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Review article

Metabolites from soil bacteria affect plant water relations

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Abstract

Water-soluble compounds move naturally in soil moisture toward roots of transpiring plants. To test for effects of rhizosphere food-web molecules on plants, low concentrations of common microbial products were supplied to bean (*Phaseolus vulgaris* L.) roots. Stomatal conductance and transpiration increased significantly (+20 to +30%, $P \leq 0.05$) 42 h after 10 nM homoserine lactone (HL) was supplied to roots. Because transpiration helps both a plant and its root-colonizing bacteria obtain diffusion-limited mineral nutrients, such as phosphorus, any increase triggered by a degradation product of *N*-acyl-homoserine lactone (AHL) regulatory signals commonly used among plant-associated bacteria may represent a mutualistic plant-microbe interaction. Results from these initial physiological tests justify further screening to identify other rhizosphere compounds that control plant functions important for root-colonizing microorganisms.

Keywords: Transpiration; Homoserine lactone; Homoserine; Lumichrome

1. Introduction

Plants use complex, signaling strategies aboveground to influence arthropods [5] and belowground to regulate genes in symbiotic [14] and pathogenic [13,26] micro-organisms. One simple comparison between aboveground and belowground signaling, however, reveals a major difference: volatile signals aboveground diffuse away to infinitely dilute concentrations where their selection value is negligible, while water-soluble compounds from rhizosphere organisms are concentrated as soil moisture moves toward roots in the transpiration stream [15]. Thus plants are exposed to a diverse pool of potential signal compounds from many rhizosphere organisms. Whether plants treat the molecules they encounter as signals depends upon their information content for the plant, e.g. linkage to beneficial variables such as N availability, soil water or particular microbial species important for plant development, and whether plants have evolved a useful response to appropriate concentrations of the compounds. Tests for plant responses can be conducted at either the physiological or transcriptional level. The former approach may supply conclusive information on one particular plant response; the latter offers a broader method for identifying many potentially active signal molecules. We report here the results of initial physiological tests.

Many water-soluble rhizosphere molecules are available as potential signals for plants, but *N*-acyl homoserine lactones (AHLs) are of special interest. Numerous plant-associated bacteria produce and respond to AHLs [17]. Soil bacteria degrade AHLs to homoserine lactone (HL) by removing the acyl group [10], and the lactone ring can be hydrolyzed by bacteria [6] or alkali [25] to produce homoserine. Eukaryotic animal hosts respond to at least one AHL [20], but whether plants recognize these compounds is unknown. AHL-mimic compounds released from plants [21] and a marine alga [7] can affect external bacteria, but possible regulatory roles for the mimics inside plants should not be overlooked. For these reasons it is logical to test for regulatory roles of AHLs and their degradation products in plants. One possible role for water-soluble rhizosphere compounds, like AHLs, involves the regulation of transpiration, a process important for both the plant and the rhizosphere microorganisms.

During the transpiration process, CO_2 used in photosynthesis moves into plant leaves through stomatal openings,

Abbreviations: AHL, *N*-acyl homoserine lactone; HL, homoserine lactone.

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while water vapor is simultaneously lost to the atmosphere. Stomatal apertures are governed by osmotically driven changes in turgor of the surrounding guard cells [2], which are linked through incompletely understood transduction pathways to three major external stimuli: light, CO_2 and humidity [1]. Little is known about how soil microorganisms can affect transpiration. Plants benefit from transpiration through an increase in CO_2 concentration inside the leaf, evaporative cooling of leaves in sunlight, and movement of minerals from roots to the leaf in the transpirational stream. Rhizosphere organisms benefit from transpiration when soil moisture carries mineral nutrients toward the root. This mass flow of soil moisture may be particularly important for enhancing the flux of diffusion-limited minerals, such as phosphorus [12], to both the plant and the rhizosphere microorganisms.

Based on this rationale, we postulated that some water-soluble bacterial metabolites should enhance stomatal opening. To test this hypothesis, we examined the effects of several ordinary rhizosphere compounds, including AHL degradation products, on stomatal conductance in common bean (*Phaseolus vulgaris* L.). Our results show for the first time that low nanomolar concentrations of natural external metabolites produced by soil bacteria can influence the internal water relations of intact plants.

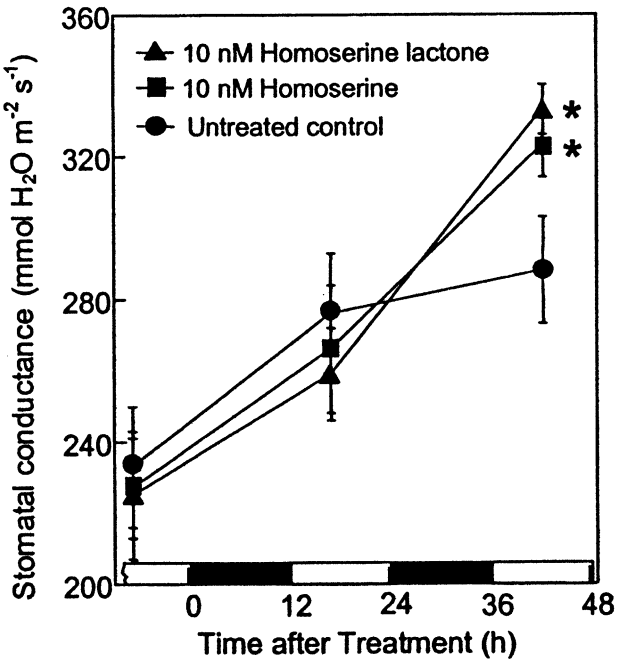

Fig. 1. HL and homoserine effects on bean leaf stomatal opening. Compounds were supplied to bean seedling roots immediately before a dark period (■), and significant (* $P \leq 0.05$) effects on leaf conductance, a measure of stomatal opening, were observed in the middle of the second subsequent light period (□).

2. Results

Tests showed repeatedly that low nanomolar concentrations of HL and homoserine increased stomatal openings, as measured by leaf conductance, on the first trifoliolate leaf of bean seedlings 42 h after the compounds were supplied to roots of intact plants growing under microbiologically and environmentally controlled conditions (Fig. 1). No significant treatment effects on stomatal conductance were measured during the first 24 h in any experiment. In most experiments the positive effects of these molecules on stomatal conductance disappeared by the third light period after treatment. Tests with HL concentrations ranging from 0.1 to 100 nM showed that 10 nM gave reproducible, 20–30% increases. The stimulatory effects of HL and homoserine on stomatal conductance were evident under two irradiance levels tested in these experiments, and similar responses also were observed in primary leaves (data not shown). Stomata in all plants functioned normally in the sense that they showed diurnal changes in stomatal conductance between dark and light periods.

Increases in stomatal conductance caused by HL treatments were associated with enhanced transpiration. For example, when transpirational water losses were quantified by weighing pots every 2 h during the light period, 37% more water was transpired ($P \leq 0.05$) by HL-treated plants during a period when their stomatal conductance was 26% greater than untreated plants (Fig. 2). The effects of HL on stomata and transpiration were most evident near the middle of the photoperiod, i.e. the 6-h time point in Fig. 2. Thus short-term

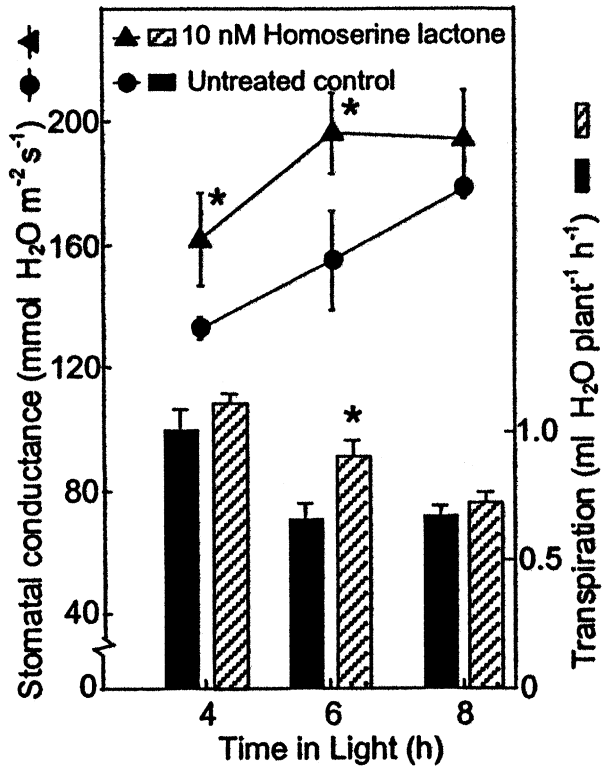

Fig. 2. HL effects on bean leaf stomatal opening and transpiration. Stomatal conductance and water loss were measured in the second light period after treating roots with 10 nM HL. The 6-h time point measured water loss from 41 to 43 h after roots were treated.

Table 1
Effects of bacterial metabolites on stomatal opening. Stomatal conductance of the first trifoliolate leaf on common bean was measured 42 h after supplying compounds to the root. Compounds were supplied to roots at 10 nM, unless noted otherwise. Data from four experiments with six replicate leaves in each treatment were normalized to mean control value. Responses followed by separate letters differed significantly ($P \leq 0.05$) based on one-way analysis of variance of the original data

Root treatment	Stomatal conductance (mmol H_2O m^{-2} s^{-1})
Untreated control	245 a
Homoserine lactone	300 b (+22%)
Homoserine	306 b (+25%)
N-(β-ketocaproyl)-homoserine lactone	255 a
Biotin	279 ab
Thiamine	260 a
Riboflavin	255 a
2,4-Diacetylphloroglucinol	245 a
Lumichrome (5 nM)	194 c (– 21%)

measurements of stomatal conductance taken after 4 h in light showed a significant increase ($P \leq 0.5$) in stomatal opening of plants treated with HL, but HL effects on water loss became evident only during the subsequent 2-h period, which were recorded as the 6-h data points (Fig. 2). The 6-h time point in Fig. 2 corresponds to the 42-h time point reported from different experiments in Fig. 1, and significant increases in transpirational losses were detected only during the second photoperiod after HL was applied to roots. Plants were not stressed for water in any experiments, but while optimizing the pre-treatment watering regimes, stomatal conductances from 150 to 350 mmol H_2O m^{-2} s^{-1} were measured. HL increased stomatal conductance under all watering regimes.

Tests with other bacterial metabolites showed that only certain compounds affected stomatal conductance in bean (Table 1). Most striking was the fact that one AHL signal compound which is available from commercial sources, *N*-(β-ketocaproyl)-homoserine lactone, had no effect on stomatal conductance. Likewise, the common rhizosphere compounds, thiamine and riboflavin [18] and the *Pseudomonas* product 2,4-diacetylphloroglucinol [9] had no effect on stomatal conductance in tests where HL served as the positive control. Lumichrome, a bacterial degradation product of riboflavin [16,27], decreased stomatal conductance by 20% ($P \leq 0.05$) in repeated tests. Thus stomata in these tests responded both positively and negatively to microbial products.

3. Discussion

The direct significance of this study is the demonstration that natural soil molecules supplied to roots can affect stomatal functioning and transpiration. Previous studies showed that stomatal opening in isolated leaf epidermal tissue is influenced within minutes by exogenous compounds, including the fungal toxin fusicoccin [22], certain diacylglycerols [11], and cytoskeletal inhibitors [8]. The novelty of the current report is that low concentrations of three compounds, HL, homoserine and lumichrome, supplied to roots affected stomatal opening many hours later (Table 1). These observations add new dimensions to the poorly understood issue of root-shoot signaling effects on stomatal functioning [3]. While the time-course of HL activity in these experiments is consistent with movement of HL to an active site in the leaf, the currently limited understanding of aquaporins and perhaps other regulators [19] leaves open the possibility for HL interactions at other sites. Homoserine is a major component of some root exudates [24] as well as a natural degradation product of HL [6], and thus without changing the overall conclusion of these experiments one can suggest that effects of HL measured here may have resulted from homoserine. Inhibitory effects of lumichrome on stomata (Table 1) support the concept that rhizosphere compounds can alter physiological functions in plants. The stomatal responses associated with this molecule, however, may reflect evolutionary forces related to the natural presence of this compound in plants [23], rather than its recently demonstrated rhizosphere role as an enhancer of root respiration and plant growth [16].

In a broader context, these results raise the possibility of other previously unrecognized plant-microbe interactions mediated by natural rhizosphere compounds. Persistence of such interactions with small effects (15–30%) would indicate their benefits for both organisms under particular ecological conditions. While these experiments monitored only foliar transpirational functions, other plant responses that benefit both plants and root-colonizing microorganisms can be imagined, including root elongation. Presumably such responses developed in the 130–355 million years when the first rootless terrestrial plants coevolved with pre-existing bacteria and fungi in primordial soil [15]. Under such conditions plants would have benefited from information on external moisture and mineral sources, and water-soluble organic compounds from other living organisms could have supplied those data. The mechanisms by which such compounds transmit information to the plant and the responses of the plant can be clarified either by focused physiological tests of the type reported here or by broader genomic methods that identify rhizosphere compounds that function as transcriptional regulators in plants.

4. Methods

Bean (*Phaseolus vulgaris L.* var. Black Turtle Soup) plants were grown with a photosynthetically active (400–700 nm) photon flux density of 250 or 400 μmol m^{-2} s^{-1} using a 12/12 h (day/night) cycle at 25/20 °C and 30–40% relative humidity. Seeds were surface sterilized with commercial bleach for 30 min, rinsed with sterile water and planted in sterile, 10-cm pots filled with vermiculite. Pots were watered every second day with 50 ml of sterile nutrient solution [4] containing 2 mM KNO_3. Immediately before the dark period on day 14, 50 ml of nutrient

solution with or without the chemical treatment was applied to the root systems. Stomatal conductance of the first trifoliolate leaves was measured with a recently calibrated LI-COR (Lincoln, NE) model 1600 leaf porometer. Transpiration was measured gravimetrically using plant-free pots as controls. All chemicals were purchased from Sigma Chemical Co. (St. Louis, MO).

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References

[1] S.M. Assmann, Signal transduction in guard cells, Annu. Rev. Cell Biol. 9 (1993) 345–375.

[2] M.R. Blatt, Ca^{2+} signalling and control of guard-cell volume in stomatal movements, Curr. Opin. Plant Biol. 3 (2000) 196–204.

[3] W.J. Davies, J.H. Zhang, Root signals and the regulation of growth and development of plants in drying soil, Annu. Rev. Plant Physiol. Plant Molec. Biol. 42 (1991) 55–76.

[4] T.M. DeJong, D.A. Phillips, Nitrogen stress and apparent photosynthesis in symbiotically grown *Pisum sativum* L, Plant Physiol. 68 (1981) 309–313.

[5] M. Dicke, J.J.A. van Loon, Multitrophic effects of herbivore-induced plant volatiles in an evolutionary context, Entomol. Exp. Appl. 97 (2000) 237–249.

[6] Y.H. Dong, L.H. Wang, J.L. Xu, H.B. Zhang, X.F. Zhang, L.H. Zhang, Quenching quorum-sensing-dependent bacterial infection by an *N*-acyl homoserine lactonase, Nature 411 (2001) 813–817.

[7] M. Givskov, R. Denys, M. Manefield, L. Gram, R. Maximilien, L. Eberl, S. Molin, P.D. Steinberg, S. Kjelleberg, Eukaryotic interference with homoserine lactone-mediated prokaryotic signaling, J. Bacteriol. 178 (1996) 6618–6622.

[8] R.F. Huang, X.C. Wang, C.H. Lou, Cytoskeletal inhibitors suppress the stomatal opening of *Vicia faba* L. induced by fusicoccin and IAA, Plant Sci. 156 (2000) 65–71.

[9] C. Keel, U. Schnider, M. Maurhofer, C. Voisard, J. Laville, U. Burger, P. Wirthner, D. Haas, G. Défago, Suppression of root diseases by *Pseudomonas fluorescens* CHA0-Importance of the bacterial secondary metabolite 2,4-diacetylphloroglucinol, Mol. Plant Microbe Interact. 5 (1992) 4–13.

[10] J.R. Leadbetter, E.P. Greenberg, Metabolism of acyl-homoserine lactone quorum-sensing signals by *Variovorax paradoxus*, J. Bacteriol. 182 (2000) 6921–6926.

[11] Y. Lee, S.M. Assmann, Diacylglycerols induce both ion pumping in patch-clamped guard-cell protoplasts and opening of intact stomata, Proc. Natl. Acad. Sci. USA 88 (1991) 2127–2131.

[12] H. Marschner, Mineral Nutrition of Higher Plants, second ed, Academic Press, London, 1995.

[13] P.F. Morris, E. Bone, B.M. Tyler, Chemotropic and contact responses of *Phytophthora sojae* hyphae to soybean isoflavonoids and artificial substrates, Plant Physiol. 117 (1998) 1171–1178.

[14] D.A. Phillips, Biosynthesis and release of rhizobial nodulation gene inducers by legumes, in: E.W. Triplett (Ed.), Prokaryotic Nitrogen Fixation: A Model System for the Analysis of a Biological Process, Horizon Scientific Press, Wymondham, 2000, pp. 349–364.

[15] D.A. Phillips, H. Ferris, D.R. Cook, D.R. Strong, Molecular control points in rhizosphere food webs, Ecology 84 (2003) (in press).

[16] D.A. Phillips, C.M. Joseph, G.P. Yang, E. Martínez-Romero, J.R. Sanborn, H. Volpin, Identification of lumichrome as a *Sinorhizobium* enhancer of alfalfa root respiration and shoot growth, Proc. Natl. Acad. Sci. USA 96 (1999) 12275–12280.

[17] L.S. Pierson, D.W. Wood, E.A. Pierson, Homoserine lactone-mediated gene regulation in plant-associated bacteria, Annu. Rev. Phytopathol. 36 (1998) 207–225.

[18] A.D. Rovira, J.R. Harris, Plant root excretions in relation to the rhizosphere effect V. The exudation of B-group vitamins, Plant Soil 14 (1961) 199–214.

[19] A.R. Schäffner, Aquaporin function, structure, and expression: are there more surprises to surface in water relations? Planta 204 (1998) 131–139.

[20] G. Telford, D. Wheeler, P. Williams, P.T. Tomkins, P. Appleby, H. Sewell, G.S. Stewart, B.W. Bycroft, D.I. Pritchard, The *Pseudomonas aeruginosa* quorum-sensing signal molecule *N*-(3-oxododecanoyl)-1-homoserine lactone has immunomodulatory activity, Infect. Immun. 66 (1998) 36–42.

[21] M. Teplitski, J.B. Robinson, W.D. Bauer, Plants secrete substances that mimic bacterial *N*-acyl homoserine lactone signal activities and affect population density-dependent behaviors in associated bacteria, Molec. Plant Microbe Interact. 13 (2000) 637–648.

[22] A.J. Travis, T.A. Mansfield, Reversal of the CO_2 responses of stomata by fusicoccin, New Phytol. 83 (1979) 607–614.

[23] G.E. Treadwell, D.E. Metzler, Photoconversion of riboflavin to lumichrome in plant tissues, Plant Physiol. 49 (1972) 991–993.

[24] A.W.S.M. van Egeraat, Exudation of ninhydrin-positive compounds of pea seedling roots: a study of the sites of exudation and of the composition of the exudate, Plant Soil 42 (1975) 37–47.

[25] E. Voelkert, D.R. Grant, Determination of homoserine as the lactone, Anal. Biochem. 34 (1970) 131–137.

[26] S.C. Winans, Two-way chemical signaling in *Agrobacterium*-plant interactions, Microbiol. Rev. 56 (1992) 12–31.

[27] T. Yanagita, J.W. Foster, A bacterial riboflavin hydrolase, J. Biol. Chem. 221 (1956) 593–607.