Endophytes: sometimes friend, sometimes foe, always present in the background

Fluidity in plant-endophyte interactions

Elske Tielens

Introduction

Endophytes are microorganisms that inhabit living plant tissues without causing symptoms of disease. Research suggests that all plants have symbiotic relationships with endophytes, with many plants containing a community of species (Aly, Debbab, & Proksch, 2011; Barrow, Lucero, Reyes-Vera, & Havstad, 2008; Faeth, 2002; Johri, 2006). It is hypothesized that endophytes have major impacts on above- and below-ground interactions and processes on multiple trophic levels (Barrow et al., 2008; Omacini, Semmartin, Pérez, & Gundel, 2011). Estimates of microbe diversity range around the million species, however precise numbers are unknown due to the difficulty of culturing these microbes. They are found in every plant organ; in seeds and leaves as well as in roots. Endophytes can be transmitted both by growing within seeds (vertically) and via spores (horizontally). They can be tightly host-associated specialists as well as generalists infecting several species, sometimes taxa-wide. Although little is certain about endophytes it is clear they are very diverse both in host (tissue) they inhabit and in life form.

Symbiosis is widely accepted to be common in the natural world. Organelles such as plastids and mitochondria have their evolutionary origin as free-living organisms before entering symbiosis and later becoming an integral part of plant physiology. This indicates that plants do not function as autonomous units but form a complex together with associated microbes, endophytes but also mycorrhizae and others. Plants provide a unique ecological niche for microbes while these microbes can strongly influence plant functioning, adaptation and evolution (Barrow et al., 2008). Some researchers argue that without endophytes plants would not be able to maintain what we perceive as their normal phenotype, resulting in strongly reduced photosynthesis levels, growth, stress tolerance and survival (Partida-Martinez & Heil, 2011).

Research on endophytes has traditionally focused on agronomic grass-endophyte relationships, and specifically on two species: tall fescue (*Festuca arundinaceae*) and perennial ryegrass (*Lolium perenne*). These species are associated with *Neotyphodium* endophytes, a group of clavicipitaceous fungi. These fungi infect aboveground structures of many grasses and reproduce asexually, with high infection rates approaching 100% as well as a high invasion rate. They reproduce through hyphae growing into seeds of maternal plants, resulting in a tight link between host and endophyte fitness. Evolutionary theory predicts that due to the presence of such a strong link the *Neotyphodium*-grass symbiosis should be mutualistic (Faeth & Sullivan, 2003). In this symbiosis the endophyte produces alkaloids that provide defense against herbivory, in return receiving photosynthase from the host. More specifically, *Neotyphodium* produces mainly ergot and indole diterpene-type in *Lolium perenne*, and pyrrolizidine and ergot alkaloids in *Festuca arundinacea* (Faeth, 2002). Research on this symbiosis was driven by the multitrophic consequences of *Neotyphodium* infection of agronomical grasses. These endophyte produced alkaloids cause toxicosis, staggers, intoxication, narcosis, gangrene and other severe negative effects on livestock (Faeth, 2002), rendering research into this phenomenon highly relevant.

Plant-pathogen interactions form the other end of the plant-microbe symbiosis spectrum. Pathogenic and parasitic microbes are widespread and often occur within a plant in a community competing with each other for resources. Pathogens can be either opportunistic symbionts and capable of surviving as saprophytes, in the soil as well as *in planta*, or obligate pathogenic depending upon living hosts for survival and reproduction. The former opportunistic strategy increases chances of colonizing new areas as it releases microbes from the pressure of immediately having to find a new

host (Barrett, Kniskern, Bodenhausen, Zhang, & Bergelson, 2009). In the latter obligate case interactions have been hypothesized to be tightly linked, with host and microbe involved in an evolutionary arms race (Denison, 2000). Research on pathogens has been done mostly on specialist species, resulting in a bias for specific (often crop related) interactions.

The above mentioned plant-microbe systems are strongly coevolved plant-mutualist/pathogen interactions, with host and pathogen involved in an arms race, or host and mutualist adapting ever tighter in response to each other. Although the latter are the most extensively researched plant-endophyte relationships, with examples as legume-rhizobial or grass-endophyte associations, coevolution is not the norm (Kari Saikkonen, Wäli, Helander, & Faeth, 2004). The majority of grasses, though associated with endophytes, have been reported to lack endophyte mycotoxins that confer defense benefits to plant hosts (Faeth, 2002; Kari Saikkonen et al., 2004). To coevolve and survive as mutualism endophyte and host must match morphologically, physiologically, reproductively and in terms of life history traits. Reproductively, an endophyte must either increase its genetic diversity or decrease host genetic diversity to remain compatible, for example through increased clonal propagation or self-pollination. In order for this tight association to evolve, strong selective forces must work on the symbiosis and it must confer high fitness benefits. This is possible in agro-ecosystems, where enhancing plant defense provides a selective advantage to intensive grazing by livestock and where resources are continuously high, thus costs of symbiosis relatively low. High degrees of inbreeding in agro-ecosystems may facilitate the accumulation of alkaloids as well. However, in natural systems selection pressures are more variable rendering such an integrated symbiosis unlikely. In natural systems this relationship can be easily destabilized by conflicts in host-endophyte reproduction, enhanced host susceptibility to other microbes, energetic costs and host control of fungal growth. These ideas on the likelihood of coevolved symbioses are supported by research on natural and domesticated grasses where infected grasses only outcompete uninfected grasses under high nutrient conditions (Faeth & Sullivan, 2003). This indicates that mutualistic plant-endophyte relationships are based on mutual exploitation instead of cooperation (Kari Saikkonen et al., 2004).

Although these specific highly specialized plant-microbe interactions certainly exist in nature and are evolutionary stable, research indicates that the majority of endophytes have a higher ecological plasticity (Aly et al., 2011; Redman, Dunigan, & Rodrigues, 2001). The variety of lifestyles that microbes can occupy within plant tissues, ranging from mutualistic and commensal to parasitic or pathogenic has been termed the symbiotic continuum (K. Saikkonen, Faeth, Helander, & Sullivan, 1998). Precise details of this continuum have seldom been tested, but factors such as genotype, environment, life stage and mode of transmission are likely to influence microbe lifestyle. This essay aims to clarify the conditions under which plant-microbe interactions result in the different lifestyles, especially with regards to neutral endophytes. Plants contain many endophytes without appearing to show benefit or harm. If these are neutral endophytes, what is their role in the host? Thus the main focus of this essay is to clarify the conditions under which microbes adopt different lifestyles and the role of commensalism in this.

To discern whether endophytes are mainly actively selected mutualists or rather coincidental commensal bypassers it may be worthwhile to compare bulk soil, rhizosphere and endophyte communities. If these are each a smaller (semi-) random subset of each other this indicates a non-selective process. If these communities and the filters selecting them differ strongly this shows that endophyte uptake is a selective process, and that endophytes are seldom accidental (commensal) passengers.

This essay will first consider the endophyte source; the bulk soil and rhizosphere. It will discuss the process through which microbes enter the plant. The process of infection is similar regardless of the beneficial or harmful effect on the plant. Within the plant a microbe can reveal or develop its ramifications for plant fitness, thus the next consideration in this essay will be the microbial behavior or response *in planta*, and the factors that may influence this microbial lifestyle. Lastly this essay will discuss the selection step between endophyte community and rhizosphere, and the plant's role (active or passive) in selecting the community.

Research on endophyte lifestyles is relevant due to endophyte abundance and diversity and their importance in plant functioning. Endophytes are widespread occurring in all studied plant species, as well as in mammals, insects and other eukaryots. Endophytes living in plants can in some cases present benefits on associated plants. These advantages range from stress tolerance, reproductive or plant growth benefits, tolerance to herbivory or pathogens to changes in the rhizosphere. Endophyte presence can lead to thermotolerance, tolerance of oxidative stress and trigger production of plant hormones (Aly et al., 2011; Johri, 2006; R J Rodriguez, White, Arnold, & Redman, 2009). Besides these benefits endophytes influence plant competition, herbivory and community composition, and may thus have complex food web effects (Saari, 2010). Besides fitness benefits in current environmental circumstances, endophytes have been hypothesized to improve host plant genetic flexibility and tolerance range under changing environmental conditions. Both the added genome, which increases the available abundance of DNA, as well as the short generation time of endophytes may increase plants adaptation and genetic flexibility (Barrow et al., 2008). Thus endophyte-plant interactions are an integral part of terrestrial ecosystems, and may well become more important in the future as a result of changed environments due to global climate change. Improving the understanding of the flexibility of these interactions and their conferred benefits as well as pathogenic damage is an essential puzzle piece towards a more complete picture of plant functioning.

The rhizosphere: source of the microbe community

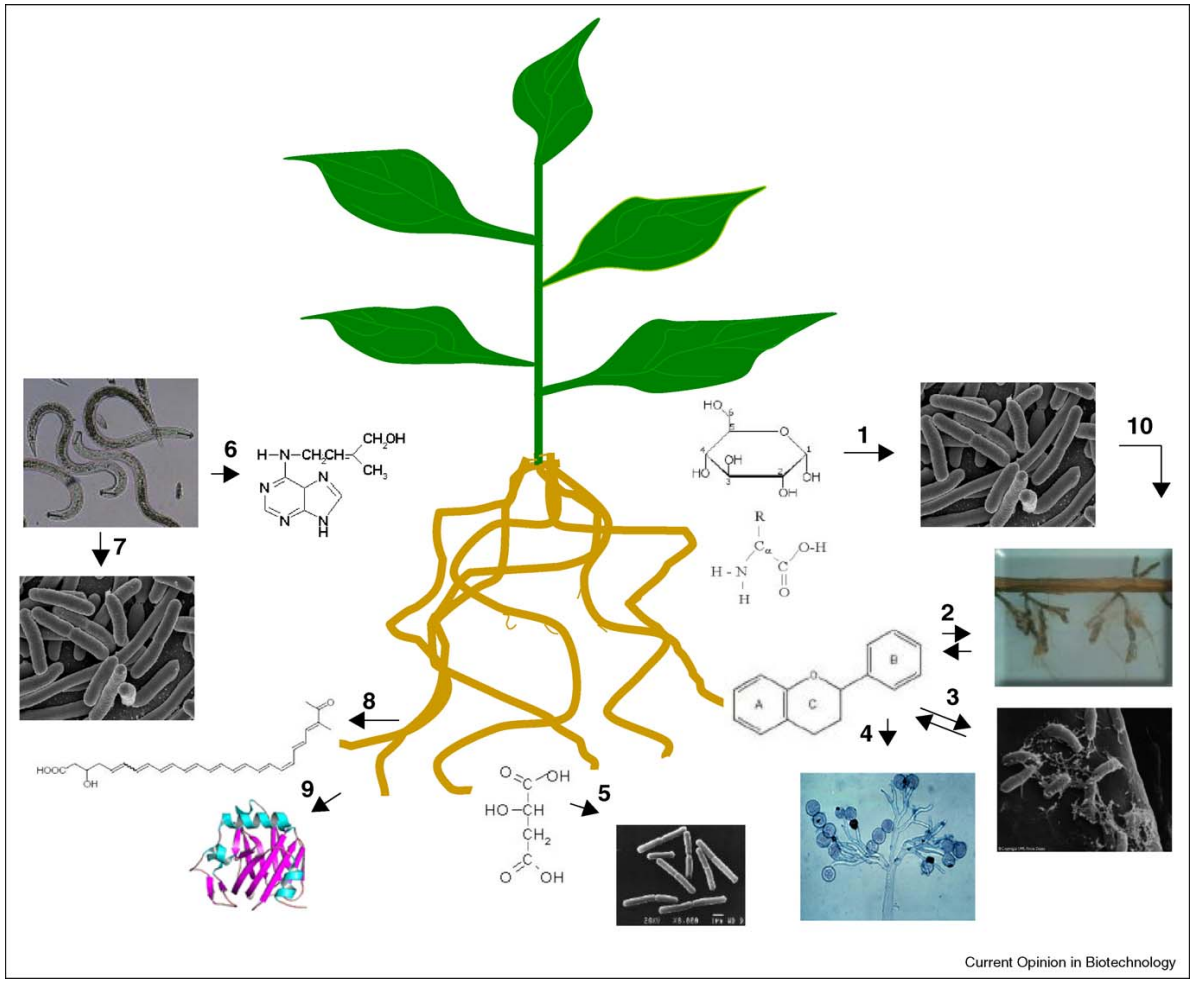
Bulk soil has been recorded to contain (in soil top layers) up to 10^9 cells per gram soil (Garbeva, Veen, & Elsas, 2004). Most of these cells are generally unculturable, which makes studying them difficult. The volume of soil surrounding plant roots and influenced by the living root differs greatly from bulk soil and is called the rhizosphere (Hiltner, 1904). Whilst organic carbon is limiting in bulk soil, in the rhizosphere nutrients are much more readily available. High nutrient content in the rhizosphere results from a number of processes: exudation of carbon compounds and specific secondary compounds, sloughing off of root cells at the root tip and root death (Hartmann, Schmid, van Tuinen, & Berg, 2009). The amount of carbon available depends on age, physiological status and environmental conditions (Miethling, Wieland, Backhaus, & Tebbe, 2000). Due to its high nutrient content the rhizosphere is a niche of high microbial activity with strong competition and even predation (Hartmann et al., 2009; Lambers, Mougel, Jaillard, & Hinsinger, 2009; Miethling et al., 2000). With such strong benefits associated with the rhizosphere there is strong selection pressure on microbes to fit the specific niche conditions (Garbeva et al., 2004; Lambers et al., 2009). Making use of resources in the rhizosphere requires adaptation to the rhizobial niche, and to do so microbes need tools for substrate acquisition, resistance mechanisms and high competition. Furthermore the plant interior is an attractive niche as well, where competition pressure is less and nutrients are even more readily available. However, persisting within plants requires well-developed resistance mechanisms and tools for plant-microbe communication (Garbeva et al., 2004).

The rhizosphere thus consists of a specific community of microbes, a subset of the microbial community in local bulk soil. To understand more about plant-microbe interactions it is interesting to ask what determines the composition of rhizosphere microbes. What conditions determine which microbes survive and colonize the rhizosphere?

Exudate composition

Literature indicates that plants are a strong selective force on rhizosphere community composition. Research on a number of species in different locations and using different methodology (cultivation-based and molecular studies) indicate that plant exudates influence microbe community structure (Garbeva et al., 2004). Plant exudates can be organic, for example carbohydrates, phenolics or amino acids, as well as inorganic, for example ion fluxes. One important group of signals in plant-microbe interactions are flavonoids, especially in communication with rhizobia (Shaw, Morris, & Hooker, 2006). Furthermore, plant exudates are highly species specific, providing an explanation for the strong host specificity of rhizosphere communities. Different microbial species respond differently to plant exudates, thus different exudate composition results in different rhizosphere communities (Garbeva et al., 2004). Some studies show that compounds exuded by plants even differ within variants of a single

species (Mazzola et al 2004, Dalmastri 1999, (Micallef, Channer, Shiaris, & Colón-Carmona, 2009)). Micallef et al. have shown that various *Arabidopsis* accessions differ in the composition of their exudates. Although this study shows accession differences in rhizosphere community and in compounds exudated, no significant correlation was found between the two. This indicates that though plant exudates and rhizosphere community are highly species specific, more information is needed on the mechanism linking the two. There are some studies showing tightly linked relations between compounds exuded by plants and taken up by microbes. An example is *Stenotrophomonas maltophilia* which requires methionine and is found mostly in the rhizosphere of cruciferous plants, which produce high levels of sulphur-containing compounds including methionine (Hartmann et al., 2009). Lastly, plant exudates are dependent on microscale environmental conditions. Calcic content of the soil influences exudate compounds such that in more acidic soil plants exudate monocarboxylic acids while plants adapted to living in more alkaline conditions exude more di- and tricarboxylic acids (Hartmann et al., 2009). Plants with phosphate deficiency often exude higher concentrations of phenolic acid, where plants in iron deficient conditions can make use of two other strategies. Depending on species or family, plants exudate either more carboxylates and phenolics or more phytosiderophores under iron-limited conditions (Hartmann et al., 2009).



Pictorial illustration of the chemical communication that exists between plant roots and other organisms in the complex rhizosphere. Plant roots secrete a wide range of compounds, among those sugars and amino acids are engaged in attracting (chemotaxis) microbes (1), flavonoids act as signaling molecules to initiate interactions with mycorrhiza (AM fungi) (2), rhizobium (3) and pathogenic fungi (oomycetes) (4), aliphatic acids (e.g. malic acid) are involved in recruiting specific plant growth promoting rhizobacteria (*Bacillus subtilis*) (5), nematodes secrete growth regulators (cytokinins) that are involved in establishing feeding sites in plant roots (6) and nematodes secrete other compounds (organic acids, amino acids and sugars) involved in attracting bacteria and in bacterial quorum sensing (7). Knowledge of the roles of other types of compounds, such as fatty acids (8) and proteins (9), secreted by roots in the rhizosphere and other multi-partite interactions (10) remains unknown.

Figure 1: Illustration showing the chemical communication between plant roots and microbes in the rhizosphere. From: (Badri, Weir, Lelie, & Vivanco, 2009)

Research on plant-driven rhizosphere community assembly

One of the factors hypothesized to 'filter' microbial community from the range of species in bulk soil to the rhizospherical subset is the genotype. Besides being species-specific, host effects have been shown to be reproducible for a given species and limited to the plants direct rhizosphere (Garbeva et al., 2004). Hartmann et al. hypothesize that microbial rhizosphere community is more than just the result of root carbon loss, it is the interplay of selective and inhibitory interactions that result in a specific rhizosphere microbial community (Hartmann et al., 2009). A large amount of research has been done identifying rhizosphere differences between plants. In a research on *Trifolium subterraneum* (clover) rhizosphere, adding 1% saponin from *Gypsophila paniculata* completely changed the microbial community. After addition of saponin the dominant microbe in the rhizosphere was *Aquaspirrillum spp*., a typical microbe associated with *G. paniculata*, while the two previously dominant microbe species decreased in abundance (Bais, Weir, Perry, Gilroy, & Vivanco, 2006; Fons, Amellal, Leyval, Saint-Martin, & Henry, 2003). This coincides with research by Narasimhan et al. (Biphenyls, Narasimhan, Basheer, Bajic, & Swarup, 2003) who found that transgenic plants engineered to secrete higher amounts of phenylpropanoid compounds directly stimulate growth of specific rhizosphere bacteria. Plants can change community composition both through positive and through negative interactions. Some possible interactions are shown in figure 1 ((Badri et al., 2009). Besides exuding carbon compounds and chemical signals, they can also secrete specific toxins, keeping some microbe species out of the rhizosphere. In the latter example, the microbial community will consist of species that are toxin tolerant or have defense mechanisms against toxins, such as efflux pumps (Hartmann et al., 2009). Sweet basil (*Ocimum basilicum*) is known to exude antimicrobial compounds against various soil-borne microorganisms, including specific opportunistic pathogens such as *Pseudomonas aeruginosa* (Bais et al., 2006). Miethling et al. compared the effects of crop species, different bulk soil communities and inoculation with an additional bacterial strain on rhizosphere community (Miethling et al., 2000). They concluded that crop species is the most significant factor determining bacterial assembly, while bulk soil origin was only a minor factor. This study confirms that a single plant species can recruit similar microbial communities from different soils (Miethling et al., 2000).

Soil type

A number of studies indicate that soil type may be important in determining bacterial species community as well. Gelsomino et al. studied rhizosphere communities in 16 different soils in different geographical locations and concluded that soil type largely determines bacterial community. Soil types studied differed in pH, available organic matter and texture (Gelsomino, Keijzer-Wolters, Cacco, & van Elsas, 1999). Da Silva et al. found similar results, concluding that soil type is more important than crop species in determining microbial community (Araújo da Silva, Salles, Seldin, & van Elsas, 2003). In their study, soils differed in texture and pH, resulting in different rhizosphere communities. Latour et al. also found that soil type, and specifically pH and $CaCO_3$ content, is the major affector of rhizosphere community, although host plant also plays a significant role. These results are supported by a number of studies with similar results (Garbeva et al., 2004; Long, Sonntag, Schmidt, & Baldwin, 2010; Micallef et al., 2009). When going into further detail on the soil characteristics that may determine community, many studies find texture to be of influence, perhaps by limiting the availability of root exudates thus influencing the community (Garbeva et al., 2004).

Studies show contradictory results on the relative importance of host plant and soil as main driver of rhizosphere community. However, one reason for this may be the lack of information about the mechanisms underlying interactions between plants and soil microbes (Garbeva et al., 2004; Hartmann et al., 2009). The importance of these factors may depend on the (a)biotic context or the relative strength of the selective pressure exercised by soil or plant (Garbeva et al., 2004). Also, rhizosphere communities can change over time. Several studies show that microbial community differs under influence of changes in plant development stage (Garbeva et al., 2004). There is evidence that microbial diversity in the rhizosphere changes over time as well (Miethling et al., 2000). It is possible that temporal factors influence the relative importance of hosts or soil type on microbial communities.

Recognition and chemotaxis

The precise mechanisms through which the microbial community locates and selects plant rhizospheres are unclear, however it seems root exudates and cues attract microbes and stimulate chemotaxis, as well as influencing flagellar motility (Bais et al., 2006; Hartmann et al., 2009). Also, some research indicates that plants can make use of quorum sensing and AHL-producing bacteria to enhance colonization (Bais et al., 2006). This can be an active or passive process, though little is known about the details. The little that has been studied about plant-microbe signaling in the rhizosphere and subsequent colonization has been studied in rhizobia. Initial plant-rhizobia signaling occurs through flavonoids. More than 4000 different flavonoids have been identified, and this specificity allows rhizobia to distinguish their host from other non-host legumes. Microbial Nod genes are induced when plant and rhizobial flavonoids match, resulting in the synthesis of Nod factor molecules; lipochitooligosaccarides. These Nod factors are very specific as well, with different sugar, acetate or sulphate groups, making these rhizobia recognizable to specific legumes. Non-rhizobial plant-microbe interactions will not include Nod factors and indeed not all microbes found in the rhizosphere may require extensive active plant-microbe interactions. Nonetheless, active selection of rhizosphere microbes by plants is likely to involve a similar pathway or cues, gene activation and recognition, resulting in chemotaxis or production of toxin or antibacteria.

Summary

Thus literature shows that microbial communities in bulk soil differ significantly from rhizosphere communities, and that the community is mostly determined by plant species and soil type. Plants both actively and passively influence microbial community; actively by secreting flavonoids and antimicrobial compounds, and passively for example through the type of carbon compounds they exudate. Thus in part specific rhizosphere communities are the result of a random match between microbial needs and plant investment and secretion, which can also result in selective pressure. Selection pressure for rhizosphere microbes is on microbes that are (genetically) compatible with plants and plant exudates, as well as being able to exist and thrive in local environmental conditions and having the tools to acquire resources in the rhizosphere and withstand high competition.

The infection and colonization process

The process of colonization or infection is species specific for both host and microbe, but this chapter will outlay the general process. Plants attract microbes, which then attach them to the emergence points of lateral roots. At this stage, communication occurs between microbe and plant mainly through means of flavenoids.

The first barrier: entering the plant

Plant defenses consist of both constitutive barriers as well as inducible defense mechanisms with the first barrier being the cell wall. Microbial entry into the plant is possible through a number of routes (Monteiro et al., 2012). One possibility is entry via plant wounds or lateral root cracks (LRC), which may be a plant-regulated process. Gough et al. (Gough et al., 1997) experimentally added flavonoids, resulting in an increase in lateral root crack colonization by *H. seropedicae* in *Arabidopsis thaliana*. Several studies have shown that its possible for various microbes to enter root xylem via LRCs (James, 2002; Monteiro et al., 2012; Schloter & Hartmann, 1998), as well as via damaged areas of roots (Gyaneshwar, James, Reddy, & Ladha, 2002). Another route of entry is by cell wall degradation. Both endophytes and pathogens have been shown to produce cell wall degrading enzymes such as cellulases and pectinases (James, 2002; Monteiro et al., 2012). However, this is not unilaterally true for all endophytes; Pedrosa et al. (Pedrosa et al., 2011) analyzed the genome of *H. seropedicae* and did not find genes coding for known cell wall degrading enzymes, and Bell et al. (Bell, Dickie, Harvey, & Chan, 1995) showed that hydrolytic enzymes occur more in soil bacteria, concluding that entry via these enzymes is unlikely for endophytic bacteria. Research indicates that results may differ even when studying the same species; even differences in strain influence the presence of cell wall degrading enzymes (Monteiro et al., 2012). Lastly, some research suggests it is

possible for bacteria to colonize a host by following rhizobium through the infection thread (Monteiro et al., 2012).

Plant defense

After entering the plant root intercellular spaces, aerenchyma and xylem are colonized, later followed by foliage and shoots. Within the plant endophytes anchor themselves through exopolysaccharides (EPS). In large amounts this can block vessels and induce disease symptoms, indicating that the extent of EPS production may be a pathogenicity trait (Monteiro et al., 2012). In the meantime, plant defenses are activated and microbes respond to them. Plant cells recognize microbe-associated-molecular-patterns (MAMPs) and instigate basal resistance (Barrett et al., 2009). Pathogens respond by virulence factors to counter basal resistance; by producing proteins that suppress MAMP perception and signaling or by producing toxins or cell wall degrading enzymes (Barrett et al., 2009). The plant in return responds to these virulence factors, often through gene-for-gene resistance, which triggers hypersensitive response (HR) and results in programmed cell death, killing pathogens along with the localized host cells. This action-reaction process can go back and forth, with microbes sometimes showing specialized responses to prevent HR, and with resistance genes (R) developing from these (Barrett et al., 2009; Monteiro et al., 2012). In some cases plants may recognize the bacterium and in the case of a non-pathogenic microbe this may lead to down-regulation of defense-related genes, however the plant remains in an alert mode of induced resistance (Monteiro et al., 2012).

Experiments on plant-endophyte defense

Through these steps of entrance, colonization and anchorage the endophyte may establish itself within the plant. Regardless of the outcome of plant-microbe interaction, invasion by microbes results in a host defense response, even after infection by a mutualistic endophyte (Monteiro et al., 2012; Schulz, Rommert, Dammann, Aust, & Strack, 1999)(Cabral, Stone, & Carroll, 1993). Even for longstanding symbioses like the legume-rhizobia system plant defense-like interactions are at first incurred. Exactly how plant defense responses differ between the further developed legume-rhizobia system and other plant-endophyte interaction is under debate, as well as at which stage defense is aborted (Mithöfer, 2002; Monteiro et al., 2012). For most species mutual antagonisms occur continuously while *in planta,* among others through production of toxic secondary metabolites (Schulz et al., 1999). For example, microbes have been shown to secrete herbicidal metabolites that harm the host by causing necroses, growth inhibition and death of plant cells. In their turn, plants secrete non-specific antifungal metabolites. Research by Schulz et al. has shown that plants infected with endophytes contain similar or sometimes higher concentrations of plant defense compounds than plants infected with pathogenic microbes, and that endophytes often synthesize more herbicidal metabolites than pathogens. They concluded that plant defense reaction is greater to endophytic than to pathogenic infection. The higher concentrations of metabolites in endophyte infected plants as opposed to pathogen infected plants is accounted to the pathogen's ability to suppress or degrade these compounds and cause disease (Schulz et al., 1999). A number of other studies support these findings; Peters et al. found higher PAL activity, greater release of H_2O_2 and higher phenol concentrations in *Lamium purpureum* infected with *Coniothyrium palmarum* than in individuals infected with the pathogen *Alternaria spp.* (Peters et al 1998, in (Schulz et al., 1999)). In a similar experiment Fengler and Schulz again found higher PAL activity (in: Schulz et al., 1999). Plant response to invasion by producing ROS (eventually leading to hyper-sensitive cell death) is observed in plant-endophyte as well as plant-pathogen interactions (Kogel, Franken, & Huckelhoven, 2006).

Balanced antagonism?

The studies discussed above show that plants respond antagonistically to endophytes in a similar manner to pathogens. As a result, Schulz et al. hypothesize that mutualism is a balanced antagonism where plant host and endophyte both excrete compounds to harm the partner (Schulz et al., 1999). One way that this balance may be maintained is through strong activation and scavenging of ROS in response to plant production (Kogel et al., 2006). Schulz et al. argue that pathogenicity is the unbalanced situation where balance is disturbed in favor of the fungus, resulting in plant fitness reduction. They note that this does not mean that it is not possible for endophytes to have a beneficial

effect on plant fitness, however they argue that regardless of the beneficial effect of endophyte on plant growth or other function, plants will produce antagonistic compounds (Schulz et al., 1999). An interesting question is whether mycorrhizal infection results in ROS production, however this is not yet well understood (Kogel et al., 2006). Regardless, research seems to indicate that presence of microbes *in planta* induces plant defense responses.

Lifestyle and conditions for its expression

Coevolution

Within the spectrum of plant-endophyte interactions, the survival and fitness of the partners can be so closely associated with each other that adaptation occurs. In the case of a negative interaction such as host and pathogen, this adaptation may head towards escape or dissolution of the partnership, in what is known as an arms race. In positive interactions, host and mutualist adapt ever tighter to each other, resulting in coevolution. Especially the latter situation is well-studied, with the bulk of plant-endophyte studies having been conducted on systems such as legume-rhizobium or grass-endophyte associations. Nonetheless, coevolution is not the norm in plant-endophyte relations (Kari Saikkonen et al., 2004). Endophyte mycotoxins have only been reported in a small portion subset of grasses, contradicting the idea that coevolution commonly leads to defense benefits in plant hosts (Faeth, 2002; Kari Saikkonen et al., 2004). For coevolution to be possible, it is essential for mutualist and host to match in a morphological, physiological and reproductive sense as well as in life history traits (Kari Saikkonen et al., 2004). The reproductive systems of host and endophyte must be compatible, which the endophyte can achieve either through increasing its own genetic diversity or decreasing host genetic diversity. Examples of this are through stimulated clonal propagation or self-pollination. Furthermore, benefits of the symbiosis in terms of fitness must be high, and selective pressure must be great for coevolution to occur. This is possible in agro-ecosystems, where nutrients or other resources are never limiting, thus costs of symbiosis are relatively low, and where enhancing plant defense provides a selective advantage to intensive grazing by livestock. Also, agro-ecosystems are characterized by high degrees of interbreeding, which may facilitate alkaloid accumulation (Faeth, 2002). Natural systems, however, have quite opposite conditions. Integrated symbiosis is less likely in natural systems because selection pressures are more variable and the relationship is easily destabilized. Examples of destabilizing situations are conflicts in host-endophyte reproduction, enhanced host susceptibility to other microbes, energetic costs and host control of fungal growth. Some evidence is available for the hypothesis that chances for coevolution to occur differ between natural and agro- ecosystems. Research on natural and domesticated grasses showed that infected grasses only outcompete uninfected grasses under high nutrient conditions (Faeth & Sullivan, 2003). This provides some indication that mutual exploitation is a more realistic model for mutualistic plant-endophyte relationships than cooperation (Kari Saikkonen et al., 2004).

Lifestyle

Though the terms ‘mutualistic’ and ‘pathogen’ have traditionally been interpreted as a fixed species characteristic, an overwhelming array of research indicates that microbes can express different lifestyles depending on circumstances (Hirsch, 2004; Hosni et al., 2011; Kogel et al., 2006; Mason, Stepien, & Blum, 2011; Redman et al., 2001; Russell J. Rodriguez, Redman, & Henson, 2005; Schulz et al., 1999). Some mycorrhizae have been demonstrated to be parasitic (Francis & Read 1995, in (Russell J. Rodriguez et al., 2005). Surveys of plant tissue that did not show signs of disease (asymptomatic tissue) revealed these to contain both pathogenic and non-pathogenic microbe species (Schulz et al., 1999). Furthermore, the initial stages of infection by pathogens and endophytes are similar (Russell J. Rodriguez et al., 2005), arguing that the division between these lifestyles occurs post-colonization under the influence of host genotype and environmental conditions. Within the genus *Colletotrichum* many species are observed that display different lifestyles in different host plants (Redman et al., 2001; Russell J Rodriguez, Redman, & Henson, 2003; Russell J. Rodriguez et al., 2005). These species and the hosts in which they occur pathogenically and endophytically are shown in table 1 (from (Russell J Rodriguez et al., 2003). The variety of forms in which the same species of endophytes are encountered *in planta* indicate that for most endophytes lifestyle is more

complicated than the labels of 'pathogen' or 'mutualist' that they have previously received. These labels are only the strategy a microbe employs at the moment of observation, without having any bearing on past strategy, future position, microbe growth, reproduction etc. Thus studies of lifestyles are snapshots of microbial diversity and life as expressed under their current conditions. This leads to the question: what are the conditions for microbes to be pathogenic, parasitic, commensal or mutualistic?

Table 1: Host range and symbiotic lifestyle expression of Colletotrichum *species, from* (Russell J Rodriguez et al., 2003)

TABLE I

Host range and symbiotic lifestyle expression of *Colletotrichum* species.

Fungus	Symptomatic hosts	Asymptomatic hosts	Symbiotic lifestyle(s) expressed
C. lindemuthianum	bean	none observed	P
C. graminicola	corn	none observed	P
C. coccodes	tomato, cucurbits, pepper, eggplant, strawberry	none observed	P
C. acutatum	strawberry	cucurbits	P, C
C. gloeosporioides	strawberry	cucurbits, tomato, pepper, eggplant	P, C
C. orbiculare	cucurbits, pepper, tomato*, eggplant*	tomato*, eggplant*	P, C, M
C. musae	banana	cucurbits, tomato, pepper, eggplant	P, C, M
C. magna	cucurbits	tomato, pepper, eggplant, bean, strawberry	P, C, M

Host range tests and pathogen bioassays were performed as previously described (Redman et al. 2001). P = pathogenic, C = commensal and M = mutualistic (based on the ability of the fungus to confer disease protection against fungal pathogens). * – depending on the plant variety fungi were either pathogenic or expressed non-pathogenic symbiotic lifestyles.

Latency

One possible explanation for the large abundance of endophtytes *in planta* is that these are saprophytes that survive *in planta* until plant senescence when they can successfully reproduce. Other endophytes may be latent pathogens that can survive *in planta* and may turn pathogenic when environmental conditions allow (Aly et al., 2011; Promputtha et al., 2007; Schulz et al., 1999). Some researches support this hypothesis while Ganley et al., based on a sequencing study, rejects it (Ganley, Brunsfeld, & Newcombe, 2004).

Some studies have reported known pathogens to occur asymptomatically (without symptoms) in non-host species, such as *Colletotrichum magna*, a fungal pathogen reported asympotamically in various species (Kogel et al., 2006). Another example is *Beauveria bassiana*, an insect pathogen that is also able to survive saprophytically in plants when insect hosts are not available, lying dormant in leaves until consumption of leaf and pathogen by their insect hosts (Torres, Nacional, & Mar, 2009). Also, a group of fungi called the *Clavicipitaceae* demonstrate evolutionary steps from insect pathogens to plant mutualistic endophytes (Torres et al., 2009). The mechanism enabling pathogens to be latent and lack pathogenicity in non-host plants may be through host-specific virulence factors. Some pathogens produce host-specific toxins that do not harm non-host genotypes. Thus it seems likely that many species are capable of colonizing multiple hosts, thus having periods in which they exist as endophytes as well as their pathogenic lifestyle.

Transmission method

The role an endophyte plays within plants is strongly dependent on transmission method. Endophytes can be transmitted horizontally through spores from one host to the next. Horizontal

transmission is sexual and can evolve microbe virulence. Vertical transmission on the other hand takes place through infection from parent to offspring, where microbial reproduction is tied up with host reproduction. This form of transmission is asexual. Some research indicates that vertical transmission reduces the ability of a microbe to transmit horizontally (Saari, 2010), however this is still under investigation. *Neotyphodium* endophytes for example are unable to spread in a contagious manner through grasses rendering them completely dependent on the host (Saari, 2010). As a result, this symbiosis is obligate for the microbe but not for the host. As such the host is able to control the relationship and microbe performance. Due to the strong connection between host and microbe evolutionary theory predicts that vertically transmitted endophytes must always be mutualistic (Faeth & Sullivan, 2003). In theory, endophyte presence must increase plant fitness or infected plants will be outcompeted. However, in practice some studies show known vertically transmitted species that appeared to have negative effects on hosts (Faeth & Sullivan, 2003; Saari, 2010). There are a number of possible theories explaining vertical transmission of harmful endophytes, explaining it through the complexity of the food web, temporal changes in selection pressure, unanticipated horizontal transmission, spatial pocketing, mutualistic benefits under rare but important conditions (such as drought etc.) or microbial control of plant reproductive organs (Faeth & Sullivan, 2003; Saari, 2010). Horizontally transmitted endophytes can reproduce independently thus experience no such limits on survival strategy.

Virulence & infectivity

Pathogen ability to survive and persist *in planta*, its virulence, is determined by a number of characteristics. Pathogenic virulence increases with production of toxins and other virulence factors, with adherence to host tissues and with avoidance of host defense mechanisms (Venturi & Silva, 2012). Microbes can respond to plant defense in a number of ways. Pathogens can produce proteins that suppress recognition of MAMPs, compounds that interfere with the ethylene-signaling pathway such as AAC deaminase or compounds that prevent the hypersensitive response such as exosaccharides or LPS (Barrett et al., 2009; Monteiro et al., 2012). Violi et al. studied pathogenic infection of avocado in presence of a mutualistic microbe. They found that infection chances increased with higher infectivity potential, determined largely by increased density of pathogens (Violi, Menge, & Beaver, 2007). Furthermore, pathogen growth, virulence and transmission rate is positively related, with positive conditions resulting in more energy for growth as well as reproduction and production of transmissible propagules (Barrett et al., 2009).

Genetics

Whether a microbe emerges as a pathogen is dependent on genetic make-up of plant and microbe (Aly et al., 2011). Rodriguez et al. report that microbes can display different lifestyles depending on plant genotype (Russell J. Rodriguez et al., 2005). Also, although mutualists and pathogens seem to have fairly similar genetics, some genes can enhance development *in planta*. An example is genes coding for the production of hydrolytic enzymes. These genes are important for pathogenic species, enabling colonization and survival with necrotrophic or saprotrophic lifestyles. These genes are lost in some mutualistic species rendering them highly dependent on their host. Another example is genes coding for production of signal molecules, chemically inhibiting plant defense (Plett & Martin, 2011). Research by Monteiro et al. shows that related plant species with different lifestyles (i.e. mutualistic or pathogenic) differ in genetics with the pathogenic species having obtained genes coding for effector proteins that related mutualistic species lack, which may aid in suppressing plant defense (Monteiro et al., 2012). Small changes in plant or microbial genome can result in a switch from mutualistic to pathogenic strategies (Kogel et al., 2006; Redman et al., 2001; Russell J. Rodriguez et al., 2005). For example, *C. magna* loses pathogenicity factors by mutation of a single gene, thus losing the capacity for the pathogenic strategy (Kogel et al., 2006). Studies on the ryegrass *Lolium perenne* showed that when it was infected with a mutant form of the otherwise endophytic *E. festucae*, the plant showed a range of disease symptoms and died. Tanaka et al. suggest that mutation of the NoxA gene in the endophyte results in limited ROS production, decreasing regulation of fungal development and resulting in excessive colonization and eventually plant death (in: (Kogel et al., 2006)). The fact that endophytes can switch lifestyle as a result of a single mutation may provide an explanation for the abundance of asymptomatic endophytes/pathogens reported in plants (Russell J. Rodriguez et al.,

2005; Schulz et al., 1999). Furthermore, this shows that the microbial lifestyle, though fluid, is constrained by fixed conditions such as plant and microbe genetic make-up.

Environment and stress

Pathogenic expression is strongly dependent on environmental conditions (Aly et al., 2011; Faeth & Sullivan, 2003). Both microbial survival and growth and plant resistance and tolerance is strongly dependent on environmental conditions. Main environmental factors of interest are moisture, temperature and nutrient availability. High moisture enhances microbe growth and transmission, as well as increasing infection rates by making it possible for microbes to enter through open leaf stomata. Pathogen infection is stronger and transmission higher under cool conditions. For example wheat that contains the pathogen *Puccinia striiformis* is resistant to infection at high temperatures, but susceptibility increases when temperatures decrease (Uauy et al., 2005) Also, under limited nutrient availability or in stressed environments plant tolerance decreases and fitness reduction following infection is higher. Presence of some cations can directly influence microbe performance, for example soil calcium concentration can negatively influence infection rates in flaxes (Barrett et al., 2009; Springer, Hardcastle, & Gilbert, 2007). Furthermore, plant-microbe interactions can turn parasitic when resources are limited because in these circumstances supporting 'foreign' microbes is more costly (Faeth, 2002; Partida-Martinez & Heil, 2011; Violi et al., 2007). Figure 2 shows this balance between beneficial and parasitic interactions. The presence of endophytes incurs a cost, and at the same time confers benefits. Depending on the environmental conditions both cost and benefit may change. For example under low nutrient conditions endophyte presence is increasingly costly (figure 2D), whereas at low stressor presence benefits conferred by endophytes decrease (figure 2C).

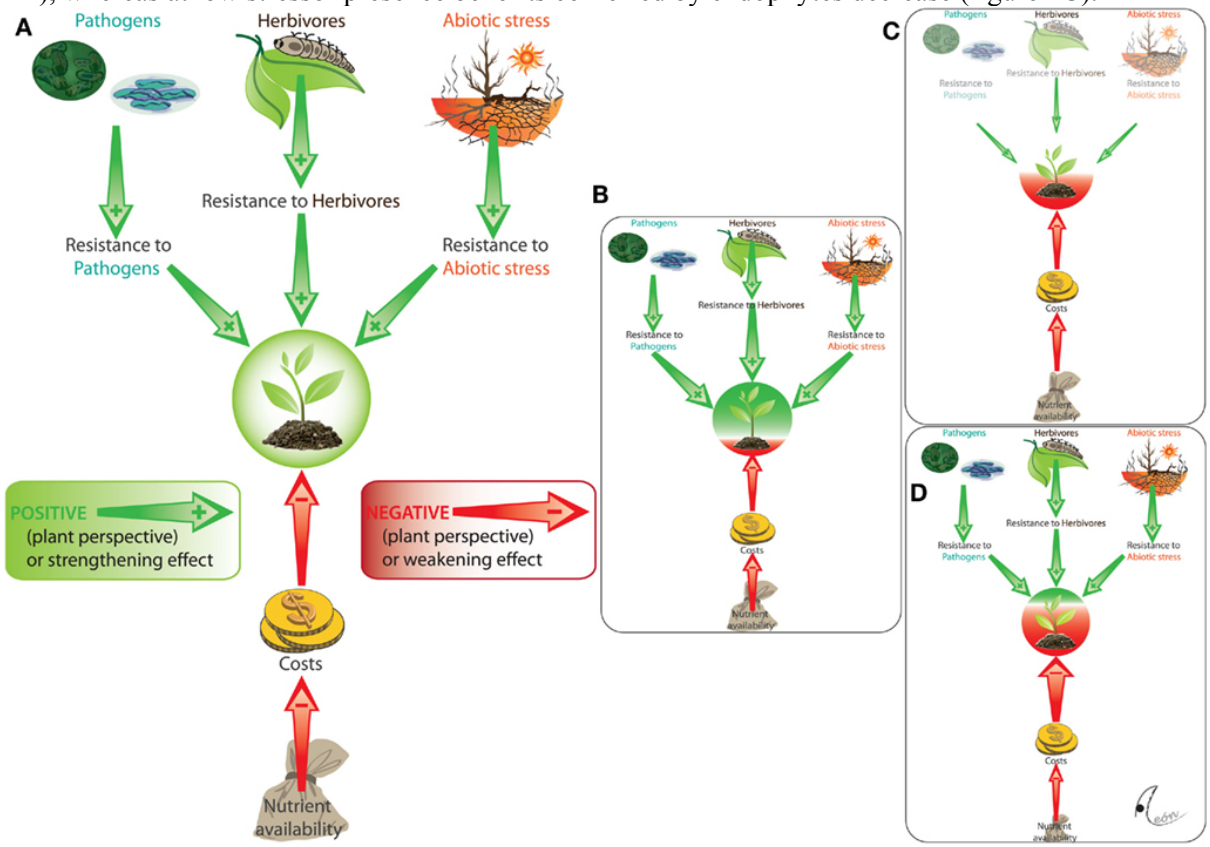


Figure 2: Conditional outcomes of plant–microbe interactions. We illustrate here the multiple factors that mediate the outcome of the presence of a resistance-enhancing endophyte (A). Resistance against biotic stressors (pathogens and herbivores) and abiotic stressors (e.g., heat) has positive effects (from the perspective of the plant: green arrows), whereas the costs that are caused by the maintenance of the resistance-enhancing endophyte represent a negative effect (red arrows). Due to this interplay of positive and negative effects, the same interaction results mutualistic in presence of those stressors to which the endophyte provides resistance (B) whereas, by contrast, the net outcome becomes negative (antagonism) either in the absence of stressors [(C) lack of benefits] or when resource limitation enhances the relative costs of maintaining the endophyte (D). Text and figure from: (Partida-Martinez & Heil, 2011)

Community effects

The surrounding microbial community can both enhance and reduce pathogenic expression. Presence of mutualistic endophytes can result in production of antibiotic or antifungal compounds reducing the effectiveness of competing pathogens. Some plants can even interact with these mutualists resulting in enhanced growth of such disease-suppressing microbes (Smith, Handelsman, & Goodman, 1999). Also, known pathogens can function as commensal associate in presence of other microbes that inhibit pathogenic expression. An example is *C. elatum* which is a weak pathogen when infecting alone, and a neutral endophyte when G. *intraradices* is present (Violi et al., 2007). Mutualists can induce systemic defense responses such as SAR and ISR resulting in lower pathogen infection rates (Barrett et al., 2009). On the other hand microbes already present *in planta* can also enhance pathogen performance. This phenomenon was first described in other organisms such as humans (gut) or insects (Hosni et al., 2011; Rio, Hu, & Aksoy, 2004; Venturi & Silva, 2012). Resident harmless bacteria can enable infection by pathogens or decrease plant tolerance. Mechanisms hypothesized to facilitate pathogens are cell–cell signaling, metabolic interactions, resident microbe assisted evasion of the immune response and a commensal-to-pathogen switch (Venturi & Silva, 2012).

Furthermore, competition can influence the outcome of plant-microbe interactions. *A. thaliana* infected with *P. syringae* have been shown to produce 10% more seeds than in controls in the absence of microbial competition, and 40% fewer seeds in the presence of intra-specific competition (Barrett et al., 2009; Korves & Bergelson, 2004). C. elatum showed similar results in a study by Venturi et al.; with the microbe interacting neutrally until intra-specific competition became high and resources were limited (Venturi & Silva, 2012). On a bigger scale, these interactions are predicted with microbes that survive using a specific substrate such as dead plants or other organic material. When these resources decline competition increases and microbes need to shift to a different lifestyle, which is often pathogenic colonization of plant roots (Violi et al., 2007). However, this shift only occurs for opportunistic pathogens, as obligate pathogens are unable to survive on different substrate.

It is important to study plant-endophyte interactions in their microbial context as this context influences the outcome of interactions, leading to different results from theory of studies done in the lab. In theory, mutualism can appear to not be an evolutionary stable strategy, as cheaters will gain higher benefits than mutualists thus destabilizing the relationship. Mueller et al. studied ant-fungi interactions in presence of a parasite and found that cooperation can be stabilized by addition of a common enemy. In presence of a parasite cheating by either fungi or ants resulted in decreased health of the fungal garden and higher parasite-induced morbidity, resulting in lower fitness than in cooperative systems. Thus the conflict between two mutualists can be reduced by addition of a third party, stabilizing the relationship. They hypothesize that this relationship can be generalized for systems in which survival and reproduction are closely connected to cooperation, as mortality in one partner then influences the other (Mueller, Ishak, Lee, Sen, & Gutell, 2010).

Summary

In terms of lifestyle endophytes exist on a continuum from mutualistic via commensal to parasitic or pathogenic. Lifestyle switches are observed for many endophytes within species, geographical ranges and even lifetimes. Also, some microbes can be obligate mutualists or pathogens. Much is still unclear about factors influencing switches in lifestyle, in part because lifestyle and especially pathogenicity is often perceived as fixed, and the strongest conclusion is that lifestyle expressed by a microbe is context dependent.

Nonetheless the most important factor determining microbe lifestyle is host genotype. It seems that microbes can become pathogenic under specific circumstances. They must contain the genetic make-up to produce virulence factors, molecules to inhibit plant defense etc. They must match with hosts susceptible to the compounds they produce. Furthermore, environmental conditions are of importance; for many pathogens optimal conditions are high moisture, low temperature and limited nutrients, as hosts in stressed environments are more susceptible and have lower tolerance. Endophytes can also be latent pathogens or saprophytes. Lastly, the microbial community can help or inhibit pathogens. The presence of mutualists often lowers pathogen infection rates by activating SAR and ISR and can result in pathogens living as commensals. However, some mutualists can enhance pathogen performance as well. Furthermore, competition seems to be important; when competition becomes greater and

resource availability is lower endophytes more often turn to pathogenicity. The opposite is also true; when conditions for pathogenicity are not met, when environmental conditions are not optimal or the plant genotype is a mismatch, an endophyte may express a commensal lifestyle.

From rhizosphere to *in planta*

The microbes able to colonize *in planta* and become endophytes are a subset of the rhizosphere microbial community similar to the way rhizosphere microbes are a subset of bulk soil microbes. A relevant question is how in this second step endophytes are 'selected' or acquired. Is the filter determining which species are acquired *in planta* similar to the filter determining rhizosphere microbes? Are endophytes a random subset of rhizosphere (already filtered) microbes or does a selection process take place, and if so which characteristics are selected on?

In some tightly associated eukaryote-microbe interactions the first step of endophytic colonization is concentrating the bacteria. In the symbiosis between *V. fischeri* and *Euprymna*, the squid excretes mucus to entrap bacteria (Nyholm et al. 2000 in: (Hirsch, 2004)). A similar process using mucilage from plant glands collects cyanobacteria in *Nostoc-Gunnera* symbiosis (Johansson and Bergman 1992 in:(Hirsch, 2004)). Rhizobia-legume interactions start with plants secreting lectins into the rhizosphere to accumulate bacteria near susceptible root hairs, which is followed by root hair curling and nodule development (Hirsch, 2004). Thus in the case of specific tightly associated symbioses plants actively secrete compounds to attract bacteria for endophytic colonization. In the case of generalist endophytes this process is likely to be less developed. Nonetheless, root tips and damaged areas leak carbon compounds resulting in conglomeration of non-specialist microbes as well.

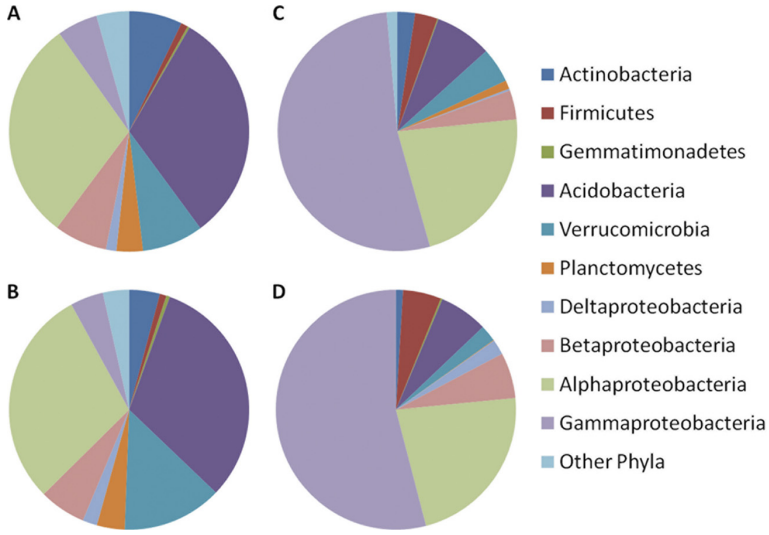


FIG. 3. Bacterial classifications using the RDP classifier at 80% identity as implemented in mothur, shown at the phylum level except for *Proteobacteria*, which are classified by class. The charts represent average results for rhizospheric (A and B) and endophytic (C and D) or communities originating from samples of either bottomland (B and D) or upland (A and C) trees. To aid in distinguishing the colors, phylogenetic groups are presented in the same order in the pie charts (clockwise) as in the legend (top to bottom) in each subchart.

Figure 3: Bacterial classification per phylum of Populus deltoid *rhizosphere bacteria (A & B) and endophytes (C & D), at two different locations. From:* (Gottel et al., 2011)

In order to identify the factors determining endophyte community composition similar factors have been studied as for rhizosphere communities. A study on the influence of soil type on endophyte community selection shows that similar to rhizosphere composition organic matter is a strong influence on endophyte community composition, (Long et al., 2010). The most important factor influencing endophyte community seems to be plant genotype. Furthermore, research indicates an active role for plants in acquiring this community. Siciliano et al. studied plants grown in petroleum hydrocarbon contaminated soil and their interaction with endophytes possessing hydrocarbon degradation genes. In contaminated soils plants contained two and four times higher abundances of such endophytes than in bulk soil or rhizosphere (Siciliano et al., 2001). These results indicate that the plant is capable of actively selecting its endophyte fauna. Also, when experimentally altering plant

genotype Siciliano et al. found that this had a larger impact on root interior microbial population than on the rhizosphere community (Siciliano et al 1998, in:(Siciliano et al., 2001)). An extensive study of bacterial and fungal communities in *Populus deltoids* showed that root endophytes consist of a distinct community of microbes rather than a random subset (Gottel et al., 2011). The distinct differences in bacterial phyla are shown in figure 3. Gottel et al. hypothesize that if the endophyte community were made up of a similar subset of rhizospheric microbes or accidental passengers, strong similarities are expected between rhizosphere and in planta community. However, the dominant phylogenetic groups and operational-taxonomic-unit (OTU) abundance patterns differ between these habitats. These results indicate that endophyte communities are not random subsets of the rhizosphere but that a selection force works on community composition.

Conclusion

Endophytes that occur in plants without increasing or decreasing plant fitness are very common. Recent research indicates that mutualists do not occur as often as has previous been thought, nor is their lifestyle as obligate as assumed. Plants contain many species whose effect (beneficial or harmful) differs with microbial community and environmental conditions, in some circumstances benefiting the plant while in others decreasing plant fitness. This has lead many researchers to postulate the lifestyle switch, arguing that microbe lifestyle is fluid and mutualism, commensalism and pathogenicity can all be temporary conditions. This in turn begs the question: if lifestyle is fluid, what conditions then determine microbe lifestyle? It is this question that this essay addresses.

Microbes, regardless of lifestyle, are drawn to the rhizosphere by both the presence of nutrients as well as plant signaling molecules such as flavonoids. The microbial community in the rhizosphere differs from the endophyte/pathogen community within the plant, indicating a selective force influencing which microbes colonize the plant. After infection plant defense mechanisms are triggered for mutualistic rhizobia, neutral endophytes and harmful pathogens alike. In some cases after a period of time plant defense response against the microbe decreases after recognition, though how this works is unclear (Cocking, 2003). In many cases both plant and microbe continue to produce antagonistic compounds.

I hypothesize that plants, through their exudes and secreted compounds, select for microbes that may be mostly beneficial. However, once *in planta* some microbes may express pathogenic lifestyles, due to environmental or community opportunities. Some microbes may be capable of having beneficial effects but become pathogenic due to high environmental stress lowering plant defenses, their own high virulence and high intraspecific competition resulting in low availability of other resources. It is possible that to counteract these lifestyle-fluid endophytes plants have a high degree of non-specific background defense, resulting in the observed antagonistic exchanges with most endophytes.

As a result I conclude that mutualists, commensals and pathogens are labels for fluid and often temporary microbe strategies. Within limits (such as microbial genotype and possession of virulence factors) microbes can assume different life strategies. Which strategy a specific microbe employs depends among others on host genotype, environmental conditions and the microbial community *in planta*. Thus I expect plants contain a large population of commensal endophytes that may at a later moment or in another host express a different lifestyle. Due to the fluidity of microbial lifestyle host plants keep up a high level of background defense resulting in antagonistic plant-microbe interactions.

Literature

Aly, A. H., Debbab, A., & Proksch, P. (2011). Fungal endophytes: unique plant inhabitants with great promises. *Applied microbiology and biotechnology*, *90*(6), 1829-45. doi:10.1007/s00253-011-3270-y

Araújo da Silva, K. R., Salles, J. F., Seldin, L., & van Elsas, J. D. (2003). Application of a novel Paenibacillus-specific PCR-DGGE method and sequence analysis to assess the diversity of Paenibacillus spp. in the maize rhizosphere. *Journal of Microbiological Methods*, *54*(2), 213-231. doi:10.1016/S0167-7012(03)00039-3

Badri, D. V., Weir, T. L., Lelie, D. V. D., & Vivanco, J. M. (2009). Rhizosphere chemical dialogues : plant – microbe interactions. *Current opinion in Biotechnology*, *20*, 642-650. doi:10.1016/j.copbio.2009.09.014

Bais, H. P., Weir, T. L., Perry, L. G., Gilroy, S., & Vivanco, J. M. (2006). The Role of Root Exudates in Rhizosphere Interactions with Plants and Other Organisms. *Annual Review of Plant Biology*, *57*, 233-266. doi:10.1146/annurev.arplant.57.032905.105159

Barrett, L. G., Kniskern, J. M., Bodenhausen, N., Zhang, W., & Bergelson, J. (2009). Continua of specificity and virulence in plant host-pathogen interactions: causes and consequences. *The New Phytologist*, *183*(3), 513-29. doi:10.1111/j.1469-8137.2009.02927.x

Barrow, J. R., Lucero, M. E., Reyes-Vera, I., & Havstad, K. M. (2008). Do symbiotic microbes have a role in plant evolution, performance and response to stress? *Communicative & Integrative Biology*, *1*(1), 69-73. Retrieved from http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=2633805&tool=pmcentrez&rendertype=abstract

Bell, C. R., Dickie, G. A., Harvey, W. L. G., & Chan, J. W. Y. F. (1995). Endophytic bacteria in grapevine. *Canadian journal of microbiology*, *41*(1), 46-53.

Biphenyls, P., Narasimhan, K., Basheer, C., Bajic, V. B., & Swarup, S. (2003). Enhancement of Plant-Microbe Interactions Using a Rhizosphere Metabolomics-Driven Approach and Its Application in the Removal of, *132*(May), 146-153. doi:10.1104/pp.102.016295.populations

Cabral, D., Stone, J. K., & Carroll, G. C. (1993). The internal mycobiota of] uncus spp .: microscopic and cultural observations of infection patterns, *97*(3), 367-376.

Cocking, E. C. (2003). Endophytic colonization of plant roots by nitrogen-fixing bacteria. *Plant and Soil*, *252*(1), 169-175. doi:10.1023/A:1024106605806

Denison, R. F. (2000). Legume Sanctions and the Evolution of Symbiotic Cooperation by Rhizobia. *The American Naturalist*, *156*(6), 567-576.

Faeth, S. H. (2002). Are endophytic fungi defensive plant mutualists ? *Oikos*, *98*(December 2001), 25-36.

Faeth, S. H., & Sullivan, T. J. (2003). Mutualistic asexual endophytes in a native grass are usually parasitic. *The American Naturalist*, *161*(2), 310-25. doi:10.1086/345937

Fons, F., Amellal, N., Leyval, C., Saint-Martin, N., & Henry, M. (2003). Effects of Gypsophila saponins on bacterial growth kinetics and on selection of subterranean clover rhizosphere bacteria. *Canadian journal of microbiology*, *49*(6), 367-73. doi:10.1139/w03-052

Ganley, R. J., Brunsfeld, S. J., & Newcombe, G. (2004). A community of unknown, endophytic fungi in western white pine. *Proceedings of the National Academy of Sciences of the United States of America*, *101*(27), 10107-12. doi:10.1073/pnas.0401513101

Garbeva, P., Veen, J. A. V., & Elsas, J. D. V. (2004). Microbial Diversity in Soil: Selection of Microbial Populations by Plant and Soil Type and Implications for Disease Suppressiveness. *Annual Review of Phytopathology*, *42*, 243-270. doi:10.1146/annurev.phyto.42.012604.135455

Gelsomino, A., Keijzer-Wolters, A. C., Cacco, G., & van Elsas, J. D. (1999). Assessment of bacterial community structure in soil by polymerase chain reaction and denaturing gradient gel electrophoresis. *Journal of Microbiological Methods*, *38*(1-2), 1-15. doi:10.1016/S0167-7012(99)00054-8

Gottel, N. R., Castro, H. F., Kerley, M., Yang, Z., Pelletier, D. a, Podar, M., Karpinets, T., et al. (2011). Distinct microbial communities within the endosphere and rhizosphere of Populus deltoides roots across contrasting soil types. *Applied and environmental microbiology*, *77*(17), 5934-44. doi:10.1128/AEM.05255-11

Gough, C., Galera, C., Vasse, J., Webster, G., Cocking, E. C., & Dénarié, J. (1997). Specific flavonoids promote intercellular root colonization of Arabidopsis thaliana by Azorhizobium caulinodans ORS571. *Molecular plant-microbe interactions : MPMI*, *10*(5), 560-70. doi:10.1094/MPMI.1997.10.5.560

Gyaneshwar, P., James, E. K., Reddy, P. M., & Ladha, J. K. (2002). Herbaspirillum colonization increases growth and nitrogen accumulation in aluminium-tolerant rice varieties. *New Phytologist*, *154*(1), 131-145. doi:10.1046/j.1469-8137.2002.00371.x

Hartmann, A., Schmid, M., van Tuinen, D., & Berg, G. (2009). Plant-driven selection of microbes. *Plant and Soil*, (321), 235-257. doi:10.1007/s11104-008-9814-y

Hiltner, L. (1904). Uber neure Erfahrungen und probleme auf dem gebeit der bodenback- teriologie und unter besonderer berucksichtigung der grundungung und brache. *Arb. Deut. Landwirsch Ges.*, *98*, 59-78.

Hirsch, A. N. N. M. (2004). Plant-Microbe Symbioses : A Continuum from Commensalism to Parasitism. *Symbiosis*, *37*, 0-19.

Hosni, T., Moretti, C., Devescovi, G., Suarez-Moreno, Z. R., Fatmi, M. B., Guarnaccia, C., Pongor, S., et al. (2011). Sharing of quorum-sensing signals and role of interspecies communities in a bacterial plant disease. *The ISME journal*, *5*(12), 1857-70. doi:10.1038/ismej.2011.65

James, E. K. (2002). Infection and colonization of rice seedlings by the plant growth- promotion bacterium Herbaspirillum seropedicae. *Molecular plant-microbe interactions*, *15*(9), 894.

Johri, B. N. (2006). Endophytes to the rescue of plants! *Current Science*, *90*(10), 1315-1316.

Kogel, K.-heinz, Franken, P., & Huckelhoven, R. (2006). Endophyte or parasite – what decides? *Current Opinion in Plant Biology*, (9), 358-363. doi:10.1016/j.pbi.2006.05.001

Korves, T., & Bergelson, J. (2004). A novel cost of R gene resistance in the presence of disease. *The American naturalist*, *163*(4), 489-504. doi:10.1086/382552

Lambers, H., Mougel, C., Jaillard, B., & Hinsinger, P. (2009). Plant-microbe-soil interactions in the rhizosphere: an evolutionary perspective. *Plant and Soil*, *321*(1-2), 83-115. doi:10.1007/s11104-009-0042-x

Long, H. H., Sonntag, D. G., Schmidt, D. D., & Baldwin, I. T. (2010). The structure of the culturable root bacterial endophyte community of Nicotiana attenuata is organized by soil composition and host plant ethylene production and perception. *The New Phytologist*, *185*(2), 554-67. doi:10.1111/j.1469-8137.2009.03079.x

Mason, K. L., Stepien, T. A., & Blum, J. E. (2011). From Commensal to Pathogen : Translocation of Enterococcus faecalis from the Midgut to the Hemocoel of Manduca sexta. *Microbiology*. doi:10.1128/mBio.00065-11.Editor

Micallef, S. a, Channer, S., Shiaris, M. P., & Colón-Carmona, A. (2009). Plant age and genotype impact the progression of bacterial community succession in the Arabidopsis rhizosphere. *Plant signaling & behavior*, *4*(8), 777-80. doi:10.1093/jxb/erp053

Miethling, R., Wieland, G., Backhaus, H., & Tebbe, C. C. (2000). Variation of Microbial Rhizosphere Communities in Response to Crop Species, Soil Origin, and Inoculation with Sinorhizobium meliloti L33. *Microbial Ecology*, (41), 43-56. doi:10.1007/s002480000021

Mithöfer, A. (2002). Suppression of plant defence in rhizobia-legume symbiosis. *Trends in plant science*, *7*(10), 440-4. Retrieved from http://www.ncbi.nlm.nih.gov/pubmed/12399178

Monteiro, R. A., Balsanelli, E., Wassem, R., Marin, A. M., Brusamarello-Santos, L. C. C., Schmidt, M. A., Tadra-Sfeir, M. Z., et al. (2012). Herbaspirillum-plant interactions: microscopical, histological and molecular aspects. *Plant and Soil*. doi:10.1007/s11104-012-1125-7

Mueller, U. G., Ishak, H., Lee, J. C., Sen, R., & Gutell, R. R. (2010). Placement of attine ant-associated Pseudonocardia in a global Pseudonocardia phylogeny (Pseudonocardiaceae, Actinomycetales): a test of two symbiont-association models. *Antonie van Leeuwenhoek*, *98*(2), 195-212. doi:10.1007/s10482-010-9427-3

Omacini, M., Semmartin, M., Pérez, L. I., & Gundel, P. E. (2011). Grass–endophyte symbiosis: A neglected aboveground interaction with multiple belowground consequences. *Applied Soil Ecology*. doi:10.1016/j.apsoil.2011.10.012

Partida-Martinez, L. P., & Heil, M. (2011). The microbe-free plant: fact or artifact? *Frontiers in Plant Science*, *2*(December), 1-16. doi:10.3389/fpls.2011.00100

Pedrosa, F. O., Monteiro, R. A., Wassem, R., Cruz, L. M., Ayub, R. a, Colauto, N. B., Fernandez, M. A., et al. (2011). Genome of Herbaspirillum seropedicae strain SmR1, a specialized diazotrophic endophyte of tropical grasses. *PLoS genetics*, *7*(5), e1002064. doi:10.1371/journal.pgen.1002064

Plett, J. M., & Martin, F. (2011). Blurred boundaries: lifestyle lessons from ectomycorrhizal fungal genomes. *Trends in genetics : TIG*, *27*(1), 14-22. doi:10.1016/j.tig.2010.10.005

Promputtha, I., Lumyong, S., Dhanasekaran, V., McKenzie, E. H. C., Hyde, K. D., & Jeewon, R. (2007). A phylogenetic evaluation of whether endophytes become saprotrophs at host senescence. *Microbial ecology*, *53*(4), 579-90. doi:10.1007/s00248-006-9117-x

Redman, R. S., Dunigan, D. D., & Rodrigues, R. J. (2001). Fungal symbiosis from mutualism to parasitism: Who controls the outcome, host or invader? *New Phytologist*, *151*(3), 705-716.

Rio, R. V. M., Hu, Y., & Aksoy, S. (2004). Strategies of the home-team : symbioses exploited for vector-borne disease control. *Trends in Microbiology*, *12*(7). doi:10.1016/j.tim.2004.05.001

Rodriguez, R J, White, J. F., Arnold, a E., & Redman, R. S. (2009). Fungal endophytes: diversity and functional roles. *The New phytologist*, *182*(2), 314-30. doi:10.1111/j.1469-8137.2009.02773.x

Rodriguez, Russell J, Redman, R. S., & Henson, J. M. (2003). The role of fungal symbioses in the adaptation of plants to high stress environments. *Mitigation and Adaptation Strategies for Global Change*, 261-272.

Rodriguez, Russell J., Redman, R. S., & Henson, J. M. (2005). Symbiotic Lifestyle Expression by the Fungal Endophytes and the Adaptation of Plants to Stress: Unravelling the Complexities of Intimacy. *Mycology Series* (pp. 683-696).

Saari, S. (2010). *Plant Endophytes in Food Chain- Friend or Foe?*

Saikkonen, K., Faeth, S. H., Helander, M., & Sullivan, T. J. (1998). Fungal Endophytes : A Continuum of Interactions with Host Plants. *Annual Review of Ecology and Systematics*, *29*, 319-343.

Saikkonen, Kari, Wäli, P., Helander, M., & Faeth, S. H. (2004). Evolution of endophyte-plant symbioses. *Trends in plant science*, *9*(6), 275-80. doi:10.1016/j.tplants.2004.04.005

Schloter, M., & Hartmann, A. (1998). Endophytic and surface colonization of wheat roots (Triticum aestivum) by different Azospirillum brasilense strains studied with strain-specific monoclonal antibodies. *Symbiosis*, *25*, 159-179.

Schulz, B., Rommert, A.-K., Dammann, U., Aust, H.-J., & Strack, D. (1999). The endophyte-host interaction: a balanced antagonism? *Mycological Research*, *103*(10), 1275-1283.

Shaw, L. J., Morris, P., & Hooker, J. E. (2006). Perception and modification of plant flavonoid signals by rhizosphere microorganisms. *Environmental microbiology*, *8*(11), 1867-80. doi:10.1111/j.1462-2920.2006.01141.x

Siciliano, S. D., Fortin, N., Mihoc, A., Wisse, G., Labelle, S., Beaumier, D., Ouellette, D., et al. (2001). Selection of Specific Endophytic Bacterial Genotypes by Plants in Response to Soil Contamination. *Applied and environmental microbiology*, *67*(6), 2469-2475. doi:10.1128/AEM.67.6.2469

Smith, K. P., Handelsman, J., & Goodman, R. M. (1999). Genetic basis in plants for interactions with disease-suppressive bacteria. *Proceedings of the National Academy of Sciences of the United States of America*, *96*(9), 4786-90. Retrieved from http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=21769&tool=pmcentrez&rendertype=abstract

Springer, Y. P., Hardcastle, B. a, & Gilbert, G. S. (2007). Soil calcium and plant disease in serpentine ecosystems: a test of the pathogen refuge hypothesis. *Oecologia*, *151*(1), 10-21. doi:10.1007/s00442-006-0566-1

Torres, M. S., Nacional, U., & Mar, D. (2009). *Clavicipitaceae : Free-Living and Saprotrophs to Plant Endophytes* (pp. 422-430).

Uauy, C., Brevis, J. C., Chen, X., Khan, I., Jackson, L., Chicaiza, O., Distelfeld, A., et al. (2005). High-temperature adult-plant (HTAP) stripe rust resistance gene Yr36 from Triticum turgidum ssp. dicoccoides is closely linked to the grain protein content locus Gpc-B1. *TAG: Theoretical and applied genetics*, *112*(1), 97-105. doi:10.1007/s00122-005-0109-x

Venturi, V., & Silva, D. P. D. (2012). Incoming pathogens team up with harmless "resident" bacteria. *Trends in microbiology*, 1-5. doi:10.1016/j.tim.2012.02.003

Violi, H. A., Menge, J. A., & Beaver, R. J. (2007). Root-colonizing fungus in avocado: Is it a mutualist, cheater, commensalistic associate, or pathogen? *American Journal of Botany*, *94*(4), 690-700.