

PATHOGEN SELF-DEFENSE: Mechanisms to Counteract Microbial Antagonism

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■ **Abstract** Natural and agricultural ecosystems harbor a wide variety of microorganisms that play an integral role in plant health, crop productivity, and preservation of multiple ecosystem functions. Interactions within and among microbial communities are numerous and range from synergistic and mutualistic to antagonistic and parasitic. Antagonistic and parasitic interactions have been exploited in the area of biological control of plant pathogenic microorganisms. To date, biocontrol is typically viewed from the perspective of how antagonists affect pathogens. This review examines the other face of this interaction: how plant pathogens respond to antagonists and how this can affect the efficacy of biocontrol. Just as microbial antagonists utilize a diverse arsenal of mechanisms to dominate interactions with pathogens, pathogens have surprisingly diverse responses to counteract antagonism. These responses include detoxification, repression of biosynthetic genes involved in biocontrol, active efflux of antibiotics, and antibiotic resistance. Understanding pathogen self-defense mechanisms for coping with antagonist assault provides a novel approach to improving the durability of biologically based disease control strategies and has implications for the deployment of transgenes (microorganisms or plants).

INTRODUCTION

The ability of naturally occurring microorganisms to inhibit the growth or metabolic activity of deleterious microorganisms has been studied intensively during the past century and continues to inspire research in many fields, including drug discovery and crop protection. Interest in biological control of plant pathogens by introducing antagonistic microorganisms onto plant surfaces, into growing media, or onto propagative material has further increased in the past decade. This increased interest is, in part, due to the desire to enhance the sustainability of agriculture and

horticulture and also because biocontrol may provide control of plant diseases that can not, or only partially, be managed by other strategies (27).

Most studies on the biocontrol of plant pathogens focus on a multitude of factors related to the behavior of the microbial antagonist (the antagonist perspective), i.e., how antagonists affect pathogens; which mechanisms, metabolites, and genes are involved; and how antagonists behave and survive after introduction into a specific environment. Consequently, substantial progress has been made in the identification of biosynthetic and regulatory genes involved in suppression of plant pathogens by antagonistic microorganisms (67), and in identifying microbial traits that contribute to disease suppression and the ecological competence of introduced biocontrol agents (99, 190). In contrast, responses of plant pathogens to biocontrol agents (the pathogen perspective) have received little attention. Studies on the efficacy of biocontrol agents often consider only one single strain of the target pathogen and, with respect to sensitivity to a particular antagonistic trait, only one specific stage in the life cycle of the pathogen. Most pathogen populations, however, are not evolutionary static entities, but instead harbor substantial genetic variation and comprise numerous structures that allow pathogens to respond rapidly to environmental changes. Thus, analogous to the relatively rapid changes observed within pathogen populations to chemical control agents, one may postulate that strong selection pressure exerted by biocontrol agents also may result in responses and changes within pathogen populations toward resistance. However, resistance development in pathogen populations to biocontrol is presumed not to develop, or at least relatively slowly. In this context, Handelsman & Stabb (62) suggested that most biocontrol agents suppress disease via more than one mechanism, and that resistance to multiple antagonistic traits should occur only at a very low frequency. Second, antagonistic microorganisms are thought to exert only limited selection pressure since they operate in microsites on the plant surface where only a fraction of the pathogen population is exposed during a short period of its life cycle. In the case of antibiosis, only minute amounts of the compound(s) are produced by the biocontrol agent as opposed to the inundative application of chemical pesticides (150). These conditions, among others discussed below, are expected to exert a relatively low selection pressure for the buildup of resistance in pathogen populations to biocontrol. To date, however, the limited amount of experimental data on resistance of pathogens to biological control makes it difficult to draw sound conclusions on the stability or, conversely, erosion of biological control systems.

This review highlights current insights and concepts in pathogen defense mechanisms to counteract microbial antagonism. Just as microbial antagonists utilize a diverse arsenal of mechanisms to dominate interactions with pathogens, pathogens have surprisingly diverse responses to cope with antagonism from coexisting microorganisms, including antagonists and, in some cases, other pathogens vying for the same substrate/host. Many of our examples are presented from a different perspective and, it is hoped, will be sufficiently provocative to stimulate new approaches to understanding and improving biocontrol. We focus primarily on

defense mechanisms of plant pathogenic fungi and bacteria. For a conceptual review of the responses and defense mechanisms of insect pests to biocontrol, we refer readers to Holt & Hochberg (69). Pathogen defense mechanisms are presented for the major biocontrol mechanisms, which include antibiosis, competition, parasitism, and hypovirulence.

LESSONS FROM PATHOGEN DEFENSE AGAINST TOXINS AND PESTICIDES

Prior to reviewing defense mechanisms of plant pathogenic fungi and bacteria against biocontrol, we first give a brief summary of defense mechanisms of pathogens described in the areas of chemical control and plant-pathogen interactions. Both areas of research have clearly shown that plant pathogens possess ample mechanisms to deal with a series of toxic compounds from various sources. [For more comprehensive reviews on these topics we refer readers to (34, 39, 124, 158, 159, 176)]. First, many plant pathogens themselves produce toxins with broad-spectrum activity, which enable them to defend their habitat and to infect plants. Second, pathogens are confronted with a range of plant defense compounds, phytoalexins, and phytoanticipins, which play a role in resisting penetration and subsequent infection. Third, many xenobiotic compounds have been introduced into the environments where pathogens reside, with deliberate application of agrochemicals for crop protection or pollution with other xenobiotics.

Various mechanisms have been described that enable plant pathogens to resist such toxic compounds. Toxins can be inactivated by enzymatic degradation and a wide variety of responsible enzymes with either narrow or broad substrate specificities have been described, like acetyltransferases, hydrolases, hydratases, demethylases, and cytochrome P450-dependent monooxygenases (78, 114, 117, 176). The latter are particularly renowned because, in addition to their sometimes wide substrate specificity, these heme proteins can perform an array of reaction types, ranging from oxidative reactions, like monooxygenations and dehydrogenations, to nonoxidative reactions, like reductions and isomerizations (104). The pathogen can also acquire resistance by modification of the toxin target sites. This was reported for phytoanticipin resistance (114, 176) and, in particular, fungicide resistance (158). Finally, toxins can be actively exported out of cells through membrane-bound pumps in order to reduce the toxin concentration at the target. Overall, membrane transporters are present from archaebacteria to man and enable both influx and efflux of many different compounds (Figure 1). In plant pathogens, efflux pumps can play an important role in the export of endogenous toxins, resistance against fungicides and insensitivity to plant defense compounds (3, 51, 65, 118, 119, 135, 147, 169, 170, 178). Resistance can be based on a combination of these mechanisms (176). Disruption of one mechanism will then only partially abolish resistance or, in some cases, be fully compensated for

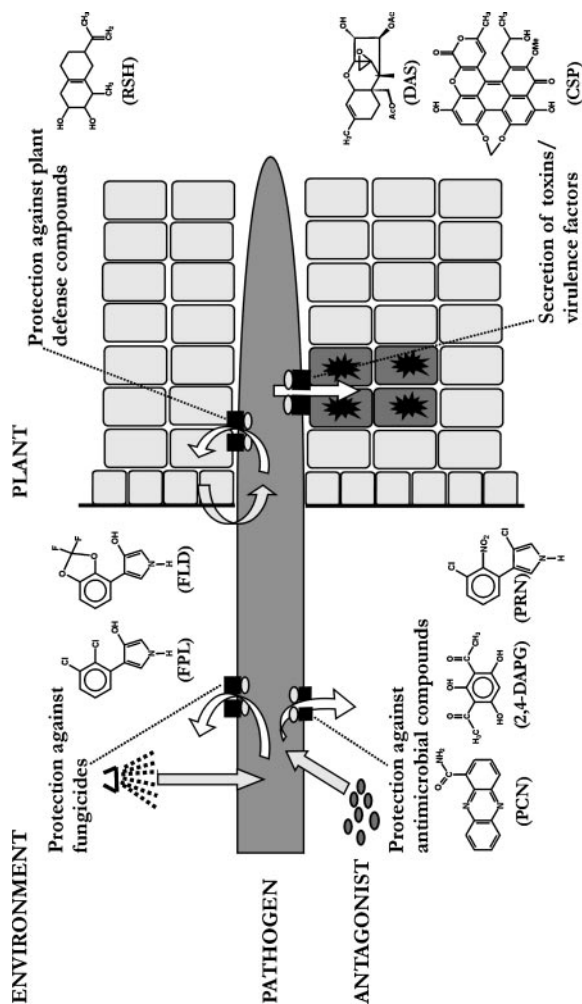


Figure 1 Schematic representation of the multifunctionality of ABC transporters and other efflux pumps in plant pathogenic fungi. Exogenous toxic compounds that enter the pathogen can be effectively exported out of the cell in order to reduce the toxin concentration at the target site. Common sources of toxins are agrochemicals, plant hosts, and antagonistic microbial competitors. For example, efflux pumps protect certain pathogens against the fungicides fenpiclonil (FPL) and fludioxonil (FLD) (118, 119, 147, 178), the plant defense compound rishtin (RSH) (51), and the antibiotic phenazine-1-carboxamide (PCN) produced by *Pseudomonas* biocontrol strains (148). Efflux pumps may also offer pathogens protection against other antimicrobial compounds produced by biocontrol agents, including 2,4-diacetylphloroglucinol (2,4-DAPG) and pyrrolnitrin (PRN). The fungicides FPL and FLD were derived from the *Pseudomonas* antibiotic PRN, but appear to be handled differently than PRN by efflux pumps (148). Efflux pumps secrete virulence factors and endotoxins produced by pathogens, such as cercosporin (CSP) and the trichothecene 4,15-diacetoxyscripenol (DAS) that damage plant cells and also have broad-spectrum antimicrobial activity (3, 36, 135, 169, 170). The figure is modified from Stergiopoulos et al. (159) and is used with permission of M.A. de Waard.

by other defense mechanisms. Resistance development against one toxin can also lead to resistance to other, chemically unrelated toxins. This phenomenon of multidrug resistance (MDR) is frequently observed in plant pathogens and may be mediated by both degradative and nondegradative resistance mechanisms (39, 149).

PATHOGEN DEFENSE AGAINST ANTIBIOSIS

Antibiotics encompass a heterogeneous group of organic, low-molecular-weight compounds that are deleterious to the growth or metabolic activities of other microorganisms. Numerous antibiotics have been isolated from fungal and bacterial biocontrol strains (62, 137). Biocontrol agents not only exhibit diversity in the type but also in the number of antibiotics produced by an individual strain. This indicates that for at least some biocontrol agents, several antibiotics may account for the suppression of specific or multiple plant diseases. Furthermore, many of the antibiotics produced by biocontrol agents have a broad-spectrum activity. For example, pyrrolnitrin, produced by strains of *Pseudomonas*, *Burkholderia*, *Enterobacter*, and *Serratia* species, has shown activity against several economically important pathogens, including *Rhizoctonia solani*, *Botrytis cinerea*, *Verticillium dahliae*, and *Sclerotinia sclerotiorum* (89). For many of the antibiotics produced by biocontrol agents, genes involved in the biosynthesis and regulation have been cloned and sequenced. This knowledge has provided tools to further enhance the efficacy of biocontrol agents, either by increasing production levels or by combining traits.

In the following sections, we discuss several examples of biocontrol systems in which defense mechanisms of pathogens against antibiotics (including volatiles) have been described. In those cases where actual data are lacking, we evaluate the potential mechanisms that exist within pathogen populations to resist antibiotic compounds produced by biocontrol agents.

Natural Diversity in Sensitivity of Pathogens to Antibiotics

There are numerous studies on antibiotics produced by antagonistic microorganisms and their role in biocontrol of plant pathogenic fungi and bacteria (137, 190). For many of these antibiotics, the activity and chemical structure have been determined, but, surprisingly, for relatively few is the mode of action known. Several studies have addressed the variation in sensitivity of pathogenic fungi and bacteria to antibiotics produced by antagonists (Table 1). Gurusiddaiah et al. (61) determined the activity of phenazine-1-carboxylic acid (PCA), an antibiotic produced by antagonistic *Pseudomonas* species, against a range of fungi and oomycetes. *Cochliobolus sativus*, *Gaeumannomyces graminis* var. *tritici*, *Pythium aristosporum*, *Pythium heterothallicum*, *Pythium volutum*, and *R. solani* were relatively sensitive to PCA, whereas other *Pythium* species, including *P. ultimum* var. *sporangiferum*, were relatively insensitive. Variation in sensitivity among

TABLE 1 Examples of the variation in sensitivity of plant pathogens to antibiosis and mechanisms involved in defense against antibiotics and volatiles produced by antagonistic microorganisms

Pathogen	Compound	(Putative) resistance mechanism	Reference
Bacterial pathogens			
<i>Agrobacterium tumefaciens</i>	Agrocin 84	Loss of Ti plasmid Horizontal resistance gene transfer	(28, 160)
<i>Erwinia amylovora</i>	Unknown antibiotic of <i>P. agglomerans</i>	Active efflux	A. Burse & M. Ullrich, unpublished
Fungal pathogens			
<i>Botrytis cinerea</i>	PCN	Active efflux	(148)
<i>B. cinerea</i>	2,4-DAPG	ND	Schouten et al., unpublished
<i>Fusarium lateritium</i>	HCN	Cyanide hydratase	(125)
<i>Fusarium oxysporum</i>	2,4-DAPG	Degradation	Schouten et al., unpublished
<i>F. oxysporum</i>	Fatty acid antibiotics of <i>S. flocculosa</i>	ND	(13, 16)
<i>Gaeumannomyces graminis</i> var. <i>tritici</i>	PCA	ND	(107)
<i>G. graminis</i>	HCN	ND	(187)
<i>Gloeocercospora sorghi</i>	HCN	Cyanide hydratase	(125)
<i>Microcyclus ulei</i>	HCN	Cyanide-resistant respiration	(125)
<i>Mycosphaerella graminicola</i>	POH	Degradation/ detoxification of active oxygen species	(86)
<i>Phanaerochaete magnoliae</i>	HCN	Upregulation of antibiotic synthesis (increased competitiveness)	(102)
<i>Phytophthora cryptogea</i>	HCN	ND	(187)
<i>Pythium</i> and <i>Rhizoctonia</i> species	Gliotoxin	Reduced uptake (possible)	(73)
<i>Pythium</i> species	Kanosamine	ND	(110)
<i>Pythium deliense</i>	2,4-DAPG	ND	(155)
<i>Pythium ultimum</i> var. <i>sporangiferum</i>	PCA	ND	(61)
<i>Stemphylium loti</i>	HCN	Cyanide hydratase	(125)

PCA, phenazine-1-carboxylic acid; 2,4-DAPG, 2,4-diacetylphloroglucinol; POH, 1-hydroxyphenazine; HCN, hydrogen cyanide; ND, not determined.

Pythium species was also recently studied (155) for the phenolic antibiotic 2,4-diacetylphloroglucinol (2,4-DAPG), with *P. volutum* as the most sensitive and *Pythium deliense* as the most insensitive. Whether the level of sensitivity within fungal and oomycete species is representative for all individual isolates of the species remains to be determined.

Mazzola et al. (107) screened 66 individual isolates of the take-all fungus *G. g. var. tritici*, collected from populations of different geographic origin, for sensitivity to both 2,4-DAPG and PCA. Radial growth assays on plates showed considerable variation in sensitivity among isolates for both antibiotics. Even among multiple isolates from a single field, substantial variation in sensitivity to these antibiotics was observed. In general, the fungal isolates included in their study were more sensitive to PCA than to 2,4-DAPG. The biological relevance of the observed variation in sensitivity was elegantly demonstrated by the fact that 2,4-DAPG- or phenazine-producing *Pseudomonas* strains failed to control take-all caused by the 2,4-DAPG- or PCA-insensitive *G. g. var. tritici* isolates, respectively. Notably, the 2,4-DAPG-producing *Pseudomonas* strain was also incapable of suppressing take-all caused by PCA-resistant isolate 1818, suggesting that cross resistance to both compounds may play a role.

Recent studies showed that substantial variation in sensitivity to 2,4-DAPG exists among pathogenic and nonpathogenic *Fusarium oxysporum* (A. Schouten, G. van den Berg, V. Edel, N. Gautheron, P. Lemanceau & J.M. Raaijmakers, unpublished data). In a collection of 70 pathogenic *F. oxysporum*, representing 13 formae speciales, and 27 nonpathogenic *F. oxysporum* isolates, 18% and 25% of the isolates, respectively, were insensitive to 2,4-DAPG. Preliminary analysis indicates that there is no clear relationship between 2,4-DAPG insensitivity and geographical origin or formae speciales of *F. oxysporum*, suggesting that the traits responsible for 2,4-DAPG-insensitivity are relatively ancient, have developed independently, or are easily transferred within and between populations. Similar levels of insensitivity to 2,4-DAPG were found among natural populations of *B. cinerea* (A. Schouten, G. van den Berg, Y. Cuesta Arenas & J.M. Raaijmakers, unpublished data). In contrast, among 20 isolates of *V. dahliae*, obtained from different host plants and geographic origins, no differences in sensitivity to 2,4-DAPG were found (A.J. Termorshuizen, L. Soesanto & J.M. Raaijmakers, unpublished data).

Bélangier & Deacon (13) and Benyagoub et al. (16) found that growth of *F. oxysporum* f.sp. *radicis-lycopersici* was not inhibited by the biocontrol agent *Sporotrichum flocculosum*. Pathogen insensitivity was attributed to tolerance to purified toxins produced by this yeast-like fungus. *S. flocculosum* was effective against both *B. cinerea* and *Cladosporium cucumerinum*, suggesting that sensitivity to antibiotics can dictate the host range of an antagonist.

Variation in sensitivity to the antibiotic gliotoxin produced by antagonistic strains of *Trichoderma* and *Gliocladium* was observed for pathogenic *Rhizoctonia* and *Pythium* species (73), and for different anastomosis groups of *R. solani* (74). Insensitive pathogens accumulated less gliotoxin than did sensitive pathogens, and the mechanism for natural insensitivity to this toxin was proposed to involve selective binding of gliotoxin to thiol groups in the cytoplasmic membrane (73). When sensitive pathogens were exposed to gliotoxin at low concentrations, toxicity was reversible, indicating active pathogen defense to concentrations that may be more typically encountered in natural ecosystems (64).

Resistance Mechanisms to Antibiotics Produced by *Pseudomonas*

Levy et al. (86) were, to our knowledge, the first to study in detail resistance mechanisms in a plant pathogen to antibiotics produced by *Pseudomonas*. They showed that addition of sublethal concentrations of 1-hydroxyphenazine to liquid cultures of *Mycosphaerella graminicola* resulted in an increase in catalase, peroxidase, and superoxide dismutase, enzymes involved in scavenging of active oxygen species. Additionally, melanin biosynthesis significantly increased by 42%. Melanins are polymers with varying composition, containing a polymeric nucleus with quinone, hydroxyquinone, and semiquinone moieties together with protein, carbohydrate, and lipid moieties. These compounds support survival under adverse conditions and can act as a "sponge" for free radicals (14). The presented data suggest that 1-hydroxyphenazine imposed oxidative stress on *M. graminicola* and that the primary defense action of the fungus against 1-hydroxyphenazine is a detoxification of active oxygen species. Since 1-hydroxyphenazine eventually disappeared from the culture medium, the authors also postulated that this fungus is capable of protecting itself against the deleterious effects of the antibiotic via degradation. Similar responses to phenazine antibiotics were found in other organisms. Mahajan-Miklos et al. (103) reported that pyocyanin, produced by human pathogenic strains of *P. aeruginosa*, contributes to the fast killing of the nematode *Caenorhabditis elegans* through the generation of active oxygen species. A *C. elegans* mutant with increased tolerance for oxidative stress mediated by increased levels of catalase and superoxide dismutase was more resistant to fast killing. Conversely, a mutant with reduced activity of superoxide dismutase was more sensitive to the antibiotic. In addition to detoxification of oxygen radicals, they found that efflux pumps play a role in the resistance of *C. elegans* to pyocyanin. Mutant NL130, disrupted in the ABC transporter genes *pgp-1* and *pgp-3*, was also very sensitive to pyocyanin-producing bacteria. This sensitivity was alleviated in the presence of bacteria defective in pyocyanin synthesis.

Recently, Schoonbeek et al. (148) demonstrated the role of efflux pumps in resistance of *B. cinerea* to antibiotics produced by antagonistic *Pseudomonas*. They showed that several phenazine antibiotics induced expression of the ABC transporter gene *BcatrB* in a dose-dependent manner. *BcatrB* replacement mutants were more sensitive than their parental strain to pure phenazines and phenazine-producing *Pseudomonas* strains. The biological relevance of the ABC transporter was further confirmed in a bioassay. When phenazine-producing *Pseudomonas* strains were sprayed onto tomato leaves, a *BcatrB* replacement mutant was significantly less capable than its parental strain of inciting disease symptoms. Biochemical analysis of the leaf confirmed the accumulation of phenazines to relatively high concentrations. When phenazine-deficient *Pseudomonas* mutants were applied, no differences in development of disease symptoms were observed between the disruption mutant and the parental strain. Uptake experiments confirmed that phenazine antibiotics act as a substrate of BcATR. BcATR also confers

decreased sensitivity to the phytoalexin resveratrol and the phenylpyrrole fungicides fenpiclonil and fludioxinil. Hence, BcATRb can be regarded as a trait that confers multidrug resistance. Another interesting aspect of the study by Schoonbeek et al. (148) was the differential effect on *B. cinerea* by pyrrolnitrin and fludioxinil, a fungicide derived from pyrrolnitrin (89). In contrast to fludioxinil, pyrrolnitrin did not elevate expression of *BcatrB* in the parental strain. In addition to phenazine antibiotics, 2,4-DAPG also induced expression of *BcatrB*. However, *BcatrB* replacement mutants showed similar sensitivity to 2,4-DAPG as their parental strain, indicating that induction of expression of a particular ABC gene does not necessarily imply that the encoded protein acts as a major transporter of that compound. Since 2,4-DAPG also induced expression of *BcatrD*, this transporter may have operated in *BcatrB* mutants, thereby compensating for deficiency in BcATRb-mediated efflux.

Bacterial Efflux Pumps and Antibiotic Resistance

Efflux pumps are present in a wide range of bacteria, and in lactic acid bacteria they have been found to play a role in resistance to bacteriocins and other antimicrobial compounds excreted by competitors (139). Recently, two multidrug efflux pumps (AcrB, NorM) were identified in *Erwinia amylovora* that play a role in pathogen tolerance to apple phytoalexins, to the synthetic antibiotic tetracycline, and to an antibiotic produced by the biocontrol bacterium *Pantoea agglomerans* (A. Burse & M.S. Ullrich, unpublished data). In *Erwinia chrysanthemi*, *ybiT* encoding a putative ABC transporter is essential for competitive fitness against endophytic bacteria during plant infection (92).

Resistance to Antibiotics Produced by Bacillus

Bacillus species are appealing candidates as biocontrol agents. They have the capability to produce effective and broad-spectrum antibiotics, like peptides, lipopeptides, aminoglycosides, and aminopolyols [(151) and references therein]. *Bacillus cereus* strain UW85 synthesizes both zwittermicin A (66) and kanosamine (110). Bacteria were insensitive to kanosamine; growth of 26 fungal species was inhibited by kanosamine, ranging from less than 30% for most species to more than 50% for *Ustilago maydis* (110). For the four oomycete species tested, significant variation in sensitivity was observed, with *Pythium aphanidermatum* and *Pythium torulosum* being less sensitive to kanosamine than *Aphanomyces euteiches* and *Phytophthora medicaginis*. Handelsman and coworkers (111, 161) have identified the *zmaR* gene in *B. cereus* that encodes ZmaR protein, which inactivates zwittermycin A by acetylation. Understanding self-resistance in antibiotic-producing biocontrol strains may provide valuable insight into potential self-defense mechanisms that could develop in pathogen populations.

Leifert et al. (83) characterized the antibiotics produced by *Bacillus subtilis* strain CL27 and *Bacillus pumilus* strain CL45. CL27 produced three compounds,

of which two were identified as peptides. One had activity against *Alternaria brassicicola* and the other had activity against both *A. brassicicola* and *B. cinerea*. The third antibiotic was not peptide based and also showed activity against *B. cinerea*. Based on TLC analysis, a similar compound was present in CL45. Although CL27 produces different compounds with antibiotic activity against *B. cinerea*, results obtained by Li & Leifert (88) suggested that the pathogen could develop resistance against the biocontrol agent after repetitive treatment of *Astilbe hybrida* plants. In glasshouse experiments, *B. cinerea* was effectively controlled in the first seven growth cycles of *Astilbe* plants, but in cycles eight and nine the biocontrol efficacy dropped dramatically. In the tenth cycle, strain CL27 was completely ineffective in controlling *B. cinerea* infection, and in vitro assays showed that culture filtrates of strain CL27 were no longer able to inhibit mycelial growth of the recovered *B. cinerea*.

Resistance to Agrocin 84

Crown gall, caused by the soilborne bacterium *Agrobacterium tumefaciens*, is a disease of many dicotyledonous plants resulting in substantial yield losses in fruit tree stocks, grapevine, and ornamentals. *Agrobacterium radiobacter* strain K84, now referred to as *A. rhizogenes* K84 (132), is the most well-characterized and commercially successful biocontrol agent for crown gall (113). Production of the antibiotic agrocin 84 is a key component of the biocontrol activity of strain K84. Agrocin 84 is believed to inhibit DNA replication and is transported into cells of *A. tumefaciens* via agrocinopine permease, a periplasmic protein encoded by genes carried on certain, but not all, types of the Ti plasmid present in sensitive strains of *A. tumefaciens* [(160) and references therein]. In strain K84, agrocin 84 biosynthetic and resistance genes are located on the conjugative plasmid pAgK84 (141). Resistance to agrocin 84 is determined by two distinct loci (*immA* and *immB*) of pAgK84, and each region alone appears to be sufficient to confer resistance (141, 183). Interestingly, mutations mapping to either region abolish production of agrocin 84, but have no effect on resistance itself (141, 183). With respect to resistance in pathogen populations to agrocin 84, van Zyl et al. (175) screened 65 strains and isolates of *A. tumefaciens*, representing each of the known biotypes, for in vitro and in vivo susceptibility to strain K84. All of the biotype 3 strains tested were resistant to K84, whereas high percentages of biotype 1 and 2 strains were susceptible. In an earlier in vitro study, Cooksey & Moore (28) showed that in three *A. tumefaciens* strains and one *A. rhizogenes* strain mutation rates for resistance to agrocin 84 were relatively high, ranging from 2.5×10^{-3} to 4.2×10^{-4} .

Several mechanisms of resistance to agrocin 84 have been described for *A. tumefaciens*, including (a) loss of the Ti plasmid carrying the genes for agrocinopine permease, (b) large deletions from the Ti plasmid, and (c) horizontal transfer of pAgK84 from strain K84 to *A. tumefaciens* (28, 160). Conjugal transfer of pAgK84 has been demonstrated in vitro and in crown gall tissue of infected plants, resulting in pathogenic *A. tumefaciens* strains that not only are resistant to agrocin 84 but

also produce agrocin 84 [references cited in (28, 160)]. The fact that agrocin 84-resistant *A. tumefaciens* strains have been isolated from different soils worldwide (175) suggests that conjugal transfer may have occurred in natural environments. To further evaluate the transfer frequency, pathogenicity, survival, and stability of transconjugants, Stockwell et al. (1996) carried out a field experiment with cherry seedlings treated with K84 and *A. tumefaciens*. Transconjugants were detected in 4 of 13 galls, and the estimated frequency of pAgK84 transfer was approximately 10^{-4} transconjugants per recipient. A transconjugant strain retained the plasmid for up to seven months in the rhizosphere of field-grown plants, colonized the rhizosphere of cherry to the same extent as its parental strain and caused crown gall.

To minimize the risk of erosion of the biocontrol activity of K84, derivative strain K1026 was constructed that still produces agrocin 84 but lacks the *tra* region necessary for horizontal transfer of pAgK84 (72). Subsequent studies by Vicedo et al. (179) revealed that K1026 colonized roots of seedlings of two peach cultivars as well as K84 and was as effective as its parental strain in biocontrol of agrocin 84-sensitive and -resistant strains of *A. tumefaciens*. Based on these results, the authors suggested that K1026 was a safer organism than K84 for biocontrol of crown gall. One may question, however, if engineering of derivative K1026 was necessary to safeguard the biocontrol efficacy of K84, given that K84 has been reported to effectively control crown gall caused by agrocin 84-resistant *A. tumefaciens* (96, 131). Recent studies have indicated that multiple other mechanisms may be involved in the biocontrol activity of K84. These mechanisms include the production of (a) another agrocin, referred to as agrocin 434 (41), (b) an antibiotic-like substance designated ALS84 (130), and (c) a hydroxamate siderophore, the latter possibly being the same compound as ALS84 (132). Collectively, these studies suggest that multiple mechanisms have, so far, precluded erosion of biocontrol of *A. tumefaciens* by *A. radiobacter* K84. Recent studies, however, have reported *A. tumefaciens* strains that are not only resistant to agrocin 84 but also to agrocin 434 and ALS84 (131). The implications of resistance to multiple compounds for the long-term stability of biocontrol by K84 remains unclear and is further discussed below in a more general context.

Pathogen Signaling to Repress Biosynthesis of Antimicrobial Compounds

The first example of phytopathogen signaling influencing interactions with an antagonist was described by Duffy & D efago (43) for the repression of *phl* biosynthetic genes in biocontrol strain CHA0 of *Pseudomonas fluorescens* by the tomato crown and root rot pathogen *F. oxysporum* f.sp. *radicis-lycopersici* (Table 2). They identified fusaric acid, a pyridine-carboxylic acid with phyto- and mycotoxigenic activity produced by *Fusarium*, as a pathogen signal that specifically repressed 2,4-DAPG biosynthesis by the biocontrol bacterium in a hydroponic tomato production system (43). Fusaric acid is a repressor of *phlA* promoter gene expression,

TABLE 2 Pathogen signaling of antagonist gene expression

Pathogen	Antagonist	Signal molecule	Biocontrol gene and/or trait affected	Reference
<i>Fusarium oxysporum</i> f.sp. <i>radicis-lycopersici</i>	<i>Pseudomonas fluorescens</i>	Fusaric acid	<i>phlA</i> , Blocked 2,4-DAPG biosynthesis	(42)
<i>F. oxysporum</i>	<i>P. fluorescens</i>	Fusaric acid	<i>phlA</i> , Blocked 2,4-DAPG biosynthesis	(120)
<i>Fusarium graminearum</i>	<i>Trichoderma atroviridae</i>	Deoxynivalenol	<i>nagI</i> , Repressed chitinase biosynthesis	(100)
<i>Fusarium culmorum</i>	<i>T. atroviridae</i>	Deoxynivalenol	<i>nagI</i> , Repressed chitinase biosynthesis	(100)
<i>Phytophthora parasitica</i>	<i>Pseudomonas putida</i>	Unknown	Stimulated putative diacylglycerol kinase and putative ABC transporter	(82)
<i>Pythium debaryanum</i>	<i>P. fluorescens</i>	Trehalose	<i>treA</i> , Stimulated utilization of fungal trehalose, and putative upregulation of other biocontrol genes	(56)
<i>Pythium ultimum</i>	<i>P. fluorescens</i>	Unknown	<i>rrmB</i> , <i>rrmC</i> , Impaired root colonization <i>glbB</i> , Impaired nitrogen assimilation	(154)
<i>P. ultimum</i>	<i>P. fluorescens</i>	Unknown	<i>phlA</i> , Stimulated 2,4-DAPG biosynthesis	(120)

with complete loss of expression at concentrations of 500 μM (146). Blocking fusaric acid production by the pathogen with zinc fertilization relieved *phlA* repression and improved the biocontrol activity of CHA0 on tomato (43), affirming the role of fusaric acid as the pathogen signal and demonstrating one practical approach to circumvent this pathogen defense. Fusaric acid specifically represses 2,4-DAPG and has no effect on expression of other antimicrobial metabolic genes, such as hydrogen cyanide (43; B. Duffy, C. Keel & G. Défago, unpublished data). Examination of an ecologically and geographically diverse collection of 2,4-DAPG producing strains of *P. fluorescens* (76) revealed two clusters with all strains that synthesize 2,4-DAPG and pyoluteorin, such as CHA0, being similarly sensitive to fusaric acid repression, and strains lacking pyoluteorin biosynthetic genes being relatively insensitive to this pathogen signal (B. Duffy & G. Défago, unpublished data). Biocontrol efficacy of these strains was positively correlated with their ability to synthesize 2,4-DAPG in the presence of fusaric acid (42), indicating that application of fusaric acid-insensitive *Pseudomonas* strains could be another practical approach to overcome this pathogen defense.

Schnider-Keel et al. (146) used a *phlA'*-*lacZ* translational fusion to elucidate the mechanism behind fusaric acid signaling in strain CHA0. In a *phlF* mutant, Reporter gene expression was unaffected by fusaric acid, indicating that PhlF, a pathway-specific repressor of 2,4-DAPG biosynthesis, is a likely target for fusaric acid repression. One possible mechanism for the repression is that fusaric acid increases the binding characteristics of the bacterial repressor-promotor complex (146). The fungal pathogen may be interfering with the bacterial mechanism for autoregulation of 2,4-DAPG biosynthesis as a defense strategy to thwart antagonism. Interestingly, salicylic acid produced by the bacterium also interacts with PhlF to repress biosynthesis of 2,4-DAPG.

This system presents compelling evidence for a coevolutionary interaction between the pathogen and antagonistic bacteria that are able not only to overcome growth inhibitory activity of fusaric acid, but in certain cases have also acquired the ability to overcome gene-repression activity. While the particular variant of *F. oxysporum* in this system may have a transient cohabitation with *P. fluorescens* in the tomato rhizosphere, in a broader ecological sense fusaric acid (and other mycotoxins), which appears to be produced by most forms of *Fusarium* (8), could have a greater influence on bacterial evolution in crop residues or on crop plants in general, where much greater population interactions can be expected. Indeed, Notz et al. (120) have demonstrated that nonpathogenic *F. oxysporum* producing fusaric acid also repress *phlA* gene expression in *P. fluorescens* CHA0.

Gaballa et al. (56) identified the disaccharide trehalose as a positive signal released by *Pythium debaryanum* that induces *P. fluorescens* genes involved in fungal antagonism (Table 2). Trehalose is a common storage molecule in many fungi (55), suggesting that this signaling may be fairly nonspecific. Notz et al. (121) used a *lacZ* reporter fusion to demonstrate that root infection with *P. ultimum* stimulated expression of the 2,4-DAPG biosynthetic gene *phlA* by *P. fluorescens* CHA0 on both maize and cucumber. Bacterial gene expression was independent

of host effects on rhizosphere colonization, suggesting that a pathogen signal may have positively affected *phlA* expression.

The first example of signaling described between a pathogen and a fungal biocontrol agent involves mycotoxigenic *Fusarium* and mycoparasitic *Trichoderma*. Grain contamination with trichothecene mycotoxins, particularly deoxynivalenol (DON), produced by *Fusarium* species results in tremendous economic losses worldwide. Although DON has some influence on pathogenicity, as with fusaric acid, its broad-spectrum toxicity hints at a wider ecological role in saprophytic survival. Antagonistic *Trichoderma* species, which produce cell wall-degrading lytic enzymes and inhibit *Fusarium*, are a primary competitor in crop residues. Défago and colleagues have recently found that DON produced by *Fusarium culmorum* and *Fusarium graminearum* acts as a negative signal repressing the expression of *nag1* chitinase gene in *Trichoderma atroviride* (formerly *T. harzianum*) by as much as 50% in vitro and in maize residues (100). Repression appeared to be specific for *nag1* with no effect observed on the expression of another chitinase gene, *ech42*. However, since chitinases act synergistically during mycoparasitism (97), repression of one antagonist gene may provide sufficient protection to the pathogen. The degree of antagonist gene repression in maize residues was cultivar dependent, with the greatest repression observed on maize cultivars that supported the highest levels of DON production by the pathogen. The antagonist in these studies did not have any effect on biosynthesis of DON by the pathogen (100). Other microorganisms coexisting in this habitat, however, do affect mycotoxin biosynthesis in *Fusarium* and could short-circuit this pathogen defense strategy, altering the outcome of multitrophic competitions. For example, DON-nonproducing strains of *Fusarium subglutinans*, which also colonize grain, suppressed DON biosynthesis in *F. graminearum* by 62% but did not degrade existent DON (30). Crop residues are the natural habitat where these fungi most intensively compete and thus where defense strategies most likely evolved. From a crop-protection perspective, pathogen reduction in residues is key to minimizing infection of subsequent crops, particularly in reduced tillage systems. Just as with 2,4-DAPG producing pseudomonads, negative pathogen signaling must be considered in designing effective biocontrol strategies for mycotoxigenic *Fusarium* using chitinase-producing *Trichoderma* strains, perhaps by combining strains or selecting DON-insensitive strains.

Pathogen Degradation of Toxins Produced by Fungal Competitors

Degradation of antimicrobial compounds produced by competitor microorganisms can provide reprieve from antagonism. Hydroxylation of hydrocarbon compounds is a fungal strategy for eliminating toxic metabolites (79). The antagonist *Trichoderma harzianum* produces the antibiotic 6-pentyl- α -pyrone, which has the dual effect of inhibiting pathogen growth and also downregulating genes in *F. graminearum* for the biosynthesis of trichothecenes, a class of mycotoxins with

broad-spectrum antimicrobial activity that likely contribute to the ecological fitness of the pathogen. The antagonist or its purified antibiotic can reduce production of deoxynivalenol (DON) by as much as 80% (30), and antagonist antibiotic genes are upregulated by the presence of the pathogen (29, 30). In its defense, *Fusarium* degrades 6-pentyl- α -pyrone, but for most isolates degradation is slow and ineffective. One *Fusarium* isolate adept at metabolizing the *Trichoderma* antibiotic also produced the most DON in the presence of the purified antibiotic or the competitor (30).

Cercosporin is a virulence factor produced by many fungi in the genus *Cercospora* that is highly toxic to most organisms, including plants, animals, and fungi (112). A surprising proportion of the microorganisms identified in screening for cercosporin degradation in disease control efforts are phytopathogenic *Xanthomonas campestris* pv. *zinniae* (112). This raises the possibility that pathogen degradation of another pathogen toxin could be a mechanism to gain a competitive advantage. A final scenario is that one pathogen may piggyback on the ability of another to degrade antifungal compounds. This has been elegantly demonstrated in the obligate lichenicolous *Nectria parmeliae*, which takes advantage of the ability of lichenicolous *Fusarium* to degrade antifungal toxins produced by the lichen (81). It is easy to imagine a succession of pathogens relying on initial detoxification of an antagonist antibiotic by *Fusarium*, such as degradation of 2,4-DAPG described above.

Resistance to Volatiles

A wide variety of microorganisms produce volatile compounds, including alcohols, aldehydes, aromatics, sulphides, and ketones (187). Several studies have shown that volatile organic compounds are produced in soil and plant-associated environments (157, 188). Wheatley (187) referred to volatile organic compounds as ideal “infochemicals” in microbial interactions because of their ability to be effective over a wide range of spatial scales. Several volatiles produced by bacterial and fungal genera, including *Serratia*, *Pseudomonas*, and *Trichoderma* spp., exert deleterious effects on the in vitro growth of diverse fungi, including wood decay fungi and plant pathogenic fungi like *G. g.* var. *tritici*, *Phytophthora cryptogea*, and *V. dahliae* (4, 188). Hydrogen cyanide (HCN) is probably one of the best known examples of a volatile compound involved in biocontrol (61a). In studies employing mutants of *P. fluorescens* strain CHA0 defective in HCN biosynthesis, it was shown that HCN accounts, at least in part, for the ability of strain CHA0 to suppress *Thielaviopsis basicola*, the causal agent of black root rot of tobacco (181). HCN production appears to be a more widespread characteristic of many antagonistic *P. fluorescens* strains, particularly those that produce 2,4-DAPG (76).

Defense strategies of plant pathogens to volatile compounds produced by biocontrol agents have, to our knowledge, not been studied in detail. Mackie & Wheatley (102) reported that volatile organic compounds produced by a diversity of root-colonizing bacteria inhibit growth of many plant pathogenic fungi but

that *P. cryptogea* and *G. graminis* apparently exploit these volatiles for directional growth toward root surfaces harboring the producing bacteria. Wheatley (187) reported that volatile organic compounds might have direct or indirect effects on the activity of specific fungal enzymes. These activities include a reduction in laccase activity and an increase in tyrosinase activity in *Phanaerochaete magnoliae* (102). Tyrosinases are involved in the biosynthesis of melanins (14). Laccases can also be involved in specific steps of melanin biosynthesis, as was demonstrated for the synthesis of 1,8-dihydroxynaphthalene (DHN)-melanin in *Cochliobolus heterostrophus*, *Magnaporthe oryzae*, and *G. graminis* (5, 46, 164). These laccases mediate the polymerizations of the immediate precursor DHN into DHN-melanin. However, DHN itself has antibiotic properties. Thus, one may speculate that the observed upregulation of tyrosinase activity and downregulation of laccase activity could result in the accumulation of the antibiotic DHN. Volatile organic compounds produced by antagonistic microorganisms may therefore act as a signal for the fungus to increase its own competitiveness via induction of structural and biochemical defense mechanisms.

With respect to defense strategies in fungi to HCN, it was suggested that prolonged application of HCN-producing biocontrol agents may select for pathogens containing cyanide-resistant respiratory pathways (62). Indeed, Osbourn (124) reported that a range of plant pathogenic fungi can tolerate HCN and that for some of these fungi, including *Microcyclus ulei*, HCN tolerance is attributed to cyanide-resistant respiration. Other pathogenic fungi, including *Fusarium lateritium*, *Gloeocercospora sorghi*, and *Stemphylium loti*, are able to detoxify HCN by conversion of HCN to formamide via the cyanide-inducible enzyme cyanide hydratase [see (124) and references therein]. A recent search in the NCBI database yielded at least two other plant pathogenic fungi, *Fusarium solani* and *Leptosphaeria maculans*, in which cyanide hydratases were discovered. Collectively, these observations suggest that specific defense mechanisms against HCN, and possibly other volatile compounds, are present in a range of plant pathogenic fungi. The significance of these defense mechanisms against volatiles in interactions between plant pathogens and biocontrol agents remains to be tested.

PATHOGEN DEFENSE AGAINST COMPETITION

Meddling with Antagonist Population Dynamics

Population growth of certain antagonists can be stimulated by plant damage caused by pathogen attack. A capacity to rapidly exploit the nutrients leaking from wounds is considered an important feature of many effective biocontrol strains (27, 185). Root damage caused by fungal pathogens has been positively correlated with rhizosphere colonization of specific bacterial biocontrol agents (185), but wounds caused by pathogens also can stimulate total populations of indigenous microorganisms nonspecifically (12, 156). In ecological terms, it would be advantageous for pathogens to reduce the competitiveness of potential antagonists, possibly by

orchestrating the indigenous microbial community to foster members that may interfere with the activity of potential antagonists.

Mazzola & Cook (106) observed differential effects of target and nontarget pathogens on populations of introduced *P. fluorescens*. The target pathogens *G. g. var. tritici*, *P. aristosporum*, and *Pythium ultimum* var. *sporangiferum*, which were inhibited by *P. fluorescens* Q72a-80, either stimulated or had no effect on populations of this bacterium in the wheat rhizosphere. In contrast, the nontarget pathogen *Pythium irregulare*, which was not inhibited by Q72a-80, reduced population growth of this strain. *P. irregulare* is also a nontarget pathogen for *P. fluorescens* 2-79, and rhizosphere populations of this biocontrol strain were reduced in the presence of this fungus. However, not all nontarget pathogens had this effect. The other two *Pythium* species stimulated populations of *P. fluorescens* 2-79 even though this strain had no inhibitory action against them (106).

Pythium ultimum is a target pathogen for *P. fluorescens* F113, which is applied for control of sugarbeet damping-off. O'Gara and colleagues screened random *lacZ*-tagged transposon insertions for response to *Pythium* mycelial macerations in an elegant approach to identify pathogen signals for bacterial genes. They found that an undetermined mycelial component(s) repressed a few genes that were important for rhizosphere colonization by the biocontrol agent (48). One of the bacterial genes repressed by the pathogen, *gltB*, encodes the large subunit of glutamine synthase, which is involved in nitrogen assimilation (Table 2). Its repression would presumably reduce bacterial competitiveness under NH_4^+ -limiting conditions. The other repressed genes identified were two *rrn* operons, which are proposed to be involved in growth under conditions that should favor rapid growth (154). Pathogen repression of these genes may reduce competition for nutrients released from lesions. Nevertheless, repression of *gltB* and *rrn* genes had no effect on the biocontrol activity of this strain, which relies on the production of antibiotics that are not repressed by this pathogen (48). The fact that such a defense strategy has evolved, however, suggests that it likely serves the pathogen well against many other potential competitors in the indigenous microbial community, just not against F113. There is likely an ongoing struggle of pathogens to suppress competitor growth and antagonists to out-compete pathogens. Lee & Cooksey (82) reported that undetermined signal molecules produced by *Phytophthora parasitica* induced *Pseudomonas putida* genes encoding a putative ABC transporter and diacylglycerol kinase. In this case, being able to sense the presence of the target pathogen enhanced the ability of the antagonist to colonize its hyphae and inhibit pathogen growth.

A recent approach to improving biocontrol is through systematic selection of biocontrol strains that have a high degree of overlap with the target pathogen in nutritional utilization patterns. Application of this approach to bacterial and yeast biocontrol agents aims to deploy strains that most effectively compete with pathogens in nutrient-limited environments like the phyllosphere (71). Pathogen populations as a whole often are adept at utilizing a wide range of nutritional compounds, which raises the question as to whether repetitive application of such

biocontrol strains could direct the evolution of pathogen strains with greater nutrient scavenging ability. Similarly, the ability of bacterial biocontrol agents to utilize heterologous siderophores produced by coexisting bacteria appears to enhance their ecological competitiveness (94, 136). Whether pathogens also exploit such cross-feeding strategies to gain a competitive advantage over antagonists and the effect of such an advantage on plant disease remains to be investigated.

Shutting Out Competitors (Lifting the Drawbridge)

Plant invasion is a basic strategy for pathogens to avoid epiphytic competitors; this works best when competitors can be shut out for as long as possible. Many *Pseudomonas syringae* pathovars produce the lipodepsipeptides, syringomycin and syringopeptin. These are considered first and foremost to be virulence factors (15). An early effect of these phytotoxins is to close stomata, a point of pathogen entry into plants (40, 116). By shutting these natural openings once it has entered the plant, the pathogen may impede its pursuit by potential antagonists inside the plant host. Whether competitor microorganisms have developed ways to overcome this escape tactic has not been investigated, but fusicoccin produced by mycotoxigenic *Fusarium* opens plant stomata (105) and can reverse the effects of syringomycin (116). A related strategy is to physically impede competitors from sharing in a food cache. The downy mildew fungus of lettuce, *Bremia lactucae*, forms callose plugs around the outer germ tube and appressorium after invading host cells, thereby preventing leakage of nutrients and entry of competitors.

Altering the Environment

Agrobacterium tumefaciens is a classic example of a pathogen escaping competition by customizing its environment, in this case by programming the host plant to produce unique carbon compounds, opines, that can be utilized by the pathogen but relatively few other organisms. The competitive advantage secured by opine utilization has been exploited in the design of model biocontrol systems where *Pseudomonas* inoculants are engineered to utilize unique opines that transgenic host plants have been engineered to excrete (143).

Certain fungi alter their physical environment to gain an ecological advantage over potential competitors. *Sclerotinia sclerotiorum* produces oxalate, which acidifies its ambient environment (44) to a pH less conducive to general bacterial growth. Reduced pH interferes with the biocontrol activity of *P. fluorescens* (126) because it drastically diminishes biosynthesis of phenazine antibiotics by the bacterium (153). Oxalate-mediated pH reduction bolsters fungal defenses against antagonism through stimulation of lytic enzyme biosynthesis and sclerotial formation (140). Reduced pH also increases iron availability and stimulates fungal siderophore biosynthesis, which would enhance fungal competitiveness for limited iron resources (191). This is in addition to the direct inhibitory effects of oxalate on growth, sporulation, and mycotoxin production by competitor fungi (44). Maize leaf tissues killed by T toxin produced by *Helminthosporium maydis* dry to an

extremely low water potential (below -100 bars), which effectively prevents growth of most epiphytic bacteria but allows for necrotrophic growth of the pathogen free of these competitors (27).

Co-Opting Plant Host Defenses

Hijacking plant host defenses may be a metabolically inexpensive mechanism for pathogens to gain advantage over competitors. *Botrytis cinerea* is a necrotrophic pathogen that triggers a plant hypersensitive response (HR) involving the release of reactive oxygen species and hypersensitive cell death. This response blocks the progress of biotrophic pathogens and at the same time facilitates plant colonization by *B. cinerea* (58). Oxidative bursts are usually associated with incompatible plant/pathogen interactions that lead to an HR. Successful plant infection by *E. amylovora* and *E. chrysanthemi*, however, is characteristically associated with such toxic oxidative bursts that appear to be triggered by pathogen *hrp* type III secretion systems (177). Whether the plant is simply wise to the ways of the pathogen, or whether the pathogen has evolved a mechanism for intentionally triggering host defenses is debatable. Many plant pathogens have self-protection systems against oxidative damage, such as manganese-containing superoxide dismutase and bacterial exopolysaccharides in the case of *Erwinia* spp. (142, 177). The bursts are short-lived and are generally considered more a plant signal for turning on other defenses like phytoalexin production, but this may be all the head start that the pathogen needs. Phytoalexins generally have greater inhibitory activity against secondary colonists (27), and by reducing microbial competition their induction may be advantageous to the inducing primary invaders. Selection for resistance to plant toxins in pathogen populations, as described above, supports this hypothesis.

Pathogen Toxins as Chemical Defense

Plant pathogenic fungi produce an astonishing array of toxins with broad-spectrum activity against animals and microorganisms that function to improve ecological competitiveness. Lewis & Lumsden (87) reported that metabolites of root pathogenic *R. solani* reduced mycelial growth and conidia production of the antagonists *Trichoderma hamatum*, *T. harzianum*, and *Trichoderma viridae*. Metabolites of pathogenic *P. ultimum* also reduced conidia production but actually stimulated mycelial growth of the antagonists (87). Sclerotia of *Rhizoctonia carotae*, *Rhizoctonia cerealis*, and *Typhula incarnate* contain antibiotic compounds that inhibit growth of antagonistic *B. subtilis* (26). One of the earliest demonstrations that antibiotics play an ecological role in nature is the classic example of *Cephalosporium graminearum*. This wheat pathogen produces broad-spectrum antimicrobial compounds in host tissues in order to prevent displacement by saprophytic microorganisms after plant death (22). Mycotoxins produced by several groups of fungal pathogens, notably members of *Aspergillus*, *Penicillium* and *Fusarium*, are multifunctional metabolites that offer protection from abiotic environmental stress, as in the UV-protecting activity of *Penicillium verrucosum* (162),

and broad-spectrum protection against animals and microorganisms. Generally, it appears that slowly growing fungi, which are less efficient than quickly growing fungi at escaping competition by entering specific niches, have a higher incidence of antagonism against competing fungi (57). Perylenequinones, most notably cercosporin, are photosensitizers produced by a wide range of phytopathogenic fungi that are important factors in plant pathogenesis, but also have high toxicity against bacteria and nonproducing fungi (36).

Pseudomonas syringae pathovars and other pathogenic *Pseudomonas* spp. produce lipodepsipeptides, notably syringomycin, syringotoxin, and syringopeptins, which are key virulence factors (15). The fact that nonpathogenic epiphytic isolates of *P. syringae* also produce these (2, 23) points to an additional ecological role of these toxins. These bacterial toxins have broad-spectrum activity against potential competitor epiphytic and parasitic microorganisms (182), with each toxin having a slightly different range of activity. For example, syringopeptin has high activity against Gram-positive bacteria, and syringomycin is especially inhibitory of fungal growth and conidial germination, particularly yeasts. Further evidence for a role in pathogen competition is the fact that lipodepsipeptides act synergistically with microbial cell wall-degrading enzymes produced by the pathogen to inhibit other microorganisms (52). *Trichoderma* cell walls are protected from the action of cell wall-degrading enzymes, and no synergism was observed against this antagonist.

PATHOGEN DEFENSE AGAINST PARASITISM

Bacteriophage Resistance

Bacteriophages are parasitic viruses that infect bacterial cells. They are found ubiquitously in nature, but are most easily isolated from aquatic environments even standing water in agricultural fields (45). Bacteriophages have been utilized for biocontrol of phytopathogenic bacteria (45, 180) and may have extended application for reducing the occurrence of human pathogenic bacteria on fresh produce (85). Phages typically exhibit a high degree of specificity, which has been exploited for bacterial typing in public health epidemiology. High specificity, however, can also give rise to rapid resistance development, a fact that has complicated bacteriophage therapy in medicine (163) and could interfere with biocontrol of plant diseases. Schnabel & Jones (145) proposed bacteriophage for biocontrol of fire blight, but they found that the ability of five phage variants isolated from field sites to infect a large collection of strains of *E. amylovora* varied dramatically. None of the pathogen strains was sensitive to all five phages, and several of the pathogen strains were resistant to multiple phage variants. In *Erwinia carotovora* subsp. *atroseptica*, nearly 75% of pathogen strains were insensitive to the newly characterized phage Φ M1 (165), and *E. c.* subsp. *carotovora* rapidly developed resistance to phage ZF40 (166). Resistance is based on several mechanisms, the most important of which stems from pathogen cell wall changes that impair phage adsorption, as seen with *Xanthomonas campestris* pv. *malvacearum* (54). To avoid problems

with bacteriophage resistance developing in bacterial pathogens, mixtures of host-range mutants (h-mutants) are recommended (50). Much of our understanding of bacteriophage resistance comes from decades of research to preserve *Lactobacillus* dairy starter cultures (25), and this expertise may be useful for preventing bacteriophage problems in bacterial inoculants used for biocontrol of plant diseases (75).

Pathogen Structural Barriers and Induced Sporulation

Pathogens have diverse responses to inclement environmental conditions, and the same strategies are employed to withstand microbial antagonism (6). *Fusarium udum*, causal agent of pigeon pea wilt, forms vacuolated mycelia and chlamydospores for protection against the mycolytic activity of *B. subtilis* (63). Melanin is a primary defense system in all organisms that produce it. Melanin and its precursor compounds enhance the ability of pathogenic fungi to resist microbial lysis, and melanized fungal biomass survives years in soil whereas hyaline spores and hyphae are quickly lysed. Melanin provides substantial protection against hydrolytic enzymes produced by many types of antagonists. The ability of cellulose, chitinase, and glucanase to degrade pathogen cell walls is inversely related to cell wall melanin content, and these cell wall-degrading enzymes are bound in a non-competitive irreversible manner with melanin (14). Even within a species such as *R. solani*, different isolates can exhibit tremendous variation in susceptibility to lysis (93). Relative resistance among varieties of *G. graminis* to microbial lysis is positively correlated with the melanin content in hyphae, and melanin extracted from these fungi strongly inhibits lysis of living mycelia of the same fungus by lytic enzymes from the bacterial antagonist *Streptomyces lavendulae* (167). In addition to providing a physical/biochemical shield, melanin from the wood-rot fungus *Phellinus weirii* has broad-spectrum antibiotic activity against potential competitor microorganisms (14). Antagonistic *P. fluorescens* induces laccase, an enzyme involved in melanin polymerization, in *R. solani* (33). Antagonistic *B. subtilis* induces laccase in the wood-rot fungus *Hypholoma fasciculare* (59). Laccase induction appears to be a cellular response to calcium influx, which is a general cellular signal of fatal stress (33). In addition to assisting in melanization, laccase-derived free radicals may also serve pathogens by detoxifying antifungal compounds (21). Fungal resistance to lytic enzymes may also be influenced by cell wall hydropolysaccharides insensitive to glucanase and chitinase, as in the case of chlamydospores of *Fusarium solani* f.sp. *cucurbitae* (172).

Attack by the obligate mycoparasite *Aphanocladium album* induces precocious teliospore formation by the rust fungi *Puccinia graminis* f.sp. *tritici*, *Puccinia dispersa*, and *Puccinia sorghi* (53). *A. album* aggressively attacks aecio- and uredospores, colonizing 100% of these, but teliospores are relatively resistant to mycoparasitism with less than 2% colonized and the rest remaining undamaged (80). Thus induction of sexual teliospores may be a mechanism for genetic escape from the mycoparasitic attack in that, while the thallus may ultimately succumb, the

genetic material for future host plant infections is conserved. Epiphytic *Pseudomonas* strains stimulate the germination of spores and formation of appressoria in *Colletotrichum* species that are pathogenic to weeds (49, 144) and crops (84, 108). By entering a plant host, the pathogen escapes bacterial competitors on the plant surface. *Colletotrichum dematium* f.sp. *spinaciae*, which also releases such nutrients into the conidiosphere, is not susceptible to *Pseudomonas*-mediated inhibition of conidia germination and in fact is stimulated to form more appressoria on both plant surfaces and on glass slides (19). Siderophore-mediated iron depletion by epiphytic *Pseudomonas* species appears to be a major mechanism for stimulation of appressorial induction (108, 152). This is clearly an advantage available only to certain pathogens.

Reverse Mycoparasitism

Mycoparasitism is a primary mechanism of biocontrol of many antagonistic fungi, particularly *Trichoderma* species (70). Several forms of plant pathogenic *F. oxysporum* have the ability to parasitize *T. hamatum*, *T. harzianum*, *T. longibrachiatum*, and *T. pseudokoningii*, sometimes mutually, and with all of the attendant parasitic hyphal interactions, including coiling, resistance sheath formation, and hyphal invasion (171). Reverse mycoparasitism is not limited to confrontation with antagonists. *Fusarium* and *Rhizoctonia* parasitize the hyphae of other pathogenic fungi that are potential competitors for the same plant hosts (11, 60, 171).

Nematode Resistance to Parasitic Bacteria

Several species in the genus *Pasteuria* are obligate parasites of nematodes. *Pasteuria penetrans* is being intensively investigated for potential biocontrol of various root-knot nematodes, particularly *Meloidogyne* spp. The bacterium is highly specific with significant pathogenic variability observed between species of *Meloidogyne*, and even among races and biotypes within single field populations (24). Much of this specificity is attributed to bacterial spore attachment and nematode cuticular heterogeneity (38). Aside from natural variation in nematode susceptibility, resistance development has been demonstrated in *Meloidogyne javanica* and *M. incognita* to repeated application of single-spore isolates of *P. penetrans* (168). Resistance was suggested to involve spore-cuticle recognition, attachment, and infection. Resistance development could be ameliorated with application of several *P. penetrans* isolates as a biocontrol mixture.

PATHOGEN DEFENSE AGAINST HYPOVIRULENCE

The ascomycete fungus *Cryphonectria parasitica* (formerly *Endothia parasitica*) causes substantial damage and yield losses in chestnut forests and orchards, especially in North America (37). *C. parasitica* grows in and underneath the bark of chestnut trees, forming cankers and causing destruction of the tree. The observation in the 1950s in Italy of "spontaneous healing" of cankers led to the

discovery of hypovirulent isolates of *C. parasitica* (37). Hypovirulence refers to the reduced virulence of isolates of the chestnut blight fungus and is caused by infection with double-stranded (ds) RNA viruses of the genus *Hypovirus* (68). Hypovirus infection of *C. parasitica* is persistent and nonlytic and is associated with specific stable traits, including the inability to effectively penetrate the host plant, reduced asexual sporulation, female infertility, and reduced pigmentation (122). Recent studies have also shown that a series of changes at the molecular level occur in *C. parasitica* upon hypovirus infection, including the expression of protein kinase, cutinase, laccase, and pheromone genes [(37, 77) and references cited therein]. In Europe, *C. parasitica* has been effectively controlled for over 40 years with hypovirulent isolates of *C. parasitica*, indicating that biocontrol of this pathogen has remained relatively stable (128). However, the effectiveness of hypovirulent strains of *C. parasitica* can be compromised by traits associated with hypovirulence, including reduced asexual sporulation and concomitant reduction in dissemination of introduced hypovirulent strains (101, 122).

To address the issue of defense strategies of *C. parasitica* against biocontrol with hypoviruses, one needs to consider the diversity of the pathogen and virus populations. A series of studies have shown that, based on vegetative compatibility (VC), there is considerable diversity in populations of *C. parasitica* (31). Similarly, substantial diversity exists in the population of the hypoviruses, some of which are widespread geographically whereas others are only found in specific countries or regions within a country (129). Peever et al. (128) recently examined variation both in tolerance of fungal hosts to hypoviruses and in virulence among hypovirus isolates. From a population of *C. parasitica*, sampled from a chestnut forest in Italy, they obtained a total of 158 isolates that were subsequently screened for the presence of hypoviruses with dsRNA-specific monoclonal antibodies. Six hypovirus-infected and six hypovirus-free isolates were selected. The unique hypovirus isolates, plus a CHV1-control isolate, were transferred into each of the six virus-free *C. parasitica* isolates, resulting in 42 hypovirus-fungus combinations. The authors showed that among fungal isolates (within each hypovirus) and among hypoviruses (within each fungal isolate) there was significant variation in both fungal tolerance and in virulence of the hypovirus. For example, significant differences in sporulation were observed among fungal isolates when infected with five of six hypovirus isolates. Similarly, the canker area on field-inoculated chestnut trees differed significantly within a fungal isolate infected with different hypovirus isolates. Given that the observed differences, although statistically different, were relatively small, the authors concluded that biological control of chestnut blight was unlikely to break down due to evolution of tolerance to hypoviruses in the fungus. In this context, they postulated that tolerance to hypovirus infection in *C. parasitica* might impose fitness costs on tolerant isolates when the hypovirus infection is absent.

A second aspect of strategies in populations of *C. parasitica* to overcome or, better, escape from hypovirus infection relates to the fact that virus transmission is primarily restricted between VC types. Vegetative compatibility results in stable

heterokaryons only if they share the same alleles at all vegetative incompatibility (*vic*) loci (32). Thus, one may postulate that rare VC types escape virus infection, resulting in a selective advantage over more commonly found VC types. To date, however, there is no support for this selective advantage. Liu & Milgroom (91) showed that hypovirus transmission between VC types decreased as the number of heteroallelic *vic* loci increased. By determining the underlying genetics of VC (32), it was further shown that transmission probabilities depended on the specific alleles at each *vic* locus (31). Additionally, Biella et al. (17) elegantly showed that the probability of virus transmission correlates negatively to the rate of cell death between incompatible isolates after anastomosis. In other words, similar to defense responses in plants and animals against pathogens, programmed cell death appears to be an essential element of the defense response in *C. parasitica* against hypovirus infection (17). Vegetative incompatibility significantly reduces hypovirus transmission, but the barrier to transmission is not complete (31). In this context, Milgroom & Cortesi (109) postulated that this incomplete inhibition of hypovirus transmission may result in transmission between vegetative incompatible individuals often enough to prevent selection. They also stated that selection might not have occurred yet, since only one generation occurs in *C. parasitica* per year and therefore relatively few generations have occurred since its introduction into Europe and North America approximately 60 and 100 years ago, respectively.

IMPLICATIONS FOR THE DURABILITY OF BIOCONTROL

In this review, we have described several mechanisms by which pathogens can counteract microbial antagonism. To date, however, most of the defense mechanisms have been studied only in laboratory trials at the organism level and almost always with single isolates. One of the major challenges is to link pathogen self-defense mechanisms found *in vitro* with processes that occur in pathogen populations *in situ*. With respect to biological control of plant pathogenic fungi and bacteria, we found very few examples that would indicate a potential breakdown of control by pathogens. The clearest example is resistance development in the crown-gall pathogen *A. tumefaciens* to agrocin 84 produced by antagonistic *A. radiobacter*, which results from horizontal gene transfer or other means (160). Further examples are the work of Li & Leifert (88) on biocontrol of *B. cinerea* by *B. subtilis* strain CL27 and the work of Mazzola et al. (107) demonstrating that variation in sensitivity of the take-all fungus to antibiotics produced by *Pseudomonas* interferes with the ability of antibiotic-producing *Pseudomonas* strains to control resistant pathogen isolates. There are several explanations for this paucity of reports of breakdown in plant disease biocontrol. First, this may be due to a bias against reporting negative results. Holt & Hochberg (69) observed that regarding insect biocontrol “if the target pests evolve very rapidly, thereby escaping control, the attempt at biological control would be deemed a failure and so would not likely

enter the published record.” We think a more plausible explanation for the lack of documented examples is that, relative to chemical control, biocontrol of plant pathogenic fungi and bacteria is still applied at small and local scales, on a less frequent basis, and is usually targeted against one or very few pathogen species. These factors reduce the chances that dramatic shifts in pathogen populations toward resistance will occur.

From a population dynamic and evolutionary perspective, Holt & Hochberg (69) articulated that changes in pathogen populations will occur relatively slowly when (a) there is a lack of genetic variation in the target pathogen, (b) there are constraints on selection, (c) selection is weak, (d) selection is temporally inconsistent, and (e) the biocontrol agent coevolves with the target pathogen. In the past decade, molecular-based techniques have clearly shown that most, if not all, pathogen populations harbor substantial genetic variation. Therefore we do not consider reason a as a major limiting factor for pathogen populations to counteract biocontrol. Whether this applies to all plant pathogenic fungi and bacteria, however, will depend on the rates of several processes such as reproduction, recombination, and horizontal gene transfer that enable reallocation of pathogen defense traits among different strains and offspring (35). There are several studies describing constraints (trade-offs, fitness costs) on selection, thereby preventing or reducing the evolution of enhanced resistance. Probably the best example is the ecology and evolution of bacteriophage resistance in *E. coli* in which different mutations lead to differences in the physiological costs associated with resistance [reviewed in (20)]. Another example of constraints on fitness is that pathogens may be able to escape the antagonist, but at the same time end up in refugia where resources for growth and reproduction are very limited. The spatial and temporal heterogeneity of the environments where both pathogens and antagonists reside are, in our opinion, important factors involved in the (lack of) development of resistance in pathogen populations. A series of studies have shown that population densities of biocontrol agents of soilborne diseases vary among root systems of different plants or among roots of single plants by several orders of magnitude; introduced biocontrol agents usually follow a lognormal distribution with decreasing densities away from the inoculum source (95). Similarly, population densities of biocontrol agents introduced onto seeds and into phyllosphere and rhizosphere environments tend to decline substantially over a prolonged period of time (189). Based on these observations one may conclude that in many biocontrol systems selection is weak and temporally inconsistent. This conclusion is supported by a series of risk-assessment studies [reviewed in (115)], which show that introduction of antibiotic-producing biocontrol agents had a transient or undetectable impact on nontarget soil microorganisms and ecosystem functioning. In the case of antibiosis, there is still little knowledge about the spatial and temporal production patterns of introduced strains. Both the place and time of antibiotic production need to be considered with respect to resistance development in pathogen populations. An antibiotic may reach threshold concentrations for activity within certain microsites while remaining below this threshold level at other sites. These sites could

serve as refugia for a substantial fraction of the pathogen population to reside unexposed to antibiosis or antagonism in general, and thus without selection pressure to hone self-defense mechanisms. Conserving refugia is a strategy recommended in the application of agrochemicals and deployment of transgenic plants to minimize development of pathogen and insect resistance. Blanket application of a synthetic or purified antibiotic derived from biocontrol agents, however, may more likely lead to selection of pathogen resistance, as has been seen with the agrochemical application of streptomycin and tetracycline. Potential interference with disease suppression afforded by native antagonist populations that produce such an antibiotic would need to be carefully considered.

A key difference between chemical and biological control is that antagonistic microorganisms are capable of evolutionary responses to changes in pathogen populations. Coevolution could be either constantly ongoing or reach a coevolutionary equilibrium where the level of control remains relatively stable (69). In this respect, it is tempting to classify the natural biocontrol that occurs in disease-suppressive soils (186) as a coevolutionary equilibrium between the pathogen and the antagonistic microflora. The substantial genotypic diversity existing among antagonists that share a common biocontrol trait (137, 138) may be regarded as an adaptation, in part, to changes in pathogen populations. In this context, the application of mixtures of genotypically different isolates that share the same biocontrol trait may allow antagonists to better respond to changes not only in abiotic conditions but also in biotic conditions, resulting in a more stable biocontrol.

INTENSIFIED APPLICATION/TRANSGENE DEPLOYMENT Special circumstances that theoretically could alter expected evolutionary interactions described above are intensified application of biocontrol agents and deployment of transgenes. Assuming that both the scale and rate of application of effective biocontrol agents will increase in the near future, especially due to the ban on a range of chemical pesticides, can we expect an increase in the erosion of biocontrol systems? Will this be exacerbated by commercial incentive to go with a limited number of isolates because of exorbitant investment costs for product registration and marketing? Another consideration is whether the application of transgenic biocontrol agents having enhanced expression of a specific trait, or in which antagonistic traits are combined, will increase the chances of resistance development in pathogen populations. Transgenic bacteria designed to overexpress or otherwise alter the expression of antibiotic biosynthetic genes could elevate the selection pressure for emergence of naturally resistant pathogens. There is currently no evidence that this occurs, either direct with isolation of resistant variants or indirect by observing a breakdown in biocontrol. Utilization of transgenic antagonists designed to express multiple modes of action may be an approach to avoid such resistance development (9), but careful consideration should be given to the mechanisms combined. Based on studies of pathogen self-defense, we can suggest that traits vulnerable to cross-resistance should be avoided, such as introducing genes into a bacterial antagonist for two antibiotics that are both substrates of the same pathogen efflux

pump. The potential value of transgenic plants expressing biocontrol traits from microbial antagonists is just now being explored. Transgenic potato plants expressing antimicrobial T4 lysozyme, for the purpose of controlling *E. carotovora* subs. *atroseptica*, selected for increased rhizosphere populations of lysozyme-resistant *P. putida* (98). Such effects tend to be transient and there is no indication of any long-term ecological consequences. Transgenic plants expressing components of bacteriophage may have lower risk of resistance development in bacterial pathogens than application of living phage (127). Biocontrol of plant viruses has not been considered in this review and the reader is referred to Falk & Bruening (47) for discussion of transgenic plants expressing antiviral traits. While there is no evidence that transgenes pose an inordinate risk, at least in terms of selection for pathogen resistance, an incentive remains to evaluate transgenes based on the traits expressed and on a case-by-case basis to demonstrate sensitivity of this still controversial issue and to ensure utmost precaution.

CONCLUDING REMARKS

Biological control of plant pathogens by application of antagonistic microorganisms to seeds or other planting material has been studied intensively over the past five decades. During most of that time it was not considered to be a commercially feasible strategy for crop protection. Significant progress has been achieved in the past 20 years, and several successful biocontrol products are now commercially available (108a). Nevertheless, variable performance remains an obstacle to the use of many more promising biocontrol agents in agriculture and horticulture. This variable performance has been ascribed to a multitude of factors, including poor survival and colonization by the introduced strain, and variable expression of key antagonistic traits. The examples given in this review indicate that self-defense mechanisms in pathogen populations may be an important factor in the variable performance by introduced biocontrol strains. Clearly, not all of the pathogen responses to antagonism are sufficiently effective to prevent biocontrol, and we suggest that there is a dynamic interaction between pathogenic microorganisms and their competitors. We also propose that many of the pathogen defenses reported here have developed to facilitate the ecological competence of pathogens. We hope the concept of pathogen self-defense will raise awareness of the dynamic structure of this interaction and will encourage further studies to elucidate pathogen responses to antagonism with the ultimate objective of exploiting this insight to develop more durable and reliable biocontrol strategies.

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LITERATURE CITED

1. Deleted in proof
2. Adetuyi FC, Isogai A, Di Giorgio D, Ballio A, Takemoto JY. 1995. Saprophytic *Pseudomonas syringae* strain M1 of wheat produces cyclic lipodepsipeptides. *FEMS Microbiol. Lett.* 131:63–67
3. Alexander NJ, McCormick SP, Hohn TM. 1999. TRI12, a trichothecene efflux pump from *Fusarium sporotrichioides*: gene isolation and expression in yeast. *Mol. Gen. Genet.* 261:977–84
4. Alstrom S. 2001. Characteristics of bacteria from oilseed rape in relation to their biocontrol of activity against *Verticillium dahliae*. *J. Phytopathol.* 149:57–64
5. Alsubaey A, Sisler HD, Matthews BF. 1996. Purification and characterization of an extracellular phenol oxidase from culture filtrates of *Pycularia oryzae*. *Can. J. Microbiol.* 42:437–45
6. Amir H, Mahdi N. 1992. Correlations between certain ecological characteristics of different strains of *Fusarium* with particular reference to their persistence in soil. *Soil Biol. Biochem.* 24:249–58
7. Deleted in proof
8. Bacon CW, Porter JK, Norred WP, Leslie JF. 1996. Production of fusaric acid by *Fusarium* species. *Appl. Environ. Microbiol.* 62:4039–43
9. Bakker PAHM, Glandorf DCM, Viebahn M, Ouwens TWM, Smit E, et al. 2002. Effects of *Pseudomonas putida* modified to produce phenazine-1-carboxylic acid and 2,4-diacetylphloroglucinol on the microflora of field grown wheat. *Antonie van Leeuwenhoek* 81:617–24
10. Deleted in proof
11. Barnett HL, Binder FL. 1973. The fungal host-parasite relationship. *Annu. Rev. Phytopathol.* 11:273–92
12. Barnett SJ, Singleton I, Ryder M. 1999. Spatial variation in populations of *Pseudomonas corrugata* 2140 and pseudomonads on take-all diseased and healthy root systems of wheat. *Soil Biol. Biochem.* 31:633–36
13. Bélanger RR, Deacon JW. 1996. Interaction specificity of the biocontrol agent *Sporothrix flocculosa*: a video microscopy study. *Phytopathology* 86:1317–23
14. Bell AA, Wheeler MH. 1986. Biosynthesis and functions of fungal melanins. *Annu. Rev. Phytopathol.* 24:411–51
15. Bender CL, Alarcón-Chaidez F, Gross DC. 1999. *Pseudomonas syringae* phytotoxins: mode of action, regulation, and biosynthesis by peptide and polyketide synthases. *Microbiol. Mol. Biol. Rev.* 63:266–92
16. Benyagoub M, Bel Rhild R, Bélanger RR. 1996. Purification and characterization of new fatty acids with antibiotic activity produced by *Sporothrix flocculosa*. *J. Chem. Ecol.* 22:405–13
17. Biella S, Smith ML, Aist JR, Cortesi P, Milgroom MG. 2002. Programmed cell death correlates with virus transmission in a filamentous fungus. *Proc. R. Soc. London Ser. B* 269:2269–76
18. Deleted in proof
19. Blakeman JP. 1993. Pathogens in the foliar environment. *Plant Pathol.* 42:479–93
20. Bohannan BJM, Lenski RE. 2000. Linking genetic change to community

- evolution: insights from studies of bacteria and bacteriophage. *Ecol. Lett.* 3:362–77
21. Bollag J, Leonowicz A. 1984. Comparative studies of extracellular fungal laccases. *Appl. Environ. Microbiol.* 48:849–54
 22. Bruehl GW. 1987. *Soilborne Plant Pathogens*. New York: Macmillan. 368 pp.
 23. Bull CT, Wadsworth ML, Sorensen KN, Takemoto JY, Austin RK, Smilanick JL. 1998. Syringomycin E produced by biological control agents controls green mold on lemons. *Biol. Control* 12:89–95
 24. Channer AG de R, Gowen SR. 1992. Selection for increased host resistance and increased pathogen specificity in the *Meloidogyne* spp.–*Pasteuria penetrans* interaction. *Fundam. Appl. Nematol.* 15:331–39
 25. Coffey A, Ross RP. 2002. Bacteriophage-resistance systems in dairy starter strains: molecular analysis to application. *Antonie van Leeuwenhoek* 82:303–21
 26. Coley-Smith JR. 1979. Survival of plant pathogenic fungi in soil in the absence of host plants. See Ref. 146a, pp. 39–57
 27. Cook RJ, Baker KF. 1983. *The Nature and Practice of Biological Control of Plant Pathogens*. St. Paul, MN: APS Press. 539 pp.
 28. Cooksey DA, Moore LW. 1982. High frequency spontaneous mutations to Agrocin 84 resistance in *Agrobacterium tumefaciens* and *A. rhizogenes*. *Physiol. Plant Pathol.* 20:129–35
 29. Cooney JM, Lauren DR. 1998. *Trichoderma*/pathogen interactions: measurement of antagonistic chemicals produced at the antagonist/pathogen interface using a tubular bioassay. *Lett. Appl. Microbiol.* 27:283–86
 30. Cooney JM, Lauren DR, di Menna ME. 2001. Impact of competitive fungi on trichothecene production by *Fusarium graminearum*. *J. Agric. Food Chem.* 49:522–26
 31. Cortesi P, McCulloch CE, Song HY, Lin HQ, Milgroom MG. 2001. Genetic control of horizontal virus transmission in the chestnut blight fungus, *Cryphonectria parasitica*. *Genetics* 159:107–18
 32. Cortesi P, Milgroom MG. 1998. Genetics of vegetative incompatibility in *Cryphonectria parasitica*. *Appl. Environ. Microbiol.* 64:2988–94
 33. Crowe JD, Olsson S. 2001. Induction of laccase activity in *Rhizoctonia solani* by antagonistic *Pseudomonas fluorescens* strains and a range of chemical treatments. *Appl. Environ. Microbiol.* 67:2088–94
 34. Cundliffe E. 1989. How antibiotic-producing organisms avoid suicide. *Annu. Rev. Microbiol.* 43:207–33
 35. Czárán TL, Hoekstra RF, Pagie L. 2002. Chemical warfare between microbes promotes biodiversity. *Proc. Natl. Acad. Sci. USA* 99:786–90
 36. Daub ME, Ehrenschaft M. 2000. The photoactive cercospora toxin cercosporin: contributions to plant disease and fundamental biology. *Annu. Rev. Phytopathol.* 38:461–90
 37. Dawe AL, Nuss DL. 2001. Hypoviruses and chestnut blight: exploiting viruses to understand and modulate fungal pathogenesis. *Annu. Rev. Genet.* 35:1–29
 38. de Gives PM, Davies KG, Morgan M, Behnke JM. 1999. Attachment tests of *Pasteuria penetrans* to the cuticle of plant and animal parasitic nematodes, free living nematodes and *srf* mutants of *Caenorhabditis elegans*. *J. Helminthol.* 73:67–71
 39. De Waard MA. 1997. Significance of ABC transporters in fungicide sensitivity and resistance. *Pestic. Sci.* 51:271–75
 40. Di Giorgio D, Camoni L, Mott KA, Takemoto JY. 1996. Syringopeptins, *Pseudomonas syringae* pv. *syringae* phytotoxins, resemble syringomycin in closing stomata. *Plant Pathol.* 45:564–71

41. Donner SC, Jones DA, McClure NC, Rosewarne GM, Tate ME, et al. 1993. Agrocin 434, a new plasmid encoded agrocin from the biocontrol *Agrobacterium strains* K84 and K1026, which inhibits biovar 2 agrobacteria. *Physiol. Mol. Plant Pathol.* 42:185–94
42. Duffy BK, Défago G. 1997. A *Fusarium* pathogenicity factor blocks antibiotic biosynthesis by *Pseudomonas fluorescens* biocontrol strains. *Phytopathology* 87:S26
43. Duffy BK, Défago G. 1997. Zinc improves biocontrol of *Fusarium crown* and root rot of tomato by *Pseudomonas fluorescens* and represses the production of pathogen metabolites inhibitory to bacterial antibiotic biosynthesis. *Phytopathology* 87:1250–57
44. Dutton MV, Evans CS. 1996. Oxalate production by fungi: its role in pathogenicity and ecology in the soil environment. *Can. J. Microbiol.* 42:881–95
45. Eayre CG, Bartz JA, Concelmo DE. 1995. Bacteriophages of *Erwinia carotovora* and *Erwinia ananas* isolated from freshwater lakes. *Plant Dis.* 79:801–4
46. Edens WA, Goins TQ, Dooley D, Henson JM. 1999. Purification and characterization of a secreted laccase of *Gaeumannomyces graminis* var. *tritici*. *Appl. Environ. Microbiol.* 65:3071–74
47. Falk BW, Bruening G. 1994. Will transgenic crops generate new viruses and new diseases? *Science* 263:1395–96
48. Fedi S, Tola E, Moënné Locozy Y, Dowling DN, Smith LM, O'Gara. 1997. Evidence for signaling between the phytopathogenic fungus *Pythium ultimum* and *Pseudomonas fluorescens* F113: *P. ultimum* represses the expression of genes in *P. fluorescens* F113, resulting in altered ecological fitness. *Appl. Environ. Microbiol.* 63:4261–66
49. Fernando WGD, Watson AK, Paulitz TC. 1996. The role of *Pseudomonas* spp. and competition for carbon, nitrogen and iron in the enhancement of appressorium formation by *Colletotrichum coccoodes* on velvetleaf. *Eur. J. Plant Pathol.* 102:1–7
50. Flaherty JE, Jones JB, Harbaugh BK, Somodi GC, Jackson LE. 2000. Control of bacterial spot on tomato in the greenhouse and field with h-mutant bacteriophages. *HortScience* 35:882–84
51. Fleissner A, Sopalla C, Weltring KM. 2002. An ATP-binding cassette multidrug-resistance transporter is necessary for tolerance of *Gibberella pulicaris* to phytoalexins and virulence on potato tubers. *Mol. Plant-Microbe Interact.* 15:102–8
52. Fogliano V, Ballio A, Gallo M, Woo S, Scala F, Lorito M. 2002. *Pseudomonas* lipodepsipeptides and fungal cell wall-degrading enzymes act synergistically in biological control. *Mol. Plant-Microbe Interact.* 15:323–33
53. Forrer HR. 1977. Der Einfluss von Stoffwechselprodukten des Mycoparasiten *Aphanocladium album* auf die Teleutosporenbildung von Rostpilzen. *J. Phytopathol.* 88:306–11
54. Freigoun SO, Elfaki HI, Gelie B, Schirmer M, Lemattre M. 1994. Phage sensitivity in relation to pathogenicity and virulence of the cotton bacterial blight pathogen of Sudan. *Plant Pathol.* 43:493–99
55. Frey P, Frey-Klett P, Garbaye J, Berge O, Heulin T. 1997. Metabolic and genotypic fingerprinting of fluorescent pseudomonads associated with the Douglas fir–*Laccaria bicolor* mycorrhizosphere. *Appl. Environ. Microbiol.* 63:1852–60
56. Gaballa A, Abeyasinghe PD, Ulrich G, Matthijs S, De Greve H, et al. 1997. Trehalose induces antagonism towards *Pythium debaryanum* in *Pseudomonas fluorescens* ATCC 17400. *Appl. Environ. Microbiol.* 63:4340–45
57. Gloer JB. 1995. The chemistry of fungal antagonism and defense. *Can. J. Bot.* 73:S1265–74

58. Govrin EM, Levine A. 2000. The hypersensitive response facilitates plant infection by the necrotrophic pathogen *Botrytis cinerea*. *Curr. Biol.* 10:751–57
59. Griffith GS, Rayner ADM, Wildman HG. 1994. Interspecific interactions and mycelial morphogenesis of *Hypholoma fasciculare* (Agaricaceae). *Nova Hedwigia* 59:47–75
60. Gupta RC, Upadhyay RS, Rai B. 1979. Hyphal parasitism and chlamydospore formation by *Fusarium oxysporum* on *Rhizoctonia solani*. *Mycopathologia* 67:147–51
61. Gurusiddaiah S, Weller DM, Sarkar A, Cook RJ. 1986. Characterization of an antibiotic produced by a strain of *Pseudomonas fluorescens* inhibitory to *Gaeumannomyces graminis* var. *tritici* and *Pythium* spp. *Antimicrob. Agents Chemother.* 29:488–95
- 61a. Haas D, Keel C, Reimann C. 2002. Signal transduction in plant-beneficial rhizobacteria with biocontrol properties. *Antonie van Leeuwenhoek* 81:385–95
62. Handelsman J, Stabb EV. 1996. Biocontrol of soilborne plant pathogens. *Plant Cell* 8:1855–69
63. Harish S, Manjula K, Podile AR. 1998. *Fusarium udum* is resistant to the mycolytic activity of a biocontrol strain of *Bacillus subtilis* AF 1. *FEMS Microbiol. Ecol.* 25:385–90
64. Harris AR, Lumsden RD. 1997. Interactions of *Gliocladium virens* with *Rhizoctonia solani* and *Pythium ultimum* in non-sterile potting medium. *Biocontrol Sci. Technol.* 7:37–47
65. Hayashi K, Schoonbeek H, Sugiura H, De Waard MA. 2001. Multidrug resistance in *Botrytis cinerea* associated with decreased accumulation of the azole fungicide oxpoconazole and increased transcription of the ABC transporter gene *BcatrD*. *Pestic. Biochem. Physiol.* 70:168–79
66. He H, Silo-Suh LA, Clardy J, Handelsman J. 1994. Zwittermycin A, an antifungal and plant protection agent from *Bacillus cereus*. *Tetrahedron Lett.* 35:2499–502
67. Heeb S, Haas D. 2001. Regulatory roles of the GacS/GacA two-component system in plant-associated and other gram-negative bacteria. *Mol. Plant-Microbe Interact.* 14:1351–63
68. Heiniger U, Rigling D. 1994. Biological control of chestnut blight in Europe. *Annu. Rev. Phytopathol.* 32:581–99
69. Holt RD, Hochberg ME. 1997. When is biological control evolutionarily stable (or is it)? *Ecology* 78:1673–83
70. Howell CR. 2003. Mechanisms employed by *Trichoderma* species in the biological control of plant diseases: the history and evolution of current concepts. *Plant Dis.* 87:4–10
71. Ji P, Wilson M. 2002. Assessment of the importance of similarity in carbon source utilization profiles between the biological control agent and the pathogen in biological control of bacterial speck of tomato. *Appl. Environ. Microbiol.* 68:4383–89
72. Jones DA, Ryder MH, Clare BG, Farrand SK, Kerr A. 1988. Construction of a *Tr*-deletion mutant of pAgK84 to safeguard the biological control of crown gall. *Mol. Gen. Genet.* 212:207–14
73. Jones RW, Hancock JG. 1988. Mechanism of gliotoxin action and factors mediating gliotoxin sensitivity. *J. Gen. Microbiol.* 134:2067–75
74. Jones RW, Pettit RE. 1987. Variation in sensitivity among anastomosis groups in *Rhizoctonia solani* to the antibiotic gliotoxin. *Plant Dis.* 71:34–36
75. Keel C, Ucurum Z, Michaux P, Adrian M, Haas D. 2002. Deleterious impact of a virulent bacteriophage on survival and biocontrol activity of *Pseudomonas fluorescens* strain CHA0 in natural soil. *Mol. Plant-Microbe Interact.* 15:567–76
76. Keel C, Weller DM, Natsch A, D efago G,

- Cook RJ, Thomashow LS. 1996. Conservation of the 2,4-diacetylphloroglucinol biosynthesis locus among fluorescent *Pseudomonas* strains from diverse geographic locations. *Appl. Environ. Microbiol.* 62:552–63
77. Kim MJ, Choi JW, Park SM, Cha BJ, Yang MS, Kim DH. 2002. Characterization of a fungal protein kinase from *Cryphonectria parasitica* and its transcriptional upregulation by hypovirus. *Mol. Microbiol.* 45:933–41
78. Kimura M, Kaneko I, Komiyama M, Takatsuki A, Koshino H, et al. 1998. Trichothecene 3-*O*-acetyltransferase protects both the producing organism and transformed yeast from related mycotoxins. Cloning and characterization of *Tri101*. *J. Biol. Chem.* 273:1654–61
79. Kinderlerer JL. 1993. Fungal strategies for detoxification of medium chain fatty acids. *Int. Biodeter. Biodegr.* 32:213–24
80. Koc NK, Forrer HR, Défago G. 1983. Hyperparasitism of *Aphanocladium album* on aecidiospores and teliospores of *Puccinia graminis* f.sp. *tritici*. *J. Phytopathol.* 107:219–23
81. Lawrey JD. 2000. Chemical interactions between two lichen-degrading fungi. *J. Chem. Ecol.* 26:1821–31
82. Lee SW, Cooksey DA. 2000. Genes expressed in *Pseudomonas putida* during colonization of a plant-pathogenic fungus. *Appl. Environ. Microbiol.* 66:2764–72
83. Leifert C, Li H, Chidburee S, Hampson S, Workman S, et al. 1995. Antibiotic production and biocontrol activity by *Bacillus subtilis* CL27 and *Bacillus pumilus* CL45. *J. Appl. Bacteriol.* 78:97–108
84. Lenne JM, Parberry DG. 1976. Phyllosphere antagonists and the appressorium formation in *Colletotrichum gleosporioides*. *Trans. Br. Mycol. Soc.* 66:334–35
85. Leverenz B, Conway WS, Alavidze Z, Janisiewicz W, Fuchs Y, et al. 2001. Examination of bacteriophage as a biocontrol method for *Salmonella* on fresh-cut fruit: a model study. *J. Food Protect.* 64:1116–21
86. Levy E, Eyal Z, Chet I, Hochman A. 1992. Resistance mechanisms of *Septoria tritici* to antifungal products of *Pseudomonas*. *Physiol. Mol. Plant Pathol.* 40:163–71
87. Lewis JA, Lumsden RD. 1995. Do pathogenic fungi have the potential to inhibit biocontrol fungi? *J. Phytopathol.* 143:585–88
88. Li H, Leifert C. 1994. Development of resistance in *Botryotinia fuckeliana* (de Bary) Whetzel against the biological control agent *Bacillus subtilis* CL27. *Z. Pflanzenkr. Pflanzenschutz* 101:414–18
89. Ligon JM, Hill DS, Hammer PE, Torke-witz NR, Hofmann D, et al. 2000. Natural products with antifungal activity from *Pseudomonas* biocontrol bacteria. *Pest Manage. Sci.* 56:688–95
90. Deleted in proof
91. Liu Y-C, Milgroom MG. 1996. Correlation between hypovirus transmission and the number of vegetative incompatibility (*vic*) genes different among isolates from natural populations of *Cryphonectria parasitica*. *Phytopathology* 86:79–86
92. Llama-Palacios A, López-Solanilla E, Rodríguez-Palenzuela P. 2002. The *ybiT* gene of *Erwinia chrysanthemi* codes for a putative ABC transporter and is involved in competitiveness against endophytic bacteria during infection. *Appl. Environ. Microbiol.* 68:1624–30
93. Lockwood JL, Filonow AB. 1987. Responses of fungi to nutrient-limiting conditions and to inhibitory substances in natural habitats. In *Advances in Microbial Ecology*, ed. M Alexander, pp. 1–61. New York: Plenum
94. Loper JE, Henkels MD. 1999. Utilization of heterologous siderophores enhances levels of iron available to *Pseudomonas*

- putida* in the rhizosphere. *Appl. Environ. Microbiol.* 65:5357–63
95. Loper JE, Suslow TV, Schroth MN. 1984. Lognormal distribution of bacterial populations in the rhizosphere. *Phytopathology* 74:1454–60
96. López MM, Gorris MT, Salcedo CI, Montojo AM, Miro M. 1989. Evidence of biological control of *Agrobacterium tumefaciens* strains sensitive and resistant to agrocin 84 by different *Agrobacterium radiobacter* strains on stone fruit trees. *Appl. Environ. Microbiol.* 55:741–46
97. Lorito M. 1998. Chitinolytic enzymes and their genes. In *Trichoderma and Gliocladium*, ed. GE Harman, CP Kubicek, 2:73–99. London: Taylor & Francis
98. Lottmann J, Heuer H, de Vries J, Mahn A, Duering K, et al. 2000. Establishment of introduced antagonistic bacteria in the rhizosphere of transgenic potatoes and their effect on the bacterial community. *FEMS Microbiol. Ecol.* 33: 41–49
99. Lugtenberg BJJ, Dekkers L, Bloemberg GV. 2001. Molecular determinants of rhizosphere colonization by *Pseudomonas*. *Annu. Rev. Phytopathol.* 39:461–90
100. Lutz M, Feichtinger G, Défago G, Duffy B. 2003. Mycotoxigenic *Fusarium* and deoxynivalenol production repress chitinase gene expression in the biocontrol agent *Trichoderma atroviridae*. *Appl. Environ. Microbiol.* In press
101. MacDonald WL, Fulbright DW. 1991. Biological control of chestnut blight: use and limitation of transmissible hypervirulence. *Plant Dis.* 75:656–61
102. Mackie AE, Wheatley RE. 1999. Effects and incidence of volatile organic compound interactions between soil bacterial and fungal isolates. *Soil Biol. Biochem.* 31:375–85
103. Mahajan-Miklos S, Tan Man W, Rahme LG, Ausubel FM. 1999. Molecular mechanisms of bacterial virulence elucidated using a *Pseudomonas aeruginosa*-*Caenorhabditis elegans* pathogenesis model. *Cell* 96:47–56
104. Mansuy D. 1998. The great diversity of reactions catalyzed by cytochromes P450. *Comp. Biochem. Physiol. C* 121: 5–14
105. Marré E. 1979. Fusicoccin: a tool in plant physiology. *Annu. Rev. Plant Physiol.* 30:273–88
106. Mazzola M, Cook RJ. 1991. Effects of fungal root pathogens on the population dynamics of biocontrol strains of fluorescent pseudomonads in the wheat rhizosphere. *Appl. Environ. Microbiol.* 57:2171–78
107. Mazzola M, Fujimoto DK, Thomashow LS, Cook RJ. 1995. Variation in sensitivity of *Gaeumannomyces graminis* to antibiotics produced by fluorescent *Pseudomonas* spp. and effect on biological control of take-all of wheat. *Appl. Environ. Microbiol.* 61:2554–59
108. McCracken AR, Swinburne TR. 1979. Siderophores produced by saprophytic bacteria as stimulants of germination of conidia of *Colletotrichum musae*. *Physiol. Plant Pathol.* 15:331–40
- 108a. McSpadden Gardner BB, Fravel DR. 2002. Biological control of plant pathogens: research, commercialization, and application in the USA. Online. *Plant Health Prog.* DOI:10.1094/PHP-2002-0510-01-RV
109. Milgroom MG, Cortesi P. 1999. Analysis of population structure of the chestnut blight fungus based on vegetative incompatibility genotypes. *Proc. Natl. Acad. Sci. USA* 96:10518–23
110. Milner JL, Silo-Suh LA, Lee JC, He H, Clardy J, Handelsman J. 1996. Production of kanosamine by *Bacillus cereus* UW85. *Appl. Environ. Microbiol.* 62:3061–65
111. Milner JL, Stohl EA, Handelsman J. 1996. Zwittermicin A resistance gene

- from *Bacillus cereus*. *J. Bacteriol.* 178: 4266–72
112. Mitchell TK, Chilton WS, Daub ME. 2002. Biodegradation of the polyketide toxin cercosporin. *Appl. Environ. Microbiol.* 68:4173–81
 113. Moore LW, Warren G. 1979. *Agrobacterium radiobacter* strain 84 and biological control of crown gall. *Annu. Rev. Phytopathol.* 17:163–79
 114. Morrissey JP, Osbourn AE. 1999. Fungal resistance to plant antibiotics as a mechanism of pathogenesis. *Microbiol. Mol. Biol. Rev.* 63:708–24
 115. Morrissey JP, Walsh UF, O'Donnell A, Moënné-Loccoz Y, O'Gara F. 2002. Exploitation of genetically modified inoculants for industrial ecology applications. *Antonie van Leeuwenhoek* 81:599–606
 116. Mott KA, Takemoto JY. 1989. Syringomycin, a bacterial phytotoxin, closes stomata. *Plant Physiol.* 90:1435–39
 117. Muhitch MJ, McCormick SP, Alexander NJ, Hohn TM. 2000. Transgenic expression of the *TR101* or *PDR5* gene increases resistance of tobacco to the phytotoxic effects of the trichothecene 4,15-diacetoxyscirpenol. *Plant Sci.* 157: 201–7
 118. Nakaune R, Adachi K, Nawata O, Tomiyama M, Akutsu K, Hibi T. 1998. A novel ATP-binding cassette transporter involved in multidrug resistance in the phytopathogenic fungus *Penicillium digitatum*. *Appl. Environ. Microbiol.* 64:3983–88
 119. Nakaune R, Hamamoto H, Imada J, Akutsu K, Hibi T. 2002. A novel ABC transporter gene, *PMR5*, is involved in multidrug resistance in the phytopathogenic fungus *Penicillium digitatum*. *Mol. Genet. Genomics* 267:179–85
 120. Notz R, Maurhofer M, Dubach H, Haas D, Défago G. 2002. Fusaric acid-producing strains of *Fusarium oxysporum* alter 2,4-diacetylphloroglucinol biosynthetic gene expression in *Pseudomonas fluorescens* CHA0 in vitro and in the rhizosphere of wheat. *Appl. Environ. Microbiol.* 68:2229–35
 121. Notz R, Maurhofer M, Schnider Keel U, Duffy B, Haas D, Défago G. 2001. Biotic factors affecting expression of the 2,4-diacetylphloroglucinol biosynthesis gene *phlA* in *Pseudomonas fluorescens* biocontrol strain CHA0 in the rhizosphere. *Phytopathology* 91:873–81
 122. Nuss DL. 1996. Using hypoviruses to probe and perturb signal transduction processes underlying fungal pathogenesis. *Plant Cell* 8:1845–53
 123. Deleted in proof
 124. Osbourn AE. 1996. Preformed antimicrobial compounds and plant defense against fungal attack. *Plant Cell* 8:1821–31
 125. Osbourn AE, Bowyer P, Daniels MJ. 1996. Saponin detoxification by plant pathogenic fungi. *Adv. Exp. Med. Biol.* 404:547–55
 126. Ownley BH, Weller DM, Thomashow LS. 1992. Influence of in situ and in vitro pH on suppression of *Gaeumannomyces graminis* var. *tritici* by *Pseudomonas fluorescens* 2–79. *Phytopathology* 82: 178–84
 127. Ozawa H, Tanaka H, Ichinose Y, Shiraishi T, Yamada T. 2001. Bacteriophage P4282, a parasite of *Ralstonia solanacearum*, encodes a bacteriolytic protein important for lytic infection of its host. *Mol. Genet. Genomics* 265:95–101
 128. Peever TL, Liu Y, Cortesi P, Milgroom MG, Liu YC. 2000. Variation in tolerance and virulence in the chestnut blight fungus-hypovirus interaction. *Appl. Environ. Microbiol.* 66:4863–69
 129. Peever TL, Liu Y-C, Wang K-R, Hillman BI, Foglia R, et al. 1998. Incidence and diversity of double-stranded RNAs occurring in the chestnut blight fungus, *Cryphonectria parasitica*, in China and Japan. *Phytopathology* 88:811–17
 130. Peñalver R, Vicedo B, Salcedo CI, López MM. 1994. *Agrobacterium radiobacter* strains K84, K1026 and K84 Agr

- produce an antibiotic-like substance, active in vitro against *A. tumefaciens* and phytopathogenic *Erwinia* and *Pseudomonas* spp. *Biocontrol Sci. Technol.* 4:259–67
131. Penyalver R, López MM. 1999. Colonization of the rhizosphere by pathogenic *Agrobacterium* strains and nonpathogenic strains K84 and K1026, used for crown gall biocontrol. *Appl. Environ. Microbiol.* 65:1936–40
132. Penyalver R, Oger P, López MM, Farrand SK. 2001. Iron-binding compounds from *Agrobacterium* spp.: biological control strain *Agrobacterium rhizogenes* K84 produces a hydroxamate siderophore. *Appl. Environ. Microbiol.* 67:654–64
133. Deleted in proof
134. Deleted in proof
135. Pitkin JW, Panaccione DG, Walton JD. 1996. A putative cyclic peptide efflux pump encoded by the *toxA* gene of the plant-pathogenic fungus *Cochliobolus carbonum*. *Microbiology* 142:1557–65
136. Raaijmakers JM, van der Sluis I, Koster M, Bakker PAHM, Weisbeek PJ, Schippers B. 1995. Utilization of heterologous siderophores and rhizosphere competence of fluorescent *Pseudomonas* spp. *Can. J. Microbiol.* 41:126–35
137. Raaijmakers JM, Vlami M, de Souza JT. 2002. Antibiotic production by bacterial biocontrol agents. *Antonie van Leeuwenhoek* 81:537–47
138. Raaijmakers JM, Weller DM. 2001. Exploiting genotypic diversity of 2,4-diacetylphloroglucinol-producing *Pseudomonas* spp.: characterization of superior root-colonizing *P. fluorescens* strain Q8r1-96. *Appl. Environ. Microbiol.* 67:2545–54
139. Rincé A, Dufour A, Uguen P, Le Pennec J-P, Haras D. 1997. Characterization of the lactacin 481 operon: The *Lactococcus lactis* genes *lctF*, *lctE*, and *lctG* encode a putative ABC transporter involved in bacteriocin immunity. *Appl. Environ. Microbiol.* 63:4252–60
140. Rollins JA, Dickman MB. 2001. pH signaling in *Sclerotinia sclerotiorum*: identification of a *pacC/RIM1* homolog. *Appl. Environ. Microbiol.* 67:75–81
141. Ryder MH, Slota JE, Scarim A, Farrand SK. 1987. Genetic analysis of agrocin 84 production and immunity in *Agrobacterium* spp. *J. Bacteriol.* 169:4184–89
142. Santos R, Franza T, Laporte ML, Sauvage C, Touati D, Expert D. 2001. Essential role of superoxide dismutase on the pathogenicity of *Erwinia chrysanthemi* strain 3937. *Mol. Plant-Microbe Interact.* 14:758–67
143. Savka MA, Dessaux Y, Oger P, Rossbach S. 2002. Engineering bacterial competitiveness and persistence in the phytosphere. *Mol. Plant-Microbe Interact.* 15:866–74
144. Schisler DA, Howard KM, Bothast RJ. 1991. Enhancement of disease caused by *Colletotrichum truncatum* in *Sesbania exaltata* by coinoculating with epiphytic bacteria. *Biol. Control* 1:261–68
145. Schnabel EL, Jones AL. 2001. Isolation and characterization of five *Erwinia amylovora* bacteriophages and assessment of phage resistance in strains of *Erwinia amylovora*. *Appl. Environ. Microbiol.* 67:59–64
146. Schnider-Keel U, Seematter A, Maurhofer M, Blumer C, Duffy B, et al. 2000. Autoinduction of 2,4-diacetylphloroglucinol biosynthesis in the biocontrol agent *Pseudomonas fluorescens* CHA0 and repression by the bacterial metabolites salicylate and pyoluteorin. *J. Bacteriol.* 182:1215–25
- 146a. Schippers B, Gams W, eds. 1979. *Soil-borne Plant Pathogens*. New York: Academic
147. Schoonbeek H, Del Sorbo G, De Waard MA. 2001. The ABC transporter *BcatrB* affects the sensitivity of *Botrytis cinerea* to the phytoalexin resveratrol

- and the fungicide fenpiclonil. *Mol. Plant-Microbe Interact.* 14:562–71
148. Schoonbeek HJ, Raaijmakers JM, De Waard MA. 2002. Fungal ABC transporters and microbial interactions in natural environments. *Mol. Plant-Microbe Interact.* 15:1165–72
 149. Scott JG. 1999. Cytochromes P450 and insecticide resistance. *Insect Biochem. Mol. Biol.* 29:757–77
 150. Séveno NA, Kallifidas D, Smalla K, van Elsas JD, Collard JM, et al. 2002. Occurrence and reservoirs of antibiotic resistance genes in the environment. *Rev. Med. Microbiol.* 13:15–27
 151. Silo-Suh LA, Lethbridge BJ, Raffel SJ, He H, Clardy J, Handelsman J. 1994. Biological activities of two fungistatic antibiotics produced by *Bacillus cereus* UW85. *Appl. Environ. Microbiol.* 60:2023–30
 152. Slade SJ, Swinburne TR, Archer SA. 1986. The role of a bacterial siderophore and of iron in the germination and appressorium formation by conidia of *Colletotrichum acutatum*. *J. Gen. Microbiol.* 132:21–26
 153. Slininger PJ, Shea-Wilbur MA. 1995. Liquid-culture pH, temperature, and carbon (not nitrogen) source regulate phenazine productivity of the take-all biocontrol agent *Pseudomonas fluorescens* 2–79. *Appl. Microbiol. Biotechnol.* 37:388–92
 154. Smith LM, Tola E, deBoer P, O’Gara F. 1999. Signalling by the fungus *Pythium ultimum* represses expression of two ribosomal RNA operons with key roles in the rhizosphere ecology of *Pseudomonas fluorescens* F113. *Environ. Microbiol.* 1:495–502
 155. Souza JT de. 2002. *Distribution, diversity, and activity of antibiotic-producing Pseudomonas spp.* PhD. thesis. Wageningen Univ., The Netherlands
 156. Souza JT de, Weller DM, Raaijmakers JM. 2003. Frequency, diversity, and activity of 2,4-diacetylphloroglucinol-producing fluorescent *Pseudomonas* spp. in Dutch take-all decline soils. *Phytopathology* 93:54–63
 157. Stahl PD, Parkin TB. 1999. Microbial production of volatile organic compounds in soil microcosms. *Soil Sci. Soc. Am. J.* 60:821–28
 158. Steffens JJ, Pell EJ, Tien M. 1996. Mechanisms of fungicide resistance in phytopathogenic fungi. *Curr. Opin. Biotechnol.* 7:348–55
 159. Stergiopoulos I, Zwiers LH, De Waard MA. 2002. Secretion of natural and synthetic toxic compounds from filamentous fungi by membrane transporters of the ATP-binding cassette and major facilitator superfamily. *Eur. J. Plant Pathol.* 108:719–34
 160. Stockwell VO, Kawalek MD, Moore LW, Loper JE. 1996. Transfer of pAgK84 from the biocontrol agent *Agrobacterium radiobacter* K84 to *A. tumefaciens* under field conditions. *Phytopathology* 86:31–37
 161. Stohl EA, Milner JL, Handelsman J. 1999. Zwittermicin A biosynthetic cluster. *Gene* 237:403–11
 162. Stormer FC, Sandven P, Huitfeldt HS, Eduard W, Skogstad A. 1998. Does the mycotoxin citrinin function as a sun protectant in conidia from *Penicillium verucosum*? *Mycopathologia* 142:43–47
 163. Summers WC. 2001. Bacteriophage therapy. *Annu. Rev. Microbiol.* 55:437–51
 164. Tanaka C, Tajima S, Furusawa I, Tsuda M. 1992. The *PrgI* mutant of *Cochliobolus heterostrophus* lacks a *p*-diphenol oxidase involved in naphthalenediol melanin synthesis. *Mycol. Res.* 96:959–64
 165. Toth IK, Mulholland V, Cooper V, Bentley S, Shih YL, et al. 1997. Generalized transduction in the potato blackleg pathogen *Erwinia carotovora* subsp. *atroseptica* by bacteriophage phiM1. *Microbiology* 143:2433–38
 166. Tovkach FI. 2002. A study of *Erwinia*

- carotovora* phage resistance with the use of temperate bacteriophage ZF40. *Microbiology* 71:72–77
167. Tschudi S, Kern H. 1979. Specific lysis of the mycelium of *Gaeumannomyces graminis* by enzymes of *Streptomyces lavendulae*. See Ref. 146a, pp. 611–15
168. Tzortzakakis EA, Gowen SR, Goumas DE. 1996. Decreased ability of *Pasteuria penetrans* spores to attack to successive generations of *Meloidogyne javanica*. *Fundam. Appl. Nematol.* 19: 201–4
169. Upchurch RG, Rose MS, Eweida M. 2001. Over-expression of the cercosporin facilitator protein, CFP, in *Cercospora kikuchii* up-regulates production and secretion of cercosporin. *FEMS Microbiol. Lett.* 204:89–93
170. Upchurch RG, Rose MS, Eweida M, Callahan TM. 2002. Transgenic assessment of CFP-mediated cercosporin export and resistance in a cercosporin-sensitive fungus. *Curr. Genet.* 41:25–30
171. Vajna L. 1985. Phytopathogenic *Fusarium oxysporum* Schlecht as a necrotrophic mycoparasite. *J. Phytopathol.* 114:338–47
172. Van Eck WH. 1978. Chemistry of cell walls of *Fusarium solani* and the resistance of spores to microbial lysis. *Soil Biol. Biochem.* 10:155–57
173. Deleted in proof
174. Deleted in proof
175. van Zyl FGH, Strijdom BW, Staphorst JL. 1986. Susceptibility of *Agrobacterium tumefaciens* strains to two agrocin-producing *Agrobacterium* strains. *Appl. Environ. Microbiol.* 52:234–38
176. Van Etten H, Temporini E, Wasmann C. 2001. Phytoalexin (and phytoanticipin) tolerance as a virulence trait: Why is it not required by all pathogens? *Physiol. Mol. Plant Pathol.* 59:83–93
177. Venisse J-S, Gullner G, Brisset M-N. 2001. Evidence for the involvement of an oxidative stress in the initiation of infection of pear by *Erwinia amylovora*. *Plant Physiol.* 125:2164–72
178. Vermeulen T, Schoonbeek H, De Waard MA. 2001. The ABC transporter *BcatrB* from *Botrytis cinerea* is a determinant of the activity of the phenylpyrrole fungicide fludioxonil. *Pest Manage. Sci.* 57:393–402
179. Vicedo B, Peñalver R, Asins MJ, López MM. 1993. Biological control of *Agrobacterium tumefaciens*, colonization, and pAgK84 transfer with *Agrobacterium radiobacter* K84 and the Tr mutant strain K1026. *Appl. Environ. Microbiol.* 59:309–15
180. Vidaver AK. 1976. Prospects for control of phytopathogenic bacteria by bacteriophages and bacteriocins. *Annu. Rev. Phytopathol.* 14:451–65
181. Voisard C, Keel C, Haas D, Défago G. 1989. Cyanide production by *Pseudomonas fluorescens* helps suppress black root rot of tobacco under gnotobiotic conditions. *EMBO J.* 8:351–58
182. Völsch B, Weingart H. 1998. Toxin production by pathovars of *Pseudomonas syringae* and their antagonistic activities against epiphytic microorganisms. *J. Basic Microbiol.* 38:135–45
183. Wang CL, Farrand SK, Hwang I. 1994. Organization and expression of the genes on pAgK84 that encode production of agrocin 84. *Mol. Plant-Microbe Interact.* 7:472–81
184. Deleted in proof
185. Weller DM. 1988. Biological control of soilborne pathogens in the rhizosphere with bacteria. *Annu. Rev. Phytopathol.* 26:379–407
186. Weller DM, Raaijmakers JM, Gardener BBM, Thomashow LS. 2002. Microbial populations responsible for specific soil suppressiveness to plant pathogens. *Annu. Rev. Phytopathol.* 40:309–48
187. Wheatley RE. 2002. The consequences of volatile organic compound mediated

- bacterial and fungal interactions. *Antonie van Leeuwenhoek* 81:357–64
188. Wheatley RE, Millar SE, Griffiths DW. 1996. The production of volatile organic compounds during nitrogen transformations in soils. *Plant Soil* 181:161–63
189. Whipps JM. 1997. Developments in the biological control of soil-borne plant pathogens. *Adv. Bot. Res.* 26:1–134
190. Whipps JM. 2001. Microbial interactions and biocontrol in the rhizosphere. *J. Exp. Bot.* 52:487–511
191. Wiebe MG. 2002. Siderophore production by *Fusarium venenatum* A3/5. *Biochem. Soc. Trans.* 4:696–98

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ERRATA

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