

PLANTS AND MICROBES TOGETHER – A SUMMARY OF OLD HERBORN UNIVERSITY SEMINAR 33

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INTRODUCTION – PLANT AS HOST

During the past 3-plus decades of the **Old Herborn University Seminar**, prior seminars focused on mammalian and vertebrate animals as hosts for microbial communities with resident microbiomes affecting healthy physiology and disease states. In this **Old Herborn University Seminar 33 (OHUS 33)**, we explored the Kingdom Plantae as hosts in order to provide a broader perspective on microbial ecology, microbiome science and environmental health. This seminar focused on land plants with elaborate roots in soil below ground and complex above-ground architecture. This edition did not include any detailed examination of aquatic plants, algae, or more ancient plant types.

Land plants first appeared approximately 500 million years ago and created new “homes” for many environmental microbes. Terrestrial ecosystems have rich soil environments that provide a fertile substrate for microbial proliferation and development of intricate microbial communities. Elaborate rhizobial (root) systems embedded in soil provide a large surface area of direct interactions between soil microbes and plants. The intricate plant anatomy extending from roots to trunks and stems to leaves and flowers permit investigations of microbial communities in different plant habitats. Consideration of microbiomes in pollen and seeds provide opportunities to explore horizontal and vertical transmission of

microbes between plants. Beyond identification of specific microbes, microbial metabolites provide signals that deliver insights into microbe:host and microbe:environment communication patterns. Transmission of signals above ground via volatile organic compounds (VOCs) and below ground in soils surrounding root structures provide obvious opportunities to understand communication between plant and microbial species within intact ecosystems. Such lessons may provide clues about optimal strategies for promoting biodiversity and considerations about plants as food for humans and as sources of medicinal compounds.

Volker Rusch started the Seminar with a compelling history of plant:microbiome science and general concepts for Seminar 33. Plant-associated microbial habitats include the gemmisphere (buds), anthosphere (flowers), carposphere (fruit), and phyllosphere (leaves) above the ground surface. Below-ground, intra-soil habitats include the rhizosphere (soil adjacent to roots) and rhizoplane (root surfaces). Combined with other plant-associated habitats, one can readily appreciate that, like animals, plants accommodate diverse microbial communities that may differ in terms of composition and function based on anatomic location. As an example, the plant pathogen *Pseudomonas syringae* pv. *glycinea* colonizes soybean plant buds and can be found in healthy seedlings and

healthy parts of diseased seedlings. The question arises as to whether and how plants develop resistance to microorganisms and the contributions of plant-associated microbial communities to disease resistance. As new concepts have emerged, the view of the land

plant as a holobiont with a complex anatomy and a multitude of plant:microbial interactions provide a fresh perspective on plant ecosystems and implications for human and environmental health.

FOUNDATIONS OF THE PLANT MICROBIOME BELOW GROUND: THE SOIL AND THE ROOTS

Some people talk about “grass roots” efforts as a strategy to galvanize popular support for human initiatives. In terms of plant microbiome science, the 33rd OHUS began with a detailed examination of plant roots (not grass) and the soil. *Roeland Berendsen* from Utrecht (**Chapter I**) explored the concept of soil-borne legacies and how soil-borne microbial communities promote plant health in successive generations (*Bakker et al., 2018*). Plant pathogens infecting leaves (above ground) or seedlings may alter microbial composition in the rhizosphere, and consortia of soil bacterial species following this “cry for help” may alter microbial composition in the rhizosphere or areas of soil adjacent to plant roots. Experiments by *Roeland Berendsen* and colleagues were performed with sugar beet seedlings, and specific soil microbes were identified that, when combined, were able to confer disease-suppression in subsequent plant generations. These concepts of induced systemic resistance (ISR) and disease-suppressive soils highlights the ability of plant-associated microbial communities to profoundly affect plant health and vertically transmit disease resistance.

An intriguing comparison of a land plant root system with the mammalian intestine emphasizes common features such as nutrient/water absorption, nutrient trafficking, and the presence of

rich, complex microbial communities adjacent to eukaryotic host cells. Colonization resistance in the mammalian gastrointestinal tract refers to the abilities of health-associated, diverse gut microbiomes to resist penetration and infection by enteric pathogens in animals. Soil microbes in the rhizosphere and rhizoplane may similarly suppress proliferation and colonization on root surfaces (rhizoplane) by plant pathogens, effectively resulting in patterns of “colonization resistance” in soil. The intestinal microbiome may have a profound impact on mammalian host immunity by conferring signals stimulating development and maturation of innate and adaptive immunity. Similarly, the soil microbiome may confer ISR by providing metabolites as signals stimulating plant cells to release key antimicrobial compounds such as coumarins, glucosinolates, and terpenoids. *Margot Schulz* (**Chapter III**) eloquently described these and other classes of plant microbial metabolites that may be modified by plant microbes. Compounds such as coumarins (p-coumaric acid) may suppress soil bacteria while enhancing soil fungal populations. These same compounds may suppress inflammation and stimulate antioxidant activity in mammals.

Andrzej Tkacz (**Chapter II**) described techniques to explore microbial counts and total microbial load in plants and the environment, instead of

simply relying on ratios or percent of total reads. By applying quantitative PCR methodologies, the rhizosphere contains vastly richer microbial communities than the bulk soil and rhizoplane. Additional insights include findings demonstrating that Proteobacteria (Gram-negative bacteria) are dominant in soil environments. Plant scientists are continuing to amass data regarding the complexity, composition and function of microbial communities in different plant tissues and soil locations. Soil associated with richer plant growth yielded greater total microbial counts than soil associated with poorer plant growth. “Rich” soil yielded greater quantities of bacteria and may explain the relative resistance to plant pathogen colonization and greater nutrient bioavailability from “plant-friendly” microbes in fertile ground. In *Margot Schulz*’ presentation (**Chapter III**), we confronted the relative abundances of soil bacterial and fungal populations. Notably, the vast majority of plant pathogens and infectious disease phytopathology are caused by fungi. So, it is important to consider the

relative impact of soil-resident microbes and microbial metabolites on soil fungal communities. *Margot Schulz* described the relative diminution of fungal and Gram-negative bacterial pathogens in soils inoculated with rapeseed extract. Rapeseed extract containing goitrin compounds derived from plants may have a dramatic impact on compositional shifts in soil microbial communities. Interestingly, rapeseed extract inoculations resulted in relative population shifts from a predominance of ascomycota (leading source of plant pathogens) to basidiomycota in inoculated soils. Perhaps plant metabolites undergo biochemical conversions to secondary metabolites by plant- or soil-associated microbes (progoitrin to goitrin and derivative metabolites) and modulate changes in microbial communities that benefit or compromise plant survival. One can easily appreciate the potential impact on plant communities as a result of more fertile soil ecosystems, and the deleterious impact on rival plant species by resisting invasion of resident ecosystems.

MICROBIAL METABOLITES – CONVERSATIONS BETWEEN PLANTS AND MICROBES

Margot Schulz (**Chapter III**) eloquently described a variety of plant-associated and microbial metabolites in plant ecosystems and emphasized that land plants produce a plethora of secondary metabolites in collaboration with plant-associated microbial communities. By modifying “older” primary metabolites through evolving enzymatic bioconversions, secondary metabolites with new biochemical functions were generated during millions of years of plant evolution. Such biochemical diversification by plant-microbiome collaboration provided ample opportunities for coevolution

with significant impacts on the development of different ecosystems containing land plants. Accumulation of specific secondary metabolites represented a later step in the evolution of land plants since their initial appearance approximately 500 million years ago. Importantly, **Seminar 33** invited parallel discussions and consideration of similar phenomena in the development of terrestrial microbiomes with their resident animal-associated microbial communities and microbial metabolites.

Various classes of plant-associated metabolites were described in **OHUS**

33, and these compounds (and classes) included coumarins (p-coumaric acid, scopoletin), salicylates (salicylic acid), glucosinolates, benzoxazinoids, terpenoids and goitrins. Coumarins or simple phenolic compounds such as p-coumaric acid in soil diminished the relative abundance of soil bacteria while simultaneously enhancing relative abundances of soil fungi. Thus, these secondary metabolites may yield dramatic shifts in soil microbial community composition and function with important consequences for plant communities. Interestingly, the same compound (p-coumaric acid) enhances the proliferation of *Lactobacillus plantarum* in the mammalian intestine. Similar metabolites can affect both land plant- and animal-associated microbial communities. Via at least two enzymatic conversions, p-coumaric acid can be converted to the secondary microbial metabolite, protocatechuic acid, and this compound has general antioxidant properties as well as serving as a precursor compound for the TCA cycle (aerobic respiration). *Roeland Berendsen (Chapter I)* told the story of another coumarin compound, scopoletin, produced in root exudates by plants as a response to iron deficiency. Scopoletin enriched in root exudates then plays a key role in soil iron mobilization and enhancement of proliferation of beneficial microbes fostering induced systemic disease resistance (ISR) in plants (*Stringlis et al., 2018*).

Another fascinating class of metabolites are the glucosinolates that are produced by various plants including the class of vegetable sources known as *Brassica* plants. *Brassica* plants include human food plants such as broccoli, cauliflower, cabbage and turnip, in addition to the seed plants yielding mustard and canola cooking oil. These plants yield elevated concentrations of

glucosinolates (thought to have originated approximately 90 million years ago) which can be converted to isothiocyanate (ITC) compounds. These metabolites can be toxic to plant-associated microbes, especially fungi, and these metabolites may protect such plants due to the prominence of fungal phytopathogens. Glucosinolates are powerful microbiome modulating agents and have been extensively used for biofumigation purposes targeting weeds and phytopathogens. Extracts with elevated concentrations of glucosinolates such as rapeseed extract suppress soil mycorrhizal fungi and Gram-negative bacteria, but the microbial survivors seem to enhance growth of other plants such as *Arabidopsis thaliana*. Perhaps the data shared by *Margot Schulz (Chapter III)* provide clues regarding microbial metabolites and pathways for suppressing infection and promoting beneficial microbes in soil environments.

Benzoxazinoids are indole-derived plant metabolites enriched in root exudates of *Poaceae* species such as maize, rye and wheat. These compounds are converted to benzoxazinones such as DIMBOA (maize) and DIBOA (rye), and these metabolites, in turn, can be converted to soil-toxic compounds, MBOA and BOA. MBOA can suppress soil microbes when administered directly, and soil microbes may detoxify the soil environment by providing microbial enzymes adept at covalently modifying these compounds. Various soil microbes may detoxify soil by converting benzoxazinones to less toxic or inert compounds via hydroxylation, glucosylation and nitration pathways. These findings emphasize the potentially beneficial role of plant-associated and soil microbes in detoxifying and revitalizing land to support healthy plant ecosystems and sustainable agriculture.

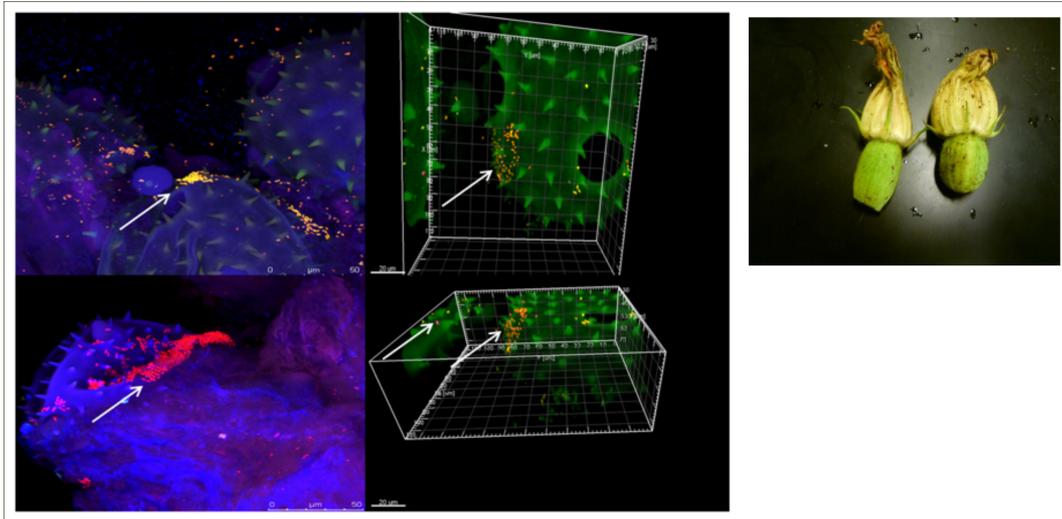


Figure 1: The oilseed pumpkin microbiome. Bacteria on the pollen grains of the pistil of withered female oilseed pumpkin flower by CLSM and FISH (credit to G. Berg and E. Adam, Graz, Austria).

MICROBES AND PLANTS ABOVE GROUND: LEAVES, FLOWERS, POLLEN (AND SEED)

OHUS 33 considered the foliar microbiome (phyllosphere), interactions between the leaf and root microbiomes, and compositional and functional aspects of plant microbiomes above ground. Microbes can travel within plant hosts, and microbial colonization in one anatomic region (roots) may affect microbial composition remotely in plant parts that are above ground level (leaves). Such colonization patterns may be secondary to microbial proliferation and motility of microbial cells within plants. This microbe-associated communication pattern is bidirectional from root to leaf and from leaf to root. Foliar colonization and establishment of plant leaf-associated microbial communities (phyllosphere) affect the root biology by modulating root exudates in the soil (metabolite production) and the composition of root-associated microbial communities (rhizosphere).

Gabrielle Berg (Chapter V) followed discussions initiated by *Volker*

Rusch of land plant biology above the ground surface by addressing plant microbial communities in leaves (phyllosphere) and flowers (anthosphere). The lettuce plant with its large leaves provided an elegant model system for exploring foliar colonization by plant-associated microbes. The lettuce microbiome demonstrated the existence of complex foliar microbial communities and the differences in microbial composition between different lettuce cultivars. A salient feature of the lettuce microbiome was the relatively greater abundance of Gram-negative bacterial species (Proteobacteria) in the lettuce phyllosphere when compared to the lettuce rhizosphere. The “resistome” of the lettuce leaf microbiome was another prominent feature among leaf-associated bacteria defending their niche against microbial competitors. Keeping in mind that many antimicrobial agents were originally derived from environmental fungi and bacteria (e.g. *Streptomyces*), it is not surprising

to see such an elaborate resistome contributing to successful colonization of plants by different bacterial species. From the lettuce to pumpkin, *Gabrielle Berg* transitioned to discussions of plant microbiomes in seeds, flowers and fruit. The pumpkin seed microbiome also showed antimicrobial resistance features that may be crucial in shaping the pumpkin seed and fruit microbiome. Consideration of seed microbiomes in plants raises the issue regarding the roles of microbes in vertical transmission between plant generations. *Gabrielle Berg* showed that both pumpkin flower and seed-associated microbes form structured communities as displayed via confocal laser scanning microscopy (CLSM) and fluorescence *in situ* hybridization (FISH) (Figure 1). The seed microbiome provides an explanation for vertical transmission of plant-associated microbes and possible enhancement of plant germination. Published findings regarding the existence of a placental microbiome in mammals including humans (*Aagaard et al., 2014; Seferovic et al., 2019*) highlights potentially common themes of vertical transmission of health-promoting microbes among land

plants and terrestrial animals. *Gabrielle Berg* commented on recent efforts in the agricultural industry to fortify plant seeds via inoculation of plant microbes, and new insights about vertical transmission of microbiomes may provide opportunities to create healthier, disease-resistant crops.

Sylvia Schnell (Chapter VI) expanded the consideration of horizontal and vertical transmission of plant-associated microbes by describing microbial communities in plant pollen. By sharing molecular and microscopic data (CLSM and FISH), bacterial communities can be easily visualized on pollen from plants such as rye, birch and rape-seed. Such pollen can transmit plant-associated microbes between plants and provide a mechanism for horizontal transfer of microbes between flowers. Specific bacterial genera, *Pseudomonas* and *Rosenbergiella*, and fungal genera, *Cladosporium* and *Aureobasidium*, are prominent members of the core pollen microbiome across different plant species. The implications for human health are readily apparent with a more highly diverse, richer pollen microbiome in plants associated with human respiratory allergies.

PLANT MICROBIOLOGY AND HUMAN HEALTH: ONE HEALTH ON ONE PLANET

The **Old Herborn University Seminar 33** provided a timely forum to consider the plant kingdom and its associated microbial partners. Concepts such as vertical metagenomic transmission and the beneficial contributions of microbial metabolism can help expand our understanding of the holobiont in both botany and zoology. The holobiont (*Sanchez-Canizares et al., 2017*) represents combinations of plant (or animal) and microbial cells that constitute intact multicellular organisms. By probing plant biology and various

plant-associated microbial habitats, we have attained a deeper comprehension of beneficial microbes, microbial pathogens, disease resistance, microbial metabolites and biochemistry.

One important topic left to the end of this summary is that of human-plant interactions and the possible roles of plant-associated microbes in such plant-mammal interspecies relationships. *Gabrielle Berg* described findings related to plants in built environments occupied by humans, and how plants in indoor environments may

promote microbial biodiversity. Such indoor biodiversity may enhance the biodiversity of the human microbiome on skin, in the respiratory tract, the oral cavity or the gastrointestinal tract. Clearly plant microbiomes in human food plants such as pumpkins and members of the *Brassica* family provide a direct connection between microbiota on plants (vegetables and fruits) and the human oral cavity and gastrointestinal tract (*Wassermann et al., 2019*). Although direct relationships between plant-based human nutrition, the human microbiome and human health were not major topics of this Seminar, such topics are ripe for discussion and considerations in future studies.

Max Moehs (Chapter IV) described chemical mutagenesis strategies impacting plant genetics that could yield significant results for human health and disease susceptibility. By using TILLING, mutants of grain crops such as wheat or barley could be generated that alter seed protein profiles. Since wheat and barley proteins such as gliadins and low molecular weight (lmw) glutenins are strong triggers for coeliac disease in humans, mutants with reduced gliadin and/or lmw glutenin content could serve as more “human-friendly” grain sources. Such grain crops could reduce the coeliac disease burden in human populations and may also positively affect the epidemiology of disorders such as gluten sensitivity and wheat allergy as well. *Max Moehs* described experiments with mutant barley (reduced gluten content) in a non-human primate coeliac disease model (*Mohan et al., 2016*). Findings showed that small intestinal pathology was ameliorated with the mutant barley-based diet, and detectable changes in the composition of the primate intestinal microbiome were also documented following gluten consumption. Clearly

alterations in plant proteins yielded effects on human pathology and such changes in diet may modulate the composition of the human microbiome. *Sylvia Schnell* showed us that that elevated plant pollen-associated microbial diversity was associated with more highly allergenic plant species such as birch, rye and mugwort. Plant microbiome proteins may have a direct impact on the susceptibility of humans to respiratory allergies.

Plants and plant-associated microbes clearly benefit human health by providing nutritious food sources and by promoting planetary biodiversity (*Hacquard et al., 2015*). Many studies have demonstrated that human health is associated with microbial biodiversity and that human disease states often are associated with less diverse microbial communities. *Margot Schulz (Chapter III)* mentioned that human nitrates released into the ambient air through exhalation may benefit plant leaf growth by providing nitrogen sources via volatile organic compounds (VOCs). Perhaps humans and plants can benefit each other by sharing microbial metabolites. Plants and humans can benefit each other, and conversely harm each other. As we have discussed, plants may generate toxic metabolites and may contribute to immune-mediated diseases in humans such as coeliac disease and respiratory allergies. Plants may serve as vehicles for food-borne disease outbreaks. Humans can play a major role in destroying plant habitats and harming or reducing plant-associated biodiversity. The final lesson of **Seminar 33** may be that we must explore nature and seek to arrive at a deeper understanding of plant and animal ecosystems to save our planet. As *Gabrielle Berg* persuasively stated, we should strive for One Health on One Planet with One Future.

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