PN-AAU-789
ISN 14513
23

WHAT'S NEW

in PLANT PHYSIOLOGY

Volume 11, Number 5 1980 May

"CRYPTIC" PLANT ROOT-MICROORGANISM INTERACTIONS[1]

David H. Hubbell and Murray H. Gaskins
Departments of Soil Science and Agronomy
University of Florida
Gainesville, Florida 32611 USA

Plants in their natural environment must co-exist and interact with many kinds of microorganisms. Where phytopathogenic organisms are involved, their detrimental effects often can be easily recognized. Beneficial effects also may be readily apparent as in the case of interactions with symbiotic microorganisms (e.g., mycorrhiza, *Rhizobium*, etc.). However, many interactions occur which involve neither obviously pathogenic nor symbiotic associations. Botanists and microbiologists are becoming increasingly aware of these less conspicuous associations, whose existence is not readily detected or measured. Gradually increasing recognition of these inconspicuous plant-microorganism associations has led to the development of some useful concepts such as "spermosphere," "rhizosphere" and "phyllosphere" to denote the botanical microhabitat of particular microbial communities. The validity of these concepts is supported by the repeated observation that microbial communities often are characteristic for particular botanical microenvironments.

Recent reviews have dealt with various aspects of plant root-microorganism (R-M) interactions, including their initiation (11), establishment (4) and manipulation (5), and their significance in the ecosystem (10). Products of soil microorganisms which may affect plant growth are discussed in detail (9). The general subject of nonpathogenic R-M interactions is discussed comprehensively in a recent volume edited by Dommergues and Krupa (8).

Cryptic Interactions and Plant Growth. The more subtle of "cryptic" R-M interactions which favorably affect plant growth are of special interest because of the possibility of manipulating the biological mechanisms involved to achieve consistent plant yield increases, as in the case when a *Rhizobium* inoculant is used to increase the yield of leguminous plants. However, many R-M interactions differ from the classical legume-*Rhizobium* N-fixing systems in various ways which reduce the probability of their recognition. Their existence is not normally signalled by characteristic morphological features, as in the case of symbiotic N-fixing nodules, and the beneficial effects which accrue to the plants are reduced in magnitude. Effects often are noted as consistent trends which in conventionally designed experiments fail to reach statistical significance.

Despite the generally low magnitude and high variability of measurable effects, plant yield increases attributable to R-M associations are noted in various types of experiments. There are at least two and probably more mechanisms whereby cryptic R-M interactions beneficially affect plant growth. The most important of these are believed to be nitrogen fixation (6) and plant growth hormone production (5). Many common soil microbes not only fix nitrogen, but also produce biologically significant quantities of hormones which affect plant growth and development. It is reasonable therefore to assume that soils supporting plant growth have an inherent, microbiologically-based potential for the establishment of beneficial R-M associations. The possible number of such associations may be large and the full range of their nature and extent is completely open to conjecture. The absence of associative microbes which fix nitrogen and/or produce plant growth substances, or their presence, alone or combined, in the rhizosphere could result in a broad spectrum of plant responses. One suspects that many such associations are operative at a low level and such "cryptic" associations may remain undetected until they are intentionally and systematically sought.

Nitrogen Fixation as a Cryptic Interaction. Dobereiner and Day (6) reviewed information related to "associative" nitrogen fixation by bacteria in the rhizosphere of tropical grasses. It is generally believed that associative nitrogen fixation is of greater significance in the rhizosphere of grasses than of other species, but definitive information on this point has yet to be presented.

[1]Florida Agricultural Experiment Stations Journal Series No. 2477.

This concept is supported by recent studies of the *Azospirillum*-grass association described initially by Dobereiner and Day (6). The infection process in an axenic ("without strangers") *Panicum-Azospirillum* system has been studied by electron microscopy (14). The bacteria readily colonized the root surface but were capable of only limited intercellular invasion of the root cortex. The presence of weak bacterial pectinase activity was implicated as a factor in the infection process. In a subsequent study (15), root surface colonization events were described. Selectivity in this R-M association was observed in that azospirilla adsorbed in significantly higher numbers to grass root surfaces in comparison with other soil bacteria. Presence of azospirilla also resulted in profuse root hair formation and increased lateral root development, suggesting a hormonal stimulus. This was verified (13) by the demonstration of production of substantial quantities of auxin and lesser amounts of cytokinins and gibberellins by the bacteria and duplication of bacteria-induced root growth effects by the addition of similar quantities of the pure compounds to aseptically grown plants.

Field inoculation experiments with several grass-azospirilla systems have resulted in reasonably predictable increased yields of plant biomass (11). However, nitrogen gains have been highly variable and usually not statistically significant. These results are perhaps consistent with what is known of colonization and infection of grass roots by azospirilla, as outlined above. The bacteria predominate on the root surfaces, so there is low efficiency in the provision of an energy substrate (photosynthate) for microbial nitrogen fixation and no direct mechanism for transfer of fixed nitrogen from the bacteria to the plant. Conversely, the bacteria are ideally situated to produce hormone-induced root growth enhancement, ultimately producing better plants and higher yields. The interactions between the bacteria and plant roots are obviously complex and not readily amenable to conventional investigations.

Recent studies of this system using callus cultures have yielded considerable information related to morphological and physiological characteristics of this rhizoplane interaction. In early studies (16), it was shown that callus cultures of tobacco and some grasses did not survive inoculation with *Azospirillum* due to overgrowth by the bacteria. The bacteria showed nitrogenase activity as long as the callus survived (about one week) if the medium had little or no combined nitrogen. Sugarcane callus cultures grown under similar conditions were maintained with regular sub-culture for more than 18 months and showed appreciable nitrogenase activity. Plantlets could be regenerated from these callus-bacteria associations. These observations suggest some specificity in the establishment of this type of association which is defined by compatibility or balance of the biochemical traits of the two organisms.

Subsequent studies (3) of the sugarcane callus-*Azospirillum* system have dealt with ultrastructure of the association. The bacteria were predominantly pleomorphic (of more than one form) on callus and were strictly extracellular on viable callus cells. It was speculated that callus cells occurring in tight groups and lacking intercellular spaces may be required for establishment of long-term associations.

In recent studies (2) it was found that *Azospirillum* which had been reisolated from inoculated sugarcane callus exhibited a severalfold increase in nitrogenase activity. Bacteria grown aerobically (on callus surface or on artificial N-free media) were capsulated and pleomorphic, leading to speculation that capsulation might protect against unfavorable pO_2 under N-fixing conditions.

Significance and Implications of Cryptic Associations. The existence of such cryptic R-M associations and our increasing awareness of possible beneficial effects on plant growth present some interesting prospects for future studies. The plant-microorganism specificity frequently observed implies that factors influencing their establishment and functioning are under *genetic* control. Indeed, it has been suggested that "associative" nitrogen fixing systems are less frequent in hybrid genotypes than in more primitive genotypes because the former have been selected for maximum response to fertilizer nitrogen. By this process, the genes controlling unknown factors favoring the association could be lost. Assuming the validity of this reasoning, it may be worthwhile to return to the primitive germplasm and breed and select for genotypes showing maximum growth in nutrient deficient natural soils.

Current efforts to obtain biological nitrogen fixation in plants by direct genetic alteration of a specific plant or microorganism have been discussed by Zobel and Wallace (17). This manipulation of plant genetics to enhance biochemical characteristics which favor the natural colonization and functioning of beneficial or "associative" microorganisms at the rhizoplane has a distinct advantage. It removes the necessity to inoculate plants with appropriate microorganisms in order to achieve the desired growth response.

The obstacles to achieving successful inoculation, even in the well-defined *Rhizobium*-legume system, are substantial. Recent experience indicates that information successfully applied to this system cannot be readily extrapolated to "associative" systems, where inoculation has thus far proved unrewarding. The problem could perhaps be solved by tailoring plants which biochemically select for beneficial microorganisms at the rhizoplane. The initial presence of such organisms may in most cases be non-limiting due to their ubiquitous occurrence in agricultural soils. The primary limitation may be the existence of soil conditions generally favorable for reasonable plant growth.

Research which will define the principles and establish the means of obtaining plant genotypes favoring specific R-M associations has additional implications. The widespread occurrence and complexity of antagonistic interactions between soil microorganisms is well documented (1). Knowledge of such interactions which result in the suppression of one organism by another have been the basis for numerous attempts to achieve biological control of phytopathogens. This approach has been unsuccessful in most cases, probably due to failure of the antagonist (control agent) to establish or function under non-controlled field conditions.

It is conceivable that many plants naturally harbor microbial communities at the rhizoplane which are significantly protective by virtue of their ability to suppress potential phytopathogens. Elucidation of the R-M interactions involved in such systems could provide the guidelines for developing plant genotypes with enhanced ability to favor protective associations. These guidelines would include identification of plant and microbial traits which are important in establishing such interactions and methods for their detection and assay in breeding and selection programs.

The concept of developing plant genotypes having the inherent ability to favor the establishment and functioning of beneficial root microflora need not be restricted to "cryptic" R-M associations. The concept may be in principle extended to enhancement of such obvious classical systems as the mycorrhizal and *Rhizobium*-legume associations.

Determination of the validity of the concept of custom-made beneficial R-M associations (new or enhanced) awaits the future close cooperative efforts of plant physiologists and microbiologists.

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9411 Warfield Road
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