

## Proposed Elimination of the Term *Endorhizosphere*

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In recent years, the term *endorhizosphere* has been used in studies of root-zone microflora. We propose that *endorhizosphere* is a semantically incorrect term that means different things to different people.

Hiltner, in 1904, originally used *Rhizosphäre* to describe the zone of soil under the influence of legume roots (12). Expansion of the rhizosphere concept to other plants soon followed; however, the term was still used to describe a niche within soil. Herein lies the semantical problem with the term *endorhizosphere*. With the base word, *rhizosphere*, referring to a soil niche, the addition of *endo* would make the word literally refer to an interior zone of the same soil niche. In other words, *endorhizosphere* describes soil, rather than a niche within the host plant. Semantically correct terms to describe the interior of roots would be *endoroot*, *endorhiza*, *hypoepidermis*, or *hyporhizoplane*. However, as we propose later, we do not believe that any specialized term is necessary.

The first use of the term *endorhizosphere* by Balandreau and Knowles in 1978 (1) reflected reports in the early 1970s that the interior of roots contained bacteria. In their discussion, Balandreau and Knowles cited work of Old and Nicholson (19,20), which in turn cited reports by Darbyshire and Greaves (5,11). In 1973, Darbyshire and Greaves (5) stated, "as microbes inside plant roots can probably affect the metabolism and particularly the exudation of the host plant, it would seem reasonable in the future to consider them as part of the rhizosphere community." Old and Nicholson (19) reported internal populations of bacteria in a study using electron microscopy, and, based on their finding of bacteria in root cortical tissues and in pits of the endodermis, they supported modification of the rhizosphere concept to include soil microorganisms that penetrate plant roots.

The first published usage of the term *endorhizosphere* occurred as a section heading in a review on the rhizosphere (1); however, no definition of *endorhizosphere* was proposed in this section, which discussed general bacterial and fungal invasion of root tissues. The first review that cited *endorhizosphere* was by Lynch (15), who stated, "the root-epidermis-cortex zone, when colonized by pathogens or non-pathogens, has been termed the 'endorhizosphere' by Balandreau and Knowles." As previously indicated, Balandreau and Knowles did not define the term. Therefore, it is unclear who should be cited as the original source of the term *endorhizosphere* and what the original definition is. In any case, the original idea of Darbyshire and Greaves (5) to consider microorganisms inside roots as part of the rhizosphere community was somehow transformed to consider only the microorganisms specifically in the root epidermis and cortex.

In addition to the semantical problem and historical confusion, we believe that the term *endorhizosphere* should not be used for the following four reasons.

First, the proposed limitation of the *endorhizosphere* to the cell layers between the epidermis and endodermis is apparently based on the premise that the endodermis forms an impenetrable barrier to bacteria. Several lines of evidence suggest that this is not the case. In the process of lateral root growth from the pericycle to the epidermis of the primary root, the endodermis and its Casparian strips are disrupted (4). Redifferentiation of endodermal cells occurs between the parent and lateral roots;

however, this process takes time (4), thereby providing a temporary apoplastic (intercellular space) pathway between the cortex and stele at sites of lateral root eruption (6). Peterson et al (21) used fluorescent dyes to trace apoplastic pathways to the stele in corn and broad bean roots at various stages of development. Tracer dyes were not found in the stele of healthy, nonwounded primary roots, but they were found in the stele when a secondary root had just emerged from the epidermis of the main root. In such cases, dyes moved through the pericycle, moved into the root xylem, and were detected in the xylem of stems. Peterson et al (21) concluded "that a continuous apoplastic pathway of transport from the root epidermis to the shoot existed in both corn and broad bean roots." The occurrence of even temporary continua in the intercellular space between the cortex and xylem would be sufficient for movement of microorganisms into the xylem and may account for recent observations of saprophytic bacteria in stems after their application to seeds or roots. Misaghi and Donndelinger (17) reisolated a rifampicin-resistant mutant of one *Erwinia* sp. strain from cotton stems, flowers, and bolls stems after inoculation by vacuum infiltration of emerged radicles.

Second, even as there is a continuum of root-associated microorganisms from the rhizosphere to rhizoplane to epidermis and cortex, so is there a continuum from the endodermis to the root xylem and to the stem. Campbell and Greaves (3) reflected a commonly held view when they stated, "microorganisms in the stele are almost invariably pathogens." However, saprophytic bacteria are also found in the stele and can move to different plant organs. Bacteria have been isolated from the interior of healthy plants, including ovules (18), seed pieces, and stems of potato (13); taproot of clover (22); roots of sugarbeet (14); and roots of alfalfa (7,8). With alfalfa, *Pseudomonas* spp., *Erwinia*-like spp., gram-positive strains, and other unidentified gram-negative bacteria were consistently isolated from root xylem (7) at populations of  $\log_{10}$  3–4 cfu/g. The effects of 33 of these vascular endophytes on plant growth were investigated in a separate study (8). One strain induced a significant reduction in root development and yield, whereas another significantly increased both parameters. Hence, bacteria within the root stele, and therefore not included in the current definition of *endorhizosphere*, may have the same positive effects on plants as rhizosphere bacteria.

The most extensive work with population dynamics of xylem-inhabiting bacteria is found in the reports from Gardner and colleagues in 1982 (10) and 1985 (9) with citrus trees. Thirteen genera were represented among 556 strains, the most frequent being *Pseudomonas* (40%); *Enterobacter* (18%); and *Bacillus*, *Corynebacterium*, and other gram-positive bacteria (40%). Inoculation of pruned roots with 19 randomly selected strains resulted in significant growth inhibition of shoots by seven strains. Two strains induced a significant increase in shoot growth. These results further demonstrate that xylem-inhabiting bacteria may also be beneficial to the plant and, therefore, are not always pathogens.

In the past few years, several reports have been published on bacterial endophytes. Sunyun and Yanxi at Beijing Agricultural University (24) reported that *Bacillus* spp., *Xanthomonas* spp., and *Erwinia* spp. were common endophytes of cotton infested with the Fusarium wilt pathogen. At another university in China, Xiao et al (27) reported that endophytic bacteria could be isolated

from stems and roots of cotton seedlings and that two endophytic strains of *Pseudomonas* spp. provided biological control of *Colletotrichum gossypii* on cotton seedlings. Misaghi and Donndelinger (17) isolated *Erwinia* sp., *Bacillus* spp., *Clavibacter* sp., and *Xanthomonas* sp. from surface-sterilized radicles, roots, and stems of cotton. McNroy and Kloepper (16) isolated 29 genera of bacteria from inside surface-disinfested cotton and sweet corn roots and stems of field-grown plants. Bacterial diversity generally declined during the growing season, and *Bacillus* spp. and *Pseudomonas* spp. predominated in mature roots and stems. Van Peer and Schippers (25) demonstrated that growth reduction of tomato seedlings in hydroponic cultures correlated with the extent of internal root colonization by endophytic fluorescent pseudomonads. Bacterization with plant-growth-promoting *Pseudomonas* sp. strain WCS417r counteracted this plant growth reduction and was accompanied by dense colonization of root interiors by WCS417r and a displacement of the indigenous endophytic pseudomonads. Lipopolysaccharide patterns, cell envelope protein patterns, and certain biochemical characteristics indicated that endophytic *Pseudomonas* strains were distinct from *Pseudomonas* strains obtained from the root surface. Endophytic strains especially were able to recolonize the interior of roots (26). Collectively, these reports suggest that bacterial colonization of internal regions of the plant, including the vascular system, is a common phenomenon.

Third, because the currently used definition of endorhizosphere is based on root anatomical boundaries, it is impractical for investigations of microbial ecology involving isolation of bacteria. Isolation of internal root bacteria involves surface disinfection followed by grinding and dilution plating. This process would obviously lead to isolation of xylem-inhabiting and cortex-inhabiting (i.e., *endorhizosphere*) bacteria, as it is impractical to dissect out or grind only the cortical tissues.

Fourth, the term *endorhizosphere* is currently being used in different ways, which adds to the confusion regarding what precise ecological niche or group of microorganisms is under investigation. One common usage of *endorhizosphere* is to describe those bacteria that are isolated from surface-disinfested roots after grinding (23), even though the definition cited by Lynch in 1982 (15) restricts the endorhizosphere to the epidermis and cortex. Bazin et al (2) recently defined *endorhizosphere* as "the region internal to the root, within which microorganisms other than pathogens or symbionts occur." This definition alters the 1982 definition in two ways: by not restricting to the epidermis and cortex and by eliminating pathogens and symbionts as legitimate members of the endorhizosphere.

### PROPOSAL

We believe that the preceding discussion adequately demonstrates that there are problems in using the term *endorhizosphere* sufficient to create confusion in reporting research results. In an effort to bring clarity to reports of investigations with root-associated microorganisms, we propose to eliminate usage of *endorhizosphere*, both as a noun describing an ecological niche and as an adjective describing microorganisms living inside that niche. We believe that there is no need to create a new term, as clarity can be gained using existing terms. Hence, we would continue to use the terms *rhizosphere* and *rhizoplane* to describe niches and microorganisms occupying niches external to roots. Microorganisms found inside roots could be referred to generally as internal root colonists and specifically as cortical colonists or vascular colonists. Alternatively, these microorganisms could be called endophytes or endorhizal microorganisms. When using the noun form for the plant niche colonized by these microorganisms, one could refer to interior tissues, the root interior, or inside roots. The precise choice of terms should be left to each researcher, and the chosen terms should be clearly defined or referenced in each report.

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