Botanica Acta

Berichte der Deutschen Botanischen Gesellschaft · Journal of the German Botanical Society

E 4953 F

1 Volume 103 February 1990 Page 1–130

Contents

Thieme

Georg Thieme Verlag Stuttgart · New York
Thieme Medical Publishers, Inc., New York

BOACEJ (1), 1–130 (1990)

Botanica Acta ISSN 0932-8629

Botanica Acta

Berichte der Deutschen Botanischen Gesellschaft
Journal of the German Botanical Society

1990

Georg Thieme Verlag
Rüdigerstraße 14
D-7000 Stuttgart 30

Postfach 104853
D-7000 Stuttgart 10

Thieme Medical Publishers, Inc.
381 Park Avenue South
New York, NY 10016

598 Figures
65 Tables

© 1990 Georg Thieme Verlag, Rüdigerstraße 14, D-7000 Stuttgart 30 – Printed in Germany

Contents

Author's Index

Subject Index

Editorial

In this first issue of 1990, BOTANICA ACTA would like to thank all readers, subscribers, members of the German Botanical Society, authors, associate editors, and reviewers for their continued interest and support. Thanks to the joint efforts of all involved, BOTANICA ACTA can start its third year optimistically. Progress has been made towards attaining international recognition as a journal serving all branches of plant science. The number of manuscripts received increased considerably: in 1987 and 1988 a total of 99 manuscripts were submitted, in 1989 alone, 121 papers were received. The quality was high; the average rejection rate at present amounts to just under 30%. However, much still remains to be done and the editors and publishers will continue to seek ways of improving our journal. We are also happy to welcome 12 new Associate Editors who will either be responsible for additional fields of plant science or will increase our expertise in areas where we receive rather more contributions.

Special thanks are due to the many referees who donated their valuable time to help us evaluate all the papers submitted to BOTANICA ACTA including solicited review articles and contributions to BOTANICA ACUTA. The members of the Editorial Board are named in each issue whereas the referees remain anonymous. While not abolishing this principle of anonymity, we would like, at intervals, to convey our sincere thanks to these people by name. Hence, after its first two years BOTANICA ACTA expresses its gratitude to the following scientists who have put their expert knowledge at our disposal to maintain and improve the high scientific quality of our journal:

W. O. Abel, Hamburg
W. Alt, Bonn
N. Amrhein, Zürich
K. Apel, Kiel
T. J. Bach, Karlsruhe
T. Baumann, Zürich
H.-D. Behnke, Heidelberg
J. Bereiter-Hahn, Frankfurt
L. Bergmann, Köln
H. Binding, Kiel
M. Blatt, Cambridge
E. Blumwald, Toronto
M. Bopp, Heidelberg
S. W. Breckle, Bielefeld
A. Bresinsky, Regensburg
K. Brinkmann, Bonn
D. P. Briskin, Illinois
T. Buckhout, Kaiserslautern
E. Bünning, Tübingen
C. Buschmann, Karlsruhe
M. Chrispeels, San Diego
H. Clojsters, Diepenbuk
D. J. Cosgrove, Pennsylvania
J. Cram, Newcastle upon Tyne
R. M. Crawford, Bristol
B. Demmig, Würzburg
J. D. Doge, Surrey
D. Dörnemann, Marburg
R. Douce, Grenoble
G. Drews, Freiburg
G. E. Edwards, Washington
E. F. Elstner, München
H. van den Ende, Amsterdam
W. Eschrich, Göttingen
S. Fink, Tübingen
D. B. Fisher, Washington
H. Fock, Kaiserslautern
G. E. Fogg, North Wales
A. C. Franco, Darmstadt
P. Gadal, Orsay
P. Galland, Marburg
P. Gardeström, Umeå
H. G. Gassen, Darmstadt
B. Gerhardt, Münster
S. P. Gibbs, Montreal
G. Giraud, Paris
P. Gogarten, Santa Cruz
P. B. Goodwin, Sydney
D. Gradmann, Gießen
P. Gräber, Berlin
J. Green, Plymouth
H. Griesebach, Freiburg
R. Grimm, Freiburg
F. Grohlig, Gießen
F. Gubler, Canberra
J. Guern, Gif-sur-Yvette
M. G. Guerrero, Sevilla
M. Guiry, Galway
A. Hager, Tübingen
V. Hamann, Bochum
U. P. Hansen, Kiel
C. Happey-Wood, Bangor
A. Hardham, Canberra
H.-P. Haschke, Darmstadt
W. Haupt, Erlangen
I. B. Heath, Ontario
R. Hedrich, Göttingen
E. Heinz, Hamburg
Z. Hejnowicz, Bonn
A. Henssen, Marburg
R. Hertel, Freiburg
W. Herth, Heidelberg
C. van den Hoek, Groningen
H. Hurka, Osnabrück
D. W. Jewson, Ulster
R. Jones, Berkeley
H. Jupin, Perpignan
H. Kasemir, Freiburg
K. Katou, Nagoya
R. E. Kendrick, Wageningen
H. Kindl, Marburg
T. B. Kinraide, Beckley
K. Kiyosawa, Osaka
D. Klämbt, Bonn
K. Kloppstech, Hannover
L. Kochian, Ithaca
R. Kollmann, Kiel
E. Komor, Bayreuth
H.-U. Koop, München
I. Kottke, Tübingen
G. H. Krause, Düsseldorf
K. Kubitzki, Hamburg
A. Kurkdjian, Gif-sur-Yvette
W. Lang, Kaiserslautern
R. Leblanc, Quebec
J. Lengeler, Osnabrück
W. Lin, Wilmington
G. Link, Bochum
C. Lloyd, Norwich
W. Lockan, Regensburg
C. Maggs, Belfast
D. Marmé, Freiburg
E. Martinoia, Zürich
F. Mayer, Göttingen
F. Meins, Basel
K. Mendgen, Konstanz
D. Menzel, Ladenburg
B. Moss, East Anglia
D. Müller, Konstanz
R. Murphy, Edinburgh
K. Napp-Zinn, Köln
F. Nienhaus, Bonn
G. A. Nimmo, Glasgow
H. Ninnemann, Tübingen
W. Nultsch, Marburg
R. Oelmüller, München
I. Ohad, Jerusalem
W. H. Outlaw, Tallahassee
E. Pahlich, Gießen
B. A. Palewitz, Athens
M. V. Parthasarathy, Cornell
D. G. Patriquin, Halifax
H. W. Pfefferkorn, Heidelberg
A. Pfitzner, München
E. Pistorius, Bielefeld
M. Popp, Münster
I. Potrykus, Zürich
H. Prehn, Gießen
H. Quader, Heidelberg
K. Raschke, Göttingen
F. Rasi-Caldogno, Mailand
T. Rausch, Frankfurt
P. Rea, Heslington
W. Reisser, Göttingen
G. Richter, Hannover
A. W. Robards, Heslington
W. Rüdiger, München
J. J. Sauter, Kiel
C. Schäfer, Bayreuth
E. Schäfer, Freiburg
H. Schat, Amsterdam
H. Scheer, München
G. Scherer, Bonn
U. G. Schlösser, Göttingen
K. Schmitz, Köln
H. Schnabl, Bonn
C. Schnarrenberger, Berlin
F. Schönbeck, Hannover
H. Schraudolf, Ulm
E. Schrott, München
A. Schulz, Heidelberg
H. J. Schweitzer, Bonn
D. Siefermann-Harms, Karlsruhe
A. Sievers, Bonn
H. Stabenau, Oldenburg
L. Stange, Kassel
E. Steudle, Bayreuth
M. Steup, Bochum
U. Stewart, Darmstadt
D. Strack, Braunschweig
R. J. Strasser, Genf
H. Strotmann, Düsseldorf
M. Tevini, Karlsruhe
W. W. Thomson, Riverside
J. E. Thomzik, Leverkusen
D. A. Thurman, Liverpool
R. Tischner, Göttingen
T. V. Tran, Gif-sur-Yvette
A. Trèmolière, Gif-sur-Yvette
C. Ullrich, Darmstadt
W. Ullrich, Darmstadt
W. Urbach, Würzburg
R. D. Viershra, Wisconsin
S. Vogel, Mainz
F. Weberling, Ulm
J. Webster, Exeter
E. W. Weiler, Bochum
M. H. Weisenseel, Karlsruhe
K. Wells, Davis
C. Westerkamp, Heidelberg
P. Westhoff, Düsseldorf
M. Westphal, Martinsried
W. Wießner, Göttingen
A. Wild, Mainz
M. B. Wilkins, Glasgow
J. Willenbrink, Köln
R. Williamson, Canberra
K. P. van Winkle-Swift, Texas
K. Winter, Würzburg
G. B. Witman, Shrewsbury
L. Wu, Davis
A. R. Yeo, Brighton
H. Ziegler, München
F. K. Zimmermann, Darmstadt

Darmstadt and Heidelberg
January 1990

Ulrich Lüttge
Eberhard Schnepf

Hypersensitive Reaction of Nodule Cells in the *Glycine* sp./ *Bradyrhizobium japonicum*-Symbiosis Occurs at the Genotype-Specific Level*

M. Parniske[1], *Christa Zimmermann*[1], *P. B. Cregan*[2], *and D. Werner*[1]

[1] Fachbereich Biologie der Philipps-Universität, Marburg/Lahn, FRG
[2] N_2-Fixation and Soybean Genetics Lab, USDA-ARS Beltsville, USA

Received: September 22, 1989; Accepted: October 30, 1989

Abstract

Three *Glycine* genotypes, *G. max* cv. Williams, *G. soja* PI 468397, and *G. soja* PI 342434 in combination with the two rhizobial strains *Bradyrhizobium japonicum* USDA 123 and *Rhizobium fredii* USDA 193 were analysed for phytoalexin concentration in the nodules. In the nodules of PI 468397/*B. japonicum* USDA 123 a very strong glyceollin I accumulation occurred around 30 d.p.i. Ultrastructural analysis of these nodules revealed several symptoms of a severe plant defense response associated with plant cell death (hypersensitive reaction): The cytoplasm of the infected cells was degraded and organelles had vanished. The cell walls of the infected cells showed remarkable thickening. This plant defense response could only be observed in this strain/genotype interaction. The same strain did not elicit a phytoalexin accumulation in the other plant genotypes tested, indicating that this response occurs at the genotype-specific level. This special character of *G. soja* PI 468397 is heritable as indicated by glyceollin I analysis of the nodules formed by F1 hybrids of PI 468397 x Williams inoculated with *B. japonicum* USDA 123. The genotype/strain specific occurrence of the hypersensitive response in root nodules resembles the race/cultivar specific incompatibility of several plant-pathogen interactions. This specificity, together with the phenomenon of the HR itself, points out the close physiological relationship between the late stages of the root nodule symbiosis and a plant/pathogen interaction.

Key words

Glycine, Rhizobium, Bradyrhizobium, glyceollin, hypersensitive reaction, phytoalexin, root nodules.

Abbreviations and Symbols

d.p.i.:	day after infection
PBM:	peribacteroid membrane
PHB:	poly-β-OH-butyrate
HR:	hypersensitive reaction
RIA:	radioimmunoassay
TEM:	transmission electron microscopy

Introduction

Glycine max forms nitrogen fixing nodule symbioses with its microsymbiont *Bradyrhizobium japonicum*. A few years ago, Keyser et al. (1982) described a new genus of fast growing rhizobia, *Rhizobium fredii*, with the ability to nodulate soybeans. As a rule one might state, that *R. fredii* strains form a less effective symbiosis with *G. max* than the slow growing *Bradyrhizobia* (Keyser and Cregan, 1984), although a few exceptions to this rule have been detected recently (Dowdle and Bohlool, 1985; Dowdle and Bohlool, 1987). Interestingly, a poorly effective strain of *R. fredii* has been shown to establish a highly effective symbiosis after mutation (Buendia-Claveria et al., 1988).

In the midwestern USA, indigenous *Bradyrhizobium* strains of serocluster 123 occupy most nodules of field grown soybeans (Ellis et al., 1984; Moawad et al., 1984). These strains of *B. japonicum* are highly competitive but form less than optimal nitrogen fixing symbioses with the commercially used cultivars of *G. max* (Caldwell and Vest, 1970; Ham, 1980). Highly effective strains of *B. japonicum* like USDA 110, are outcompeted by these strains even when inoculated at very high rates (Caldwell and Vest, 1970; Ham et al., 1971). Several approaches have been tried to circumvent this problem. Cregan and Keyser (1986) and Cregan et al. (1989) reported genotypes of *G. max* that restrict nodulation of the indigenous strains and thereby enable inoculum strains to nodulate better (Cregan et al., 1988; Keyser et al., 1988). Another strategy was based on the use of *G. soja* genotypes that form ineffective symbioses with slow growing rhizobia (Keyser and Cregan, 1984). These genotypes form highly effective symbioses with *R. fredii* strains. Using the genetic factors responsible for this "inverse" nodulation behaviour in combination with *R. fredii* inoculum might be a strategy to improve nitrogen fixation of *G. max* in the field even in the presence of *Bradyrhizobium* serocluster 123.

One of these *G. soja* genotypes is PI 468397 from the People's Republic of China. This genotype forms an effective symbiosis with *R. fredii* USDA 193, originally isolated from this genotype (Keyser and Cregan, 1984). On the other hand, the symbiosis with *B. japonicum* USDA 123 is Fix^-. In addition, *R. fredii* USDA 193 has been shown to

Bot. Acta 103 (1990) 143–148

* Dedicated to Professor André Pirson on the occasion of his 80th birthday.

be a much better competitor for nodulation on this genotype than *B. japonicum* strains (Cregan and Keyser, 1988).

In order to use the genetic factors of this genotype for breeding new *G. max* cultivars one has to understand better the physiological and genetic reasons for the observed change in compatibility. In this paper we give evidence that the incompatibility of PI 468397 with *B. japonicum* is accompanied by a severe plant defense reaction in the nodules. This plant reaction has striking similarities with a hypersensitive reaction (HR) described for several host-pathogen interactions (Klement,1982) in terms of phytoalexin accumulation, plant cell death and degradation. The ability of PI 468397 to produce an HR when infected with USDA 123 is heritable as suggested by observation of the nodules of PI 468397 crossed with *G. max* cv. Williams.

Materials and Methods

White plastic pots (18 cm diameter at top) which had been soaked overnight in a sterilizing solution of Rocall were filled with autoclaved vermiculite. Experimental design consisted of a randomized complete block with a split plot arrangement of treatments and two replications. Whole plots (pots) were a factorial of two inoculation treatments (USDA 123 and USDA 193) × 9 harvest dates and subplots were the three *Glycine* genotypes Williams, PI 342434 and PI 468397. The vermiculite was wetted with a nitrogen-free nutrient solution (Cregan and Keyser, 1988) and one "hill" of Williams, and two hills each of the *G. soja* genotypes were planted per pot at a depth of 1.5–2 cm. The seeds were subsequently inoculated with 1 ml of stationary broth culture per hill of either USDA 123 or USDA 193 and covered. The surface of the vermiculite was then covered with a 1.0 cm layer of autoclaved perlite to serve as a dry barrier to contamination by extraneous rhizobia. Four pots, otherwise treated as described above, were planted and left uninoculated. Nodules were harvested at 19, 21, 25, 27, 31, 33, 35, 38, and 40 d.p.i.

The radioimmunoassay specific for glyceollin I was performed as described by Hahn et al. (1985) except that solid-phase-bound anti-rabbit serum (Tecova, F.R.G.) was used to precipitate the glyceollin I-antibody. For electron microscopic studies, plants were grown in modified Leonard jar assemblies filled with perlite (Leonard, 1943). Nodules were fixed with glutaraldehyde, stained with osmium tetroxide, embedded in Epon and post-stained with uranyl acetate and lead citrate as described previously (Werner and Mörschel, 1978).

Results

Glyceollin I content of root nodules does not correlate with ineffectiveness of a given genotype/strain interaction

Three *Glycine* genotypes and two *Rhizobium/Bradyrhizobium* strains were included in this study (Table 1).

G. max cv. Williams formed effective nodules when inoculated with *B. japonicum* USDA 123 (Keyser and Cregan, 1984) (Table 1). These nodules contained only very low amounts of the phytoalexin glyceollin I (Fig. 1). When cv. Williams was inoculated with the fast growing *R. fredii* USDA 193, only small, ineffective nodules were formed. The nodules developed very late, i.e. there were no nodules found on the 19th d.p.i. The nodules of this host/strain combination contained very low amounts of glyceollin I (Fig. 1). *G. soja* PI 342434, a genotype that has a nodulation behaviour similar to commercially grown *G. max* cultivars, formed effective nodules with *B. japonicum* USDA 123. Glyceollin I content of these nodules was low (Fig. 1). *G. soja* PI 342434 in combination with *R. fredii* USDA 193

Table 1 Effectiveness and glyceollin I content of nodules of the genotype/strain interactions used in this study.

Interaction	Nitrogen fixation	Glyceollin I accumulation
G. max cv. Williams		
/ *B. japonicum* USDA 123	+	–
/ *R. fredii* USDA 193	–	–
G. soja PI 342434		
/ *B. japonicum* USDA 123	+	–
/ *R. fredii* USDA 193	–	–
G. soja PI 468397		
/ *B. japonicum* USDA 123	–	+
/ *R. fredii* USDA 193	+	–

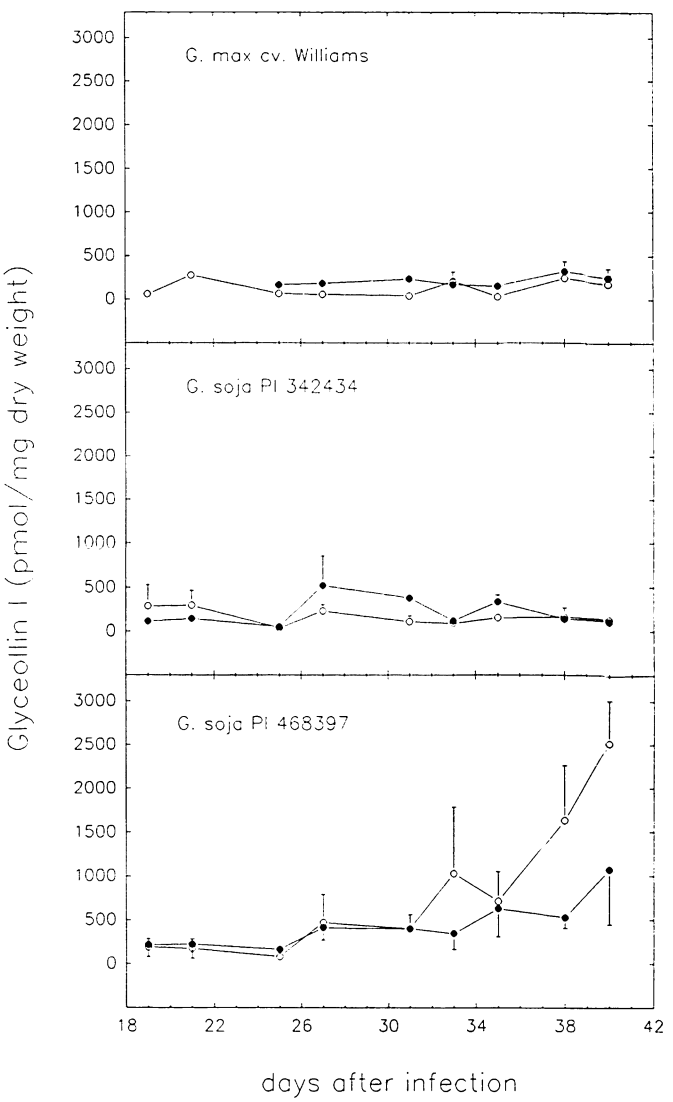


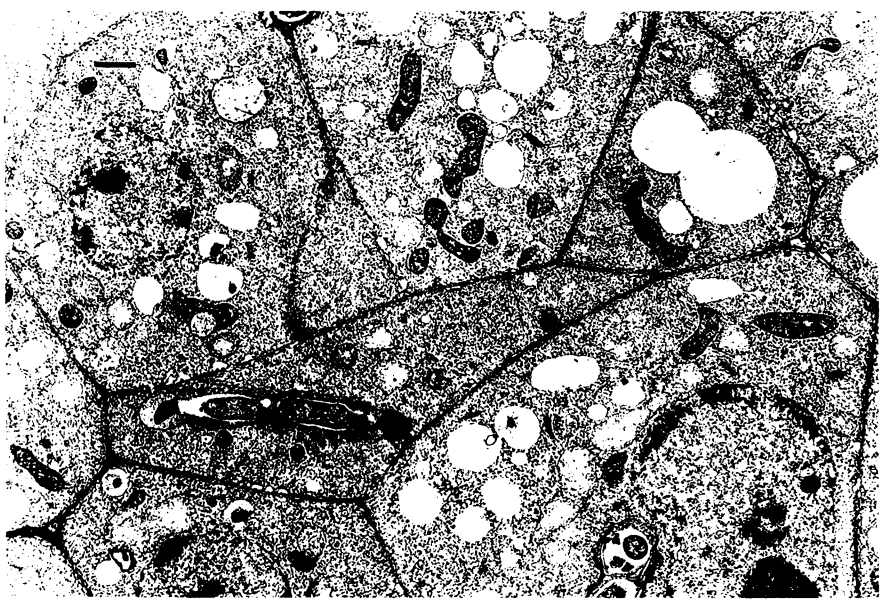
Fig. 1 Glyceollin I content of nodules formed by three *Glycine* genotypes in combination with *B. japonicum* USDA 123 (○) or *R. fredii* USDA 193 (●).

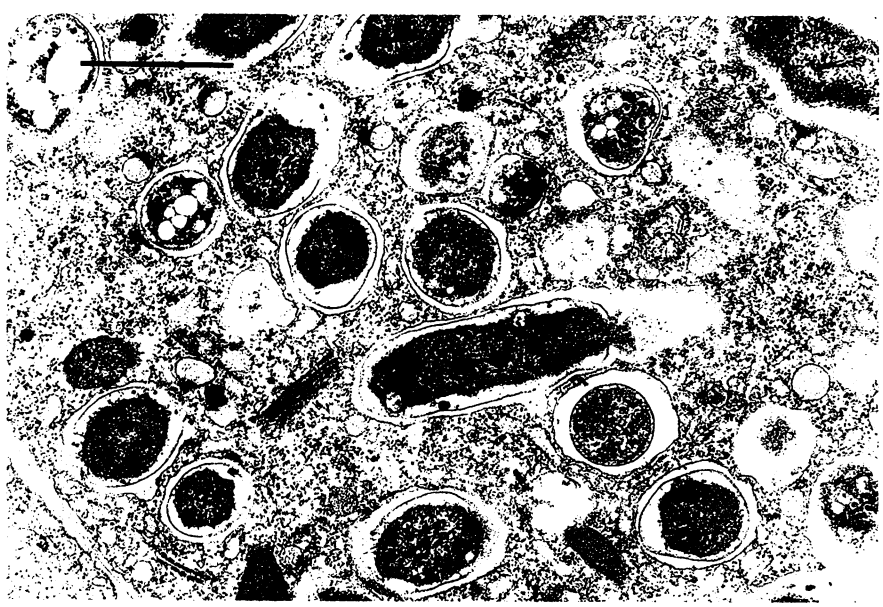
formed an ineffective symbiosis. Only low levels of glyceollin I were detected in these nodules (Fig. 1).

The symbiotic effectiveness and glyceollin I accumulation by *G. soja* PI 468397 contrasted with the two other *Glycine* genotypes. This genotype formed Fix^--nodules with the slow growing *B. japonicum* USDA 123. These nodules had a smooth surface and a green color, indicating the lack of leghemoglobin. In these nodules very high concentrations of glyceollin I could be found 33 days p.i. indicating a plant defense reaction. In contrast, this accumulation did not occur in nodules formed with *R. fredii* 193, rather normal effective nodules resulted from inoculation with this strain.

Nodules of G. soja PI 468397/B. japonicum USDA 123 exhibit several symptoms of a plant-pathogen interaction

We studied the developing nodules of the PI 468397/*B. japonicum* USDA 123 interaction in more detail by using TEM. At day 12 after infection, many infection threads were found in the meristematic plant cells (Fig. 2). Bacteria were enclosed by a thick layer of matrix material. Only few, if any, released bacteria could be detected in the plant cells at this developmental stage. Instead, vacuolar structures were observed in the meristematic cells. At day 15 p.i. (Fig. 3) many released bacteria could be found in the plant cells. They were surrounded by a peribacteroid membrane (PBM). In these early stages, no obvious differences to a fully compatible interaction could be observed at the fine structural level (for TEM analysis of effective nodule development see Bassett et al. (1977a); Werner and Mörschel (1978); Roth and Stacey (1989)).





Figs. 2–5 Electron micrographs of nodules of the *G. soja* PI 468397/*B. japonicum* USDA 123 interaction at different developmental stages. Each bar represents 1 μm.

Fig. 2 Meristematic nodule cells at the 12th d.p.i. contained infection threads with thick matrix material around the bacteria. Little or no released bacteria were visible.

Fig. 3 Part of an infected plant cell at the 15th d.p.i. Bacteroids surrounded by a PBM and plant organelles such as dictyosomes and mitochondria were visible in the plant cells.

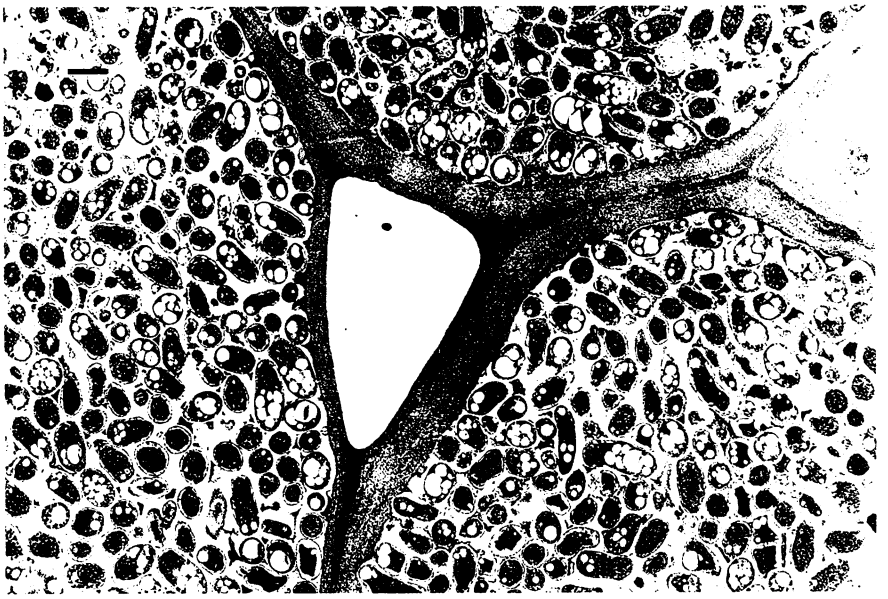


Fig. 4 Infected plant cells at the 29–33th d.p.i. showed massive cell wall thickening. Bacteria inside the plant cells accumulated large amounts of PHB and had reached higher cell densities than in the earlier stages. They were no longer surrounded by a PBM nor by any other intact plant structure indicating that plant cells were dead.

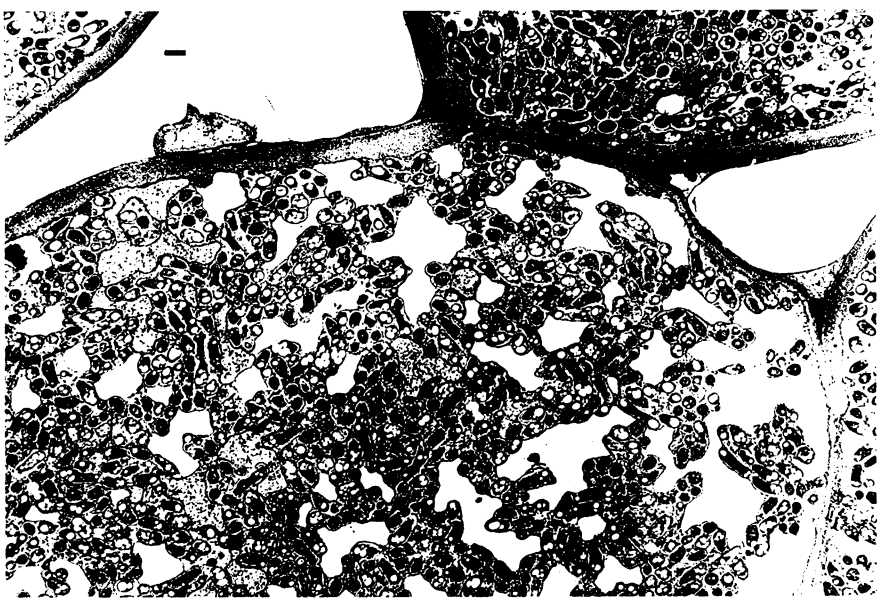


Fig. 5 Infected plant cell at the 29–33th d.p.i. contained bacteroids, plant organelles and vacuolar structures indicating plant cell lysis. Bacteroids contained large PHB-granules.

In contrast to an effective interaction, at day 29–33 p.i. a number of infected plant cells had been subjected to severe degradation as indicated by the complete lack of plant organelles (Fig. 4). The bacteria were no longer enclosed by a PBM. We conclude from these observations, that these plant cells are dead at this stage of development. Cell death and degradation are typical features of the hypersensitive reaction of a plant cell (Klement, 1982). Another marker of a plant defense response on the ultrastructural level is the observed thickening of cell walls. Interestingly, a major portion of the bacteria inside the degraded plant cells looked structurally intact suggesting that they were still alive.

Apart from this cell type another type of development occurred in nodules of this age (Fig. 5). Here, most of the plant organelles remained present but some irregular vacuolar structures were seen. We interpret this cell type as a developmental precedent of the terminal stage described in Fig. 4.

Hypersensitive plant reaction to B. japonicum USDA 123 in PI 468397 is heritable

In order to determine if the nodulation features of PI 468397 are inherited when hybridised with cultivar Williams, we analysed the glyceollin I content of the nodules of the F1-generation of this cross (Table 2). Interestingly, all plants of the F1-generation produced two types of nodules on their root systems when inoculated with *B. japonicum* USDA 123: small green ineffective (nodule type 2 in Table 2) and normal looking effective ones (type 1). Visual estimates made at harvest indicated that 60 to 90 percent of the nodules on F_1 plants were of the green ineffective type. The ineffective nodules contained glyceollin I in

Table 2 Glyceollin I content of nodules of *B. japonicum* USDA 123 formed with *G. soja* PI 468397, *G. max* cv. Williams and F1-hybrids.

Genotype	Nitrogen fixation	Glyceollin I content (pmol/mg dw)
G. max cv. Williams	+	175
G. soja PI 468397	–	2518
F1, nodule type 1	+	79
F1, nodule type 2	–	2323

the same order of magnitude as the nodules of the PI 468397 parent genotype indicating that this character is heritable.

Discussion

The *G. soja* genotype PI 468397 has some unusual nodulation properties in comparison to the commercial cultivars of *G. max* and several other genotypes of *G. soja*, e.g. PI 342434. First, it forms ineffective nodules with all *B. japonicum* strains tested so far (Keyser and Cregan, 1984). Second, it forms a highly effective symbiosis with *R. fredii* USDA 193. Third, it nodulates preferentially with *R. fredii* USDA 193 instead of *B. japonicum* USDA 123.

In this paper we demonstrate that the ineffective symbiosis of PI 468397 with *B. japonicum* USDA 123 leads to a plant defense reaction in the later stages (i.e. about day 30 p.i.) of the symbiosis. Until day 15 p.i. no obvious difference to a fully compatible interaction could be observed by TEM or by glyceollin I analysis. Only in later stages of the interaction is *B. japonicum* USDA 123 recognized as a pathogen. The phytoalexin glyceollin I is accumulated in the nodules and a hypersensitive reaction of the infected plant cells could be observed at the ultrastructural level. Glyceollin accumulation is believed to be an important early defense reaction of *G. max* towards the fungal pathogen *Phytophthora megasperma* f. sp. *glycinea* (Ebel and Grisebach, 1988). Glyceollin I concentration in the nodules of *G. soja* PI 468397/*B. japonicum* USDA 123 is in the same order of magnitude as in soybean root tissue infected with *P. megasperma* (compare to Hahn et al., 1985). It should be mentioned that phytoalexin accumulation may not only be elicited by biotic factors but also by abiotic stresses such as the presence of heavy metals (Darvill and Albersheim, 1984; Ebel, 1986).

However, phytoalexin accumulation was only observed in the PI 468397/USDA 123 interaction and in none of the other genotype/strain combinations tested. The elicitation of the plant defense response seems to be a highly specific event for which a certain strain/genotype combination is needed.

Cultivars of *G. max* accumulate large amounts of glyceollin I in the nodules only when infected with specific strains of *B. japonicum* like USDA 24 or a *nifA* deletion mutant of USDA 110 (Werner et al., 1980; Werner et al., 1985; Parniske et al., in preparation). Other ineffective strains fail to elicit a hypersensitive response of the infected nodule cells. These results confirm the observations of Werner et al. (1985) that phytoalexin accumulation is not simply a consequence of the ineffectivity of the symbiosis. The strong genotype/strain dependence of the HR-phenomenon in nodule cells suggests a very specific recognition process between the two partners in the later stages of nodule development.

The genotype/strain specific occurrence of the HR in nodule cells resembles the cultivar/race specific incompatibility of several host-pathogen-interactions. The close physiological relationship between the early recognition process in the *Rhizobium*-legume symbiosis and that of a pathogenic interaction has been mentioned by several authors (Vance, 1983; Djordjevic et al., 1987; Djordjevic et al., 1988; Keen and Staskawicz, 1988; Rolfe and Gresshoff, 1988). The recognition process that decides between a symbiotic and a pathogenic development does not only work in the early stages (i.e. infection of the root hairs) of the symbiotic interaction. Even infected nodule cells may perform a hypersensitive reaction. Compatible rhizobia must have evolved mechanisms to circumvent the elicitation of a HR in the early as well as in the late stages of the symbiotic interaction.

The term "hypersensitive response" with respect to soybean root nodule tissue was first used by Bassett et al. (1977b). These authors describe a "selective autolysis" of the infected cells which has a greater effect on the bacteria than on the host cell. The latter remains alive during this process. They also compared this specific type of autolysis with that occurring during senescence. In order to better distinguish between different types of ineffective nodule developments, we suggest that the term "hypersensitive response" should only be used if host cell death occurs. As a consequence, we think that the term "early senescence" would better fit the reaction type described by Bassett, because host cells stay alive and that "hypersensitive response" more appropriately describes the reaction of *G. soja* PI 468397 to *B. japonicum* 123 that results in host cell death.

Factors that lead to advantages in competition act in the early stages of the host-microsymbiont interaction. Cregan and Keyser (1988) observed a better competitiveness of *R. fredii* USDA 193 in comparison to *B. japonicum* USDA 123 on PI 468397. We think that this phenomenon cannot be explained by the observed defense reaction of this genotype towards USDA 123 because it occurs in the later stages of the symbiosis. However, we have not yet determined whether *B. japonicum* elicits a higher glyceollin I accumulation in the root hairs during the infection process. Using the very sensitive RIA-technique we will attempt to answer this next question.

Acknowledgements

We thank Prof. Dr. H. Grisebach and Dr. A. Bonhoff for the gift of the glyceollin I antibody and initial instructions concerning the RIA. We acknowledge the continuous support by the Deutsche Forschungsgemeinschaft (DFG).

References

Bassett, B., Goodman, R. N., and Novacky, A. – Ultrastructure of soybean nodules. I: release of rhizobia from the infection thread. Can. J. Microbiol. 23 (1977a), 573–582.

Bassett, B., Goodman, R. N., and Novacky, A. – Ultrastructure of soybean nodules. II: deterioration of the symbiosis in ineffective nodules. Can. J. Microbiol. 23 (1977b), 873–883.

Buendia-Claveria, A. M., Cubo-Sanchez, T., Romero, F., Perez-Silva, J., and Ruiz-Sainz, J. E. – Mutants of *Rhizobium fredii* strains that are effective on American soybean cultivars are also better nitrogen fixers on some plants of the cowpea group. In: H. Bothe, F. J. de Bruijn, and W. E. Newton, eds., Nitrogen Fixation: Hundred Years After, p. 781. G. Fischer Verlag, Stuttgart – New York, 1988.

Caldwell, B. E. and Vest, G. – Effect of *Rhizobium japonicum* strains on soybean yields. Crop Sci. 10 (1970), 19–21.

Cregan, P. B. and Keyser, H. H. – Influence of Glycine spp. on competitiveness of *Bradyrhizobium japonicum* and *Rhizobium fredii*. Appl. Environ. Microbiol. 54 (1988), 803–808.

Cregan, P. B., Keyser, H. H., and Sadowsky, M. J. – Soybean genotypes which reduce the competitiveness of the *Bradyrhizobium japonicum* serotype strains comprising serocluster 123. In: H. Bothe, F. J. de Bruijn, and W. E. Newton, eds., Nitrogen Fixation: Hundred Years After, p. 782. G. Fischer Verlag, Stuttgart – New York, 1988.

Cregan, P. B., Keyser, H. H., and Sadowsky, M. J. – Soybean genotype restricting nodulation of a previously unrestricted serocluster 123 bradyrhizobia. Crop. Sci. 29 (1989), 307–312.

Cregan, P. B. and Keyser, H. H. – Host restriction of nodulation by *Bradyrhizobium japonicum* strain USDA 123 in soybean. Crop Sci. 26 (1986), 911–916.

Darvill, A. G. and Albersheim, P. – Phytoalexins and their elicitors – a defense against microbial infection in plants. Ann. Rev. Plant Physiol. 35 (1984), 243–275.

Djordjevic, M. A., Gabriel, D. W., and Rolfe, B. G. – *Rhizobium* – the refined parasite of legumes. Ann. Rev. Phytopathol. 25 (1987), 145–168.

Djordjevic, S. P., Ridge, R. W., Chen, H., Redmond, J. W., Batley, M., and Rolfe, B. G. – 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 (1988), 1848–1857.

Dowdle, S. F. and Bohlool, B. B. – Predominance of fast-growing *Rhizobium japonicum* in a soybean field in the People's Republic of China. Appl. Environ. Microbiol. 50 (1985), 1171–1176.

Dowdle, S. F., and Bohlool, B. B. – Intra- and inter-specific competition in *Rhizobium fredii* and *Bradyrhizobium japonicum* as indigenous and introduced organisms. Can. J. Microbiol. 33 (1987), 990–995.

Ebel, J. – Phytoalexin synthesis: The biochemical analysis of the induction process. Ann. Rev. Phytopathol. 24 (1986), 235–264.

Ebel, J. and Grisebach, H. – Defense strategies of soybean against the fungus *Phytopthora megasperma* f. sp. *glycinea:* a molecular analysis. TIBS 13 (1988), 23–27.

Ellis, W. R., Ham, G. E., and Schmidt, E. L. – Persistence and recovery of *Rhizobium japonicum* inoculum in a field soil. Agron. J. 76 (1984), 573–576.

Hahn, M. G., Bonhoff, A., and Grisebach, H. – Quantitative localisation of the phytoalexin glyceollin I in relation to fungal hyphae in soybean roots infected with *Phytophtora megasperma* f. sp. glycinea. Plant Physiol. 77 (1985), 591–601.

Ham, G. E. – Interactions of *Glycine max* and *Rhizobium japonicum*. In: R. J. Summerfield, and A. H. Bunting, eds., Advances in legume science. Royal Botanical Gardens, Kew, England, 1980.

Ham, G. E., Cardwell, V. B., and Johnson, H. W. – Evaluation of Rhizobium japonicum inoculants in soils containing naturalized populations of rhizobia. Agron. J. 63 (1971), 301–303.

Keen, N. T. and Staskawicz, B. – Host range determinants in plant pathogens and symbionts. Ann. Rev. Microbiol. 42 (1988), 421–440.

Keyser, H. H., Bohlool, B. B., Hu, T. S., and Weber, D. F. – Fast-growing rhizobia isolated from root nodules of soybean. Science (Washington, DC) 215 (1982), 1631–1632.

Keyser, H. H. and Cregan, P. B. – Interactions of selected *Glycine soja* Sieb. & Zucc. genotypes with fast- and slow-growing soybean rhizobia. Crop Sci. 24 (1984), 1059–1062.

Keyser, H. H., Cregan, P. B., and Sadowsky, M. J. – Soybean genotypes which restrict nodulation of strains of *Bradyrhizobium japonicum* serocluster 123. In: H. Bothe, F. J. de Bruijn, and W. E. Newton, eds., Nitrogen Fixation: Hundred Years After, p. 784. G. Fischer Verlag, Stuttgart – New York, 1988.

Klement, Z. – Hypersensitivity. In: M. S. Mount and G. H. Lacy, eds., Phytopathogenic procaryotes, vol. 2, p. 149–177. Academic Press, Inc., New York, 1982.

Leonard, L. T. – A simple assembly for use in the testing of cultures of rhizobia. J. Bacteriol. 45 (1943), 523–527.

Moawad, H. A., Ellis, W. R., and Schmidt, E. L. – Rhizosphere response as a factor in competition among three serogroups of indigenous *Rhizobium japonicum* for nodulation of field grown soybeans. Appl. Environ. Microbiol. 47 (1984), 607–612.

Rolfe, B. G. and Gresshoff, P. M. – Genetic analysis of legume nodule initiation. Ann. Rev. Plant Physiol. Plant Mol. Biol. 39 (1988), 297–319.

Roth, L. E. and Stacey, G. – Bacterium release into host cells of nitrogen-fixing soybean nodules: the symbiosome membrane comes from three sources. Eur. J. Cell Biol. 49 (1989), 13–23.

Vance, C. P. – *Rhizobium* infection and nodulation: a beneficial plant disease? Ann. Rev. Microbiol. 37 (1983), 399–424.

Werner, D. and Mörschel, E. – Differentiation of nodules of *Glycine max*. Planta 141 (1978), 169–177.

Werner, D., Mörschel, E., Stripf, R., and Winchenbach, B. – Development of nodules of *Glycine max* infected with an ineffective strain of *Rhizobium japonicum*. Planta 147 (1980), 320–329.

Werner, D., Mellor, R. B., Hahn, M. G., and Grisebach, H. – 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 (1985), 179–181.

M. Parniske

Fachbereich Biologie
der Philipps-Universität
Karl-von-Frisch-Straße
D-3550 Marburg/Lahn
Federal Republic of Germany