Fitness costs of induced resistance: emerging experimental support for a slippery concept

Martin Heil and Ian T. Baldwin

Fitness costs can explain the evolution and maintenance of induced resistance in plants. However, the methods currently used to gather evidence for such costs do not allow decreases in fitness to be cleanly attributed to individual traits responsible for induced resistance, because the studies are plagued by multiple confounding responses. Reproductive performance provides an overview of physiological performance and is thus a useful currency to understand the function of induced responses. Integrated molecular, physiological and ecological studies are needed to identify the mechanisms responsible for the decrease in fitness and to evaluate fully the usefulness of the cost paradigm.

Many plants have evolved induced resistance and hence coordinate the development of resistance with the need for resistance. These responses can be directed, with some degree of specificity, against herbivores, pathogens or abiotic stresses, and can be elicited by attack from herbivores, pathogens and non-pathogenic root microorganisms [1]. The same, or at least similar, responses can be elicited by salicylic acid and jasmonic acid treatments or by factors in salivary and oviposition fluids. These phenomena have been called induced resistance (IR) when directed against herbivores [2] and systemic acquired resistance (SAR) or induced systemic resistance (ISR) when mainly directed against pathogens [3]; the two terms SAR and ISR are often used interchangeably.

All varieties share the common feature that resistance traits are expressed only in response to a first, initial attack or elicitation. Because this 'just-in-time' defence strategy has the disadvantage of leaving plants unprotected until resistance is induced (which can take hours to days), its selective advantage over constitutively expressed defence traits, which lack this drawback, demands an explanation. If resistance or its elicitation incurs significant fitness costs (i.e. if resistant plants have a lower reproduction than less resistant ones when compared under enemy-free conditions that prevent the resistance from having beneficial effects) [4] then the disadvantages of the delay might be offset by the fitness benefit of not incurring these costs when resistance is not needed. This cost–benefit explanation has been widely applied to IR against herbivores [2,5,6] and has recently been extended to IR against pathogens [3,7].

Martin Heil*†
Centre d'Ecologie Fonctionelle et Evolutive (CEFE-CNRS, UPR 9056), 1919 Route de Mende, F-34293 Montpellier Cedex 5, France.
*e-mail: martin_heil@hotmail.com
†Present address: Max-Planck-Institut für Chemische Ökologie

Ian T. Baldwin
Max-Planck-Institut für Chemische Ökologie, Beutenberg Campus, Winzerlaer Str. 10, D-07745 Jena, Germany.

Costs can arise from processes both internal and external to the plant

- Allocation costs can occur if large quantities of fitness-limiting resources are allocated to resistance traits. Such allocations might not be quickly recycled [8] and hence are unavailable for fitness-relevant processes such as growth or reproduction [9].
- Constitutive costs of inducible resistance occur because plants must have wound-detection pathways, defence precursors and storage vesicles, which all require constitutive allocation of both energy and resources [10].
- Autotoxicity costs: some resistance traits are toxic to the plant and their constitutive expression might impose a significant metabolic burden [11].
- 'Ecological' or 'environmental' costs [12] result from the myriad of interactions that a plant has with its environment. The best studied are cases in which defence compounds are sequestered by herbivores and used to protect them from their enemies [13]. Similarly, resistance directed against a herbivore or pathogen might have negative effects on symbiotic insects such as pollinators or on microorganisms such as mycorrhiza and root-nodulating bacteria [14].
- Tradeoffs with other defences: resistance against one group of enemies might be a liability when the plant is attacked by another group [15].

Ecological and pharmacological studies

Most studies that detected fitness costs of induced resistance have found them by manipulating the jasmonic acid signalling pathway, which plays an important role in IR against herbivores [16,17]. These responses might function either as direct resistance (traits that act directly against further attack or reduce herbivore performance) or as indirect resistance [2]. Indirect defence is based on attracting 'enemies of the plant's enemies' [13] via volatile compounds [13,18] or extrafloral nectar [19].

Indirect resistance does not cause high allocation costs (see Refs [20,21] for volatiles and Ref. [22] for extrafloral nectar) but several studies have revealed evidence for fitness costs of direct resistance traits. All these studies have compared induced to uninduced plants of the same species and did not take into account the constitutive costs resulting from the necessity of being inducible [10]. They thus represent conservative estimates of overall costs. Application of jasmonic acid (or its methyl ester, MeJA) to native wild tobacco (*Nicotiana attenuata*) growing in a variety of natural sites that differed in herbivore pressure, reduced lifetime seed production compared with untreated plants under herbivore-free conditions. By contrast, the fitness costs of jasmonic acid induction paid off for plants growing under moderate levels of herbivore pressure [23]. These findings clearly support the selective 'cost–benefit' scenario developed for the evolution and maintenance of inducible defences. In wild tobacco, jasmonic acid elicits large increases in nicotine production and accumulation, a defence response that makes 8% of the whole-plant

http://plants.trends.com 1360-1385/02/$ – see front matter PII: S1360-1385(01)02186-0

nitrogen pool unavailable for fitness-related processes [8] and reduces a plant's ability to compete with uninduced conspecifics (plants belonging to the same species) for uptake of soil nitrogen [24].

Eliciting proteinase inhibitor (PI) production and other defensive proteins in tomato produced mixed results. In a greenhouse study, high-dose foliar sprays of jasmonic acid that increased PI and peroxidase levels caused plants to produce fewer, larger fruits and so caused an overall decrease in lifetime seed production [25]. However, a similarly designed field study found no effect on fruit production [26]. Another greenhouse study with tomato that used chitosan to elicit PI production found no fitness effects [27]. The detection of fitness costs in crop plants whose fitness parameters have been selected to be buffered from leaf loss might be particularly problematic.

The jasmonate cascade is activated by wounding, and two studies have been designed with controls for the fitness effects of leaf removal to examine the fitness costs of resistance elicited by wounding [28,29]. Indole acetic acid (IAA) treatment of plant wounds suppresses wound-induced jasmonic acid production and subsequent nicotine accumulation in native *Nicotiana* [30]. Leaf wounding decreased lifetime seed production, but these negative fitness consequences could be fully reversed when IAA was applied to leaf wounds in a field experiment [28]. When the fitness effects in wild radish plants (*Raphanus raphanistrum*) induced by caterpillar feeding (induction and leaf tissue removal) were compared with those resulting from chemical induction (induction without tissue removal) and artificial leaf removal by clipping (only marginal induction), the effects of induction and tissue removal could be statistically separated. In this design, induction did not affect the number or weight of seeds but only the time to the first flower and the quantity of pollen [29].

Evidence is also emerging that salicylic acid-dependent ISR might incur fitness costs. The salicylic acid mimic BION® (benzothiadiazole) induces several 'wheat chemically induced' (WCI) genes in wheat and elicits a systemic resistance against pathogens [31]. Potted wheat plants growing under pathogen-free conditions in competition and under limited nitrogen were treated with BION. This strongly reduced aboveground growth and seed set, a result that is consistent with a resource allocation cost associated with elicitation [32]. No yield increases in wheat in response to BION treatment could be observed in an agronomic field study, although disease symptoms were reduced [33]. However, no defensive proteins were measured in these studies and therefore no estimates of the amount of fitness-limiting resources could be made. Much more effort will be required to explain and quantify 'the limiting effect of disease resistance on yield' [34]. An additional problem arises from the fact that salicylic acid can inhibit jasmonic acid synthesis and thereby JA-induced responses [35–37]. Fitness effects visible after the induction of one pathway might thus be the result of effects on the other pathway.

Methodological problems

None of the above-mentioned studies have clearly shown that a particular biochemically characterized induced defence incurs a fitness cost. Jasmonic acid and salicylic acid can elicit many physiological and morphological changes that seem not to be related to resistance but that nevertheless affect fitness parameters [16,38]. The induction of resistance can reduce protein biosynthesis in important parts of the primary metabolism. For example, ribulose bisphosphate carboxylase (RuBPCase) levels and the expression of histone-encoding genes are strongly reduced after pathogen infection or elicitor treatment [39,40]. Similarly, MeJA inhibits protein biosynthesis, which is essential for growth, including that of RuBPCase and chlorophyll *a/b* binding proteins [41]. This 'metabolic competition' is likely to result in fitness costs when plants grow under resource-limiting conditions [9] but it has still to be proved that these effects translate into negative consequences for plant growth or reproduction under natural conditions.

Many of the 'side effects' are strongly dosage dependent, and exogenous treatments probably do not faithfully mimic the endogenous pools, which are frequently highly tissue specific and transient. This methodological problem limits the value of those studies that have applied elicitors exogenously and at physiologically unrealistic concentrations. We know little about the traits that are necessary and sufficient for establishing resistance. Some of the 'side effects' might actually be necessary for the expression of resistance. For example, a reduction in growth and the degradation of photosynthetic proteins might be necessary to free up the resources required for the *de novo* production of resistance traits [40,41]. However, other effects might not be related to resistance induction.

Mutants

These experimental difficulties can be more elegantly addressed with mutants that constitutively express resistance or have lesions in genes that are directly involved in resistance or in the eliciting signal cascades. The phenotypic descriptions of many of these mutants are consistent with the hypothesis that constitutive expression of resistance leads to a decrease in fitness, whereas suppression of induced signalling under enemy-free conditions can increase fitness (Table 1). For example, the mutants expressing constitutive pathogen resistance owing to constitutive salicylic acid production (*cpr1*) exhibit a stunted phenotype but, when the mutation is placed in the *NahG* background (which relieves the salicylic acid overproduction phenotype), the stunted growth phenotype disappears [42]. Other mutants (e.g. *cev1*) that have constitutively active jasmonic acid and ethylene pathways also exhibit stunted growth [43]. Even fitness data from transgenic plants affected in salicylic acid content point to the same result: *Arabidopsis* plants expressing a novel hybrid enzyme with salicylate synthase (SAS) activity, and thus having elevated salicylic acid levels, have a strongly dwarfed phenotype (Fig. 1) and produce few seeds when

Table 1. Morphological and fitness traits of mutants affected in induced resistance[a]

Mutant	Species	Resistance-related phenotype	Biological resistance	Morphological traits	Refs
Constitutive expressors of (or parts of) salicylic acid signalling					
cpr1	*Arabidopsis*	Constitutively elevated levels of SA and PR-1, PR-5 and BGL2 gene expression	Resistant to *Peronospora parasitica* and *P. s.* pv. *maculicola*	Stunted growth	[42]
cpr5	*Arabidopsis*	Constitutively elevated levels of SA and PR-genes and PDF1.2 expression	Enhanced resistance to bacterial pathogens	Reduced plant size	[68]
cpr6-1	*Arabidopsis*	Constitutively elevated levels of SA and PR-1, BGL2, PR-5 and PDF1.2 expression	Enhanced constitutive resistance to *P. s.* pv. *maculicola* and *P. parasitica* cotyledons, delayed flowering	Reduced plant size, loss of apical dominance, earlier senescence of	[69]
cpr22	*Arabidopsis*	Spontaneous lesion formation, constitutively elevated levels of SA and SAG, and of PR-1, PR-2 and PDF1.2 expression	Enhanced resistance to *P. parasitica*	Stunted growth and development of curly leaves, lethal in homozygous plants	[70]
dnd1	*Arabidopsis*	Constitutively elevated levels of SA and SAG and PR-1 and BGL expression, no HR	Reduced growth of *P. s.* pv. *tomato* in spite of absence of HR	Reduced rosette size	[57]
cpr1, *cpr5*, *cpr6*, *dnd1*, *dnd2*	*Arabidopsis*	Spontaneous lesion formation, constitutively elevated levels of SA and PR-1 expression	Reduced growth of *P. s.* pv. *maculicola* in *cpr6*, *dnd1*, *dnd2*	Reduced rosette size	[55]
acd5	*Arabidopsis*	Spontaneous development of lesions starting at week 5. Constitutively elevated levels of camalexin, free and total SA and PR-1 expression starting at week 5 (but not before)	Enhanced disease symptoms after *P. s.* pv. *maculicola* and *P. s.* pv. *tomato* infection at week 3	Before onset of cell death, rosette sizes are normal, mature plants are shorter than wild type and yield fewer seeds	[56]
acd6	*Arabidopsis*	Small spontaneous patches of cell death, constitutively elevated levels of PR-1, GST1 and AIG1 expression	Enhanced resistance to virulent and avirulent *P. s.* pv. *maculicola*	Homozygous plants considerably smaller than wild type, heterozygous plants intermediate in size	[46]
ssi1 (in *npr1-5* background)	*Arabidopsis*	Spontaneous development of HR-like lesions, constitutively elevated levels of SA, SAG and PR-1, BGL2, PR-5 and PDF1.2 expression	Resistance to a *P. s.* pv. *tomato* strain restored that is pathogenic in *npr1-5*	Strongly reduced plant size, effect more pronounced in homozygous than in heterozygous plants	[54]
mpk4	*Arabidopsis*	Inactivated MAP Kinase 4 (normally negatively regulating ISR) Constitutively elevated levels of SA and SAG, and of PR-1, PR-5, chitinase, BGL2 and BGL3 expression No spontaneous lesion formation	Resistance to *P. s.* pv. *tomato* and *P. parasitica*	Strongly dwarfed plants, curled leaves, flowers with reduced pollen production and fertility, decreased cell size	[71]
mpk4 npr1-1 double mutant	*Arabidopsis*	Constitutive PR-1 expression as in *mpk4*	Resistance against *P. s.* pv. *tomato* as in *mpk4*	Full *mpk4* dwarfism	

the gene product is targeted to the chloroplast (p-SAS plants) [44]. When SAS was targeted to the cytosol (c-SAS plants), increases in free and conjugated SA, and in resistance, were observed, and the increased resistance of these lines correlated with reduced seed production [44].

Salicylic acid has several other effects in plant metabolism and is involved in the regulation of leaf senescence [45] and cell growth [46,47]. Jasmonic acid signalling is involved in anther development and mutants blocked in jasmonic acid signalling (e.g. *opr3*, which lacks 12-oxophytodienoic acid reductase) can be male sterile [48,49]. Not surprisingly, mutants constitutively expressing the hypersensitive response with its associated overexpression of localized cell death [50–53] are typically stunted [54–56]. In the case of p-SAS plants (Fig. 1), it remains unclear whether the dwarfed phenotype results from the extreme upregulation of resistance traits or from a nearly complete shunting of chorismate to salicylic acid synthesis in the chloroplast [44]. The appropriate experiments to determine whether the growth phenotypes described in Fig. 1 and Table 1 are due specifically to particular resistance traits have not been done, but the rapidly accumulating evidence points in this direction. *Arabidopsis* plants carrying the *dnd1* mutation and exhibiting elevated salicylic acid levels and enhanced disease resistance without HR have reduced rosette sizes [57]. When potato plants are transformed with a lipoxygenase gene in the antisense orientation (which inhibits induced PI accumulation), they show increased susceptibility to an adapted herbivore but gain the apparent fitness advantage of producing larger tubers [58]. Plant sizes of double mutants between *pad4* (inhibited salicylic acid accumulation after infection) and *cpr1*, *cpr5*, *cpr6*, *dnd1* and *dnd2* (all showing constitutively high levels of salicylic acid, defence gene expression and resistance) suggest that 'dwarfism is a result of constitutive defence gene expression' [55]. *Arabidopsis* mutants such as *edr1*, which do not display constitutive PR-1 production and enhanced resistance but become resistant more rapidly than the wild type in response to infection, are phenotypically normal in the absence of pathogens [59,60]. In short, morphological and fitness data from mutants constitutively expressing resistance, and from mutants defective in resistance, are consistent with the existence of fitness costs of resistance expression when plants are grown under enemy-free conditions (Table 1).

Ecological costs

The occurrence and potential importance of ecological costs adds further complexity to the characterization

Table 1. continued

Mutant	Species	Resistance-related phenotype	Biological resistance	Morphological traits	Refs
Double mutants with suppressed constitutive resistance					
ssi1 nahG (in *npr1-5* background)	*Arabidopsis*	Normal *npr1-5* phenotype	Normal *npr1-5* phenotype	No different to *npr1-5* (*ssi1* phenotype thus suppressed)	54
acd6 nahG	*Arabidopsis*	No spontaneous cell death patches, no elevated levels of PR-1, GST1 and AIG1 expression	Enhanced resistance to virulent and avirulent *P. s.* pv. *maculicola*	*acd6 nahG* homozygous plants normal in stature	46
acd6 npr1	*Arabidopsis*	Occurrence of spontaneous cell death patches delayed, modest increase in level of PR-1 expression	*P. syringae* susceptibility intermediate between *npr1* and *acd6*	Rosettes intermediate in size between *npr1* and *acd6*	46
cpr1 pad4	*Arabidopsis*	Total levels of SA and PR-1 mRNA similar to that in *pad4* and wild type	Growth of *P. s.* pv. *maculicola* similar to that in *pad4* and wild type	Rosette size as *pad4* and wild-type plants	55
dnd1 pad4, dnd2 pad4	*Arabidopsis*	Spontaneous lesion formation as in *dnd1* and *dnd2*, total levels of SA, PR-1 mRNA and PDF1.2 mRNA similar to that in *dnd1* and *dnd2*, respectively	Growth of *P. s.* pv. *maculicola* similar as in *pad4*	Reduced rosette size compared to wild type and *pad4* mutant	55
cpr22 nahG	*Arabidopsis*	No spontaneous lesion formation, no constitutive expression of PR-1 and PDF1.2	No information available	No stunted growth or curled leaves	70
mpk4 nahG	*Arabidopsis*	No constitutive PR-1 expression	Resistance against *P. s.* pv. *tomato* as in *nahG*	Partial suppression of *mpk4* dwarfism	71
Constitutive expressors of (or parts of) jasmonic acid or ethylene signalling					
cev1	*Arabidopsis*	Constitutive expression of VSP1, VSP2, Thi2.1, PDF1.1, CHI-B, accumulated anthocyanin	Enhanced resistance to powdery mildew	Plants smaller, stunted roots with long root hairs	43
cet1	*Arabidopsis*	Spontaneous lesion formation, constitutively elevated levels of JA and Thi2.1. Levels of SA and SAG twice as high as in wild type	No information available	Significantly smaller than wild type	72
Deficient in (or parts of) jasmonic acid or ethylene signalling					
ein2	*Arabidopsis*	Blocked ethylene signalling	No information available	Plants taller and yield more seeds than wild type	56
acd5 ein2 double mutant	*Arabidopsis*	Spontaneous cell death later. Spontaneous cell death of old plants reduced relative to *acd5* single mutant, reduced camalexin production compared to *acd5* single mutant	No reduced growth of *P. syringae* relative to *acd5* single mutant	Plants taller and yield more seeds than *acd5* single mutant	56
LOX-H3 antisense	*Solanum tuberosum*	Antisense-mediated depletion of lipoxygenase, no wound-induced expression of pin2 and cathepsin D inhibitor gene, wound-induced JA levels not reduced	Higher weight gain of Colorado potato beetle larvae and beet armyworm caterpillars	Higher tuber yield	58

[a]Morphological traits of mutants constitutively expressing resistance or deficient in resistance signalling are consistent with the hypothesis that resistance expression under enemy-free conditions leads to reductions in growth or seed set and thus can incur fitness costs.
Abbreviations: HR, hypersensitive response; JA, jasmonic acid; *P. s.*, *Pseudomonas syringae*; pv., pathovar; SA, salicylic acid; SAG, glucoside-bound SA. Mutants: *acd*, accelerated cell death; *cet*, constitutive expressor of thionine; *cev*, constitutive expressor of *VSP1*; *cpr*, constitutive expressor of PR proteins; *dnd*, defence no death; *mpk4*, affected in MAP kinase; *npr1*, nonexpressor of PR1; *ssi*, suppressor of SA insensitivity. Defence-related proteins and genes: AIG, avr-induced gene; BGL, β-glucanase; CHI-B, basic chitinase; GST, glutathione-transferase; LOX, lipoxygenase; PDF, plant defensin; pin, proteinase inhibitor; PR, pathogenesis-related protein; Thi, thionine; VSP, vegetative storage protein.

of fitness costs. These additional environmental costs will only be seen in particular environments and yet might have played an important role in the evolution of inducibility. The large allocation costs observed in *Nicotiana* were only found when plants were growing with, and not without [24], conspecific competitors, underscoring the importance of testing cost under environmentally realistic conditions. Many induced defences appear to be tailored specifically to the attacking herbivore and this tailoring might, in part, be a response to minimizing the fitness costs of particular defence components.

For example, *N. attenuata* recognizes attack from one of its specific herbivores, *Manduca sexta*, by the introduction of particular fatty-acid–amino-acid conjugates from the herbivore's saliva into wounds produced by the feeding herbivore [61]. This recognition includes a transcriptional downregulation of a direct defence, nicotine, which is, in turn, mediated by an ethylene burst and an upregulation of an indirect defence (a release of volatile compounds). An additional consequence of the ethylene burst for an attacked plant growing with a conspecific competitor is that the large fitness costs associated with jasmonic acid elicitation are nullified [61]. Hence, the plant's response to the recognition of a particular herbivore entails an adjustment of its defence responses and a reduction of the associated fitness costs. Detailed knowledge of a specific system is required to test all relevant forms of fitness costs that might be associated with a particular defence response. Hence, it is difficult to disprove the existence of fitness costs without resorting to studies of plants growing in their natural habitats.

Alternative explanations

Alternative hypotheses for the evolution of induced resistance have been proposed. Among others [62], the 'moving target' theory [2] proposes that it is the change

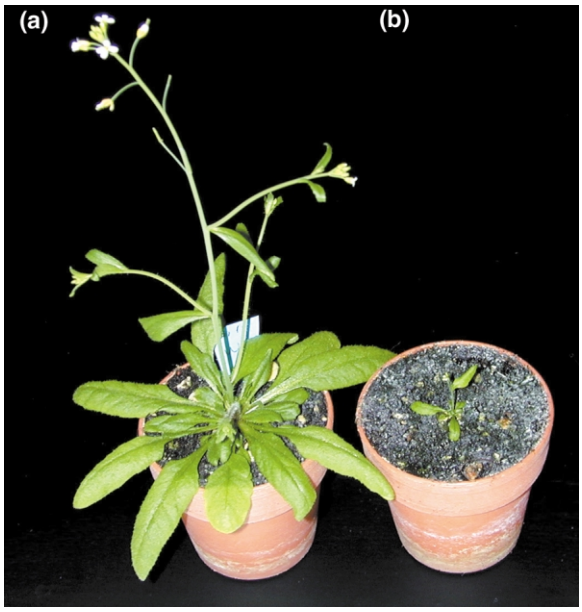

Fig. 1. 'Dwarfed' phenotype of a salicylic-acid-overproducing *Arabidopsis* plant. Comparison between (a) a wild-type *Arabidopsis* plant and (b) an *Arabidopsis* plant constitutively expressing a novel bacterial enzyme, salicylic acid synthase, with targeting of the gene product to the chloroplast [44]. The mutant plants show elevated salicylic acid levels and enhanced resistance to the pathogen *Peronospora parasitica*, but remain much smaller than their wild-type counterpart. Photograph courtesy of Felix Mauch.

in food quality itself and not necessarily the direction of the change that is the defensive trait. Herbivores might have difficulty adjusting their digestive systems to food that varies greatly in chemical composition, and the evolutionary adaptation of herbivores or pathogens is probably slowed by resistance that is 'unstable' and 'unpredictable' owing to its inducibility. This theory does not require resistance to incur any costs. The discovery that the activity of transposable elements is increased by attack, jasmonic acid elicitation and wounding provides a potential mechanism for the generation of random variability [63]. Finally, a delay in defence activation might allow a herbivore to grow until a particularly mobile stage, enabling it to move to a neighbouring, and therefore competing, plant [64].

Outlook

The concept of costs provides the most powerful explanation for the evolution of induced direct resistance and is consistent with most, if not all, empirical findings published to date on different aspects of induced resistance (discussed in Ref. [3]). Allocation costs of defence traits have even been used to explain tradeoffs between pathogen and herbivore resistance [65]. However, it is unclear whether induced resistance is largely a laboratory phenomenon and whether plants growing in natural populations are permanently in the induced state. Data are lacking for ISR [7,14] but several field experiments on IR against herbivores have shown that field-grown plants can be elicited to produce significant increases in secondary metabolites or extrafloral nectar, or increases in resistance [19,23,66].

Improved biochemical and genetic methods should allow researchers to dissect the association between resistance and fitness costs, and thus to understand the underlying mechanisms. Although the use of exogenous elicitors is plagued by pleiotropic effects that confound fitness measures, their use in studies with mutants defective in the endogenous production of the elicitors (Table 1) should provide a powerful experimental approach to understand causal relations between induced resistance traits and fitness effects. In these studies, broad, ecologically relevant measures of cost should be used. Costs might be defined as 'all negative effects on plant fitness that result from the expression of a defence trait when a plant grows under evolutionarily relevant conditions'. Such a definition includes both costs arising from internal processes (e.g. allocation, autotoxicity) and ecological costs. Costs appearing only under artificial conditions, resulting from (for example) the toxic effects of exogenous applications of resistance elicitors, are, by contrast, excluded. For self-pollinating species, measures of fitness through the female function (i.e. seed set) are appropriate. For out-crossing species, measures of both male and female function are necessary, and, for tuber- and stolon-producing species, the mass, number and viability of these organs should be quantified.

More empirical studies on different forms of induced defence in different plant species should be conducted under different growing conditions and on different levels (molecular, physiological, whole plant and ecological) to evaluate the utility of the cost–benefit model fully. Although several examples of morphological or fitness traits of mutants affected in resistance signalling are listed in Table 1, most studies focusing on molecular aspects of resistance signalling do not report factors such as plant growth rate or seed set. There are likely to be many more examples and they should be reported to allow a more complete overview of the genetic backgrounds and growing conditions under which constitutive expression of resistance pathways leads to reductions in plant growth and fitness.

Although allocation costs might not result in yield reductions in intensively managed agricultural production systems, they can reduce yield under less favourable growing conditions. Moreover, one of the most important genetic advances in agronomy in the past 50 years was the discovery of wheat varieties that increase grain yield at the expense of straw biomass [67]. The underlying mutation probably disrupted an ancient adaptive ecological response to competitors: the elongation of stems to gain canopy dominance. Hence, this trait could be viewed as a resource-allocation cost of competition. If this was indeed the case, the disruption of an ecological response to competitors was in part responsible for the dramatic increases in yields during the 'green revolution'. Understanding and manipulating other ecological responses, including potentially costly responses to herbivore or pathogen attack, might provide insights for future yield increases in our crop plants. Understanding the physiological and molecular mechanisms of how resistance is elicited, and the environmental conditions under which resistance expression becomes deleterious for fitness, would greatly increase our knowledge of whole-plant function and would allow more reliable risk assessments when this strategy is used as part of an 'integrative' crop-protection programme.

Acknowledgements
We thank Jean T. Greenberg, Doyle McKey and three anonymous referees for valuable comments on earlier drafts of the manuscript, and Evelyn Claussen and Birgit Baumann for much bibliographic help. Special thanks to Brigitte Mauch-Mani and Felix Mauch for providing us with Fig. 1. Financial support by the DFG (grant He3169/1-1 and 1-3,4 to M.H.) and the Max-Planck Society (I.T.B.) is gratefully acknowledged.

References

1 Walling, L.L. (2001) Induced resistance: from the basic to the applied. *Trends Plant Sci.* 6, 445–447
2 Karban, R. and Baldwin, I.T. (1997) *Induced Responses to Herbivory*, University of Chicago Press
3 Heil, M. (2001) The ecological concept of costs of induced systemic resistance (ISR). *Eur. J. Plant Pathol.* 107, 137–146
4 Simms, E.L. and Fritz, R.S. (1990) The ecology and evolution of host–plant resistance to insects. *Trends Ecol. Evol.* 5, 356–360
5 Tollrian, R. and Harvell, C.D. (1999) *The Ecology and Evolution of Inducible Defenses*, Princeton University Press
6 Baldwin, I.T. and Preston, C.A. (1999) The eco-physiological complexity of plant responses to insect herbivores. *Planta* 208, 137–145
7 Heil, M. (1999) Systemic acquired resistance: available information and open ecological questions. *J. Ecol.* 87, 341–346
8 Baldwin, I.T. and Ohnmeiss, T.E. (1994) Swords into plowshares? *Nicotiana sylvestris* does not use nicotine as a nitrogen source under nitrogen-limited growth. *Oecologia* 98, 385–392
9 Herms, D.A. and Mattson, W.J. (1992) The dilemma of plants: to grow or to defend. *Q. Rev. Biol.* 67, 283–335
10 Purrington, C.B. (2000) Costs of resistance. *Curr. Opin. Plant Biol.* 3, 305–308
11 Baldwin, I.T. and Callahan, P. (1993) Autotoxicity and chemical defense: nicotine accumulation and carbon gain in solanaceous plants. *Oecologia* 94, 534–541
12 Tollrian, R. and Harvell, C.D. (1999) The evolution of inducible defenses: current ideas. In *The Ecology and Evolution of Inducible Defenses* (Tollrian, R. and Harvell, C.D., eds), pp. 306–321, Princeton University Press
13 Price, P.W. *et al.* (1980) Interactions among three trophic levels: influence of plants on interactions between insect herbivores and natural enemies. *Annu. Rev. Ecol. Syst.* 11, 41–65
14 Heil, M. (2001) Induced systemic resistance (ISR) against pathogens – a promising field for ecological research. *Perspect. Plant Ecol. Evol. Syst.* 4, 65–79
15 Bostock, R.M. *et al.* (2001) Signal interactions in induced resistance to pathogens and insect herbivores. *Eur. J. Plant Pathol.* 107, 103–111
16 Creelman, R.A. and Mullet, J.E. (1997) Biosynthesis and action of jasmonates in plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 48, 355–381
17 Wasternack, C. and Parthier, B. (1997) Jasmonate-signalled plant gene expression. *Trends Plant Sci.* 2, 302–307
18 Dicke, M. (1999) Evolution of induced indirect defense of plants. In *The Ecology and Evolution of Inducible Defenses* (Tollrian, R. and Harvell, C.D., eds), pp. 62–88, Princeton University Press
19 Heil, M. *et al.* (2001) Extrafloral nectar production of the ant-associated plant, *Macaranga tanarius*, is an induced, indirect, defensive response elicited by jasmonic acid. *Proc. Natl. Acad. Sci. U. S. A.* 98, 1083–1088
20 Dicke, M. and Sabelis, M.W. (1989) Does it pay plants to advertise for bodyguards? Towards a cost–benefit analysis of induced synonome production. In *Variation in Growth Rate and Productivity of Higher Plants* (Lambers, H. *et al.*, eds), pp. 342–358, SPB Academic Publishing, The Hague, The Netherlands
21 Halitschke, R. *et al.* (2000) Ecophysiological comparison of direct and indirect defenses in *Nicotiana attenuata*. *Oecologia* 124, 408–417
22 Heil, M. *et al.* (2000) Temporal, spatial and biotic variations in extrafloral nectar secretion by *Macaranga tanarius*. *Funct. Ecol.* 14, 749–757
23 Baldwin, I.T. (1998) Jasmonate-induced responses are costly but benefit plants under attack in native populations. *Proc. Natl. Acad. Sci. U. S. A.* 95, 8113–8118
24 van Dam, N.M. and Baldwin, I.T. (2001) Competition mediates cost of jasmonate-induced defenses, N acquisition and transgenerational plasticity in *Nicotiana attenuata*. *Funct. Ecol.* 15, 406–415
25 Redman, A.M. *et al.* (2001) Fitness costs of jasmonic acid-induced defense in tomato, *Lycopersicon esculentum*. *Oecologia* 126, 380–385
26 Thaler, J.S. (1999) Induced resistance in agricultural crops: effects of jasmonic acid on herbivory and yield in tomato plants. *Environ. Entomol.* 28, 30–37
27 Brown, D.G. (1988) The cost of plant defense: an experimental analysis with inducible proteinase inhibitors in tomato. *Oecologia* 76, 467–470
28 Baldwin, I.T. *et al.* (1990) The reproductive consequences associated with inducible alkaloidal responses in wild tobacco. *Ecology* 71, 252–262
29 Agrawal, A.A. *et al.* (1999) Costs of induced responses and tolerance to herbivory in male and female fitness components of wild radish. *Evolution* 53, 1093–1104
30 Baldwin, I.T. *et al.* (1997) Quantification, correlations and manipulation of wound-induced changes in jasmonic acid and nicotine in *Nicotiana sylvestris*. *Planta* 201, 397–404
31 Görlach, J. *et al.* (1996) Benzothiadiazole, a novel class of inducers of systemic acquired resistance, activates gene expression and disease resistance in wheat. *Plant Cell* 8, 629–643
32 Heil, M. *et al.* (2000) Reduced growth and seed set following chemical induction of pathogen defence: does systemic acquired resistance (SAR) incur allocation costs? *J. Ecol.* 88, 645–654
33 Stadnik, M.J. and Buchenauer, H. (1999) Control of wheat diseases by a benzothiadiazole-derivative and modern fungicides. *Z. Pflanzenkr. Pflanzenschutz J. Plant Dis. Prot.* 106, 466–475
34 Smedegaard-Petersen, V. and Tolstrup, K. (1985) The limiting effect of disease resistance on yield. *Annu. Rev. Phytopathol.* 23, 475–490
35 Pena-Cortes, H. *et al.* (1993) Aspirin prevents wound-induced gene-expression in tomato leaves by blocking jasmonic acid biosynthesis. *Planta* 191, 123–128
36 Baldwin, I.T. *et al.* (1996) Effects of octadecanoid metabolites and inhibitors on induced nicotine accumulation in *Nicotiana sylvestris*. *J. Chem. Ecol.* 22, 61–74
37 Gupta, V. *et al.* (2000) *Arabidopsis thaliana* EDS4 contributes to salicylic acid (SA)-dependent expression of defense responses: evidence for inhibition of jasmonic acid signaling by SA. *Mol. Plant–Microbe Interact.* 13, 503–511
38 Raskin, I. (1992) Role of salicylic acid in plants. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 43, 439–463
39 Longemann, E. *et al.* (1995) Gene activation by UV light, fungal elicitor or fungal infection in *Petroselium crispum* is correlated with repression of cell-cycle-related genes. *Plant J.* 8, 865–876
40 Somssich, I.E. and Hahlbrock, K. (1998) Pathogen defence in plants – a paradigm of biological complexity. *Trends Plant Sci.* 3, 86–90
41 Reinbothe, S. *et al.* (1994) JIPs and RIPs: the regulation of plant gene expression by jasmonates in response to environmental cues and pathogens. *Plant Cell* 6, 1197–1209
42 Bowling, S.A. *et al.* (1994) A mutation in *Arabidopsis* that leads to constitutive expression of systemic acquired resistance. *Plant Cell* 6, 1845–1857
43 Ellis, C. and Turner, J.G. (2001) The *Arabidopsis* mutant *cev1* has constitutively active jasmonate and ethylene signal pathways and enhanced resistance to pathogens. *Plant Cell* 13, 1025–1033
44 Mauch, F. *et al.* (2001) Manipulation of salicylate content in *Arabidopsis thaliana* by the expression of an engineered bacterial salicylate synthase. *Plant J.* 25, 67–77
45 Morris, K. *et al.* (2000) Salicylic acid has a role in regulating gene expression during leaf senescence. *Plant J.* 23, 677–685
46 Rate, D.N. *et al.* (1999) The gain-of-function *Arabidopsis acd6* mutant reveals novel regulation and function of the salicylic acid signaling pathway in controlling cell death, defenses, and cell growth. *Plant Cell* 11, 1695–1708
47 Vanacker, H. *et al.* (2001) A role for salicylic acid and NPR1 in regulating cell growth in *Arabidopsis*. *Plant J.* 28, 209–216
48 Sanders, P.M. *et al.* (2000) The *Arabidopsis DELAYED DEHISCENCE1* gene encodes an enzyme in the jasmonic acid synthesis pathway. *Plant Cell* 12, 1041–1061
49 Stintzi, A. and Browse, J. (2000) The *Arabidopsis* male-sterile mutant, *opr3*, lacks the 12-oxophytodienoic acid reductase required for jasmonate synthesis. *Proc. Natl. Acad. Sci. U. S. A.* 97, 10625–10630
50 Heath, M.C. (1998) Apoptosis, programmed cell death and the hypersensitive response. *Eur. J. Plant Pathol.* 104, 117–124
51 Kombrink, E. and Schmelzer, E. (2001) The hypersensitive response and its role in local and systemic disease resistance. *Eur. J. Plant Pathol.* 107, 69–78
52 Lamb, C. and Dixon, R.A. (1997) The oxidative burst in plant disease resistance. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 48, 251–275
53 Greenberg, G.T. (1997) Programmed cell death in plant–pathogen interactions. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 48, 525–545
54 Shah, J. *et al.* (1999) The *Arabidopsis ssi1* mutation restores pathogenesis-related gene expression in *npr1* plants and renders defensin gene expression salicylic acid dependent. *Plant Cell* 11, 191–206
55 Jirage, D. *et al.* (2001) Constitutive salicylic acid-dependent signaling in *cpr1* and *cpr6* mutants requires PAD4. *Plant J.* 26, 395–407
56 Greenberg, J.T. *et al.* (2000) Uncoupling salicylic acid-dependent cell death and defense-related responses from disease resistance in the *Arabidopsis* mutant *acd5*. *Genetics* 156, 341–350
57 Yu, I.C. *et al.* (1998) Gene-for-gene disease resistance without the hypersensitive response in *Arabidopsis dnd1* mutant. *Proc. Natl. Acad. Sci. U. S. A.* 95, 7819–7824
58 Royo, J. *et al.* (1999) Antisense-mediated depletion of a potato lipoxygenase reduces wound induction of proteinase inhibitors and increases weight gain of insect pests. *Proc. Natl. Acad. Sci. U. S. A.* 96, 1146–1151
59 Frye, C.A. and Innes, R.W. (1998) An *Arabidopsis* mutant with enhanced resistance to powdery mildew. *Plant Cell* 10, 947–956
60 Frye, C.A. *et al.* (2001) Negative regulation of defense responses in plants by a conserved MAPKK kinase. *Proc. Natl. Acad. Sci. U. S. A.* 98, 373–378
61 Baldwin, I.T. *et al.* (2001) Merging molecular and ecological approaches in plant–insect interactions. *Curr. Opin. Plant Biol.* 4, 351–358

62 Agrawal, A.A. and Karban, R. (1999) Why induced defenses may be favored over constitutive strategies in plants. In *The Ecology and Evolution of Inducible Defenses* (Tollrian, R. and Harvell, C.D., eds), pp. 45–61, Princeton University Press
63 Grandbastien, M.A. (1998) Activation of plant retrotransposons under stress conditions. *Trends Plant Sci.* 3, 181–187
64 van Dam, N. *et al.* (2001) Instar-specific sensitivity of specialist *Manduca sexta* larvae to induced defences in their host plant *Nicotiana attenuata*. *Environ. Entomol.* 26, 578–586
65 Felton, G.F. and Korth, K.L. (2000) Trade-offs between pathogen and herbivore resistance. *Curr. Opin. Plant Biol.* 3, 309–314
66 Thaler, J.S. (1999) Jasmonate-inducible plant defences cause increased parasitism of herbivores. *Nature* 399, 686–688
67 Peng, J. *et al.* (1999) 'Green revolution' genes encode mutant gibbereline response. *Nature* 400, 256–261
68 Bowling, S.A. *et al.* (1997) The *cpr5* mutant of *Arabidopsis* expresses both NPR1-dependent and NPR1-independent resistance. *Plant Cell* 9, 1573–1584
69 Clarke, J.D. *et al.* (1998) Uncoupling *PR* gene expression from NPR1 and bacterial resistance: characterization of the dominant *Arabidopsis cpr6-1* mutant. *Plant Cell* 10, 557–569
70 Yoshioka, K. *et al.* (2001) Environmentally sensitive, SA-dependent defense responses in the *cpr22* mutant of *Arabidopsis*. *Plant J.* 26, 447–459
71 Petersen, M. *et al.* (2000) *Arabidopsis* MAP kinase 4 negatively regulates systemic acquired resistance. *Cell* 103, 1111–1120
72 Hilpert, B. *et al.* (2001) Isolation and characterization of signal transduction mutants of *Arabidopsis thaliana* that constitutively activate the octadecanoid pathway and form necrotic microlesions. *Plant J.* 26, 435–446
73 Cipollini, D. *et al.* Costs of induced responses. *Basic Appl. Ecol.* (in press)

Note added in proof

Recently, Don Cipollini and co-workers [73] have presented an alternative point of view on the costs of induced responses, which might be an interesting addition to what is presented here.

Balancing selection favors guarding resistance proteins

Renier A.L. Van der Hoorn, Pierre J.G.M. De Wit and Matthieu H.A.J. Joosten

The co-evolutionary arms race model for plant–pathogen interactions implies that resistance (*R*) genes are relatively young and monomorphic. However, recent reports show *R* gene longevity and co-existence of multiple *R* genes in natural populations. This indicates that *R* genes are maintained by balancing selection, which occurs when loss of the matching avirulence (*Avr*) gene in the pathogen is associated with reduced virulence. We reason that balancing selection favors R proteins that function as guards, monitoring changes in the virulence target mediated by the Avr factor, rather than recognizing the Avr factor itself. Indeed, the available experimental data support the notion that guarding is prevalent in gene-for-gene interactions.

Renier A.L. Van der Hoorn
Pierre J.G.M. De Wit
Matthieu H.A.J. Joosten*
Laboratory of Phytopathology, Wageningen University, The Netherlands.
*e-mail: matthieu.joosten@fyto.dpw.wag-ur.nl

Classical resistance breeding has long been used to suppress plant diseases. Many resistance genes have been introduced into crop plants, resulting in new pathogen-resistant cultivars that quickly became popular and were grown as homogeneous crops. However, in many cases, pathogens were eventually able to overcome resistance, resulting in outbreaks of large epidemics. The plant cultivar that was once 'booming' but now 'busts', forced breeders to introduce a cultivar with a new resistance trait. Repeated boom-and-bust cycles in agriculture have provided material for extensive studies on various plant–pathogen interactions [1]. Genetic studies of the flax–flax-rust pathosystem led to the development of the gene-for-gene model, which states that, for every dominant resistance (*R*) gene in the plant, there is a matching dominant avirulence (*Avr*) gene in the pathogen [2]. After this classic work, it became evident that matching gene pairs control the outcome of the interaction for many other pathosystems [3].

A logical prediction of the gene-for-gene model is that *R* genes encode receptors that interact physically with products of matching *Avr* genes, enabling recognition of the pathogen and subsequent elicitation of an array of plant defense responses that eventually lead to resistance [4]. The structure and predicted location of R and Avr proteins are usually consistent with this model [5]. For example, most R proteins carry leucine-rich repeats (LRRs), which are thought to form a versatile binding domain that could fulfill the receptor role of the R protein. In addition, membrane-anchored R proteins mediate the perception of extracellular Avr factors, whereas cytoplasmic R proteins mediate the perception of Avr factors that are produced in or injected into the host cytoplasm by the pathogen. Although these observations agree with the ligand–receptor model, a direct physical interaction between Avr and R proteins has only been shown for the AvrPto–Pto and AvrPita–Pi-ta pairs [6–8]. In most other cases (e.g. AVR9–Cf-9 [9]), in spite of extensive and detailed studies, no evidence for a direct interaction between the two gene products has been found.

Guard model

Lack of evidence for direct Avr–R interactions stimulated scientists to propose new models for Avr perception by resistant plants. One interesting model is that R proteins confer recognition of Avr factors only when these Avr factors are complexed with their host virulence targets. This model was initially proposed [10] to explain the role of Prf in AvrPto–Pto signaling and was later referred to as the guard model [11]. In this model, Pto is considered to be the virulence target of AvrPto, which is guarded by the 'real' R protein, Prf [10].

Although the guard model needs to be proved experimentally, it has gained increasing support from experimental data obtained for most of the intensively studied gene-for-gene pairs [12]. Table 1 shows nine examples in which the R protein seems to guard the virulence target and monitors changes of this target mediated by the Avr factor. In general, three observations support the guard model. First, no

http://plants.trends.com 1360-1385/02/$ – see front matter PII: S1360-1385(01)02188-4