

Induced systemic resistance (ISR) against pathogens – a promising field for ecological research

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Abstract

Local infection by single strains of pathogenic bacteria, fungi or viruses can induce plants to develop a plant-wide resistance against future attack by a variety of microbial pathogens. This phenomenon, called induced systemic resistance (ISR), has been intensively studied with respect to the underlying signalling pathways and to its potential use in crop protection, while ecological or evolutionary studies are rare. However, understanding the selective advantages of an induced as compared to constitutive resistance requires ecological and evolutionary research. The same holds true for the questions of how important this form of resistance is in natural plant populations, and how it affects other plant-insect and plant-microbe interactions.

Putative fitness costs provide an explanation for why ISR is induced instead of constitutive, and they might constrain the use of ISR as preventative protection of cultivated plants. Though ISR is mainly elicited by and effective against pathogens, further biotic agents such as leaf-chewing herbivores, leaf miners, aphids and even non-pathogenic root-colonising bacteria can induce systemic pathogen resistance, while some ISR traits can have a defensive effect against herbivores. 'Cross-resistance' elicited by and effective against non-microbial plant enemies thus might add significantly to the function of ISR. On the other hand, 'trade-offs' have been reported, i.e. increased susceptibility to herbivores in ISR-expressing plants. Finally, ISR is a rather unspecific response, being active against different microbes. It thus might have effects on mutualistic bacteria and fungi, too. The question of how expression of ISR affects the large variety of mutualistic and antagonistic plant-microbe and plant-insect interactions cannot yet be answered. This knowledge is, however, needed to obtain a risk assessment for the use of chemically induced or genetically engineered ISR in crop protection. This review aims to provide an overview and to highlight some of the many open questions which require intensive ecological research.

Key words: costs of resistance, crop protection, induced defence, plant disease, plant-pathogen interaction, systemic acquired resistance

Introduction

Induced plant resistance against pathogen infection has been described for more than 100 years (early studies reviewed by Chester 1933). Over the past decades, research on induced systemic resistance (ISR) against

microbial pathogens has concentrated (i) on the specificity of interactions (Jackson & Taylor 1996; Somssich & Hahlbrock 1998), (ii) on the underlying signalling pathways (Ryals *et al.* 1994; Hunt *et al.* 1996; Hunt & Ryals 1996;

Schneider *et al.* 1996; Kuc 1997; van Loon 1997), and (iii) on how these findings might be used in crop protection (Karban & Kuc 1999). Few studies have focused on ecological or evolutionary aspects of ISR (Heil 1999). Therefore, important questions are still unresolved. For example, it is not understood why an induced rather than a constitutive resistance has evolved. Moreover, the high number of biotic and abiotic inducing agents leads to the question of whether there exists any plant in nature which has not been already induced. Finally, the interactions between ISR and phenomena such as induced herbivore resistance (IRH) and effects on various plant-insect and plant-microbe interactions, along with the potential influence on these interactions when ISR is used in crop protection, require intensive ecological research.

Elicitation, signalling and the ISR phenotype

Local infection by single strains of bacteria, viruses or fungi induces many plant species to synthesise a signal at the site of infection which then spreads systemically throughout the plant. Salicylic acid (SA, Raskin 1992) is involved in this pathway (Malamy *et al.* 1990; Métraux *et al.* 1990; Lawton *et al.* 1995; Hammerschmidt & Smith-Becker 1999; Cameron 2000; Métraux 2001), but it is probably not the translocated signal, and if it is, then it is probably not the only signal (Hammerschmidt & Smith-Becker 1999). The systemically translocated signal induces the expression of a broad-spectrum, long-lasting immunity against further pathogen infection in both infected and non-infected plant parts, called systemic acquired resistance (SAR) (Ross 1961) or induced systemic resistance (ISR, for reviews see, e.g. Kuc 1982; Ryals *et al.* 1994; Hammerschmidt & Kuc 1995; Kuc 1995a; Hunt *et al.* 1996; Sticher *et al.* 1997; Hammerschmidt & Smith-Becker 1999). The efficacy of ISR against a variety of plant diseases has been demonstrated convincingly, and it is clear that the specificity of the induced resistance is not directly dependent on the type of inducing pathogen (Ryals *et al.* 1994; Jackson & Taylor 1996; Schneider *et al.* 1996; Sticher *et al.* 1997). Initially, this phenomenon was thought to be ubiquitous, and resistance was thought to be equally effective against many different pathogens, but recent

studies suggest intra- and interspecific variability and specificity (see e.g. Sticher *et al.* 1997 for an overview). In general, resistance seems to be most active against fungi, less effective against bacteria and least effective against viruses (Kuc 2001).

A local oxidative burst (Lamb & Dixon 1997), along with a hypersensitive response characterised by programmed cell death and leading to necrosis at the site of infection, is regularly associated with (and might be causally related to) ISR (Greenberg 1997; Kombrink & Schmelzer 2001). The systemic response involves the *de novo* production of cell-wall components (Hammerschmidt 1999a; Hammerschmidt & Nicholson 1999), of phytoalexins (Kuc 1995b; Hammerschmidt 1999b), and of so-called pathogenesis-related (PR) proteins, some of which show chitinase or β -1,3-glucanase activity (van Loon 1997; Neuhaus 1999; van Loon & van Strien 1999).

The importance of local alteration of cell wall composition in inhibiting penetration by pathogens has been described for many plant species. Both monocotyledonous and dicotyledonous plants respond by forming cell wall appositions (so-called papilla) at sites of attempted pathogen penetration (Aist 1976). Elicitation of ISR can lead to a higher rate of papillae formation in challenged, previously uninfected leaves (Stumm & Gessler 1986). Phytoalexins are antimicrobial compounds that are induced after infection (Hammerschmidt & Nicholson 1999). Chitinases play an important role in the defence of plants against fungal pathogens with chitin-containing cell walls (Sahai & Manocha 1993; Iseli *et al.* 1996; Jackson & Taylor 1996). Purified chitinases exhibit pronounced antifungal activity, particularly in combination with β -1,3-glucanases (Schlumbaum *et al.* 1986; Mauch *et al.* 1988). Plants which over-express chitinase can show decreased susceptibility to infection by some fungi that have a chitin-containing cell wall (Broglie *et al.* 1991; Datta & Datta 1999).

Though these results point to a function of PR-proteins in resistance, members within the same family of PR-proteins can differ greatly in their enzymatic activity, and the function of several PR-proteins is still unknown (van Loon & van Strien 1999). Overall, the functional significance of PR-proteins with respect to plant defence is unresolved. In the case of rhizobacteria-mediated induced

pathogen resistance, no enhanced expression of PR-proteins can be observed before the plant is challenged by infection (Pieterse *et al.* 2001). The expression of genes encoding typically 'defence-related' proteins, such as PR-1 and β -glucanase 2 (which are often used as ISR markers), can be uncoupled from pathogen resistance itself (Greenberg *et al.* 2000), indicating that these compounds are at least not absolutely necessary for an effective phenotypic resistance.

Inducing agents

ISR is mainly induced by pathogenic microorganisms but can be elicited by other organisms and by exogenous application of several different, chemically unrelated compounds. Exogenous SA or MeSA application or over-expression of SA (Bowling *et al.* 1994; Mauch *et al.* 2001) can elicit ISR or lead to constitutive ISR. The same holds true for dichloroisonicotinic acid (INA) and other chemicals such as benzothiadiazole ('acibenzolar', 'BTH', or 'BION', see below) which substitute for SA in the signalling pathway (Kessmann *et al.* 1994; Görlach *et al.* 1996; Oostendorp *et al.* 2001). Further elicitors of ISR include substances such as several organic acids, potassium and sodium phosphates, silica, reactive oxygen species and so on. Chemical ISR inducers are structurally very different, but most lead to oxidative stress and local cell death (Kuc 2001). Several chemicals as well as natural pathogen infection have been reported not to elicit ISR directly, but rather to 'prime' or 'potentiate' plants, thus leading to a faster and stronger response in case of pathogen attack (Conrath *et al.* 2001; Jakab *et al.* 2001). ISR can also be induced by the plant hormone ethylene (Linthorst *et al.* 1996) or by nitric oxide (NO), which seems to be an internal signal as well (Klessig *et al.* 2000). Plant-wide pathogen resistance can be induced even by abiotic stress, including mechanical wounding (Ignatius *et al.* 1994), wind-induced mechanical stress (Moran & Cipollini Jr. 1999) or cold stress (Tronsmo *et al.* 1993).

A phenotypically similar, systemic resistance against pathogens can be induced by non-pathogenic rhizobacteria (so-called plant growth promoting bacteria, see Ramamoorthy *et al.* 2001). This 'rhizobacteria-mediated resistance' does, however – at least in some cases – not include the synthesis of PR-pro-

teins; it is not associated with major changes in gene expression at all, and relies on jasmonic acid (JA) signalling rather than on SA (van Loon *et al.* 1998; Pieterse *et al.* 2001). However, induction in other rhizobacteria-plant combinations seems to depend on a functional SA signalling. Obviously, there are several possibilities, and rhizobacteria-mediated resistance can be SA-dependent or independent, JA-dependent or independent, both SA- and JA-dependent, or might rely on a novel pathway (C.-M. Ryu, pers. comm). Non-pathogenic bacterial endophytes can induce pathogen resistance, too (Benhamou *et al.* 2000). Further biotic inducers of PR-proteins and/or of phenotypically efficient ISR include a large range of non-microbial organisms such as herbivorous beetle larvae (Hatcher & Paul 2000), caterpillars (Padgett *et al.* 1994; Stout *et al.* 1998a), whiteflies (Mayer *et al.* 1996; Inbar *et al.* 1999), aphids (Russo *et al.* 1997; Stout *et al.* 1998a; van der Westhuizen *et al.* 1998; Forslund *et al.* 2000), thrips (Russo *et al.* 1997) and leaf miners (Inbar *et al.* 1999).

Interactions with other forms of induced resistance

Several studies have focused on cross-resistance or trade-offs between ISR and induced herbivore resistance. While 'cross-resistance' describes resistance against one group of plant enemies induced by another, the term 'trade-off' has been used to describe an increased susceptibility of plants to one group when being in the induced stage of resistance against the other. Several examples of cross-resistance have been reported. For example, some PR-proteins typically involved in ISR (especially chitinases) can be induced by the action of insects (Mayer *et al.* 1996; Inbar *et al.* 1998, 1999), and can have detrimental effects against herbivores as well (Broadway *et al.* 1998; Inbar *et al.* 1998, 1999). It has, therefore, already been hypothesised that chitinases might have negative effects on mutualistic insects (Picard-Nizou *et al.* 1997; Heil *et al.* 1999), though conclusive evidence is still missing. Damage by herbivores can make plants more resistant against fungi (Hatcher *et al.* 1995; Hatcher & Paul 2000) and bacteria (Stout *et al.* 1998a). Aphids in general seem to elicit ISR rather than IRH (Fidantsef *et al.* 1999; Stout *et al.* 1999), which makes

some ecological sense since they often function as vectors for fungal or viral diseases. Chemical elicitors of ISR can protect plants against leaf-miners and whiteflies (Inbar *et al.* 1998). Methyl-jasmonate/JA, a typical elicitor of herbivore resistance, can induce PR-proteins (Xu *et al.* 1994). Galling herbivores can elicit a hypersensitive response which might be a widespread plant response effectively decreasing gall survival (Fernandes & Negreros 2001), while it is regularly associated with ISR (Greenberg 1997; Kombrink & Schmelzer 2001).

On the other hand, trade-offs have been described (Felton *et al.* 1999; Thaler 1999b). In tomato, *Lycopersicon esculentum*, chemical induction of ISR decreased the plant's ability to express wound-inducible proteinase-inhibitors (Doares *et al.* 1995; Fidantsef *et al.* 1999), and treating leaves with a chemical ISR elicitor increased their suitability for caterpillars (Thaler *et al.* 1997; Stout *et al.* 1999). SA-treatment inhibited wound- and JA-induced responses (Stout *et al.* 1998b), and application of JA reduced the efficacy of chemical ISR elicitors (Thaler *et al.* 1997). SA or related substances can inhibit the synthesis of JA and thus the expression of JA-dependent genes (Pena-Cortes *et al.* 1993; Baldwin *et al.* 1996, 1997; Engelberth *et al.* 2001), and an inhibition of JA-responsive genes by SA acting downstream of JA has been reported, too (Doares *et al.* 1995; Niki *et al.* 1998). For a general review on the role of JA in plants see Creelman & Mullet (1997).

The system is additionally complicated by the multiple action of secondary compounds such as phenolics, terpenoids, alkaloids, furanocoumarins (Berenbaum & Zangerl 1999) and many other substance classes which are well-known to exhibit biological activity against herbivores and/or pathogens (Luckner 1990; Dey & Harborne 1997). In general, these substances are not considered in studies on ISR, which are based often on the detection of some selected PR-proteins to characterise the induced stage. For many substances it is not yet known whether or not their induction depends on the SA-dependent signalling pathway at all (Constabel 1999). However, some studies – and especially those investigating induced resistance phenomena at the phenotype level by challenging plants with pathogens – might be strongly biased by the multiple effects of all these substances. Difficulties and misunderstandings can result from

interpreting phenotypic resistance on the basis only of widely used molecular markers. It is important that studies on cross-resistance and trade-offs take account of all the chemical changes in the plant which can affect further pathogen infection. They therefore need to consider the 'classical' secondary compounds instead of concentrating on PR-proteins.

Costs of ISR

Why induced resistance?

Why has ISR evolved as an induced rather than as a constitutive defence? Given the evidence for the ability of a plant to establish resistance against many different pathogens at the same time, it is difficult to understand why a first, challenging infection is required, which might be even harmful if the pathogen spreads too rapidly within the plant (Heil 1999). Induced defences leave the plants unprotected for the time span until resistance traits have been established, and thus should have a lower beneficial effect on plant fitness as compared to constitutive defence. However, resistance might be too costly to be expressed continuously (Heil 2001). The concept of fitness costs of resistance assumes a reduced fitness of the more resistant as compared to the less resistant phenotype, when both are compared under enemy-free conditions which prevent the resistance from having any beneficial effects. This concept has been well developed for resistance against insect herbivores (Rhoades 1979; Simms & Rausher 1987; Simms & Fritz 1990; Herms & Mattson 1992), but has rarely been applied to ISR (Heil 2001). Several empirical studies have tried to quantify costs of induced resistance against herbivores (Brown 1988; Baldwin 1998; Agrawal *et al.* 1999; Thaler 1999a; Redman *et al.* 2001). Phenotypes of plants that are positively or negatively affected in their ISR expression are in general consistent with the proposition that ISR can have negative effects on plant growth and fitness when expressed under pathogen-free conditions (M. Heil & I.T. Baldwin, unpubl. data).

Fitness costs can arise in different ways. For example, 'allocation costs' might occur since limited resources, once allocated to the production of defensive compounds, cannot be used for other fitness-relevant processes such as growth and reproduction (Herms &

Mattson 1992). However, costs might result also from autotoxic properties of defensive compounds ('self-damage costs'), or from other 'side effects' of molecules that are involved in signalling or causing the resistant phenotype. Furthermore, detrimental influences on plant mutualists and other negative effects appearing when defences interact with the plants' environment can lead to 'ecological' or 'environmental' costs (Tollrian & Harvell 1999).

Allocation costs of ISR

Several studies have produced results which are consistent with the suggestion that ISR expression can cause fitness costs by using up limited resources which then cannot be allocated to other processes such as growth and reproduction (see discussion in Heil 2001). In order to detect allocation costs of ISR directly we used spring wheat (*Triticum aestivum* cv. 'Hanno') and treated it with BION® (active component benzo(1,2,3)thiadiazole-7-carbothioic acid-S-methyl ester; Novartis, now Syngenta, Basel, Switzerland). BION® elicits resistance in wheat against several pathogens and induces expression of a set of so-called 'wheat chemically induced' genes (Görlach *et al.* 1996). Plants that were protected passively against fungal infection by the application of traditional fungicides were grown under a range of nutrient conditions and were either treated with BION® or left untreated as controls; for detailed methods description see Heil *et al.* (2000a).

BION® treatment led to a reduced number of lateral shoots and thus to a reduced above-ground biomass gain and seed set under most growing conditions (Fig. 1a). Effects were most pronounced for plants treated during lateral shoot production, while later BION® application had no detectable effect on seed set as compared to controls (Fig. 1b). Though some phytotoxic effects of BION® occur when the compound is applied in high concentrations, these results suggest that there may be fitness costs of ISR. We tried to avoid phytotoxicity by using low concentrations of BION® as recommended by the provider for agricultural field use. Moreover, we compared different nitrogen regimes and found negative fitness effects which were consistent with them being consequences of allocation costs of ISR. Largest effects occurred in plants suffering from a strong shortage of nitrogen, while

there was only a slight trend in those plants which had been fertilised with high amounts of nitrogen (Fig. 1c). This is not the result we would have expected from direct effects of BION®, which should have occurred under all conditions. Using different growing conditions (which affect the outcome of allocation costs caused by a given level of induction) rather than different concentrations of the inducing agent (thus affecting the level of the induced resistance) provides a good tool to separate fitness effects of allocation costs from other effects of the inducing agent. However, it cannot be excluded that 'side effects' of BION® have not biased these findings. Much more research is required to decide whether the results obtained from this first, exploratory study indicate fitness-relevant allocation costs of ISR, and whether these effects occur in other species and from 'natural' as well as chemical elicitation of ISR.

Physiological 'side effects' of ISR

Apparent costs of ISR could also be due to side-effects of induced compounds, of inducing agents, or of signalling molecules (i.e. effects unrelated to the defensive function of these compounds). Both JA and SA are plant hormones involved in developmental processes such as flower and fruit development (Raskin 1992; Creelman & Mullet 1997) and several fitness-relevant effects of increased JA- or SA-levels might result from these functions rather than from their defensive role. For example, following pathogen infection or elicitor treatment inhibitory effects of SA on nitrate reductase (Jain & Srivastava 1981; Ramanujam *et al.* 1998), reduced RUBISCO levels, and reduced expression of histone-encoding genes have been reported (Longemann *et al.* 1995; Somssich & Hahlbrock 1998). For all these phenomena, it is unknown: (i) whether the effects appear only in the unnatural experimental situation of eliciting ISR chemically, (ii) whether they are necessary or only incidental consequences of resistance elicitation, and (iii) whether they also occur at the whole-plant level. In any case, the physiological relevance of some of the observed effects is doubtful since very high concentrations of SA were used in the experiments (Raskin 1992).

No evidence has been reported to indicate that there are costs associated with the systemic resistance elicited by rhizobacteria. It is interesting that this resistance appears, at

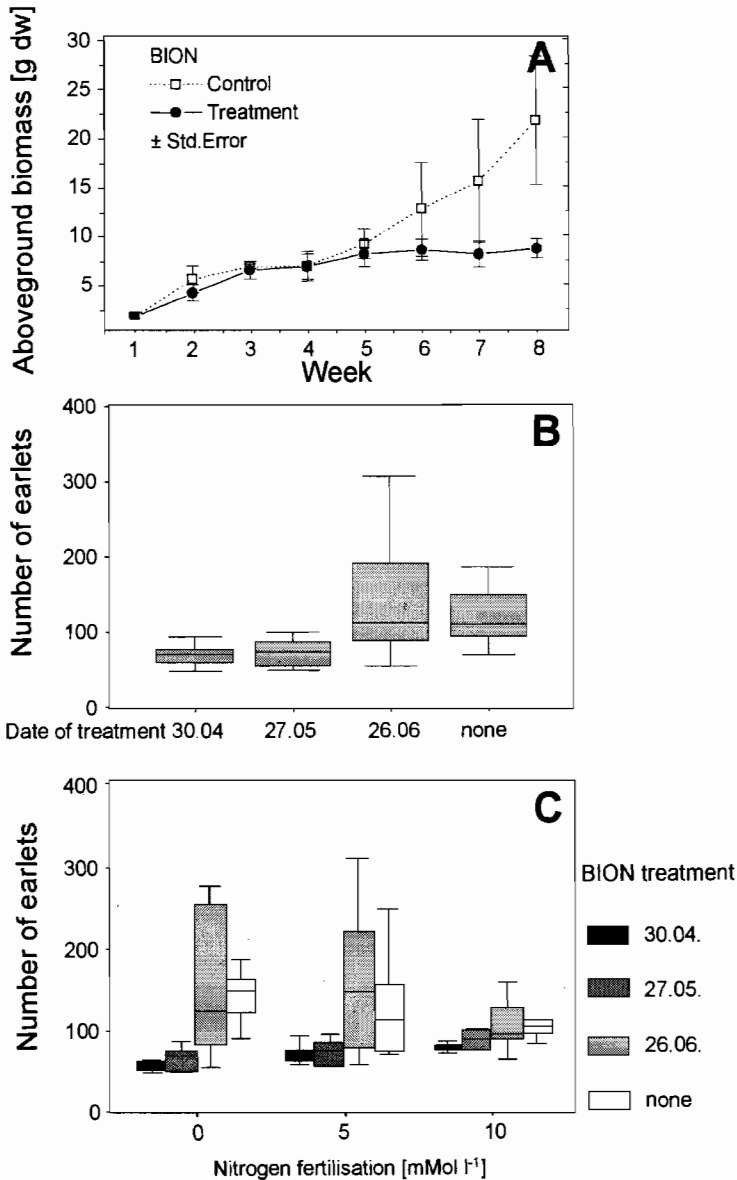


Fig. 1. Effects of chemically induced ISR on growth and reproduction in wheat. Box-whisker plots (parts B and C) represent 5% percentile (lower horizontal line), 1st and 3rd quartile (lower and upper margin of the box), median (line within the box) and 95% percentile (upper horizontal line). Figures 1a, b redrawn from Heil *et al.* (2000a); data presented in Fig. 1c from Table 4 in Heil *et al.* (2000a). **A.** Time course in above-ground growth [dry biomass produced by four plants growing in one pot] after BION[®] treatment at the beginning of week 1 ($n = 12$ pots per treatment and harvest). **B.** Seed set [number of earlets produced by seven plants grown in one pot] in dependence of date of BION[®] treatment. The two first applications (30 April and 27 May) were done during lateral shoot production, the last (26 June) after the end of lateral shoot production (controls are not treated; $n = 24$ pots per application date). **C.** Effect of date of BION[®] treatment on seed set [number of earlets produced by seven plants grown in one pot] in dependence of nitrogen fertilisation. For each temporal application pattern (see B), plants were treated with nutrient solutions containing either no (0), 5 mM, or 10 mM nitrogen ($n = 8$ pots per treatment date-fertilisation combination).

least in some cases, to be independent of the induction of PR gene expression (Pieterse *et al.* 2001), which is a possible source of allocation costs. Induction or 'priming' of resistance via interactions with rhizobacteria might incur relatively low costs which are strongly outweighed by the positive effects of these interactions on plant nutrition. The outcome of these interactions depends strongly on the specificity of the interacting partners as well as on abiotic factors; ecological studies are needed in natural ecosystems to understand better the significance of these interactions for plant populations.

Ecological costs

Induced susceptibility

For IRH the resistance stage can be followed by an induced susceptibility (Underwood 1998), but this phenomenon is unlikely to be detected in experimental studies using a single challenge infection to check for the existence of resistance. It will be even overlooked in most time-course studies, since these normally are terminated as soon as the plant has reached the original level of resistance (Underwood 1998). The induced resistance of ISR can persist for a considerable period – even until the reproductive stage – though there is known to be strong interspecific variability in the duration of the resistant stage (Oostendorp *et al.* 2001). Induced susceptibility would represent a strong environmental

cost of ISR. Time-course studies using different plants and focusing on the phenotypically expressed resistance would help to find out whether this phenomenon occurs in induced pathogen resistance, too.

Effects on mutualists

Effects on mutualists can easily be overlooked, but they might have a strong influence on the net outcome of ISR. Some studies have already reported inhibitory effects of chemically induced ISR on the development of root nodules (Fig. 2; see also Martínez-Abarca *et al.* 1998; Ramanujam *et al.* 1998; Lian *et al.* 2000). Does this occur in nature as well, or is it a consequence of artificial, chemical elicitation rather than a 'real' effect of ISR itself? Experiments reported by Russin *et al.* (1990) indicate that herbivory and fungal infections can indeed inhibit nodule development and N_2 -fixing activity. It is, however, not clear whether these effects have resulted from ISR elicited by the plant enemies, or from a reduced allocation of assimilates to nodules; Russin *et al.* (1990) propose the latter interpretation. Are there comparative influences on other forms of plant-microbe mutualisms such as mycorrhizae or interactions with endosymbiotic fungi and bacteria? Given the apparently low specificity of ISR, and the fact that ISR components are involved in the establishment and stabilisation of root nodules and mycorrhiza (which have to be permanently stabilised at a stage between too

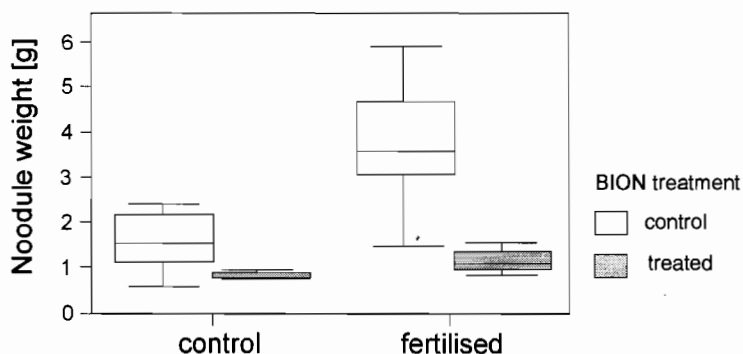


Fig. 2. Effect of chemical ISR elicitation on root nodulation of faba bean (*Vicia faba* cv. 'Hang down'). Total nodule dry weight was measured on roots of bean plants six weeks after BION[®] application to both shoot and roots. Different nutrient conditions were obtained by either fertilising plants weekly with a standard nutrient solution (see 'solution 1b' in Table 1, Heil *et al.* 2000a) or growing them in the same soil without any fertilisation. Sample size is five plants per BION[®] treatment for the unfertilised and 10 plants per treatment for the fertilised plants (M. Heil, unpubl. data).

heavy infection and a too effective defence, see Vierheilig *et al.* 1994; Dumas-Gaudot *et al.* 1996; Martínez-Abarca *et al.* 1998; Schultze & Kondorosi 1998; Ruiz-Lozano *et al.* 1999), interactions are likely to be of great importance. For example, Vierheilig *et al.* (1995) and Glandorf *et al.* (1997) reported negative effects on colonisation of tobacco roots by *Glomus mosseae* in plants constitutively expressing β -1,3-glucanase, while constitutive expression of several other PR-proteins did not affect colonisation by this mycorrhizal fungus. Ruiz-Lozano *et al.* (1999) investigated early events in mycorrhiza and nodule establishment and reported the induction of seven defence-related genes. Mycorrhizal fungi and nodulating bacteria have to overcome these defence responses, and strains and species are likely to differ in their ability to suppress or neutralise their hosts' defences, leading to a large variability in the outcome of these interactions. More research is required, using both physiological methods and ecologically realistic study systems, into the relative importance of the different possible interactions between plant defence against pathogenic microorganisms and plant mutualisms with other microorganisms.

Trade-offs with IRH

The contradictory evidence concerning trade-offs and cross-resistances between ISR and induced herbivore resistance (see above, and recent reviews by Bostock 1999; Stout & Bostock 1999; Stout *et al.* 1999; Bostock *et al.* 2001) makes any general statement impossible at present, and no general answer may even exist. This problem clearly requires further research on many different study systems. From the current knowledge it can just be stated that the former idea of two main pathways, one leading to herbivore resistance (the JA-mediated octadecanoid signalling cascade) and the other one leading to pathogen resistance (the SA-dependent ISR pathway) is over-simplified. Several recent studies have tried to find new, integrating descriptions (Bostock 1999; Genoud & Métraux 1999; Pieterse & Van Loon 1999; Bostock *et al.* 2001; Genoud *et al.* 2001; Thomma *et al.* 2001), but there is still no generally accepted model. Separating the 'elicitation level' from the 'signalling' level and the 'production' level (i.e. gene expression leading to the synthesis of resistance compounds), and assuming independent interactions at all three levels, al-

lows us to integrate most published data within one unifying framework (M. Heil & R.M. Bostock, unpubl. data); however, further studies are required to test the applicability of this model. While molecular work on interactions between the signalling pathways is proceeding rapidly, ecological studies are urgently needed to establish the significance of these processes for plant fitness in natural situations where plants are vulnerable to attack by many different enemies.

Interactions with further trophic levels

Chemical compounds which have apparently evolved as defences are not always effective in protecting a plant from attack. Several specialised insect herbivores are known to use defensive chemicals for host plant detection or nutrition, and some can even incorporate defensive compounds to use them for their own protection from predators and parasitoids (Boethel & Eikenbary 1986) or entomopathogens (Hoover *et al.* 1998). In this way, defensive compounds may also affect organisms at higher trophic levels. The situation can be complicated if the physiological effects of defensive compounds are non-linear. Such dose-dependent effects are well known for insects on plants. For example, in studies using transgenic tobacco plants which over-express proteinase inhibitor activity, it was shown that a compound having detrimental effects on insects and preventing further insect feeding when produced at one intensity can improve larval performance when expressed at lower levels (de Leo *et al.* 1998). It seems very probable that similar non-linear responses also occur with ISR.

ISR as a promising field for ecological research

Pathogen pressure under natural conditions

The role of plant pathogens in natural plant populations has been studied much less than in agricultural crops. The existing studies present very different pictures of the importance of pathogens. García-Guzmán & Dirzo (2001) investigated severity of pathogen infection in a natural tropical rainforest, along with the potential importance of different infection pathways. They reported generally low levels of pathogen damage: in only 2% of the sample

species was >12% of the leaf surface visibly affected by fungi attack. Some 70% of the species had damage levels <6%, and about half of these were not visibly damaged by fungi at all. The authors observed no evidence of bacterial infections or damage caused by viruses. These results might be interpreted either as demonstrating low pathogen pressure or an effective resistance. However, only 1.4% of the leaves were damaged by fungi alone (i.e. not also damaged by herbivores), and experimental inoculation in the absence of simulated herbivory failed in nearly all cases (García-Guzmán & Dirzo 2001). This finding leads to the interpretation that pathogen pressure on intact plants was indeed low in the investigated system.

In contrast, other studies have reported that pathogens destroy more leaf area than herbivores (Marquis *et al.* 2001). Roy *et al.* (2001) found up to 100% of the plants investigated to be infected by pathogens, though they could find no indication of negative fitness effects of pathogen infection. These different outcomes might result in part from the different methods employed; whereas some studies have attempted to measure the leaf area damaged by pathogens (e.g. García-Guzmán & Dirzo 2001; Marquis *et al.* 2001), other studies report only the presence or absence of pathogen infection (e.g. Roy *et al.* 2001). However, a considerable part of the differences between studies on pathogen infection under natural conditions (see references in Roy *et al.* 2001 for further examples) probably reflects differences between natural ecosystems. Much more work is required to obtain a realistic picture of the importance of pathogen attack in natural ecosystems. Future studies should include not only quantitative measurements of infection such as those presented by García-Guzmán & Dirzo (2001), but also experimental work on the effects of infection on plant fitness. Total lifetime reproduction is the most desirable measure of plant fitness and could be estimated as total seed production in the case of annual plants and by measuring growth rates and annual fruit or seed set for longer living plant species.

Natural occurrence of ISR

A wide range of ISR elicitors, both biotic and abiotic, occur in nature (see above). This prompts the question of whether any plant can exist under natural conditions without

being induced – at least to some degree – by the action of some ISR elicitor or other (Heil 1999). Another question which arises is: how important is ISR as compared to other resistance mechanisms?

To answer these questions, ISR could be detected both by molecular and/or biochemical and by 'biological' methods. Molecular ISR markers are widely used for monocotyledonous and dicotyledonous plants belonging to different families. They are likely to respond to gene products expressed in other, related species as well. Methods based on the quantification of enzymatic activity of, e.g. chitinases (e.g. Boller 1992) are independent of the molecular structure of the enzymes. Thus, they are suitable for investigations in species or genera for which established molecular markers are not likely to give a reliable response. Measurements of chitinase activity can even be conducted under field conditions (Heil *et al.* 1999, 2000b) and thus are a promising tool for first screenings of ISR-related responses in natural ecosystems. With these methods, levels of ISR-related traits in plants growing under natural conditions could be compared with conspecifics grown under sterile conditions (in order to provide a zero-control for the 'uninduced stage') and with plants experimentally challenged by wounding or inoculation. These data would help to answer the question whether, and to which degree, plants growing under natural conditions are already in the induced stage. 'Biological' studies (comparing the phenotypically expressed resistance against pathogens in response to different experimental treatments such as wounding or pathogen inoculation) are another tool that could be easily conducted under field conditions. These studies would allow to detect whether and to which degree different plants can be (further?) induced when growing under natural conditions.

ISR in general is believed to be ubiquitous, since it has been described in monocotyledonous as well as in dicotyledonous plants. Therefore, the signalling pathway is assumed to be phylogenetically 'old' and highly conserved. However, while representing some taxonomic diversity, the species investigated so far exhibit a much lower diversity with respect to their ecological behaviour. Research has been conducted mainly on crop plants and *Arabidopsis* (see overviews in Schneider *et al.* 1996 and Sticher *et al.* 1997). The wild

ancestors of most of these species exhibit the same main life history traits, being fast growing annuals that occur typically on nutrient-rich soils in unshaded habitats. Much less evidence exists for long-lived plants or for plants growing under resource-poor conditions. Comparative studies involving plants from different taxonomic groups, different ecosystems, and exhibiting different life strategies, would help to prove whether ISR is indeed as widespread as appearing from the current literature, or whether it rather represents a strategy evolved mainly by one functional group of plant species growing under specific ecological conditions and thus facing the same selective forces.

Natural pathogen infection and practical use of ISR

Theoretical considerations lead to the expectation that induced resistance should be favoured over constitutive strategies only under conditions of low and unpredictable enemy pressure (Agrawal & Karban 1999; Järemo *et al.* 1999; Tollrian & Harvell 1999; and literature cited therein). This situation does clearly not apply to agricultural systems which in general are characterised by large monospecific stands of genetically similar plants suffering quite often from a strong pathogen pressure. A strong pathogen pressure affecting otherwise intact plants – as it is observed regularly in crop populations – might be an artificial situation resulting from, e.g. reduced resistance of cultivated plants and genetic uniformity, rather than being a natural phenomenon. More research on pathogen infection rates and ISR under natural conditions would help to figure out whether the assumptions and predictions formulated by Agrawal & Karban (1999), Järemo *et al.* (1999) or Tollrian & Harvell (1999) can be applied successfully to this resistance form.

This knowledge would not only be of ecological and evolutionary interest, but would allow to estimate the chances to use ISR as plant protection tool in agriculture. ISR might be less effective in systems that suffer from a high, predictable pathogen pressure than in natural systems characterised by low and unpredictable intensities of pathogen attacks. On the other hand, many annual plants tend to grow in comparably large and nearly monospecific populations, thus resembling the agricultural situation much closer than the

species-rich 'mature' ecosystem investigated by García-Guzmán & Dirzo (2001). If ISR turns out to be an effective strategy especially in these plant populations (which might be indeed the case according to the large number of fast growing annuals which exhibit ISR, see above), this would be in contrast to the evolutionary concerns mentioned above, but would strongly improve its value for applied plant protection. Comparative studies thus should not remain restricted to different plant species, but should be extended to the comparison of different plant populations and ecosystems as well. The paper by García-Guzmán & Dirzo (2001) provides a good example how this work could be done even under difficult field conditions.

Conclusions

Though the physiological and molecular understanding of processes leading to induced systemic resistance has increased impressively during the last decades, many questions are still open. How widespread and how important is ISR in plants belonging to different taxonomic and ecological groups? Does it provide relevant selective advantages under natural conditions? – Given the many potential interactions with different mutualistic and antagonistic plant-pathogen and plant-herbivore interactions, the fitness-relevant net outcome of ISR in natural ecosystems might differ strongly from that as estimated by laboratory or agricultural field experiments conducted under more or less controlled conditions and using over-simplified study systems. Physiological and ecological costs along with other constraints that might have played a role in the evolution of an induced instead of a constitutive resistance are not yet quantified or even not detected at all. Phylogenetic studies on ISR (comparable to that presented by Thaler & Karban 1997 on induced and constitutive mite resistance of several *Gossypium* species) are missing as well. Therefore, the evolution of ISR is not understood thus far. For example, Thaler & Karban (1997) demonstrated for the investigated study system that being inducible represents a derived form of defence, and that this can change rapidly during evolution, thus adding a further level of evolutionary plasticity to the phenotypic plasticity achieved by inducible resistance traits. Comparable data on induced pathogen resis-

tance are obviously missing.

Concerning the agricultural use of ISR, the potential impact of ecological research on this phenomenon goes beyond its meaning for a better ecological and evolutionary understanding of induced pathogen resistance. When ISR is to be used in crop protection by either chemical elicitation or transforming plants to exhibit stronger or faster ISR, or to express ISR compounds constitutively, possible problems arising from allocation costs of ISR (which can translate to yield losses under certain growing conditions) or ecological costs (negative effects on, e.g. mutualistic microbes or insects) must be taken into account and require careful investigation. Research on ecologically realistic study systems and ecologically intended research in the agricultural systems thus is needed to obtain a reliable risk assessment of this new, 'integrative' tool in plant protection.

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References

- Agrawal, A.A. & Karban, R. (1999) Why induced defenses may be favored over constitutive strategies in plants. *The Ecology and Evolution of Inducible Defenses* (eds. R. Tollrian & C.D. Harvell), pp. 45–61. Princeton University Press, Princeton.
- Agrawal, A.A., Strauss, S.Y. & Stout, M.J. (1999) Costs of induced responses and tolerance to herbivory in male and female fitness components of wild radish. *Evolution*, **53**, 1093–1104.
- Aist, J.R. (1976) Papillae and related wound plugs of plant cells. *Annual Review of Phytopathology*, **14**, 11–21.
- Baldwin, I.T. (1998) Jasmonate-induced responses are costly but benefit plants under attack in native populations. *Proceedings of the National Academy of Sciences of the USA*, **95**, 8113–8118.
- Baldwin, I.T., Schmelz, E.A. & Zhang, Z.-P. (1996) Effects of octadecanoid metabolites and inhibitors on induced nicotine accumulation in *Nicotiana sylvestris*. *Journal of Chemical Ecology*, **22**, 61–74.
- Baldwin, I.T., Zhang, Z.-P., Diab, N., Ohnmeiss, T.E., McCloud, E.S., Lynds, G.Y. & Schmelz, E.A. (1997) Quantification, correlations and manipulation of wound-induced changes in jasmonic acid and nicotine in *Nicotiana sylvestris*. *Planta*, **201**, 397–404.
- Benhamou, N., Gagné, S., Le Quééré, D. & Dehbi, L. (2000) Bacterial-mediated induced resistance in cucumber: beneficial effect of the endophytic bacterium *Serratia plymuthica* on the protection against infection by *Pythium ultimum*. *Phytopathology*, **90**, 45–56.
- Berenbaum, M.R. & Zangerl, A.R. (1999) Coping with life as a menu option: inducible defenses of the wild parsnip. *The Ecology and Evolution of Inducible Defenses* (eds. R. Tollrian & C.D. Harvell), pp. 10–32. Princeton University Press, Princeton.
- Boethel, D.J. & Eikenbary, R.D. (1986) *Interactions of Plant Resistance and Parasitoids and Predators of Insects*. Ellis Horwood, New York.
- Boller, T. (1992) Biochemical analysis of chitinases and β -1,3 glucanases. *Practical Methods in Molecular Plant Pathology* (eds. D.J. Bowles & S. Gurr), pp. 23–30. Oxford University Press, Oxford.
- Bostock, R.M. (1999) Signal conflicts and synergies in induced resistance to multiple attackers. *Physiological and Molecular Plant Pathology*, **55**, 99–109.
- Bostock, R.M., Karban, R., Thaler, J.S., Weyman, P.D. & Gilchrist, D. (2001) Signal interactions in induced resistance to pathogens and insect herbivores. *European Journal of Plant Pathology*, **107**, 103–111.
- Bowling, S.A., Guo, A., Cao, H., Gordon, A.S., Klessig, D.F. & Dong, X. (1994) A mutation in *Arabidopsis* that leads to constitutive expression of systemic acquired resistance. *The Plant Cell*, **6**, 1845–1857.
- Broadway, R.M., Gongora, C., Kain, W.C., Sanderson, J.P., Monroy, J.A., Bennett, K.C., Warner, J.B. & Hoffmann, M.P. (1998) Novel chitinolytic enzymes with biological activity against herbivorous insects. *Journal of Chemical Ecology*, **24**, 985–998.
- Brogliè, K., Chet, I., Holliday, M., Cressman, R., Biddle, P., Kowiton, S., Mauvais, C. & Brogliè, R. (1991) Transgenic plants with enhanced resistance to the fungal pathogen *Rhizoctonia solani*. *Science*, **254**, 1194–1197.
- Brown, D.G. (1988) The cost of plant defense: an experimental analysis with inducible proteinase inhibitors in tomato. *Oecologia*, **76**, 467–470.
- Cameron, R.K. (2000) Salicylic acid and its role in plant defense responses: what do we really know? *Physiological and Molecular Plant Pathology*, **56**, 91–93.
- Chester, K.S. (1933) The problem of acquired physiological immunity in plants. *The Quarterly Review of Biology*, **8**, 129–154, 275–324.
- Conrath, U., Thulke, O., Katz, V., Schwindling, S. & Kohler, A. (2001) Priming as mechanism in induced systemic resistance of plants. *European Journal of Plant Pathology*, **107**, 113–119.
- Constabel, C.P. (1999) A survey of herbivore-inducible defensive proteins and phytochemicals. *Induced Plant Defenses against Pathogens and Herbivores: Biochemistry, Ecology, and Agriculture* (eds. A.A. Agrawal, S. Tuzun & E. Bent), pp. 137–166. The American Phytopathological Society Press, St. Paul.
- Creelman, R.A. & Mullet, J.E. (1997) Biosynthesis and action of jasmonates in plants. *Annual Review of Plant Physiology and Plant Molecular Biology*, **48**, 355–381.
- Datta, K.N. & Datta, S.K. (1999) Expression and function of PR-protein genes in transgenic plants. *Pathogenesis-related Proteins in Plants* (eds. S.

- Datta & S. Muthukrishnan), pp. 261–277. CRC Press, Boca Raton.
- De Leo, F., Bonadé-Bottino, M.A., Ceci, L.R., Gallerani, R. & Jouanin, L. (1998) Opposite effects on *Spodoptera littoralis* larvae of high expression level of a trypsin proteinase inhibitor in transgenic plants. *Plant Physiology*, **118**, 997–1004.
- Dey, P.M. & Harborne, J.B. (1997) *Plant Biochemistry*. Academic Press, London.
- Doares, S.H., Narváez-Vásquez, J., Conconi, A. & Ryan, C.A. (1995) Salicylic acid inhibits synthesis of proteinase-inhibitors in tomato leaves induced by systemin and jasmonic acid. *Plant Physiology*, **108**, 1741–1746.
- Dumas-Gaudot, E., Slezack, S., Dassi, B., Pozo, M.J., Gianinazzi-Pearson, V. & Gianinazzi, S. (1996) Plant hydrolytic enzymes (chitinases and beta-1,3-glucanases) in root reactions to pathogenic and symbiotic microorganisms. *Plant and Soil*, **185**, 211–221.
- Engelberth, J., Koch, T., Schüler, G., Bachmann, N., Rechtenbach, J. & Boland, W. (2001) Iaa channel-forming Alalmethicin is a potent elicitor of volatile biosynthesis and tendrill coiling. Cross talk between jasmonate and salicylate signaling in lima bean. *Plant Physiology*, **125**, 369–377.
- Felton, G.W., Korth, K.L., Bi, J.L., Wesley, S.V., Huhman, D.V., Mathews, M.C., Murphy, J.B., Lamb, C. & Dixon, R.A. (1999) Inverse relationship between systemic resistance of plants to microorganisms and to insect herbivory. *Current Biology*, **9**, 317–320.
- Fernandes, G.W. & Negreiros, D. (2001) The occurrence and effectiveness of hypersensitive reaction against galling herbivores across host taxa. *Ecological Entomology*, **26**, 46–55.
- Fidantsef, A.L., Stout, M.J., Thaler, J.S., Duffey, S.S. & Bostock, R.M. (1999) Signal interactions in pathogen and insect attack: expression of lipoxygenase, proteinase inhibitor II, and pathogenesis-related protein P4 in the tomato, *Lycopersicon esculentum*. *Physiological and Molecular Plant Pathology*, **54**, 97–114.
- Forslund, K., Pettersson, J., Bryngelsson, T. & Jonsson, L. (2000) Aphid infestation induces PR-proteins differently in barley susceptible or resistant to the birdcherry-oat aphid (*Rhopalosiphum padi*). *Physiologia Plantarum*, **110**, 496–502.
- García-Guzmán, G. & Dirzo, R. (2001) Patterns of leaf-pathogen infection in the understory of a Mexican rain forest: incidence, spatiotemporal variation, and mechanisms of infection. *American Journal of Botany*, **88**, 634–645.
- Genoud, T. & Métraux, J.-P. (1999) Crosstalk in plant cell signaling: structure and function of the genetic network. *Trends in Plant Science*, **4**, 503–507.
- Genoud, T., Santa Cruz, M.B.T. & Métraux, J.P. (2001) Numeric simulation of plant signaling networks. *Plant Physiology*, **126**, 1430–1437.
- Glandorf, D.C.M., Bakker, P.A.H. & van Loon, L.C. (1997) Influence of the production of antibacterial and antifungal proteins by transgenic plant on the saprophytic soil microflora. *Acta Botanica Neerlandica*, **46**, 85–104.
- Görlach, J. et al. (1996) Benzoethiadiazole, a novel class of inducers of systemic acquired resistance, activates gene expression and disease resistance in wheat. *The Plant Cell*, **8**, 629–643.
- Greenberg, G.T. (1997) Programmed cell death in plant-pathogen interactions. *Annual Review of Plant Physiology and Plant Molecular Biology*, **48**, 525–545.
- Greenberg, J.T., Silverman, F.P. & Liang, H. (2000) Uncoupling salicylic acid-dependent cell death and defense-related responses from disease resistance in the *Arabidopsis* mutant *acd5*. *Genetics*, **156**, 341–350.
- Hammerschmidt, R. (1999a) Induced disease resistance: how do induced plants stop pathogens? *Physiological and Molecular Plant Pathology*, **55**, 77–84.
- Hammerschmidt, R. (1999b) Phytoalexins: what have we learned after 60 years? *Annual Review of Phytopathology*, **37**, 285–306.
- Hammerschmidt, R. & Kuc, J. (1995) *Induced Resistance to Disease in Plants*. Kluwer, Dordrecht.
- Hammerschmidt, R. & Nicholson, R.L. (1999) A survey of defense responses to pathogens. *Induced Plant Defenses against Pathogens and Herbivores: Biochemistry, Ecology, and Agriculture* (eds. A.A. Agrawal, S. Tuzun & E. Bent), pp. 55–71. The American Phytopathological Society Press, St. Paul.
- Hammerschmidt, R. & Smith-Becker, J.A. (1999) The role of salicylic acid in disease resistance. *Induced Plant Defenses against Pathogens and Herbivores: Biochemistry, Ecology, and Agriculture* (eds. A.A. Agrawal, S. Tuzun & E. Bent), pp. 37–53. The American Phytopathological Society Press, St. Paul.
- Hatcher, P.E. & Paul, N.D. (2000) Beetle grazing reduces natural infection of *Rumex obtusifolius* by fungal pathogens. *New Phytologist*, **146**, 325–333.
- Hatcher, P.E., Ayres, P.G. & Paul, N.D. (1995) The effect of natural and simulated insect herbivory, and leaf age, on the process of infection of *Rumex crispus* L. and *R. obtusifolius* L. by *Uromyces rumicis* (Schum.) Wint. *New Phytologist*, **130**, 239–249.
- Heil, M. (1999) Systemic acquired resistance – available information and open ecological questions. *Journal of Ecology*, **87**, 341–346.
- Heil, M. (2001) The ecological concept of costs of induced systemic resistance (ISR). *European Journal of Plant Pathology*, **107**, 137–146.
- Heil, M., Fiala, B., Boller, T. & Linsenmair, K.E. (1999) Reduced chitinase activities in ant plants of the genus *Macaranga*. *Naturwissenschaften*, **86**, 146–149.
- Heil, M., Hilpert, A., Kaiser, W. & Linsenmair, K.E. (2000a) Reduced growth and seed set following chemical induction of pathogen defence: does systemic acquired resistance (SAR) incur allocation costs? *Journal of Ecology*, **88**, 645–654.
- Heil, M., Staehelin, C. & McKey, D. (2000b) Low chitinase activity in *Acacia myrmecophytes*: a potential trade-off between biotic and chemical defences? *Naturwissenschaften*, **87**, 555–558.
- Hermes, D.A. & Mattson, W.J. (1992) The dilemma of plants: to grow or to defend. *The Quarterly Review of Biology*, **67**, 283–335.
- Hoover, K., Stout, M.J., Alaniz, S.A., Hammock, B.D. & Duffey, S.S. (1998) Influence of induced plant defenses in cotton and tomato on the efficacy of baculoviruses on noctuid larvae. *Journal of Chemical Ecology*, **24**, 253–271.

- Hunt, M.D. & Ryals, J.A. (1996) Systemic acquired resistance signal transduction. *Critical Reviews in Plant Sciences*, **15**, 583–606.
- Hunt, M.D., Neuenschwander, U.H., Delaney, T.P., Weymann, K.B., Friedrich, L.B., Lawton, K.A., Steiner, H.-Y. & Ryals, J.A. (1996) Recent advances in systemic acquired resistance research – a review. *Gene*, **179**, 89–95.
- Ignatius, S.M.J., Chopra, R.K. & Muthukrishnan, S. (1994) Effects of fungal infection and wounding on the expression of chitinases and β -1,3 glucanases in near-isogenic lines of barley. *Physiologia Plantarum*, **90**, 584–592.
- Inbar, M., Doostdar, H., Sonoda, R.M., Leibe, G.L. & Mayer, R.T. (1998) Elicitors of plant defensive systems reduce insect densities and disease incidence. *Journal of Chemical Ecology*, **24**, 135–149.
- Inbar, M., Doostdar, H., Leibe, G.L. & Mayer, R.T. (1999) The role of plant rapidly induced responses in asymmetric interspecific interactions among insect herbivores. *Journal of Chemical Ecology*, **25**, 1961–1979.
- Iseli, B., Armand, S., Boller, T., Neuhaus, J.-M. & Henrissat, B. (1996) Plant chitinases use two different hydrolytic mechanisms. *FEBS letters*, **382**, 186–188.
- Jackson, A.O. & Taylor, C.B. (1996) Plant-microbe interactions: life and death at the interface. *The Plant Cell*, **8**, 1651–1668.
- Jain, A. & Srivastava, H.S. (1981) Effect of salicylic acid on nitrate reductase activity in maize seedlings. *Physiologia Plantarum*, **51**, 339–342.
- Jakab, G., Cottier, V., Toquin, V., Rigoli, G., Zimmerli, L., Métraux, J.-P. & Mauch-Mani, B. (2001) β -Aminobutyric acid-induced resistance in plants. *European Journal of Plant Pathology*, **107**, 29–37.
- Järømo, J., Tuomi, J. & Nilsson, P. (1999) Adaptive status of localized and systemic defense responses in plants. *The Ecology and Evolution of Inducible Defenses* (eds. R. Tollrian & C.D. Harvell), pp. 33–44. Princeton University Press, Princeton.
- Karban, R. & Kuc, J. (1999) Induced resistance against pathogens and herbivores: an overview. *Induced Plant Defenses against Pathogens and Herbivores: Biochemistry, Ecology, and Agriculture* (eds. A.A. Agrawal, S. Tuzun & E. Bent), pp. 1–16. The American Phytopathological Society Press, St. Paul.
- Kessmann, H., Staub, T., Hofmann, C., Maetzke, T., Herzog, J., Ward, E., Uknes, S. & Ryals, J. (1994) Induction of systemic acquired resistance in plant by chemicals. *Annual Review of Phytopathology*, **32**, 439–459.
- Klessig, D.F. *et al.* (2000) Nitric oxide and salicylic acid signaling in plant defense. *Proceedings of the National Academy of Science of the USA*, **97**, 8849–8855.
- Kombrink, E. & Schmelzer, E. (2001) The hypersensitive response and its role in local and systemic disease resistance. *European Journal of Plant Pathology*, **107**, 69–78.
- Kuc, J. (1982) Induced immunity to plant disease. *BioScience*, **32**, 854–860.
- Kuc, J. (1995a) Induced systemic resistance – an overview. *Induced Systemic Resistance to Disease in Plants* (eds. R. Hammerschmidt & J. Kuc), pp. 169–175. Kluwer, Dordrecht.
- Kuc, J. (1995b) Phytoalexins, stress metabolism, and disease resistance in plants. *Annual Review of Phytopathology*, **33**, 275–297.
- Kuc, J. (1997) Molecular aspects of plant responses to pathogens. *Acta Physiologiae Plantarum*, **19**, 551–559.
- Kuc, J. (2001) Concepts and direction of induced systemic resistance in plants and its application. *European Journal of Plant Pathology*, **107**, 7–12.
- Lamb, C. & Dixon, R.A. (1997) The oxidative burst in plant disease resistance. *Annual Review of Plant Physiology and Plant Molecular Biology*, **48**, 251–275.
- Lawton, K., Weymann, K., Friedrich, L., Vernoij, B., Uknes, S. & Ryals, J. (1995) Systemic acquired resistance in *Arabidopsis* requires salicylic acid but not ethylene. *Molecular Plant-Microbe Interactions*, **8**, 863–870.
- Lian, B., Zhou, X., Miransari, M. & Smith, D.L. (2000) Effects of salicylic acid on the development and root nodulation of soybean seedlings. *Journal of Agronomy and Crop Sciences*, **185**, 187–192.
- Linthorst, H.J.M., Knoester, M., van Loon, L.C. & Bol, J.F. (1996) Ethylene in the expression of tobacco PR genes. *Journal of Experimental Botany*, **47** (Suppl.), 85.
- Longemann, E., Wu, S.-C., Schröder, J., Schmelzer, E., Somssich, I.E. & Hahlbrock, K. (1995) Gene activation by UV light, fungal elicitor or fungal infection in *Petroselinum crispum* is correlated with repression of cell-cycle-related genes. *The Plant Journal*, **8**, 865–876.
- Luckner, M. (1990) *Secondary Metabolism in Microorganisms, Plants, and Animals*. Fischer, Jena.
- Malamy, J., Carr, J.P., Klessig, D.F. & Raskin, I. (1990) Salicylic acid: a likely endogenous signal in the resistance response of tobacco to viral infection. *Science*, **250**, 1002–1004.
- Marquis, R.J., Diniz, I.R. & Morais, C. (2001) Patterns and correlates of interspecific variation in foliar insect herbivory and pathogen attack in Brazilian cerrado. *Journal of Tropical Ecology*, **17**, 127–148.
- Martínez-Abarca, F., Herrera-Cervera, J.A., Bueno, P., Sanjuan, J., Bisseling, T. & Olivares, J. (1998) Involvement of salicylic acid in the establishment of the *Rhizobium meliloti*-alfalfa symbiosis. *Molecular Plant-Microbe Interactions*, **11**, 153–155.
- Mauch, F., Mauch-Mani, B. & Boller, T. (1988) Antifungal hydrolases in pea tissue II. Inhibition of fungal growth by combination of chitinase and β -1,3 glucanase. *Plant Physiology*, **88**, 936–1042.
- Mauch, F., Mauch-Mani, B., Gaille, C., Kull, B., Haas, D. & Reimmann, C. (2001) Manipulation of salicylate content in *Arabidopsis thaliana* by the expression of an engineered bacterial salicylate synthase. *The Plant Journal*, **25**, 67–77.
- Mayer, R.T., McCollum, T.G., McDonald, R.E., Polston, J.E. & Doostdar, H. (1996) *Bemisia* feeding induces pathogenesis-related proteins in tomato. *Bemisia 1995: Taxonomy, Biology, Damage Control and Management* (eds. D. Gerling & R.T. Mayer), pp. 179–188. Intercept Ltd., Andover.
- Métraux, J.-P. (2001) Systemic acquired resistance and salicylic acid: current state of knowledge. *European Journal of Plant Pathology*, **107**, 13–18.

- Métraux, J.-P. *et al.* (1990) Increase in salicylic acid at the onset of systemic acquired resistance. *Science*, **250**, 1004–1006.
- Moran, P.J. & Cipollini Jr., D.F. (1999) Effect of wind-induced mechanical stress on soluble peroxidase activity and resistance to pests in cucumber. *Journal of Phytopathology*, **147**, 313–316.
- Neuhaus, J.M. (1999) Plant Chitinases (PR-3, PR-4, PR-8, PR-11). *Pathogenesis-related Proteins in Plants* (eds. S.K. Datta & S. Muthukrishnan), pp. 77–105. CRC Press, Boca Raton.
- Niki, T., Mitsuura, I., Seo, S., Ohtsubo, N. & Ohashi, Y. (1998) Antagonistic effects of salicylic acid and jasmonic acid on the expression of pathogenesis-related (PR) protein genes in wounded mature tobacco leaves. *Plant Cell Physiology*, **39**, 500–507.
- Oostendorp, M., Kunz, W., Dietrich, B. & Staub, T. (2001) Induced resistance in plants by chemicals. *European Journal of Plant Pathology*, **107**, 19–28.
- Padgett, G.B., Russin, J.S., Snow, J.P., Boethel, D.J. & Berggren, G.T. (1994) Interactions among the soybean looper (Lepidoptera: Noctuidae), threecorned alfalfa hopper (Homoptera: Membracidae), stem canker, and red crown rot in soybean. *Journal of Entomological Science*, **29**, 110–119.
- Pena-Cortes, H., Albrecht, T., Prat, S., Weiler, E.W. & Willmitzer, L. (1993) Aspirin prevents wound-induced gene-expression in tomato leaves by blocking jasmonic acid biosynthesis. *Planta*, **191**, 123–128.
- Picard-Nizou, A., Grison, R., Olsen, L., Pioche, C., Arnold, G. & Pham-Delegue, M. (1997) Impact of proteins used in plant genetic engineering: toxicity and behavioral study in the honeybee. *Journal of Economic Entomology*, **90**, 1710–1716.
- Pieterse, C.M.J. & Van Loon, L.C. (1999) Salicylic acid-independent plant defence pathways. *Trends in Plant Science*, **4**, 52–58.
- Pieterse, C.M.J. *et al.* (2001) Rhizobacteria-mediated induced systemic resistance: triggering, signalling and expression. *European Journal of Plant Pathology*, **107**, 51–61.
- Ramamoorthy, V., Viswanathan, R., Raguchander, T., Prakasam, V. & Samiyappan, R. (2001) Induction of systemic resistance by plant growth promoting rhizobacteria in crop plants against pests and diseases. *Crop Protection*, **20**, 1–11.
- Ramanujam, M.P., Abdul Jaleel, V. & Kumara Velu, G. (1998) Effect of salicylic acid on nodulation, nitrogenous compounds and related enzymes of *Vigna mungo*. *Biologia Plantarum*, **41**, 307–311.
- Raskin, I. (1992) Role of salicylic acid in plants. *Annual Review of Plant Physiology and Plant Molecular Biology*, **43**, 439–463.
- Redman, A.M., Cipollini Jr., D.F. & Schultz, J.C. (2001) Fitness costs of jasmonic acid-induced defense in tomato, *Lycopersicon esculentum*. *Oecologia*, **126**, 380–385.
- Rhoades, D.F. (1979) Evolution of plant chemical defense against herbivores. *Herbivores: their Interaction with Secondary Plant Metabolites* (eds. G.A. Rosenthal & D.H. Janzen), pp. 4–53. Academic Press, New York and London.
- Ross, A.F. (1961) Systemic acquired resistance induced by localized virus infection in plants. *Virology*, **14**, 340–358.
- Roy, B.A., Kirchner, J.W., Christian, C.E. & Rose, L.E. (2001) High disease incidence and apparent disease tolerance in a North American Great Basin plant community. *Evolutionary Ecology*, **14**, 421–438.
- Ruiz-Lozano, J.M., Roussel, H., Gianinazzi, S. & Gianinazzi-Pearson, V. (1999) Defense genes are differentially induced by a mycorrhizal fungus and *Rhizobium* sp. in wild-type and symbiosis-defective pea genotypes. *Molecular Plant-Microbe Interactions*, **12**, 976–984.
- Russin, J.S., Layton, M.B., Boethel, D.J., McGawley, E.C., Snow, J.P. & Berggren, G.T. (1990) Growth, nodule development, and N₂-fixing ability in soybean damaged by an insect-fungus-herbivore-nematode pest complex. *Journal of Economic Entomology*, **83**, 247–254.
- Russo, V.M., Russo, B.M., Peters, M., Perkins-Veazie, P. & Cartwright, B. (1997) Interaction of *Colletotrichum orbiculare* with thrips and aphid feeding on watermelon seedlings. *Crop Protection*, **16**, 581–584.
- Ryals, J., Uknes, S. & Ward, E. (1994) Systemic acquired resistance. *Plant Physiology*, **104**, 1109–1112.
- Sahai, A.S. & Manocha, M.S. (1993) Chitinases of fungi and plants: their involvement in morphogenesis and host-parasite interaction. *FEMS Microbiology Reviews*, **11**, 317–338.
- Schlumbaum, A., Mauch, F., Vögeli, U. & Boller, T. (1986) Plant chitinases are potent inhibitors of fungal growth. *Nature*, **324**, 365–367.
- Schneider, M., Schweizer, P., Meuwly, P. & Métraux, J.P. (1996) Systemic acquired resistance in plants. *International Review of Cytology* (ed. K.W. Jeon), pp. 303–340. Academic Press, San Diego.
- Schultze, M. & Kondoroski, A. (1998) Regulation of symbiotic root nodule development. *Annual Review of Genetics*, **32**, 33–57.
- Simms, E.L. & Fritz, R.S. (1990) The ecology and evolution of host-plant resistance to insects. *Trends in Ecology and Evolution*, **5**, 356–360.
- Simms, E.L. & Rausher, M.D. (1987) Costs and benefits of plant resistance to herbivory. *The American Naturalist*, **130**, 570–581.
- Somssich, I.E. & Hahlbrock, K. (1998) Pathogen defence in plants – a paradigm of biological complexity. *Trends in Plant Science*, **3**, 86–90.
- Sticher, L., Mauch-Mani, B. & Métraux, J.-P. (1997) Systemic acquired resistance. *Annual Review of Phytopathology*, **35**, 235–270.
- Stout, M.J. & Bostock, R.M. (1999) Specificity of induced responses to arthropods and pathogens. *Induced Plant Defenses against Pathogens and Herbivores: Biochemistry, Ecology, and Agriculture* (eds. A.A. Agrawal, S. Tuzun & E. Bent), pp. 183–209. The American Phytopathological Society Press, St. Paul.
- Stout, M.J., Workman, K.V., Bostock, R.M. & Duffey, S.S. (1998a) Specificity of induced resistance in the tomato, *Lycopersicon esculentum*. *Oecologia*, **113**, 74–81.
- Stout, M.J., Workman, K.V., Bostock, R.M. & Duffey, S.S. (1998b) Stimulation and attenuation of induced resistance by elicitors and inhibitors of chemical induction in tomato (*Lycopersicon esculentum*) fo-

- liage. *Entomologia Experimentalis et Applicata*, **86**, 267–279.
- Stout, M.J., Fidantsef, A.L., Duffey, S.S. & Bostock, R.M. (1999) Signal interactions in pathogen and insect attack: Systemic plant-mediated interactions between pathogens and herbivores of the tomato, *Lycopersicon esculentum*. *Physiological and Molecular Plant Pathology*, **54**, 115–130.
- Stumm, D. & Gessler, C. (1986) Role of papillae in the induced systemic resistance of cucumber against *Colletotrichum lagenarium*. *Physiological and Molecular Plant Pathology*, **29**, 405–410.
- Thaler, J.S. (1999a) Induced resistance in agricultural crops: Effects of jasmonic acid on herbivory and yield in tomato plants. *Environmental Entomology*, **28**, 30–37.
- Thaler, J.S. (1999b) Jasmonic acid mediated interactions between plants, herbivores, parasitoids, and pathogens: a review of field experiments in tomato. *Induced Plant Defenses against Pathogens and Herbivores: Biochemistry, Ecology, and Agriculture* (eds. A.A. Agrawal, S. Tuzun & E. Bent), pp. 319–334. The American Phytopathological Society Press, St. Paul.
- Thaler, J.S. & Karban, R. (1997) A phylogenetic reconstruction of constitutive and induced resistance in *Gossypium*. *The American Naturalist*, **149**, 1139–1146.
- Thaler, J.S., Fidantsef, A.L., Duffey, S.S. & Bostock, R.M. (1997) Trade-offs in plant defense against pathogens and herbivores: a field demonstration of chemical elicitors of induced resistance. *Journal of Chemical Ecology*, **25**, 1597–1609.
- Thomma, B.P.H.J., Penninckx, I.A.M.A., Broekaert, W.F. & Cammue, B.P.A. (2001) The complexity of disease signalling in *Arabidopsis*. *Current Opinion in Immunology*, **13**, 63–68.
- Tollrian, R. & Harvell, C.D. (1999) The evolution of inducible defenses: current ideas. *The Ecology and Evolution of Inducible Defenses* (eds. R. Tollrian & C.D. Harvell), pp. 306–321. Princeton University Press, Princeton.
- Tronsmo, A.M., Gregersen, P., Hjeljord, L., Sandal, T., Bryngelsson, T. & Colinge, D.B. (1993) Cold induced disease resistance. *Mechanisms of Plant Defense Responses* (eds. B. Fritig & M. Legrand), pp. 215–221. Kluwer, Dordrecht.
- Underwood, N.C. (1998) The timing of induced resistance and induced susceptibility in the soybean-Mexican bean beetle system. *Oecologia*, **114**, 376–381.
- Van der Westhuizen, A.J., Qian, X.M. & Botha, A.M. (1998) β -1,3-Glucanases in wheat and resistance to the Russian wheat aphid. *Physiologia Plantarum*, **103**, 125–131.
- Van Loon, L.C. (1997) Induced resistance in plants and the role of pathogenesis-related proteins. *European Journal of Plant Pathology*, **103**, 753–765.
- Van Loon, L.C. & van Strien, E.A. (1999) The families of pathogenesis-related proteins, their activities, and comparative analysis of PR-1 type proteins. *Physiologia and Molecular Plant Pathology*, **55**, 85–97.
- Van Loon, L.C., Bakker, P.A.H.M. & Pieterse, C.M.J. (1998) Systemic resistance induced by rhizosphere bacteria. *Annual Review of Phytopathology*, **36**, 453–483.
- Vierheilig, H., Alt, M., Mohr, U., Boller, T. & Wiemken, A. (1994) Ethylene biosynthesis and activities of chitinase and β -1,3-glucanase in the roots of host and non-host plants of vesicular-arbuscular mycorrhizal fungi after inoculation with *Glomus mosseae*. *Journal of Plant Physiology*, **143**, 337–343.
- Vierheilig, H., Alt, M., Lange, J., Gutreilla, M., Wiemken, A. & Boller, T. (1995) Colonization of transgenic tobacco constitutively expressing pathogenesis-related proteins by the vesicular-arbuscular mycorrhizal fungus *Glomus mosseae*. *Applied and Environmental Microbiology*, **61**, 3031–3034.
- Xu, Y., Chang, P.F.L., Liu, D., Narasimhan, M.L., Raghothama, K.G., Hasegawa, P.M. & Bressan, R.A. (1994) Plant defense genes are synergistically induced by ethylene and methyl jasmonate. *Plant Cell*, **6**, 1077–1085.

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