

COMMUNICATION AND SIGNAL EXCHANGE IN THE *RHIZOBIUM/BRADYRHIZOBIUM-LEGUME* SYSTEM

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Summary: A new comprehensive communication concept in the *Rhizobium/Bradyrhizobium* legume symbiosis was developed. It includes a root zone specific flavonoid exudation, the differential activity of phenylpropane/acetate pathway derivatives on Chemotaxis, nod-gene inducing activity and phytoalexin resistance induction on the microsymbiont side (*Bradyrhizobium*). Nod factor production from the microsymbiont affects the host plant in root hair curling and meristem induction. Phytoalexin production in the host plant is also an early response, however repressed to a low level after a few hours. Another strategy of the microsymbiont to overcome phytoalexin effects is degradation of phytoalexins in *Rhizobium leguminosarum* bv. *viciae*. Competitiveness within the same infection group of the microsymbiont was studied with *gus*-gene fusion, using the blue coloured nodules to easily discriminate marked strains from unmarked competitors. New exopolysaccharide (EPS) mutants of *Bradyrhizobium japonicum* were reconstructed homologous with a DNA region to *exoB* gene of *Rhizobium meliloti*. Their clearly reduced competitiveness of nodulation, demonstrates that exopolysaccharides of *Bradyrhizobium japonicum* also have an important function during the early stages of this symbiotic interaction.

1. FLAVONOID ROOT EXUDATION

With soybean seeds 4-day-old seedlings were placed between cellulose acetate filters. Flavonoid exudation onto those filters was used to identify the special pattern of flavonoids along the root axes (Kape et al., 1992). The flavonoids exuded by

different areas were analysed by capillary electrophoresis. The ratio of the five compounds separated differs significantly in the root hair zone, the elongation zone and also the root tip. From the identified compounds, genistein and daidzein decreased markedly from the root hair zone to the root tip. Daidzein and genistein were found also in different ratios in the root exudate (14:1) and in the seed exudate (3.2:1). Significant differences in the composition of flavonoids in seeds and root exudates were also found in alfalfa (Phillips et al., 1990). The different flavonoids found in those seeds and root exudates had also different effects on the microsymbiont of alfalfa, *Rhizobium meliloti* (Hartwig et al., 1990). The authors used the term 'ecochemical zones' to describe those effects.

2. PRODUCTION OF THE PHYTOALEXIN GLYCEOLLIN I BY SOYBEAN ROOTS IN RESPONSE TO SYMBIOTIC INFECTION

Bradyrhizobium japonicum induces the production of low but significant amounts of glyceollin I in *Glycine max* seedling roots (Schmidt et al., 1992). These glyceollin I levels remain low even at high inoculum densities, indicating that the microsymbiont is capable of suppressing or preventing this plant defense response. Such a reduction in the plant defense response could be a prerequisite for a successful infection. We were interested whether rhizobia that are unable to nodulate soybean differ from *Bradyrhizobium japonicum* with respect to the amounts of glyceollin I/seedling they induce. When soybean roots were infected with suspensions of *Rhizobium meliloti* (unable to nodulate *Glycine max*), highly variable responses were observed. These ranged from no response up to a production of 3 nmol/seedling in the root hairs. It was determined that the variation was not due to the length of the incubation time. However, the glyceollin I concentration seemed to be dependent on the batch of bacterial cells used. In order to analyse the factors responsible for the variation, we tested crude cell extracts for their inducing capacity. Interestingly, incubation in such a preparation of *Rhizobium meliloti* cells always led to very high glyceollin I concentrations in the nmol-range.

Since it was found that the use of cell extracts led to more reproducible reactions by plant roots, these conditions were chosen to test two additional rhizobial strains. *Sinorhizobium fredii*, another *Glycine max* nodulating bacterium, and *Rhizobium leguminosarum*, a bacterium which is unable to nodulate *Glycine max*, were used. The two strains tested induced different glyceollin I levels. The glyceollin I levels increased significantly after incubation with the supernatant from a crude *Rhizobium meliloti* cell extract, whereas no enhancement could be observed after incubation with a comparable preparation from *Rhizobium leguminosarum* cells. In

addition, there were strong differences between the two soybean nodulating rhizobia analysed in this study. Disrupted *Bradyrhizobium japonicum* cells failed to induce any more glyceollin I accumulation than whole cells. Crude extract of *Sinorhizobium fredii* cells induced glyceollin I concentrations in soybean root exudate which are in the same order of magnitude as those found after compatible infection with zoospores of the root pathogen *Phytophthora megasperma* f. sp. *glycinea* race 1. No correlation was found between the ability of the rhizobial strains to infect soybeans and the glyceollin I levels induced by cell preparations from these strains.

3. ISOFLAVONOID-INDUCIBLE RESISTANCE TO THE PHYTOALEXIN GLYCEOLLIN IN SOYBEAN RHIZOBIA

From the results summarized in section 2 the next question arose whether *Bradyrhizobium japonicum* and *Sinorhizobium fredii* are sensitive towards the phytoalexin glyceollin. The antibacterial effect of the soybean phytoalexin glyceollin was assayed using a liquid microculture technique (Parniske et al., 1991). Log-phase cells of *Bradyrhizobium japonicum* and *Sinorhizobium fredii* were sensitive to glyceollin. As revealed by growth rates and survival tests, these species were able to tolerate glyceollin after adaptation. Incubation in low concentrations of the isoflavones genistein and daidzein induced resistance to potentially bactericidal concentrations of glyceollin. This inducible resistance is not due to degradation or detoxification of the phytoalexin. The inducible resistance could be detected in *B. japonicum* 110*spc4* and 61A101, representing the two taxonomically divergent groups of this species, as well as in *S. fredii* HH103, suggesting that this trait is a feature of all soybean-nodulating rhizobia. Glyceollin resistance was also inducible in a *nodD₁D₂YABC* deletion mutant of *B. japonicum* 110*spc4*, suggesting that there exists another recognition site for flavonoids besides the *nodD* genes identified so far. Exudate preparations from roots infected with *Phytophthora megasperma* f. sp. *glycinea* exhibited a strong bactericidal effect towards glyceollin-sensitive cells of *B. japonicum*. This killing effect was not solely due to glyceollin since purified glyceollin at concentrations similar to those present in exudate preparations had a much lower toxicity. However, glyceollin-resistant cells were also more resistant to exudate preparations than glyceollin-sensitive cells. Isoflavonoid-inducible resistance must therefore be ascribed an important role for survival of rhizobia in the rhizosphere of soybean roots.

The results of this study show that rhizobia which form symbioses with soybeans are susceptible to the phytoalexin glyceollin produced by their host plant. More interestingly, all soybean rhizobia tested are able to adapt to the presence of the

phytoalexin and tolerate previously bactericidal concentrations. Low concentrations of isoflavones can induce glyceollin resistance. These symbiotic signal compounds are constitutively present in the host plant rhizosphere (Graham, 1991; Parniske et al., 1988; Parniske et al., 1990).

Furthermore, the exudate from phytoalexin-producing soybean roots contains a toxic mixture of compounds that are able to kill cells of the microsymbiont. The killing effect of this exudate is not only due to the presence of glyceollin. However, glyceollin-resistant cells are much more resistant to this mixture than sensitive ones. We conclude that one important function of the inducible resistance is to increase the survival of the microsymbiont in the rhizosphere of challenged host roots.

Phytoalexin accumulation upon contact with incompatible pathogenic microorganisms is a local response. Only plant cells at or in the vicinity of the infection site react with a massive production of phytoalexins (Hahn et al., 1985). Field-grown soybean roots should have multiple sites of phytoalexin accumulation due to the presence of pathogenic organisms and other environmental stress factors that elicit defense responses. It is therefore plausible that the rhizosphere of soybeans consists of temporal as well as spatial concentration gradients of phytoalexins. An inducible resistance would allow cells of the microsymbiont the physiological plasticity to adapt to changes in phytoalexin concentration.

It is not yet clear whether phytoalexin resistance confers any advantages other than the ability to survive in the rhizosphere of phytoalexin-producing plants. Reports of enhanced flavonoid or phytoalexin concentrations at several different steps of the symbiotic legume-*Rhizobium* interaction after infection with compatible (Recourt et al., 1990), incompatible (Werner et al., 1985) or mutant (Djordjevic et al., 1988) rhizobia suggest that phytoalexin resistance could also be important at later stages of the interaction.

B. japonicum 110 and 61A101 represent two highly divergent groups within this bacterial species. The fast-growing microsymbiont of soybeans, *S. fredii*, is very little related to the bradyrhizobia (Chen et al., 1988). Yet, all soybean rhizobia tested exhibited the phenotype of inducible resistance. The apparent conservation of this phenotype between these relatively unrelated rhizobia suggests an essential role in the symbiotic interaction with the host plant.

In the rhizosphere of *G. max*, isoflavones are constitutively present (Kosslak et al., 1987). These isoflavones have at least two functions in the interaction between *G. max* and its microsymbionts: a) they induce the common *nod* genes (Djordjevic et al., 1987) as well as *B. japonicum* genes for host-specific nodulation of soybeans (Banfalvi et al., 1988) and b) they apparently also induce the resistance against the phytoalexin glyceollin. The *nodD* gene product is believed to interact with flavonoids, thereby activating the transcription of the common *nod* genes. However, in the

common *nod* mutant 1240, tolerance to glyceollin is still inducible, indicating that these genes are not required for the expression of the resistance. Thus, a recognition process specifically activated by isoflavonoids not involving the previously described *nodD* genes has to be postulated in *B. japonicum*.

4. DEGRADATION OF WYERONE, THE PHYTOALEXIN OF FABA BEANS BY *RHIZOBIUM LEGUMINOSARUM* BV. *VICIAE*

Wyerone is the predominant phytoalexin produced by *Vicia faba*. At a concentration of 100 μM , wyerone prolonged the lag phase before the onset of exponential growth of different strains of *Rhizobium leguminosarum* and *Bradyrhizobium japonicum*. The response to wyerone was dose dependent, with a shorter lag phase occurring at lower concentrations. Wyerone was only moderately inhibitory towards the phytopathogenic bacterium *Erwinia carotovora* cv. *atroseptica*. HPLC analysis of the medium during bacterial growth indicated that *Rhizobium leguminosarum* was able to metabolize wyerone (Görge and Werner, 1991). The identification of the product as hydroxyester wyerol was confirmed by GC/MS analysis.

5. ISOLIQUIRITIGENIN, A STRONG *NOD* GENE- AND GLYCEOLLIN RESISTANCE-INDUCING FLAVONOID FROM SOYBEAN ROOT EXUDATE

The following new biological activities of isoliquiritigenin were found:

1. Induction of *nod* genes. Isoliquiritigenin (2',4',4'-trihydroxychalcone) was identified as a strong *nod* gene inducer. This induction was compared to that of other *nod* gene inducers (Kape et al., 1992). Isoliquiritigenin exhibited an inducing activity already at 1 nM. This concentration is about 1 order of magnitude below those required of the previously described strong *nod* gene inducers daidzein and genistein. The stronger *nod* gene-inducing activity of isoliquiritigenin at extremely low concentrations is in contrast to its reduced inducing activity at higher concentrations; the opposite is true for daidzein or genistein. The maximal *nod* gene induction of all three compounds was achieved at a concentration of 10 μM . The concentrations required for half-maximum activities of *nod* gene induction were 52, 144, and 159 μM for isoliquiritigenin, daidzein, and genistein, respectively.

2. Chemotactic effect of isoliquiritigenin. No chemotactic effect of isoliquiritigenin could be detected, while the positive control, caffeic acid, caused a strong chemotactic response. A chemotactic ratio of 31 was obtained for caffeic acid at a concentration of 10 mM.

Table 1. Nodule occupancy in different *Rhizobium leguminosarum* bv. *phaseoli* and *Rhizobium tropici* strains when coinoculated with the *Rhizobium phaseoli* strain KIM5s *gus+*

<i>Rhizobium</i> spp. strain	Nodule occupancy/KIM5s (%)	
	pH 6.4	pH 5.2
CIAT 2510	4± 2	ND
H2C	4± 4	ND
CIAT 323	9± 5	10± 3
<u>CFN 1500</u>	11± 11	67± 20*
CE 3	23± 2	ND
<u>C-05-II</u>	25± 17	82± 20*
CIAT 7004	30± 4	23± 8
<u>CIAT 899</u>	41± 4	76± 18*
CIAT 895	54± 10	33± 10*
Tal 182	68± 4	72± 15
CIAT 652	79± 4	73± 22
Costa 6	79± 11	ND
CIAT 7014	82± 7	67± 12
CFN 227	85± 11	ND
CIAT 151	89± 10	90± 10
CIAT 904	91± 4	86± 4
CIAT 163	91± 8	95± 5
CIAT 956	93± 6	93± 6
CFN 1600	94± 8	86± 13
CIAT 611	96± 4	96± 2

Means ± SD of two replicates of five plants each. *R. tropici* strains are underlined. Assays were performed in growth pouches at two pH levels. * P<0.05, versus nodule occupancy at pH 6.4. (From Streit et al., 1992)

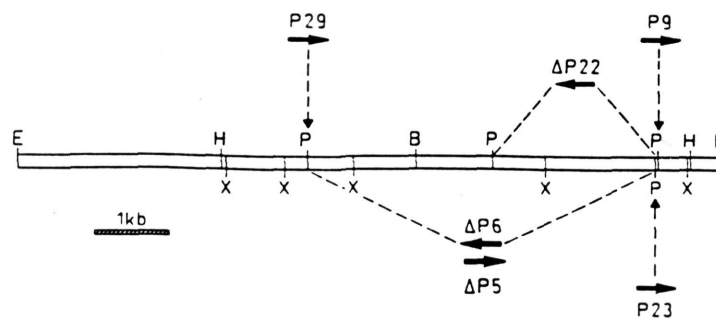


Fig. 1: Restriction map of the 9.1-kb EcoRI fragment of *Bradyrhizobium japonicum* 110spc4 DNA. The two internal PstI fragments of 2.1 and 2.4 kb hybridized with a *exoB* specific probe of *R. meliloti*. The genotypes of the insertion mutants P9, P23, and P29 and of the deletion mutants DP5, DP6, and DP22 are indicated by the insertion points and orientation of the *aph* gene of pUC4K. Abbreviations: E, EcoRI; H, HindIII; P, PstI; X, XhoI; B, BamHI. (From Parniske et al., 1993)

3. Induction of glyceollin resistance. Isoliquiritigenin proved to be a strong inducer of glyceollin resistance in *B. japonicum* 110*spc4* harboring pRJ458. The applied concentration of 10 μ M resulted in a resistance of 90% of all cells in the test culture against a glyceollin concentration of 300 μ M. The degree of resistance is as high as that obtained by genistein, the best inducer of glyceollin resistance described up to now.

6. NODULATION COMPETITIVENESS OF *RHIZOBIUM LEGUMINOSARUM* BV. *PHASEOLI* AND *RHIZOBIUM TROPICI* STRAINS MEASURED BY GLUCURONIDASE (*GUS*) GENE FUSION

The nodulation competitiveness of 17 *R. leguminosarum* bv. *phaseoli* and 3 *R. tropici* strains was studied at pH 6.4 and 5.2 (Streit et al., 1992). These 20 strains were each coinoculated with a *gus*⁺ mutant of *R. leguminosarum* bv. *phaseoli* strain KIM5s. A range of nodulation competitiveness was obtained. The least successful strains occupied 4% of nodules (*R. leguminosarum* bv. *phaseoli* strains CIAT 2510 and H2C) and 96% were formed by the most competitive strain CIAT 611 (Table 1). The competition tests showed that the three *R. tropici* strains (CIAT 899, C-05-II, and CFN 1500) were poorly competitive in nodulation at the higher (near neutral) pH value; *R. tropici* nodule occupancy increased significantly ($P < 0.05$) at pH 5.2 compared to pH 6.4. Under these slightly acid conditions, the *R. tropici* strains were almost as competitive in nodulation as the competitive *R. leguminosarum* bv. *phaseoli* strains. Other authors have also observed an increase in nodulation by *R. tropici* strains under acid soil conditions (Vargas and Graham, 1989). However, soil type can override the effect of low pH on *R. tropici* strains (Streit et al., 1990; Wolff et al., 1991). In contrast, the *R. leguminosarum* bv. *phaseoli* strains were generally not affected by pH. Only strain CIAT 895 was significantly less competitive ($P < 0.05$) at pH 5.2. The results obtained for this strain are consistent with its failure to nodulate in acid soils. In acid field sites the *R. leguminosarum* bv. *phaseoli* strains CIAT 151, KIM5s, CE3 and H2C also showed decreased nodule occupancy. These results indicate that low nodule occupancy, under acid soil conditions, is more likely due to Mn or Al toxicity or other environmental stress factors than to the low pH itself.

7. EXOB MUTANTS OF *BRADYRHIZOBIUM JAPONICUM* WITH REDUCED COMPETITIVENESS FOR NODULATION OF *GLYCINE MAX*

Exopolysaccharide (EPS) mutants of *Bradyrhizobium japonicum* defective within a DNA region homologous to the *Rhizobium meliloti* *exoB* gene were con-

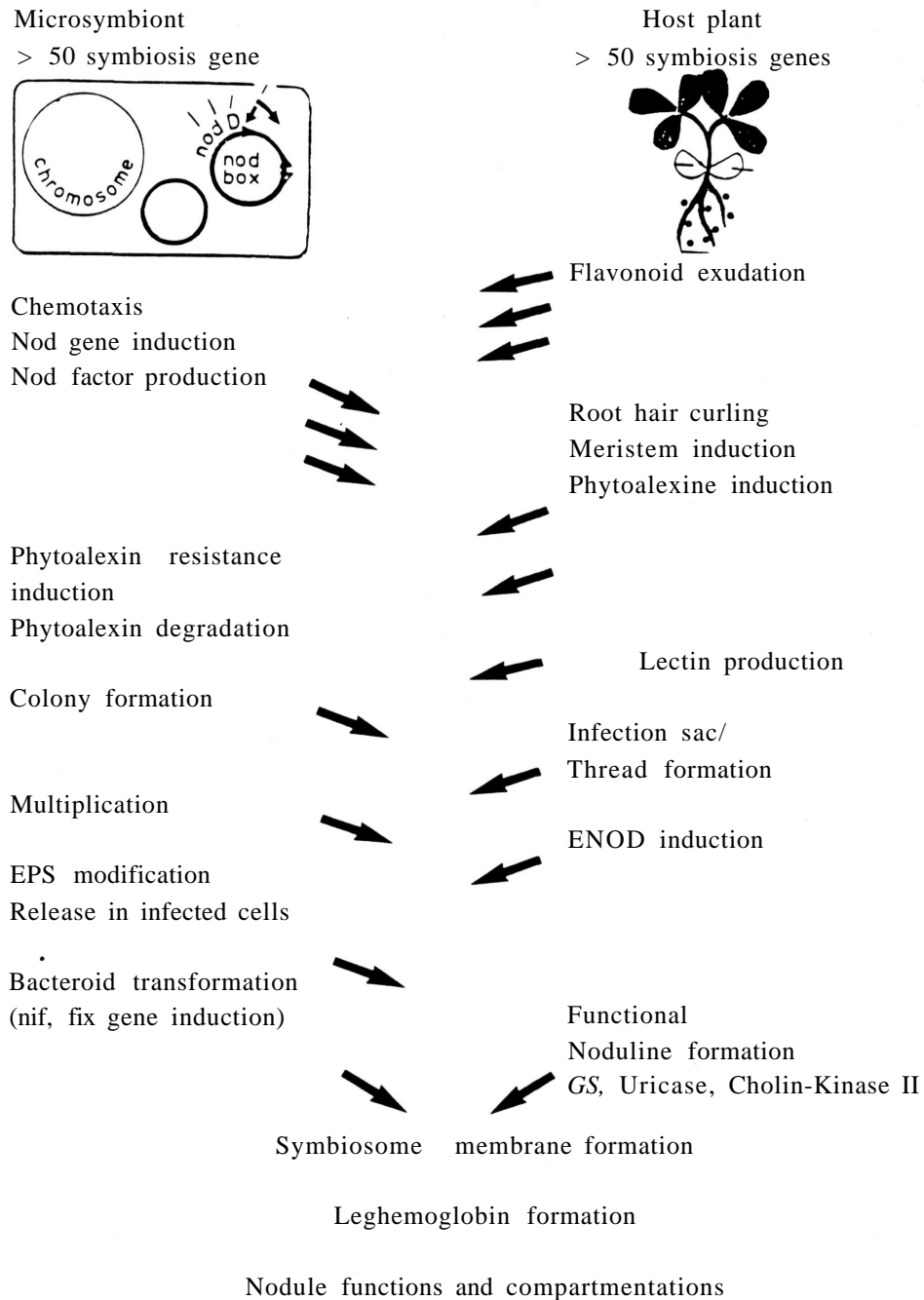


Fig. 2: Communication in the Rhizobium/Bradyrhizobium Legume Symbiosis

structed (Parniske et al., 1993). Using an interspecies complementation approach, two overlapping cosmid clones of *B. japonicum* Z)NA were isolated. A 9.1 kb-*EcoRI* subclone common to both cosmids was found to restore the ability of the *exoB* mutant *R. meliloti* H36, to induce effective nodules on alfalfa plants and to form fluorescent colonies on agar media containing cellufluor white. Km^r -deletion as well as insertion derivatives of this fragment were introduced into *B. japonicum* 110*spc4* by marker exchange. The resulting deletion mutants DP5, DP6 (4.5 kb each) and DP22 (2.1 kb) were designated *exoB*-mutants, because they lacked UDP-glucose 4'-epimerase activity and the deleted regions hybridized with an *exoB* DNA probe of *R. meliloti* (Fig. 1). The mutants had a non-mucoid colony morphology. In contrast to the wild type EPS, no galactose could be detected in the residual EPS produced by the mutant strains, indicating an altered EPS composition. The mutant strains exhibited a wild-type lipopolysaccharide pattern on Polyacrylamide gels. Although the mutants induced effective nodules on soybean, the early stages of the symbiotic interaction were disturbed. Nodulation was delayed by about 5 days, and the mutants exhibited a greatly reduced competitiveness. When the mutants DP5 or DP22 were coinoculated together with the parent strain 110 at similar titers, almost all of the nodules were occupied by the wild-type. An at least 100-fold excess of DP5 or DP22 cells was necessary to obtain half of the nodules occupied by the mutant strains. This effect is not due to the presence of the *aph* gene in these mutants, as revealed by the unaffected competitiveness of the insertion mutants and P29. The reduced competitiveness of *B. japonicum* *exoB*-mutants for nodulation of soybean clearly demonstrates that exopolysaccharides of *B. japonicum* carry out important functions during the early stages of the symbiotic interaction.

8. THE MICROSymbiont-HOST PLANT COMMUNICATION SCHEME

The results reported in the 7 paragraphs of this review are summarized in Fig. 2. It demonstrates the continuing communication between the two symbiotic partners, starting from the specific signal exudation from the roots, down to the final nodule functions and compartmentations (see also Quispel, 1992; Stacey et al., 1992; Werner 1992).

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REFERENCES

- Banfalvi Z, Nieuwkoop A, Schell M, Besl L, Stacey G, 1988. Regulation of nod gene expression in *Bradyrhizobium japonicum*. Mol. Gen. Genet. 214:420-424.
- Chen WX, Yan GH, Li JL, 1988. Numerical taxonomic study of fast-growing soybean rhizobia and a proposal that *Rhizobium fredii* be assigned to *Sinorhizobium* gen. nov. Int. J. Syst. Bacteriol. 38:392-397.
- Djordjevic MA, Gabriel DW, Rolfe BG, 1987. *Rhizobium* - the refined parasite of legumes. Ann. Rev. Phytopathol. 25:145-168.
- Djordjevic SP, Ridge RW, Chen H, Redmond JW, Batley M, Rolfe BG, 1988. Induction of pathogenic-like responses in the legume *Macroptilium atropurpureum* by a transposon-induced mutant of the fast-growing, broad-host-range *Rhizobium* strain NGR234. J. Bacteriol. 170:1848-1857.
- Görge E, Werner D, 1991. Degradation of wyerone, the phytoalexin of faba beans by *Rhizobium leguminosarum*. Curr. Microbiol. 23:153-157.
- Graham TL, 1991. Flavonoid and isoflavonoid distribution in developing soybean seedling tissues and in seed and root exudates. Plant Physiol. 95:594-603.
- Hahn MG, Bonhoff A, Grisebach H, 1985. Quantitative localization of the phytoalexin glyceollin I in relation to fungal hyphae in soybean roots infected with *Phytophthora megasperma* f. sp. *glycinea*. Plant Physiol. 77:591-601.
- Hartwig UA, Maxwell CA, Joseph CM, Phillips DA, 1990. Effects of alfalfa *nod* gene-inducing flavonoids on *nodABC* transcription in *Rhizobium meliloti* strains containing different *nodD* genes. J. Bacteriol. 172:2769-2773.
- Kape R, Parniske M, Brandt S, Werner D, 1992. Isoliquiritigenin, a strong *nod* gene- and glyceollin resistance-inducing flavonoid from soybean root exudate. Appl. Environ. Microbiol. 58:1705-1710.
- Kape R, Wex K, Parniske M., Görge E., Wetzel A, Werner D, 1992. Legume root metabolites and VA-mycorrhiza development. J. Plant Physiol. 141:54-60.
- Kosslak RM, Bookland R, Barkei J, Paaren HE, Appelbaum ER, 1987. Induction of *Bradyrhizobium japonicum* common nod genes by isoflavones isolated from *Glycine max*. Proc. Natl. Acad. Sci. USA 84:7428-7432.
- Parniske M, Pausch G, Werner D, 1988. Changes in flavonoid pattern of root hairs of *Glycine max* in response to symbiotic infection with *Bradyrhizobium japonicum*. In H Bothe, FJ de Bruijn, WE Newton, eds, Nitrogen Fixation: Hundred Years After, G. Fischer Verlag, Stuttgart, p. 466.
- Parniske M, Zimmermann C, Cregan PB, Werner D, 1990. Hypersensitive reaction of nodule cells in the *Glycine sp./Bradyrhizobium japonicum*-symbiosis occurs at the genotype-specific level. Bot. Acta 103:143-148.
- Parniske M, Ahlborn B, Werner D, 1991. Isoflavonoid-inducible resistance to the phytoalexin glyceollin in soybean rhizobia. J. Bacteriol. 173:3432-3439.
- Parniske M, Kosch K, Werner D, Müller P, 1993. ExoB mutants of *Bradyrhizobium japonicum* with reduced competitiveness for nodulation of *Glycine max*. MPMI 6:99-106.
- Phillips DA, Hartwig UA, Maxwell CA, Joseph CM, Wery J, Hungria M, Tsai SM, 1990. Host legume control of nodulation by flavonoids. In PM Gresshoff, LE Roth, G Stacey, WE Neston, eds, Nitrogen Fixation: Achievements and Objectives, Chapman & Hall, New York, London, pp. 331-338.

- Recourt K, van Brüssel AAN, Kijne J, Schripsema J, Lugtenberg BJJ, 1990. Inoculation of *Vicia sativa* sssp. *nigra* with *Rhizobium leguminosarum* bv. *viciae* increases the number of nod gene inducing flavonoids released by the roots of the host plant. In PM Gresshoff, LE Roth, G Stacey, WE Neston, eds, Nitrogen Fixation: Achievements and Objectives, Chapman & Hall, New York, London, p. 271.
- Quispel A, 1992. A search for signals in endophytic microorganisms. In DPS Verma, ed, Molecular Signals in Plant-Microbe Communications, CRC Press, Boca Raton, pp. 471-491.
- Schmidt PE, Parniske M, Werner D, 1992. Production of the phytoalexin glyceollin I by soybean roots in response to symbiotic and pathogenic infection. Bot. Acta 105:18-25.
- Stacey G, Gresshoff PM, Keen NT, 1992. Friends and foes: New insights into plant-microbe interactions. The Plant Cell ? 1173-1179.
- Streit W, Kipe-Nolt J, Werner D, 1991. Competitive growth of *Rhizobium leguminosarum* bv. *phaseoli* strains under oligotrophic conditions. Curr. Microbiol. 23:159-163.
- Streit W, Kosch K, Werner D, 1992. Nodulation competitiveness of *Rhizobium leguminosarum* bv. *phaseoli* and *Rhizobium tropici* strains measured by glucuronidase (*gus*) gene fusion. Biol. Fertil. Soils 14:140-144.
- Vargas AAT, Graham PH, 1989. Cultivar and pH effects on competition for nodule sites between isolates of *Rhizobium* in beans. Plant & Soils 117:195-200.
- Werner D, 1992. Symbiosis of Plants and Microbes, Chapman & Hall, London, New York, 389p.
- Werner D, Mellor RB, Hahn MG, Grisebach H, 1985. Soybean root response to symbiotic infection. Glyceollin I accumulation in an ineffective type of soybean nodules with an early loss of the peribacteroid membrane. Z. Naturforsch. 40c:179-181.
- Wolff AB, Streit W, Kipe-Nolt JA, Vargas H, Werner D, 1991. Competitiveness of *Rhizobium leguminosarum* bv. *phaseoli* strains in relation to environmental stress and plant defense mechanisms. Biol. Fertil. Soils 12:170-176.