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Importancia de los metabolitos secundarios de plantas y hongos en las enfermedades de las plantas

ABSTRACT

Importance of Plant and Fungal Secondary Metabolites in Plant Diseases

The processes that follow the encounter of a fungal pathogen with a potential plant host are remarkably complex. Non-hosts must be discarded at once and hosts must be chosen carefully at the stage they are vulnerable to attack. Whether the invader is a necrotroph or a biotroph, these actions must be performed on a timely basis. While the targeted host is displaying defensive strategies, germinating and developing the subsequent structures for infection will cost the potential pathogen to fail. Somehow the infective units must recognize its host, avoid the initial defensive barriers and start developing the appropriate infection processes. Environmental factors seem to play a definitive role in the interplay, not only by providing the general conditions for germination, but also by doing so at the adequate time. Once the infection unit germinates, the following task is to encounter a site for penetration and this usually involves a delicate physical and chemical signaling process between the germination unit and the host epidermis. After penetration, the invader must find ways to reach the target cells, either by dissolving the host cell wall or by developing structures to further invade and spread within the host tissue; then the pathogen reaches the site where nutrients are stored to establish firmly originating the disease. On the other hand the host, passively or actively displays mechanisms of defense. Passive mechanisms like physical barriers or pre-formed compounds are common to plants. Active mechanisms, like the turning up of normal and secondary biosynthetic processes to counterattack the invader, are also observed. Different compounds, for attacking as well as for defending, are also found. Some of them are unique to genera, species, varieties, forms and even strains of plants and/or fungi. Regarded as not common to all organisms (not necessary for life) these are'"secondary" compounds, but only in the sense of distinguishing them from the otherwise common "primary" metabolites. The role of secondary metabolites in pathogen-host interactions has been proved remarkably for several fungal plant diseases. The purpose of this review is to describe and present some examples pertaining to their importance for plant diseases.

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RESUMEN

Los procesos que siguen al encuentro de un hongo fitopatógeno con su hospedero potencial son extraordinariamente complejos.

Las especies fuera del rango de hospederos deben ser descartadas inmediatamente y los hospederos potenciales deben ser escogidos coincidiendo con el correcto estado de desarrollo durante el cual éstos son vulnerables. Independientemente del hábito de crecimiento del patógeno (necrotrófico o biotrófico), el proceso de infección debe ser operado con base en el tiempo. La germinación y el desarrollo de las unidades infectivas mientras el hospedero está desplegando sus mecanismos de defensa llevarían al patógeno a su desaparición. De alguna manera, la unidad infectiva del patógeno debe reconocer a su hospedero, eludir las barreras iniciales de defensa e iniciar el proceso de infección. Las condiciones ambientales juegan un papel importante durante el intercambio hospedero-patógeno. La germinación de la unidad infectiva es determinada por el ambiente y debe coincidir con el momento más adecuado para alcanzar el sitio de penetración. El proceso ulterior induce un delicado sistema de intercambio de señales físicas y químicas entre la unidad de germinación y la epidermis del hospedero potencial. Una vez ocurrida la penetración, el patógeno procede a ubicar las vías y a desarrollar los medios adecuados para llegar hasta las células apropiadas para su desarrollo; bien sea disolviendo enzimáticamente la pared celular del tejido del hospedero o generando la turgencia necesaria para penetrar la epidermis, el patógeno se disemina intra o extracelularmente hasta alcanzar las células de donde derivará su sustento, causando la infección. Durante el proceso de infección, el hospedero desplegará mecanismos pasivos y activos de defensa. Mecanismos pasivos como barreras físicas o configuraciones especializadas en el tejido epidermal, o la presencia de compuestos fito-patogénicos en las células sujetas a la invasión son comunes entre las plantas. Respuestas activas incluyen la producción de sustancias fungi-tóxicas al encuentro con patógenos potenciales, producto de metabolismos primarios y secundarios. Se han estudiado diferentes compuestos desarrollados tanto para el ataque del patógeno, como para la defensa del hospedero. Algunos de ellos son únicos entre ciertos géneros, especies, variedades, formas y aún cepas específicas en plantas y hongos. El hecho de no ser esenciales para la vida de las plantas y de los hongos en general, les ha valido su denominación de secundarios. Su extraordinario papel en el conjunto de procesos que gobiernan las relaciones hospedero-patógeno ha sido demostrada en varios sistemas en particular y esta revisión se propone, con ejemplos, demostrar su importancia para el mejor entendimiento de las enfermedades de las plantas.

INTRODUCTION

Secondary metabolites are all those compounds that do not form from the major metabolic pathways of all organisms (Stoessl, 1983). While in primary metabolism reactions are similar in all groups of living organisms and are characteristic for life in earth, a number of metabolic pathways lead to the formation of peculiar chemical compounds. The distinction between primary and secondary compounds has been largely based on knowledge about their function and distribution in nature. Primary metabolites have known function in cellular structure, energy metabolism, and regulation of metabolism or are intermediates in their biosynthesis, and are widely distributed (Griffin, 1994). Several features characteristic of secondary compounds are: a taxonomically restricted distribution; formation by specific enzymes from specific genes; compartmentalization of pre-

cursors, enzymes, intermediates and products; biosynthetic control by regulation of enzyme dosage and activity; expression as an aspect of cell specialization, differentiation and development; derivation of diverse chemical structures from products of primary metabolism; apparently lack of importance for the cell life but regarded as important for the organism. Though not necessarily all the secondary compounds are restricted to a few members in a given group and few enzymes primarily involved in main metabolic pathways are also utilized (*cysteine synthase*, p.e.), the described characteristics are still maintained for simplicity. Nevertheless, there have been many attempts to re-formulate the definition since initially proposed by Kossel in 1891 (cited by (Luckner, 1990)). Terms like "special", "natural", "excessive", "idiolyts", "luxury", "allelochemicals" among others, have been subsequently disregarded as not being precise enough. Particularly interesting from the discussion are all the attempts made to avoid separations between "primary" and "secondary" fields in metabolism. The term "secondary" is yet in general use, although it remains with a feeling of an unfortunate choice of words. The importance that these compounds have to the ecology of several organisms has been widely recognized.

One striking feature of secondary metabolisms is the number of compounds with enormously diverse chemical structure found. While several hundreds of primarily derived low molecular weight products are known so far, tens of thousands of secondary metabolites have been already described. This figure is constantly increasing due to the refinement of analytical techniques and the growing number of microorganisms, plants and animals involved nowadays. Plants and fungi are amongst the best well known producers of secondary metabolites. Several medical, agricultural and industrial processes rely upon the release of specific compounds like in the production of antibiotics, herbicides, preservatives, colorants and perfumes. Plants compete with others for moisture, light and nutrients by the aid of secondary products brought to serve on chemical interactions (allelopathy). Compounds that act as grazer deterrents and protectants against microbial infections are also well known. Secondary compounds intervening in plant protection against microbial infections have been recognized to function as pre-infectious barriers, pre-infectious toxins, prototoxins and phytoalexins. Cell wall polysaccharides, alkanes, alkenes, alkanols, wax, cutin, suberin and lignins accumulate as pre-infectious barriers to impede the entrance to pathogens.

Toxic compounds accumulate in the extra cellular space (essential oils) as well as in the cell itself, or cover the leaf surface and other over-ground parts (glucosinolates in *Brassica* spp.) that function as invader deterrents. Amongst the diverse array of plant secondary metabolites with antifungal activity there are those that are constitutive (existing in healthy plants) while others occur as inactive precursors and are activated in response to tissue damage or pathogen attack. Toxic compounds are formed during infection by breakdown of compartmentalized nontoxic substances named prototoxins (glycosidases and oxidases) while Phytoalexins are plant antibiotics formed after infection by de *novo* synthesis or by synthesis rate increase on pre-formed compounds, which function as to "ward off" the intruder. Synthesis is triggered by physical or chemical signals produced at stress as in wounding, but also by certain peptide and polysaccharide fractions (elicitors) constitutive of the pathogen infective units or the plant cell wall. The vast majority of secondary chemical compounds involved in defense are highly lipophilic phenolics with *in planta* ability to mask OH- groups by methylation or formation of methylenodioxy rings. A variety of fungal secondary metabolites with the capability to damage or kill plants are employed to help in the infection process: compounds with hormone activity modify plant metabolism and produce abnormal growth (gibberelins); host selective and non-specific phytotoxins which are poisonous to the plant; products with the ability to help infective units to trace host plants or stimulate germination; compounds that help on detoxification of defense compounds produced by the plants (Ward, 1986; Luckner, 1990).

Plant compounds

Structural characteristics provide plants with a first line of defense against pathogens, although not powerful enough like chemical substances formed in the cells before and after the infection. The different reactions observed from varieties inoculated with a virulent strain is a good indication of differences that go beyond the limits displayed by the external surface. So are the reduction in the infection rate on resistant varieties and the absence of infection in non-hosts. Several plant species release exudates with fungitoxic effects from their parts above the ground and the roots. These function as inhibitors, like in tomato and sugar beet leaves against species of *Botrytis* and *Cercospora*. Others, like red onions, release phenolic compounds (protocatechuic acid and catechol) against *Colletotrichum circinans* (the causal organism of onion smudge), which are absent on susceptible white scale onions. Phenolic tannins and fatty acid-like dienes in young fruits, leaves or seeds of several species seem to have an active role against *Botrytis*. Glycosylated steroidal, or triterpenoid saponins (tomatin, avenacin) in tomato and oats, have fungal membranolytic activity capable to kill or exclude fungi lacking the enzymes for the breakdown of saponin (Agrios, 1997).

Preinfectional inhibitors (secondary metabolites in passive defense)

A diverse group of secondary compounds with antifungal activity are constitutive. Some of them are in biologically active forms in healthy plants. Others are activated from inactive precursors in response to pathogen infection or tissue damage. Activation is caused by enzyme involvement released after breakdown of the cell integrity. The term "phytoanticipin" has been proposed to separate this group from the "phytoalexins". Differences between the two groups are defined based upon their origin and synthesis. "Phytoalexins" are regarded as low molecular weight antimicrobial compounds, which are synthesized de novo after infection from remote precursors with expenditure of plant energy. While "phytoanticipins" are antimicrobial compounds present in plants (in active form and effective concentration) before challenge or produced solely after infection from preexisting constituents (VanEtten *et al.*, 1994). However, no matter how precise the definitions are, the boundaries remain diffused as in the case of pterocarpens and stilbens which exist as phytoalexins in fruits, leaves and other aerial tissues but also as normal constitutes in lignified tissue and roots.

Probably transformed from pre-existing compounds in roots as a reaction to challenge by rhizosphere microorganisms or in heartwood after tissue death. Others, such as gossypol is a phytoalexin in the xylem tissue of cotton, but also a normal major constituent of its pigmented glands. But an even more experimentally intriguing situation has been observed on several compounds that increase on infection

after small changes and are more toxic to some fungi before but to others only after their concentrations increase. A probably better logically defined situation would be to regard these compounds as factors in static or dynamic resistance mechanisms. Nevertheless, there are reasons to think that dynamic defense is much more important than static defense as pointed out by the observation that sapwood with little inhibitory compounds is less prone to fungal diseases than heartwood which contains more antifungal factors in living trees (Stoessl, 1983).

Plant preformed inhibitors are generally tissue specific and concentrate in the outer layers of plant organs, confined into vacuoles or organelles. Exposure of the invading organism to damaging doses will depend on the extent of injury caused to the cell. Biotrophs would be able to avoid exposure by minimizing cell damage while necrotrophs would be less advantageous at this scenario. Compound concentration will also depend on the organ involved, its developmental stage, its age, the host genotype and the environmental conditions. Such scenario diversity has led to discourage attempts to associate variations in preformed inhibitors with resistance specificity. The results so far attained have not been conclusive. Although proved effective against a broad spectrum of potential pathogens, successful infection has proven to be accomplished by avoidance, tolerance or detoxification by the invaders (Osbourn, 1996; VanEtten *et al.*, 1995).

A wide array of plant-preformed metabolites with antifungal activity has been reported. More commonly cited are phenols, phenolic glycosides, unsaturated lactones, sulphur compounds, saponins, cyanogenic glycosides, glucosinolates and more recently dienes and 5-alkylated resorcinols. Based upon the metabolic pathway of origin these can be classified as Shikimatic acid derivatives, tricarboxilic acid derivatives, fatty acid derivatives (acetate-polymalonate route) and mevalonic acid derivatives, as summarized in Table 1.

Although the list of secondary compounds with suspected antifungal activity is considerable, only a few of them have

Table 1. Plant preformed fungal inhibitory secondary plant metabolites (compiled from Stoessl, 1983 and Luckner, 1990).

Metabolic origin	Product	Source and general properties
Shikimic acid		
Simple phenols	Gallic acid	Toxic to the red maple decay fungus
Lignans	Pinoresinol	From conifers and tropical woods
	Hordatines	In *Gramineae* enzyme inhibitor, affect fungal morphology
Stilbenoids	Stilbenes	Pre- and postinfectional compounds, have plant growth regulating and photosynthesis inhibition properties
	Stilben oligomers	Viniferins, constitutive in lignified tissue
	Phenanthrenoids	In *Leguminosae*, *Moraceae* and orchid species
	Benzofurans	Epidermal components in shoots of *Moraceae* species with minor antifungal properties
Flavonoids	Isoflavonoids and neoflavonoids	Ubiquitous, many active against fungal lytic enzymes and generally subject to metabolic turn-over
Naphthoquinones	Juglon	From walnut
Nitrogen-containing aromatics	Capsaicinoids	In red peppers, degraded by *Aspergillus niger*
	Benzoxazinone glucosides	From *Gramineae*, positive against *Cochliobolus* (*Helminthosporium*) *turcicum* but negative to *C. sativum*
Tricarboxilic acid	Juglone	Succinic acid precursor
	Tulipalins	In tulip bulbs against *Fusarium oxysporum*
Fatty acid		
Podoblastins	Podoblastin A	From *Podophyllum peltatum* against *Pyricularia grisea*
Olefins	Triticenes	In wheat
	Diene esters	Suspected involvement on latency of *Colletotrichum gloeosporoides*
Polyacetylenes	Falcarindiol	In *Araliaceae* leaves, young shoots of *Umbelliferae* and carrot roots (apparently induced in roots by contact to rhizosphere organisms)
Mevalonic acid		
Monoterpenes	Pinene, humulene	Volatile antifungal, from glandular hairs in tomato (but growth stimulant in *Pinus radiata*)
Sesquiterpenes	Ionone	In tobacco, depress conidiophore formation in virulent but not in normal strains of *Peronospora tabacina* if applied to cotyledons before inoculation; the reverse when applied after inoculation
Diterpenes	Gossypol	From cotton.
	Sclareol	From tobacco leaves against *Peronospora* but also phytotoxic
Triterpenoids and Steroids	Tomatine	Solanaceous alkaloids that cause electrolytic leakage to *Cladosporium fulvum*, inhibit growth and germination of *Fusarium oxysporum* but also stimulates its sporulation
	Solanine	Apparent assimilation and/or synthesis by *Phytophtora infestans*
	Avenacosides	In oat leaves

been studied extensively. Among these are the saponins, the cyanogeic glycosides and the glucosinolates. Saponins are widely distributed glycosilated compounds, which include triterpenoids, steroids or steroidal glycoalkaloids. Most triterpenoid saponins are found in dicotyledonous plants while the steroids are mainly from monocotyledonous. Oats contain both of them and a group of steroidal glycoalkaloids is found in members of the Solanaceae family. The genus Avena concentrate triterpenoid avenacins in the root epidermal cells while the steroidal avenacosides are found in the epidermis of leaves and shoots. Avenacosides are activated to antifungal compounds by hydrolases which cleavage one of their D-glucose molecules (Osbourn, 1996).

Attention to avenacins is derived from their interactions with the fungus *Gaeumannomyces graminis var. tritici,* the causal organism of "take all" disease. Severe yield losses caused by infections with this fungus are observed in wheat and barley but not in oat. Additionally, the strain of G. *graminis* attacking oat (var. *avenae*) is less sensitive to avenacins. One oat species, *Avena longiglumis* lacks avenacin and is also susceptible to *G. graminis var. tritici.* Unfortunately, this species does not hybridize well with other oat species that produce avenacin to test the co-segregation of avenacin inheritance with resistance to *G. graminis var. tritici.* Mutants of A.. strigosa lacking avenacin have been identified and their increased susceptibility to both G. graminis isolates, but also to some *Fusarium* species have been initially confirmed.

Fungi like *Cladosporium fulvum* infects species that accumulate saponin and is believed to grow intercellularly, as a developed means to avoid contact by the toxin. Saponins are toxic to fungi because of their ability to produce pores in the intruder hyphae after forming complexes with membrane sterols. This may be a reason for *Pythium* and *Phytophtora* to have evolved having little sterol. The membraneolytic activity is pH dependant in the steroidal glycoalkaloid α-tomatine. *Alternaria solani* acts apparently to lower the pH at the infection site. *Fusarium solani* sterol deficient mutants have showed increased resistance to the steroidal glycoalkaloid a-tomatine and have developed ability to infect tomatoes. Segregation analysis with crosses between these mutants and the wild type have shown that pathogenicity, low sterol contents and insensitivity are inherited together. Avecinase, a glycosil hydrolase saponin-detoxifying enzyme, has shown to be essential for the interaction between oats and *G. graminis* var. *avenae.* Enzyme deficient mutants obtained by gene disruption were increasingly sensitive to avenacin and no longer infected saponin-containing oats. However, pathogenicity towards wheat, the alternate non-containing saponin host, was retained. The ability to detoxify preformed inhibitory compounds appeared to be host dependant but not a general pathogenicity factor (Osbourn, 1996; Bowyer *et al.*, 1995).

Hydrocyanic acid (HCN) is toxic to most organisms functioning as a respiratory poison. Although formed in a wide array of plants, HCN is not at detectable levels in healthy plant tissues but released after degradation of cyanogenic glycoside precursors by hydrolytic enzymes in response to wounding. Cyanoglycosides are found often compartmentalized in outer cells layers of plant tissues. Cyanogenic glycosides are responsible for the toxicity of bitter almonds and other rosaceous seeds that contain amygdalin. Linamarin and lotaustralin are found in cassava, flax, rubber, lima bean, white clover and bird's-foot trefoil besides a number of cereals including sorghum that also produces dhurrin. There is considerable evidence that HCN may act as deterrent for grazing animals but its role in fungal pathogen protection has proven not so clear. Rubber trees (*Hevea brasiliensis*) high cyanogenic varieties appear more susceptible to fungal attack than low cyanogen containing varieties in their interaction with *Microcyelus ulei.* This reaction has apparently originated from inhibition of active defense responses like the production of the phytoalexin scopolin. In addition, *M. ulei* has shown ability to tolerate HCN.

Fungi, including saprophytes, with HCN tolerance are able to synthesize the cyanide-inducible enzyme cyanide hydratase. The sorghum pathogen *Gloeocercospora sorghi* is a well-known cyanide hydratase synthesizer on which the responsible gene has been cloned. Transformation-mediated disrupted mutants of *G. sorghi* lacked enzyme activity and showed sensitivity to HCN *in vitro.* However, the mutants remained fully pathogenic to their cyanogenic host. As the possibility for alternative detoxifying enzymes expressed only *in planta* was ruled out from the test, alternative means of tolerance like cyanide-resistant respiration could account for the results (Osbourn, 1996; VanEtten *et al.*, 1995; Poulton and Chun, 1994).

Glucosinolates are found among members of the family *Cruciferae* like in the genus *Brassica* and in the weed Arabidopsis. These are sulphur-containing glucosides with known functions as feeding deterrents to vertebrates and pests. Three major classes of glucosinolates are recognized according to the nature of their side chains. Glucosinolates are tissue specific within individual plants and become active in response to wounding. Highly reactive products like volatile isothiocyanates (mustard oils), nitriles and thiocynates are formed from an unstable aglycone generated by the action of a thioglucosidase enzyme on the glucosinolates. In addition to their fungitoxicity, the isothiocyanates may be used as auxin precursors by fungi infecting *Brassicas*, like the root gall fungus *Plasmodiophora brassicae* that causes abnormal growth. The *Brassica* pathogens *Leptosphaeria maculans*, *Peronospora parasitica*, *Mycosphaerella brassicae* and *Alternaria sp.* have been reported to be sensitive to glucosinolate breakdown toxins (Osbourn, 1996).

Postinfectional inhibitors (secondary metabolites in active response)

Normally absent in all but negligible traces in living tissue, a group of secondary compounds accumulate in the course and as a specific consequence of microorganism contact. Their formation can occur by degradation of ordinary components, by newly induced enzymatic modification (oxidation, hydroxylation, etc) of primary compounds and by de *novo* synthesis from basic precursors. Products of the first two groups are mostly phenols, quinones, benzoic acids, coumarins, and other derivatives of the phenylpropanoid pathway that act in lignification processes as well as direct fungitoxicans. The third group comprises products better defined as "phytoalexins" (Stoessl, 1983).

Wound healing is the result of a series of metabolic activities set in motion by the stimulus of wounding. Membrane regeneration, enhanced respiration, protein synthesis, bio-synthesis of phenylpropanols and changes in hormone activity are common activities leading to the final restoration of cell tissues to their typical metabolic states. Within some extent, basically determined by the duration of the stimulus and the contributions made by pathogen metabolites, the response to infection would have the same objective in restoring tissues. Plants otherwise resistant are rendered susceptible to pathogens after disruption of protein synthesis by heat

treatment or by the action of protein inhibitors. However, no disease resistant mechanisms directly involved in protein synthesis have been identified different to those related to the synthesis of enzymes intervening in secondary metabolism or in healing. Peroxidase contents increase in plant cells after wounding. Its action appears to be associated to lignin synthesis, oxidation of indoleacetic acid, ethylene and flavonoid biosynthesis and hydroxylation of proline. Enhanced peroxidase activity and accumulation in the tissues surrounding induced lesions have been observed in wheat leaves inoculated with *Botrytis cinerea*. The response has been attributed to effects of a diffusible stimulus from the infection process rather than caused by simple physical damage. However, no differences in peroxidase enhancement were reported by action of compatible or incompatible races of *Phytophthora megasperma* f. sp. glycinea in soybean (Ward, 1986).

Low molecular weight phenolic compounds derived from cinnamic acid concentrate in wounded tissues, although their function is not clear. It has been proposed that they serve as reserve for the synthesis of more complex derivatives like caffeinic acid, which is formed from chlorogenic acid. Biosynthesis of cinnamic acid is performed after the elimination of NH_3 in phenylalanine from the shikimate pathway by the phenylalanine ammonia-lyase enzyme (PAL). Increased levels of PAL activity have been demonstrated in several plant species and convincing evidence of de novo synthesis has been also documented. Greater PAL activity has been demonstrated from incompatible reactions to different fungi in soybeans (apparently no so clear with *Phytophthora megasperma* f. sp. *glycinea*), in potato tuber tissue with *Phytophthora infestans*, in beans with *Cochliobolus (Helminthosporium) carbonum* or races of *Colletotrichum lindemuthianum*, in wounded wheat leaves with *Botrytis cinerea* and in oat leaves with incompatible races of crown rust, among others. PAL activity is also stimulated by UV light and a wide range of elicitors including chitosan and culture filtrates of *P. infestans* and has been regarded as a sensitive indicator of cell disturbance (Nicholson and Hammerschmidt, 1992; Ward, 1986).

PAL activity is involved in the cinnamic acid metabolism that leads to the production of the isoflavonoid phytoalexins. These phenolic compounds are characteristic of the *Leguminosae* while in the *Solanaceae* the mostly terpenoid phytoalexins derive from mevalonic acid rather than from shikimic acid. Phytoalexins accumulate after wounding but particularly after damage caused by localized freezing and can be greatly enhanced by the action of elicitors, some of them reported without involvement in cell damage. Restriction of pathogen growth in necrotic or hypersensitive lesions correlated with accumulated levels of toxic phytoalexins has been difficult to attain *in vivo*. Localized accumulation of glyceollin in soybean or avenalumin in oats has not yet been determined as the cause or the effect of hyphal growth halt. Potato tubers retained their pathotype specific resistance in conditions where phytoalexin accumulation has been greatly reduced. High levels of glyceollin accumulated in lesions were not capable to deter the growth of compatible races of *P. megasperma* f. sp. *glycinea*. These pathogens were not only able to survive but also to spread on the plant, after restoring the favorable conditions. *Aphanomyces euteiches* is apparently sensitive *in vitro* but in diseased plants appears unaffected by high concentrations of pisatin. However, the occurrence of unidentified mechanisms of detoxification that may occur *in vivo* might not be ruled out (Ward, 1986).

A number of non-pathogens and most pathogens of pea are able to detoxify the phytoalexin pisatin. Among them, *Nectria haematococca* is a well-known pisatin demethylase synthesizer from which its demethylase activity-coding gene has been cloned. Since all naturally occurring isolates that lacked the enzyme and all pisatin demethylase defective mutants assayed were essentially non-pathogenic on pea, detoxification of pisatin was considered a decisive step for pathogenicity. Surprisingly, enzyme defective mutants created by gene disruption retained their virulence in pea. The gene is located on a small dispensable chromosome together with other genes needed for pathogenicity and the fungus becomes non-pathogenic only if loosing the entire chromosome, which is what probably happened to the naturally occurring defective isolates. The findings have suggested another possible strategy for pathogen specification: analogous to bacterial plasmids, fungal dispensable chromosomes may serve to broaden the habitat spectrum (Schäfer *et al.*, 1989; Knogge, 1996).

Within the solanaceas, the occurrence of late blight was not reduced by spraying rishitin and other potato phytoalexins on leaves. Spraying capsidiol on tomato leaves however, controlled development of *Phytophthora infestans*. In separate studies rishitin inhibited spore formation applied to the surface of cut tubers and leaves. Rishitin and lubimin have been considered factors of specific resistance in potatoes to *P. infestans* (Kumar *et al.*, 1991)

In general, different models have been suggested for phytoalexin-pathogen interactions: phytolaexins synthesize in response to all invaders and successful organisms survive by being insensitive to them or by metabolizing the released toxins; either pathogens are able to avoid contact with the release mechanism compartments while non pathogens stimulate their production; or phytoalexin synthesis is triggered by limited death of the infected host cells that initiates defense responses as the observed in hypersensitive reactions in a general non-specific mechanism occurring to all types of pathogens. A striking feature is the similarities observed on the return of physically wounded tissues to their normal state. In fact, most plants are resistant to a vast majority of potential pathogens and wounds not too severe heal. But also, while susceptibility is rare, pathogencity requires great specificity. The evidence accumulated so far indicates that phytoalexins are important in defense and the conclusive determination of their role seems a matter of time, particularly with the advent of molecular approaches to deal with this task (Ward, 1986; Hammerschmidt, 1999).

Fungal compounds

Genetics and field observations have at large documented the high degree of specificity between many pathogens and their plant hosts but not so well at the level of the involved biochemistry and physiology. Although colonization, cell damage and symptoms in a number of host-pathogen interactions have been regarded as caused or influenced by low-molecular weight secondary metabolites known as toxins, recent cloning of genes has come in handy but contributed little to enlighten all the issues in this regard. Having cloned the genes has not led to provide insights about their function, as expected (Dunkle, 1984; Walton and Panaccione, 1993).

Fungal toxins can be differentiated within host selective and host non-selective. Host selective toxins are ordinary natural products, mostly low molecular weight secondary metabolites. These compounds are of diverse chemical structure, come from different biosynthetic path-

ways and have no known biological functions. These small molecules act like "diffusible substances" that move faster, and cause symptoms at distance from their site of synthesis, than much larger proteins (in biological systems 100 larger molecules move 10,000 - 100,000 slower). Such as the released fusicoccin in almond tree wilt (*Fusicoccum amygdali*), which move with the transpiration stream once capable of reaching the stomata. Several others have limited solubility and are restricted to the hydrophobic cellular membrane or the intra- or extra cellular aqueous apoplast. Others are non-specifically metabolized in vascular tissues, like the HC-toxin from *Cochiobolus carbonum* race 1. However, large elicitors are also capable of movement, like the endoxylanase derived from *Trichoderma vinide* in the xylem. The diverse array of chemical structures comprise compounds like cyclic peptides, terpenoids, oligosacharides, polyketides, etc., and occurs in families of closely related compounds from single pathogens or from different species like in *Alternaria* (Walton and Panaccione, 1993).

The most important attribute for toxins is to serve as agents on virulence or pathogenicity, like when a fungus is able to cause more disease by having a toxin than when not. Plant insensitivity to a toxin confers also increased resistance to the toxin-producing organism. However, toxins are not found in many diseases. Not even when the causal organism belongs to a family of relative well known host specific toxin producers like *Setosphaeria* (*Exserohilum*) *turcicum*. This is a member of the *Pleosporaceae* which causes Northern corn blight and is also related to the teleomorphs of *Alternaria*, *Stemphyllium*, *Drechslera, Bipolaris* and *Curvularia* (Walton and Panaccione, 1993; Alexopoulos *et al.*, 1996).

Although there is not a single genetic pattern of host response neither to the toxin nor to the fungus, sensitivity is generally controlled mono-genetically among host plants. Sensitivity can be inherited cytoplasmatically or as a dominant, a semi-dominant or a recessive trait, and so are variable the specific responses. URF 13, a protein that locates in the inner membrane of sensitive plants and is encoded by the mitochondrial maize genome, mediates responses to T-toxin from *Cochliobolus heterostrophus* (*Bipolaris maydis*). Differential detoxification instead of site related response is the mode of action against HC-toxin (*C. carbonum*) in maize (leaf spot disease). Resistance performs as a dominant inheritable trait in this case. Covalent or differential binding to a membrane-localized protein is the apparent mechanism towards victorin (*C. victoriae*), and oat sensitivity to this pathogen appears as a dominant trait. The powerful phytotoxic and selective Victorin, is toxic to sensitive oats at 10 pg/ml (13 pM) but not at one million fold higher concentrations in other resistant oat cultivars besides other plant species. The single Mendelian locus *Hv*-1 which confers susceptibility to *C. victoriae* and sensitivity to victorin is tightly linked to *Pc*-2, a locus which confers dominant resistance to many races of *Puccinia coronata*, the causal organism of crown rust (Walton and Panaccione, 1993; Schäfer *et al.*, 1989).

Complex host-pathogen interactions contrast with single gene inheritance and the need for a fast response at infection. The mediation of complex molecules requiring complicate enzymatic pathways, as prime actors in host-pathogen interactions should be ruled out as general mechanisms of defense. The production of plant secondary metabolites like flavonoid phytoalexins or gibberellins requires the involvement of many individual enzymatic steps and so the involvement of several genes. However, monogenic specificity has been now firmly demonstrated for a number of pathogens like *Cladosporium fulvum* and the *avr*9 gene in tomato. In fact, microorganisms have their genes for secondary metabolism tightly cluster, like the genes for the synthesis of actinorhodin and tetracenomycin C in the *Streptomyces*, or for the synthesis of bacterial and fungal penicillins or for the metabolism of β-lactam antibiotics in prokaryotes and eukaryotes. Similarly, the genes for non-specific toxins like syringomycin and phaseolotoxin in *Pseudomonas syringae*, or the genes for enniatin in *Fusarium osysporum*, which form as a result of synthesis by multifunctional enzymes. Both, genetic coding of multifunctional enzymes and clustering segregate as a single locus. Nevertheless, it is little clear how very specific entities like T-toxin, HC-toxin and victorin, the toxins in *Cochliobolus heterostrophus*, *C. carbonum* and *C. victoriae* are genetically controlled. Probably by a diversion in a single enzymatic step from the production of a primary metabolite or a divergent step from a secondary pathway common to all races, as it has been suggested (Walton and Panaccione, 1993).

The mode of action of host selective toxins has several different effects. In general, toxins are important to plant diseases as a whole but they are not necessarily toxic, particularly at the cellular level. Victorin and T-toxin cause cell collapse while HC-toxin inhibits root growth of maize and promotes survival of non-dividing leaf mesophyll protoplasts. While most toxins stimulate ion leakage, HC toxin stimulate uptake of certain ions. The phytoalexin avenalumin is induced by victorin in oats but the responsible fungus remains insensitive to the toxin. Other "non-toxic" compounds may be required for inhibiting defensive constitutive or inductive enzymes, functioning as host-selective repressors (Ward, 1986).

A great deal of difficulties is experienced when bio-assaying fungal produced toxins due to the small quantities and the selective techniques required. *Alternaria* and *Cochliobolus* produce large amounts of metabolites but only in special media. The PC toxin from *Periconia circinata* and HC toxin from *C. carbonum* need yeast added to the medium. ACT and ACR toxins from *Alternaria citri* (rough lemmon and tangerine) need zinc ions. In general, secondary metabolism is tightly developmentally and/or nutritionally regulated and controlled. Several metabolites are expressed only *in planta,* regulated or induced by physiological or chemical signals at specific developmental stages. Only a few toxins can be obtained directly from infected tissues or require a carefully chosen path like with *Cladosporium fulvum*, which requires homozygous recessive *cf*9/*cf*9 tomato leaves or in culture if nitrogen is limiting. And their mode of action may be highly organ or tissue specific such as that a chloroplast specific toxin will not inhibit root growth or others may be retarded or suppressed by light if expressed in leaves because of photosynthesis relatedness (Ward, 1986).

Conclusions

There is no doubt that secondary metabolites contribute to resistance to some extent. It is clear that they are varied enough and contribute to different mechanisms of microbial attack and plant defense. Host specific metabolites and plant preformed and post-infectional compounds are critically involved in several diseases. Many more interactions remain still uncovered and will be discovered with more effective and sensitive techniques. Whether their involvement is a general predominant or particularly effective means of defense is not clear yet. But it is clear that a unique defense model with secondary metabolites as broad actors in

plant disease appears unlikely. Fungi and plants are widely diverse and so are their interactions. Non-induced disease resistance relying on any single plant metabolite lacks still of a rigorous demonstration. The separation between plant pre-existing and post-infection compounds is also not completely clear and several constitutive compounds close resemble active phytoalexins. Available information does not still explain specificity in host-pathogen interactions, nor the mode of action or the need for structurally diverse compounds. Phytoalexins form as a response to a variety of physiological stress. Even more, they concentrate as well in susceptible as in resistant plants and both pathogens and plants appear to be able to degrade them. Phytoalexins and pre-existing inhibitors do not seem to afford the versatility of pathogens to adapt.

In addition, a complete array of molecules for producing a single compound does not appear to be very efficient. The mechanisms of plant response and fungal adaptation to plant-fungus interactions are more dependent in the speed of action than in the process of recognition itself. Common "primary" macromolecules can be more easily and rapidly processed. Not in vain, their metabolisms rely in fewer actors widely available within the organisms. It is clear that phytoalexin detoxification is a virulence factor to some fungi, but the toxic activity is also occasionally directed toward organisms that bear little relationship to the producer fungi. Certainly, genes involved in resistance must be not only involved in resistance mechanisms but also in modulating compatibility. Secondary metabolites appear to play a definitive role in this regard. However, to claim that they are of effective general use, as a means for disease control, remains to be demonstrated yet. The exact contribution of fungal and plant secondary metabolites to plant disease remains still unresolved for several host-pathogen interactions.

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