DO MICROBES INFLUENCE SEEDBANK DYNAMICS?

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The abundance and persistence of seeds of many annual weed species in soil offer a variety of potential interactions to occur between seeds and native soil microorganisms. These interactions may include mechanisms of neutralism, mutualism, or antagonism, where combinations of various biotic and abiotic factors could influence both the development and outcome of these relationships. Generally, microbial interactions with seeds have not been considered within the context of ecological relationships involving plants and the surrounding multi-trophic community. Soil microorganisms (bacteria and fungi) are known for their importance in processes such as nutrient and mineral cycling, residue turnover, xenobiotic degradation, and plant health and development. A number of microbial species involved in these processes have been well-studied, however, it is estimated that 95-99% of all microbial species that are present in the environment remain uncharacterized, with their corresponding ecological functions unknown. Much of the challenge in studying microbial communities in natural environments has been due to the lack of suitable methods available to examine microorganisms at relevant scales of measure. Previous studies that investigated in particular, microbial associations with seeds, relied on cultivation-based methods that often selected for cultivatable species with faster rates of growth or those present in higher abundance. This approach frequently missed important species and underestimated the diversity of microorganisms that were present, but did allow limited population sets to be identified and examined for their physiological function. More contemporary molecular-based methods have been developed in recent years to circumvent the limitations of cultivation bias, and provide some useful and more accurate tools to investigate important microbe-seed relationships that may be missed using traditional methods. To address the question of whether microbes influence weed seedbank dynamics, we need a more thorough characterization of the microorganisms involved in seed interactions, and to understand the fundamental factors and mechanisms that regulate and drive seed-related microbial processes in soil. Further, we need to consider seed-microbe interactions as mutually dynamic processes, thus there is also a need to investigate any intrinsic qualities possessed by seeds that may also significantly influence these relationships.

Microbial-mediated seed decay has received recent attention as a mechanism of interest that may be exploited and aimed at promoting depletion of weed seedbanks. Current studies in our lab are looking at aspects of seed decay. For example, we recently reported studies conducted to determine the potential extent of seed decay under conditions where seeds provided the major source of carbon nutrition for microbial populations. As a continuing working hypothesis, seeds provide rich carbon nutritional resources for the general extant soil microbial community, available for cell metabolic use under the following conditions: 1) if other essential nutrient limitations are absent, 2) if key microbial species are present with enzymatic capabilities that enable access to seed components, and 3) if intrinsic mechanisms of seed protection, whether present, are overcome. We generally observed a range of seed decay following exposure to soil microorganisms, with up to 99% of velvetleaf seeds undergoing decay under conditions of both nutrient selection and non-selection, and significantly more recalcitrance (≤10%) in seeds of giant ragweed, common ragweed, Pennsylvania smartweed, shattercane, wild buckwheat, wild oats, wooley cupgrass, and jimsonweed. Using molecular microbial analyses, we found the dominant bacteria identified in association with velvetleaf and other weed seeds were related to species common in soil, including predominant species belonging to the major phyla *Bacteroidetes* and *Proteobacteria*, many of which have key characteristics of surface attachment and polymer degradation abilities. Further, regardless of soil inoculum, decay of velvetleaf seeds was consistently extensive, but microbial communities varied on individual seeds, suggesting that different assemblages can fill a similar functional niche (seed decay). High resolution microscopic analysis has

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also been a critical tool in providing direct visual examination of microbial community assembly and seeds during the decay process, as well as seeing the spatial topography of cellular attachment and growth related to the biochemical and structural complexities of seed surfaces. For example, we can observe dense microbial biofilms even on non-decayed seeds of velvetleaf, suggesting a seed-derived source of nutrition is provided for some microbes in the absence of any obvious detriment to the seed. While our studies support a high potential for velvetleaf seed decay to occur in soil, extensive seed longevity and survival is known to occur for this weed species in natural seedbanks, suggesting that factors, as yet undefined, are present in soil that control and regulate microbial seed decay processes. Fungi are also considered in microbial associations with seeds. While there are no apparent specific relationships yet emerging between species of bacteria and seeds, we observed contrasting results with soil fungal populations. A predominance of members of the major fungal phylum *Ascomycota* have been found on seed surfaces of velvetleaf, wooley cupgrass, and Pennsylvania smartweed following microbial exposure, and these were specific relationships regardless of soil origin and the presence of diverse bacterial communities also on the seed surfaces. Species closely related to members of the genera *Chaetomium* and *Cephaliophora*, were found predominant on wooley cupgrass and velvetleaf seeds, respectively, with well-characterized species of these fungi known to occur in soil. Seeds of Pennsylvania smartweed were dominated by close relatives of *Cordyceps sinensis*, a species known for its production of bioactive substances and primarily found as an entomoparasite in other studies. The specific role of these fungi in weed seed associations is unknown and is presently being further investigated.

The types of studies we are currently conducting investigate the fundamental interactions between native soil microorganisms and weed seeds. Knowledge of the presence, identity, distribution, and relative abundance of important microbes and the activities that can affect seed fate is needed, along with understanding the consequent effects on plant development and weed demography. We also have initiated studies to examine the role of seed architecture and production of exudates on microbial populations and community assembly on seed surfaces. An ongoing effort is also being made using parallel molecular and cultivation strategies to identify key microbial species that specifically initiate seed decay processes. By understanding the mechanisms of action between seeds and naturally occurring soil microorganisms, and how these actions are affected by changes in their environment, we may be able to more accurately predict effects on plant development or the extent of seed loss that can be attributed to microbial activity across a broad range of natural soil systems. As our understanding increases, we can evaluate the potential for enhancing microbial processes that target desireable effects on seeds, and as a long term goal, develop practical strategies for seedbank modification as a component of biological-based weed management.

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