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Inducing resistance: a summary of papers presented at the First International Symposium on Induced Resistance to Plant Diseases, Corfu, May 2000

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The First International Symposium on Induced Resistance to Plant Diseases, organized by Eris Tjamos, brought together over 150 participants to discuss the complexities, questions and future direction of research on the mechanisms by which plants can become better able to defend themselves against pathogen attack. Although the term 'immunization' has been used to denote treatments that enhance the defensive capacity of plants, the correspondence to vaccination in vertebrates is far-fetched: the induced state is by no means specific, but rather constitutes a more general increase in plant resistance to various types of pathogens. Moreover, it seldom prevents disease from occurring but generally reduces its extent or severity. These characteristics make induced resistance a powerful mechanism to exploit for enhancing the overall resistance in crop plants. Indeed, the first commercial chemical triggering induced resistance in plants, acibenzolar-S-methyl (BTH) was recently introduced on the market by Novartis under the tradenames Actigard (USA) and BION (Europe).

The first talk of the meeting was presented by Professor Joe Kuć, who provided an overview of the phenomenon of induced resistance. The many different biological and chemical agents that can lead to a state of induced resistance were discussed and the question of how different stimuli can cause a similar increase in resistance was addressed. The similarity of defenses expressed in genetic resistance and in epigenetically induced resistance was highlighted to illustrate what we know and do not know about the regulation of all forms of disease resistance. The overall contribution of Kuć's work on the establishment of induced resistance as an important area of research, as well as a strategy for disease control, was clearly evident in his talk. The pioneering research of Frank Ross on the discovery and establishment of the characteristics of systemic acquired resistance in tobacco against tobacco mosaic virus (TMV) was described, along with a biographical sketch of this important figure in induced resistance research (A. Ellingboe).

The phenomenon of induced resistance has been variously described as systemic acquired resistance (SAR) and induced systemic resistance (ISR), the term 'systemic' stressing the point that protection is not confined to treated plant parts but extends into non-treated, and often even newly developing, plant parts. However, certain agents confer localized protection only, which raises the question whether this localized induced resistance is similar to SAR/ISR except for lack of the production of an emanating signal, or whether it reflects a totally separate mechanism. SAR/ISR is part of the inducible defense responses elicited by avirulent pathogens and, therefore, some workers have stressed that this type of resistance needs to be induced; hence: induced systemic resistance. Others have argued that induced resistance reflects a physiological state that is acquired by the plant as a result of prior stimulation; hence: systemic acquired resistance. Although in the past, propositions were made for an all-compassing term, both SAR and ISR are being used, often depending on the 'school' to which researchers belong. In a discussion session during the meeting it was agreed that 'induced resistance' is the general term by which all types of elicited responses that lead to enhanced protection against disease – including both locally and systemically induced resistance – can be designated. This term is the same as

the one used by ecologists to denote, e.g. reduced plant damage by herbivorous insects after a previous attack. To refer to the systemic protection resulting from for example infection by an avirulent pathogen, the terms ISR and SAR may be used synonymously. This compromise does justice to the traditions of both terms, as well as to their present use in the existing literature.

A less studied induced resistance phenomenon, localized acquired resistance (LAR), was described in detail for TMV and bacterial pathogens. The relationship of LAR as part of localized disease resistance responses to TMV in tobacco, such as the hypersensitive reaction (HR) and how LAR differs from the systemic induced resistance responses at the molecular and functional levels was discussed. In tobacco LAR is expressed in a ring of cells surrounding the HR site. Salicylic acid (SA) is important for the establishment of both LAR and SAR, but active oxygen species probably do not play a significant role in LAR (S. Kauffmann). Local induced resistance can also be activated by live and dead bacteria, as well as bacterial lipopolysaccharide (LPS). The bacterial local induced resistance occurs in two distinct temporal phases: an early induced resistance phase (6–20 h) that can be inhibited by protein synthesis inhibitors, and a late phase that requires light and 24 h to develop (Z. Klement).

The phenomenon of induced disease resistance was discussed in relation to currently existing ecological theories of plant defense, and the lack of studies evaluating data from induced disease resistance in ecological or evolutionary terms was pointed out. Questions on why systemically induced resistance is not constitutive and the allocation of fitness costs of induced resistance were raised. Potential costs of induced resistance to the plant were illustrated, in that wheat plants induced with BION were shown to produce fewer lateral shoots and yield a smaller amount of grain. This negative response was related to the developmental stage of the plants when treated, as well as to the available nitrogen supply. Faba bean plants, after treatment with BION, were reported to be smaller than controls with fewer root nodules (M. Heil).

Induction of resistance by biotic and abiotic agents

Induced resistance has been demonstrated in many plant species and various presentations extended the range investigated by showing induction by fungi, bacteria, microbial elicitors and chemicals. In addition to biotic stresses, the environinent can also alter the host response to infections. Cold acclimation treatment of winter wheat that results in a state of cold hardiness induces resistance to the snow mold *Microdochium nivale.* Cold-hardened plants expressed higher levels of chitinase and peroxidase transcripts, and inoculation of these cold-hardened plants resulted in a further increase in expression of PR-la and several defense-associated enzymes as compared to non-hardened plants (A.M. Tronsmo). Less research has been reported over the years on induced resistance to nematodes. Treatment of wheat and barley with the non-amino acid β-aminobutyric acid (BABA) induced resistance to two *Heterodera* species as measured by reduction of cyst formation. Other known chemical disease resistance activators were not effective. BABA treatment also reduced egg mass production by a cereal-specific species of *Meloidogyne.* Effects on the development of *Heterodera* juveniles into adults were discussed as part of an induced resistance mechanism (Y. Oka).

The development of BION as an inducer of resistance has led to ample information on its efficacy under field conditions. BION activates disease resistance in many crops to a wide variety of pathogens. It has a lasting effect that is more pronounced in monocots than in dicots (T. Staub). Alone or in combination with insecticides, BION was used successfully on tomato crops against *Bemisa tabaci*, the vector of the tomato leaf curl virus, resulting in better yields and less disease incidence (E. Moriones). BION was included in a large number of crop management programs and showed added benefits for the farmer (M. Oostendorp). Arabidopsis overexpressing the *NIM1* gene shows hypersensitivity to BION and resistance is induced at 10-fold lower concentrations than in untransformed lines. This suggests that BION interacts directly or indirectly with NIM1 in activating SAR (B. Dietrich).

Oxycom TM, a combination of fertilizer and an active oxygen generator, is another commercial inducer of resistance. In bean, Oxycom TM is marginally fungicidal and induced defense-related genes encoding proteins involved in phenolic metabolism and plant cell wall strengthening. Similar patterns of gene induction were observed after induction by SA or hydrogen peroxide (A. Anderson). DF-391, a novel non-fungicidal synthetic pyridine derivative (produced by Dainippon Ink & Chemicals), was presented that is active against cucumber anthracnose (H. Ishii).

Verticillium wilts are notoriously difficult to control, making this disease a worthwhile target for new control measures. *Verticillium albo-atrum* culture filtrates applied to the foliage of cucumber leaves protect root-inoculated plants. RNAse A sprayed on cucumber leaves before or after inoculation with *V. albo-atrum* also triggered a resistance response to *Verticillium.* The inducing activity of RNAse was lost upon heating, and cysteine, an amino acid present in RNAse A was also active (E. Tjamos). Some chemicals, such as dipotassium phosphate, when applied to the foliage, trigger localized cell death and, thereby, elicit SA-dependent SAR (J. Siegrist).

Genetic engineering of pathogen resistance using genes encoding pathogenesis-related proteins (PRs) or other antifungal proteins, R genes, switches *(NDR, EDS)* or *Avr* genes was discussed. While many encouraging results were presented, the limiting factor for most of these approaches remains the availability of inducible promoters that are specifically responding to the presence of a pathogen (M. Stuiver).

Recognition and signal transduction

A large number of studies with Arabidopsis have opened the way to a molecular understanding of induced resistance. It became apparent that depending on the initial stimulus (leaf pathogen, rhizobacteria inoculation or wounding) different signal transduction pathways are set in motion. These pathways rely on endogenous regulators such as SA, ethylene and jasmonic acid (JA) to induce defense reactions, and do or do not require NPR1/NIM1. Sets of defense reactions operate against specific sets of pathogens. Cross-talks between these pathways are suggestive of a network structure in which various external signals are integrated. For instance, the signal transduction pathway for light is required for induction of PR-1 by SA, leading to the notions of 'dark' versus 'light' defenses. The logical representation of pathways and cross-talks can be reduced to a relatively simple network of boolean operators (J.-P. Métraux).

Data supporting the presence of two different induced resistance pathways in barley to *Blumeria graminis* f. sp. *hordei* and *Bipolaris sorokiniana* were presented. SA and its functional analogs 2,6-dichloroisonicotinic acid (INA) and BION induce resistance to *Blumeria,* but not *Bipolaris,* while JA only induces resistance to *Bipolaris.* Suppression-subtractive hybridization was used to isolate nine new genes (named *'BCI'* for 'barley chemically induced') from barley that are induced by INA or BION. While some of these had homology to known defense or signal transduction genes, several exhibited no homology to previously characterized genes (G. Langen). All but one of these genes were also induced by JA. The data suggest that cross-talk between the two pathways leads to induced resistance (K. Besser).

Plants in the field are attacked by pathogens and insect herbivores. These different types of attacks can lead to expression of defenses that are controlled by different signaling pathways, and thus it is possible that induction of different signaling pathways by herbivores and pathogens leads to unexpected patterns of defense expression. Both conflicts and synergies between SA- and JA-mediated defense response were described and both greenhouse and field experiments were used to show the trade-off that can occur when both pathways are induced simultaneously or at different times (R. Bostock). Along these same lines, treatment of cotton plants with BTH resulted in both local and systemic increases in PRs. However, there was no effect on whitefly feeding preference or feed efficiency of cotton bollworms on induced as compared to control cotton plants (R. Mayer).

Cell wall-degrading enzymes of the bacterial soft rot pathogen *Erwinia carotovora* trigger an SA-independent pathway in Arabidopsis that is dependent on the synergistic actions of ethylene and JA and require neither SA nor NPR1. However, the pathway can be potentiated by SA (T. Palva). SA has a dual role, since it enhanced the effect of JA/ethylene, while suppressing the activation of genes induced by JA alone (G. Brader). The complexity of interactions between the signal transduction pathways was explored in Arabidopsis using DNA microarrays (made of 2400 ESTs). Measurement of relative RNA abundance (2–5-fold induction over background levels) was determined in duplicate plants after infection with an incompatible pathogen or treatment with various signals. Cluster analysis showed an overlap in gene induction between treatments. The largest one was observed after treatment with SA and JA, where 55 genes were commonly induced (J. Manners).

BABA was shown to be a powerful conditioner of plant defense mechanisms. In Arabidopsis, BABA protects against *P. parasitica* independently of SA, JA and ethylene and induces a rapid production of cell wall deposits, thus blocking the fungus. BABA

can also protect against *P. syringae*, but in this case the protection depended on SA and the induction of PRs (B. Mauch-Mani). In tobacco, BABA protected tobacco against downy mildew independently of SA and against TMV in a SA-dependent fashion (Y. Cohen; J. Siegrist). BABA either as a foliar spray or as a soil drench was also shown to have a widespread action against air-borne or soil-borne fungi, as well as nematodes (Y. Cohen).

Components of the early signal transduction pathway include nitric oxide (NO) that activates G proteins and opens Ca^{++} channels. Aconitase is a possible target of NO and may regulate the iron availability required for the production of the toxic hydroxyl radical that could be involved in HR cell death. SA is proposed to act through binding to a receptor with subsequent activation of a SA-inducible protein kinase (SIPK), which is a MAP kinase family member and may function in a MAPK cascade leading to the activation of the defense responses. Two new SA-binding proteins (SABPs) were identified, one localized in the chloroplast, the other in the mitochondria. The affinity of these SABPs for SA is high and even higher for its functional analog BION. (D. Klessig). A novel target for BION was identified in the form of a protein that phosphorylates NIM. BION, but also SA and INA, were found to inhibit phosphorylation of this NIM kinase (C. Pillonnel). SA inhibited both multiplication and cell-to-cell movement of tobacco mosaic virus (TMV) in tobacco, but interfered only with systemic transport of cucumber mosaic virus (A. Murphy). The inhibitor of alternative oxidase, SHAM, inhibited resistance to TMV, whereas the inhibitor of the cytochrome pathway, cyanide, induced resistance to TMV. A role for the alternative oxidase in SA-dependent resistance to viruses was proposed (J.P. Carr).

In spite of numerous attempts, the nature of the systemically transported signal(s) has not been elucidated. An Arabidopsis mutant, *dir1* (defective in induced resistance), is only impaired in its ability to induce systemic defense responses. dir1 is a lipid transfer protein that is absent in phloem exudates from *dir1* mutants. Exudates from wild-type plants were perceived normally by leaves of *dir1*, resulting in the induction of PR-1. Thus, a lipid transfer protein might be the phloem mobile signal or might be associated closely with its production (R. Cameron). Neither the biosynthetic pathway nor the role of SA transport is clear. The role of phloem-translocated SA in the transmission of SAR was tested using transgenic NN tobacco plants (NahG) expressing salicylate hydroxylase under a phloem-specific sucrose synthase promoter. Such plants have strongly reduced SA levels in the phloem and reduced or abolished SAR, while still responding with a HR to TMV. SA translocated or synthesized in the phloem might therefore be essential for SAR (R. Darby).

Expression of resistance

The question of how plants, once induced, are able to stop the development of pathogens in host tissue was discussed along with a compilation of the putative defenses that are expressed as a result of resistance induction or after a challenge infection with the pathogen. It is clear that there are numerous host responses to resistance induction, but the relative contribution of each to plant defense has not been established (R. Hammerschmidt). All processes in plants are under genetic control, and this, of course, includes all forms of resistance. The more ‘traditional’ route of understanding host resistance mechanisms was briefly compared with the approach of using genetic analysis to dissect resistance and susceptibility. The use of genetic analysis to address the nature of disease resistance including the multiple genes that are likely to be involved in the expression of major gene as well as induced resistance was discussed. The complexity of resistance was illustrated by the recent observations that interpretations of gene-for-gene resistance may be an oversimplification of the actual situation as multiple genes may be involved in a specific plant-pathogen interaction (A. Ellingboe). The relationship and similarities between multigenic and induced resistance was illustrated by data showing that multigenic resistance in some host-pathogen systems could be correlated with constitutive expression of enzymes, such as chitinase, that have been associated with the systemic induced resistance state. The hydrolases expressed in plants expressing multigenic resistance to a specific pathogen were also reported to be most active against that specific pathogen (S. Tuzun).

The use of SAR to control *Rynchosporiurn secalis* and barley yellow dwarf virus in barley was described in relation to studies on the genetics of induced resistance in barley. Use of molecular breeding techniques and QTL analysis as a means of optimizing induced resistance were described (M. Krämer). A genetic basis for induced resistance was further illustrated by studies on the rhizobacteria-mediated induced systemic resistance response in Arabidopsis. It was reported that not

all Arabidopsis ecotypes express this type of ISR to the same level. Genetic analysis demonstrated that this ability was associated with a single locus, *ISR1*, in the host plant (J. Ton).

The induction of ISR in soybean might involve elicitation competency for glyceollin accumulation through an oxidative pathway and induction of isoflavone accumulation via nuclear receptor ligands. This illustrates that several complementary signaling pathways govern secondary product defense pathways during ISR in soybean (T.L. Graham). In rice, BTH induced a lipoxygenase, but its role in induced resistance is not yet clear (U. Schaffrath). In Arabidopsis, BTH increased sensitivity to *Pseudomonas syringae*, by potentiating the expression of PAL genes. PAL genes and callose deposition were also potentiated by BTH after wounding, an effect that depends on a functional *NPR1* gene. NPRl may thus modulate both the SAR and a wound-induced signaling pathway (U. Conrath). In cucumber hypocotyls, pretreatments with BTH or INA condition the tissue for stronger elicitor-induced accumulation of chitinase mRNA or production of hydrogen peroxide. Specific proteins as well as the activity of the ubiquitin proteasome system are required for conditioning, indicating that the acquisition of competence requires proteolysis of proteins yet to be defined (H. Kauss). SA potentiates the oxidative burst in tobacco, thereby accelerating cell death and defense gene expression. All these responses were delayed in transgenic NahG plants. Such plants showed that resistance to TMV and bacteria depends on events occurring in the pre-necrotic phase of the HR, highlighting the importance of the kinetic parameters for resistance (L. Mur).

The role of abscisic acid (ABA) in the resistance to pathogens has been studied in ABA-deficient *sitiens* tomato plants. Such plants were more resistant to *Botrytis cinerea*, *Sclerotinia sclerotiorum* and *Alternaria solani* than wild-type plants. While BTH was effective in inducing resistance to *B. cinerea* in wild-type plants, the *sitiens* plants were ten times more responsive to this inducer. Taken together the data indicate that endogenous ABA might be a negative regulator of the SAR response (K. Audenaert).

The incompatible interaction pepper/*Xanthomonas campestris* was used to study the localized protective effect of bacterial LPS against the HR induced by avirulent bacteria. Treatment with LPS does not activate *PR* genes directly but potentiates their expression after bacterial inoculation (M. Dow). LPS also potentiates the phenol metabolism after bacterial infection. Interestingly, two antimicrobial phenolic conjugates, coumaroyl tyramine (CT) and feruloyl tyramine (FT), accumulated more rapidly after inoculation in peppers pretreated with LPS (M.A. Newman).

One of the most prominent induced responses in incompatible interactions is the accumulation of PRs. Because their induction extends to non-inoculated plant parts that develop SAR, their occurrence is associated with the state of induced resistance. Some of these PRs have antifungal or antibacterial activities, suggestive of a role of these proteins in the enhanced protection against disease in induced plants. Therefore, PRs are often used as markers for SAR. During resistance reactions, necrotic lesions may develop, but necrosis is not required for triggering SAR and systemic gene activation. Conversely, necrosis and the concomitant induction of SAR can also occur in compatible interactions. Systemic signalling and induction of PRs requires an interplay of locally produced signals which may involve mechanical pressure, as well as reactive oxygen species (E. Kombrink). Moreover, expression of different PRs is regulated by different signals and several PRs are also induced in a developmentally-controlled, organ-specific manner, suggesting that they may act not only in defense against pathogens or similar stresses, but also play a role in plant morphogenesis (K. Van Loon). Some fungal elicitors can induce PRs through their toxic action on plants, whereas others appear to specifically trigger their induction by activating the signalling pathways involved (M. Guardiola). Relatively little is known about induced disease resistance in woody plants. Birch (*Betula pendula*) is being used because of its small genome and great genetic diversity. Birch leaves, when challenged with *Erwinia carotovora*, expressed a HR and were used to clone several HR-induced genes. In addition, local induction of chitinase, PR-1 and PR- 10 was reported (M. Karls).

Besides PRs, phytoalexin synthesis and cell wall strengthening are defense responses that are induced during incompatible interactions or upon treatment with elicitors. These responses involve increases in the activities of oxidative enzymes, as well as synthesis of terpenoid and/or aromatic precursors (J. Greyerbiehl). Some of these responses occur beyond the site of invasion/treatment and reduce the activity of subsequent attackers. For instance, peroxidase activity is increased systemically, thereby providing greater capacity for lignification after challenge inoculation. A rapid oxidative burst appears to be instrumental in rapid cell death and increased lignification (S. Shetty). In leaf discs from SAR-expressing cucumber plants challenged with *Colletotrichum orbiculare*,

cycloheximide reduced hydrogen peroxide generation and increased fungal penetration and disease development while also increasing the expression of PRs. Such observations support the importance of active oxygen species in the expression of resistance (L. Velasquez). In general, a relationship between induced resistance and accumulation of PRs is less apparent (D. Silué).

Resistance induced by non-pathogenic micro-organisms

Not only avirulent pathogens but also non-pathogenic rhizobacteria can induce systemic resistance in plants. This rhizobacteria-mediated ISR has been reported to be effective against fungi, bacteria and viruses, but appears to involve different signalling pathways and mechanisms. In Arabidopsis, selected bacterial strains trigger a SA-independent but JA- and ethylene-dependent pathway that, nevertheless, is dependent on the regulatory factor NPR1, that is also part of the SA-dependent pathway. Two non-inducible ecotypes of Arabidopsis are impaired in the same gene (*ISR1*) and have reduced sensitivity to ethylene, confirming the importance of ethylene sensitivity in ISR signalling (C. Pieterse). The non-dependency of resistance induction in Arabidopsis on SA was further illustrated by the protection against *Peronospora parasitica* by strain *Pseudomonas fluorescens* CHA0, which is itself capable of producing SA. Induction of resistance was maintained in SA-degrading NahG plants and not associated with the production of PRs (E. Boutet). However, several strains selected for rhizobacteria-mediated ISR against *Colletotrichum orbiculare* on cucumber, when tested on tobacco transformants containing a chimaeric PR-1a-GUS construct, induced GUS activity, as well as hydrogen peroxide formation and lignification of the roots (K. Park). A *Paenibacillus* strain (K165) induced resistance against *Verticillium dahliae* in eggplant and Arabidopsis. Resistance induction in Arabidopsis appeared to involve both the SA and the JA/ethylene signalling pathways (S. Tjamos).

In field-grown cucumber, several rhizobacterial strains induce resistance against bacterial wilt and also reduce numbers of the cucumber beetle vectors of the causative bacteria. Reduced beetle feeding and transmission of wilt disease were associated with significantly reduced levels of cucurbitacin, a secondary plant metabolite and beetle feeding stimulant. Certain rhizobacterial strains also protected tomato against cucumber mosaic virus and whitefly-transmitted tomato mottle virus. Several combinations of bacterial strains with chitosan as a formulation carrier are being evaluated for their protective effects against various pathogens in vegetable transplant potting media (G. Zehnder). Particularly organic farming systems are a rich source for soil microorganisms that may induce ISR (T. Sicard).

Symbiotic arbuscular mycorrhizal fungi seem to suppress defense reactions when colonizing host roots. Nevertheless, they can protect plants locally against root pathogens through defense-related gene activation in arbuscule-containing cells. But in tomato split-root systems, mycorrhiza do induce systemic resistance against *Phytophthora parasitica*. This ISR is expressed as a stronger cell wall fortification at the site of attempted penetration of the pathogen (V. Gianinazzi). Culture filtrates of several plant growth-promoting fungi also protect cucmber against *Colletotrichum orbiculare* by promoting lignification (N. Koike). *Penicillium oxalicum* appears to protect tomato plants systemically against *Fusarium oxysporum* f.sp. *lycopersici* by preventing blocking or collapse of xylem vessels in infected plants. These histological changes could be related to the production of phytohormones (P. Melgarejo).

It is not clear how root colonization by these biocontrol agents is perceived by the plant and gives rise to ISR. The biocontrol bacterium *Pseudomonas putida* WCS358 appears to possess multiple determinants for inducing resistance in Arabidopsis against *Pseudomonas syringae* pv. *tomato*, adding to the complexities of this type of ISR (P. Bakker). In contrast, ISR in cucumber against *Colletotrichum orbiculare* elicited by *Serratia marcescens* was found to be solely based on siderophore production, because no protection occurred when the inducing bacterium lacked the ability to produce the catechol moiety required for iron chelation by the compound (C. Press).