Plant Physiol. (1989) 90, 1347-1352
0032-0889/89/90/1347/06/$06.00/0

Received for publication December 6, 1988 and in revised form March 16, 1989

Lack of Systemic Suppression of Nodulation in Split Root Systems of Supernodulating Soybean (Glycine max [L.] Merr.) Mutants[1]

Jane E. Olsson, Patricia Nakao, B. Ben Bohlool, and Peter M. Gresshoff*

Botany Department, Australian National University, Canberra, ACT 2600, Australia (J.E.O.), Niftal Project, University of Hawaii, 1000 Holomua Avenue, Paia, Maui, Hawaii 96779-0744 (P.N., B.B.B.), and Plant Molecular Genetics, College of Agriculture, University of Tennessee, Knoxville, Tennessee 37901-1071 (P.M.G.)

ABSTRACT

Wild-type soybean *(Glycine max [L]* Merr. cv Bragg) and a nitrate-tolerant supernodulating mutant (nts382) were grown in split root systems to investigate the involvement of the autoregulation response and the effect of timing of inoculation on nodule suppression. In Bragg, nodulation of the root portion receiving the delayed inoculation was suppressed nearly 100% by a 7-day prior inoculation of the other root portion with *Bradyrhizobium Japonicum* strain USDA110. Significant suppression was also observed after a 24-hour delay in inoculation. Mutant nts382 in the presence of a low nitrate level (0.5 millimolar) showed little, if any, systemic suppression. Root fresh weights of individual root portions were similar for both wild type and nts382 mutant. When nts382 was grown in the absence of nitrate, a 7-day delay in inoculation resulted in only 30% suppression of nodulation and a significant difference in root fresh weight between the two sides, with the delayed inoculated side always being smaller. Nodulation tests on split roots of nts382, nts1116, and wild-type cultivars Bragg, Williams 82, and Clark demonstrated a difference in their systemic suppression ability. These observations indicate that (a) autoregulation deficiencies in mutant nts382 result in a reduction of systemic suppression of nodulation, (b) some suppression is detectable after 24 hours with a delayed inoculation, (c) the presence of low nitrate affects the degree of suppression and the root growth, and (d) soybean genotypes differ in their ability to express this systemic suppression.

Legumes regulate the degree of nodulation by a variety of mechanisms (3, 6, 12). The major one is the developmental suppression of further nodulation by previously formed or forming nodules. This occurs along the tap root (18) as well as in split root systems (14). This process of internal nodulation control is termed "autoregulation of nodulation" (3, 12, 17). Suppression of nodulation in split root systems of *Glycine* max (L.) Merr. cv Davis caused by a 48-h prior inoculation of the other root half was first described by Singleton (19). This was examined in detail by Kosslak and Bohlool (14), who showed that a suppressive effect in cultivar Lee was evident when inoculation of the second side was delayed by as little as 96 h. Total suppression of nodulation was seen in a 7-d delayed inoculation and was claimed to be related to the photosynthetic potential of the host plant. Bohlool *et al.* (4) indicated that the suppressive effect was more pronounced in winter-grown plants, when dry matter production was lower.

Carroll *et al.* (6, 7) described soybean mutants that nodulate profusely in the absence or presence of nitrate. These mutants have a supernodulation phenotype characterized by extensive nodulation over a large proportion of the root system. Supernodulation is controlled by a single Mendelian recessive gene acting through the shoot of the plant (10, 11), as confirmed by reciprocal grafts between wild-type and supernodulation genotypes. Line nts382 exhibits lessened autoregulation of nodulation, most likely through the loss of a yet unidentified inhibitory substance normally produced in the shoot of wildtype plants after successful infection (13). The loss of this infection-related, shoot-derived inhibitor leads to the observed nitrate tolerance. Plant growth analysis (8) showed that *nts* plants grow like wild-type plants, if grown on nitrate in the absence of the bacterial inoculum, but that inoculation negatively affected growth of both plant shoot and root.

Since the systemic nodule suppression described by Kosslak and Bohlool (14) seemed to be related to the host's mechanism of nodule regulation, a study was conducted on the behavior of the supernodulation mutant using time-separated inoculations in split root systems. Because genetic analysis confirmed that one single mutational unit caused the absence of autoregulation, supernodulation, and nitrate tolerance (9, 11), the present study allowed the analysis of the autoregulation mechanism in the nodule suppression phenomena as described by Kosslak and Bohlool.

Partial support for this project was provided by U.S. Department of Agriculture grant 85-CRCR-1-1602 and U.S. Agency for International Development cooperative agreement DAN-4177-A-00-603500 (Niftal Project) and by Australian Government (DITEC) project grant (S39-PIP). J.O. was a recipient of an ANU postgraduate award.

MATERIALS AND METHODS

Bacterial Strains and Culture

Bradyrhizobium japonicum strain USDA110 was used throughout this study. Inoculum was grown on BMM medium (2) to midlogarithmic phase and diluted in nutrient solution (5) to the required titre (10^8 to 10^9 cells/mL).

Plant Material

Two lines of soybean *(Glycine max* [L.] Merr.) were used predominantly throughout this study: wild-type cultivar Bragg

(maturity group VII) and a supernodulating mutant nts382 (6, 7) derived directly from Bragg by EMS[2] mutagenesis. The intermediate nodulation mutant nts1116, which was isolated in the same mutant screen and most likely contains a leaky allele at the genetic locus controlling the supernodulation phenotype of nts382 (I1, 12), was also used. Intermediate nodulation (also termed hypernodulation) was shown to be shoot controlled (10, 13). Mutant material is the direct progeny from selected families and may contain other mutated genes. However, general growth analysis (8), genetic segregation analysis (11), and physiological tests (9) allow us to conclude that supernodulation and nitrate tolerance in modulation are caused by the same genetic unit and that any background mutations have a minimal effect on plant growth and the observed symbiotic phenotype. Two other wild-type cultivars, Williams 82 (maturity group III) and Clark (maturity group IV), were used. Bragg was derived from germplasm leading back to the Peking accession, whereas Clark and Williams were derived from the Mandarin accession. Both gene pools are quite distinct (1).

Split Root Apparatus and Detailed Experimental Design

Slight differences in nutrient solutions and of inoculation procedures between the two localities are detailed with each experiment. Data are presented for single, but representative, experiments conducted over an 18-month period. In general, the experiments used similar equipment, procedures, and harvesting methods. In short, the split root culture system was a modification of that used by Singleton (19). PVC piping elbows were attached to two PVC pipes (40 mm internal diameter x 250 mm length) packed with vermiculite. The purpose of using the elbow was to guide the lateral roots in opposite directions so that they could be separated into two growth compartments (i.e. split root tubes) for use in a variety of autoregulation studies.

The ends of the compartments were sealed with two layers of Parafilm, and the bottom of the elbows were secured with two Suba seal rubber plugs to prevent vermiculite spillage. The units were taped together to maintain rigidity. Plants were watered through microtubing (2 mm diameter) inserted into the Parafilm at the top of the pipes. Tubing attached to drainage holes about 5 mm above the bottom of each pipe directed excess nutrient solution away from the chambers, thereby preventing water logging of the root systems. The PVC parts were sterilized in 3% sodium hypochlorite for several hours and rinsed extensively in sterile distilled water. Vermiculite and nutrient solutions were sterilized by autoclaving. Detailed plans of the split root assembly are available from the authors.

Seeds were selected for uniformity of size and rinsed with 70% ethanol for 2 min to kill *Bradyrhizobium* contaminants. Such seeds were unable to nodulate when planted in uninoculated control experiments, although they still contained some seed-borne *non-Bradyrhizobium contaminants*. Hand-harvested seeds were free of such seed-borne bacterial contaminant. Ethanol was rinsed off with two washings of sterile distilled water, and seeds were covered with 3% (v/v) sodium hypochlorite solution and swirled for 3 min. The hypochlorite was drained off and the seeds were washed thoroughly with up to 10 washings of sterile distilled water. This extended procedure removed all bacterial contaminants on seeds, but caused some germination losses. Seeds were planted hilum down in moistened vermiculite.

Thirty-six hours after planting and incubation at 30°C in the dark, the tips (about 5-7 mm) of the radicles were removed, and the seedlings were planted into PVC elbows as described (19, 20). Lateral root formation was observed from the ends of the elbows after 5 to 7 d of culture in 25°C and dim light. At this stage, seedlings were selected for uniformity of root formation and placed on top of the PVC pipes containing vermiculite premoistened with about 50 mL of Herridge's nutrient solution (10) containing 0.5 mm $KN0_3$. This level of nitrate did not inhibit nodulation in the soybean genotypes used in these experiments. Inoculation with strain USDA 110 was as follows. Root E refers to the root portion inoculated early. At the time of elbow attachment to the pipes, 30 mL of bacterial suspension, containing about 10^8 to 10^9 viable cells in total, were added to the appropriate root portion. Root portions not receiving the inoculum were treated with nutrient solution alone. The second inoculation on root D (for delayed) occurred at 1, 2, 4, or 7 d after inoculation of root E. Each root portion was irrigated with about 30 mL of nutrient solution, with or without low-level nitrate, every 3 to 5 d as required. Split root assemblies were cultured under full sunlight in temperature-regulated (19°C to 31 °C night/day) glasshouse space receiving additional lighting to assure vegetative growth. The nutrient solution used on Maui was as described in ref. 5, whereas in Canberra quarter-strength Herridge's solution was used for the first 2 weeks, with full strength afterwards. Footnotes below tables indicate where the experiment was carried out and thus which nutrient solution was used.

Total root portions were harvested 24 to 33 d after the initial inoculation. All plants were dried at 60°C for at least 72 h prior to dry weight determination.

Detailed Procedures

(A) Systemic Suppression in a Supernodulation Mutant

Split root systems of Bragg and nts382 plants with three inoculation patterns were used: both sides were inoculated early (early/early), root E was inoculated early and root D 7 d later (early/delayed), and both sides were inoculated delayed (delayed/delayed). Both roots were supplied with a noninhibitory concentration of nitrate (0.5 mm $KN0_3$). Plants were scored 26 d after the inoculation of root **D.**

(B) Timing of Systemic Suppression in Bragg

Root E was inoculated early; inoculation of root D was either concurrent or with a 1-, 2-, 4-, or 7-d delay. Both root portions were supplied with 0.5 mm $KN0_3$. Harvesting occurred 21 d after the second inoculation.

(C) Nitrate Effect on Systemic Suppression in nts382

Split roots of Bragg and nts382 were grown as described above in section (A), but lacking the nitrate supplementation. Harvest and culture conditions were similar to those in (A).

[2]Abbreviations: EMS, ethyl methane sulfonate; PVC, polyvinylchloride, NFW, nodule fresh weight.

(D) Genotypic Variation in Systemic Suppression

Split roots of Bragg, Williams 82, Clark (all wild-type cultivars), supernodulation mutant nts382, and hypernodulation mutant nts 1116 were inoculated with a 7-d delay. Plants were grown as described in section (A) and received low-level nitrate. Plants for this experiment were harvested 17 d after inoculation of root D.

RESULTS

Lack of Systemic Suppression of Nodulation in nts382 Grown on Low Nitrate

Table I compares the nodule number per root portion of cultivar Bragg and nts382 grown in the presence of 0.5 mm KNO_3. Plants were either inoculated simultaneously or delayed as described in "Materials and Methods." Root halves of Bragg with early/early inoculations produced about 15 to 20 nodules per root half, in contrast to about 30 nodules for delayed/delayed inoculations. Early inoculation of root E severely inhibited nodule appearance on root D. Total nodule number per root portion in early/delayed plants was similar to the sum of nodules in early/early plants. Delayed/delayed inoculations repeatedly showed increased nodule numbers, presumably caused by the increased size of the root system.

In clear contrast to Bragg were the data from mutant nts382. Nodule number per root portion was about 150 to 170 in early/early inoculations, indicating the supernodulation phenotype. Nodules were found over a large proportion of the root system in nts382, in contrast to clustered nodulation in Bragg. Delayed/delayed inoculation did not significantly increase nodule number per root half compared with early/ early inoculations, suggesting that the maximum nodulation capacity under these

attained. A striking difference to Bragg was that early/delayed inoculation did not result in systemic suppression on the delayed inoculated side.

Table I also presents supporting plant growth data for the nodule number observations. Mutant nts382 had increased nodule mass compared with Bragg in early/early inoculations (0.29 g NFW per root portion *versus* 0.065 g NFW.per root portion). Both Bragg and nts382 had very similar nodule mass, root fresh and dry weight, and nodule number per root mass, if comparing root E and root D of early/early and delayed/delayed treatments.

Nodule mass per plant was constant among all treatments within a genotype (about 0.13 to 0.16 g per plant for Bragg *versus* 0.50 to 0.70 g per plant for nts382). Nodule mass in delayed/delayed inoculations of nts382 was the lowest in the set, because of the shorter available time to accumulate nodule mass (28 d for early, 21 d for delayed).

Systemic suppression in early/delayed treatments of Bragg (Table I) was also reflected in nodule mass and nodule number per root mass. Significantly, root masses of root E and root D within each subset of data did not differ. However, nts382 roots showed reduced root development in all cases relative to Bragg (cf. ref. 8).

Time Course of Systemic Suppression in Bragg

Table II shows that systemic suppression of nodulation can be detected in soybean as early as 24 h after the initial inoculation of root E. Nodule number compensation in Bragg was detected in early/uninoculated control plants and early/ early plants (23 nodules per plant in both cases). Nodule mass and root mass (roots without nodules) followed a similar trend, although with a greater variability. A 24-h delay in

Table I. *Nodulation and Root Growth of Split Roots cv Bragg and nts382 Grown in the Presence of Low Nitrate.*

Experiment was conducted in Canberra as described in "Materials and Methods." Plants were grown in the presence of 0.5 mM KNO_3. NN, nodule number; NFW, nodule fresh weight; RFW, root fresh weight without nodules.

Genotype	Inoculation Pattern (*n*)[a]	NN	NFW	RFW
			mg[c]	*g*[c]
Bragg	Early (4)	19 ± 3[b]	60 ± 10	1.31 ± 0.33
	Early (4)	17 ± 1	70 ± 30	1.47 ± 0.31
	Early (5)	31 ± 6	130 ± 14	2.12 ± 0.25
	Delayed (5)	0	0	1.99 ± 0.28
	Delayed (7)	33 ± 6	80 ± 12	2.04 ± 0.60
	Delayed (7)	30 ± 4	80 ± 8	2.10 ± 0.50
nts382	Early (4)	165 ± 30	300 ± 60	0.57 ± 0.28
	Early (4)	153 ± 18	280 ± 70	0.56 ± 0.38
	Early (8)	173 ± 20	380 ± 32	0.91 ± 0.17
	Delayed (8)	150 ± 14	320 ± 35	0.93 ± 0.37
	Delayed (3)	191 ± 32	230 ± 24	1.14 ± 0.40
	Delayed (3)	196 ± 31	270 ± 38	1.04 ± 0.46

[a] Early means inoculation occurred at time of split root apparatus establishment about 7 to 9 d after seed imbibition; delayed means 7 d delayed inoculation; *n* = number of replicates. [b] All data expressed as means ± SE. [c] Per root portion.

Table II. *Time Course of Nodule Suppression in a Split Root System of Cultivar Bragg.*

Experiments were conducted in Canberra as described. Abbreviations are as in Table I. All plants were grown in the presence of KNO_3.

Inoculation Pattern (*n*)[a]	NN	NFW	RFW
		mg[c]	*g*[c]
Early (5)	23 ± 5[b]	70 ± 9	0.86 ± 0.08
Uninoculated (5)	0	0	0.68 ± 0.05
Early (5)	13 ± 2	56 ± 9	0.99 ± 0.10
Early (5)	10 ± 2	40 ± 9	0.93 ± 0.16
Early (4)	18 ± 7	70 ± 35	0.79 ± 0.30
24-h delay (4)	4 ± 2	40 ± 15	0.55 ± 0.11
Early (6)	17 ± 7	50 ± 14	0.99 ± 0.17
48-h delay (6)	7 ± 2	40 ± 18	0.55 ± 0.10
Early (7)	26 ± 6	70 ± 25	1.17 ± 0.14
4-d delay (7)	3 ± 1	40 ± 18	0.85 ± 0.16
Early (9)	17 ± 3	80 ± 23	0.94 ± 0.13
7-d delay (9)	0	0	0.65 ± 0.08
Uninoculated (4)	0	0	1.34 ± 0.22
7-d delay (4)	18 ± 4	99 ± 19	1.15 ± 0.34

[a] Early inoculation means that the root portion was inoculated when the split root apparatus was set up (*i.e.* about 7 to 9 d after the seed imbibition. Timepoints for delayed inoculations are given; *n*, number of replicates. [b] All data are means ± SE. [c] Per root portion.

inoculation on root D caused a suppression of nodule number of as much as 70% on root D (although ranges from 40% to 60% suppression were found in related experiments). This degree of suppression was also observed for delayed inoculations over the first 4 d, whereas a 7-d delay caused complete suppression. These findings supported those in Table I and indicated that systemic suppression is a rapid process.

Increased Systemic Suppression in nts382 Grown without Nitrate

Because the preparation of split root systems means that inoculation, and thus nitrogen fixation, were substantially delayed relative to plants inoculated at germination, plants were more subject to variation of nitrogen supply from cotyledonary reserves. Low nitrate in the culture medium stimulated the degree of root growth in split root systems of soybean (16). The effect of low-level nitrate on systemic suppression was tested in an experiment parallel to that presented in Table I, except that nitrate was totally absent from the culture system. Nodule number compensation *(i.e.* the maintenance of a constant nodule number per plant), as shown in Tables I and II, was again demonstrated for Bragg. Synchronous inoculations either at the early or delayed time points gave similar nodule number per root portion. Nodule dry weight was decreased in delayed/delayed treatments, presumably because of the decreased time (1 week) available for nodule growth. Root dry weights of early/early and delayed/delayed inoculated plants were similar, being in contrast to results from Table I, which showed an advantage of delayed inoculation on root growth. Uninoculated root halves did not grow as well as inoculated halves, supporting the suggestions of Singleton and Van Kessel (20) regarding preferential allocation of photosynthate to nodulated roots. Suppression of nodulation in Bragg without nitrate supplementation was as pronounced as that in the presence of 0.5 mm $KN0_3$ (cf Table I).

Nodulation suppression for nts382 (Table III) differed according to the presence of nitrate. Because of reduced nodule suppression, early/early inoculated plants showed nearly twice as many nodules as early/delayed plants. Nodule dry weight in early/uninoculated plants was compensated with 126± 15 mg per root E being similar to 143±30 mg for the combined root portions on early/early treatments. The lowered nodule mass on uninoculated/delayed treated plants *(i.e.* 89 mg per plant) was the result of a decreased time available for complete nodule growth compared to the early/uninoculated control *(i.e.* 126 mg per plant). Numbers of nodules per half root (253 on root E *versus* 56 on root D) in early/delayed inoculated plants were indicative of some systemic suppression occurring in nts382 in the absence of nitrate. Although nodule number and nodule mass could be slightly discounted on the delayed root, because of the decreased time to complete nodule formation and growth, it was still clear that some suppression occurred.

The supernodulation mutant nts382 thus showed minimal, if any, suppression in the presence of nitrate but showed some in the absence of nitrate (compare Tables I and IV with Table III). Moreover, the extent of suppression in the early/delayed inoculated plants of nts382 was more pronounced, when nodule dry weights were compared (100 mg per plant compared with 7 mg per plant). This showed that specific nodule weight decreased, suggesting that the observed suppression resulted from a more general systemic mechanism extending beyond the early control of nodule initiation. In view of the data of Singleton and Van Kessel (20), the altered allocation of photosynthate may be a plausible explanation of this finding.

Root fresh weights supported the findings in Tables I and

Table III. *Nodulation and Root Growth of Split Roots of cv. Bragg and nts382 Grown in the Absence of External Nitrate.*

Experiments were conducted in Maui as described in "Materials and Methods."

Genotype	Inoculation Pattern[a]	NN	NDW	RDW
			mg[c]	*mg*[c]
Bragg	Early	53 ± 5[b]	59 ± 19	123 ± 23
	Uninoculated	0	0	86 ± 12
	Early	23 ± 4	31 ± 5	106 ± 13
	Early	26 ± 4	34 ± 4	95 ± 14
	Early	53 ± 5	49 ± 3	119 ± 15
	7-d delay	0	0	70 ± 3
	7-d delay	44 ± 5	20 ± 2	78 ± 7
	7-d delay	60 ± 4	24 ± 3	83 ± 4
	Uninoculated	0	0	49 ± 5
	7-d delay	120 ± 13	42 ± 4	104 ± 6
nts382	Early	268 ± 23	126 ± 6	56 ± 7
	Uninoculated	0	0	54 ± 10
	Early	230 ± 45	66 ± 14	48 ± 12
	Early	269 ± 65	77 ± 10	55 ± 8
	Early	253 ± 21	110 ± 9	67 ± 10
	7-d delay	56 ± 12	7 ± 1	60 ± 9
	7-d delay	260 ± 39	51 ± 19	76 ± 10
	7-d delay	192 ± 28	40 ± 5	58 ± 7
	Uninoculated	0	0	72 ± 6
	7-d delay	342 ± 3	89 ± 2	67 ± 6

[a] Abbreviations are as in Tables I and II; number of replicates ranged from 8 to 14. [b] All data are expressed as means ± SE. [c] Per root portion.

Table IV. *Nodulation Suppression and Root Fresh Weights (without Nodules) of Various Genotypes of Soybean in Split Root Systems.*

Both sides were inoculated with *B. japonicum* strain USDA110; plants were grown in the presence of 0.5 mM KNO_3. Root fresh weight did not include nodules. Experiments were carried out in Canberra as described.

Genotype	Inoculation Pattern (*n*)[a]	NN	RFW
			g[c]
Bragg	Early (6)	38 ± 3[b]	1.58 ± 0.25
	7-d delay (6)	0.4 ± 0.3	0.86 ± 0.15
Clark	Early (7)	25 ± 3	1.71 ± 0.25
	7-d delay (7)	9 ± 2	0.81 ± 0.18
Williams 82	Early (9)	33 ± 14	1.45 ± 0.11
	7-d delay (9)	8 ± 1	0.86 ± 0.10
nts1116	Early (4)	105 ± 14	0.73 ± 0.15
	7-d delay (4)	29 ± 10	0.55 ± 0.10
nts382	Early (6)	108 ± 10	0.69 ± 0.11
	7-d delay (6)	83 ± 7	0.69 ± 0.03

[a] Abbreviations are as in Table I; *n* = number of replicates. [b] All data are expressed as means ± SE. [c] Per root portion.

III. In the presence or absence of 0.5 mm, nitrate-nodulated roots of nts382 were smaller than those on Bragg plants. Uninoculated and inoculated roots from early as well as delayed treatments of Bragg had different masses, whereas the same treatments resulted in similar root masses for nts382.

Systemic Suppression in Different Soybean Genotypes

As shown previously, nts382, when grown on low nitrate, did not develop the pronounced suppression of nodulation on root D, as observed for Bragg following a 7-d delayed inoculation (Table IV). Wild-type cultivars Clark and Williams 82 demonstrated a substantial degree of autoregulation, giving around 70% suppression on root D. The lowered amount of suppression in these two cultivars may reflect their different genetic background or the fact that growth conditions were optimized for Bragg. Control experiments with unsplit root systems showed that total nodule numbers per plant in Bragg, Clark, and Williams 82 were similar, with a similar nodulation pattern.

Mutant nts1116 was characterized by a hypernodulation phenotype (7, 10) and also had an intermediate level of systemic suppression (about 70% compared with Bragg, showing 100%, and nts382, at 10% to 20%). The hypernodulation character was also reflected in the elevated nodule number on root E (38 for Bragg *versus* 105 for nts1116). Mutant nts1116 thus appeared to have a systemic suppression similar to Clark and Williams 82, although its nodulation pattern and nodulation interval (percentage of the root nodulated) was different *(i.e.* nts1116 showed nodulation over a larger proportion of the root system as compared with clustered nodulation near the region of the root tips at the time of inoculation in Bragg). Weights of the delayed inoculated root portions tended to be smaller than those of root E (Table IV).

DISCUSSION

Supernodulation and wild-type genotypes maintained their previously reported nodulation phenotypes in split root assemblies. It was possible to confirm the systemic suppression of nodulation in wild-type soybean cultivar Bragg by prior inoculation of one side, as reported by Kosslak and Bohlool (14). The autoregulation-deficient mutant nts382 was shown to have a substantially lowered systemic suppression response if grown on low nitrate, providing strong evidence that the systemic suppression phenomenon, at least in part, is affected by the shoot-derived autoregulation system in soybean.

Mutant nts382 in the absence of nitrate, however, showed a significant increase in the suppression effect. A likely explanation for this nitrate effect is that split roots grown without nitrate allocated "structural resources" (photosynthate, hormones, vitamins, and other shoot-derived substances) preferentially to the first inoculated side, so that these became a limiting factor for root growth and nodulation potential. For nts382, in the absence of nitrate, root growth may have preferential demand on such limiting resources compared with nodules, leading to a reduction in observed nodule formation. However, if low-level nitrate was applied, the allocation of "structural resources," which may be more than photosynthetic carbohydrates, to both root portions was equal (see also ref. 20). Hence, the limitation seen in nitrate-free plants did not occur and thus the nodulation capacity of nts382 was completely expressed. This restriction was more clearly seen in the amount of nodule mass developed on the delayed root portion of nts382 grown without nitrate. As nodule initiation preceded nodule growth, and small nodule primordia were not easily detectable with the naked eye, the interpretation of nodulation data from roots with apparently different sink demands was difficult.

These data highlight the need for appropriate physiological control between treatments, when precise nodulation characteristics are to be compared. For example, molecular analyses of nodulin profiles frequently use inoculated *versus* uninoculated tissues. Not all differences detected with such comparisons may relate to the direct ontogeny of nodule development, but may reflect tissue responses to altered photosynthate translocation as well as tissue age.

Inoculation of root D, 24 h after the inoculation of root E, caused a detectable suppression of nodule number and nodule fresh weight compared with concurrent inoculations of Bragg plants. The extent of suppression increased to 100% after a 7-d delay, comparing well with the findings of Kosslak and Bohlool (14), who observed partial suppression after 48 h and complete suppression after 10 d in a short-day, dry-season experiment.

The observed time of systemic suppression fell well into the time scale found for single root analyses in soybean by Pierce and Bauer (17), who used pouch-grown plants and double inoculations to propose the basic tenets of autoregulation of nodulation. Microscopic analysis of thin sections of nts382 and Bragg roots showed that autoregulation affected the transition of cellular divisions through the "window of nodulation competence," which itself was regulated by the physiological condition of the plant as well as by nitrate (15).

Control experiments, in which root E was inoculated early and root D was left uninoculated, confirmed the lack of cross-contamination by the inoculum between sides. Total nodule number per plant was equal for the early/early and the early/uninoculated split roots for Bragg. This agreed with the results of Bohlool et al. (4), who found that, under nitrate-free conditions, the total nodule number per wild-type plant remained constant regardless of whether one or both sides were inoculated. For nts382 plants, there were twice as many nodules, if both sides were concurrently inoculated. Total nodule mass per plant, however, remained constant for both genotypes, indicating that, although nts382 did not regulate nodule initiation and initial growth to a detectable size, ultimately, nodule development as measured by nodule mass was regulated by plant growth.

Singleton and Van Kessel (20) used split roots of soybean with controlled root atmospheres to ascertain whether the root portion that was involved in nitrogen fixation received more photosynthate. They showed that dry matter and current photosynthate were selectively partitioned to nodules and roots when nitrogen gas was available for nitrogen fixation (instead of argon controls), and that associated roots were larger than those associated with nonfixing nodules. Carbon flow was postulated to be controlled by the nitrogen fixation needs of a particular root portion. If an uninoculated root system was grown with ammonium nitrate on the one half and no nitrogen source on the other, photosynthate was

preferentially translocated to the side receiving the nitrate. The conclusion was drawn that the plant allocated carbon to the root portion directly involved in nitrogen supply to the plant.

The experiment involving low supplies of nitrate to both sides of a split root system and differential inoculation was not reported. Likewise, an experiment in which low nitrate was supplied to one half and the other was inoculated and nodulated was not carried out. Such data would have direct relevance to the findings reported here in the presence or absence of nitrate.

Olsson (16) injected "C-labeled sucrose into stems of split roots of Bragg plants and monitored the allocation of label. She showed that concurrently inoculated roots of Bragg grown on nitrate (0.5 mm) had similar specific nodule radioactivity (49 ± 18 cpm/g NFW *versus* 58 ± 21 cpm/g NFW) and root radioactivity (4168 zi: 810 cpm/g root fresh weight *versus* 4074 ± 1247 cpm/g root fresh weight). Root portions, which were inoculated with a 7-d delay, had complete nodule suppression, yet the percentage of label in the root portions was similar (40.5% *versus* 45.3% of total label in the roots). This suggested that the roots grown in the above investigation on systemic nodulation suppression allocated injected sucrose to a similar level, even if roots were differentially nodulated, provided that low-level nitrate was present. Whether this holds true for recently fixed carbon remains to be investigated.

Soybean genotypes were shown to express the systemic suppression response differently. This may be a direct reflection of inherent differences in the nodulation development or of secondary responses caused by differential interaction with a common set of experimental conditions, which, in the present case, were optimized for the culture of cultivar Bragg. Quantitative differences in the nodulation pattern and number of different nts mutants of Bragg were also reflected in their degree of suppression.

The fact that a single genetic event leading to supernodulation, lack (or substantial decrease) of autoregulation nitrate tolerance in nodulation, and lack (or substantial decrease) of shoot-derived inhibitor production (13) in soybean also led to the lack of systemic nodule suppression by prior inoculation of one side in a split root system suggests that these processes are functionally linked through a common process involving the coordinated regulation of plant cell divisions by systemic means. This further substantiates the claim that nodulation in legumes not only involves complex plant-bacterial interactions, but that plant-organ to plant-organ interactions and cell-to-cell signals within the plant are of similar significance.

ACKNOWLEDGMENTS

Paul Singleton is thanked for the original design of the split root system as are Janet Hudson and Angela Higgins for excellent technical help. Janice Crockett arid Joanne Perks helped with the preparation of the manuscript. Drs. B. J. Carroll, D. A. Day and A. C. Delves are thanked for discussion and advice.

LITERATURE CITED

1. Allen FJ, Bhardwaj HL (1987) Measured and predicted response of soybeans to simulated acid rain. Soybean Genet Newsletter 14: 148-154
2. Bergersen FJ (1961) The growth of *Rhizobium in* synthetic media. Aust J Biol Sci 14: 349-360
3. Bhuvaneswari TV, Turgeon BG, Bauer WD (1980) Early events in the infection of soybean (Glycine max L. Merr.) by *Rhizo*bium japonicum. Plant Physiol 66: 1027-1031
4. Bohlool BB, Nakao P, Singleton PW (1986) Ecology determinants of interstrain competition in *Rhizobium-legume sym*biosis. AIAS Occasional Publ 25: 145-148
5. Broughton WJ, Dilworth MJ (1971) Control of leghemoglobin synthesis in snake beans. Biochem J 125: 1075-1080
6. Carroll BJ, McNeil DL, Gresshoff PM (1985) A supemodulation and nitrate tolerant symbiotic (nts) soybean mutant. Plant Physiol 78: 34-40
7. Carroll BJ, McNeil DL, Gresshoff PM (1985) Isolation and properties of soybean (Glycine max (L) Merr.) mutants that nodulate in the presence of high nitrate concentrations. Proc Natl Acad Sci USA 82: 4162-4166
8. Day DA, Lambers H, Bateman J, Carroll BJ, Gresshoff PM (1986) Growth comparisons of a supemodulating soybean (Glycine max) mutant and its wild-type parent. Physiol Plant 88:375-382
9. Day DA, Carroll BJ, Delves AC, Gresshoff PM (1989) The relationship between autoregulation and nitrate inhibition of nodulation in soybean. Physiol Plant 75: 37-42
10. Delves AC, Mathews A, Day DA, Carter AS, Carroll BJ, Gresshoff PM (1986) Regulation of the soybean-Rhizobium symbiosis by shoot and root factors. Plant Physiol 82: 588-590
11. Delves AC, Carroll BJ, Gresshoff PM (1988) Genetic analysis and complementation studies on a number of mutant supernodulating soybean lines. J Genet 67: 1-8
12. Gresshoff PM, Delves AC (1986) Plant genetic approaches to symbiotic nodulation and nitrogen fixation in legumes. *In* AD Blonstein, PJ King, eds, Plant Gene Research III. Springer Verlag, Wien, pp 159-206
13. Gresshoff PM, Krotzky AJ, Mathews A, Day DA, Schuller KA, Olsson J, Delves AC, Carroll BJ (1988) Suppression of the symbiotic supernodulation symptoms of soybean. J Plant Physiol 132: 417-423
14. Kosslak RM, Bohlool BB (1984) Suppression of nodule development on one side of a split root system of soybean caused by a prior inoculation on the other side. Plant Physiol 75: 125130
15. Mathews A, Carroll BJ, Gresshoff PM (1989) Development of *Bradyrhizobium infections* in supemodulating and nonnodulating mutants of soybean *(Glycine max (L)* Merr.). Protoplasma (in press)
16. Olsson JE (1988) Systemic communication in soybean nodulation. PhD dissertation. Australian National University, Canberra, Australia
17. Pierce M, Bauer WD (1983) A rapid regulatory response governing nodulation in soybeans. Plant Physiol 73: 286-290
18. Ridge RW, Rolfe BG (1986) Sequence of events during the infection of the tropical legume *Macroptilium atropurpureum* Urb. by the broad host range, fast-growing *Rhizobium* ANU240. J Plant Physiol 122: 121-137
19. Singleton PW (1983) A split root growth system for evaluating the effect of salinity on components of the soybean-Rhizobium symbiosis. Crop Sci 23: 259-263
20. Singleton PW, Van Kessel C (1987) Effect of localized nitrogen availability to soybean half root systems on photosynthate partitioning to roots and nodules. Plant Physiol 83: 552-556