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Symposium

Do microorganisms influence seed-bank dynamics?

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Reduction of seed-bank persistence is an important goal for weed management systems. Recent interest in more biological-based weed management strategies has led to closer examination of the role of soil microorganisms. Incidences of seed decay with certain weed species occur in the laboratory; however, their persistence in soil indicates the presence of yet-unknown factors in natural systems that regulate biological mechanisms of seed antagonism by soil microorganisms. A fundamental understanding of interactions between seeds and microorganisms will have important implications for future weed management systems targeting seed banks. Laboratory studies demonstrate susceptibility to seed decay among weed species, ranging from high (velvetleaf) to very low (giant ragweed). Microscopic examinations revealed dense microbial assemblages formed whenever seeds were exposed to soil microorganisms, regardless of whether the outcome was decay. Microbial communities associated with seeds of four weed species (woolly cupgrass, jimsonweed, Pennsylvania smartweed, and velvetleaf) were distinct from one another. The influence of seeds on microbial growth is hypothesized to be due to nutritional and surface-attachment opportunities. Data from velvetleaf seeds suggests that diverse assemblages of bacteria can mediate decay, whereas fungal associations may be more limited and specific to weed species. Though microbial decay of seeds presents clear opportunities for weed biocontrol, limited success is met when introducing exogenous microorganisms to natural systems. Alternatively, a conservation approach that promotes the function of indigenous natural enemies through habitat or cultural management may be more promising. A comprehensive ecological understanding of the system is needed to identify methods that enhance the activities of microorganisms. Herein, we provide a synthesis of the relevant literature available on seed microbiology; we describe some of the major challenges and opportunities encountered when studying the in situ relationships between seeds and microorganisms, and present examples from studies by the ARS Invasive Weed Management Unit.

Nomenclature: Giant ragweed, *Ambrosia trifida* L.; jimsonsweed, *Datura stramonium* L.; Pennsylvania smartweed, *Polygonum pensylvanicum* L.; velvetleaf, *Abutilon theophrasti* Medic.; woolly cupgrass, *Eriochloa gracilis* (Fourn) A. S. Hitchc.

Key words: Seed–microorganism interaction, weed seed decay, soil microbiology, microbial communities, seed-bank ecology, multitrophic systems, integrated weed management.

It has long been recognized that microorganisms play key roles in many soil-related processes (Garbeva et al. 2004; Singh et al. 2004; Somers et al. 2004; van Elsas et al. 1997; Waksman 1927). Familiar activities mediated by soil microorganisms include organic matter and mineral cycling, biodegradation and detoxification of compounds, rhizosphere influences on plant growth and health, and plant diseases (Figure 1). The aim of reducing tillage and pesticide use in agriculture has made the important role that microorganisms play in these processes even more apparent.

The growth and survival of soil microorganisms are inextricably linked to the fluctuating physical and chemical conditions of the environment in which they are found. A more complete census of microbial species awaits, and the full functional diversity of natural soil microbial communities is yet undiscovered. A relatively small number of populations have been characterized, and we presently claim only minimal mechanistic understanding of microbial activities and interactions within complex, multitrophic ecosystems. Further, the number of trophic-level interactions involving microorganisms in soil is unknown, and there is little knowledge of the degrees of complexity involved in these relationships. There is increased recognition that a holistic approach to the study of natural microbial communities is needed (Torsvik and Øvreås 2002; Paerl and Steppe 2003), involving better characterizations of the environmental surroundings and the multitude of possible interactions involving microorganisms that comprise, in part, whole biotic community structure and function.

Microbial interactions with plants have been intensively studied (Garbeva et al. 2004; Johansson et al. 2004; Singh et al. 2004). These include mutualistic partnerships, such as those occurring in plant root zones that benefit the nutritional status of the plant and microorganisms, as well as antagonistic relationships involving host and pathogen. Recent studies also suggest soil microbial populations influence plant community structure, including plant species abundance and seedling recruitment (Klironomos 2002; Mills and Bever 1998; Schafer and Kotanen 2004; van der Heijden et al. 1998). Although numerous studies have shown

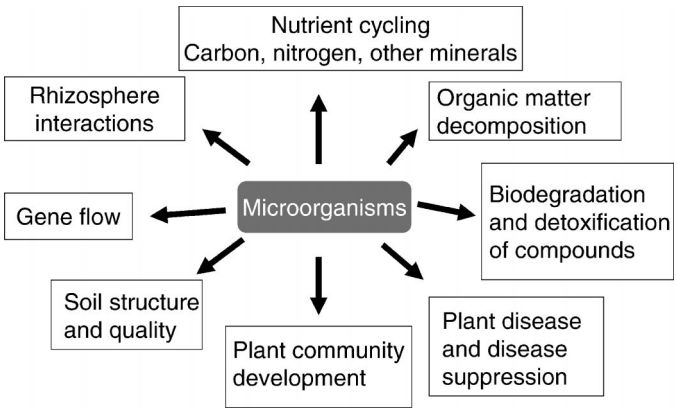

FIGURE 1. Key ecological processes mediated by soil microorganisms.

important microbial associations within the zones of the rhizosphere or spermosphere (i.e., the zone surrounding a germinating seed) (Nelson 2004; Simon et al. 2001), less focus has been made on interactions that occur specifically with long-lived seeds that persist in seed-bank systems.

Seed banks play a key role in the population dynamics of numerous weed species. For annual weeds, seeds are dispersed through both space and time, and seed banks are the source of future cohorts (Fenner 1995). Even very low weed densities can produce sizeable additions to the seed bank, ensuring species survival, and creating a continuing need for weed management. Reducing seed-bank persistence is an important goal for weed management systems (Davis 2006, Davis et al. 2004). High fecundity and the production of long-lived seeds are features of many weeds (Booth et al. 2003). Velvetleaf, for example, produces one of the longest-lived seeds among annual weed species (Lueschen et al. 1993) and along with species such as common waterhemp (*Amaranthus rudis* Sauer), produce persistent seed banks (Buhler and Hartzler 2001). The abundance, persistence, and seasonal replenishment of seeds of many annual weeds in soil rich with resident microorganisms would conceivably give rise to many interactions between seeds and microorganisms. The conditions that influence both the development and outcome of these relationships have yet to be discovered, including any attributes directly related to the seeds themselves that are intrinsic to these relationships.

There is increased interest in investigating weed management through exploiting natural seed-bank processes in both agricultural and natural area systems (Buhler et al. 1998). One major area of consideration is in the development of weed management strategies that exploit useful biological properties of plants and other resident biota, including natural predators and antagonists of weeds and their seeds. Beyond the mechanisms of interest that specifically target seed-bank depletion are those that may also benefit seed longevity, seedling viability, and other seed-related processes that lead to plant success. Accessible surfaces of all higher organisms are thought to be colonized by microorganisms, and a healthy state of various plant and animal tissues is often associated with characteristic assemblages of microorganisms (Ellis et al. 1995; Smith and Goodman 1999; Tannock 1995). Thus, teleological assessments of exposed surfaces and excretions from higher organisms now include the possibility of promoting beneficial microbial communities. Some known plant–microorganism interactions, for example, help to regulate disease suppression or provide protection against microbial antagonists. As a result, the concept of probiotic therapy used in human and animal health has been applied to plant health as well (Haas and Keel 2003; Misra 2005). Moreover, it has become clear that the plant is an active participant (Smith and Goodman 1999) and dynamic in the relationship, as evidenced by successional changes in microbial associations with plant development (Ellis et al. 1995) and in some instances, conversion from a microbial saprobe to pathogen in response to various plant signals. Understanding potentially beneficial relationships in seed-bank ecology may result in a useful strategy to promote seed or seedling mortality by disrupting particular plant–microorganism interactions.

The concept of targeting the weed seed bank using natural soil biotic processes is not new; weed seed decay and the notion of "weed-suppressive soil" have been investigated in the past (Kennedy 1999; Kennedy and Kremer 1996; Kremer 1993). These promising areas of study have yet to be fully investigated, particularly in the context of a complex ecological system that closely links both above- and below-ground processes and considers the whole developmental cycle of the plant (Figure 2) (Davis 2006). There is a need to examine individual subsets of important seed–microorganism interactions, but there also exists the more difficult challenge of identifying the ecological drivers in these interactions that might lead to better predictions of site-specific outcomes.

The focus of this discussion is to address the seemingly simple question of whether soil microorganisms influence seed-bank dynamics. The question is inherently biased, because it implies the role of the microorganism to be the more active participant in microbial-related seed-bank processes. Thus, it is just as valid to ask, do seed banks influence soil microorganisms? Addressing these questions will require equal consideration of the ecological role that both microorganisms and seeds play, and significant progress in this area of research will require the collective and interactive efforts of microbiologists, plant biologists, and ecologists, among others. The objectives here are to (1) describe some prior knowledge of soil microbiology and weed seed banks in the context of natural mechanisms that can affect microbial communities, plant development, and seed fate, including the meaningful significance at different spatial and trophic-level scales; (2) describe the characteristics of the relationship between seeds and microorganisms, including mutual influences exerted in these interactions, and (3) discuss the concept of exploiting useful (micro-)biological processes, including among others, seed decay, in future weed management strategies. This article also describes some of the major challenges and opportunities encountered when studying the relationship between seeds and microorganisms in natural and managed environments, and presents examples that demonstrate some of the current approaches being taken by the ARS Invasive Weed Management Unit.

Complexity of Seed Banks and Soil

The characteristics of seed banks, their size and composition, seed distribution in the soil, and seed fate can be highly variable (Baskin and Baskin 2006). Land-management practices can affect all of these characteristics. Pro-

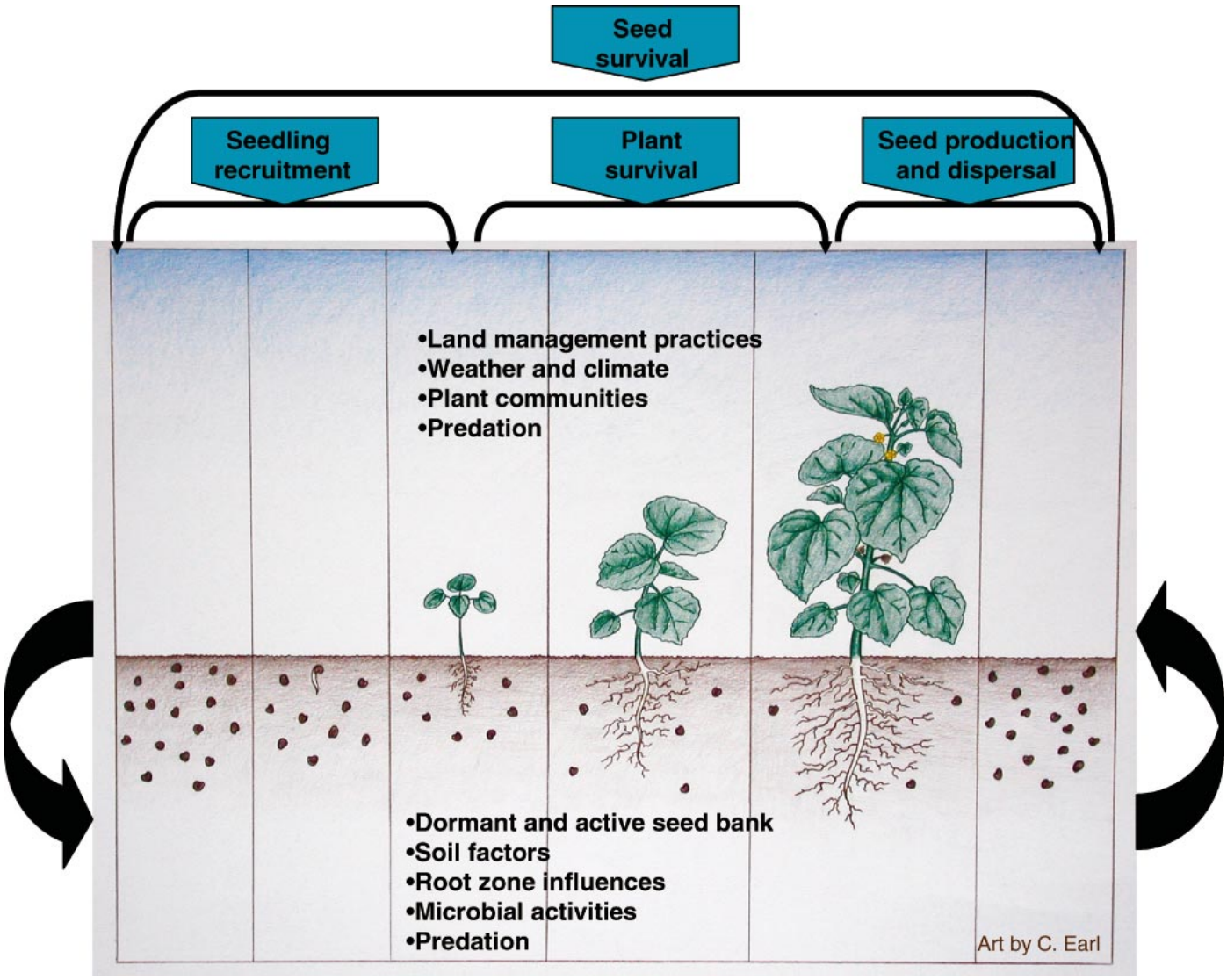

FIGURE 2. Seed-bank dynamics of an annual weed with emphasis on the interacting relationship between above- and below-ground processes.

cesses affecting seed-bank dynamics, particularly in crop systems, are complex and vary greatly depending on production practices and timing (Buhler et al. 2001). Knowledge of the processes that occur in seed banks is essential for predicting weed persistence in both managed and unmanaged land, and understanding the consequences for plant succession and evolution. The ecological framework of soil seed banks and seed persistence has been correlated to seed characteristics, their distribution in soils, land-management practices, and soil/environmental conditions (Ghersa and Martínez-Ghersa 2000; Guérif et al. 2001; Roger-Estrade et al. 2001). Seldom in the past have microorganisms been a major consideration in seed-bank ecology, due in part to the inherent challenges in studying soil microbiology.

Soil is a highly complex system that is comprised of a seemingly infinite number of discrete microhabitats that can each be described by a set of unique chemical and physical features. These microsites harbor microorganisms, the activities of which (and interactions with one another) are highly responsive to their local environment, generally thought to be characterized by a continuum of physicochemical gradients. What is also commonly found in many types of soil are discontinuous sets of physicochemical conditions that create spatially isolated microhabitats and may result in uneven distribution of both numbers and species of microorganisms (Mummey and Stahl 2004; Nunan et al. 2002; Tiedje et al. 2001; Torsvik and Øvreås 2002). Factors such as soil particle sizes, aggregate characteristics, soil depth, and nutritional content have also been found to influence microbial population distribution and community structure (Sessitsch et al. 2001; Smit et al. 2001; Zhou et al. 2002). Physical disturbances to soil from, for example, insect movement, tillage practices, and formation of cracks or fissures, would presumably lead to redistribution of seeds (Westerman et al. 2006) and either limit or increase the chance encounters between seeds and microorganisms. The presence of pesticides may also influence temporal changes in soil microbial communities (Girvan et al. 2004; Johnsen et al. 2001; Martin-Laurent et al. 2003).

Many of the environmental factors that govern the release of dormancy and germination in seeds, such as soil temperature, moisture, and oxygen concentration (Benech-Arnold

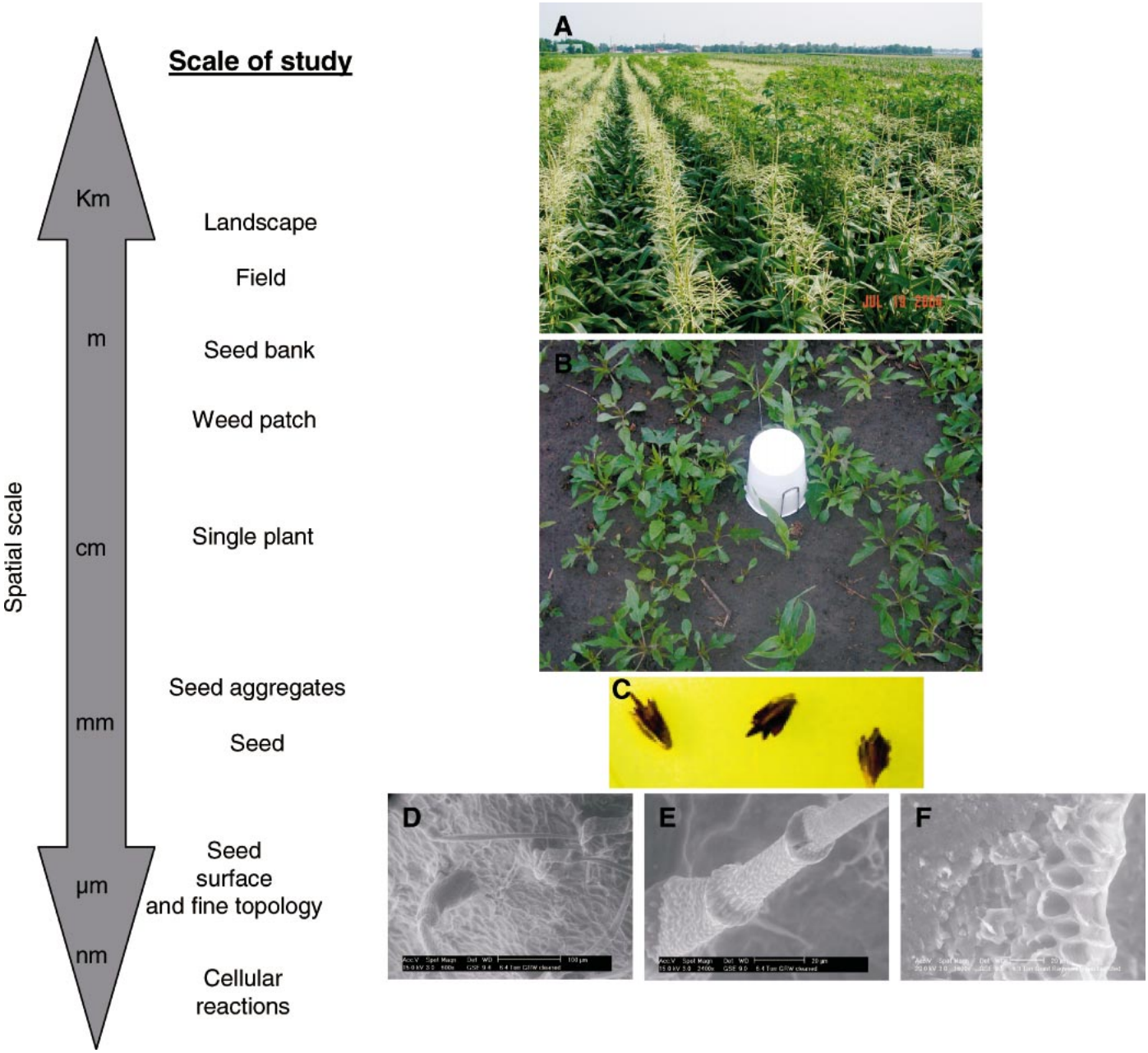

FIGURE 3. Scales of study of giant ragweed represented by (A) complex plant communities in a landscape or field, (B) patch of weeds or individual plants, (C) seed aggregates or individual seed, and (D–F) giant ragweed seed surfaces at multiple spatial scales imaged by environmental scanning electron microscopy (ESEM). Spatial scales of measure are indicated in the left arrows and potential scales of study are listed in descending order of relative size.

et al. 2000; Reuss et al. 2001), can also influence the presence, survival, and function of microorganisms, as well as other soil biota, in the same environment. Besides the relatively brief period of time associated with the onset of germination, it is not known how seeds in other physiological stages, dormant or otherwise, may influence surrounding microbial interactions and growth. Soil microorganisms have been suggested as factors in seed dormancy and germination; however, little data are available to support this (Baskin and Baskin 2000). What constitutes the favorable conditions in soil that promote microbial interactions with seeds needs to be systematically investigated.

Relevant Scales of Study

Meaningful interpretation of the data generated in studies of plant ecosystems and their constituent interactions depends largely on the scale of study. Factors that are critical to processes at one scale may not be important to others, and the methods selected for measure are also scale driven. Microscale fluxes in the environment may very well result in profound changes at the scale of individual microbial cells but would not likely be observed at the field scale, where the measurement is usually only the net effect of numerous localized and lower-level processes. Likewise, landscape-level

processes (e.g., land management, climate, etc.) directly affect the soil environment, and consequently, numerous microhabitats are influenced along with their corresponding microbial populations. Using giant ragweed as an example, Figure 3 illustrates the magnitude of spatial scale that requires consideration in studies of plant communities and seed-bank relationships; however, it should be made clear that there is presently little knowledge about how these scales are linked. This point can also be illustrated by listing potential scales of study that may focus on complex whole plant communities, or smaller groups of individuals defined by arbitrary spatial boundaries (e.g., a square meter of a weed patch or an aggregate seed deposit), or single individuals. The finest spatial scale might be represented by specific molecular and biochemical reactions occurring at the seed surface or at the individual cellular level. Incorporation of multiple scales into experimental designs is exceedingly difficult, particularly when the intent is to span several orders of magnitude in spatial range. This is increasingly evident even when the microbial community is the sole focus in a large ecosystem study, where defining what constitutes a representative sample, maintaining the biological and physicochemical integrity of the sample to be measured, and the availability of suitable methods, already pose difficult challenges in often ill-defined natural systems.

The term *landscape* is often used in reference to a portion of land surface which can be comprehended in a single view; however, the term can be equally applied, from the perspective of a microbial cell, as the surrounding environment with which the organism interacts. For example, high magnification of a giant ragweed seed reveals a nonhomogeneous surface comprised of a complex architecture associated with the involucre, the outer bur-like structure surrounding the achene (Figure 3D–3E). The nonuniform surface and large area available for interaction could presumably allow for differential microbial assemblages to form, depending on the nature of the existing microbial community. Attachment to surfaces is a common attribute of many microbial species, where such associations often confer an advantage in nutrient access and protection from surrounding biota and the environment. High-magnification images have revealed a diversity of surface features, ranging from relatively smooth seed surfaces of woolly cupgrass to the complex topologies of giant ragweed and velvetleaf seeds. Predatory grazing by small free-living amoeba (FLA) are thought to be the main control of soil bacterial populations (Rodriguez-Zaragoza 1994), and FLA tend to be concentrated at highly colonized interfaces, such as the rhizosphere and spermosphere. Although colonization of seed surfaces may be specific and advantageous to certain soil bacteria, these relationships may also be the result of an adaptation by many microbial species that takes advantage of seed architecture to offer protection against predation by FLA, as well as other protozoa and soil nematodes. A closer cross-sectional view of the involucre further reveals a highly porous structure (Figure 3F) that may confer varying degrees of challenge to microorganisms attempting to penetrate through to the seed interior. The physiological role of these seed structures is not well known, and their involvement in microbial interactions can only be hypothesized. Nonetheless, the high complexity at even the smallest spatial scales presents no lesser challenge for mechanistic studies of seed–microorganism interactions, and will very likely require special tools to measure.

Associations between Seeds and Microorganisms

Soil Microbiology

We frequently encounter in the literature discussions of soil microorganisms referenced collectively as both bacteria and fungi. The practical reason for this consolidated view may be their similar range of cellular size, their relatively high abundance in soil compared to other trophic-level community members, and some similarities in metabolic lifestyles. Bacteria and fungi also pose similar challenges in identification, possessing limited distinguishing morphological features, unlike most species of macrofauna and -flora that allow visual taxonomic assignments to be made. In truth, bacteria and fungi are very distinct; unrelated through different evolutionary lineages, and differing in degrees of genetic and physiological diversity. Bacteria comprise an entire domain of prokaryotic life, with a breadth of diversity that far exceeds the known collective of fungi, the latter of which are represented in one small phyletic branch of the eukaryotic domain (Pace 1997; Woese 1987). Fungi have a closer evolutionary relationship to plants and animals than many bacterial species have to each other. Thus, it may not be surprising if studies should uncover a much higher complexity associated with the relationships of seeds with bacteria, contrasted to those between seeds and fungi.

Beyond the microbial species that have been well studied for their recognized roles in important plant- and soil-related processes, the full diversity, functional types, and the ecological relationships among soil microorganisms are poorly understood (Rondon et al. 1999; Sessitsch et al. 2001; Smit et al. 1999; Smit et al., 2001). Microbiologists are challenged with the study of systems that are far from being fully described in terms of microbial diversity, community composition, species distribution, and function. This is exceedingly evident in soil environments. Much of the present understanding of soil microorganisms has been limited to species that have been successfully isolated as pure cultures from their environments, and only a relative few have been studied in depth in their natural habitats.

Estimates of 4,000 to 10,000 species of bacteria can be present in only a single gram of soil and in fact, an estimated 99% of all microbial species are yet unclassified or uncharacterized, owing to our inability to cultivate the majority of microorganisms for detailed examination (Rondon et al. 1999; Torsvik et al. 1990; Ward et al. 1990). Members of the kingdom *Proteobacteria*, for example, are ecologically important and widespread in nature, with numerous cultivatable species from soil having been studied in depth. In contrast, only one member of the phylogenetically distinct *Acidobacterium* kingdom has been cultivated, yet molecular-based data suggest members of this bacterial kingdom are in high abundance in many soils, possessing genetic and metabolic diversity similar to the *Proteobacteria* and other well-known soil bacteria (Barns et al. 1999). Although their dominance in many soils has been made obvious, the exact functional roles of *Acidobacterium* in soil are not known. In general, the extent of bacterial diversity is yet unknown, particularly in complex natural environments. Estimates of

about 1.5 million species of fungi, in total, have been made; however, only about 70,000 species have been described (Borneman and Hartin 2000). Fungi have important ecological roles in their relationship to other microorganisms, contribution to soil structure and quality, and plant diseases (protection and pathogenesis) (Thorn 1997; van Elsas et al. 2000). The study of fungi in interactions with plant hosts is widespread, and fungi are known to comprise a large portion of the biomass in many soils, leading to predictions that evolved interactions with seeds are likely. The richness of species and metabolic diversity thought to be present among soil bacteria, however, presents an equally compelling reason to focus on their involvement in seed interactions as well.

Although distinct, bacteria and fungi pose many similar methodological challenges in detection, identification, and characterization of their functional activities. Recent development of molecular biology techniques, better physiological-based approaches, and advanced instrumentation have facilitated both in vitro and in situ studies of environmental microorganisms. The available databases that describe genetic and metabolic characteristics of soil bacteria, and especially of fungi from natural environments, remain relatively small; but as more information is added, new methods and refinements of existing ones used to characterize populations are made. The need for an integrated strategy that combines genetic and physiological approaches to study microbial ecology and trophic level interactions is apparent. Even with ever increasing development of new tools, researchers are faced with a continuously challenging paradox: How do we characterize the vast unknown with methods based primarily on what is presently known?

The microbial diversity and species dynamics related specifically to agricultural soils, which can undergo fairly frequent and varied physical and chemical disturbances, have been the subject of numerous studies, yet agroecosystems remain far from being well characterized. The need to integrate microbial processes and interactions into the overall ecology of the soil seed-bank system is apparent, and among the important processes, microbial functions related to seed depletion and other mechanisms affecting seed fate in soil remain largely undetermined.

Decay of Weed Seeds by Microbial Activity

Given the presence of seed banks and the high diversity of microorganisms likely to be present in agricultural soils, adaptation and the evolution of new function may indeed have led to a number of microbial mechanisms targeting seeds. Of the possible mechanisms, seed decay has been one focus of previous and current research. Instances of velvetleaf seed deterioration by *Fusarium*, a common soil-borne fungus, were reported by Kremer and Schulte (1989). The hypothesis that seeds of some species may provide a major source of carbon or nitrogen nutrition for microorganisms seems logical in the absence of other explanatory relationships. Microbial-targeted activities involving seed components may be analogous to the evolution of many well-characterized microbial catabolic genes that allow a variety of complex organic (xenobiotic or natural) compounds to be broken down to support diverse microbial food webs. In examining the composition of velvetleaf seeds recovered from plants grown in the absence of competition in a greenhouse, we found the velvetleaf seed embryo has a carbon and nitrogen content (by wt.) of 48% and 3%, respectively (J. C. Chee-Sanford, unpublished). Further significant are the carbon (43%) and nitrogen (1%) contents specifically associated with the seed coat, which is presumed to be the initial point of access for microbial degradation to occur. Similar carbon (47%) and nitrogen (1%) contents were associated with the involucre of giant ragweed seeds. Although the specific compounds comprising the carbon and nitrogen of the seeds is not characterized here, seeds of velvetleaf and others do appear to present a potentially significant nutritional resource for the extant soil microbial community, if accessed. Although this suggests that seeds of velvetleaf and giant ragweed have the potential to nutritionally select for the growth of certain microbial species in soil, it may further suggest a larger impact on soil microbial community structure in seed-bank soils due to numerous localized (i.e., small-scale area of influence around a decaying seed) nutritional turnovers at the expense of high numbers of seeds. Although the outcome of this activity presumably has an impact on microbial community structure at the small scale, the cumulative effects may be measurable at the larger ecosystem level.

In determining the relative susceptibility of different species of seeds to decay, we have employed simple assays con-

TABLE 1. Decay of weed seeds following 3-mo exposure to soil microbial inocula.[a]

Weed species	Total no. seeds assayed	No. seeds decayed[b]	Percent seed decay[b]
Velvetleaf	224	222 a	99 a
Pennsylvania smartweed	208	21 b	10 b
Wild buckwheat	448	18 b	4 b
Jimsonweed	352	14 b	4 b
Giant ragweed	60	4 c	7 b
Woolly cupgrass	288	3 c	1 b
Common ragweed	288	0 c	0 b
Shattercane	240	0 c	0 b
Wild oat	256	0 c	0 b

[a] The assay consisted of a carbon-free mineral salt (pH 7.0) agar medium (modified from Fries et al. 1994) with seeds embedded into the agar surface. Seed lots used in these experiments were assayed for average viability (tetrazolium test). Species other than velvetleaf and giant ragweed were purchased commercially (Azlin Seed Service, Leland, MS) and were collected from mature plants in 2000–2001, rinsed in sterile water, air dried, and stored at 4 C until use. Seeds of velvetleaf and giant ragweed were collected by the authors locally (Champaign, IL) in the fall of 2001 and stored similarly. Velvetleaf, shattercane, and woolly cupgrass are classified as hardseeded species (Baskin and Baskin 1998; Buhler and Hoffman 1999) as defined by Meisert et al. (1999). Each seed lot (n = 96) underwent germination tests with the same agar medium described above. For the seed decay assay, the agar was inoculated with microorganisms derived from soil obtained locally from sites around Champaign and Urbana, Illinois. Soil was a silty clay loam texture (comprised of approximately 20% sand, 50% silt, 30% clay, organic matter content 4–7%, pH 6.1–6.2) typical of the region. One gram of soil was homogenized in 1× phosphate-buffered saline (pH 8.0), and an aliquot (0.1 ml) of the soil suspension was spread onto the surface of the agar. Seeds were incubated in the dark at 25 C for up to 3 mo, and periodically inspected for signs of decay. For lack of any standardized method of determining seed decay, visible inspection of the seeds allowed only the scoring of those specimens that underwent extensive deterioration (excluding from the count those that followed seed germination).

[b] Values followed by the same letter within a column are not different at alpha = 0.05. For values ≥ 5, chi-square tests were used to determine differences among weed species. Species with values less than 5 are assumed to be similar.

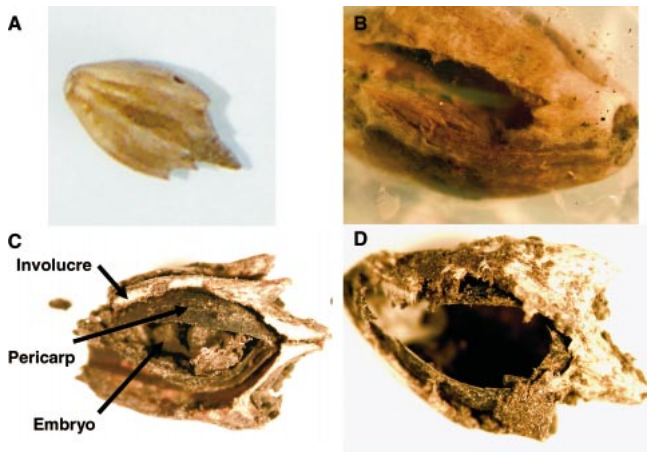

FIGURE 4. Diaspores of giant ragweed in various stages of decay. (A) intact and viable diaspore, (B) diaspore inoculated with a mixed culture associated with decay activity during enrichment cultivation experiments, (C) diaspore from a field site that appeared intact, but after bifurcation, a cross-sectional view revealed extensive embryo decay occurred, and (D) a deteriorated diaspore recovered from a field site, with only part of the pericarp and involucre remaining.

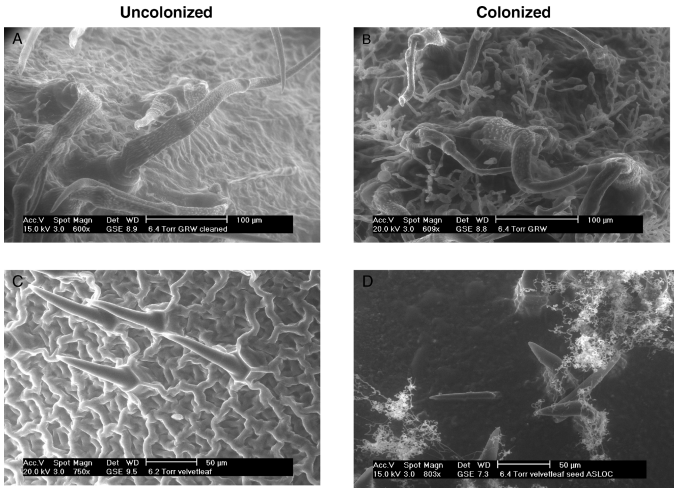

FIGURE 5. Environmental electron-scanning micrographs contrasting uncolonized (left panels) and microbial-colonized (right panels) seeds of giant ragweed (A and B) and velvetleaf (C and D).

sisting of well-defined incubation conditions in the laboratory. Although the results of such assays cannot be interpreted in the context of natural soil environments, it does allow useful comparisons among weed species. In laboratory screening assays in which different soil microbial communities were exposed to seeds of a variety of weed species, a range of susceptibility and initial rates of seed degradation were observed (Table 1) (Chee-Sanford et al. 2003). Removal of limiting factors in soil, such as availability and types of nutrients, fluctuating temperature and moisture, spatial contact, and low microbial number, allowed a general survey of the range and extent of potential seed decay that could be mediated by soil-borne microorganisms. Seeds of velvetleaf were highly susceptible to microbial-mediated decay, with 99% of the seeds assayed undergoing decay in a 3-mo period. The extent of decay was similar regardless of soil-derived microbial population the seeds were exposed to, which included soils from both agronomic and undisturbed fields. In contrast, Pennsylvania smartweed, wild buckwheat (*Polygonum convolvulus* L.), and jimsonweed lost a significant number of seeds, whereas other species demonstrated little or no decay following identical time of exposure to soil microbial populations. Velvetleaf seeds can persist for many years in seed banks, and their high susceptibility to decay under well-defined laboratory conditions suggests there are factors residing in soils that limit their decay in natural environments. Such studies demonstrate simply that the relative susceptibility of seeds to microbial decay processes differs between weed species, and for some weeds like velvetleaf, the potential for seed decay to occur may be more broadly distributed in nature. These studies suggest a closer examination of soil factors and the microbial populations involved in the seed decay process.

The ability to directly observe seed decay is an important component in data collection, and the use of a variety of visual and microscopic techniques can facilitate the viewing of seeds during the formation of interactions and the progression of decay. Nondestructive techniques that can allow examination of a seed over real time would be ideal, but not yet possible. Environmental scanning electron microscopy (ESEM), for example, is particularly effective for visualizing seeds at high resolution and magnification. Minimal sample preparation using ESEM allows a specimen to retain its structural integrity, thus allowing true in situ examination, for example, of microbial attachment or collapse of seed structure. The sample, however, cannot be recovered for further reliable analyses. To demonstrate, in laboratory studies, the microbial decay of giant ragweed seed can occasionally include dense microbial colonization with the formation of a deep cavity (Figure 4B), presumed then to allow further successional access by other microorganisms to the nutritional resources that the seed can provide. The ability to view less-visible evidence of seed deterioration would be advantageous in attempting to characterize the initial stages of decay, along with the ability to characterize microbial associations as they form. In contrast, some seeds of giant ragweed recovered from a field site appeared visually intact, with no obvious signs of damage, until a cross-sectional view indicated extensive decay of the embryo had already occurred (Figure 4C). Further contrasting this are other field-collected seed that have clearly been subjected to extensive decay or predation, with large openings in the exterior structures and absent embryos (Figure 4D). The visible pathology of deteriorating seeds is very useful in demonstrating an array of seed loss mechanisms possible in natural soil; included in this is the potential for diverse microbial processes as major mechanisms of seed decay, detectable even at this relative scale of measure.

Seeds and Their Microbial Assemblages

Many of our current efforts in characterizing seed–microorganism relationships are focused on identifying the corresponding microbial populations. The outcome of microbial associations with seeds is not always detrimental. Following exposure to soil microbial inocula, seed surfaces are often observed to be densely colonized by diverse microbial morphotypes (Figure 5), and the seed may still be intact and even viable (i.e., proceed to germinate). Once formed, the associations between the microorganisms and the seed surfaces are often difficult to physically disrupt. In soil, similar

interactions may occur, but contact may be limited due to conditions of the environment and the structure of the extant microbial community. Further, because of the high microbial diversity and functional redundancy expected in natural soil, colonization of seeds is likely to be driven by competitive factors and interactions between the microbial species present in the seed zone, with a range of fates then possible as an outcome for any one seed.

Besides providing the rich nutrition available in the embryo and a large surface area for microbial attachment to occur, seeds like those of velvetleaf produce diffusible phenolic compounds that were demonstrated to have antimicrobial properties (Kremer 1986). Numerous plant species reportedly produce a range of compounds, many associated with antimicrobial activity (Broekaert et al. 1995). In the context of seeds, these types of compounds may be intrinsically important in the regulatory mechanisms of plant development and in maintaining seed-bank longevity, providing protection of seeds against microbial (or other) antagonists. Given the functional diversity anticipated among soil microorganisms, one might expect that along with the large number of microbial species that might respond with negative feedback to seed-associated compounds, others will likely be attracted or respond favorably to seed surfaces or seed-derived chemical gradients that form in the seed zone. Many bacteria and fungi also produce antimicrobial compounds, leading to the consideration that microorganisms may play a secondary but critical role in protection of the seed from other antagonists. The hypothesis that seeds express an active role in directing beneficial microbial interactions leads to an interesting new aspect of plant–microorganism relationships.

Analysis of Microbial Communities

A multitactic research approach that includes methods of cultivation and physiological characterization, along with recently developed molecular-based techniques, is being used more frequently to address the complexities associated with microbial ecological studies. There is intensive interest in identifying the microbial species in true association with seeds, particularly those species that are key to initiating seed decay processes. In addition to ascertaining soil-borne relationships, similar approaches can be used to study seed-borne associations. Once the associated microbial populations are identified, there is a need to determine what their functional roles are and the mechanisms by which the activities occur. A major challenge in studying soil-related seed–microorganism interactions is in accurately discerning true relationships apart from the background "noise" generally encountered whenever highly complex and diverse microbial communities are present. Experiments that examine successional colonization on seeds occurring over real time would be useful, for example, to distinguish between the species that may specialize in seed decay and other generalists or saprobic species. One important aspect will be in defining the scope of the small microbial community surveys (i.e., seed) needed to provide sufficient data to describe seed associations accurately, with the larger goal in mind to inform models that can more accurately predict the outcome of processes within the broader context of entire seed banks.

A traditional approach to identifying microorganisms is cultivation based. Attempting to use artificial media to isolate microbial agents from their natural environment presents an enormous challenge to researchers. This is primarily due to a lack of knowledge about the set of growth conditions that allow specific microorganisms to be cultivated, or the lack of ability to artificially recreate the appropriate conditions in the laboratory. Consequently, microbial species abundance and diversity are seriously underestimated in natural samples when cultivation approaches are used solely. In addition to the limitations in retrieving many environmental species, these approaches are generally tedious, time consuming, and often serendipitous in their outcome. The major advantage of success is having a defined culture or consortia with confirmed targeted abilities to examine and develop strategies that exploit these species for possible field use.

Ongoing efforts are being made in our laboratory to cultivate microorganisms from seeds, particularly those involved in seed decay. The main strategy being used employs successive transfers and enrichment of cultures originating from soil that relies on seeds to provide a major selective (nutritional or otherwise) pressure. In one specific study, a consortium of fungi and bacteria associated with giant ragweed seed decay was obtained (Figure 4B) and is currently undergoing characterization and further enrichment. Similar strategies are currently being used to isolate cultures associated with velvetleaf seeds.

Along with cultivation, various nucleic-acid–based methods and genetic sequencing are routinely used to characterize microbial community structure and to identify specific taxons. These molecular-based methods are frequently more rapid than physiological-based approaches, preclude the need for cultivation, and result in a more thorough characterization of microbial community structure (Amann et al. 2001; Anderson and Cairney 2004; Forney et al. 2004; Lord et al. 2002; Lu et al. 2005; Muyzer 1999; von Wintzingerode et al. 1997). More recently developed molecular techniques can further allow characterization of functional activity without the need to grow cells (Lueders et al. 2004; Ye et al. 2001). These techniques are usually sensitive enough for detecting populations of low numerical abundance. Following the use of molecular methods to identify critical species in the microbial community, cultivation strategies may then be designed for more effective isolation of microorganisms, as well as allow development of methods to rapidly track and account for specific species in natural environments.

One major use of molecular community analysis in our seed studies is to examine the microbial assemblages that form on seeds, and identify the corresponding bacterial or fungal taxa and their relative abundances. For example, using the small subunit ribosomal 16S RNA gene and the technique of terminal restriction fragment length polymorphism (T-RFLP) (for review, see Kitts 2001; Marsh 1999), bacterial diversity and community structure can be determined in a relatively complex system. The ability to discriminate patterns of complex bacterial communities allows comparative analyses to be made of the microbial assemblages on different seeds. To demonstrate, seeds of four different annual weed species, woolly cupgrass, jimsonweed, Pennsylvania smartweed, and velvetleaf, were exposed to the same soil-derived microbial inoculum, and the microbial communities associated with each seed was analyzed by T-

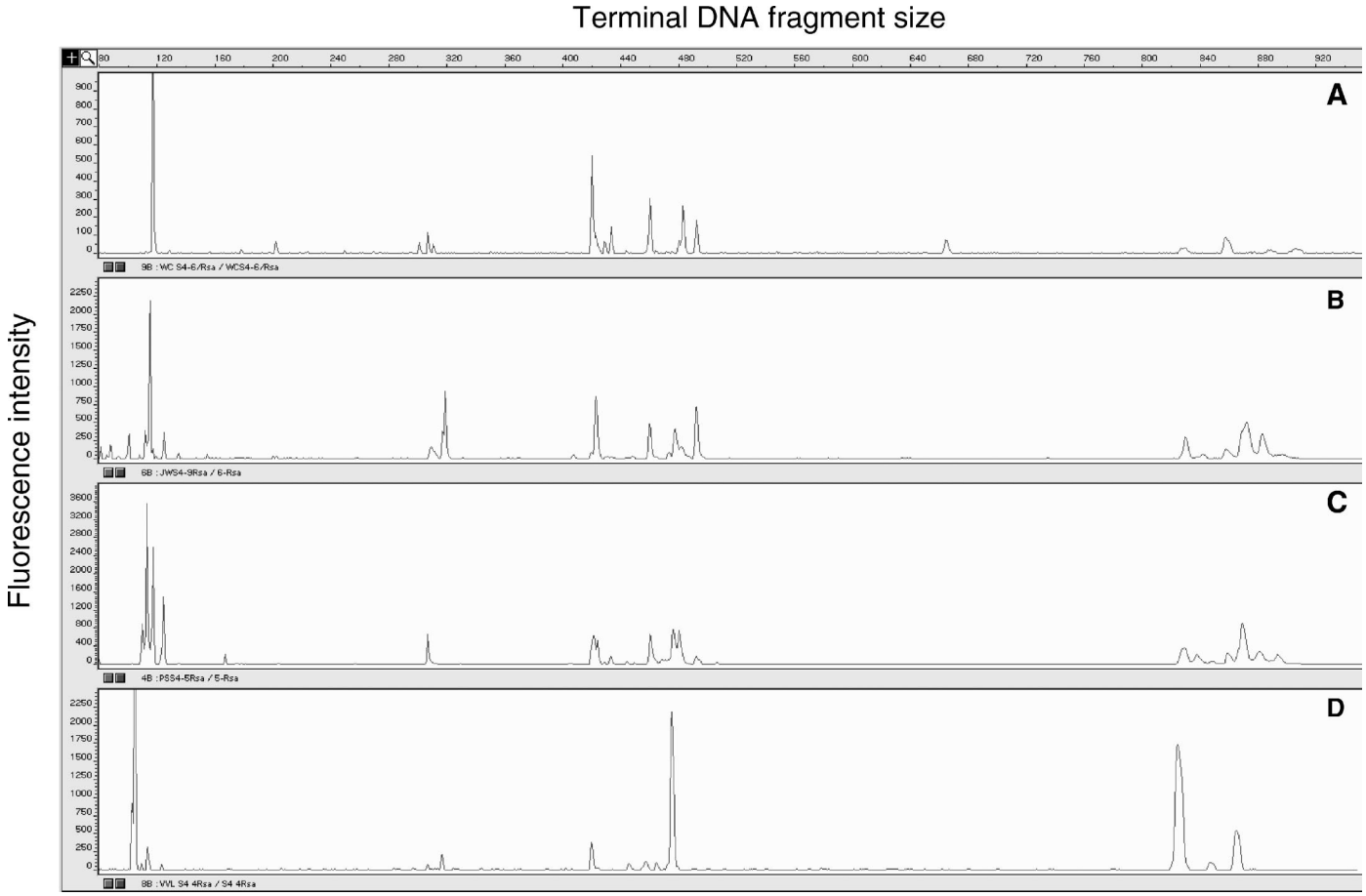

FIGURE 6. Bacterial community profiles associated with seed surfaces following colonization of microorganisms that were derived from the same soil inoculum, and analyzed with the use of T-RFLP analysis of 16S rDNA digested with *Rsa* I. (A) woolly cupgrass, (B) jimsonweed, (C) Pennsylvania smartweed, and (D) velvetleaf. Each peak is a discrete DNA fragment size that may represent a unique operational taxon (species) of bacteria.

RFLP. Different bacterial assemblages formed in correspondence to the weed species, each assemblage being represented by a characteristic set of data peaks, where each peak represents an operational taxon that may be attributed to a unique bacterial species (Figure 6A–6D). These data suggest that seeds of different weed species host distinct microbial communities, and further studies can reveal whether these associations are species specific and common to a broad range of soil. Regarding seed decay, molecular microbial community analysis will be highly useful for helping to identify key populations, because it would be expected that these species are consistently present in instances where seed decay occurred. Similar techniques can be applied to characterize associated fungal communities, and the use of more discriminating molecular probes can allow further detection of specific phyla or functional groups. Targeted genes might, for example, include group-specific phylogenetic genes of the *Basiodiomycota*, or functional genes encoding lignin- or aromatic compound degradation. Interestingly, in contrast to the frequent finding on seeds of a relatively diverse community comprised of numerous bacteria commonly found in soil, such as members of the *Proteobacteria* and *Bacteroidetes*, the fungal species found associated with seeds of specific weed species were predominantly genera members of *Ascomycota* (Chee-Sanford et al. 2004).

Because seed coats of velvetleaf and other malvaceous seeds contain cellulose and lignin (Reeves 1936), one could hypothesize the role of lignin-degrading microorganisms in seed decay processes. Genera of *Basidiomycota* such as the white rot fungus, *Phanerochaete chrysosporium*, are commonly found in soil and have been well studied for their lignin-degrading abilities (for reviews, see Martínez et al. 2005; ten Have and Teunissen 2001). To a lesser extent, ascomycetes (Lyons et al. 2003) and even bacteria (Céspedes et al. 1997) have also been associated with lignin degradation. The initiation of seed decay through the depolymerization of lignin or other complex structures would then result in ready access to structurally simpler organic compounds that could support a relatively complex microbial community. Similarly, the localized tannin-like phenolic compounds in velvetleaf seed coats that are thought to protect against microbial degradation may also serve as compounds for growth by numerous bacteria capable of metabolizing a wide range of aromatic organic compounds under a variety of environmental redox conditions (for reviews see Diaz 2004; Gibson and Harwood 2002; van der Meer et al. 1992). Chemotactic responses of bacteria to aromatic compounds have also been reported (Shingler 2003) and may have interesting implications for mechanisms involving seed-related attractants in the regulation of bacterial (or fungal) interactions with seeds. In the context of seed decay, one might hypothesize, for example, the role of certain bacteria or fungi in initiating the decomposition of seed coats or other structural barriers surrounding seeds, and the consequent niche development

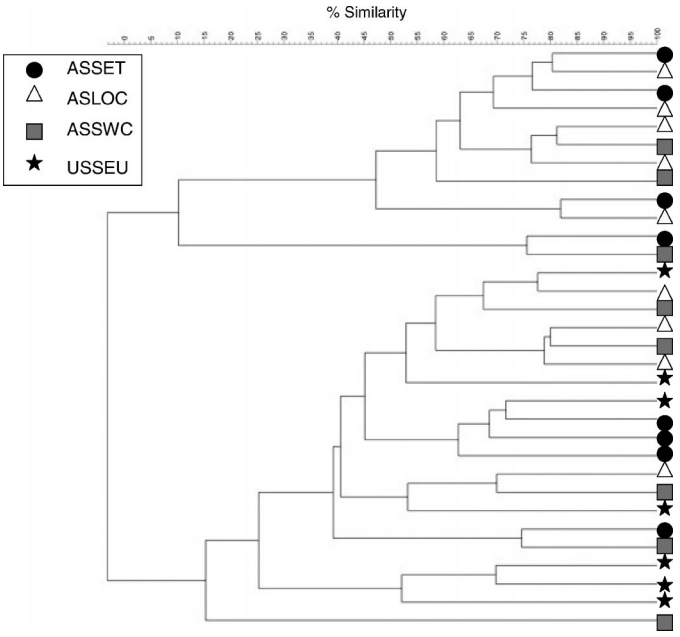

FIGURE 7. Cluster analysis of bacterial communities associated with decaying seeds of velvetleaf. Each symbol at the end of a branch corresponds to a terminal restriction fragment length polymorphism (T-RLFP) profile from a seed following exposure to one of four soil-derived inocula (ASSET, ASLOC, ASSWC, USSEU). The soils were obtained locally from sites around Champaign and Urbana, Illinois and were all silty clay loams (comprised of approximately 20% sand, 50% silt, 30%clay, organic matter content 4–7%, pH 6.1–6.2) typical of the region. Seven to nine replicate seeds corresponding to each soil inoculum were analyzed. The similarities were calculated with the use of the Dice correlation coefficient, and the best tree was drawn with the unweighted pair group method with arithmetic averages (UPGMA).

for assemblages of other bacteria and fungi. This outcome is consistent with our frequent findings of mixed bacterial and fungal communities associated with seeds. The intertwined roles of fungi and bacteria and their coexistence in competitive and mutualistic relationships have only recently been explored (de Boer et al. 2005).

The useful information derived from microbial community analysis is further demonstrated in studies focused on seeds of velvetleaf, which consistently undergo extensive decay in laboratory studies. Analysis of similarity was made between the bacterial assemblages (T-RFLP profiles) that were associated with decaying seeds of velvetleaf following incubation with one of four different soil inocula, using replicate sets of seeds. A statistical cluster analysis of T-RFLP community profiles demonstrated that not only did the bacterial assemblages on individual seeds vary with respect to the different soil-derived bacterial inoculum used, but there were significant variations in communities even on replicate seeds identically exposed to same single inoculum (Figure 7). These data suggest the hypothesis that different bacterial assemblages can fill a similar functional niche (defined here as velvetleaf seed decay) and may likely reflect the high diversity and functional redundancy among many bacterial species in soil. This further suggests that the potential for velvetleaf seed decay may indeed be widely distributed in many soils, which so far has been supported by limited data sets in our laboratory studies. Testing this hypothesis with velvetleaf and other species will require more extensive sampling along with further studies that include a broad range of soil and investigation into the characteristics of the key microbial species that are involved.

Conclusions

The symposium on seed-bank dynamics on which the series of articles in this issue is based explored possibilities for managing weed seed banks, with this article addressing specifically the potential role of microorganisms. Evidence supports varying susceptibilities of weed species to seed decay processes. For seeds of species like velvetleaf, the potential for decay is high, and this activity may be widely distributed in soil environments, in contrast to seeds of giant ragweed, which support dense microbial assemblages, but resulting decay is limited. Seeds can influence the growth of soil microorganisms by providing opportunities for nutrition and surface attachment, with consequent effects on the localized microbial community structure. Speculations on the specific factors that influence the susceptibility of seeds to undergo initial stages of decay include the role of microorganisms in seed protection. Besides the intensive focus described here on the microbiology of seed-bank systems, there also emerges a clear need for new investigations on characteristics pertaining directly to seed biology that may be intrinsically important to mechanisms of seed–microorganism interactions and the seed-bank dynamics of certain weed species.

Traditional biological control has been defined in broad terms as the use or management of biological agents to regulate pest populations and their effects (Lewis et al. 1997; Quimby Jr. et al. 2003). Previous research on the use of microbial agents to control weeds has primarily focused on known fungal plant pathogens (Chandramohan et al. 2002), where a number of formulations have been developed, but few reports of their broad success. In contrast to classical and augmentative approaches to biological control where microbial agents are released into a target environment, less attention has been paid toward a conservation approach, which entails the promotion of natural enemies and their function through habitat or cultural management. Renewed interest in examining the use of biological control for weed management, and more specifically, seed-bank management, relies on recognizing that a more comprehensive ecological understanding of the total system is needed (for review of concept, see Lewis et al. 1997). In keeping with the similar notion of a microbiological-based conservation approach to manage certain weed species, the development of useful microbial agents is only one potential aspect of the main study objective. Although the discovery of useful microbial cultures from soil is important, their application does not necessarily reside in their development as a bioherbicide. As soil residents, the microbial cultures anticipated here are presumed to be well-adapted native participants in the biotic interactions that take place naturally in their environment. Exploiting these useful interactions to promote antagonism against seeds may take the form, rather, of soil management or augmentation methods that enhance the distribution and activities of specific microorganisms of interest.

We can presently only speculate on why certain seeds are more susceptible to microbial-mediated decay than others. It is likely that the interactions between seeds and microorganisms are driven by multiple (biotic or abiotic) factors,

and involve both the characteristics of the seed and the microbial populations present. Our data suggests close physical associations can occur, and depending on the species present, the outcome may or may not be antagonistic to the seed. We might even speculate that, as with most higher organisms, seeds commonly interact with microorganisms, with perhaps most relationships being neutral or beneficial to the seed, microorganism, or to both. The associated microbial assemblages are dependent on the extant microbial community to which the seeds are exposed and their competitive relationships with one other. Within this community are microbial species whose activities are dependent on the conditions of the environment, including those conditions defined by the seed zone. In turn, the characteristics of the seed, such as their physical structure and composition (especially seed coats and other outer seed integuments), dormancy status, maternal effects on seed development, seed exudates, and possibly a host of other features, are likely to influence microbial colonization. One might also speculate that mutual adaptation between microorganisms and resident weed species is important in determining the outcome of seed-microorganism relationships.

Seeds of many annual weeds persist for years in soils, indicating the presence of factors that regulate seed antagonism by soil microorganisms. The nature of these inhibiting factors is not yet known and may involve a combination of biotic and abiotic factors. A fundamental understanding of the specific interactions that can occur between seeds and microorganisms is needed, but there is also a need to understand the underlying mechanisms that control microbial communities in soil in general, with important implications for future weed managements targeting seed banks. Although the role of soil microorganism in weed management systems has yet to be defined, there is promising potential emerging at these early stages of research. Major challenges in the design of multiscale (spatial- and trophic-level) experiments and limitations in methodology are being recognized, and as these are addressed, more meaningful links between fundamental biological processes and application to the larger dimension can be made.

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