

Review article**Pea-Rhizobial and Mycorrhizal Symbiotic Systems:
A Review of their Commonalities with Other Plant-
Microbe Systems**

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Abstract

In this review some of the mechanisms peas (*Pisum sativum* L.) have evolved for coping with microorganisms are covered. These adaptations are then compared with ways in which other host plants cope with microorganisms. The aim is to show that there are similarities between symbiotic and pathogenic host-microbe interactions and that a more profound understanding of these mechanisms and their side effects may lead to the development of new agricultural practices which will increase sustainability by taking advantage of already evolved and functioning mechanisms for nutrient supply and biological control.

Keywords: Pea (*Pisum sativum* L.), *Rhizobium*, mycorrhizae, symbiosis, host-microbe interactions, induced resistance, biological control, pathogens.

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1. Introduction

Many kinds of interactions exist between organisms. In the course of evolution, mutual or one-sided dependencies have developed between organisms with various metabolic pathways. As the microorganisms reached the present stage of development and so called higher forms of life began to emerge, these also became available as potential habitats for microorganisms. Animals and plants, thus, evolved in and had to adjust to an environment in which many types of prokaryotic metabolism were already present. Partnership associations between microorganisms and animals or plants are called symbioses, where several categories can be distinguished according to the benefits that the partners obtain from the association (Table 1).

Because of their economic importance, some of these forms of symbioses have been the objects of intensive study for a long time. One such example is the rhizobia-legume symbiosis with its ability to fix atmospheric nitrogen (Beyerinck, 1888). A leguminous crop can thus both supply a harvest and fertilise the field in which it is grown. Other symbiotic systems of interest are the plant-mycorrhiza symbioses. These types of symbioses occur in most land plants. That mycorrhizal fungi accumulate mineral nutrients especially N and P from the rhizosphere and deliver them to the root in return for photosynthetic products they require for growth and reproduction has been known for some time (Frank, 1885; Mosse, 1957; Baylis, 1959). Reports reviewed below indicate that the fungi are also capable of increasing their host's defence against plant pathogens, adding another aspect to the evolution of coadaptation.

A large number of chemical signals are involved in interactions between symbiotic and pathogenic microorganisms. However, these studies have been carried out independently by a number of different laboratories, each specialising on a specific group of organisms (Gil and Gay, 1977; Green et al., 1992; Keel et al., 1992; Downie, 1994; Gurusiddaiah et al., 1994; Meyer et al., 1994; Orenstein et al., 1994; Perotto et al., 1994a; Perotto et al., 1994b; Bécard et al., 1995). As a result, inadequate attention has been given to how the various symbionts/pathogens can cooperate with or counteract each other and thereby influence the development of their respective symbioses or pathogeneses on a specific host plant. The study of the combination of these types of interactions not only provides a fascinating opportunity for scientific investigation, but also opens the door to future scientific developments pertaining to long-term, low input, sustainable agricultural production and increased food security for future generations.

Table 1. Examples of associations between different kinds of organisms

Relationship	Effect	Examples
Mutualism	Beneficial for both partners	<p>Between microorganisms: Lichens (fungus, alga and cyanobacteria) <i>Desulfovibrio</i> and <i>Chromatium</i></p> <p>Between microorganisms and plants: <i>Rhizobium</i> and legumes <i>Azotobacter</i> and some tropical grasses Mycorrhiza – a mutualistic fungal association between fungi and plants</p> <p>Between microorganisms and animals: Rumen of ruminants – bacteria, fungi, protozoa Protozoa and bacteria <i>Pogonophora</i> (tube worms) and H₂S-oxidising bacteria</p>
Commensalism	One partner gains, the other one not damaged	<p>Between microorganisms: Aerobic and anaerobic bacteria in soil <i>Penicillium</i> and bacteria in cheese production Cellulose-degrading fungi and bacteria using by-products from the fungi</p> <p>Between microorganisms and plants: Deleterious rhizosphere bacteria such as <i>Pseudomonas</i> and several arable crops</p> <p>Between microorganisms and animals: Mammalian gastro-intestinal tract and eg. lactic acid bacteria</p>
Neutralism	No considerable influence on each other	<p>Between microorganisms: Bacteria colonising fungal hyphae Fungi and bacteria in sour dough associations</p> <p>Between microorganisms and plants: Bacteria in the rhizosphere and phyllosphere</p> <p>Between microorganisms and animals: Skin flora of humans (bacteria as mycobacteria, streptococci, staphylococci, propionibacteria)</p>
Parasitism	One partner gains, the other is damaged	<p>Between microorganisms: <i>Bdellovibrio</i> and bacteria eg. <i>Erwinia</i></p> <p>Between microorganisms and plants: <i>Aphanomyces</i> – several arable crops</p> <p>Between microorganisms and animals: <i>Legionella</i> and humans</p>

¹Note there may be either necrotrophic associations (one partner gets organic compounds from dead cells of the other) or biotrophic associations (one partner gets organic compounds from living cells of the other).

2. Background

Soil bacteria and fungi play an important role in plant nutrient supply and, as indicated above, they can also stimulate their host's resistance to pathogens. For someone familiar with only the pathogenic aspects of bacteria and fungi, this may seem slightly peculiar. A short review of a simplistic model of plant and biotrophic microbe interactions based on that proposed by van de Rhee (1994) will illustrate where symbiotic microbes "fit in" and will also illuminate some of the aspects which need further investigation (Fig. 1). Some workers call into question this conventional view and suggest that new ways of interpreting data must be sought (McKhann and Hirsch, 1994). Furthermore, the model is not intended to be a complete picture of how plants and microbes interact. For instance, necrotrophic interactions are not covered.

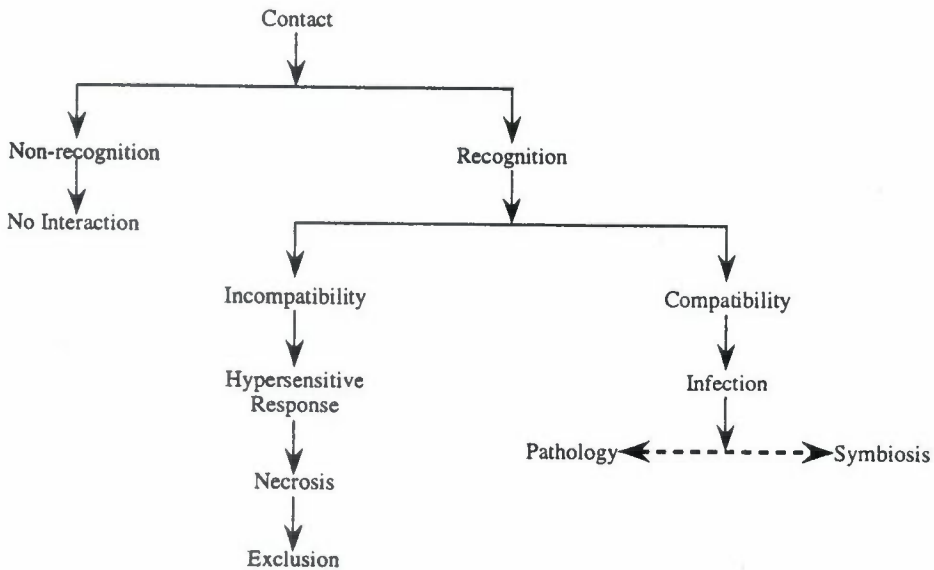


Figure 1. A simplistic model for plant-microbe interaction. The dashed line indicates that the result can be anywhere between the two points.

As plant roots penetrate the soil, they will come into contact with various soil microbes. Most of these contacts will not result in interactions and are indicated in Fig. 1 by the generic term 'Non-recognition'. An example of this would be contact between a bacterium and a plant which is outside the bacteria's host range. In such a case there will be no interaction between the two. Should the two recognise each other, however, opportunities for

interaction present themselves. One distinct result is an incompatibility reaction. This often includes the hypersensitive response (HR) which leads to the activation of a variety of defence reactions such as pathogenesis related (PR) protein synthesis, necrosis and exclusion of the recognised pathogen. The elicitors responsible for plant responses to pathogens, including HR, and the PR protein synthesised are reviewed in a number of recent articles (Stintzi et al., 1993; Ebel and Cosio, 1994; McKhann and Hirsch, 1994; van de Rhee et al., 1994). Another distinct result of plant-microbe recognition is a compatibility reaction which leads to infection. At this point there can be any number of outcomes which fall somewhere between mutualism and parasitism. In other words the same general sequence of events: contact, recognition, compatibility and infection, may lead to either a positive or a negative outcome, as far as the host plant is concerned, depending on which microbe it is faced with.

The above model not only encompasses symbiosis and pathogenesis together by simply following a sequence of events which leads to interaction between the plant and the microbe, but also provides other insight. First, there is a range of variation in how well symbiotic microbes such as rhizobia cooperate with a host plant. On the same cultivar of pea, for instance, various strains of *Rhizobium* will fix different amounts of nitrogen (Mårtensson and Rydberg, 1996). Similar variations can be found in symbiotic interactions of plants with mycorrhizal fungi (Azcón et al., 1991; Sekhon et al., 1992). Additionally, Azcón et al. (1991) found that in plants inoculated simultaneously with rhizobia and mycorrhizal fungi, the rhizobial strain which was best at fixing nitrogen will not necessarily collaborate best with the mycorrhizal fungus which was best at delivering nutrients to the same plant. This wide spectrum of symbiotic effects on host plants is mirrored in the variations of the effect that parasitic microbes have, which varies from slightly detrimental to lethal.

Mutualism and parasitism can be viewed as a variation on a theme, because biotrophic microbes appear to use the same or similar mechanisms for interacting with their host regardless of whether they are parasitic or mutualistic in action (Djordjevic et al., 1987; Bonfante-Fasolo et al., 1992; Green et al., 1992; Bonfante-Fasolo and Perotto, 1995). A more complete understanding of host-microbe interactions requires an investigation into not only how a particular set of microbes and plants interact. An examination of pea-rhizobial and pea-mycorrhizal symbioses follows. The information presented on these two systems will then provide the basis for a discussion of other plant-microbe interactions and some of the side effects of these interactions.

3. The Pea-*Rhizobium* Model

As indicated in the model below (Fig. 2), peas (and other legumes) exude (iso)flavonoids into the rhizosphere (Spaink, 1995). The (iso)flavonoids are unique evolutionary products as they generally have a negative action on the soil microbiota, but serve as recognition signals to *Rhizobium* bacteria indicating that a root is nearby and that it is ready to enter into a symbiotic relationship with the right *Rhizobium*. The bacteria respond by emitting nodulation factors, lipo-chitin oligosaccharides (LCO) and if there is a root hair at just the right stage of development in the vicinity, it will form the shepherd's crook, as described more thoroughly by Kannenberg and Brewin (1994). Thus, the process of infection is initiated. A great deal of work has been done on the nodulation genes which control the production of nodulation factors (Downie, 1994). Other chemicals have been found which also induce nodulation including triiodobenzoic acid (TIBA) and zeatin (Spaink, 1995) indicating that this is an extremely complex system in which many genes interact using a variety of chemical and hormonal signals in the developmental processes leading to nodulation.

As the bacteria infect the cell, an infection thread is formed by the cell. This infection thread is a tunnel made of plant membrane analogous to the plasma membrane (in the future it will be referred to as the peribacterial membrane or PBM); it has the same phospholipid bilayer composition and also contains transmembrane proteins for transport of both nutrients and signal molecules, including cyclic glucans, exopolysaccharides (EPS) and lipo-oligosaccharides and lipo-polysaccharides (LOS and LPS, respectively) (Kannenberg and Brewin, 1994).

Not only the formation of the infection thread, but also the development of the symbiotic organelle, called symbiosome (Kannenberg and Brewin, 1994), requires a series of signals orchestrated by the bacteria. In a sense, the bacteria 'subvert' the plant cell (Brewin, 1990). This does not happen without a certain degree of host control: infected cells possess assessment mechanisms which monitor the development of the infection and ensure that it is moving towards symbiosis (Spaink, 1995). Exactly which chemicals are exchanged is yet unknown, but the current state of knowledge of these apparent signals is reviewed in several recent articles (Leigh and Walker, 1994; Perotto et al., 1994b). Membrane surface polysaccharides, such as capsular polysaccharides (CPS), LPS and EPS have been shown to have significant influence on the progression and stability of infection (Djordjevic et al., 1987). Without attempting to complicate matters unnecessarily, it has been suggested that EPS is essential to nodule development (Leigh and Walker, 1994) and that LPS is,

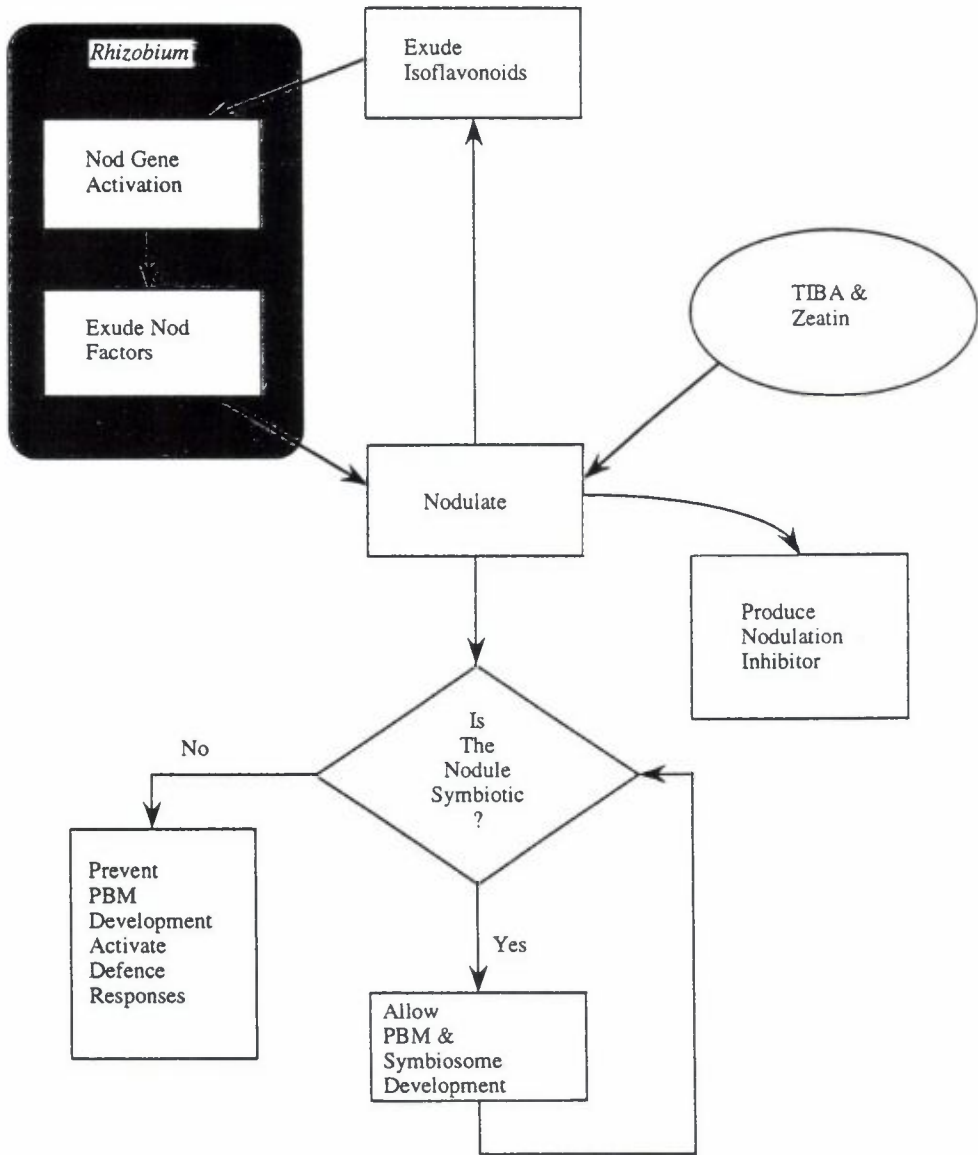


Figure 2. The flow chart indicates some of the more important aspects of the legume-rhizobia interaction. Nod gene activation and the release of Nod factors occurs in the rhizobia. TIBA is triiodobenzoic acid and PBM is the peribacteroid membrane.

at least in part, responsible for the suppression or avoidance of immune reactions (Perotto et al., 1994a).

In any event, the information which is passed back and forth controls the expansion of the PBM, host recognition and potential plant defence responses. In case the incipient symbiosome does not respond in the correct manner, the bacteroids will not be allowed to separate into mature single celled symbiosomes. Instead of dividing, the bacteroids will be bunched up in a single pocket (Kannenberg and Brewin, 1994). It has been suggested that the bacteria seem to hide from the cell within the PBM by mimicking plant cell signals (Spaink et al., 1993). If the integrity of the PBM is lost, a hypersensitivity like response will result in cell death and the termination of the root nodule (Werner et al., 1985). However, when the infection is compatible, the bacteria will differentiate and multiply into bacteroids which will each have their own PBM.

Finally, the plant has several systemic reactions to nodulation as well. An interesting response is that more (iso)flavonoids are synthesised and exuded into the soil presumably as a mechanism for protecting the root from infection by other compatible bacteria (Spaink, 1995). At the same time, a 'non-nodulation' signal is synthesised within the plant to prevent excessive nodulation (Djordjevic et al., 1987). Nodulation and the production of 'non-nodulation' factors are independent of infection and can be induced by the introduction of killed bacteria, the correct bacterial nodulation factor as well as TIBA and zeatin (Spaink, 1995). This indicates that the process is host controlled and plant hormones are currently the major candidates in the search for chemical signals (Clarke et al., 1992).

4. The Pea-Arbuscular Mycorrhizal Model

The strategies arbuscular mycorrhizal fungi employ when infecting host plants are reviewed very extensively by Bonfante-Fasolo and Perotto (1995). A slightly different flow chart than theirs is proposed here in Fig. 3. Note also that there are a number of similarities to the proposed flow chart for rhizobial infection given in Fig. 2.

Here we see that when the arbuscular mycorrhizal fungus and the plant come in contact and recognise each other, infection can occur. The infection leads to what is termed a 'mycorrhizal root'. It is no surprise that a fungal hypha can penetrate the cell wall, but the changes which occur subsequently are remarkable. Citing Bonfante-Fasolo and Perotto directly: "Detailed observations show that development of the arbuscle inside a cortical cell correlates with dramatic modifications of host cell architecture such as invagination of the plasmalemma, fragmentation of the vacuole, disappearance of amyloplasts and the increase in the number of organelles such as

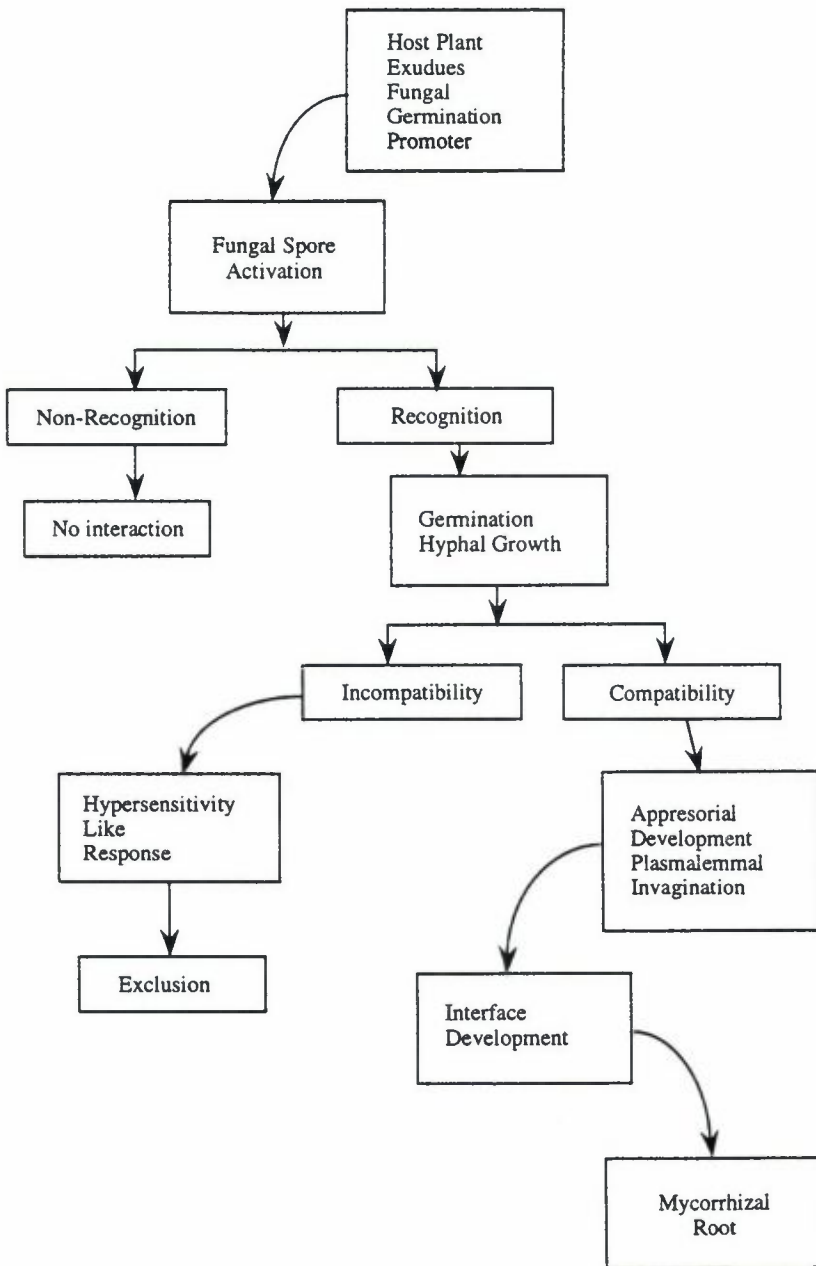


Figure 3. Flow chart, inspired by that of Bonfante-Fasolo and Perotto (1995), indicating some of the major aspects of plant-mycorrhizal interactions.

Golgi bodies". The combination of plasmalemma invagination and the increase in the number of Golgi bodies which supply the material necessary for the extension of the plasmalemma indicate that the plant and the fungal hypha inside the apoplastic 'tunnel' (to use a word familiar from the above discussion of incipient rhizobial infection) are communicating with each other and that each has mechanisms for accommodating the other's presence. In other words, the fungal hypha is capable of signalling to the cell it is invading and thus redirecting the cell's development. This is highly reminiscent of how compatible rhizobial bacteria invade the plant cell and redirect plasmalemmal development to produce symbiosomes. A short account of some of the controls which prevent mycorrhizal root development will help define what successful infection includes.

Appresorial development is apparently induced by compatible plant exudates. However, it seems that (iso)flavonoids are not solely responsible for the establishment of mycorrhizal symbiosis (Bécard et al., 1995). In a recent study, exudates from non-mycorrhizal lupines did stimulate hyphal elongation, but not true appresorial development (Giovannetti et al., 1993). So, plant exudates are, at least in part, responsible for the recognition reactions, but the lack of appresorial development indicates that this is a process which includes several steps. An imperfect match results in the termination of interaction.

The cell wall is, of course, one of the major barriers to host colonisation. To penetrate the cellulosic layer arbuscular mycorrhizae appear to employ methods which resemble those of *Uromyces viciaefabae*. Acidic cellulases followed by pectin esterase, neutral cellulases and polygalacturonate are produced to soften the cell wall (Deising and Mendgen, 1992). The action of these enzymes is coordinated with mechanical action so as to ensure that host viability is maintained at the same time as defence responses are not triggered (Bonfante-Fasolo and Perotto, 1995). This is, in fact, more complicated than it may seem since the cell wall contains plant defence elicitors which may be released as the wall is degraded (Ebel and Cosio, 1994).

Non-nodulating (*nod*⁻) pea mutants are another example of how important 'cross talk' is to successful interaction as they have been shown to be resistant to colonisation by arbuscular fungi. These mutants are also called (*myc*⁻) (Duc et al., 1989; Gianinazzi-Pearson et al., 1991). As more knowledge of the genetic variation of nodulating pea varieties accumulated, it was found that there are considerable differences in how these mutants interacted with mycorrhizae. One example of this is the 'late mutant' which nodulates, but does not fix nitrogen (*nod*⁺*fix*⁻). When this mutant is first pre-inoculated with *Rhizobium* it will allow superficial mycorrhizal colonisation by compatible fungi, but will not form fully differentiated arbuscles (Gianinazzi-Pearson et al., 1995).

Interestingly, this seems to indicate that fully functional rhizobial and mycorrhizal symbioses depend on similar, if not the same, host genes.

Unsuccessful interaction between mycorrhizal fungi and pea (cv. Frisson) can result in the production of PR proteins and rejection of the mycorrhizae as reported by Gollotte et al. (1994). Moreover, (*myc*⁻) mutants induced both β -1,3-glucans and the PR protein classed as 1b at contact. (*myc*⁺) isogenic mutants also induced β -1,3-glucans at the point of penetration and PR 1b proteins were found around arbuscles as well as around senescent arbuscles, but this happened at much later stages and to a lesser degree than for the (*myc*⁻) mutants. Thus, roots capable of symbiosis with mycorrhizae delay defence reactions. New results suggest that mycorrhizae are themselves capable of suppressing defence responses (Volpin et al., 1995). It has been proposed that arbuscular mycorrhizal fungi were instrumental in the colonisation of land by ancient plants (Pirozynski and Malloch, 1975) and it is thus surprising that the (*myc*⁺) peas produced PR proteins at all. Perhaps even closer symbiotic interactions may be found where there elicitation of the defence response is totally suppressed.

Because rhizobial and mycorrhizal symbiotic interactions demonstrate such a complexity of signalling and feed-back it can be assumed that their coevolution with plants has a long history. For this reason, a closer look at how these two biotrophs interact with the host plant may yield new ways of evaluating symbioses and, especially, new ways of comparing biotrophic and necrotrophic interactions with a plant.

5. Signal Substances and Membranes in Symbiosis

In the attempt to discover the means by which symbionts interact with their hosts, a number of researchers have begun to study the contents of apoplastic space created between the membranes of the microbe and the host cell (Kannenbergh and Brewin, 1994; Bonfante-Fasolo and Perotto, 1995; Perotto et al., 1994a; Perotto et al., 1994b). One of the more effective methods for the identification of the chemical signals used in interaction involves monoclonal antibodies. Produced in cell cultures taken from animals such as rats or hamsters and with iodine-125 or colloidal gold as markers, they act as any other antibody, binding to target chemicals and thus revealing their target's presence (Brewin et al., 1987).

As already indicated, the chemical substances and membrane components responsible for signalling in rhizobial symbiosis have been the object of intensive study. It is believed that they and the genes which code for them are responsible for nodule morphogenesis and a variety of other functions such as

the avoidance or suppression of immune responses. Considering that both *Rhizobium* and mycorrhizal fungi develop interfaces with the host cells, it is imaginable that some of the signals are similar for both systems. Indeed, a recent article showed that antibodies targeted both rhizobial and mycorrhizal membrane components in seven out of eight cases (Perotto et al., 1994a). Figs. 4 and 5 present some of the known interface compounds in rhizobial and mycorrhizal symbiosis. No doubt, more compounds will be added as our knowledge increases.

Symbiotic bacteria and fungi are not alone in having evolved the mechanisms necessary to penetrate the wall and displace the plasmalemma: compatible biotrophic parasites such as the powdery mildew *Erysiphe pisi* also survive within an apoplastic cavity created in the plasmalemma of the cells pea leaves (Green et al., 1992). While the effect on the host is negative in this case, many of the basic elements of this interaction are the same. Subsequent to recognition, the hypha swells into an appressorium 'from which an extremely fine hyphal peg grows directly through the cell wall of the host cell, producing the specialised absorption structure, termed the haustorium' (Green et al., 1992). The haustorium is surrounded by an extrahaustorial matrix, presumed to be a gel consisting of pectins and/or hemicelluloses (Gil and Gay, 1977). The indeterminate nature of Gil's characterisation of the contents of the extrahaustorial matrix needs to be clarified. The definitive work on the powdery mildew extrahaustorial matrix seems to be lacking. But what is striking is that another fungus, *Verticillium dahliae*, produces protein-lipopolysaccharides. These phytotoxins are routinely used to test cultivar resistance to the pathogen (Meyer et al., 1994; Orenstein et al., 1994). They also have a high bonding affinity to cotton membranes (Meyer and Dubery, 1993). While there is still too little evidence to support a categorical statement concerning signalling similarities, the large number of coincidences clearly begs for further investigation.

6. Side Effects of Plant-Microbe Interactions

In addition to the improved nutritional status which results from symbiotic interactions with mycorrhizae there are some side effects which are difficult to explain solely in terms of improved plant nutrition (Caron et al., 1986). In a series of experiments performed on peas, infection with the mycorrhizal fungus *Glomus fasciculatum* was accompanied by an increased resistance to root rot caused by *Aphanomyces euteiches* (Rosendahl, 1985). Three different techniques were used to investigate the nature of the induced resistance to root rot observed in mycorrhizal peas.

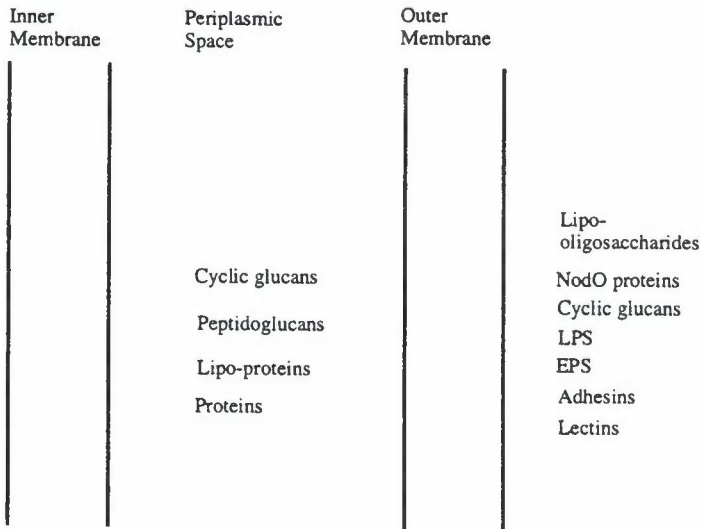


Figure 4. Diagram inspired by that of Kannenberg and Brewin (1994) of the membrane-apoplast-membrane morphology of the rhizobial interfaces and some of the chemicals known to be found there. EPS, LPS and CPS are exopolysaccharides, lipopolysaccharides and neutral capsular polysaccharides, respectively.

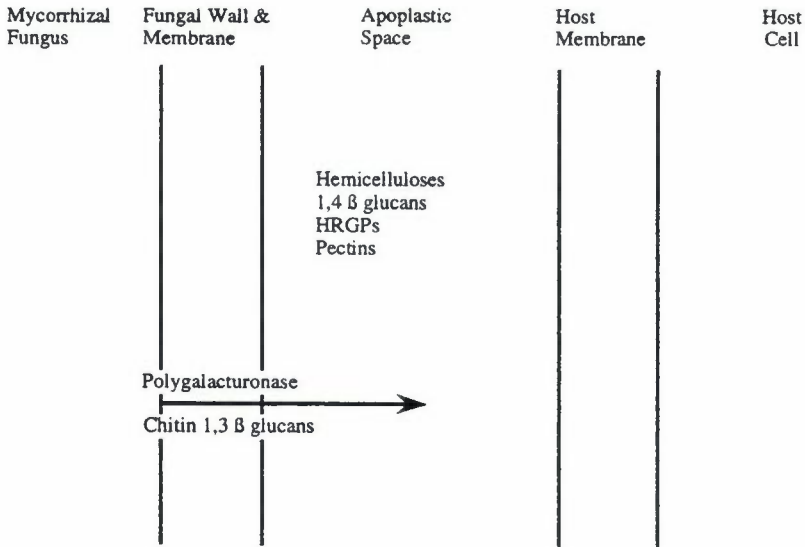


Figure 5. Diagram inspired by that of Bonfante-Fasolo and Perotto (1995) of the membrane-apoplast-membrane morphology of the mycorrhizal interfaces and some of the chemicals known to be found there. HRGPs are hydroxyproline-rich glycoproteins.

In all three series of experiments roots infected with a mycorrhizal fungus had locally enhanced resistance to attack by *A. euteiches*. In the first series, a time lapse experiment, the plants which were allowed the longest time to establish mycorrhizal infection before the introduction of the challenge were most resistant. Simultaneous inoculation did not produce a significant improvement over roots inoculated with *A. euteiches* alone. In the second, split root, series of experiments, the best control of root-rot was obtained when both fungi were present in the same pot. Non-mycorrhizal roots on mycorrhizal plants were as susceptible as roots of plants without mycorrhizal fungi, suggesting that mycorrhizae-stimulated resistance is non-systemic. The effect may be due to a fungal signal which cannot be transported to other parts of the plant. In the third series of experiments it was found that non-mycorrhizal plants became more thoroughly infected with *A. euteiches* the more zoospores were used. At the same time, the mycorrhizal plants showed insignificant increases in infection due to increased zoospore inoculation.

These experiments leave out one important aspect of fungal infection, namely, age. A close examination of the data especially in the first series of experiments, indicate that susceptibility to *A. euteiches* decreases significantly with increasing age. Plants inoculated at two weeks of age showed infection on about half of their roots while those of four and six weeks of age showed infections on a sixth and a tenth of their roots, respectively. Thus, while mycorrhizal roots were less susceptible than non-mycorrhizal roots, further investigations are necessary if the mycorrhizal effect is to be separated from the side effects of maturity.

In another series of experiments on tobacco plants, the mycorrhizal fungus *Glomus monosporum*, induced similar tolerance enhancement towards the tobacco root rot pathogen *Thielaviopsis basicola* (Giovannetti et al., 1991). Here again, the mycorrhizae was allowed a head start. At the end of the experiment, the dry weights of the roots and shoots were determined. The number of chlamydiospores and percentage of root length infected with root rot were also determined. In each case the plants with mycorrhizae were at an advantage over the non-mycorrhizal plants. These results add additional weight to Rosendahl's findings that mycorrhizae stimulate their host plants' resistance to infection by necrotrophic root rot fungi, if only in a localised fashion.

There are other examples of resistance response enhancement in the literature. Davis (1963) showed that the root nematode *Meloidogyne incognita acrita*, induced resistance to *A. euteiches* in peas. Additionally, *M. incognita acrita* was shown to have at least local inhibitory effects against the breakdown of cells due to *Fusarium solani*. The deterioration this fungus normally causes was not observed in cells associated with the mature nematode

females. This effect seems to be species specific i.e. if *M. incognita acrita* was replaced with *Pratylenchus penetrans* different effects were registered.

In experiments where the effects of metabolites from different mutants of the bacterium *Pseudomonas fluorescens* were studied, induced resistance to fungal pathogens was found (Gurusiddaiah et al., 1994). Here a supernatant consisting of "chromopeptides and other peptides, fatty acid esters, and a lipopolysaccharide matrix" derived from the *P. fluorescens* D-7 strain was found to be fungistatic towards the pathogenic fungus *Gaeumannomyces graminis* var. *tritici* in wheat bioassays. In another series of experiments testing *P. fluorescens* induced resistance, 2,4-diacetylphloroglucinol was determined to play a significant role in the induction of resistance to *G. graminis* var. *tritici* in wheat and *Thielaviopsis basicola* in tobacco (Keel et al., 1992). In this case, a mutant which did not produce the metabolite 2,4-diacetylphloroglucinol was found to have reduced antifungal effect. But, when an 11-kb DNA fragment from the wild type CHA0 strain was inserted into the mutant, metabolite production and fungal suppression were largely restored. While these experiments may seem distant from rhizobial and mycorrhizal symbiotic relations with peas, there is the common thread of induced-resistance enhancement via chemical signalling to the host plant.

7. Conclusions and Future Prospects

Evolutionary theory focuses a lot of attention on fitness and selective pressure. Any adaptation that increases an organisms' fitness increases its chances of persisting through time. Selective pressure, on the other hand, culls out the organisms least adept at adapting to and managing a niche. Keeping this in mind, a discussion of biotrophic and necrotrophic microbe interactions with a host plant is a fascinating illustration of the complexity and dynamism of life.

Successful biotrophic infection depends on a high level of genetic compatibility between the host and the infecting microbe. Recognition reactions, cell wall degradation, plasmalemmal invagination or subversion and the avoidance or suppression of defence reactions during infection and subsequent interaction all require genetic interaction and extensive collaboration which is no doubt the result of long term coevolution.

It comes as no surprise that symbiotic microbes such as rhizobia and mycorrhizae take advantage of the plant's limited access to mineral nutrients to create and sustain a niche. The mitochondrion which also has a double bilayer membrane is supposed to be a former foreign symbiotic microbe. Its niche as supplier of energetic ATP has become so closely coupled to cell functions that

it has become a permanent organelle. Over time it has evolved to either avoid the production of cell surface components that might be recognised as 'not self' or, at least, to hide them from the host cell.

The symbiotic behaviour of mycorrhizae is not limited to trading mineral nutrients for photosynthates with a host plant. It is also to the mycorrhizae's advantage that the plant with which it is interacting symbiotically can survive attacks by necrotrophic fungi. So, the mycorrhizae is hardly altruistic when it induces host resistance to root rot in pea. Instead it is simply managing its niche as a result of natural selection. The nematode discussed above is even more dependent on its host and it also actively manages its niche by inducing resistance to root rot as a result of natural selection and thereby improving its reproductive success. Based on this sort of argument, it is easy to hypothesise that rhizobia also induce local resistance to infection by *A. euteiches*.

The similarities between the interactions of biotrophic organisms such as mycorrhizae, *Rhizobium*, nematodes and mildew and their hosts are striking. These biotrophs can send and receive chemical signals across plant membranes. These signals redirect the host cell's functions and either suppress defence responses or perhaps so completely camouflage the presence of the foreign organism that the host cell never has a chance to react to the invasion. They also enable the biotrophs to obtain the photosynthates which they require and in some cases have been shown to induce resistance to pathogens.

The mechanisms discussed are dynamic in that they are not only evolved, but are continually evolving. This aspect represents an opportunity. Once these mechanisms are understood, perhaps it will be possible to produce healthy crops by promoting the conditions which lead to the beneficial 'side effects' of plant-microbe interactions such as induced resistance to pathogens. Because these side effects are the result of a continuing natural process i.e. evolution, the microbes themselves will be responsible for 'product development' and 'testing'. This promises to provide the opposite to other forms of pest control, like agro-chemicals to which target organisms are selected to develop acquired immunity.

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REFERENCES

- Azcón, R., Rubio, R., and Barea, J.M. 1991. Selective interactions between different species of mycorrhizal fungi and *Rhizobium meloti* strains and their effects on growth, N₂-fixation (¹⁵N) N nutrition of *Medicago sativa* L. *The New Phytologist* **117**: 399-404.

- Baylis, G.T.S. 1957. The effect of vesicular-arbuscular mycorrhizas on growth of *Griselinia littoralis* (Cornaceae). *The New Phytologist* **58**: 274-280.
- Bécard, G., Taylor, L.P., Douds, D.D., Pfeffer, P.E., and Doner, L.W. 1995. Flavonoids are not necessarily plant signal compounds in arbuscular mycorrhizal symbioses. *Molecular Plant-Microbe Interactions* **8**: 252-258.
- Beyerinck, M.W. 1888. Die Bakterien der Papilionaceenknöllchen. Five parts in: *Botanische Zeitung* **46**: 726-736; **47**: 741-750; **48**: 757-771; **49**: 781-790; **50**: 797-804.
- Bonfante-Fasolo, P., Perotto, R., and Perotto, S. 1992. Cell surface interactions in endomycorrhizal symbiosis. In: *Perspectives in Plant Cell Recognition*. Society for Experimental Biology Seminar Series 48. J.A. Callow and J.R. Green, eds. Cambridge University Press, Cambridge, pp. 239-255.
- Bonfante-Fasolo, P. and Perotto, S. 1995. Strategies of arbuscular mycorrhizal fungi when infecting host plants. *The New Phytologist* **130**: 3-21.
- Brewin, N.J. 1990. The role of the plant plasma membrane in symbiosis. In: *The Plant Plasma Membrane*, Larsson, C.; Moller, I.M. eds. Springer-Verlag GmbH, Berlin, pp. 351-375.
- Brewin, N.J., Bradley, D.J., Wood, E.A., Kannenberg, E.L., Van den Bosch, K.A., and Butcher, G.W. 1987. The use of monoclonal antibodies to investigate plant-microbe interactions in pea root nodules containing *Rhizobium leguminosarum*. In: *Cell Biology NATO ASI Series, Series H*, 1988, No. 17, Berlin, pp. 373-383.
- Caron, M., Fortin, J.A., and Richard, C. 1986. Effect of phosphorus concentration and *Glomus intraradices* on *Fusarium* crown and root rot of tomatoes. *Phytopathology* **70**: 447-452.
- Clarke, H.R., Leigh, J.A., and Douglas, C.J. 1992. Molecular signals in the interaction between plants and microbes. *Cell* **71**: 191-199.
- Davis, R.A. 1963. Interactions of nematodes and pea (*Pisum sativum*) diseases. *Dissertation Abstracts* **24** (2646).
- Deising, H. and Mendgen, K. 1992. Development and control of enzyme production and cell wall modification in rust fungi, and defence reactions of the host plant. In: *Molecular Biology of Filamentous Fungi*. Proceedings of the EMBRO workshop, Berlin, August 24-29, 1991. P. Tudzynski and U. Stahl eds. Weinheim, VCH, New York, pp. 27-44.
- Djordjevic, M.A., Gabriel, D.W., and Rolfe, B.G. 1987. *Rhizobium* - the refined parasite. *Annual Review of Phytopathology* **25**: 145-168.
- Downie, J.A. 1994. Signalling strategies for nodulation of legumes by rhizobia. *Trends in Microbiology* **2**: 318-324.
- Duc, G., Trouvelot, A., Gianinazzi Pearson, V., and Gianinazzi, S. 1989. First report of non-mycorrhizal plant mutants (Myc⁻) obtained in pea (*Pisum sativum* L.) and faba bean (*Vicia faba* L.). *Plant Science, Irish Republic* **60**: 215-222.
- Ebel, J. and Cosio, E. 1994. Elicitors of plant defense responses. In: *International Review of Cytology*, Academic Press, San Diego, CA **148**: 1-36.
- Frank, A.B. 1885. Ueber die auf Wurzelsymbiose beruhende Ernaehrung gewisser Baeume durch unterirdische Pilze. *Berichte der Deutschen Botanischen Gesellschaft* **3**: 128.
- Gianinazzi-Pearson, V., Gianinazzi, S., Guillemain, J.P., Trouvelot, A., Duc, G., Hennecke, H., and Verma, D.P.S. 1991. Genetic and cellular analysis of resistance to vesicular arbuscular (VA) mycorrhizal fungi in pea mutants. In: *Advances in Molecular Genetics*

- of *Plant Microbe Interactions*. Interlaken, Switzerland, 9–14 September, 1990. Kluwer, Dordrecht, pp. 336–342.
- Gianinazzi-Pearson, V., Gollote, A., Lherminier, J., Tisserant, B., Franken, P., Dumas-Gaudot, E., Lemoine, M.C., Van Tunien, D., and Gianinazzi, S. 1995. Cellular and molecular approaches in the characterization of symbiotic events in functional arbuscular mycorrhizal associations. *Canadian Journal of Botany* **73**: S526–S532.
- Gil, F. and Gay, J.L. 1977. Ultrastructural and physiological properties of the host interfacial components of haustoria of *Erysiphe pisi* *in vitro* and *in vivo*. *Physiological Plant Pathology* **10**: 1–12.
- Giovannetti, M., Tosi, L., Della Torre, G., and Zizzerini, A. 1991. Histological, physiological and biochemical interactions between vesicular-arbuscular mycorrhizae and *Thielaviopsis basicola* in tobacco plants. *Journal of Phytopathology* **131**: 265–274.
- Giovannetti, A., Avio, M., Sbrana, C., and Citernes, A.S. 1993. Factors affecting appressorium development in the vesicular-arbuscular mycorrhizal fungus *Glomus mosseae* (Nicol. and Gerd.) Gerd. and Trappe. *The New Phytologist* **123**: 115–122.
- Gollote, A., Gianinazzi-Pearson, V., and Gianinazzi, S. 1994. Immunocytochemical study of plant-arbuscular mycorrhizal fungus interfaces in isogenic peas (*myc*⁺) or mycorrhiza resistant peas (*myc*⁻). *Acta Botanica Gallica* **141**: 449–454.
- Green, J.R., Mackie, A.J., Roberts, A.M., and Callow, J.A. 1992. Molecular differentiation and development of the host-parasite interface in powdery mildew of pea. In: *Perspectives in Plant Cell Recognition*. Society for Experimental Biology Seminar Series 48. J.A. Callow and J.R. Green, eds. Cambridge University Press, Cambridge, pp. 193–212.
- Gurusiddaiah, S., Gealy, D.R., Kennedy, A.C., and Ogg, A.G., Jr. 1994. Isolation and characterization of metabolites from *Pseudomonas fluorescens*-D7 for control of downy brome (*Bromus tectorum*). *Weed Science* **42**: 492–501.
- Kannenberg, E.L. and Brewin, N.J. 1994. Host-plant invasion by *Rhizobium*: The role of cell-surface components. *Trends in Microbiology* **2**: 277–283.
- Keel, C., Ursula, S., Maurhofer, M., Voisard, C., Lavielle, J., Burger, U., Wirthner, P., Haas, D., and Défago, G. 1992. Suppression of root diseases by *Pseudomonas fluorescens* CHA0: importance of bacterial secondary metabolite 2,4-diacetylchloroglucinol. *Molecular Plant-Microbe Interactions* **5**: 4–13.
- Leigh, J.A. and Walker, G.C. 1994. Exopolysaccharides of *Rhizobium*: Synthesis, regulation and symbiotic function. *Trends in Genetics* **10**: 63–67.
- McKhann, H.I. and Hirsch, A.M. 1994. Does *Rhizobium* avoid the host response?. *Current Topics in Microbiology and Immunology* **192**: 139–162.
- Meyer, R. and Dubery, I.A. 1993. High-affinity binding of a protein-lipopolysaccharide phytotoxin from *Verticillium dahliae* to cotton membranes. *FEBS Letters* **335**: 203–206.
- Meyer, R., Slater, V., and Dubery, I.A. 1994. A phytotoxic protein-lipopolysaccharide complex produced by *Verticillium dahliae*. *Phytochemistry* **35**: 1449–1453.
- Mosse, B. 1957. Growth and chemical composition of mycorrhizal and non-mycorrhizal apples. *Nature* **179**: 922.
- Mårtensson, A.M. and Rydberg, I. 1996. Cultivar x rhizobial strain interactions in peas with respect to early symbiosis, nodule initiation and N uptake. *Plant Breeding* **115**, in press.

- Orenstein, J., Nachmias, A., Colon, L.T., and Hoogendorn, J. 1994. The effect of a *Verticillium dahliae* phytotoxin on germination of potato pollen. *Israel Journal of Plant Sciences* **42**: 29–36.
- Perotto, S., Brewin, N.J., and Bonfante, P. 1994a. Colonization of pea roots by the mycorrhizal fungus *Glomus versiforme* and by *Rhizobium* bacteria: Immunological comparison using monoclonal antibodies as probes for plant cell surface components. *Molecular Plant-Microbe Interactions* **7**: 91–98.
- Perotto, S., Brewin, N.J., and Kannenberg, E.L. 1994b. Cytological evidence for a host defense response that reduces cell and tissue invasion in pea nodules by lipopolysaccharide-defective mutants of *Rhizobium leguminosarum* strain 3841. *Molecular Plant-Microbe Interactions* **7**: 99–112.
- Pirozynski, K.A. and Malloch, D.W. 1975. The origin of plants: a matter of mycotrophism. *Biosystems* **6**: 153–164.
- Rosendahl, S. 1985. Interactions between the vesicular-arbuscular mycorrhizal fungus *Glomus fasciculatum* and *Aphanomyces euteiches* root rot of peas. *Phytopathologische Zeitschrift* **114**: 31–40.
- Sekhon, G.K., Gupta, R.P., Pandher, M.S., and Arora, J.K. 1992. Symbiotic effectiveness of Hup⁺ *Rhizobium*, VAM fungi and phosphorus levels in relation to nitrogen fixation and plant growth of *Cajanus cajan*. *Folia Microbiologica* **37**: 210–214.
- Spaink, H.P., Wijffes, A.H.M., von Vliet, T.B., Kijne, J.W., and Lugtenberg, B.J.J. 1993. Rhizobial lipo-oligosaccharide signals and their role in plant morphogenesis; are analogous lipophilic chitin derivatives produced by the plant? *Australian Journal of Plant Physiology* **20**: 381–92.
- Spaink, H.P. 1995. The molecular basis of infection and nodulation by rhizobia: the ins and outs of pathogenesis. *Annual Review of Phytopathology* **33**: 345–368.
- Stintzi, A., Heitz, T., Prasad, V., Wiedemann-Merdinoglu, S., Kauffmann, S., Geoffroy, P., Legrand, M., and Fritig, B. 1993. Plant 'pathogenesis-related' proteins and their role in defense against pathogens. *Biochemie* **75**: 687–706.
- van de Rhee, M.D., Linthorst, J.M., and Bol, J.F. 1994. Pathogen-induced gene expression. In: *Stress Induced Gene Expression in Plants*. A.S. Basra, ed. Harwood Academic Publishers, Chur, Switzerland, pp. 249–284.
- Volpin, H., Philips, D.A., Okon, Y., and Kapulnik, Y. 1995. Suppression of an isoflavonoid phytoalexin defense response in mycorrhizal alfalfa roots. *Plant Physiology* **108**: 1449–1454.
- Werner, D., Mellor, R.B., Hahn, M.G., and Griesbach, H. 1985. Glyceollin 1 accumulation in an ineffective type of soybean nodule with an early loss of peribacteroid membrane. *Zeitschrift für Naturforschung* **40c**: 179–181.