BACTERIAL SPECIES IN RELATION TO FOREST TREE DECLINE

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Abstract. Bacterial species colonize trees endophytically without causing apparent damage, but can act as facultative parasites by utilizing tissues altered by abiotic factors. Bacteria, especially anaerobic species, can cause wetwood *per se* and act as pioneer microorganisms that prepare substrates for decay fungi. *Xylella fastidiosa*, a xylem-limited bacterium, acts as a primary pathogen inciting leaf scorch and dieback in some trees. Bacterial penetration occurs via wounds in the aerial part of the plant or in the roots. Some bacteria can act as agents of ice formation (ice nucleation active bacteria) also promoting the entry of other organisms.

Decline of forest trees does not appear to be caused by pathogens acting singly. Environmental stresses (i.e., drought, insect depredations, acid precipitation to foliage and soils) assist by unbalancing tree metabolism and, eventually, causing death (15, 25). In fact, in declining species, facultative parasites, especially fungi, are dangerous only when the normal tree functions have been seriously compromised by stress factors (12, 13). In these situations bacteria and fungi clearly play a decisive role in the preparation of substrates for use by decay fungi as well as other roles still under study (26). In this paper we will review the current knowledge on the relationships between bacteria and the decline process in forest trees.

Facultative Parasites in Healthy Tissues

Although bacteria have been associated with the healthy tissues of forest trees, and serve as facultative parasites, their specific role(s) in decline has not been determined. Bacteria colonize trees endophytically without causing apparent damage but some species have the potential to react with tree tissues that have been altered physiologically. Aerobic, anaerobic, facultative anaerobic, and microaerophilic bacteria have been found in the woody tissues of apparently healthy trees (*Acer saccharum*, *Populus tremuloides*) in the absence of diebacks, wounds, wetwood, or discolored zones (1, 3), in the sapwood of *Liriodendron tulipifera* and *Liquidambar styraciflua* (36), or on healthy-appearing trees (*Quercus rubra, Q. velutina, Ulmus americana, U. rubra*, and *Populus deltoides)* containing some form of "shake" (tangential or radial separation in xylem tissues of the lower trunk) (39). The presence of such microorganisms in the tissues of apparently aseptic organs is not rare (4, 14). Knutson (16) assumed that this endophytic microflora might be utilizing tissues that were altered by wetwood caused by abiotic factors.

Bacteria *per se* cause permanent changes in the heartwood inciting (a) wetwood, with consequent slime flux on the bark and decline of the host or (b) discoloration of the heartwood. A close relation is supposed to exist between bacteria and wetwood (23); however, the direct role of bacteria in this process has not been proven experimentally with pure cultures of bacteria (16, 28). Many bacterial species, most of them anaerobic, have been isolated from forest trees showing symptoms of wetwood (*Abies alba, Populus deltoides*, and *Ulmus americana*) and from other species with discolored woody tissues (*Quercus* sp.); *Clostridium* is the genus most frequently isolated (2, 29, 35, 38). Other genera of anaerobic or facultative anaerobic groups have been recovered, such as *Bacteroides*, *Erwinia*, *Lactobacillus, Klebsiella, Edwardsiella* (29), and *Bacillus cereus* (38). Aerobic genera isolated from wetwood (the techniques used did not allow isolation of anaerobic species) include *Erwinia* and *Bacillus* in *Populus tremuloides* (16), *Xanthomonas, Agrobacterium*, *Erwinia*, and species of the coryneform group in *P. nigra* (37). The following species were isolated from discolored subcortical woody tissues of declining *Q. cerris*

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trees showing epicormic twigs and bleeding on the trunk: *Erwinia herbicola* (the most common), *Bacillus cereus, Staphylococcus sciuri, Micrococcus luteus*, and *Kluyvera criocrescens*. The apparently healthy tissues near the discolored areas contained *E. herbicola, M. luteus*, and *S. sciuri*; *Bacillus* sp. were isolated from the slime flux (30).

The association of anaerobic species (*Clostridium* sp.) with facultative anaerobes (*Bacillus* sp., *Erwinia* sp.) does not seem to be casual. One hypothesis, advanced by Schink *et al.* (29) is that the facultative anaerobic species present in the sapwood decrease the oxygen concentration, making conditions suitable for anaerobes, which probably entered through the roots. The anaerobes are then able to decompose and ferment the pectins and hemicelluloses of the cell walls forming methane, alcohols, and acids as well as wetwood. This hypothesis is supported by the fact that methane-producing bacteria of the genus *Methanobacterium* have been isolated from the same microenvironments (41). Some species associated with wetwood, such as *Clostridium* sp. and *Bacillus* sp., are able to fix atmospheric nitrogen and survive in an environment with a high carbon:nitrogen ratio (29). The recent discovery of an anaerobic nonspore-forming species of bacteria fermenting cellulose and using it as a source of energy to fix nitrogen (19) opens up new fields of research on wetwood decay.

Bacteria also may act as pioneer microorganisms which, together with some nonhymenomycetous fungal species, colonize wounds on trunks and branches leading to changes in pH, moisture, CO_2 and O_2 levels, and permitting the establishment of a succession of organisms which, in different ways and at different times, are responsible for subsequent stages of discoloration, decay, and death. Hymenomycetes, subsequently occurring, cause the most visible symptoms of tree decay.

Bacteria are commonly associated with wounds on trunks although they are usually not identified but the techniques used suggest the presence of aerobic species. All of the studies reported the presence of nonhymenomycetous fungi (24, 32, 33, 34, 36) though a recent paper states that in *Q. phellos* it is impossible to recognize a typical succession for every fungus causing decay (27). However, Cosenza (6) isolated *Bacillus*, *Pseudomonas* (the most common), and other bacteria in the family Enterobacteriaceae and in the coryneform group from trees (*Acer rubrum, A. saccharum, Betula papyrifera, B. alleghaniensis*, and *Fagus grandifolia*) with clear wounds on the trunk and with consequent wood chromatic alterations.

Primary Pathogens

Leaf scorch and diebacks of twigs and branches are aspecific symptoms associated with declining forest trees. These symptoms have been found on several urban tree species infected with *Xylella fastidiosa*, a xylem-limited bacterium (11, 17). Isolates of *X. fastidiosa* have been proved to fulfill Koch's postulates in *Q. rubra, Platanus* sp, and *Morus rubra* (14, 18, 31). Surveys for ascertaining the presence of this bacterium in declining forest trees are essential to determine if this microorganism is involved.

Bacterial Penetration

Many observations suggest that there are two main mechanisms for entry into trees by bacterial species: (a) penetration through wounds on the aerial parts of the plant (passive penetration through wounds caused by breakage of branches, coppicing, lightning, and insects), and (b) penetration through the roots (especially by anaerobic species). This initiates the subsequent establishment of bacteria in the woody parts of the tree for a very long period.

Bacterial species might also actively enter into trees as agents for ice formation (ice nucleation activity [INA] positive). In fact, some bacterial species, mainly among isolates of *Pseudomonas syringae* pv. *syringae, P. fluorescens*, and *Erwinia herbicola*, which are widespread in decaying vegetation and as epiphytic microflora, act in nature as biological sources of ice nuclei (20, 21). These nuclei catalyze the liquid to solid transition of the water (ice formation) at temperatures (-1°C) warmer than the determined temperature limits of plant supercooling (21). INA-positive bacteria might induce tiny wounds under freezing conditions

on the bark and phloem tissues of forest trees, thus opening the way for subsequent colonization by themselves, other bacterial species, and pioneer and/or pathogenic fungi.

A relationship between INA-positive bacteria and damage on trunks has been shown for peach (40) and pear (9) trees. Specific studies of INA-positive bacteria in forest trees are lacking; however, they do not seem to colonize conifer needles (22). The frequent isolation of fluorescent *Pseudomonas* sp. (105 out of 108 isolates) on declining trees was reported (6), but their capacity to nucleate ice (20, 21) has not been studied. Recently, however, INA-positive *Pseudomonas fluorescens* and *Erwinia herbicola* were isolated from declining *Quercus cerris* (Scortichini, Stead, and Rossi, in preparation). Moreover, *Pseudomonas syringae* pv. *syringae*, has been isolated from a stem canker on *Pinus radiata* (8) and from *Quercus robur* (7). Haworth and Spiers (10) reported the presence of an INA-positive *P. syringae* pv. *syringae* within leaf spots and stem cankers on poplars and willows. Finally, the frequent association of *Pseudomonas fluorescens* with anaerobic microflora in the rhizosphere of declining white fir trees (*Abies grandis*) (2) leads us to hypothesize that there may be a synergistic relationship between INA-positive species making wounds in the root system and the penetration of anaerobic bacteria.

Conclusion

Bacteria may play several important roles in declining trees, especially when pruning activities and insect vectors facilitate their penetration. Isolation and identification of the anaerobic, facultative anaerobic, aerobic, and INA-positive microflora and the subsequent inoculation with individual bacterial isolates, or in association with fungi into healthy and stressed trees, might clarify some of the relationships within woody tissues. Moreover, electrophoretic analysis of the pectolytic enzymes present in healthy and diseased woody tissues (23) might reveal the role played by anaerobic bacteria in wetwood, especially in poorly drained and/or heavy soil subjected to wounds at the collar and/or to the roots (39). Finally, it would be interesting to ascertain a correlation between the presence of INA-positive bacteria and the anaerobic microflora in the rhizosphere and wetwood development.

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Résumé. Les Bactéries peuvent coloniser les plantes de façon endophytique, sans aucun sumptome d'altération et, souvent, ils utilisent des tissue qui sont déjà altérés par des facteurs abiotiques; ils peuvent provoquer le coeur humide, surtout les espèces anaérobics et agissent comme micro-organismes "pionniers" en préparant des substrats qui, par la suite, sont dégradables par les champignons agents de pourriture. D'autre part, *Xylella fastidiosa,* une bactérie confinée dans le xylème, est un pathogène primaire, en causant des nécroses du feuillage, desséchèment et flétrissement des rameaux. Les Bactéries peuvent pénétrer dans les plantes passivement par des blessures sur la partie aérienne de l'arbre ou par les racines; en plus, un role actif a été envisagé car certaines bactéries peuvent être les noyaux pour la formation de glace.

Zusammenfassung: Beim Verfall von Forstbäumen können Bakterienarten einige Rollen spielen. Sie besiedeln Bäume durch Endophyten ohne scheinbare Schaden zu verursachen. Sie dienen als fakultative Parasiten durch die Benutzung von Geweben, die von abiotischen Faktoren verändert sind. Sie verursachen nasses Holz (wetwood) oder sie dienen als Pionier-Mikroorganismen für die Zersetzungfungi. *Xylella fastidiosa*, ein Xylem-limitiertes Bakterium, kann vielleicht als primäres Pathogen dienen, um Blattdörre anzuregen. *Bacillus spp.* hemmt das *in vitro* Wachsen von manchen Fungi, die mit Baumverfall associiert sind.