The Rhizosphere

Biochemistry and Organic Substances at the Soil-Plant Interface

Second Edition

edited by

Roberto Pinton
Zeno Varanini
Paolo Nannipieri

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Preface to the First Edition

The research on plant – soil interaction is focused on the processes that take place in the rhizosphere, the soil environment surrounding the root. Many of these processes can control plant growth, microbial infections, and nutrient uptake.

The rhizosphere is dominated by organic compounds released by plant roots and microorganisms. Furthermore, stable components of soil organic matter, namely, humic and fulvic substances, can influence both plant and microorganism. A variety of compounds are present in the rhizosphere, and they range from low-molecular-weight root exudates to high-molecular-weight humic substances. The chemistry and biochemistry of these substances is becoming increasingly clear, and their study promises to shed light on the complex interactions between plant and soil microflora.

The aim of the book is to provide a comprehensive and updated overview of the most recent advances in this field and suggest further lines of investigation. As an interdisciplinary approach is necessary to study such a complex subject, the book provides a good opportunity to summarize information concerning agronomy, soil science, plant nutrition, plant physiology, microbiology, and biochemistry. The book is therefore intended for advanced students, and researchers in agricultural, biological, and environmental sciences interested in deepening their knowledge of the subject and developing new experimental approaches in their specific field of interest.

The first chapter defines the spatial and functional features of the rhizosphere that make this environment the primary site of interaction between soil, plant, and microorganisms. Among the multitude of organic compounds present in the rhizosphere, those released by plant roots are the most important from a qualitative and quantitative point of view; furthermore, the relationships with soil components of any released compound need to be considered (Chapter 2). The release of these compounds strongly depends on the physiological status of the plants and is related to the ability of plant roots to modify the rhizosphere in order to cope with unfavorable stress-inducing conditions. These aspects are discussed in Chapter 3, with particular emphasis on water and physical, and nutritional stresses. A thorough analysis of how root exudates may influence the dynamics of microbial populations at the rhizosphere will be provided in Chapter 4. However, the importance of the role played by biologically active substances produced by microbial populations cannot be underrated, and the organic compounds acting as signals between plants and microorganisms must be identified and characterized (Chapter 7). In this context the biochemistry of the associations between mycorrhizae and plants (Chapter 9) and the interaction between rhizobia and the host plant (Chapter 10) is also considered.

It has been long recognized that both roots and microorganisms compete for iron at the rhizosphere; a wealth of literature is already available on this subject, and many studies on the production of siderophores by microbes are being carried out. Their potential use by plants and the relationship with other plant-borne iron chelating substances is still a matter of debate (Chapter 8). The fulfillment of the nutritional requirement of plants and microorganisms also depends on the processes leading to mineralization and humification of organic residues (Chapter 6). The presence of humic and fulvic substances can have a considerable effect on root habitability, plant growth, and mineral nutrition (Chapter 5). Knowledge of these aspects needs to be reconsidered at the rhizosphere (Chapter 5 and Chapter 6). The development of specific models can shed light on the events taking place at the rhizosphere (Chapter 11). Validation of the models and a better understanding of these phenomena may come from the correct use and development of new experimental approaches (Chapter 12).

We realize that the information in the book is still largely descriptive and that the interdisciplinary view of the causal relationships in the rhizosphere is still in its infancy. Nevertheless, we do hope that our efforts and these high-quality scientific contributions will stimulate further interest in and work on this fascinating topic.

Roberto Pinton
Zeno Varanini
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Udine, Florence

Preface to the Second Edition

After the first edition of this book published in 2001, interest in rhizosphere research very much increased, becoming a major area of scientific research, and some important steps ahead have been taken in the area covered by the book. This is testimonied by the vast array of publications and the number of meetings focusing on this subject. On this basis we developed the idea to edit a second edition of the book. The book structure remained essentially the same, reflecting the multidisciplinary approach of the first edition. Most of the original chapters were maintained, expanded, and updated. The second edition contains new information obtained since the first edition was published, which integrates material coming from the first edition that still remains valid.

Furthermore, some new chapters have been included that deal with areas gaining increasing importance for understanding the complex biochemistry of soil–microbe–plant interactions. Subjects have been added that describe the role of nutrient availability in regulating root morphology and architecture (Chapter 5), and the involvement of root membrane activities, besides the well-known release of exudates, in determining (and responding to) the nutritional conditions at the rhizosphere (Chapter 6). Molecular signals between root–root (including allelopathy) and root–microbe, excluding those involving rhizobia and mychorriza, have been discussed (Chapter 10). Manipulation of microbial population for biocontrol and rhizosphere management has been also considered (Chapter 11). Gene flow in the rhizosphere has been discussed for its important role in the evolution of rhizosphere microorganisms and their coevolution with plants (Chapter 14), and in relation to the fate of genetically modified organisms added to the soil–plant system.

All the chapters contain new information deriving from a molecular approach, which contribute to a better understanding of the biochemical processes occurring in the rhizosphere.

We do hope that the efforts made by the editors and by the different contributors can help to make more vigorous the interconnection among scientists interested in rhizosphere biochemistry and molecular biology.

Roberto Pinton
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The Editors

Roberto Pinton is full professor of plant nutrition and head of the Department of Agricultural and Environmental Sciences at the University of Udine, Italy. The author or coauthor of over 160 articles, abstracts, proceedings, and book chapters, he is a research fellow of the Alexander von Humboldt Foundation and a member of the steering committee for the International Symposium on Iron Nutrition and Interactions in Plants. He is topic editor for the section on root and rhizosphere and a member of the editorial advisory board to the *Encyclopedia of Plant and Crop Science* (Dekker).

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Paolo Nannipieri is full professor of agricultural biochemistry and head of the Department of Soil Science and Plant Nutrition at the University of Florence, Italy. The author or coauthor of over 200 articles, abstracts, proceedings, and book chapters, he is the editor of four international books, editor-in-chief of *Biology and Fertility of Soils*, and a member of the editorial boards of *Archives of Agronomy and Soil Science* and *Arid Soil Research and Rehabilitation*. He has been president of the Italian Society of Agricultural Chemistry, and he is chairman of soil biology division in the International Union of Soil Sciences.

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Table of Contents

1 Types, Amounts, and Possible Functions of Compounds Released into the Rhizosphere by Soil-Grown Plants

Nicholas C. Uren

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I. INTRODUCTION

The rhizosphere is defined here as that volume of soil affected by the presence of the roots of growing plants. The overall change may be deemed biological, but chemical, biological, and physical properties of the soil in turn are affected to varying degrees. A multitude of compounds are released into the rhizosphere of soil-grown plants, most of which are organic compounds and are normal plant constituents derived from photosynthesis and other plant processes (Table 1.1). The relative and absolute amounts of these compounds produced by plant roots vary with the plant species, cultivars, plant's age, and environmental conditions including soil properties, particularly, the level of physical, chemical, and biological stress and so on [1,2,15–18].

An impression one gets from the voluminous literature on the rhizosphere and related topics is that the rhizosphere is deemed by many as a feature of not only soil-grown plants but also of those plants grown *in vitro* in any sort of medium. If that is the case, then a new name other than *rhizosphere* is required for those media other than soil. Similarly, one gets the impression that each and every compound released has a specific role or function, but the reality is that very few proposed effects are established; some are feasible, and some, probably the majority, must remain speculative and unproven. From the time this chapter was first completed in 1999 [19], nothing seems to have changed in the sense that there is no shortage of enthusiastic speculative reviews or of fanciful titles (for example, see Reference 20 and Reference 21).

It is salutary to read the review of Rovira [22], a renowned rhizosphere researcher, where he records significant progress in the understanding of the rhizosphere and how his optimism, born in the 1960s, has transformed into frustration in the 1990s. Admittedly, the complexity of the system

TABLE 1.1
Organic Compounds Released by Plant Roots

Sugars and Polysaccharides

Arabinose, desoxyribose, fructose, galactose, glucose, maltose, mannose, mucilages of various compositions, oligosaccharides, raffinose, rhamnose, ribose, sucrose, xylose

Amino Acids[a]

α-alanine, β-alanine, α–amino adipic, γ-amino butyric, arginine, asparagine, aspartic, citrulline, cystathionine, cysteine, cystine, deoxymugineic, 3-epihydroxymugineic, glutamine, glutamic, glycine, homoserine, histidine, isoleucine, leucine, lysine, methionine, mugineic, ornithine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, valine

Organic Acids[a]

Acetic, aconitic, aldonic, ascorbic, benzoic, butyric, caffeic, citric, *p*-coumaric, erythonic, ferulic, formic, fumaric, glutaric, glycolic, glyoxilic, lactic, malic, malonic, oxalacetic, oxalic, *p*-hydroxy benzoic, piscidic, propionic, pyruvic, succinic, syringic, tartaric, tetronic, valeric, vanillic

Fatty Acids[a]

Linoleic, linolenic, oleic, palmitic, stearic

Sterols

Campesterol, cholesterol, sitosterol, stigmasterol

Growth Factors

p-amino benzoic acid, biotin, choline, *n*-methyl nicotinic acid, niacin, pantothenic, vitamins B_1 (thiamine), B_2 (riboflavin), and B_6 (pyridoxine)

Enzymes

Amylase, invertase, peroxidase, phenolase, phosphatases, polygalacturonase, protease

Flavonones and Nucleotides

Adenine, flavonone, guanine, uridine or cytidine

Miscellaneous

Auxins, *p*-benzoquinone, scopoletin, hydrocyanic acid, 8-hydroxyquinoline, glucosides, hydroxamic acids, luteolin, unidentified ninhydrin-positive compounds, unidentified soluble proteins, reducing compounds, ethanol, glycinebetaine, inositol and myo-inositol-like compounds, Al-induced polypeptides, dihydroquinone, quercetin, quercitrin, sorgoleone

[a] The pKa's of these acids and the pH of the solution will determine the form that these acids adopt.

Source: From Rovira, A.D., *Bot. Rev.*, 35, 35, 1969; Hale, M.G. et al., *Interactions between Non-Pathogenic Soil Micro-Organisms and Plants,* Dommergues, V.R. and Krupa, S.V., Eds., Elsevier, Amsterdam, 1978, p. 163; Kraffczyk, I. et al., *Soil Biol. Biochem*., 16, 315, 1984; Curl, E.A. and Truelove, B., *The Rhizosphere*, Springer-Verlag, New York, 1986; Schönwitz, R. and Ziegler, H., *Z. Pflanzenernähr. Bodenk.*, 152, 217, 1989; Einhellig, F.A. and Souza, I.F., *J. Chem. Ecol*., 18, 1, 1992; Basu, U. et al., *Plant Physiol.,* 106, 151, 1994; Fan, T.W.M. et al., *Anal. Biochem.,* 251, 57, 1997; Shinmachi, F., *Plant Nutrition for Sustainable Food Production and Environment,* Ando, T., Fujita, K., Mae, T., Matsumoto, H., Mori, S., and Sekiya, J., Eds., Kluwer Academic Publishers, Dordrecht, 1997, p. 277; Bais, H.P. et al., *Plant Physiol*., 128, 1173, 2002; Dakora, F.D. and Phillips, D.A., *Plant Soil*, 245, 35, 2002; Inderjit, S. and Weston, L.A., in *Root Ecology*, de Kroon, H. and Visser, E.J.W., Eds., Springer-Verlag, Berlin, 2003, chap. 10; Rumberger, A. and Marshner, P., *Soil Biol. Biochem*., 35, 445, 2003; Vivanco, J.M. et al., *Ecol. Lett.*, 7, 285, 2004. With permission.

is great, daunting at times, and a source of some frustration. But another source of frustration has been born out of unfulfilled expectations — expectations that were, as it turned out, unrealistically high. A period of rationalization may be arising as, for example, Vaughan and Ord [23] prefer to refer to phenolic acids as *phytotoxins* rather than allelochemicals because the proof of allelopathy is difficult to establish; other authors may not be quite so circumspect. Similarly, Jones et al. [24] state: "Root exudates released into the soil surrounding the root have been implicated in many mechanisms for altering the level of soluble ions and molecules within the rhizosphere, however, very few have been critically evaluated." Further similar cautionary statements have been made [25–29] and none more apt than the following by Farrar and Jones [30]:

> Root exudation cannot be simply explained by a single mechanism but is moreover a combination of complex multidirectional fluxes operating simultaneously. While we currently possess a basic understanding of root exudation, its overall importance in plant nutrition, pathogen responses, etc., still remains largely unknown. Future research should therefore be directed at quantifying the significance of root exudates in realistic plant–soil systems.

Many of the problems arise out of the extrapolation of what happens in solution cultures to soils. Although solution cultures have served, and continue to serve, very useful functions in basic research of plant science, they differ from soils in several important ways: (1) the surface area available in soils for processes such as sorption is much greater than in solution cultures, (2) solution cultures are mixed continuously, (3) the microbial ecology differs greatly between the two media, and (4) the status of water and O_2 in the two systems is usually quite different. It is not difficult to find quotes from eminent plant scientists that indicate their support for this general view. For example, "Laboratory studies blind us to the complexity found by careful study of roots in soil" [31] and "The idea, however, that the laboratory control is the norm is false and can lead to misunderstanding and poor predictions of behavior" [32].

In this chapter I have continued to take a skeptical view, as I did previously [19], of the rosy pictures so commonly painted in the current literature. The role of the devil's advocate has been adopted to raise issues that are too readily disregarded or assumed glibly to be true. Although some attention will be given to the quantitative aspects of the release of root products, the main purpose is to classify types of root products on the basis of their known properties and perceived roles in the rhizosphere.

Because most phytoactive compounds released by plants do not persist in soil in a free and active form for very long, it appears implausible that they should be implicated in a process of infection or nutrient acquisition, for example. However, circumstances in the rhizosphere or at the root–soil interface may arise, through normal root growth through soil that preserve the compound and its activity, and thus facilitate the process. Because the theory of Mn uptake in soils of neutral and alkaline pH failed to adequately explain the observed facts, the "right set of circumstances" was invoked by Uren and Reisenauer [25] to explain how labile reducing agents secreted by roots may be protected physically from O_2 and microbial degradation, and thus be preserved to react with insoluble oxides of Mn at the root–soil interface. Provided that the right set of circumstances occurs sufficiently and frequently, then the Mn needs of the plant will be met, but if not, then deficiency prevails. The right set of circumstances may have relevance in other situations where other types of root exudates remain phytoactive, in what appears to be a hostile environment. Such possibilities are discussed later in this chapter.

This chapter considers the various types of root products with a potential functional role in the usually tough environment of soil. Only direct effects of immediate benefit to plant growth, e.g., an increase in nutrient solubility, will be considered here. Although root products of a plant species may have a direct effect on important groups of soil organisms such as rhizobia and mycorrhizae, their effect on the plant is not immediate; these and other aspects related to microbial activity in the rhizosphere will not be considered here (see Chapter 3, Chapter 9, and Chapter 11). For some reviews of the microorganisms in the rhizosphere, the reader is referred to Bowen and Rovira [33] and to more recent works [34–38].

II. ROOT GROWTH, THE RHIZOSPHERE, AND ROOT PRODUCTS

Roots are linear underground organs of plants that grow through soil with a complex architecture, a three-dimensional configuration, which in turn secures the plant and facilitates the exploitation of soil for nutrients and water [39,40]. The complexity of root growth and the architecture of root systems are illustrated in several reviews [41–43] (see also Chapter 5), whereas more recent emphasis has been on the influence of nutrients on root morphology and architecture [44–46]. The pattern of root growth, given adequate supplies of nutrients, is determined largely by the type of plant, the soil water potential, and its interaction with soil structure. The growth of roots is such that in soil under ideal conditions, favoring full exploitation of nutrients and water, roots avoid one another and rarely do neighboring roots interfere with one another; the heterogeneity of soil structure tends to separate roots spatially [47]. Under suboptimal soil conditions where yield is decreased, any configuration of the root system imposed by the conditions cannot make good the deficit.

The successful models of nutrient uptake have included the parameter for root surface area, or proxies for surface area, such as root length and radius or rate of root growth and radius (see Chapter 12). The sensitivity analysis carried out by Silberbush and Barber [48] reinforced what the modelers perceived to be important. Those soil conditions that inhibit root elongation, such as Al toxicity, high bicarbonate activities, and heavy metal toxicity, restrict the uptake of the least mobile nutrients and so, for example, iron chlorosis is a common symptom of heavy metal toxicity.

The rhizosphere forms around each root as it grows because each root changes the chemical, physical, and biological properties of the soil in its immediate vicinity. The rhizosphere along the axis of each root can be described in terms of the longitudinal and radial gradients that develop as a result of root growth, nutrient and water uptake, rhizodeposition, and subsequent microbial growth. In solution cultures most of these gradients tend to be obliterated by the active mixing, such that a phytoactive compound released at one point on the root axis may have an impact on or at another more distal (nearer the root apex) point, a situation that may have little or no relevance for soil-grown plants. Further, although reabsorption of sugars and amino acids occurs in solution-based systems [49], it seems unrealistic that root exudates can play a major role in plant growth, because they are highly biodegradable and assimilated by microbes in the rhizosphere.

The evaluation of rhizodeposition in soil in terms of not only the quantities of compounds released by roots and their identification but also the sites of release, their fate, and their impact remains a major difficulty faced by researchers in this field [50–56], see Chapter 13. When faced with such an apparently intractable situation, one might ask, “Does it matter?” Maybe for most root products it does not matter as it is really quite remarkable that, given adequate light and support, healthy vigorous plants can be grown in sterile nutrient solutions. The point here is that it is possible, that in most respects plants can manage quite well without the majority of effects that have been proposed to occur in the rhizosphere.

Rhizodeposits stimulate microbial growth because of their high energy and C content, and thus they will tend to decrease the availability of nutrients in the region of the rhizosphere where they are released from the root. Because the apical regions of the root (i.e., from the root hair zone to the root apex) have extracted most nutrients that are available for uptake before there is extensive colonization of the rhizosphere by saprophytic microorganisms, there is no impact on plant growth but microbial growth is likely to be limited. Merckx et al. [57] found that microbial growth in the rhizosphere of maize was limited by the depletion of mineral nutrients. Similarly, it was found that microbial respiration was not limited in the rhizosphere of winter wheat by available C [58], which in turn indicates, as one would expect, that perhaps some other nutrient element is limiting. The high C-to-N ratio of root products leads to the suggestion that N may be significant [59], and so it is not a surprise that rhizodeposition by maize plants enhanced microbial denitrification and immobilization of N in the rhizosphere [60]. Immobilization of other nutrients may occur also, but, as suggested earlier, it may not be a problem for the plant because root extension and absorption of nutrients occurs at a more rapid rate than the colonization of the rhizosphere with competing

microorganisms. Ultimately, nutrients immobilized by microbial assimilation are likely to become available (recycled) following the death and degradation of microbes and microfauna, but the timing and location of such events in relation to the nutrient-absorbing regions of the root has not been pursued, probably because of the difficulties involved. For example, Mary et al. [61] measured the recycling of C and N during the decomposition of root mucilage, glucose, and roots by simply mixing the substrates with soil at low rates of addition. Although mixing by soil fauna and cultivation may occur in some circumstances such as annual plants, is it realistic where, say, perennial plants are growing?

High root densities may lead to the overlapping of rhizospheres but not in a consistent way, and extensive overlapping is more likely to be the exception rather than the rule. Some poor soil structures, e.g., cloddiness, may cause clumping of roots, but such an arrangement would not appear to be the preferred way, and it is unlikely to confer any benefits upon the plant's growth. Even if there are any benefits, they are not sufficient to make good the decrease in plant yield brought about by poor structure. Although some of the contrived systems of studying plant root exudates utilize clumping of roots in an attempt to mimic the rhizosphere (see Reference 62), the effects measured in those systems may reflect what happens when roots are clumped together, nothing more, and not what happens in more normal and desirable circumstances in soil-grown plants in the field. In some pot and field experiments, the Fe status of peanuts was increased by close intercropping with maize [63], and although the cause is no doubt due to an increase in the availability of Fe in the intermingling rhizospheres, there is no direct evidence to support the contention that phytosiderophores were responsible [64].

A common situation that requires some consideration is the emergence of lateral roots. The apices of these roots must grow through the rhizosphere of the superior axis from which they originate, and thus may experience specific effects related to the type of exudates produced by the main axis and the microbial population. If the situation in nonsterile solution cultures has relevance in soil, then the high level of bacterial colonization of the rhizoplane observed near emerging laterals of maize in solution cultures [65] may be significant. However, any effects on the new lateral root apex, which may be significant and quite specific, have not been investigated.

Root products are all the substances produced by roots and released into the rhizosphere (Table 1.2) [25]. Although most root products are C compounds, the term includes ions, sometimes O_2, and even water. Root products may also be classified on the basis of whether they have either a perceived functional role (excretions and secretions) or a nonfunctional role (diffusates and root debris). Excretions are deemed to facilitate internal metabolism such as respiration, whereas secretions are deemed to facilitate external processes such as nutrient acquisition [25]. Both excretion and secretion require energy, and some exudates may act as either. For example, protons derived from CO_2 production during respiration are deemed *excretions*, whereas those derived from an organic acid involved in nutrient acquisition are deemed *secretions*.

TABLE 1.2
Root Products: A Classification

Product	Compound
Root exudates	
Diffusates	Sugars, organic acids/anions, amino acids, water, inorganic ions, oxygen, riboflavin etc.
Excretions	Carbon dioxide, bicarbonate ions, protons, electrons, ethylene, etc.
Secretions	Mucilage, protons, electrons, enzymes, siderophores, allelochemicals, etc.
Border cells	Root cap cells separated from the root apex
Root debris	Cell contents, lysates, etc.

Source: From Uren, N.C. and Reisenauer, H.M., *Adv. Plant Nutr.,* 3, 79, 1988. With permission.

The name *root border cells* has been adopted for those root cap cells that separate during growth from the root apex [66]. Most plant species, but not all, appear to exhibit this release of border cells *in vitro*, particularly in free water [67]. In soil, maize border cells have been observed to remain intact and alive among root hairs in the rhizosphere [68–70] and to continue secretion of mucilage for up to 3 weeks after separation [71]. In 1988, Uren and Reisenauer [25] suggested that "if these cells had fulfilled their functions of protection, secretion of mucilage, and geotropic response near the root cap, then it appears wasteful of photosynthate that they should not serve another purpose in association with more proximal regions of the root." Although many other roles have been proposed for border cells [67,69,72–74], it would seem, thus far, "to date, research on root cap biology and its relationship with the rhizosphere has raised more questions than it has answered" [67].

Most root debris comes from the senescence of cortical cells, an event which may be the trigger for infection with mycorrhizae, but it is probably of little, real, direct consequence for plant growth in fertile soil. The possibility that phytohormones such as indole acetic acid, cytokinin, and abscisic acid produced by rhizosphere bacteria of field-grown maize [75] do have a consistent effect on plant growth has yet to be established. The production of plant growth-regulating substances in the rhizosphere has been reviewed extensively by Arshad and Frankenberger [76], and they conclude that there are many unresolved aspects when it comes to the consideration of *in situ* events and circumstances.

Root products as defined by Uren and Reisenauer [25] represent a wide range of compounds. Only secretions are deemed to have a direct and immediate functional role in the rhizosphere. CO_2, although labeled an excretion, may play a role in rhizosphere processes such as hyphal elongation of vesicular-arbuscular mycorrhiza [77]. Also, root-derived CO_2 may have an effect on nonphotosynthetic fixation of CO_2 by roots subject to P deficiency and thus contribute to exudation of large amounts of citrate and malate as observed in white lupins [78]. The amounts utilized are very small and, in any case, are extremely difficult to distinguish from endogenous CO_2 derived from soil and rhizosphere respiration.

III. AMOUNTS RELEASED

The bulk of root products are C compounds derived from products of photosynthesis. The root products that are not C compounds are few (H^+, inorganic ions, water, electrons, etc.), but nevertheless they are deemed to be highly significant. Both H^+ and electrons may be secreted as C compounds in the form of undissociated acids and reducing agents, respectively, but plasma membrane-bound entities are believed to be the main sites of H^+ and electron transport [79–81]. The origins of root-mediated pH changes in the rhizosphere have been discussed by Hinsinger et al. [82], whereas Ryan et al. [83] do not exclude the possibility of exudation of undissociated organic acids. The reducing capacity of roots has been known ever since it has been discovered that they require oxygen. Schreiner et al. [84] found that selenite was reduced to metallic Se by wheat roots in solution culture, and Lund and Kenyon [85] showed that onion roots reduced methylene blue, but nonsterile conditions prevailed in these and other similar experiments. Uren [86] found that sterile sunflower roots reduced an insoluble higher oxide of Mn impregnated into filter paper and showed unequivocally that roots in their own right secreted reducing agents.

Estimates of the amounts and proportions of photosynthate committed to roots and to root products vary considerably, and the shortcomings associated with measurements have been critically and realistically reviewed [27,87,88] (see also Chapter 13). The units used, or those which might be used, need closer attention. In relation to the cycling of C rhizodeposition, rates of kg C ha^{-1} y^{-1} are appropriate, whereas micromoles per unit root length per hour might be more appropriate in relation to the secretion of phytosiderophores. Because uptake, for example, may be restricted to a specific region of each root, then the units of micromoles per unit root length per hour might be even more relevant still. Darrah [27] concludes that the major challenge to quantify the individual flux components of rhizodeposition remains. Obviously, further huge challenges exist as so little is known of the timing of the release (in relation to the stage of growth and development), the sites of the release, and other aspects of individual secretion.

TABLE 1.3
Rough Estimates of the Fates of Carbon Fixed by Soil-Grown Plants

Photosynthesis = 100%
- Shoots = 50%
  - Shoots = 45%
  - Respiration = 5%
- Roots = 50%
  - Root biomass = 25%
  - Root products = 25%
    - Respiration = 15%
    - Root debris including border cells = 10%
    - Diffusates <1% (guess)
    - Secretions <1% (guess) includes mucilage — may be more

Note: Amounts and relative proportions depend on species, cultivars, environmental conditions, health, age, level of chemical, physical and biological stress, and so on.
Source: From Darrah, P.R., *Plant Soil,* 187, 265, 1996; Whipps, J.M., *The Rhizosphere*, Lynch, J.M., Ed., John Wiley and Sons, Chichester, U.K., 1990, p. 59; Lynch, J.M. and Whipps, J.M., *The Rhizosphere and Plant Growth,* Keister, D.L. and Creagan, P.B., Eds., Kluwer Academic Publishers, Dordrecht, 1991, p. 15. With permission.

All the variables aside, approximately 50% of fixed C is committed to roots (Table 1.3). Fifty percent of this C is retained as root tissue and the other 50% is root products. Three fifths (15% of the net fixed C) is used in root respiration and two fifths (10%) make up border cells, root debris, diffusates, and secretions. Of the latter, border cells and root debris predominate ahead of secretions (largely mucilage), with diffusates making up the difference [27,87,88]. The contribution of root border cells is difficult to estimate, but as Griffin et al. [89] estimated that for sterile peanuts grown in solution culture "95 to 98% of the sloughed organic matter plus total sugars lost by roots is sloughed organic matter," the contribution might be significant. By comparison, 5 to 10% of C deposited by maize roots in sand culture was attributed to sloughed cap cells [90]. More recent estimates (for example, see Reference 91) are not greatly different, and so at present we have to accept these rough estimates in much the same way that we acknowledge that many factors affect the proportion released by roots and that sick or stressed plants make a larger commitment than healthy plants [25]. It is often construed without much evidence for soil-grown plants that such an extra commitment is a controlled response to stress, which in turn enables the plant to overcome the stress, the so-called *stress response*. Ryan et al. [83] emphasize the need for caution when stressed plants are involved: "It is important to remember that P-deficient plants are stressed plants, and every metabolic perturbation that they display will not necessarily be directed toward increasing P availability in the rhizosphere." In field soil, the environment is not nearly as friendly as in solution cultures, and incontinent roots are likely to encourage infection by pathogens as much as by beneficial or saprophytic microorganisms. There must be a limit to what quantities and proportions of the fixed C can be lost in stress responses but, as with quantitative estimates of exudation, they must remain as uncertain at best.

In an investigation of the phytotoxicity and antimicrobial activity of (±)-catechin exuded by knapweed roots, extraction of the soil with absolute methanol for 24 h gav concentrations ranging from 292 to 390 μg/g [10]. Assuming a bulk density of 1 g cm^{-3} and a volumetric water content of 0.25, the concentration of catechin in the soil solution would be of the order of 1200 μg/ml (approximately, 4 m*M*), which is to be compared with the 50 to 60 μg/ml of (–)-catechin required to give an allelopathic response in *Arabidopsis* seedlings. The extracted concentrations in the soil would

amount to about 1% of the total soil organic matter and as such appear abnormally high. However, as there have been so few studies of the chemical forms and activities of potential allelochemicals in soils, we have little idea of what is normal or abnormal, and thus caution is required in evaluating new data.

For wheat plants grown to maturity under irrigation [92] in a soil of neutral pH [93], one can calculate that a mature wheat plant (yield 13.2 g dm [dry matter]) with a Mn concentration of 42 mg kg^{-1} took up 556 μg of Mn (i.e., 556/55 × 2 equivalents of Mn). If ascorbic acid (M_{AA} = 176), or another reducing agent of similar equivalent weight and C content, is assumed to reduce insoluble Mn oxides at the root–soil interface, then it can be calculated that an amount of ascorbic acid equivalent to about 0.01% of the total C in the mature plant needs to be secreted to give the concentration of 42 mg Mn/kg in the mature plant. In this calculation it is assumed that all the Mn in the plant comes from the reduction of insoluble Mn oxides and that every molecule of reductant hits its target with concomitant uptake of the Mn^{2+} formed. As the root system is made up only 1.7% of the total dry matter in these mature wheat plants, it is difficult to give a more precise estimate of the proportion of the total C attributed to Mn mobilization. Nevertheless, 0.01% is near the maximum, and values less than 0.01% are realistic.

Similar calculations might be performed for other secretions, which are neither changed nor consumed in their interaction with soil entities. For example, complexation of Fe^{3+} by a ligand secreted by the root involves first the diffusion of the ligand away from the root to the insoluble oxides of Fe, a complex forms between the ligand and Fe^{3+}, and then, if the appropriate activity gradient exists, the complex diffuses to the root. At the plasmalemma, if the Fe^{3+} is complexed by a phytosiderophore [59] it is absorbed by the root, but if the Fe^{3+} is separated from the ligand, then the ligand is free to diffuse back into the soil, and so the process may continue. The quantities of root secretion required in such a "search and fetch" role are likely to be much less than the earlier case for Mn, where the secretion is destroyed, or in cases such as Al toxicity, where it is important that the secretion stays associated with the metal (if not permanently, then at least until the root tip has progressed beyond the point of interaction).

IV. TYPES OF ROOT PRODUCTS: SECRETIONS AND THEIR ROLES

Root products contain probably every type of compound that exists in plants, except for chlorophyll and other specific compounds associated with photosynthesis (Table 1.1). The range of compounds is increasing with the increasing sensitivity and analytical capabilities of modern equipment. For example, Fan et al. [8] analyzed comprehensively the root exudates of iron-stressed barley plants with multinuclear or multidimensional nuclear magnetic resource (NMR) and silylation gas chromatography/mars spectrometry (GC-MS), not only bypassing tedious traditional methods but also detecting unknown and unexpected ligands.

Most root products are by-products, which represent some of the costs of growth and development, and, except in the development of symbiotic relationships, they simply become the substrate for attendant microorganisms. With time the organic C compounds are converted progressively to either CO_2 or into recalcitrant forms of organic matter (e.g., humins). There may be indirect effects associated with heterotrophic activity, which may be either harmful or beneficial, but these will not be discussed here (see Chapter 3). Of the other root products, secretions that facilitate external processes are our primary interest here, and they are discussed in the following text.

Root products may be classified on the basis of their (1) chemical properties such as composition, solubility, stability (e.g., hydrolysis and oxidation), volatility, molecular weight, etc., (2) site of origin, and (3) established, not just perceived, functions. The chemical properties in turn determine their biological activity and how the compounds will behave in soils; their persistence in soil is very much an outcome of their chemical behavior, particularly sorption and biodegradability. Root products as chemical signals, and issues relating to their persistence, etc., are discussed in Chapter 11.

The persistence of a secretion and the likelihood that it will reach an appropriate nutrient source and be effective in its role is a prime concern. A secretion must be free to diffuse through a portion of the rhizosphere, but a sort of tyranny of distance exists. The longer it takes, or the further it must travel, then the greater is the chance that it will be rendered ineffective by either microbial degradation or assimilation, or chemical degradation or reaction, or by sorption, or by a combination of these processes. Low-molecular-weight exudates (sugars, amino acids, and other organic acids), the so-called diffusates, may be more mobile, but usually they are more readily assimilated by a wider range of microorganisms than are high-molecular-weight compounds such as mucilage, although Mary et al. [61] found the mineralization rate of C from mucilage of maize roots to be comparable with that of glucose.

Secretions may be classified also on the basis of their biological activity. Some of the classes are phytohormones, ectoenzymes, phytoalexins, allelochemicals, and phytotoxins, or referred to as "chemical signals" (see Chapter 11). However, whether or not they can exert their potential activity, which so often has been illustrated in solution cultures or under axenic conditions, depends on their survival in the soil, being at the right place, and for long enough at appropriate concentrations. All these preceding classes of compounds, except for phytoalexins and protectors against toxic Al, are usually secreted during normal plant growth in the absence of stress. Phytoalexins are secreted in response to an external stimulus (infecting organisms), which presumably is a chemical compound, whereas in the case of Al toxicity the plant response in soil occurs if the Al is present in a mobile and active form [83]. The possible roles of some different types of root secretions are given in Table 1.4.

The growth of roots through soil is perceived often as improving soil structure for plant growth. In the context of this review, the question is whether or not a plant's root products directly improve the soil structure for the growth of that plant. However, it is a difficult question to answer, because in addition to the release of root products there may be shearing and compression that together, in turn, may tend to destabilize aggregates [94], perhaps with some benefits (e.g., mineralization of physically protected organic N). Accompanying root elongation and radial expansion, there is the

TABLE 1.4
Possible Roles of Some Different Types of Root Secretions

Role	Action
Acquisition of nutrients	
Fetchers	Seek and fetch, e.g., phytosiderophores
Modifiers	Modification of the rhizosphere soil with, e.g., protons, reductants
Ectoenzymes	Convert unusable organic forms to usable ones, e.g., phosphatase
Acquisition of water	Modification of the rhizosphere soil with mucilage
Protection against physical stress	Response to high soil strength through modification of interface through lubrication and amelioration of rhizosphere soil
Protection against pathogens	Defensive response to invasion, e.g., phytoalexins
Protection against toxic elements	Response to toxic entity, e.g., complexation of Al^{3+}
Protection against competition	Modification of rhizosphere soil with phytoactive compounds, e.g., allelochemicals
Establishment of symbiotic relationships	Chemotactic response
Rhizobia	
Endomycorrhizae	
Ectomycorrhizae	

permeation of soil with mucilage that has been shown *in vitro*, and when accompanied by wetting and drying, to confer stability on soil aggregates [95]. Other than permeation of soil with mucilage, most effects on structure would appear to be indirect effects, and thereby of little benefit to the plant whose roots brought about the change in structure.

Whiteley [96] found that remolded soil near (300 to 600 μm from the root surface) the roots of peas showed evidence of orientation of the clay fraction. He argues that the change could only have come about if the soil water potential had increased because of mucilage secretion by the root tip; the lower soil strength then allowed deformation by the growing root. Also, he believes that the lower soil strength may then predispose the remolded soil in the rhizosphere to penetration by root hairs and lateral roots. The secretion of water by roots as observed by McCully [97] and Young [98] adds weight to his argument.

It is impossible to discuss the possible effects of mucilage associated with the root apex without including border cells, although there are some species of plants which do not produce border cells [67]. Bengough and Mckenzie [99] suggested that the mucilage assists root cap cells, or acts in concert with them, to decrease the friction between the growing root tip and soil or, conversely, the mucilage acts as a lubricant. Iijima et al. [100] showed that the border cells of maize roots tips were more effective than mucilage at decreasing soil resistance to root elongation. Presumably, then, those plant species that do not produce either much mucilage or border cells are not at a great disadvantage when it comes to root growth in soil.

The permeation of soil at the root–soil interface by mucilage from the root cap may affect structure, and it may oppose the damaging effects of compression and shearing. Read et al. [101] found that the mucilages of maize, lupin, and wheat contained phospholipids that indirectly were shown to decrease the surface tension of water and thus facilitate possibly the permeation of soil with mucilage. And, attempts to measure the development of water repellency in the rhizosphere of barley, oil-seed rape, potato, and Italian ryegrass were not convincing [102]. Whalley et al. [103] attributed a decrease in the infiltration of water into rhizosphere soil to deformation of the soil, rather than to any other changes due to mucilage or the development of water repellency. Also, they found that neither natural mucilage from maize nor polygalacturonic acid affected the soil water characteristic between 0 and −15 kPa.

It is sometimes claimed that mucilage and similar gels may help to maintain hydraulic conductivity between root and soil [104]. However, the hydraulic conductivity of soils is often substantially decreased when soils are irrigated with wastewater, which is largely due to the production of microbial biomass, particularly extracellular polysaccharides. For example, Wu et al. [105] found that the hydraulic conductivity of sand was decreased by one to one-half orders of magnitude 3 weeks after treatment with a mixture of dextrose and nutrient solution. These extracellular polysaccharides form gels which may store large quantities of water and allow water and ions to diffuse through them at rates not much less than free water, but they could be expected to restrict mass flow of water, and thus some nutrients, to roots [106].

Another apparent paradox exists as mucilage is most easily seen on roots when they are immersed in water, and yet the evidence of its secretion in soil, apart from electron micrographs [71], is the development of rhizosheaths in soil-grown plants, particularly at relatively low water potentials [107]. The explanation may be simple in that at high water potentials the cohesive and adhesive properties of mucilage are low and bonds are easily broken, whereas in drier conditions the bonds are much stronger and so soil sticks more readily to the root. An alternative suggestion is that mucilage and water is secreted as a gel when conditions favor guttation, that is, at night and at water potentials from −120 to −500 kPa [97].

The release of mucilage from the periplasmic space can be triggered by contact with water at high potential [108]. In soil, growing roots are only exposed to high soil–water potential when the soil is saturated after rain or irrigation, but they make most of their growth at water potentials between −10 and −1000 kPa when most intra-aggregate pores are full of water, and aggregate surfaces are covered by a thick water film [106]. When a root tip makes contact with the aggregates,

the mucilage will tend to be secreted and to form a gel on the surfaces of aggregates [109] and in those pores that favor accommodation of the macromolecules of mucilage, that is, pores that are full of water, big enough, and do not repel the molecules. If the diameter of the mucilage molecule is taken as 68 nm [110], and diffusion of molecules is severely restricted in pores up to one order of magnitude larger than the molecule [111], then mucilage will move into pores whose diameters are greater than about 680 nm, which is equivalent to a soil water potential of about −500 kPa. Pores of such size are large enough to accommodate bacteria, and so the mucilage molecules may not be safe from microbial degradation. We obviously need to know more about the secretion of mucilage from the periplasmic space, its physical and chemical properties, its interaction with soil, and the consequences.

In soil, the chances that any enzyme retains its activity are very slim, indeed, because inactivation can occur by denaturation, microbial degradation, and sorption [112,113], although it is possible that sorption may protect an enzyme from microbial degradation or chemical hydrolysis and retain its activity. The nature of most enzymes, particularly size and charge characteristics, is such that they would have very low mobility in soils, so that if a secreted enzyme is to have any effect, then it must operate close to the point of secretion, and its substrate must be able to diffuse to the enzyme. Secretory acid phosphatase was found to be produced in response to P-deficiency stress by epidermal cells of the main tap roots of white lupin and in the cell walls and intercellular spaces of lateral roots [114]. Such apoplastic phosphatase is safe from soil but can only be effective when presented with soluble organophosphates, which are often present in the soil solution [115]. However, because the phosphatase activity in the rhizosphere originates from a number of sources [116], mostly microbial, and is much higher in the rhizosphere than in bulk soil [117], it seems curious that plants would have a need to secrete phosphatase at all. The role of phosphatases in the rhizosphere remains uncertain [118].

Of enzymes that appear to acquire or retain their activity in soils, urease is an example. It is a microbial product, bound to soil, and causes very rapid hydrolysis of urea upon its addition to soil.

By contrast when phytase from seedlings of transgenic *Arabidopsis thaliana* was added to soil, it was rapidly immobilized and deactivated by adsorption, which was favored by low soil pH [119]. The phytase could be desorbed by increasing the pH but gradually the overall activity decreased, possibly due to proteinase activity.

Many phytoalexins and allelochemicals have much in common, in that they are designed to protect plants from either injury by other organisms or competition from other plants, and they are usually aromatic compounds. Chemical signals are discussed in Chapter 11. The phytoalexins are usually larger and more complex molecules [120] than those deemed to be allelochemicals [23]. Compounds of this type that are secreted by roots have to run the usual gauntlet in the rhizosphere, and if any of the secretions are likely to survive the trip, then the phenolics implicated in allelopathy have some chance. Not only do the phenolics have low molecular weights and usually have some negative charge, but they also have some antimicrobial activity. In spite of these attributes, benzoic and cinnamic acid derivatives are easily degraded microbially in soils [121], and they are chemically oxidized by Mn oxides [122,123] and adsorbed by soils as well [123,124]. Further, when nine different phenolics (caffeic acid, chlorogenic acid, *p*-coumaric acid, ellagic acid, ferulic acid, gallic acid, *p*-hydroxybenzoic acid, syringic acid, and vanillic acid) were added to three different soils, they had no effect on seed germination, on seedling growth, or on early plant growth of several species, even when added to soils at rates well above the concentrations detected in soils [125]. The case for these types of compounds as having an important role in root secretions is not strong, in spite of the case argued earlier, except perhaps in the right set of circumstances, discussed later. The situation presented here is supported by the statement that "it is important to demonstrate that phenolics, released by the plant, should have enough bioactive concentration and persistence in the rhizosphere in order to argue their probable involvement in allelopathy" [126]. Other potential allochemical compounds, such as momilactone B found in rice exudates [127] and sorgoleone in sorghum spp. [128], appear to be better candidates but very much depends on their fate and behavior in soil.

Inderjit and Duke [129] indicate that there is a lack of consensus in identifying any compound as an allelochemical, and they discuss the requirements for a chemical compound to be deemed an allelochemical. The difficulty associated with the isolation, identity, activity, etc., of suspected allelochemicals is illustrated by the work of Wu et al. [130], who isolated significant bioactive phenolic and hydroxamic acids exuded into agar growth medium by wheat seedlings, and they found that the so-called allelochemicals inhibited the growth of annual ryegrass (*Lolium rigidum*). Annual ryegrass is one of the worst weeds in cereal growing in Australia, and so one can only wonder about the relevance of the finding, particularly in the context of the difficulties faced in the search for allelochemicals.

The roots of canola plants and decaying residues of canola crops release 2-phenylethylisothiocyanate, which is believed to inhibit soilborne pathogens [13], but its microbial degradation is rapid. In a Luvisol (20% clay) the concentration decreased 50% from the initial concentration of 3382 pmol g^{-1} in close to 2 h. Such rapid degradation suggests that if the compound is to have an effect it will be close to the root, and it will need to be secreted in a specific zone and not be released along with cortical degeneration.

Nonaromatic organic acids such as citric have been implicated in nutrient acquisition since the 19th century [131] and, in spite of the certainty with which some authors assert that these acids play an unquestionable role, there is still uncertainty [132,133]. The process involves secretion of the acid in either an undissociated form (H_3X) or a dissociated form (H_2X^-, HX^{2-}, or X^{3-}). Although it would seem that, at the pHs that prevail in the cytoplasm and in the soil solution, the form secreted is anionic and not as the acid [83], it is possible that the undissociated acids are secreted or that there is a concomitant efflux of H^+ [133]. The form of the acid in the rhizosphere depends on the pH and on the availability of metals and their tendency to form complexes of varying stability. The acid *per se* may mobilize metals by dissolution (e.g., $Fe(OH)_3$) or cation exchange, whereas the anion through its tendency to form stable soluble complexes may protect metals from precipitation, or it may cause insoluble sources to dissolve, or it even may precipitate a cation such as Ca^{2+} [134]. These respective reactions for Fe can be represented as follows, where X^{3-} represents an anion such as citrate:

$$H_3X = H_2X^- + H^+,\ H_2X^- = HX^{2-} + H^+,\ HX^{2-} = X^{3-} + H^+,$$

$$Fe(OH)_3 + 3\ H^+ = Fe^{3+} + 3H_2O$$

$$Fe^{3+} + X^{3-} = FeX$$

$$Fe(OH)_3 + H_3X = FeX + 3H_2O$$

$$3Ca^{2+} + 2X^{3-} = Ca_3X_2$$

It is conceived that the soluble complexes once formed diffuse back to the root where the complex is absorbed or the metal is separated from the ligand and then absorbed [29]. The ligand is then released back into the rhizosphere and, as the organic acids are not prone to reabsorption [135], they are fully available to run the gauntlet once more. Because citrate is adsorbed by soil surfaces and rapidly degraded microbially [136], there are serious doubts about the role of citric acid and similar organic acids in the acquisition of nutrients except, perhaps, in the right set of circumstances, where some form of protection is proffered by the spatial arrangement of root and soil surfaces. Further, the soil diffusion rates of soluble exudates such as amino acids are several orders of magnitude less than, say, nitrate [137,138], and so the chances that they are assimilated by microbes in the rhizosphere rather than absorbed by roots are high.

It is curious that white lupins, which have the capacity to produce relatively large quantities of citric acid [134], do not grow as well as other species on calcareous soils [139,140] that cannot produce such large quantities of citric acid [141]. Hinsinger [142] highlights in a table some data from Dinkelaker et al. [134], in which the concentration of citrate in the rhizosphere accompanies an eightfold increase in diethylenetriamine pentaacetic acid (DTPA)-extractable concentration of Fe, and

yet white lupins suffer from Fe chlorosis on calcareous soils. Dinkelaker et al. [134] estimated that the quantity of citric acid produced was 23% of the total plant dry weight at harvest. Such a huge release should probably be regarded as abnormal rather than as a result of a stress response mechanism.

The role of the secretion from the root apex of organic acids such as citric and malic in the resistance of maize and wheat respective to Al toxicity [143,144] has emerged recently as one with plausibility [83,145]. These studies have been carried out in solution cultures, but how does the suggestion hold up in soil? The first and probably greatest difficulty is that the toxic species of Al, probably hydrated Al^{3+}, must diffuse to some site in the root apex and stimulate the production and subsequent release of the organic acid. The site may be extracellular or intracellular, but whichever it is, the production of the organic acid must be intracellular, and after its release it must inactivate the toxic Al species in the apoplasm as beyond the apoplasm rapid microbial degradation is likely [146]. The relative freedom of the root apex from microbial colonization and the production of mucilage both help to create the right set of circumstances that allows the detoxification of the Al to take place in the protection provided by the apoplasm. The observation that phosphate was released by the root apex of Al-tolerant cultivars of maize [144] and wheat [147] is of interest in this context, but it may be an unusual situation restricted to solution cultures, because those soils in which the activity of Al in the soil solution is high are usually P deficient as well. As plausible as the role of organic acids may be, there is evidence to suggest that organic acids, and polypeptides, arise out of Al-induced failure of membranes rather than *de novo* synthesis and secretion [7].

Similarly, the pivotal role of the so-called stress response in the acquisition of Fe by plants grown in calcareous soils [59] may have been overrated [118]. The key role of the inhibitory effect of bicarbonate on root growth of calcifuge plant species [148] has been overlooked in most considerations of the acquisition of Fe, the least soluble of all nutrients. Also, it is likely that Fe acquisition in normal healthy plants is a constitutive process, such as Mn uptake in barley [149], so that normal healthy roots acquire their Fe as a matter of course. By the time the Fe stress response is triggered, it is possible that cell membranes are losing their integrity and that compounds normally involved in metabolism requiring or involving Fe are released. It is likely, though, that some of the compounds released as a result of Fe stress are involved in the constitutive process, and their production is related to a species prowess in acquiring Fe from calcareous soils. The inability of the Fe response in so-called iron-efficient sunflowers to overcome Fe stress in a calcareous soil [150] suggests that the Fe stress response in this case may be restricted to culture solutions and of little relevance in calcareous soils. Another reason to question the theory of Fe stress response is that in soil the Fe-active compounds are rapidly decomposed by microorganisms [151–153]. The early reviews on root exudates by Rovira [1] and others [2,15] all drew attention to the numerous factors that affect membrane permeability and cause roots to leak; they are just as relevant today as they were 20 or more years ago [83]. Once again, the right set of circumstances may overcome the problem of microbial decomposition, but it cannot overcome membrane failure.

V. THE RIGHT SET OF CIRCUMSTANCES

Contact reduction was proposed to explain how plants obtain Mn from soils of neutral and alkaline pH [154]. The evidence that sterile roots of sunflower roots could directly reduce insoluble reactive oxides of Mn strengthened the theory [86]. Nevertheless, the idea of the "right set of circumstances" was developed to explain how labile reductants produced by roots may be protected physically from microorganisms and O_2 and be directed toward insoluble oxides of Mn instead [25]. The right set of circumstances are thought to arise where roots contact soil and an interface is created that is saturated with water, and that, with physical blockage, creates a zone with low activity of O_2 [155,156] and, presumably, of microorganisms. If the right set of circumstances arise sufficiently and frequently enough, then the mechanism remains a plausible one [25,157].

The absence of suitable reducing agents among root exudates has been taken as a flaw in this theory, but their absence is explained readily because either they are not looked for nor are adequate

precautions taken to exclude O_2; also, the oxidized products are not recognized as derivatives of reducing agents. Evidence that this sort of thing could happen easily is provided by Einhellig and Souza [6], who analyzed for sorgoleone rather than its precursor dihydroquinone, a major root exudate of sorghum seedlings, as dihydroquinone was rapidly oxidized by ambient O_2 to sorgoleone. Also, the right of set circumstances that exist in soil would never occur in well-aerated solution cultures, although ascorbic acid, a suitable but labile reducing agent, has been found in solution cultures of healthy cucumber and tomato [9]. Many phenolic compounds discussed earlier in reference to allelopathy have reducing activity toward Mn oxides. For example, Park et al. [158] found in the root exudates of sunflowers hydroquinone, β-resorcyclic acid, vanillic acid, caffeic acid, salicylic acid, quercetin, gentisic acid, and ferulic acid, some of which readily reduce reactive Mn oxides, e.g., hydroquinone.

The complexity of plant root interactions with soil are such that even the right set of circumstances as described earlier is a simplification. For example, in the case of Mn acquisition, the right set of circumstances must arise at the right time and frequently enough for the plant to acquire sufficient quantities of Mn. It depends on (1) the constitutive properties of the roots to produce reducing compounds, (2) the growth of roots through soil so that the parts of the root producing the reductant and those involved in Mn absorption come into contact with soil, (3) the location of Mn oxides on the soil surfaces contacted by the root, (4) the reactivity of the Mn oxides (their ability to accept electrons) and (5) soil properties such as pH and structure. Although the number of variables involved is high, the probability of the right set of circumstances occurring frequently is also high in most soils: it becomes less so as the opportunities of roots making contact with active oxides becomes less.

VI. CONCLUSIONS

It is likely that of the vast array of compounds released by plant roots very few have a direct effect on the growth of soil-grown plants. One must ask whether or not they serve any useful purpose at all. The fears and uncertainty about what is physiologically normal were expressed by Ayers and Thornton in 1968 [159]; their concerns are as valid today as they were then. The absolute and relative quantities released are at present no more than approximations based largely on a very narrow selection of short-lived agricultural plants.

In attempting to classify types of secretions on the basis of what might happen in the rhizosphere, the foregoing discussion has taken a fairly distrusting view of data derived from *in vitro* experiments such as solution cultures, those using sterilized soil, and those using highly contrived situations where the reality of normal soil-grown plants is disregarded. The discussion has also highlighted the difficulties faced by some secretions and how these difficulties will decrease their likelihood of bringing about the process that they are purported to bring about. However, arguments in cases such as tolerance of Al toxicity and the acquisition of Fe and Mn can be strengthened by invoking the right set of circumstances.

Root products represent a vast array of predominantly organic compounds. Of these, secretions represent a small proportion, but they are deemed the most likely of all root products to have a direct effect on the growth of the plant that produced them. When a secretion is released by a root, all the following are likely to affect its behavior:

1. Site of secretion appropriate or inappropriate
2. Microbial assimilation or degradation
3. Chemical alteration or degradation, for example, oxidation
4. Sorption and persistence with or without activity, for example, ectoenzymes
5. Diffusion and reaction with the target, for example, complex with Al^{3+} or Fe^{3+}
6. Mechanism of uptake, for example, reabsorption by the root

Very few root secretions can be expected to be effective unless the right set of circumstances arise sufficiently often. Further, research involving soil-grown plants is required to establish whether or not the right set of circumstances as discussed earlier do make a real contribution to the well-being of field-grown plants. Similarly, close scrutiny of all research must be made, particularly when results obtained in solution cultures and other contrived situations are believed to be relevant for plants growing in soil.

Finally, if the understanding of the rhizosphere and the functional roles of compounds therein is to increase, then it is important that the enthusiasm shown in recent times continues, but that enthusiasm must be curbed at times by reality checks and frequent reference to what might actually happen in normal soil. One should also heed the words of Tinker and Nye [118]:

> It is not surprising that the large literature on the rhizosphere in the past has produced rather little in the way of firm generalizations and mechanisms on the nutritional effects, bearing in mind its complexity, constant variation, and difficulty of access. It is interesting to note the list of processes in the rhizosphere that have been proposed in the past, but that have only slowly and often partially been proven to occur. The critical question is whether they are important for plant growth, and it is only recently that we have gained some insight into this.

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2 The Release of Root Exudates as Affected by the Plant Physiological Status

Günter Neumann and Volker Römheld

CONTENTS

I. INTRODUCTION

Apart from the function of plant roots as organs for water and nutrient uptake and anchorage in soils, roots are able also to release a wide range of organic and inorganic compounds into the rhizosphere. Soil–chemical changes related to the presence of these compounds and products of their microbial turnover are important factors affecting microbial populations, availability of nutrients, solubility of toxic elements in the rhizosphere, and thereby, the ability of plants to cope with adverse soil–chemical conditions [1]. Organic rhizodeposition includes lysates, liberated by autolysis of sloughed-off cells and tissues, intact root border cells, as well as root exudates, released passively (diffusates) or actively (secretions) from intact root cells (Table 2.1; see also Chapter 1). In annual plant species, 30 to 60% of the photosynthetically fixed carbon is translocated to the roots, and a considerable proportion of this carbon (up to 70%) can be released into the rhizosphere [2,3] as pointed out in Chapter 1, Chapter 3, and Chapter 13 of this book. This rhizodeposition is affected by multiple factors such as light intensity, temperature, nutritional status of the plants, activity of retrieval mechanisms, various stress factors, mechanical impedance and sorption characteristics of the growth medium, and microbial activity in the rhizosphere. This chapter will focus on the release of root exudates, and highlight effects of the physiological status on root exudation and its significance for adaptations to adverse soil conditions and nutrient efficiency. Because the methods employed for collection and analysis of root exudates play an important role for the qualitative and quantitative interpretation of measured exudate data, methodological aspects will also be discussed in the introductory section.

TABLE 2.1
Root Exudates Detected in Higher Plants

Class of Compounds	Single Components
Sugars	Arabinose, glucose, fructose, galactose, maltose, raffinose, rhamnose, ribose, sucrose, xylose
Amino acids and amides	All 20 proteinogenic amino acids, aminobutyric acid, homoserine, cysrathionine, mugineic acid phytosiderophores (mugineic acid, deoxymugineic acid, hydroxymugineic acid, epi-hydroxymugineic acid, avenic acid, distichonic acid A)
Aliphatic acids	Formic, acetic, butyric, popionic, malic, citric, isocitric, oxalic, fumaric, malonic, succinic, maleic, tartaric, oxaloacetic, pyruvic, oxoglutaric, maleic, glycolic, shikimic, cis-aconitic, trans-aconitic, valeric, gluconic
Aromatic acids	p-Hydroxybenzoic, caffeic, p-coumaric, ferulic, gallic, gentisic, protocatechuic, salicylic, sinapic, syringic
Miscellaneous phenolics	Flavonols, flavones, flavanones, anthocyanins, isoflavonoids
Fatty acids	Linoleic, linolenic, oleic, palmitic, stearic
Sterols	Campestrol, cholesterol, sitosterol, stigmasterol
Enzymes	Amylase, invertase, cellobiase, desoxyruibonuclease, ribonuclease, acid phosphatase, phytase, pyrophosphatase apyrase, peroxidase, protease
Miscellaneous	Vitamins, plant growth regulators (auxins, cytokinins, gibberellins), alkyl sulfides, ethanol, H^+,K^+ Nitrate, Phosphate, HCO^-_3

II. COLLECTION OF ROOT EXUDATES: METHODOLOGICAL ASPECTS

A. Collection Techniques with Trap Solutions

Water-soluble root exudates are most frequently collected by immersion of root systems into aerated trap solutions for a defined time period (Figure 2.1A). The technique is easy to perform and allows kinetic studies by repeated measurements using the same plants. Although it is possible to get a first impression about qualitative exudation patterns and even quantitative changes in response to different preculture conditions, the technique also includes several drawbacks, which should be taken into account for the interpretation of experimental data. Application should be restricted to plants grown in nutrient solution, because removal of root systems from solid media (soil, sand) is almost certainly associated with mechanical damage of root cells, resulting in overestimation of exudation rates. On the other hand, it has been frequently demonstrated that the mechanical impedance of solid growth media leads to alterations in root morphology and stimulates root exudation [4,5]. In liquid culture media, simulation of the mechanical forces imposed on roots of soil-grown plants may be achieved by addition of small glass beads [5–7]. Alternatively, exudate collection from plants grown in solid media (sand, vermiculite) may be performed by percolating the culture vessels with the trap solution for a defined time period (Figure 2.1B), after removal of

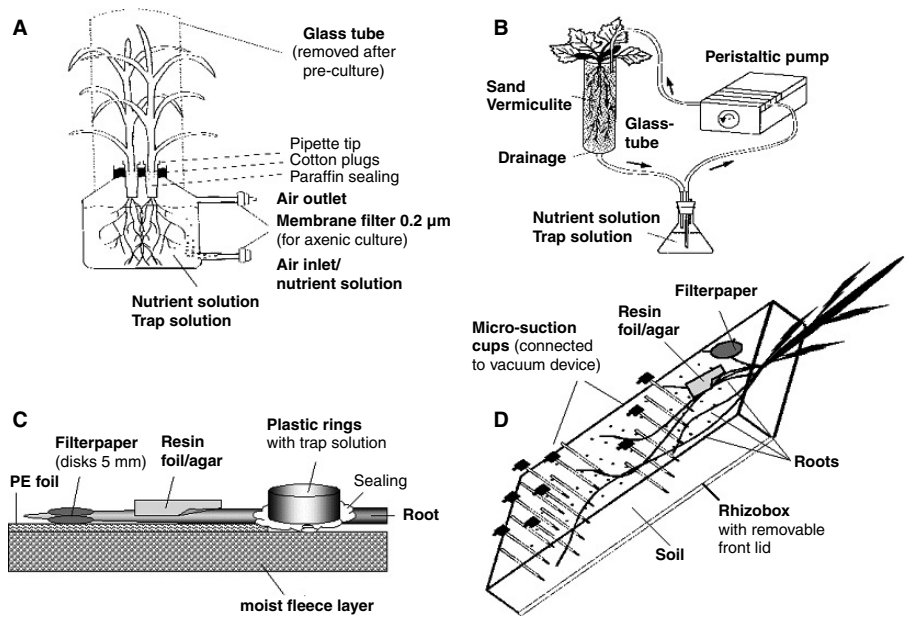

FIGURE 2.1 Techniques for collection of root exudates: (A) Solution culture system [396]; root exudates collected from the whole root system by immersion into aerated trap solutions under sterile conditions (optional). (B) Plant culture in solid media (vermiculite, sand); root exudates collected from the whole root system by percolation of the culture vessels with trap solution [9,11]. (C) Localized root exudate sampling from plants grown in solution culture. Exudates collected into trap solution inside of sealed plastic rings straddling the root [29], or by application of sorption media (filter paper, agar, ion-exchange resins) onto the root surface [18,46]. (D) Localized collection of rhizosphere soil solution from plants grown in soil culture. Rhizoboxes with removable front lids for plant culture (root windows under field conditions). Collection by insertion of microsuction cups (made from HPLC capillaries, 1 mm in diameter) connected to a vacuum collection device [52] or by application of sorption media (filter paper, agar, ion-exchange resins) onto the root surface [28,46].

rhizosphere products accumulated during the preceeding culture period by repeated washing steps [8–11]. For this approach, however, recovery experiments and comparison with results obtained from experiments in liquid culture are essential, because incomplete leaching and sorption of certain exudate compounds to the matrix of solid culture media cannot be excluded [12]. As a modification of the percolation technique, cartridges filled with selective adsorption media (e.g., XAD resin for hydrophobic compounds, anion-exchange resins for carboxylates), which are installed in the draining tube below the plant culture vessel, can be employed for the enrichment of distinct exudate constituents [13,14]. After adsorption to a resin, exudate compounds are also protected to a certain extent against microbial degradation (see Subsection II.C.1).

Trap solutions employed for collection of water-soluble root exudates are nutrient solutions of the same composition as the culture media [8,10,11], solutions of 0.5 to 2.5 m*M* $CaSO_4$ or $CaCl_2$ to provide Ca^{2+} for membrane stabilization [15] or simply distilled water [9,16–18]. Because the osmotic strength of nutrient solutions is generally low, short-term treatments (1 to 2 h) even with distilled water are not likely to affect membrane permeability by osmotic stress. Accordingly, comparing exudation of amino acids from roots of *Brassica napus* L. into nutrient solution, 20 m*M* KCl, or distilled water, respectively, revealed no differences during collection periods between 0.5 and 6 h [19]. In contrast, Cakmak and Marschner [20] reported increased exudation of sugars and amino acids from roots of wheat and cotton during a collection period of 6 h when distilled water, instead of 1 m*M* $CaSO_4$, was applied as trap solution. Thus, for longer collection periods or for repeated measurements, only complete or at least diluted nutrient solutions should be employed as trap solutions to avoid depletion of nutrients and excessive leaching of Ca^{2+}. Long-term exposure of plant roots to external solutions of very low ionic strength is also likely to increase exudation rates due to an increased transmembrane concentration gradient of solutes [21,22]. Prior to further sample preparation, solids, microorganisms, and root border cells in trap solutions should be removed by filtration or centrifugation steps.

Exudate collection in trap solutions usually requires subsequent concentration by vacuum evaporation or lyophilization, due to the low concentration of exudate compounds. Depending on the composition of the trap solution, the reduction of sample volume can lead to high salt concentrations, which may interfere with subsequent analysis, or may even cause irreversible precipitation of certain exudate compounds (e.g., Ca-citrate, Ca-oxalate, proteins). Therefore, if possible, removal of interfering salts by use of ion-exchange resins prior to sample concentration is recommended. Alternatively, solid-phase extraction techniques may be employed for enrichment of exudate compounds from the diluted trap solution [11,23]. High-molecular-weight (HMW) compounds may be concentrated by precipitation with organic solvents (methanol, ethanol, acetone 80% [v/v] for polysaccharides and proteins) or acidification (trichloroacetic acid 10% [w/v], perchloric acid 5% [w/v] for proteins; [24]). Alternatively, ultrafiltration of the trap solutions or even cultivation of plant roots enclosed in dialysis bags is possible [25]. Mucilage polysaccharides adhering to the root surface have been collected by application of vacuum suction [26], by abrasion with a soft brush and subsequent transfer to cellulose acetate filters [27], or simply by collection with forceps [28].

B. Localized Sampling Techniques in Solution and Soil Culture Systems

In many plants, root exudation is not uniformly distributed over the whole root system. Considerable spatial variation has been reported for the exudation of carboxylates and protons in P-deficient oilseed rape [29,30], the exudation of protons and phenolics in many dicotyledonous plant species in response to Fe deficiency [1,31], or for the release of phytosiderophores in Fe-deficient barley [31]. In all these cases, exudation was mainly confined to apical root zones. Various plant species adapted to low fertile soils, such as members of the Proteaceae and Casuarinaceae, but also white lupin (*Lupinus albus*) are characterized by the formation of cluster roots (proteoid roots; see Figure 2.3A) mainly under conditions of P deficiency or Fe deficiency [32,33]. Exudation of large amounts of carboxylates and protons involved in the mobilization of mineral nutrients such as P and Fe (see