A Successful Bacterial Coup d’État: How *Rhodococcus fascians* Redirects Plant Development

Elisabeth Stes,1,2 Olivier M. Vandeputte,3 Mondher El Jaziri,3 Marcelle Holsters,1,2 and Danny Vereecke4,*

1Department of Plant Biotechnology and Genetics, Ghent University, 9052 Gent, Belgium; email: elisabeth.stes@psb.vib-ugent.be, marcelle.holsters@psb.vib-ugent.be
2Department of Plant Systems Biology, VIB, 9052 Gent, Belgium
3Laboratoire de Biotechnologie Végétale, Université Libre de Bruxelles, 6041 Gosselies, Belgium; email: ovdeputt@ulb.ac.be, jaziri@ulb.ac.be
4Department of Plant Production, University College Ghent, Ghent University, 9000 Gent, Belgium; email: danny.vereecke@hogent.be

**Keywords**
Actinomycete, autoregulation, cytokinin, auxin, polyamine, organogenesis

**Abstract**
*Rhodococcus fascians* is a gram-positive phytopathogen that induces differentiated galls, known as leafy galls, on a wide variety of plants, employing virulence genes located on a linear plasmid. The pathogenic strategy consists of the production of a mixture of six synergistically acting cytokinins that overwhelm the plant’s homeostatic mechanisms, ensuring the activation of a signaling cascade that targets the plant cell cycle and directs the newly formed cells to differentiate into shoot meristems. The shoots that are formed upon infection remain immature and never convert to source tissues resulting in the establishment of a nutrient sink that is a niche for the epiphytic and endophytic *R. fascians* subpopulations. Niche formation is accompanied by modifications of the transcriptome, metabolome, physiology, and morphology of both host and pathogen. Here, we review a decade of research and set the outlines of the molecular basis of the leafy gall syndrome.
INTRODUCTION

In the order of the high G+C gram-positive Actinomycetales, members of the genus Rhodococcus are notorious for their medical, veterinary, environmental, and especially industrial implications (11, 46, 50, 65, 96, 99). With an ever-growing number of new and reclassified species assigned to the genus Rhodococcus, the systematics of the genus remain cumbersome (38). However, despite the expansion of the genus and several reports documenting the plant-associated occurrence of Rhodococcus species (6, 13, 30, 97), Rhodococcus fascians remains the only plant pathogenic member.

R. fascians is a biotrophic phytopathogen that causes hyperplastic outgrowths through the production of cytokinins (90). Secretion of phytohormones is a common strategy among plant-associated microbes (3, 33, 98), and the responsible genes are often located on large circular plasmids (67). However, to date, R. fascians is the only phytopathogen described that harbors virulence genes on a linear plasmid (17, 32). Moreover, R. fascians is unique among the hyperplasia-inducing bacteria because it provokes differentiated galls, known as leafy galls (105). Other gall-inducing pathogens, such as stunting, deformed leaves, witches’ brooms, and fasciated shoots, are commonly observed on plants infected by R. fascians (Figure 1a–e). When R. fascians hits ornamentals plants, they lose their commercial value because symptomatic tissues are malformed compared to unaffected ones and flower formation is impaired. Consequently, economic damage is mainly situated in the globally expanding ornamentals industry (79). In this horticultural sector, R. fascians infections are a persistent problem because of the lack of efficient eradication methods and little knowledge of the epidemiology of the disease (24). Indeed, leafy galls are often mistakenly diagnosed as symptoms inflicted by A. tumefaciens, viruses, or eriophyid mites, or as shoot proliferations caused by exposure to growth hormones or herbicides (24, 61, 77). Moreover, R. fascians can persist on the plant surface for months before symptoms arise and thus be established without the grower’s awareness (16, 48). As a result, propagation of (latently) infected plant material seems to be the primary mode of transmission of the disease (63). An early diagnosis of new plant material via the polymerase chain reaction (PCR) (34, 59, 69, 85, 88), preferably combined with isolation of the bacterium from PCR-positive plants, can prevent introduction of R. fascians into greenhouses. Upon disease establishment, nurseries have to rely on strict sanitary measures to control bacterial spreading (24, 63).

RHODOCOCCUS FASCIANS POSES A THREAT TO THE ORNAMENTALS INDUSTRY

Since the first report of fasciated peas (10), the host range of R. fascians has expanded continuously (52, 60, 62, 80) and currently encompasses 164 species in 43 plant families. R. fascians primarily affects dicotyledonous herbaceous plants, but several woody and monocotyledonous plants are sensitive as well. Incidences of infection have been recorded throughout the world, mainly in temperate regions (24, 79).

Other than leafy galls, aerial malformations, such as stunting, deformed leaves, witches’ brooms, and fasciated shoots, are commonly observed on plants infected by R. fascians (Figure 1a–e). When R. fascians hits ornamentals plants, they lose their commercial value because symptomatic tissues are malformed compared to unaffected ones and flower formation is impaired. Consequently, economic damage is mainly situated in the globally expanding ornamentals industry (79). In this horticultural sector, R. fascians infections are a persistent problem because of the lack of efficient eradication methods and little knowledge of the epidemiology of the disease (24). Indeed, leafy galls are often mistakenly diagnosed as symptoms inflicted by A. tumefaciens, viruses, or eriophyid mites, or as shoot proliferations caused by exposure to growth hormones or herbicides (24, 61, 77). Moreover, R. fascians can persist on the plant surface for months before symptoms arise and thus be established without the grower’s awareness (16, 48). As a result, propagation of (latently) infected plant material seems to be the primary mode of transmission of the disease (63). An early diagnosis of new plant material via the polymerase chain reaction (PCR) (34, 59, 69, 85, 88), preferably combined with isolation of the bacterium from PCR-positive plants, can prevent introduction of R. fascians into greenhouses. Upon disease establishment, nurseries have to rely on strict sanitary measures to control bacterial spreading (24, 63). Although the increased incidence of R. fascians infections...
Figure 1

Typical symptoms induced on ornamental plants and characteristic features of Rhodococcus fascians and the interaction with its host. (a) Leafy gall (arrow) on Erysimum spp. (b) Leafy galls (arrow) on Viola spp. (c) Stunted growth on Iberis spp. (d) Leaf deformation (arrow) in Ipomoea spp. (e) Fasciation (arrow) on Iberis spp. (f) R. fascians orange-colored colonies. (g) Epiphytic R. fascians colony covered by a slime layer on tobacco; rod-shaped (asterisk) and elongated (arrow) bacteria are indicated. (h) Axillary activation (asterisk) and de novo meristem formation (arrow) in Arabidopsis thaliana.

in nurseries is best documented for the United States (60, 78), the worldwide dispersal of R. fascians and the extraordinarily wide host range of this phytopathogen present a potential global threat in horticultural practices (24).

EPiphytic colonization induces changes in the primary metabolism of the plant

R. fascians has several characteristics that are typical of a successful epiphyte. The orange carotenoid pigments (76) (Figure 1f) provide protection against UV irradiation (91) during the establishment of epiphytic colonies on the stems and leaves of a host. Moreover, colonies are often formed in excavations and epidermal cell wall junctions, thus evading harsh environmental conditions, whereas colocalization with hydathode-rich leaf margins may be associated with nutrient availability (16, 53). R. fascians also produces indole-3-acetic acid (IAA) (101), an auxin hormone that may trigger nutrient release from plant cells (53) and suppress their defense responses (25), a strategy commonly deployed by biotrophic pathogens (5). R. fascians is not motile and to facilitate spreading, epiphytic bacteria shift from initially rod-shaped cells to extended hyphae-like cells (16) (Figure 1g). The colonies resemble biofilms in that they are covered by a slime layer (Figure 1g) possibly improving attachment to the plant surface, preventing desiccation, and playing a role in epiphytic fitness (16). The epiphytic colonization capacity of nonvirulent linear plasmid-free strains is comparable to that of wild-type strains (16), implying that all these features are encoded by chromosomal genes.

Although symptom development is not initiated during the initial epiphytic phase (16), the plant responds to the bacterial presence by modulating its primary metabolism (25). This reaction occurs with pathogenic strains as well as with their nonpathogenic derivatives, but it is much stronger during colonization by the virulent bacteria (25). The bacterial signals that trigger this response have not yet been identified, but possibly they could be low levels of phytohormones produced from chromosomal

IAA: indole-3-acetic acid

Auxin: plant hormone that promotes and regulates growth and development by affecting cell division, differentiation, phototropism, geotropism, and apical dominance
**Autoregulation:** cell-to-cell communication that enables a population density–based control of gene transcription via production, release, and sensing of low-molecular weight compounds

**att:** attenuation

genes (73, 101) or microbe-associated molecular patterns (8). In *Arabidopsis thaliana*, plant metabolic processes are redirected upon *R. fascians* infection toward an enhanced synthesis of specific amino acids, such as proline, valine, phenylalanine, and tryptophan, and sugars, such as trehalose, glucose, and fructose (25), likely providing extra carbon and nitrogen sources for bacterial proliferation. Moreover, increased tryptophan levels may stimulate bacterial auxin biosynthesis and, thus, epiphytic fitness because IAA biosynthesis by *R. fascians* is tryptophan-dependent (101). Other modifications include a downregulation of arginine and ornithine levels and a rise in succinate and pyruvate concentrations (25). Together with the increased glucose, proline, valine, and phenylalanine levels, a physiological condition is created that is perceived by the bacteria as a signal for the initiation of the pathogenic lifestyle by favoring the activation of autoregulation processes for virulence (55).

**AUTOREGULATION TRIGGERS THE ONSET OF THE PATHOLOGY**

The success of pathogens often depends on the coordinated expression of their virulence functions, allowing them to act as a community rather than as single cells. This phenomenon, known as quorum sensing, can be accomplished by autoregulatory compounds, such as N-acyl homoserine lactones in gram-negative or oligopeptides and γ-butyrolactones in gram-positive bacteria. Typically, an autoregulatory compound accumulates in the surroundings of the growing bacterial population, and its concentration correlates positively with the cell density. As soon as the extracellular concentration of the autoinducer is high enough, that is, when a quorum is reached, concerted target gene expression occurs. Consequently, such autoregulatory compounds can be detected in spent medium of high-density bacterial cultures (9, 68). Although in *R. fascians* positive autoregulation of virulence functions also takes places, the process has not been described as genuine quorum sensing because the presence of the plant is required for an optimal production of the autoregulatory compound (55).

Autoregulation of virulence in *R. fascians* strain D188 is mediated by nine genes that constitute the *attenuation (att)* operon, located on the linear plasmid (55). The gene products are homologous to proteins implicated in the biosynthesis and secretion of arginine and β-lactam–like compounds (Figure 2). Although the structure of the *att*-autoregulatory molecule remains to be determined, several data point to a positively charged acylated cyclic amino acid derivative (54, 55, 100, 104).

Expression of the *att* genes is controlled by a LysR-type transcriptional regulator, AttR,

<table>
<thead>
<tr>
<th>AttR</th>
<th>LysR-type regulator</th>
</tr>
</thead>
<tbody>
<tr>
<td>AttX</td>
<td>Translocator</td>
</tr>
<tr>
<td>AttA</td>
<td>Argininosuccinate lyase</td>
</tr>
<tr>
<td>AttB</td>
<td>Argininosuccinate synthase</td>
</tr>
<tr>
<td>AttC</td>
<td>Formyltransferase</td>
</tr>
<tr>
<td>AttD</td>
<td>Enoyl-CoA hydratase/isomerase (CarB)</td>
</tr>
<tr>
<td>AttE</td>
<td>β-lactam synthetase (CarA)</td>
</tr>
<tr>
<td>AttF</td>
<td>Clavaminic acid synthase (CarC)</td>
</tr>
<tr>
<td>AttG</td>
<td>AMP-binding acetyl-CoA synthetase</td>
</tr>
<tr>
<td>AttH</td>
<td>Ornithine acetyltransferase</td>
</tr>
<tr>
<td>FasR</td>
<td>AraC-type regulator</td>
</tr>
<tr>
<td>Mtr1</td>
<td>SAM-dependent methyltransferase</td>
</tr>
<tr>
<td>Mtr2</td>
<td>SAM-dependent methyltransferase</td>
</tr>
<tr>
<td>FasA</td>
<td>P450 monooxygenase</td>
</tr>
<tr>
<td>FasB</td>
<td>Ferredoxin/pyruvate decarboxylase α subunit</td>
</tr>
<tr>
<td>FasC</td>
<td>Pyruvate decarboxylase β subunit</td>
</tr>
<tr>
<td>FasD</td>
<td>Isopentenyltransferase</td>
</tr>
<tr>
<td>FasE</td>
<td>Cytokinin oxidase/dehydrogenase</td>
</tr>
<tr>
<td>FasF</td>
<td>Phosphoribohydrolase</td>
</tr>
</tbody>
</table>

**Figure 2**

Organization and homologies of the *fas* and *att* loci on pFiD188, the linear plasmid of *Rhodococcus fascians* strain D188.
and is activated in response to specific combinations of carbon and nitrogen sources that reflect the physiological state of epiphytically colonized plant tissues (Figure 3). An initially low concentration of the *att* compound is sufficient to activate the expression of the *att*-biosynthetic genes, thus mediating a positive feedback mechanism that results in the production of high amounts of the *att* compound required to trigger the expression of essential virulence genes, including the *fasciation* (*fas*) genes for cytokinin production (55, 94). The *att* compound is also involved—directly or indirectly—in breaching the plant’s cuticula, thus facilitating endophytic colonization (55).

As a result, few epidermis cells collapse and large ingression sites are formed beneath the epiphytic colonies on leaves, stems, and axils (16, 90). During the endophytic colonization, the bacteria undergo a series of adaptations to the new environment (Figure 3). Their cell wall is modulated (16) or even completely lost (49), and their metabolism is shifted toward the use of C2 compounds (31, 107). Surprisingly, in the endophytic population, *att* gene expression is shut down without affecting *fas* gene expression (15). This observation implies that, upon invasion of the internal plant tissues, *fas* gene regulation is controlled by other (unknown) factors.

**Figure 3**
Overview of the different steps of the *Rhodococcus fascians*–plant interaction leading to symptom development and niche establishment. A detailed description of the events is given throughout the text. (a) Bacterial responses (epiphytic and endophytic bacteria are depicted in orange and green, respectively); (b) molecular crosstalk between bacteria (orange text) and plant (green text); (c) timeline and the phenotypical responses in *Arabidopsis*. 

*fas*: fasciation
Interestingly, the **att** compound appears to be highly diffusible: It can be detected throughout the tissues of infected plants and even seems to be exuded by the roots (100, 104). This feature could undoubtedly contribute to the aggressiveness of the *R. fascians* population as a whole. Indeed, the **att** operon determines the transition from a harmless epiphytic lifestyle to a pathogenic endophytic one by controlling the onset of penetration and virulence (15) (**Figure 3**). The significance of autoregulation in the *R. fascians* pathology has recently been documented in the incompatible interaction between a tropical legume, *Dalbergia pervillei*, and *R. fascians*. Although the recalcitrance of this plant to *R. fascians* infection was found to be multifactorial, an important cause for the lack of symptom formation was the production of perbergin, a newly identified prenylated isoflavone that acts as a competitive inhibitor of the natural autoinducer of AttR (81).

**RHODOCOCCUS FASCIANS PRODUCES A MIX OF CYTOKININS THAT IS PERCEIVED BY THE HOST AND ACTIVATES HOMEOSTASIS MECHANISMS**

The major virulence strategy of *R. fascians* is the production of shoot-inducing cytokinins (90). Since the discovery of isopentenyladenin (iP) in the supernatans of *R. fascians* (95), several other cytokinin derivatives have been isolated from different isolates grown under diverse culture conditions (37). However, despite major efforts by several generations of researchers (2, 28, 39, 47, 82, 84), a positive correlation between a specific cytokinin and virulence was not found (17, 28, 64). Undoubtedly, the very stringent regulation of **fas** gene expression contributes to this problem. Indeed, **fas** expression is subjected to the **att** compound (55) and other environmental conditions (94) and controlled by two transcriptional regulators, AttR (72) and the AraC-type regulator FasR (94), and an unidentified posttranscriptional mechanism (72, 94).

More clarity came when the biosynthetic pathway encoded by the six genes of the **fas** operon (**Figure 2**) was solved for strain D188, based on biochemical data and cytokinin profiles of several **fas** mutants (74). Instead of producing a single cytokinin, the Fas proteins proved to mediate the synthesis of six distinct cytokinin bases: iP, cis-zeatin (**cZ**), trans-zeatin (**tZ**), and their methylthio-derivatives (2MeSiP, 2MeScZ, 2MeStZ) (73, 74) (**Figure 3**). The isopentenyltransferase (IPT) FasD, the key enzyme of the pathway, synthesizes iP (and possibly **tZ**) that is a precursor for other Fas enzymes. FasA is a putative P450 monooxygenase hydroxylating iP to form **tZ** and **cZ**. FasB is a putative bifunctional enzyme with a ferredoxin-like domain and a pyruvate decarboxylase α subunit domain, whereas FasC is homologous to the β-subunit of pyruvate decarboxylase. It is hypothesized that FasB and FasC are accessory proteins of FasA and deliver the energy for the hydroxylation reaction by using pyruvate as an electron donor (18, 37). FasA can also hydroxylate chromosomally produced 2MeSiP, yielding 2MeScZ and probably 2MeStZ. The absolute requirement of FasA for virulence (18) illustrates the central position of the **Z**-type cytokinins in the modulation of plant development (73, 74). The enzymes involved in the direct methylthiolation of iP, tZ, and **cZ** to yield their 2MeS derivatives remain to be identified. FasF, a prerequisite for symptom maintenance, is a phosphoribohydrolase capable of releasing cytokinin bases directly from their nucleotide precursors in a complementary route for the production of **Z**-type cytokinins (74). FasE is a cytokinin oxidase/dehydrogenase (CKX) with a strong affinity for iP-type cytokinins and is probably required for the optimal functioning of the FasD enzyme (74). A similar reaction mechanism presumably occurs in *Streptomyces turgidiscabies*, a scab-causing phytopathogen that is the only other organism known until now to carry a **fas** operon (see sidebar, Phytopathogenic Streptomycetes) (42).

**IPT:** isopentenyltransferase  
**CKX:** cytokinin oxidase/dehydrogenase
Unexpectedly, the linear plasmid-free non-virulent derivative of strain D188 secretes the same array of cytokinins as the wild-type strain, albeit at much lower levels (73). Whereas tRNA degradation is believed to be the source of this basal level (1, 29, 57), the increased pathogenicity-related levels are the consequence of de novo biosynthesis by the Fas machinery (74). These findings suggest that \textit{R. fascians} virulence depends on exposure of the host to particular cytokinin concentrations and ratios rather than on the production of specialized molecules (73, 74) and clarify why no specific virulence-associated cytokinins have been identified.

At the onset of the interaction, bacterially produced cytokinins are perceived by the plant (73) and trigger substantial transcriptomic and metabolomic changes (25). In \textit{Arabidopsis}, homeostatic mechanisms are activated that are directed toward the reduction of the cytokinin levels in the infected tissues (Figure 3). For instance, plant cytokinin biosynthesis is switched off, as illustrated by the downregulation of \textit{IPT3}, \textit{IPT5}, and \textit{IPT7} expression and the decrease in cytokinin monophosphates. Moreover, expression of all \textit{CKX} genes is induced correlating with a net reduction of iP and N-glucosides at later time points of the interaction (23). Also, in infected pea cytokinin nucleotide levels are reduced (28, 34), and in tobacco plants \textit{CKX} gene expression is upregulated upon infection (35). The modulation of the cytokinin metabolism explains why no increased cytokinin levels have been detected in infected tissues (20, 23, 34). Importantly, however, because generally little biological relevance had been attributed to cZ and 2MeS-cytokinins, these compounds have been overlooked in the past.

With the identification of the \textit{fas}-produced cytokinins and the recent development of very sensitive analytical methods (92), the enigmatic lack of an increased cytokinin content in infected plant tissues has been reassessed. As such, depending on the plant species, specific \textit{fas} cytokinins have been found to accumulate in symptomatic plants (73).

**PHYTOPATHOGENIC STREPTOMYCETES**

Just like \textit{Rhodococcus fascians}, the genus \textit{Streptomyces} belongs to the high G+C gram-positive Actinomycetales. The majority of the Streptomyces are renowned producers of bioactive secondary metabolites and antibiotics (12, 56). However, several species are devastating phytopathogens that are neither host nor tissue specific (7). The most important global economical impact is caused by potato scab, a disease characterized by the formation of lesions resulting from the bacterial interference with the plant’s celllose biosynthesis (45). Although the main pathogenicity factor is the production of thaxtomin, a peptidic phytotoxin, \textit{Streptomyces turgidiscabies} also secretes cytokinins that are encoded by a \textit{fas} operon that is similar to that of \textit{R. fascians}. Wild-type \textit{S. turgidiscabies} strains do not induce shoot formation, but scab lesions with an erumpent phenotype. In contrast, a thaxtomin-deficient mutant induces leafy galls in tobacco (\textit{Nicotiana tabacum}) and \textit{Arabidopsis thaliana} that are indistinguishable from those induced by \textit{R. fascians} (42). The role of cytokinin in this pathosystem is postulated to be multifactorial, as an apoptosis inducer in the plant and as a trigger of secondary metabolites in the pathogen.

**THE TRICK-WITH-THE-CYTOKININ-MIX: THE SYNERGISTIC ACTION OF BACTERIALLY PRODUCED CYTOKININS OVERTAKES PLANT GENE EXPRESSION AND CAUSES ECTOPIC AND PERSISTENT PLANT CELL DIVISIONS**

Bacterial production of a mixture of cytokinins instead of a large amount of a single cytokinin provides multiple advantages. First of all, the generation of a cytokinin mixture ensures an enhanced in planta stability because of the restricted substrate specificity of \textit{CKX} enzymes in different plant tissues and species (36, 109). With the de novo production of 2MeS-cytokinins in addition to the classical iP and Z, the cytokinin-degrading capacity of the plant is challenged but defeated and hence, irrespective of the host, always at least some of the bacterial cytokinins will accumulate in the infected tissues (73) (Figure 3). Indeed, in \textit{Arabidopsis...
the activation of the apoplastic CKXs (CKX2, CKX4, and CKX6) effectively reduces the in planta levels of most of the bacterially produced cytokinins, but these enzymes are unable to efficiently degrade cZ and 2MeScZ. In tobacco, however, the CKX enzymes seem to be ineffective against iP (73).

Secondly, the R. fascians cytokinin mixtures modulate the sensitivity of plants for these morphogens. In Arabidopsis, throughout the interaction, all secreted cytokinins are perceived by ARABIDOPSIS HISTIDINE KINASE (AHK) 3 (73, 74), a broad-spectrum cytokinin receptor that plays an important role in shoot development (83). However, at the onset of the interaction, iP strongly accumulates and induces ectopic expression in the shoots of the root-specific, narrow-spectrum cytokinin receptor AHK4 (40, 73, 87). Consequently, even with a relatively low amount of secreted cytokinins, R. fascians manipulates the plant so that its cytokinin sensitivity is the highest at the moment when symptoms need to be initiated (73, 74) (Figure 3).

Thirdly, the individual compounds appear to act synergistically on plant development. Bioassays that assess the activity of the six cytokinins secreted by R. fascians show that compared with an equal final concentration of the individual molecules, an equimolar mix has a stronger effect on proliferation of tobacco callus and in Arabidopsis, on cytokinin receptor activation, bleaching, anthocyanin accumulation, de-etiolation, and shoot regeneration (73).

Moreover, the cytotoxicity of the 2MeS-cytokinins is considerably lower than that of the classical cytokinins in a tobacco callus bioassay (73). The continuous presence of the bacteria is essential for sustaining symptoms (105) and the constant secretion of cytokinins (15) possibly results in locally very high accumulation levels. Therefore, the production of less active and less toxic 2MeS-derivatives in addition to iP, tZ, and cZ may be required to avoid deleterious effects on plant development.

Finally, the ratio of locally secreted cytokinins in the plant tissues is most probably not the same throughout the interaction because the cytokinin spectrum is produced in a very controlled and dynamic fashion (74). At the onset of symptom formation, R. fascians launches a cytokinin initiation wave consisting mainly of iP, tZ, and cZ via the combined action of FasD and FasA. Later, the bacteria shift their metabolism toward the production of a cytokinin maintenance flow containing tZ, 2MeStZ, cZ, and 2MeScZ, through the action of FasF (74). At the same time, the activated CKX machinery of the plant contributes to the relative concentrations of the six bacterial cytokinins (73).

Altogether, the cytokinin mixture eventually triggers a signal transduction cascade in the host that leads to an elaborate modification of gene expression. The effect on the transcriptome has been assessed with different techniques, including differential display (70, 86, 102), cDNA-amplified fragment length polymorphism (27, 103), and microarray hybridization (25), applied to different hosts, such as Atropa belladonna (70), Arabidopsis (25), and tobacco plants (86) and plant cell cultures (102, 103).

Because R. fascians infection leads to the dedifferentiation and reactivation of cortical cells to generate new shoot meristems (20), the cell cycle genes have drawn a lot of attention (Figure 3). Infection of Arabidopsis (105) and tobacco (20) reporter lines for cell cycle markers illustrated the anticipated stimulation of cell division. The effect of R. fascians signals on cell cycle progression was studied in greater detail in S-phase synchronized cell cultures of tobacco cv. Bright Yellow 2 (BY-2). Depending on the treatment, an extended prophase, resulting in a widening of the mitotic index peak (93), or an acceleration of the progression of the plant cells through the successive phases of the cell cycle (103) were observed. Finally, the effect of R. fascians on different phases of the cell cycle was demonstrated in Arabidopsis. The induced transcription of the mitotic cyclin-dependent kinases (CDKS) CDKB1;1 and CDKA;1 and of the cyclins CYCB1;1 and CYCA2;1 promotes the G2-to-M phase transition, pushing progression through mitosis (22). Additionally, the bacterial
cytokinins are postulated to directly promote the G2-to-M transition by dephosphorylating CDKs (22). Moreover, transition through the G1-to-S phase is stimulated via the transcriptional activation of the three CYCD3 genes (22). The central position of the CYCD3 signal transduction pathway in symptomatology has been revealed by the strongly reduced response of the Arabidopsis cycd3;1–3 triple knockout mutant toward infection (22).

DE NOVO SHOOT MERISTEM FORMATION AND PREVENTION OF TISSUE MATURATION LEAD TO NICHE ESTABLISHMENT

One of the earliest visible symptoms of R. fascians infection in many hosts is the activation of dormant axillary meristems (19, 20, 24, 77, 79, 105). This overruling of apical dominance has been correlated with a local upregulation of abscisic acid and gibberellic acid breakdown upon infection (86). Other than their effect on the activity of existing meristems, the induced hormone imbalances, together with the secreted bacterial cytokinin mix, create physiological conditions that are optimal for the de novo formation of meristems (23, 86) (Figure 3). Indeed, the reentry of cortical cells into a stage of active cell division is followed by their differentiation into shoot meristems (20), but the newly formed shoots generated from these meristems do not develop normally. Analysis of the DNA ploidy level distribution of symptomatic leaves of Arabidopsis demonstrated that most cells have a 2C content irrespective of their age, suggesting that the tissues retain a meristematic identity (22). Uninfected leaves grow in two phases: initially driven by cell division and subsequently by cell expansion resulting from endoreduplication (26). However, cell cycle gene expression profiles of symptomatic leaves exhibited a biphasic pattern, indicating that endoreduplication does not occur but is replaced by continuous growth via cell proliferation (22). The undifferentiated state of the symptomatic plant organs was illustrated by the ectopic expression of class I KNOTTED-like homeobox (KNOX) genes, such as SHOOT MERISTEMLESS (STM) and BREVIPEDICELLUS/KNOTTED-LIKE1 (BP/KNAT1) (23) (Figure 3).

Analysis of infected Arabidopsis tissues by microarray hybridization and primary metabolite profiling pointed to the establishment of a niche. Because symptomatic tissues do not mature, they remain sink tissues for photosynthates that do not convert into source tissues. This feature is demonstrated by an increased hexose/sucrose ratio and an enhanced invertase gene transcription and enzyme activity in Arabidopsis (25). In accordance with the source-to-sink transition, the photosynthetic activity in the symptomatic leaves is repressed via end-product inhibition (25) (Figure 3). Also, in infected tobacco BY-2 cells, an acidic invertase gene is upregulated (103). Most likely, the accumulating hexoses serve as fuel for the energy-demanding process of symptom formation and for supporting bacterial growth (25). In addition to the modulation of the carbohydrate profile of symptomatic tissues, a significant increase in the amino acid content has been measured, which may provide a nitrogen source for the colonizing bacteria (25). Altogether, it is clear that R. fascians infection strongly affects the physiology of the plant to its advantage, an aspect that has been termed metabolic habitat modification (107) (Figure 3).

CYTOKININ-ACTIVATED SIGNALING IS AMPLIFIED BY PLANT-DERIVED PUTRESCINE

Although the dominant role of cytokinins in symptom development is obvious and the advantageous production of a mixture of cytokinins may be the reason for the significant impact of bacterial infection on plant development, metabolite profiling revealed the accumulation of putrescine and trehalose, two potential secondary signaling molecules (25). This observation implies the intriguing alternative (although not mutually exclusive) possibility that plant messengers may reinforce the pathology by amplification of the
cytokinin signaling. Recent research in *Arabidopsis* cytokinins, the transcription of ARGinine DEcarboxylase (*ADC*) genes is induced. Because ADC enzymes mediate the rate-limiting step of polyamine biosynthesis, this overexpression results in the accumulation of the diamine putrescine (89). Interestingly, the plant growth stimulation obtained by exogenous putrescine seems to be accomplished by the direct stimulation of the cell cycle through the activation of *CYCD3;1* expression (89). Because the *CYCD3* genes are also the primary targets of the bacterial cytokinins (22), *R. fascians*-induced putrescine production feeds into the same signal transduction pathway (Figure 3). The importance of the additional putrescine signaling is evidenced by the strongly reduced response toward *R. fascians* infection of plants treated with inhibitors of polyamine biosynthesis. Moreover, exogenously applied putrescine significantly enhances *R. fascians*-induced symptom development (89). Consequently, the strong effect of *R. fascians* infection on plant development would not only be achieved through the production of a mixture of synergistically acting cytokinins, but also through deviation of the plant metabolism to reinforce the proliferative and organogenic effect of its morphogens.

**PATHOGEN AND HOST CONTRIBUTE TO NICHE MAINTENANCE**

The role of plant-derived putrescine in the establishment of the niche illustrates that both pathogen and host play an active role in symptom development and maintenance. An intriguing observation is that whereas decaying leaves are quickly macerated, *R. fascians* is unable to overgrow healthy symptomatic plant tissue (16). Therefore, the plant seems to control the bacterial multiplication. Indeed, after the primary epiphytic colonization, the bacteria penetrate through the cuticula, proliferate in the epidermal cells, and spread from there, mainly intercellularly, in a few cortical cell layers. The colonization of the internal tissues is accompanied by an increased tissue autofluorescence, indicative of a mild defense reaction (16), which is confirmed by microarray hybridization (25). However, no extensive tissue necrosis occurs (16), and there is no hypersensitive reaction to prevent bacterial spread (66). Although an initial defense reaction is mounted, it is very quickly alleviated and eventually suppressed (25). Interestingly, ectopic expression of *BP/KNAT1* is known to decrease cell wall lignification (58) and consequently, the cytokinin-induced ectopic KNOX gene expression upon *R. fascians* infection has been postulated to contribute to the formation of ingress sites (23). Whereas this is an appealing hypothesis, the timing of this expression suggests a role rather late in the interaction, but formation of secondary ingressions sites at later time points cannot be ruled out. Altogether, these observations suggest that pathogen and host balance each other’s responses from the beginning of their encounter. Moreover, in the only reported example of an incompatible interaction, *Dalbergia* tissues contain perbergin, a prenylated isoflavanone that specifically inhibits AttR functioning and, therefore, onset of virulence gene expression and symptom development (81). Thus, plants apparently manage *R. fascians* activity despite its very broad host range (Figure 3).

Because symptomatic tissues never mature, they remain sinks and thus represent a nutrient-rich niche for *R. fascians* (22, 25). Whereas the epiphytic subpopulation profits from the accumulating hexoses and amino acids, the endophytic subpopulation responds to this metabolic habitat modification by converting its metabolism toward the use of C2 compounds as specific carbon and nitrogen sources (31, 107). Indeed, for endophytic survival, *R. fascians* requires a functional malate synthase encoded by the chromosomal *vicA* gene (Figure 3). Together with isocitrate lyase, the VicA protein forms the glyoxylate shunt of the Krebs cycle (107). Moreover, downregulation of two Krebs cycle genes, *pyruvate dehydrogenase* and *fumarate hydratase*, in *R. fascians* grown on leafy gall extract supports the metabolic shift toward
the glyoxylate shunt (31). Interestingly, in the related animal pathogen *Mycobacterium tuberculosis*, an operational glyoxylate pathway is also needed for persistence because it allows the pathogen to feed on the abundant fatty acids of the host (41). As it is unlikely that *R. fascians* uses the rather scarce fatty acids from plants, it has been proposed that the intercellular population would utilize photorespiration intermediates, such as glycine, glycolate, and glyoxylate (106). In support of this hypothesis, a glycine dehydrogenase gene residing in the same chromosomal locus as *vicA* is also necessary for full virulence (14, 107) and metabolic and transcriptional analysis in *Arabidopsis* and tobacco suggest the acceleration of photorespiration in symptomatic plants (25, 103). Importantly, symptoms are not maintained when the endophytic population deteriorates (105), implying that bacterial cytokinin production by this subpopulation is essential for symptom persistence.

At the late stages of infection, bacterial proliferation remains tightly controlled by the plant even though transcript profiling has shown that defense-related genes, typically upregulated during biotic interactions (21, 43), are downregulated upon symptom establishment in *R. fascians*-infected *Arabidopsis* plants (25). Moreover, no signs of plant defense reactions have been observed by microscopic analysis of infected tobacco and *Arabidopsis* plants (19, 20), and no relevant differences in secondary metabolite production have been detected upon infection of *Pratia nummularia* (51). In contrast, a few reports point to some level of defense activation in infected plants. For instance, the phenolic profile of tobacco leafy galls differs from that of control plants and although none of the identified compounds seemed to be toxic to *R. fascians* grown in culture media (108), it cannot be ruled out that they affect the epiphytic and/or endophytic population. In *Atropa belladonna* leafy galls, the expression reduction of three genes possibly involved in plant defense has been correlated with bacterial colonization (70). Finally, in tobacco the upregulation of an annexin during leafy gall formation has been linked to stress responses (and/or plant development) (102). These seemingly conflicting observations may indicate that a moderate defense reaction occurs throughout the *R. fascians* pathology and that its main function is to keep the bacterial population size within limits (Figure 3). However, no full defense response seems to be set up that would eliminate the bacteria. Auxin, from bacterial or plant origin, is a plausible candidate to be implicated in this process. Indeed, increased auxin signaling has long been recognized as a downregulator of plant defense (5).

**CONCLUDING REMARKS AND PERSPECTIVES**

In the past decade, a lot has been unraveled about the mechanisms that lead to the formation of a leafy gall. Nevertheless, several important questions remain to be answered. The shooty phenotype of the induced malformations is clearly caused by the bacterial cytokinin mixture, but the regulation of *fas* gene expression remains largely unsolved, as does the question as to which genes are involved in the de novo biosynthesis of the 2MeS-cytokins. Upstream of the *fas* operon, two genes are present that are homologous to methyltransferases (MTRs) (Figure 2) and these genes are conserved in the *fas* operon of *S. turgidiscabies* (42). Interestingly, *mtr* mutants of *R. fascians* are nonpathogenic, implying a role in cytokinin biosynthesis (72). The possibility that *R. fascians* produces highly modified adenine derivatives has been opted for in the past (34, 37), so one can speculate that something has been overlooked or missed. Cytokinin profiling of infected tissues of different hosts and of *S. turgidiscabies* may shed more light on these issues.

One can also wonder if leafy gall formation is really only about cytokinins. *R. fascians* produces a considerable amount of IAA via chromosomally located genes under control of the linear plasmid (101). A dual function for auxin has been postulated in epiphytic fitness and as a virulence factor (101), but the auxin biosynthesis genes await identification. To clarify the
role of bacterial auxin production in the interaction, a bacterial IAA mutant needs to be generated and its virulence and colonization capacity investigated.

The very broad host range of \textit{R. fascians} implies that it can efficiently suppress the defense systems of an extensive variety of plants. The question as to which strategy the pathogen employs to avoid plant defense remains open. Possibly, \textit{R. fascians} modulates the plant’s auxin metabolism to achieve this goal, as reported for other plant microbial pathosystems (44).

Finally, plants that do not respond to \textit{R. fascians} merit more attention because they are potential sources of antivirulence or antidesease compounds. As currently no eradication methods exist for \textit{R. fascians}, molecules such as perbergin isolated from \textit{Dalbergia} species (81) could be developed as control agents to manage plant damage in infested ornamentals nurseries.

In conclusion, in Figure 3 we summarize the \textit{R. fascians} story and provide an overview of the molecular, physiological, and morphological changes in pathogen and host at the different stages of leafy gall formation. Although the major steps of symptom development have probably been outlined, with the genome sequence of strain D188 under way, the study of the more subtle aspects of this intriguing pathosystem are within reach.

**SUMMARY POINTS**

1. Although phytohormones and plasmids seem to be used by many, \textit{R. fascians} is unique among the phytopathogens because it induces the neoformation of differentiated tissues, collectively called the leafy gall syndrome, through the activity of linear plasmid-encoded virulence factors.

2. The interaction starts with the epiphytic nonpathological colonization of the host that triggers the modification of the plant’s primary metabolism, which, in turn, signals to the bacteria when conditions are appropriate to initiate the expression of the virulence genes.

3. The transition from an epiphytic life style to an endophytic pathogenic one is controlled by autoregulation via the \textit{att} operon. Interference with optimal functioning of the Att mechanism strongly impedes virulence.

4. Pathogenicity strictly relies on the \textit{fas} operon that codes for the production of a mixture of six synergistic cytokinin bases: isopentenyladenine, \textit{cis}-zeatin, \textit{trans}-zeatin, and their methylthio-derivatives. The trick-with-the-cytokinin-mix has several advantages, such as a high in planta stability, the broad recognition by the cytokinin receptors, and the overruling of the host’s cytokinin-degrading mechanisms, assuring the activation of a signal transduction pathway leading to shoot formation.

5. The bacterially produced cytokinins, reinforced by secondary signals from the host, target key cell cycle genes, such as CDKs and cyclins, reactivating the cell division machinery in differentiated cortical cells. The newly dividing cells develop into shoot meristems that produce shoots in which the continuous expression of the CYCD3 signal transduction pathway prevents tissue maturation and the concomitant sink-to-source transition.

6. The establishment of the leafy gall is mediated by significant alterations of the transcriptome and the metabolome of the host. These changes modify the metabolic habitat in which carbon and nitrogen sources accumulate providing a nutrient-rich niche for the inhabiting \textit{R. fascians} population.
7. Whereas the epiphytic bacterial subpopulation triggers the onset of symptom development, the maintenance of the symptoms depends on the endophytic population. During niche formation, the bacteria that colonize the plant’s apoplast adapt to the changing environment by modifying their cell wall, transcriptome, and metabolism.

FUTURE ISSUES

1. Analysis of the \( \text{fas} \) operon and the produced cytokinins of \( S. \text{turgidiscabies} \) should confirm and complete the knowledge obtained with \( R. \text{fascians} \) on the composition and generation of the cytokinin mixture.

2. \( R. \text{fascians} \) has a very important impact on the development of an extremely broad range of plants, whereas the plant is seemingly unable to protect or inefficiently defend itself against the activity of the virulence factors. Which are the bacterial signals that mediate the apparent defense suppression? Investigation of the role of the bacterial auxin or of the putative modulation of the plant auxin metabolism would shed more light on this important issue.

3. Is the plant really a defenseless victim surrendered to the will of \( R. \text{fascians} \)? The restriction of bacterial proliferation throughout the interaction may suggest that the plant has some level of control over the bacteria. By assessing the responsiveness of plants with defective defense pathways, a better insight would be obtained into the relevance of the transient induction of defense genes.

4. The expansion in global movement of ornamentals and the lack of eradication methods increase the threat posed by \( R. \text{fascians} \) to horticultural practices. Therefore, from a practical point of view, it would be interesting to explore the mechanisms of recalcitrance toward \( R. \text{fascians} \) infection.

5. Determination of the genomic sequence of \( R. \text{fascians} \) will allow a thorough investigation of all uncovered secrets of this pathogen.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

ACKNOWLEDGMENTS

We thank Tita Ritsema for the scanning electron microscope pictures, Melodie Putnam for the images of symptomatic ornamental plants, Karel Spruyt for the photography of \( \text{Arabidopsis} \), and Martine De Cock for help in preparing the manuscript. E.S. is a research fellow of the Research Foundation-Flanders. O.M.V. is a postdoctoral researcher of the Fonds de la Recherche Scientifique (F.R.S.-F.N.R.S., Belgium).
LITERATURE CITED


www.annualreviews.org • Origin of the Leafy Gall Syndrome 85


97. Unequivocal confirmation of the central role of bacterial cytokinins during the Rhodococcus fascians–plant interaction. Cytokinin profiling of bacterial supernatants led to the identification of six secreted cytokinins.

98. First report on a mechanism of plant resistance toward Rhodococcus fascians infection. Dalbergia pervillei produces perbergin, an isoflavanone that quenches virulence of R. fascians by interfering with autoregulation.

99. Report on the activation by bacterial signals of putrescine-mediated secondary signaling in the host that eventually assists in the symptom formation.
## Contents

- Not As They Seem
  *George Bruening* ................................................................. 1

- Norman Borlaug: The Man I Worked With and Knew
  *Sanjaya Rajaram* ................................................................. 17

- Chris Lamb: A Visionary Leader in Plant Science
  *Richard A. Dixon* .................................................................. 31

- A Coevolutionary Framework for Managing Disease-Suppressive Soils
  *Linda L. Kinkel, Matthew G. Bakker, and Daniel C. Schlatter* .............. 47

- A Successful Bacterial Coup d’État: How *Rhodococcus fascians* Redirects Plant Development
  *Elisabeth Stes, Olivier M. Vandeputte, Mondher El Jaziri, Marcelle Holsters, and Danny Vereecke* .................................................. 69

- Application of High-Throughput DNA Sequencing in Phytopathology
  *David J. Studholme, Rachel H. Glover, and Neil Boonham* ..................... 87

- *Aspergillus flavus*
  *Saori Amaike and Nancy P. Keller* ................................................ 107

- Cuticle Surface Coat of Plant-Parasitic Nematodes
  *Keith G. Davies and Rosane H.C. Curtis* ........................................ 135

- Detection of Diseased Plants by Analysis of Volatile Organic Compound Emission

- Diverse Targets of Phytoplasma Effectors: From Plant Development to Defense Against Insects
  *Akiko Sugio, Allyson M. MacLean, Heather N. Kingdom, Victoria M. Grieve, R. Manimekalai, and Saskia A. Hogenhout* ............................. 175

- Diversity of *Puccinia striiformis* on Cereals and Grasses
  *Mogens S. Hovmøller, Chris K. Sørensen, Stephanie Walter, and Annemarie F. Justesen* ............................................................ 197
Emerging Virus Diseases Transmitted by Whiteflies


Evolution and Population Genetics of Exotic and Re-Emerging Pathogens: Novel Tools and Approaches

Niklaus J. Grünwald and Erica M. Goss ................................................................. 249

Evolution of Plant Pathogenesis in Pseudomonas syringae:
A Genomics Perspective

Heath E. O’Brien, Shalabh Thakur, and David S. Guttman ........................................ 269

Hidden Fungi, Emergent Properties: Endophytes and Microbiomes

Andrea Porras-Alfaro and Paul Bayman ........................................................................ 291

Hormone Crosstalk in Plant Disease and Defense: More Than Just JASMONATE-SALICYLATE Antagonism

Alexandre Robert-Seilaniantz, Murray Grant, and Jonathan D.G. Jones ...................... 317

Plant-Parasite Coevolution: Bridging the Gap between Genetics and Ecology

James K.M. Brown and Aurélien Tellier ...................................................................... 345

Reactive Oxygen Species in Phytopathogenic Fungi: Signaling, Development, and Disease

Jens Heller and Paul Tudzynski .................................................................................. 369

Revision of the Nomenclature of the Differential Host-Pathogen Interactions of Venturia inaequalis and Malus


RNA-RNA Recombination in Plant Virus Replication and Evolution

Joanna Sztuba-Solińska, Anna Urbanowicz, Marek Figlerowicz, and Jozef J. Bujarski ................................................................. 415

The Clavibacter michiganensis Subspecies: Molecular Investigation of Gram-Positive Bacterial Plant Pathogens

Rudolf Eichenlaub and Karl-Heinz Gartemann ............................................................ 445

The Emergence of Ug99 Races of the Stem Rust Fungus is a Threat to World Wheat Production

Ravi P. Singh, David P. Hodson, Julio Huerta-Espinó, Yue Jin, Sridhar Bhavani, Peter Njau, Sybil Herrera-Foessel, Pawan K. Singh, Sukhwinder Singh, and Velu Govindan ................................................................. 465

The Pathogen-Actin Connection: A Platform for Defense Signaling in Plants

Brad Day, Jessica L. Henty, Katie J. Porter, and Christopher J. Staiger ......................... 483
Understanding and Exploiting Late Blight Resistance in the Age of Effectors

Water Relations in the Interaction of Foliar Bacterial Pathogens with Plants
Gwyn A. Beattie 533

What Can Plant Autophagy Do for an Innate Immune Response?
Andrew P. Hayward and S.P. Dinesh-Kumar 557

Errata
An online log of corrections to Annual Review of Phytopathology articles may be found at http://phyto.annualreviews.org/