slykhuis, he too recognized the distinct and unique nature of spermosphere microorganisms and speculated that they might contribute to rhizosphere microbial communities. The spermosphere concept was expanded in 1966 to include the seed surface, a zone that Watson termed the spermoplane (238).

During the early 1960s, a number of scientists, apparently unaware of the observations of Slykhuis and Verona, were independently establishing the ecological importance of the spermosphere in regulating the preinfection stages of pathogenesis by fungal and oomycete plant pathogens (30, 31, 158, 187–191, 207). These studies confirmed the speculations of both Slykhuis and Verona that seed exudates were an important factor regulating pathogenesis by seed and seedling pathogens.

Since the 1960s, there have been relatively few studies in spermosphere biology with only small incremental advances in our knowledge base. Much of the work has been descriptive in nature, and few contemporary and detailed studies of spermosphere microbiology, biochemistry, and ecology are available. However, despite the slow progress over the past decades, the spermosphere is recognized today as a dynamic site of microbial interactions, governed largely by the nature and flux of materials released from seeds during germination. The significance of this habitat to plant microbiology is becoming more apparent and research efforts in spermosphere biology are likely to increase, thus contributing to a better fundamental understanding of spermosphere and rhizosphere ecology as well as plant pathogenesis.

DEVELOPMENT OF THE SPERMOSPHERE DURING SEED GERMINATION

To gain a better understanding of the dynamic associations between plants and microbes around a germinating seed, it is important to recognize the many factors that give rise to the spermosphere and shape its characteristics. These factors range from intrinsic genotypic properties of the seed to a myriad of extrinsic factors ranging from temperature and moisture characteristics of the soil to the site-specific biotic environment in which a seed exists. In the end, it is both intrinsic and extrinsic factors that influence seed germination characteristics, beginning with the uptake of water by the quiescent seed and ending with the protrusion of the radicle from the seed coat that ultimately shape the character of the spermosphere.

When a seed is sown in soil, its germination rate is determined largely by the water uptake characteristics of the seed. Such hydration characteristics directly affect the carbon deposition of the germinating seed. The carbon released from seeds during germination represents the major driving force behind plant-microbe and microbe-microbe interactions in the spermosphere. Additionally, the differential manner in which monocot and most dicot seeds germinate (i.e., hypogeal or epigeal germination, respectively) will also influence the carbon deposition of the seed and microbial behavior in the spermosphere. In epigeal germination, the cotyledons and seed coat are pushed out of the soil as the seed germinates, thus removing the seed from further colonization of and control over soil organisms. In contrast, with hypogeal germination, the seed remains in the soil. Because the spermosphere is shaped largely by seed germination behavior, it is appropriate to detail some of the more important physical and biochemical events that precede radicle emergence and seedling development and serve to establish the nature and dynamic properties of the spermosphere.

Water Imbibition and Seed Exudation

Seed germination progresses through three rather distinct phases (17, 18, 141) (Figure 2). The period of these phases is determined by a variety of seed and environmental factors (18), each influencing microbiological associations with the seed. Immediately following sowing, water rapidly moves from the surrounding soil into the seed. This Phase I hydration, also known as imbibition, is strictly a physical process, driven largely by the protein, lipid, and starch composition of

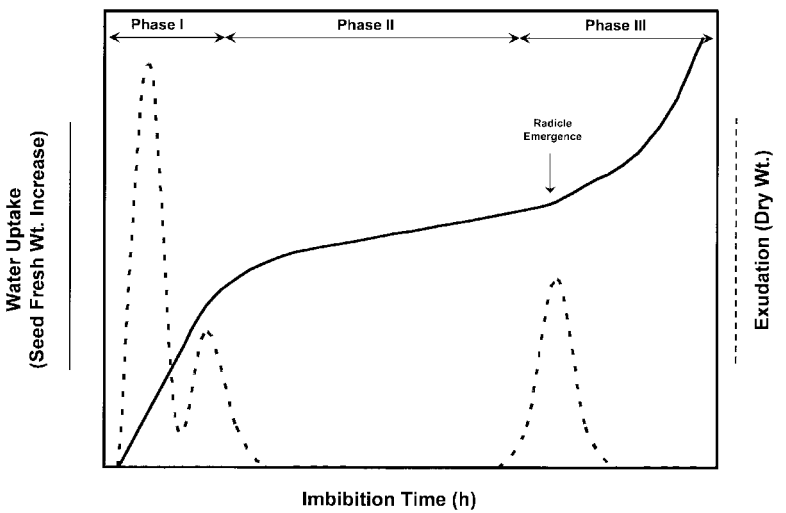

Figure 2 Temporal relationships between water imbibition and seed exudation. Modified from References (17, 204).

the seed, the permeability of the seed coat, and the differential water potential between the inside and outside of the seed. Water potentials inside the seed may be in the order of –350 to –50 MPa (180), making the water potential gradient from the inside to the outside of the seed quite large and the imbibition rate quite high. These imbibition properties may be modulated by extrinsic factors such as the extent of contact between the seed and soil water films.

The amount of water taken up by the seed may reach 150% or more of the seed weight, with germination occurring at an internal seed water potential of around –2 to 0 MPa (92). Accompanying this rapid water uptake is the hydration of seed storage proteins, resulting in considerable swelling of the seed. Generally, seeds with high protein contents (e.g., many legumes) will imbibe water more rapidly and swell to a greater degree than seeds containing mostly starch or lipids. The comparative composition of lipids, starches, and proteins of various seeds is shown in Table 1.

The swelling that results from the rapid influx of water leads to considerable internal hydrostatic pressures, often exceeding several MPa. This leads not only to rupture of the seed coat, but also to leakage of internal substances from the seed, because of the temporary structural damage to tonoplast and plasmalemma membranes when in a dried state (164, 165). This rapid leakage of cellular and vacuolar constituents is referred to as seed exudation.

TABLE 1 Chemical composition of seeds of selected plant species

	Mean composition (%)			
Species	**Lipids**	**Starch**	**Sugars**	**Proteins**
Zea mays	4–6	50–70	1–4	10–12
Pisum sativum	2	30–40	4–6	20
Arachis hypogea	40–50	8–21	4–12	20–31
Helianthus annuus	20–50	0	2	25–40
Triticum aestivum	2	60–75	13–14	
Citrullus vulgaris	46–52			38
Cucurbita pepo	47–48			35
Linum usitatissimum	24–43		23	23–26
Cannabis sativa	30–41		21	18–31
Cucumis sativus	38–40			28–30
Brassica rapa	34–48		25	20–35
Lactuca sativa	33–37			24
Gossypium hirsutum	15–33			25–39
Glycine max	13–24			36–38
Papaver somniferum	40–55		19	20

Data compiled from References (92, 118, 129).

The highest levels of exudation occur in the minutes and hours immediately after imbibition is initiated (112, 203, 204), followed by a secondary increase around 6 h (204). By 8–12 h of imbibition, when membranes are transformed from the dried gel phase to the fully hydrated liquid crystalline phase, imbibition and subsequent exudation ceases (17, 165). For seeds of most plant species under typical soil conditions, the bulk of exudation is complete within the first 12 h of sowing.

In addition to simple imbibition and leakage processes, Phase I of seed germination also marks the resumption of active but partial cellular metabolism, relying on preexisting ribosomes, proteins, and nucleic acids. New ribosomes are synthesized within hours of imbibition initiation (17, 141), and preformed proteins are rapidly degraded to provide amino acids for new protein assembly. By the time membranes are rehydrated and imbibition ceases, the seed is in a state of active cell metabolism.

When seeds are fully hydrated and water uptake and exudation cease, seeds enter the second phase of germination (Phase II) during which major metabolic events take place that prepare the seed for expansion and emergence of the radicle (18). During this period new mitochondria, mRNAs, and proteins are synthesized to support metabolism within the expanding radicle. The extension and protrusion of the radicle through the seed coat ends the germination process and begins the active growth of the seedling (Phase III). This is accompanied by another burst of metabolites and low-molecular-weight exudates that are released during this extension and protrusion phase. These materials arise largely from the mobilization of storage reserves that serve as energy for the nonphotosynthesizing and developing seedling. At this stage, triacylglycerols are broken down by lipases into fatty acids and subsequently by β-oxidation enzymes into acetate, proteins are broken down into amino acids by proteinases and peptidases, and starch and other polysaccharides are broken down into simple sugars by α- and β-amylases, α-glucosidases, and dextrinases. More details of storage reserve mobilization may be found in several comprehensive treatments of this subject (16–18, 92, 141).

The Nature of Seed Exudates and Exudation

The imbibitional processes described above largely drive the exudation of molecules from the seed and into the surrounding soil and serve to define the types of molecules that are released. Since seed exudation is greatest during the first minutes after imbibition commences, the types of molecules released during this period are generally low-molecular-weight compounds, which were preformed during seed development and maturation. Additionally, larger molecules such as peptides and proteins can be released because of the hydrostatic pressures in the inside of the seed (211, 212). Ultimately, however, differences in size, mass, morphology, composition of storage reserves, and other features of seeds of different plant species affect the quantity and quality of exudates released during germination.

The types of molecules that have been detected in seed exudates are listed in Table 2. A wide range of sugars, amino acids, organic acids, phenolic compounds,

TABLE 2 Components of seed exudates

Compound	Reference(s)
Sugars and sugar alcohols	
Arabinose, cellobiose, deoxyribose, fructose, galactose, glucose, glycerol, lactose, maltose, mannitol, mannose, raffinose, rhamnose, ribose, sorbose, stachyose, sucrose, trehalose, xylose	(3, 4, 19, 20, 24, 69, 70, 90, 110, 173, 189–191, 230–232, 245, 246)
Amino acids	
α-alanine, β-alanine, α-aminoadipic acid, α-γ-glutamylalanine, α-aminobutyric acid, γ-aminobutyric acid, asparagine, arginine, aspartic acid, citrulline, cysteic acid, cystathionin, cysteine, cystine, α,ε-diaminopimelic acid, dihydroxyphenylalanine, glutamine, glutamic acid, α-γ-glutamylalanine, glycine, histidine, homocysteic acid, homocystine, isoasparagine, isoleucine, isoxazolin-5-one, leucine, lysine, methionine, ornithine, phenylalanine, pipecolic acid, proline, β-pyrazolylalanine, serine, threonine, tryptophan, tyrosine, uracil-alanines, valine	(3, 4, 6, 10, 19, 38, 69, 99, 127, 143, 149, 159, 190, 221, 230–232, 244, 245)
Aliphatic organic acids	
Acetic acid, aconitic acid, aminocyclopropane-1-carboxylic acid, citric acid, fumaric acid, glycolic acid, α-ketoglutaric acid, lactic acid, malic acid, malonic acid, oxalic acid, succinic acid, tartaric acid	(3, 4, 21, 24, 97, 159, 221, 232)
Aromatic organic acids	
Caffeic acid, chlorogenic acid, trans-cinnamic acid, p-coumeric acid, 3,4-dihydroxybenzaldehyde, ferulic acid, gentisic acid, p-hydroxybenzoic acid, protocatechuic acid, salicylic acid, syringic acid, vanillic acid	(19, 94, 97, 103, 170)
Fatty acids and other lipids	
Azaleic acid, linoleic acid, myristic acid, oleic acid, palmitic acid, 4-(2,2,4-trimethylpentyl)-phenol, 5-(12-heptadecenyl)-resorcinol	(123, 183)
Flavonoids and other phenolic compounds	
Apigenin, catechin, chrysoeriol, cyanidin, daidzein, delphinidin, dihydroxyflavonols, 4′,7-dihydroxyflavone, flavonols, flavones, genistein, kaempferol, luteolin, luteolin-7-O-glucoside, malvidin, myricetin, naringenin, petunidin, phenolic acids, proanthocyanidins, quercetin aglycone, quercetin-3-O-galactoside, 7-O-α-L-rhamnopyranosyl-4′-O-rutinosylapigenin, stachydrine, trigonelline, condensed tannins	(14, 15, 27, 36, 48, 59, 64–68, 79, 80, 94, 122, 161–163, 220, 224, 227)
Volatiles	
Acetone, acetaldehyde, ethane, ethanol, ethylene, formaldehyde, formic acid, hydrogen cyanide, methane, methanol, propionaldehyde, propylene,	(57, 58, 131, 149, 156, 157, 233)
Other miscellaneous compounds	
Canavanine, various enzymes, lepidimoic acid, lepidimoide, nucleoside diphosphate kinase, unknown proteins, vicilin	(37, 42, 102, 114, 211, 212, 243)

and volatiles has been identified and, in some cases, quantified. Essentially any component of a plant cell can find its way into seed exudates. However, they all may not be present in exudates at the same time because some molecules may be released early in imbibition, whereas others may be released a considerable time after the seed has been sown. Unfortunately, there have been no standards in collecting, analyzing, and quantifying seed exudate components so it is difficult to deduce the details of the exudation process or compare quantitative or qualitative analyses from study to study. It is critical, however, that inferences about the biological activity of specific exudate molecules be made only after the quantitative temporal release characteristics of the molecules are established and synchronized with the response behavior of the organism or organisms under study. Unfortunately, the temporal release characteristics of specific exudate compounds during the first 12 to 24 h of seed germination are virtually unknown. Similarly, response behavior of microorganisms during this period is also generally not known.

Factors Affecting Seed Exudation

The concentration of specific exudate components in the spermosphere is particularly important for microbial growth and development. This is particularly well illustrated by seed-infecting pathogens such as *Pythium, Rhizoctonia*, and *Fusarium* species for which the amount of exudation has been correlated directly with disease incidence (30, 44, 53, 90, 91, 115, 116, 160, 167, 187, 191, 199). However, accurate concentrations of specific molecules are generally not known. This is usually not because of problems associated with their extraction and detection, but rather because of problems associated with their temporal release, their concomitant degradation by spermosphere microorganisms, the concentration gradient away from the seed surface, and the heterogeneous nature of the soil surrounding the germinating seed. As a result, the timing of exudate collection, the manner in which the spermosphere is sampled, and the microbial properties of the soil can all influence the quantitative estimates of specific exudate molecules.

Another significant problem associated with the quantitation of seed exudate molecules is the units by which concentrations are expressed. Numerous attempts have been made to quantify specific seed exudate molecules. These have been based exclusively on in vitro collections where imbibition characteristics differ from those in a solid matrix and where the concentrations are expressed as amounts per seed, amounts per seed per hour, or amounts per ml or liter (or an expression of molarity) (e.g., 24, 38, 110, 173). Although these expressions do not provide useful estimates of the concentrations experienced by a microbial cell in the spermosphere, they provide a means for comparative analysis of the factors that influence the relative amounts of exudate molecules released into the spermosphere.

Nevertheless, quantitative estimates of exudation from seeds exposed to different environmental variables have provided some insight as to the types of factors that may influence the concentrations of molecules in the spermosphere. Most

extensively studied has been the influence of temperature on the exudation of sugars and amino acids from germinating seeds (69, 191, 198, 206, 221, 231). Although temperature clearly influences the amount and type of exudate molecules released, no consistent response has been seen with different plant species. With some plants, an increase in exudation is observed with increasing temperature whereas with other plants, just the opposite is observed. This illustrates a general problem in interpreting many studies on seed exudation since the lack of experimental standards has made comparisons from study to study quite difficult, often leading to conflicting conclusions.

In one of the more comprehensive studies of temperature on seed exudation, Short & Lacy (198) examined carbohydrate exudation at hourly intervals over the first 96 h of pea seed germination. They found that the bulk of the carbohydrates released from seeds occurred during the first 18 h at temperatures of 22–30°C. However, at 10°C, significant exudation persisted for 48 h. Within the first 5 h of imbibition, nearly three times the amount of carbohydrate was released from seeds germinating at 30°C as opposed to those germinating at 22°C. Most significant of their findings was that even though the pattern of exudation was temperature dependent, the total amount of exudate collected over 48–96 h was not.

Even more important is the role of temperature on the release characteristics of specific exudate compounds. Vancura (231) observed the increasing release of some sugars over 48 h of seed germination with increasing temperature whereas the release of other sugars declined. Similar observations have been made with amino acids (221) and volatile compounds (131).

Factors other than temperature also influence seed exudation, including plant species or cultivar (25, 26, 38, 191, 198, 206, 232, 233), oxygen tension (20), seed age (58, 198), seed coat integrity (186, 187), and soil moisture (30, 91, 197). Again, it is difficult to draw conclusions from many of these studies because of differences in methods of exudate collection and in collection times. Our understanding of exudation dynamics and the factors that influence these dynamics are rudimentary at best, and more detailed studies under ecologically relevant conditions are necessary to define relationships and predict microbial responses and behavior.

Spermosphere Size

Because of the critical impact of seed exudates on spermosphere properties, any factor that influences exudation may also influence the size and dynamic of the spermosphere. Several attempts have been made to measure the extent of the spermosphere and its influence on microbial behavior (197, 200, 216). By determining the germination response of propagules of seed- and root-infecting pathogens placed at various distances from the seed, it is possible to begin to develop a better understanding of the temporal and spatial dynamic of the spermosphere.

Stanghellini & Hancock (216) studied the influence of soil moisture on the germination of chlamydospores of *Fusarium solani* f. sp. *phaseoli* at various distances

from the seed 24 h after sowing. Germination within the first 2 mm of germinating bean seeds ranged from 26% to 48.5% and declined with increasing distance from the seed. In soils held at 50 mbar (−5 kPa) matric potential, no germination was observed beyond 10–12 mm from the seed whereas in the drier soil held at 100 mbar (−10 kPa), no germination was observed beyond 6–8 mm. In the same study, sporangia of *Pythium ultimum* germinated within the first 2 mm of the seed by 1.5 h and by 12 h germination was observed up to 12 mm from the seed surface.

Short & Lacy (197) expanded upon this study to examine the influence not only of soil moisture, but also of temperature, cultivar, time, and area of the seed on the germination of chlamydospores of *Fusarium solani* f.sp. *pisi* (*Fspi*) in the spermosphere of pea. The results from this study clearly demonstrate the dynamic nature of the spermosphere and its influence on seed-associated pathogens. Germination of *Fspi* chlamydospores were always greater near the site of radicle emergence than at a position opposite the radicle. In general, the greater the soil moisture and the lower the temperature, the greater was the extent of the spermosphere. Additionally, germination was observed earliest next to the seed, but eventually germination levels increased at greater distances from the seed. The greatest spermosphere size determined in this study was 6–7 mm, with smaller zones associated with different temperatures, moistures, or adjacent to different locations on the seed surface.

These results are important for several reasons. First, they point to the lack of uniformity in exudation across the seed surface and potentially to the corresponding microbiological responses in the spermosphere. Greater microbial activity would be predicted near the emerging radicle than at other locations across the seed surface. These results also indicate some level of spermosphere specificity. That is, the traits of the spermosphere and the particular impacts on particular microorganisms are time-, cultivar-, and environment-specific.

Additionally, the properties of the spermosphere should also be dependent on the physical characteristics of the soil. As exudates are released during imbibition, they diffuse through soil, establishing a concentration gradient away from the seed. The diffusion properties and the steepness of the gradient will be greatly influenced by the pore size distribution and the proportion of air-filled to water-filled pores. Although water-soluble molecules would be expected to diffuse through water-filled pores, volatiles would most likely diffuse through air-filled pores. Other compounds may partition between water and vapor states and be regulated mostly by temperature.

Reported estimates of spermosphere size are likely to underestimate the true spermosphere size. This is because the sensitivity of the reporter organism is critical for establishing the width of these zones around a seed. In a study with *Macrophomina phaseolina* in which sclerotium germination was monitored in the soybean spermosphere (200), spermosphere sizes were estimated to be no larger than 2–3 mm from the seed surface. Perhaps the concept of a pathozone (54, 55) for pathogenic organisms or a response zone for other organisms is more meaningful for estimating the extent of the spermosphere that influences microbial responses.

A level of detail, not yet developed for most plant-microbe association, is clearly necessary for a more comprehensive understanding of how microbial activities in spermosphere habitats are regulated, particularly those related to pathogenesis and biological disease control.

MICROBIOLOGY OF THE SPERMOSPHERE

Indigenous Spermosphere Microbial Communities

Numerous studies have provided evidence that seeds harbor a diverse microbial community, not only on their surfaces but also within the embryo (8, 29, 32, 60, 81, 89, 101, 128, 138, 168, 236). During germination, the proliferation of these and other soil microorganisms is stimulated (23, 104, 181, 205). The changes in microbial communities are illustrated by shifts seen in the activities of specific functional groups of organisms that develop in response to germinating seeds (83, 150). However, the development and ecology of specific seed-colonizing microorganisms in the spermosphere, particularly those colonizing seeds in the hours immediately following sowing, have rarely been studied, and few contemporary examples of research in this area are available.

Based on a small number of observations, it appears that the types of microorganisms that colonize seeds during the early stages of germination are determined largely by the composition of the soil microbial community (23). This was recently corroborated in a study of microbial colonization of seeds germinating in *Pythium*-suppressive and nonsuppressive composts (120). However, despite the important role of soil microbial communities in establishing spermosphere communities, the seed genotype can certainly affect the quantitative levels of indigenous bacterial populations that colonize the spermosphere (78, 205) and that associate endophytically with seeds and radicles (2). Seeds may also select specific groups of organisms since those that proliferate in the spermosphere appear to differ from those colonizing the rhizosphere (138, 235).

Aside from plant pathogenic species, the identities of indigenous seed-colonizing microbial species have generally not been determined. Species of *Fusarium* and *Pythium* were the dominant spermoplane/spermosphere fungi recovered from turnip seeds germinated for 72 h in soil. High frequencies of the oomycetes *Achlya* and *Thraustotheca* were also detected (237, 238). *Rhizoctonia solani* and species of *Penicillium, Trichoderma, Gliocladium, Cylindrocarpon*, *Cephalosporium, Cunninghamella*, *Mucor*, and *Helicocephalum* were recovered sporadically and at low frequency. These same fungi were also isolated in roughly the same proportions from tomato, onion, cabbage, bean, mustard, and melon spermospheres (237).

Among the bacteria colonizing barley seeds during the early stages of germination are species of *Acinetobacter, Bacillus, Burkholderia, Pantoea*, and *Pseudomonas* (138), whereas cottonseeds were colonized by species of *Xanthobacter, Enterobacter, Microbacterium, Paracoccus, Curtobacterium, Micrococcus,*

Agrobacterium, Paenibacillus, and unidentified coryneform bacteria (120). In the latter study, bacteria and actinobacteria were the only organisms detected on surface disinfested seeds within 12 h of sowing in various composts (120).

Germinating seeds are colonized by indigenous microbial populations within a few hours of sowing (104, 120, 138, 153, 181, 205). Populations may reach densities of 10^5 to 10^7 cells/seed within 12 h after a seed is planted (120, 153). Within 2 h of sowing surface-disinfested cottonseeds, populations of bacteria and actinobacteria increased from 10^1 to 10^2 cells/seed to over 10^6 cells/seed (120). Many (over 10^5 cells/seed) of those bacteria and actinobacteria colonizing cottonseeds were fatty acid-metabolizing bacteria, previously shown to suppress *Pythium* infections (228, 229). Despite the *Pythium* suppressiveness that developed within 4 to 8 h of sowing, no antibiotic-producing organisms were detected (120).

Indigenous spermosphere microbial communities are still poorly understood and represent perhaps the greatest need for research. The nature and activities of the organisms colonizing germinating seeds would be expected to significantly affect the performance of microbial strains introduced for the purpose of nitrogen fixation, plant growth promotion, or biological disease control. Furthermore, indigenous seed-colonizing microbial communities can have significant effects on plant health (120) and on longer-term seedling establishment (126).

SPERMOSPHERE REGULATION OF MICROBIAL BEHAVIOR

As noted above, the types, quantities, and temporal release of seed exudate molecules largely govern the microbial dynamics in the spermosphere. Because of the rapid changes in seed exudation that take place during the first few hours after sowing, microbial responses during this period are equally rapid and changing. Rapid changes occur in the development of seed- and seedling-infecting pathogens, the natural successions of indigenous spermosphere microorganisms, and in the accelerated growth, proliferation, and activity of microorganisms introduced to the spermosphere. These dynamic changes must be understood if specific microbial activities in the spermosphere are to be predicted and manipulated. The impacts of such rapid changes are best illustrated by the responses of seed-infecting fungal and oomycete pathogens to seeds in the early stages of germination.

Spermosphere Responses of Oomycete and Fungal Pathogens

As early as the 1960s, seed exudates were known to stimulate propagules of oomycete and fungal pathogens (30, 31, 189, 190). However, the true significance of this response to disease development and biological control was not then fully understood and appreciated. We now realize that these responses represent critical stages in pathogenesis. If these responses are altered, subsequent disease development is greatly affected (62, 146, 147, 199).

Of particular importance are the sequence and timing of pathogenesis-related developmental responses to germinating seeds and the exudate molecules that elicit such responses. The preinfection events in pathogenesis, such as spore activation and germination, tactic and tropic responses, and infection structure development, provide an important reporting system on the molecules present in the spermosphere that may elicit rapid developmental responses. This knowledge allows one to predict when microbial interactions with pathogens are likely to occur, facilitating the synchronization of introduced biological control organisms with susceptible periods of pathogen development. The timing of such events also aids in understanding how and when exudate molecules might regulate biological control processes in the spermosphere.

Temporal responses of pathogens to seeds or roots are rarely studied within an ecologically meaningful time-frame and there are few examples of temporal responses reported in the literature. Some of our best examples come from seed-infecting pathogens such as the oomycete species *P. ultimum* and *P. aphanidermatum*, and from selected form species of *Fusarium solani*.

PYTHIUM ULTIMUM Our best understanding of temporal responses to seeds comes from studies of *P. ultimum*. Both oospores and sporangia serve as important soilborne propagules of this species. Oospores of *P. ultimum* form abundantly and rapidly in infected plant tissues (121) and serve as important survival propagules and primary inoculum. Germination of oospores can occur either directly by the formation of a germ tube, or indirectly through the formation of a zoosporangium, followed by the release of zoospores (35, 226). An essential step in the germination of oospores of *P. ultimum* is the thinning of the oospore wall (7, 86, 111). This process can take up to 10 weeks when incubated in soil or soil extracts (86, 111) but a high degree of conversion can occur within 15 days (depending on the age of the oospore). The conversion of oospores to thin walls may be enhanced in the presence of oxygen and at pH above 6.5 (85) and at increasing soil moistures and temperatures around, at, or above 25°C (105, 111). Although high soil moisture tends to favor oospore wall thinning, no thinning occurs in saturated soils (87). Once converted, oospores can germinate within 2 h (111).

Surprisingly few studies have examined oospore germination in *P. ultimum* in association with plants, especially in the spermosphere. Our only knowledge comes from one study in the rhizosphere, where direct germination of oospores was observed in the cotton rhizosphere (86). Greatest germination occurred within 1.5 mm of the root tip or root hair region with germ tubes all oriented tropically toward the root surface. The greatest germination occurred when oospores were in direct root contact. Germination has also been shown indirectly to occur in the spermosphere (41, 219), but direct temporal and developmental details are lacking.

Much more is known about the behavior of sporangia of *P. ultimum* (both zoosporangia and hyphal swellings) in the spermosphere. Sporangia of *P. ultimum* germinate directly in the spermosphere within 1–1.5 h with maximum germination occurring 3–4 h after exposure to seeds (88, 106, 120, 135, 136, 216, 217, 228).

Subsequent germ tube growth may exceed 300 μm/h (217). Because of their rapid germination responses to plants, there has been much interest in determining the factors that trigger germination. Although much of the early literature indicated that sugars and amino acids were the primary exudate components responsible for stimulating sporangium germination and initiating *Pythium*-seed interactions in soil (133), it is now clear that long-chain unsaturated fatty acids present in seed exudates serve as the primary elicitors of sporangium germination in *P. ultimum* (183), especially when sporangia have been produced on living plant tissues (135, 136). In fact, sporangia produced on living plant tissue, which most likely reflects the manner by which they form in nature, fail to germinate in response to sugars, amino acids, or other organic acids, but respond to long-chain unsaturated fatty acids as well as unfractionated seed exudates.

The release of zoospores from sporangia of *P. ultimum* (i.e., *P. ultimum* var *sporangiferum*) has not been studied in any detail in spermosphere or rhizosphere habitats since Drechsler's first descriptions of the phenomenon (35, 35a). Although there are no observations on zoospore release in spermosphere habitats, insights into the process can be gleaned from a limited number of observations in rhizosphere habitats.

Zoospores of *P. ultimum* are attracted to roots of a number of plant species (33a, 36). Accumulation occurs typically in the root hair region and the zone of cell elongation just behind the root cap (212a). Presumably zoospores are attracted to these sites because of elevated levels of glutamic acid (212a). Zoospores accumulate rapidly on roots within 1–2 min (33a), encyst within 10–15 min (212a), and germinate within 40–45 min (33a). Few differences between the proportion of swimming and encysted zoospores were seen across a range of plant species (125a).

Observations of zoospore cysts on artificially inoculated pea roots reveal that the spatial distribution of cysts across the root surface can change with inoculum density (33). At low and intermediate densities cysts were either randomly or uniformly distributed over the root surface whereas at high inoculum densities, cysts aggregated over the root surface. Such aggregation has been described previously in other oomycetes and in other species of *Pythium* (170a). Whereas the reasons for the aggregation are not entirely clear, it is believed to induce chemotropic growth of germ tubes emerging from zoospore cysts, enhance zoospore accumulation on root surfaces and thereby increase inoculum potential for infection, and enhance zoospore survival.

Once propagules have germinated in response to seed exudates, the seeds may be colonized by *P. ultimum* as early as 2–4 h after planting, with nearly 100% seed colonization occurring within 12–24 h of planting (62, 106, 108, 120, 132, 134, 145–148, 153, 216, 219) and high frequency of embryo infection by 48 h (46, 47, 219, 242). If early seed colonization is prevented or the size of the spermosphere is reduced by pregerminating seeds (62, 146, 147) or by the presence of active spermosphere organisms (120), seeds do not become infected. Populations of *P. ultimum* also increase around germinating seeds within 48 h of sowing (207).

Increases of 188% to 344% have been observed within 10 mm from the seed surface with greater populations around wheat and pea seeds than around seeds of corn or barley.

PYTHIUM APHANIDERMATUM Unlike *P. ultimum*, oospores of *P. aphanidermatum* typically do not require a thinning of the oospore wall before germination can occur and are generally considered to be exogenously dormant (22, 215). They germinate rapidly when provided with an appropriate stimulus (218, 223) at relatively high soil moistures and temperatures (1, 214, 223).

Oospores germinated directly (1–3 germ tubes/oospore) within 1.5 h in response to bean seed exudate added to soil (215). When placed adjacent either to bean seeds, sugarbeet seeds, or 2-week-old sugarbeet seedlings, greatest oospore germination (direct) was observed within 6–10 h. Although indirect germination (zoospore release) was observed at low frequencies in water-saturated soils, only direct germination was observed in the presence of host plants or exudates. This suggests that it is unlikely that zoospores are formed from oospores germinating in the spermosphere. As with sporangia of *P. ultimum*, the germination behavior of *P. aphanidermatum* oospores is strongly influenced by other microorganisms in the rhizosphere (40, 223).

The germination of sporangia of *P. aphanidermatum* in the spermosphere or rhizosphere has not been studied extensively. Much of the research focus has been on zoospore behavior as opposed to zoospore release characteristics of sporangia. Stanghellini and Burr (215) observed that, along with oospores, *P. aphanidermatum* sporangia germinated within 1.5 h of amending soils with bean seed exudate. Sporangia germinated directly by the production of 1–3 germ tubes, even when soils were saturated. However, in the absence of seed exudate, 90% of the sporangia released zoospores in saturated soils. Once released, zoospores of *P. aphanidermatum* are attracted to seed exudates (73) presumably to facilitate seed colonization and infection.

FUSARIUM SOLANI F.SP. PHASEOLI Nearly all plant pathogenic species of *Fusarium* survive in soils as chlamydospores (119, 130), which serve as primary inoculum. The behavior of chlamydospores, therefore, provides significant insights into the nature of disease development and the possible spermosphere regulators of pathogenesis. Studies with various form species of *Fusarium solani* have provided a critical understanding of the important role seed exudates play in regulating germination and pathogenesis of *Fusarium* species in general.

Seeds of various plants have been shown to stimulate the germination of *Fusarium solani* f.sp. *phaseoli* (*Fsph*) chlamydospores (82, 188). Chlamydospores within the first two millimeters of the bean seed surface germinated within 4–5 h after sowing (216); maximum germination occurred within 16–24 h after sowing seeds (189). This occurred with seeds of both susceptible and nonsusceptible plant species (188). The spermosphere extended up to 12 mm away from the seed surface 24 h after sowing in moist soil whereas it was much less extensive in dryer

soil (216). Germination of chlamydospores of *Fsph* in the spermosphere has been correlated with the presence of particular sugars and amino acids in bean seed exudates, including glucose, sucrose, fructose, asparagine, aspartic acid, glutamine, glutamic acid, glycine, and phenylalanine (189, 190). This has not been confirmed, however, with chlamydospores produced on plant tissues.

FUSARIUM SOLANI F.SP PISI In studies similar to those with *Fsph*, chlamydospores of *Fusarium solani* f.sp. *pisi* (*Fspi*) germinated maximally in response to pea seeds or seedlings 12–42 h after sowing (30, 197, 239). A careful and comprehensive study by Short & Lacy (197) revealed details of the spatial relationships of chlamydospores in the spermosphere with their germination responses. Chlamydospore germination of *Fspi* was always greater near the emerging radicle than in any other location around the seed. Whereas the extent of the spermosphere as measured by the germination of chlamydospores of *Fspi* was typically in the range of 5–7 mm from the seed surface (197), this was greatly modulated by soil temperature, moisture, and pea cultivar. Chlamydospore germination was observed at greater distances from the seed of the more susceptible wrinkle-seeded cultivar than of the less susceptible smooth-seeded cultivar. Cooler temperatures and wetter soils also increased the extent of the spermosphere within 24–48 h after sowing (197). Generally the amount of exudation was coupled to the degree of chlamydospore germination and germling survival (30). Therefore, reducing the size of the spermosphere by presoaking seeds for 48 h prior to sowing was shown to dramatically decrease chlamydospore germination (197) as well as reduce seed rot induced by *Fspi* (199).

Similar to the observations of Short & Lacy (197) on differential chlamydospore germination in the spermospheres of resistant and susceptible cultivars, Kraft (98) had earlier observed less germination of macroconidia of *Fspi* in the spermospheres of resistant pea cultivars than in the spermospheres of more susceptible cultivars.

Although the spermosphere molecules that elicit germination responses of *Fspi* chlamydospores are unknown, direct correlations between carbohydrate exudation, chlamydospore germination, and pea seed and root rot have been observed (198). More recently a number of exudate flavonoids were shown to possess high levels of stimulatory activity to macroconidia of *Fspi* and *Fsph* (182). Micromolar concentrations of a number of different flavanones, flavones, and pterocarpans were highly stimulatory to *Fspi*, inducing high levels of germination within 3 h of exposure. Isoflavones and pterocarpans were the most stimulatory to macroconidia of *Fsph*. Pisatin, hesperitan, naringenin, luteolin, and apigenin were also highly stimulatory to chlamydospore germination of both form species. Although macroconidia and chlamydospores also germinated in response to various sugars and amino acids (182), the flavonoid-induced germination was shown indirectly to be mediated by cAMP whereas the sugar-responsive germination was not. Flavonoids are believed to transiently elevate cAMP levels in chlamydospores and macroconidia by inhibiting cAMP phosphodiesterase (9). It is possible that these two distinct modes of germination response (flavonoid-induced and sugar-induced) to host

plants may provide some level of specific recognition as well as general modes of carbon maintenance in the spermosphere and rhizosphere.

Significance of Pathogen Responses for Biological Control in the Spermosphere

These studies that describe the temporal pattern of pathogen response to germinating seeds provide important insights into the nature of the spermosphere molecules that elicit such developmental responses. They also point to important mechanisms by which indigenous or introduced seed-associated microorganisms might suppress seed infections by pathogenic organisms. For example, observations described above point to a rather short period of vulnerability of most germinating seeds to seed-infecting pathogens, generally within 12–24 h (62, 134, 146, 147). Therefore, it is critical that organisms used for biological disease control express biological control traits within the first 12–24 h of germination. Thus, either microbial traits necessary for pathogen suppression or plant defense response must be activated and expressed within this narrow time frame. Often this must occur well before a seedling emerges from the soil. Investigations of microbial behavior and interactions occurring well beyond this time frame are likely to be of little ecological relevance to the biological system under investigation.

These observations also point to the importance of studying microbial interactions with each partner in the ecologically correct developmental stage. For example, it seems inappropriate to study interactions of spermosphere organisms with the mycelium of a fungal or oomycete pathogen if the pathogen exists solely as chlamydospore germlings or zoospores in the spermosphere. Finally, in attempting to study the influence of various exudate components on microbial behavior in the spermosphere, it is important to collect exudates for analysis within this important 12–24 h period. Analysis of exudates collected one week after sowing, for example, will have little relevance to questions being addressed.

Chemotaxis in the Spermosphere

In highly competitive habitats such as the spermosphere, rapid occupation of substrates is essential to establishment and activity of microorganisms. The ability of both indigenous and introduced microbes to locate and exploit spermosphere resource can facilitate their persistence and activity. Chemotaxis may be an especially important trait in this regard, particularly in light of the observation that some spermosphere bacteria can swim over a 2 cm distance in as little as 24 h to reach a germinating seed (13).

Studies with *Bacillus* and *Pseudomonas* species have provided much of the basis for our understanding of chemotaxis in the spermosphere. For example, *Bacillus megaterium* strain B153-2-2 has been shown to be positively chemotactic to soybean seed exudates (247), largely in response to alanine, asparagine, glutamine, malate, serine, and threonine present in the exudate. Chemotactic responses to amino acids present in soybean seed exudates have been observed with some

Rhizobium species (11). However, with *B. megaterium*, chemotaxis was also observed in response to malate, malonate, pyruvate, and succinate but not in response to sugars. Chemotaxis occurred over a broad temperature and pH range and cells at an exponential growth stage were more chemotactic than stationary stage cells. This chemotactic response to soybean seed exudates is significantly correlated with seed colonization and subsequent antagonism to *Rhizoctonia solani* (248).

Earlier studies with strains of *Pseudomonas fluorescens* and *P. putida* support the results with *B. megaterium*. Both species have shown positive chemotaxis to soybean (185) and tomato (49) seed exudates. Again, the chemotactic response was largely due to exudate amino acids and not to sugars. In studies with solarized and nonsolarized soils (49, 50), chemotactic and growth responses were greater in solarized soils than in nonsolarized soil, indicating that other components of the soil microbial community may regulate chemotactic responses.

As with bacteria, zoospores of oomycete pathogens also display positive chemotaxis to seed exudates (73). Zoospores of *P. aphanidermatum* are attracted to both amino acids and sugars present in seed exudates (34). *Aphanomyces euteiches*, on the other hand, has been shown to be attracted to flavones and isoflavones (192, 193).

Spermosphere Colonization by Introduced Strains

The ability of bacterial and fungal strains to colonize the spermosphere and reach high population densities during the first 12–24 h of seed germination are important for their abilities to induce growth responses and protect seeds from seed-infecting pathogens (47, 148, 151, 172, 179) as well as to subsequently colonize the rhizosphere (63, 71, 78, 93, 109, 110, 113, 152). Spermosphere colonizing traits vary considerably among species and also among strains within the same species (205). Some bacterial species are more adapted than others to colonize the spermosphere directly from the soil than from the seed (95). However, species introduced directly on seeds most commonly are more competitive with indigenous seed-colonizing organisms and better able to proliferate in the spermosphere than those colonizing from soil (95, 151), particularly when they are fast-growing strains (47, 148, 151) or population densities are maintained at high levels (10^7–10^8 cells/seed) for the first 12–24 h of seed germination (47, 148, 151).

It is often not clear whether the distribution of microbial cells on the seed surface is sufficient for timely and efficient interactions with seed-infecting pathogens. Cells of various bacteria may be randomly distributed over the seed surface at low population density ($\sim 10^4$ cell/seed). However, their distribution is patchy at 24 h after sowing (only 10–40% of the seed surface colonized) when population density exceeds 10^6–10^7 cells/seed (45, 75, 225). This may be influenced by the availability of particular seed exudate compounds or may be related to the surface architecture of imbibing seeds. Furthermore, this could be important in

biological control systems where quorum sensing is critical for disease-suppressive activities.

Properties of the host influencing spermosphere colonization by individual microbial strains can be quite significant. For example, over a range of recombinant inbred lines of tomato, the 48 h growth increase of a number of strains of *Bacillus cereus* on seed surfaces ranged from 1.14 to 1.83 log cfu/seed (205, 209). This indicates that bacterial growth can be inhibited in association with some lines and strongly stimulated by others. Similar effects have been observed with *Pseudomonas* species and indigenous seed-colonizing bacteria (205). Furthermore, in studies with different plant species, population development of strain EcCT-501R3 of *Enterobacter cloacae* was significantly greater in the spermosphere of some plants than in others (107, 171, 173, 174, 177, 178).

The composition and temporal release of specific seed exudate components can exert a major influence on the metabolic activities and growth dynamics of microorganisms in the spermosphere and ultimately influence population size and interaction with pathogens. This has been most clear with studies of *E. cloacae*, a prolific spermosphere-colonizing biological control organism and competitor with other seed-associated microorganisms (75, 169, 172).

The ability of *E. cloacae* to control Pythium damping-off is related, in part, to its ability to proliferate in the spermosphere. *E. cloacae* populations increase in the spermospheres of many different plant species within a relatively short period of time (179) by selectively utilizing mono- and oligosaccharides for growth (107, 173, 174, 178) and in other plant spermospheres, also on amino acids and peptides (171, 176, 177). Carbohydrates found in seed exudates supported growth of strain EcCT-501R3 of *E. cloacae* as did major monosaccharide constituents of seed storage carbohydrates and various seed-associated oligosaccharides (178), inducing increases in α-galactosidase, α-glucosidase, β-glucosidase, and β-xylosidase activities.

Studies in which carbohydrate catabolism in *E. cloacae* has been impaired reveal the important role of exudate carbohydrates for the colonization and biological control activities of introduced bacterial strains. For example, mutations in the phosphofructokinase gene (*pfkA*) (173, 174) impair the ability of *E. cloacae* to grow on certain seed exudate carbohydrates, to proliferate in various plant spermospheres, and to suppress Pythium damping-off. *pfkA* mutants of *E. cloacae* are unable to grow on most sugars commonly found in seed exudates, including arabinose, galactose, glucose maltose, raffinose, ribose, and sucrose. Growth on fructose, glycerol, amino acids, and organic acids, however, is unaffected (173). This deficiency greatly reduces the ability of *E. cloacae* to proliferate in the cucumber and radish spermospheres. However, proliferation in pea, soybean, sunflower, and sweet corn spermospheres is not impacted within 24–45 h of sowing (173, 174, 178).

The *pfkA* mutation has the greatest impact on growth rate of *E. cloacae* in the spermospheres of seeds such as cucumber and radish that released low quantities of fructose, other carbohydrates, and amino acids (173). Mutants are less affected in

the spermospheres of pea, soybean, sunflower, and sweet corn whose seeds release relatively high levels of carbohydrates, particularly fructose (up to 4000-fold) and amino acids, during the first 96 h of seed germination. Such high concentrations of fructose support the growth of *pfkA* mutants at wild-type levels. For example, adding fructose to cucumber and radish seeds at quantities similar to those released from pea seeds over a 96 h period resulted in spermosphere populations of the *pfkA* mutant equivalent to wild-type levels. Furthermore, complementation of the *pfkA* mutation with a homolog cloned from strain 501R3 of *E. cloacae* restored the nutritional phenotype as well as spermosphere colonization to near wild-type levels (174). Other catabolic mutants have been described that show similar reductions in spermosphere colonization (175). Most likely these catabolic genes and pathways play key roles in the competitiveness of *E. cloacae* in the spermosphere.

Mutations in anabolic pathways in *E. cloacae* have also been shown to affect spermosphere colonization (107). Mutations in the ribose-5-phosphate isomerase gene (*rpiA*) gene result in an inability of *E. cloacae* to grow on ribose and other pentose sugars, which can ultimately influence its ability to synthesize nucleic acids. *rpiA* mutants are deficient in the colonization of cucumber, sunflower, and wheat seeds and significantly reduced in the colonization of corn and cowpea seeds relative to the wild-type strain of *E. cloacae* (107). These phenotypes were also expressed as reduced populations in the rhizosphere of cucumber, wheat, and sunflower. In 42-day-old plants, populations of the *rpiA* mutant of *E. cloacae* were not detected in the rhizosphere of any plant, whereas populations of the wild-type strain persisted at high densities in the rhizospheres of all plants. Complementation of the *rpiA* mutant with a wild-type copy of the *rpiA* gene restored ribose phosphate isomerase activity, seedling colonization, and disease suppression to wild-type levels. Unlike catabolic functions, anabolic genes and pathways are likely to be important in supplying key amino acids, vitamins, and nucleotide precursors that regulate spermosphere colonization.

Whereas the role of carbohydrate metabolism in spermosphere colonization and biological control is readily apparent, the role of amino acids in affecting these activities in *E. cloacae* has not been elucidated. *E. cloacae* is able to grow in vitro and in soil on several amino acids commonly found in seed exudates (177). Several mutants auxotrophic for seven different seed exudate amino acids were reduced in their ability to proliferate in the spermosphere of corn, cucumber, and pea. This reduced colonization could be rescued in some mutants by applying casamino acids along with the bacteria to the spermosphere (176). Some of these mutants did not differ from the wild-type in bean, cowpea, radish, and sunflower spermospheres whereas other mutants did not differ from the wild-type only in pea and radish spermospheres.

These results demonstrate the complex regulation of microbial behavior in the spermospheres of different plant species by components of seed exudates. They also point to the need for a more complete biochemical analysis of spermosphere habitats and the molecular regulation of metabolic functions in microbial populations.

Spermosphere Regulation of Gene Expression

Much of the work on gene regulation in the spermosphere has been done with various biological control species of *Pseudomonas* and more recently with *B. cereus*. Genes involved in sugar and amino acid metabolism are commonly induced by seed exudate components. For example, canola seed exudates were shown to induce the expression of an ABC sugar transporter in *P. putida* GR12-2R3 (12). Similarly, the expression of an aminotransferase gene involved in lysine catabolism was increased in the presence of corn seed exudate (43). More recently, it has been shown that sugar beet seed exudate can trigger the GacS/GacA regulatory system in a *Pseudomonas* species that is involved in the biosynthesis of a fungal inhibitory cyclic lipopeptide, amphisin (96). This is significant because the GacS/GacA system is important to many functional attributes of gram-negative bacteria, including the biosynthesis of secondary metabolites and plant colonization (61, 72). Research such as this is beginning to shed light on some of the molecular details of the regulatory role of seed exudates in microbial behavior in the spermosphere and providing evidence of the complexity of such regulatory processes in spermosphere habitats.

The complexity of this regulation is further illustrated with a study by Dunn et al. (38) in which they developed a promoter trap strategy for identifying genes that were either up-regulated or down-regulated by tomato seed exudate components. From among clones expressing exudate-regulated genes, one was identified in which the expression of a gene encoding a lipoprotein of unknown function, designated *lipA*, was increased in the presence of seed exudate from a specific tomato recombinant inbred line designated RIL37. Most of the inducing activity was present in seed exudates within the first 24 h of germination. Intriguingly, the *lipA* promoter was not affected by seed exudate from another tomato inbred line designated RIL55. Although the nature of the inducing compound or compounds is not known, they do not appear to be individual sugars, amino acids, organic acids, or volatiles (38). Although the *lipA* gene does not seem to affect any significant fitness traits, a more exhaustive screen of this and other such libraries will likely begin to reveal more of the complex microbial behavior and dynamics in the spermosphere.

Plasmid Transfer in the Spermosphere

A growing body of evidence is now pointing to the spermosphere as a particularly active habitat for conjugative plasmid transfer among bacterial strains. Recent evidence has shown that plasmids can be transferred at extremely high rates in spermospheres of pea and barley (194, 210, 222). Such transfer is facilitated by the rapid bacterial growth stimulated in this carbon-rich environment.

In studies with *Burkholderia cepacia* and *P. fluorescens*, the more rapid the cell growth of both the donor and the recipient strains in the spermosphere, the more efficient was the transfer of plasmid R388::Tn1721 (222). Transfer of plasmid RP4 from strain sp127 of *P. putida* or strain AS12 of *P. fluorescens* in the

spermosphere and rhizosphere of barley occurs at an unusually high rate in the spermosphere ($10^{-2.8}$) (210). This and similar transfer efficiencies that have been reported in other studies (194) are among the highest reported from any natural environment. Transfer has been observed not just between introduced organisms, but also between introduced and indigenous spermosphere bacteria (210). Despite high plasmid transfer efficiencies, no horizontal transfer of chromosomally encoded genes is known to occur in the spermosphere.

Regulation of Antibiotic Biosynthesis in the Spermosphere

The level and timing of antibiotic biosynthesis may influence the suppression of seed and seedling pathogens by biological control organisms (166). However, little direct evidence exists for the biosynthesis of antibiotics in the spermosphere of seeds inoculated with specific bacteria. As early as 1956, studies revealed that antibiotics could be detected on seeds sown in soil (241), providing the first evidence that they can play important roles in nature. Only a limited number of follow-up studies have occurred since. Nonetheless, these studies reveal some important insights into the potential regulatory role of the spermosphere in affecting the biological control activities of antibiotic-producing microorganisms.

PSEUDOMONAS SPECIES Much of the work on antibiotic biosynthesis in the spermosphere has focused on antibiotics produced by *P. fluorescens* and *P. aureofaciens* that suppress seed infection by *P. ultimum*. These include oomycin A, pyoluteorin, 2,4-diacetylphloroglucinol (DAPG), and phenazine antibiotics. In studies with strain Hv37A of *P. fluorescens*, Howie & Suslow (77) demonstrated that an oomycin A biosynthetic gene (*afuE*) was expressed in the cotton spermosphere within 24 h after sowing. The fact that this is a glucose-regulated gene (84) suggests that the levels of oomycin A found in the spermosphere could be tightly linked to the temporal release of glucose from the seed. Similarly, a pyoluteorin biosynthetic gene (*plt*) of strain Pf-5 of *P. fluorescens* was expressed in the spermosphere of both cotton and cucumber within the first 72 h of seed germination (100). However, expression in the cucumber spermosphere was delayed in comparison with expression in the cotton spermosphere where *plt* expression peaked at about 12 h after sowing. Similar trends in pyoluteorin biosynthesis in association with cucumber and cress have been described for strain CHA0 of *P. fluorescens* (117). More recently, it has been reported that other strains of *P. fluorescens* produce other antifungal compounds such as vicosinamide preferentially in the spermosphere and rhizosphere as compared to bulk soil (137). This suggests that the carbon precursors for such biosynthesis are more commonly found in the phytosphere than in plant-free soil.

Phenazine biosynthesis in the spermosphere of various plant species by strain PGS12 of *P. aureofaciens* has also been investigated (52). The expression of a phenazine biosynthetic gene (*phz*) was first detected 12 h after planting on seeds of a number of different plant species and increased up to 48 h after sowing, at which

time different levels of *phz* expression were observed among the different plant species. The highest level of expression was observed on wheat seeds, whereas the lowest expression level was observed on cottonseeds. Expression did not appear to be affected by different cell densities, soil matric potentials, or soil type.

As with oomycin A, the sugar regulation of antibiotic biosynthesis has also been observed with other antioomycete and antifungal antibiotics. For example, sucrose, fructose, and mannitol have been shown to enhance the biosynthesis of DAPG in strain F113 of *P. fluorescens*, whereas glucose and sorbose repress DAPG production (195). In other strains of *P. fluorescens*, glucose can promote DAPG biosynthesis (140). Although glucose is not known to up-regulate the biosynthesis of pyoluteorin, it can down-regulate its biosynthesis in some strains of *P. fluorescens* (140). This could reflect a fundamental difference in DAPG regulation among different strains of *P. fluorescens* and may explain some of the strain-to-strain variability in biological control efficacy.

Collectively, these observations highlight the regulatory role of seed exudate in controlling important bacterial traits related to biological control. A repeating theme from these studies is the importance of the timing of exudation of specific molecules as it related to the timing of antibiotic biosynthesis and biological control expression.

BACILLUS CEREUS Strain UW85 of *B. cereus* produces at least two known antibiotics, zwittermicin A and kanosamine, both of which play a role in the suppression of *Pythium* species (125, 196, 201, 213). Zwittermicin A is a broad host range antibiotic (201) effective against a wide range of fungi, oomycetes, and bacteria (202), whereas kanosamine is most toxic to oomycetes but has some activity against fungi and bacteria (125). Seed and seedling exudates from alfalfa seedlings enhance the production of zwittermicin A and kanosamine in culture (124, 125). Although the component or components of seed exudates that regulate the biosynthesis of these antibiotics are unknown, the different levels of biological control observed on different recombinant inbred lines of tomato (209) indicate the utility of these lines for assessing the exudate molecules responsible for this regulation.

Inactivation of Seed Exudate Regulators of Pathogen Development

Plant-associated microorganisms must prevent pathogen development prior to infection to effectively protect seeds from pathogens such as *Pythium*, *Fusarium*, and *Rhizoctonia*. This can be accomplished either by producing inhibitors such as antibiotics that stop pathogen development, or by eliminating essential carbon, energy, and nutritional resources. This must all happen within the narrow 12–24 h window following the sowing of seeds because of rapid pathogen responses to germinating seeds.

There is now a growing body of empirical as well as direct experimental evidence to suggest that *Pseudomonas* species (41, 46, 155–157), *Trichoderma*

species (5, 58, 76), *E. cloacae* (88, 228, 229), *Burkholderia cepacia* (73), or indigenous seed-colonizing microorganisms (39, 40, 120, 139) metabolize exudate compounds that regulate preinfection growth and propagule germination responses of pathogens. This may play a significant role in preventing seed and root infections. This concept is best exemplified by work on *E. cloacae* and its interaction with *P. ultimum*.

A key element to the interaction of *P. ultimum* with germinating seeds is the dependency of rapid propagule germination on the early release of long chain unsaturated fatty acids during seed germination (183). In association with certain plant species, *E. cloacae* can very rapidly metabolize these exudate fatty acids rendering the seed exudate nonstimulatory to sporangia of *P. ultimum*, whereas on other plant species this exudate inactivation does not occur (88, 228, 229). Early studies demonstrated that *E. cloacae* could protect cucumber, cotton, and ryegrass from *P. ultimum*-incited damping-off, but was ineffective in protecting seeds of snapbean, lima bean, soybean, corn, and pea (134). This was believed to be related to the carbohydrate levels present in seed exudates, an observation confirmed in more recent studies (88).

In subsequent experiments involving several different plant species, *E. cloacae* readily reduced the stimulatory activity of 2-h-old exudates from carrot, cotton, cucumber, lettuce, sunflower, and tomato within 3 h of exposure. No inactivation of exudates from corn, pea, radish, and wheat was observed within 3 h. By 6 h, however, significant reductions in the stimulatory activity of exudates were observed with all plants except corn. When tested in soil systems, *E. cloacae* failed to reduce the stimulatory activity of corn and pea seed exudates.

The differential responses of sporangium germination to seeds treated with *E. cloacae* translated directly into reductions in biological control efficacy. The suppression of Pythium damping-off was only effective on seeds that would support the ability of *E. cloacae* to reduce sporangium germination and not in association with seeds of plants such as corn or pea where no such reduction in germination responses was evident.

The reasons for these differential responses of *E. cloacae* to seed exudates of corn and pea are likely due to the levels of sugars that are coreleased with fatty acids during seed germination. Sugars released from corn, pea, and a number of other plant species during the first 24 h of seed germination are known to consist largely of glucose, sucrose, and fructose (173, 174), with galactose and stachyose dominant in some species (173). Glucose and possibly other hexoses can repress β-oxidation in *E. coli* (154). Current evidence suggests that similar repression may occur in *E. cloacae* in the presence of exudate sugars (240). In the presence of increasing concentrations of glucose, sucrose, or fructose, the ability of *E. cloacae* to metabolize linoleic acid was correspondingly reduced. For example, in the absence of any sugar, the stimulatory activity of linoleic acid was eliminated in as little as 4 h. However, concentrations of glucose, sucrose, or fructose as low as 1–2 mM were sufficient to reduce linoleic acid metabolism. Concentrations of 4 mM and higher eliminated fatty acid metabolism entirely. Additionally, adding increasing

concentrations of glucose to cottonseed exudate reduced and eventually prevented *E. cloacae* from metabolizing exudate fatty acids. For example, as concentrations of glucose increased in a 4-h collected seed exudate, the ability of *E. cloacae* to inactivate the stimulatory activity of the exudate decreased, again demonstrating that exudate sugars, if present in high enough levels, can regulate the fatty acid metabolism by *E. cloacae* and thus affect biocontrol efficiency.

Although current evidence points to seed exudate sugars as regulating β-oxidation in *E. cloacae* and thus regulating biological control efficacy, many important questions remain. It is not clear what the concentrations of exudate sugars or exudate fatty acids are in the spermosphere that may regulate the biological control behavior of *E. cloacae*. The timing of the release and metabolism of both sugar and fatty acid exudate components are also unknown and believed to be critical to this biological control interaction since the ability of *E. cloacae* to rapidly metabolize any stimulatory fatty acids is critical to its biocontrol success. A more detailed quantitative examination of these important seed exudate components is warranted.

CONCLUDING REMARKS

The spermosphere represents an important plant-associated microbial habitat that is ephemeral yet rich in microbial form and function. Despite the short-lived nature of the spermosphere, the microbial activities taking place in this 5–10 mm zone of soil surrounding the germinating seed may have long-lasting impacts on plants, ultimately influencing their distribution, development, and health. Associations with pathogenic microorganisms, nitrogen-fixing and other nutrient-transforming organisms, and other stimulatory and deleterious organisms most frequently begin in the spermosphere. The ecological importance of the spermosphere cannot be denied and numerous examples of its significance have been presented to illustrate this point. Yet research in spermosphere biology has lagged noticeably behind studies of the rhizosphere. Many concepts gained from our study of the rhizosphere will likely aid in our understanding of the spermosphere. However, because of the uniqueness of the spermosphere habitat, many new concepts might await discovery.

The seed has been an important delivery vehicle for a variety of beneficial microorganisms, including inoculants such as *Rhizobium, Bradyrhizobium, Sinorhizobium, Mesorhizobium*, and *Azospirillum*, for plant growth enhancement to *Pseudomonas* and *Bacillus* species for biological disease control. Inconsistencies in performance of these inoculants have been largely unexplained. Future research in spermosphere biology may very well provide answers to such unexplained behavior and contribute to a better understanding of why inoculants succeed under some conditions or on particular plant species but fail on others. The interactions of these organisms with indigenous populations of microorganisms may influence the expression of biological control traits or the subsequent colonization of the rhizosphere.

One of the greatest deficiencies in our knowledge of the spermosphere is the lack of data about the nature, succession, and activities of indigenous spermosphere microbial communities. Since the first recognition of the ecological significance of the rhizosphere, questions of the origin of the rhizosphere community have arisen from time to time. It is evident that the seed preferentially stimulates populations of indigenous soil organisms. However, we lack the knowledge of which populations are stimulated and how they relate to other plant-associated microbial communities.

The regulation of microbial activities through the types and concentrations of exudate molecules is a significant finding that will likely open up new avenues of ecological research. The very basis by which spermosphere microorganisms associate amensalistically, mutualistically, and antagonistically is influenced by the biochemical environment molded by the germinating seed. A more detailed understanding of how such interactions are regulated will greatly affect the success of introduced inoculants and provide better insight into relationships with seed- and seedling-infecting pathogens. Furthermore, future research may focus on efficient means of manipulating spermosphere habitats, either through plant breeding efforts alone or in conjunction with microbial manipulations that facilitate microbial associations that may not otherwise occur in nature (142, 144, 184).

One of the more significant and least understood aspects of spermosphere biology is the temporal dynamic that characterizes all seed-associated processes. The rapid transformation of the seed is reflected in the astonishingly rapid changes in exudation and microbial activity that shape the character of the spermosphere. As experimental models, spermosphere systems offer a simple yet rapidly changing habitat in which questions about microbial succession, microbial behavior, and plant or microbial developmental processes can be addressed in relatively short time frames. Furthermore, because of the tight linkage between microbial behavior and exudation, the synchronization of the two activities offers the opportunity to gain insights into the biochemical processes regulating various microbial functions in terrestrial habitats.

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