Ecological costs of induced resistance

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There has been rapid progress in detecting the genetic or allocation costs of induced resistance. In addition to these 'internal' costs, ecological costs may result from external mechanisms, that is, from the detrimental effects of resistance on the plant's interactions with its environment. All evolutionarily relevant costs affect a plant's ability to perform under natural conditions. The conceptual separation of different forms of resistance costs simplifies the study of mechanisms by which these costs arise. Yet, integrative measures of fitness must be applied under natural conditions so that researchers can fully understand the costs and benefits of induced resistance.

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Current Opinion in Plant Biology 2002, **5**:

1369-5266/02/$ – see front matter

DOI 10.1016/S1369-5266(02)00267-4

Abbreviations

IR	induced resistance against herbivores
ISR	induced systemic resistance
JA	jasmonic acid
MeJA	methyljasmonate
SA	salicylic acid
VOC	volatile organic compound

Introduction

Plant resistance against herbivores and pathogens is subject to extensive phenotypic plasticity: many resistance traits are expressed only, or to a higher degree, in response to a first 'eliciting' attack. *De novo* synthesis of phytoalexins and pathogenesis-related (PR) proteins, and changes in cell wall composition, are associated with induced systemic resistance (ISR) or systemic acquired resistance (SAR) against pathogens [1•,2,3]. Increased or *de novo* production of secondary compounds is correlated with induced resistance against herbivores (IR) [4,5]. IR can also be achieved indirectly by the attraction of 'enemies of the plant's enemies' [6] by volatile organic compounds (VOCs) [7] or extrafloral nectar [8•]. Recent studies have demonstrated the defensive effects of VOCs [9••] and the induction of extrafloral nectar flow [8•] under natural conditions. Most resistance traits can be artificially induced by the application of 'elicitors', and plants can be genetically engineered to express these traits constitutively, particularly those that are correlated with ISR. Induced defences are therefore receiving increasing attention, especially in the context of crop protection.

To explain the evolution of induced defences, as distinct from constitutive (i.e. constant) resistance, costs of induced resistance have been postulated: resistance traits are assumed to reduce the fitness of the plant when expressed under enemy-free conditions [10]. 'Just-in-time' mechanisms such as induced resistance are believed to have evolved, at least in part, to 'save' costs when defence is not required. Different forms of costs have been defined (Box 1), but they are by no means mutually exclusive. Recent studies have focused on 'allocation costs' and have revealed negative fitness effects of induced responses under enemy-free conditions [11–14,15••]. Most of these studies have used chemical elicitation of resistance, which may cause effects beyond those that are associated with induced resistance (Box 2). Yet, there is increasing agreement that inducing resistance does cause relevant allocation costs [16•,17,18].

The evolutionarily relevant costs of resistance include all of the negative effects on plant fitness that may be caused by a resistance trait under natural growing conditions [16•,19]. Costs may result from 'internal' mechanisms (e.g. genetic costs, allocation costs, or autotoxicity), but can also result from 'external' mechanisms. 'Ecological costs' arise when one of the interactions between a plant and its biotic and abiotic environment is affected in a way that negatively affects plant fitness (Figure 1). These costs are not apparent in experiments on isolated plants that are cultivated

Box 1

Glossary: definitions of 'costs'.

Costs. In evolutionary terms, costs can be any trade-off between resistance and another fitness-relevant process. The costs of a particular trait are normally outweighed by its benefits, and therefore can be quantified only in an environment in which the beneficial effects cannot affect fitness.

Genetic costs. Negative pleiotropic effects, that is, heritable effects of a resistance trait that correlate negatively with plant fitness. This definition does not claim a mechanism by which fitness reductions are caused at the phenotypic level.

Allocation costs result from the allocation of limited resources to resistance instead of to other fitness-relevant processes such as growth or reproduction.

Autotoxicity costs result from negative effects of a resistance trait on the plant's own metabolism (see Wittstock and Gershenzon, this issue, for a discussion of autotoxicity).

Opportunity costs. Short-term reductions in growth resulting from the synthesis of resistance compounds do not represent important costs on their own. Yet, they might lead to a reduced ability to compete for soil nutrients or light, giving neighbouring plants an advantage that is never made up for. 'Missed opportunities' can severely compromise plant fitness.

Ecological costs (i.e. environmental costs) result from negative effects of resistance on one of the myriad of interactions between a plant and its environment that affect a plant's fitness under natural growing conditions.

Box 2

Measuring allocation costs: methodical problems.
Costs of resistance are often searched for using the natural or artificial removal of leaf tissue to induce resistance. This tool causes relevant costs itself and is not perfect. Other experiments induce resistance using chemicals. However, the chemical elicitors that are used are known either to affect fitness-relevant processes, such as fruit development and ripening, directly (e.g. JA) or to have phytotoxic effects when applied in high concentrations (e.g. SA and BION®). JA and SA can elicit many physiological and morphological changes that are not related to resistance, but that nevertheless affect fitness parameters [54,55]. SA can inhibit the activity and expression of important enzymes, such as nitrate reductase or ribulose bisphosphate carboxylase, and can inhibit the growth of cell cultures (reviewed in [36,45]). These extensive pleiotropic effects constrain the meaningfulness of studies that are based on the exogenous application of elicitors [52"]. In particular, the widely used treatments in which elicitors are sprayed onto plants ensure neither within-plant nor within-cell elicitor concentrations that are physiologically realistic, nor a realistic within-plant distribution of the elicitor (nor, thus, realistic changes in the patterns of gene activity). Although many phenomena occur as a result of artificial elicitor treatments, it is not known whether these effects are induced only by the experimental elicitation of resistance by chemicals or whether they are necessary consequences of resistance responses. Thus, it can be argued that whereas the costs of SA- or JA-induced responses are proven, it is still unclear whether induced resistance itself has a relevant cost (T Mitchell-Olds, personal communication). In general, the phenotypes of mutants that are affected in resistance expression are consistent with the interpretation that the induction of resistance causes relevant fitness costs [16']. However, further efforts are required to demonstrate whether fitness costs can be attributed clearly to the elicitation of plant defence. Recently, Mitchell-Olds [52"] suggested that expression profiling could be used to answer this and other ecologically important questions.

indoors under artificially optimised growing conditions, and thus are likely to be hidden in most of the studies conducted to far. To provide a starting point for further research, this review lists the few recent examples that have identified ecological costs of induced resistance and highlights some of the resistance traits that are likely to be associated with such costs.

Delayed flowering and impaired pollination

Several studies have identified reduced numbers of flowers [20], or delayed flowering, fruit set, or fruit ripening [11,14], when plants were treated with jasmonic acid (JA) or methyljasmonate (MeJA) to induce IR. The fitness consequences of these manipulations were, however, inconsistent under the chosen experimental conditions. Though not inevitably reducing seed production, delays in flowering, fruit set, or fruit ripening can severely compromise plant fitness under natural conditions. Particularly in temperate regions, a delay in fruit ripening can lead to a complete failure to produce ripe fruit during the growth season. Strauss *et al.* [21] demonstrated that alterations in flower morphology and numbers of open flowers in *Brassica rapa* plants selected for (or induced to express) increased myrosinase concentrations had consequences for pollinator behaviour. These effects can cause fitness costs during periods when pollinator numbers are limiting.

Allocation costs and reduced competitive ability

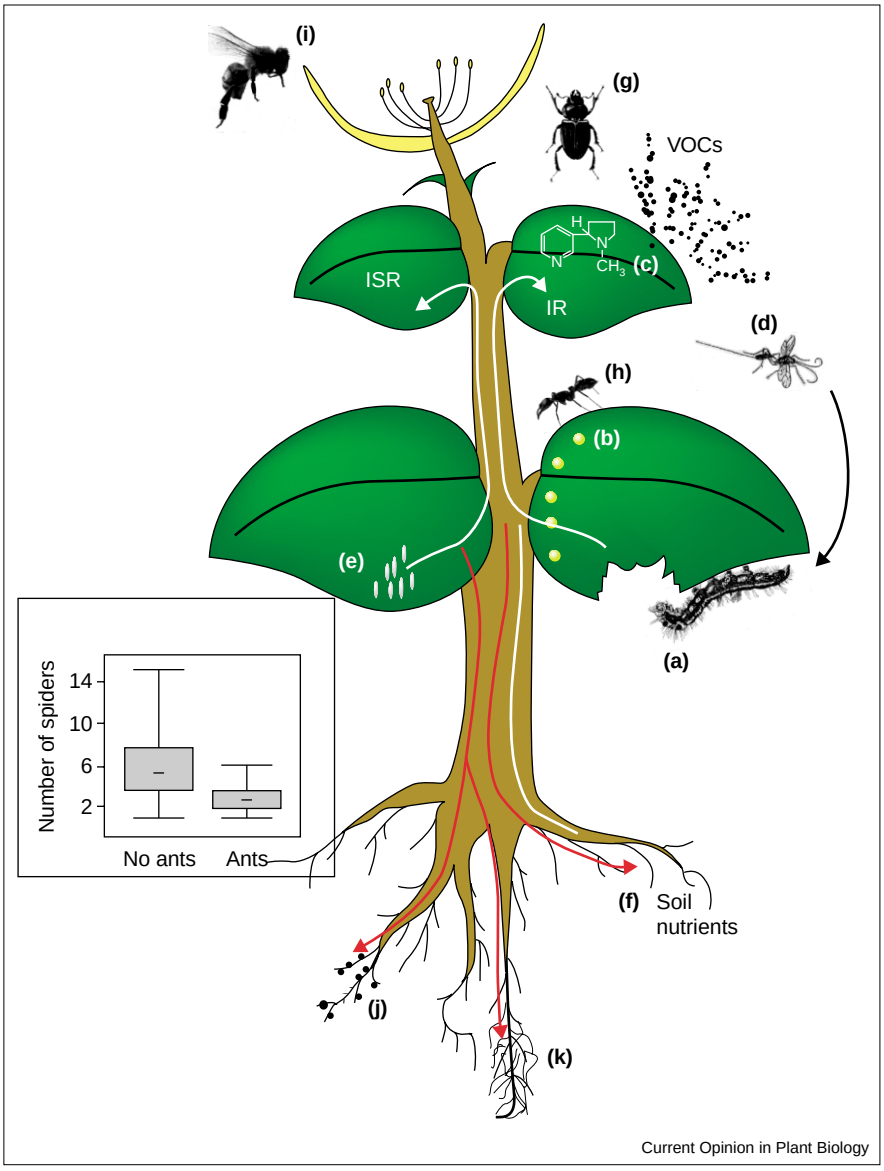
The allocation costs of chemically (i.e. MeJA-) induced nicotine production in native tobacco (*Nicotiana attenuata* and *N. sylvestris*) translate to fitness costs only when the plants compete with uninduced conspecific neighbours, when induced plants suffer from an impaired ability to compete for nitrogen [22]. Herbivore-induced *Lepidium virginicum* (Brassicaceae) plants showed reductions in root biomass when growing at high densities [23]. In wheat plants, the negative effects on seed set of chemically elicited pathogen resistance were most pronounced when potted plants (suffering root competition) were grown under severe nitrogen shortage [13]. Competition reduced both constitutive and induced levels of proteinase inhibitors in *Brassica napus* in a density-dependent manner, yet the levels of these inhibitors increased in a dosage-dependent fashion after nutrient supplementation [24]. Similarly, no resistance responses could be induced in potted *Nicotiana sylvestris* plants that suffered from root competition [25]. Van Dam and Baldwin [22] and Agrawal [23] even reported opportunity benefits for uninduced plants competing with induced neighbours. Recently, Cipollini [15••] elicited resistance in *Arabidopsis* lines that differed from each other in their competence to express resistance. Overall, induced resistance reduced seed set in these lines, and thus caused obvious relevant costs. Competition significantly reduced the plants' ability to express ISR. However, Cipollini [15••] detected no increase in the costs of eliciting resistance as a result of competition.

Most of these studies used chemicals to elicit resistance (but see [23]), and thus might have suffered from side-effects of these treatments (Box 2). These studies suggest (but do not prove) that competition and nutrient availability can affect both the degree to which a resistance trait is induced and the fitness-relevant costs incurred by plants in which resistance is elicited. Conversely, the data are consistent with the hypothesis that elicitation of resistance can, at least transiently, reduce a plant's competitive ability and thus generate severe 'opportunity costs'.

Negative effects on mutualists and positive effects on enemies

Strauss *et al.* [21] reported negative effects of induced herbivore resistance on pollinator visitation rates. Herbivore-induced volatiles may have deterrent effects on some of the plants' insect mutualists, as the information provided by volatiles released from an infested plant is highly complex [7]. Moreover, indirect defence strategies involve a third partner that cannot be controlled perfectly by the plant. Mutualistic ants that inhabit obligate ant-plants (myrmecophytes) are highly mobile and form an effective indirect defence that is quickly inducible: ants

Figure 1



Putative sources of the ecological costs of induced resistance. **(a)** Feeding by a non-specialised herbivore will normally elicit a JA-dependent signalling cascade leading to IR [4,5]. IR can occur both locally (i.e. in the affected leaf) and systemically. **(b)** Induced flow of extrafloral nectar has so far been reported only as a local response [8•]. Systemic responses comprise **(c)** the induction of chemical compounds that prevent herbivore feeding directly (e.g. nicotine) [4], and **(d)** VOCs that, for example, attract parasitoids or carnivorous arthropods that are capable of attacking the herbivore [6,7,9••]. **(e)** Local infection by bacteria or other pathogens elicits an SA-dependent signalling cascade that leads to ISR, which is achieved phenotypically by a change in cell-wall composition and the induced synthesis of phytoalexins and defensive (pathogenesis-related [PR]) proteins [1•,2,3]. **(f)** The intensity of IR-expression can depend on competition and/or soil nutrient availability [24,25]. Conversely, eliciting IR can affect the plant's ability to compete for limited soil nutrients [22]. Similarly, the costs and intensity of ISR elicitation can depend on growing conditions [13,15••]. The costs of synthesising resistance compounds and the inhibitory effects of SA on JA signalling are putative reasons for trade-offs between IR and ISR [45]. Such tradeoffs may lead to compromised resistance against one class of plant enemies in plants expressing resistance against another class. **(g)** VOCs that are released by induced plants may repel herbivores [9••], but VOCs or induced defensive chemicals can also be used by specialised herbivores to locate their food plants [7,30•,31]. IR against some herbivores thus may increase susceptibility to others or even, in the long run, to conspecific herbivores [42]. **(h)** Ants that are attracted to extrafloral nectar may deter other putative defending arthropods such as flies, wasps, and spiders (see insert). **(i)** Further putative 'non-target' or 'side' effects of IR include reduced attractiveness to pollinators [21] and negative effects of ISR on **(j)** nodulating *Rhizobium* bacteria [36–40] or, more probably, **(k)** mycorrhiza. Insert: The numbers of spiders on extrafloral-nectar-producing *Pseudocedrela kotschyi* (Meliaceae) shrubs growing in Comoé Park, Ivory Coast. Plants from which ants have been excluded for one month (left) are compared with untreated control plants (right). (n = 44 plant pairs, $p < 0.001$; K Mody, unpublished data.) See text for further details. Insert courtesy of Karsten Mody, Würzburg.

can rapidly concentrate at sites that actually require defence (see [26,27]). Yet, ants and other animals can also 'abuse' this system and might even parasitise their host tree [28,29]. Ants that are attracted to extrafloral nectaries may deter other putative defending arthropods, and thus decrease rather than increase the total number of defenders available on a plant. Negative correlations of ant numbers with numbers of defending flies have been observed on *Macaranga tanarius* (M Heil, A Hilpert, unpublished data). Similarly, higher numbers of spiders were found on *Pseudocedrela kotschyi* (Meliaceae) shrubs from which ants were excluded than on those with ants (K Mody, unpublished data; see insert in Figure 1). Different indirect defences may compete, but ecological costs can also arise from trade-offs between direct and indirect defences. An example of this has been found during very recent studies on cucumber plants. When infested with herbivorous spider mites, 'bitter' plants (i.e. those high in cucurbitacins) attracted less predatory mites than 'sweet' plants [30•].

Volatiles and extrafloral nectar can also be 'abused' by herbivorous insects or otherwise detrimental organisms. Dicke and van Loon [7] have listed several studies which show that herbivores can be attracted by induced plant

volatiles. The same is true for constitutive defences. Many specialist herbivores use secondary plant metabolites as cues to locate their food plants. Thus, increased levels of defensive compounds can make plants more attractive to specialist herbivores, which can even sequester plant-derived defence compounds and incorporate them into their own defensive system (see examples in [31,32]). Increased resistance against generalist herbivores can thus concomitantly cause increased susceptibility to specialists, and may even lead to the increased resistance of plant enemies against their predators.

The resistance responses of *Nicotiana attenuata* can be specifically 'tailored' to suit the attacking herbivore species. Although this species normally responds to herbivore damage with a JA-dependent increase in nicotine accumulation, damage by a specialist (nicotine-tolerant) caterpillar (*Manduca sexta*) elicits an ethylene burst that leads to induced volatile production [33]. This response reduces allocation costs by suppressing nicotine production while concomitantly eliciting alternative defence mechanisms to which the specialist is more vulnerable [34•]. However, it leaves the plant with the risk of a compromised resistance against generalist herbivores while responding to its specialist.

Because of the broad-spectrum activity of many resistance traits, it has repeatedly been assumed that ISR, in particular, might negatively affect mutualistic plant–microbe interactions [35,36]. Empirical evidence for this is still anecdotal. Some studies have identified inhibitory effects of chemically induced ISR on the development of root nodules [37–39]. It is not clear whether this is a consequence of chemical elicitation or a 'real' effect of ISR, though Russin *et al.* [40] reported that herbivory and fungal infections can inhibit nodule development and N_2-fixing activity. Are there comparable influences on other forms of plant–microbe mutualism such as mycorrhiza or interactions with endosymbiotic fungi? Martínez-Abarca *et al.* [37] and Ruiz-Lozano *et al.* [41] investigated early events in the establishment of mycorrhiza and nodules. Seven known defence-related genes were induced during these events, making reciprocal effects (i.e. negative influences of the induced expression of defence genes on these plant–microbe interactions) likely. More research is required to evaluate fully the putative negative effects of ISR on plant–microbe mutualisms and of IR on mutualistic or antagonistic plant–insect interactions.

Induced susceptibility

Further ecological costs occur when plant defence mechanisms cause resistance to one species but also result in susceptibility to other natural enemies. Herbivory-induced susceptibility to herbivores has rarely been reported (probably because of the lack of long-term studies such as that by Underwood [42]), but many studies have addressed the question of whether and how induced resistance against herbivores affects ISR against pathogens and *vice versa* (for reviews see [43–46]). Evidence for positive (i.e. cross-resistance) and negative (i.e. trade-offs) reciprocal interactions is mixed, though some general patterns have recently been elucidated [47]. Many studies using induced biological resistance have reported cross-resistance, that is, induced resistance against pathogens that is elicited by herbivore attack or *vice versa*. Chemical elicitation of resistance often leads to trade-offs, that is, compromised resistance against one class of enemies when plants are expressing resistance to another group (see examples in [45–47]). As already discussed, a plant's ability to synthesise defensive compounds could be restricted by resource availability, thus compromising its ability to express, for example, ISR when IR is already activated [35,36]. There is clear evidence for inhibitory effects of salicylic acid (SA) on JA biosynthesis (reviewed in [45]). If SA produced endogenously after pathogen infection has similar effects on JA biosynthesis, then octadecanoid signalling will be strongly affected and the ability of a plant to synchronously express IR and ISR compromised.

Trade-offs between resistance and tolerance

Tolerance is a phenotypically plastic characteristic that has been defined as 'a decrease in the per unit effect of herbivory on plant fitness' [48], and thus is another strategy by which plants may cope with damage. Theoretical considerations show that highly resistant plants do not need to be tolerant, whereas highly tolerant plants are not forced to be resistant. Hence, negative correlations between resistance and tolerance are likely to have evolved [49]. Empirical data supporting this expectation have been presented by Fineblum and Rausher [50] and by Stowe [51]. It remains to be proven whether complex, and in part negative, correlations between tolerance and resistance against multiple enemies, such as those reported by Pilson [48], form a general source of putative ecological costs of resistance.

Conclusions

Allocation costs may have an important opportunity aspect if they reduce a plant's future competitive ability. Even such 'internal' costs may therefore depend on — and affect — a plant's environment and thus are, in fact, 'ecological' costs. Such interactions point to the close linkage between 'internal' and 'external' mechanisms that give rise to costs of induced resistance. Yet, it is by no means true that 'ecological costs are simply a special case of allocation costs' (as stated in [48]). Research has just begun to detect 'typical' ecological costs such as reduced pollination rates, attracted enemies, or trade-offs among different types of resistance. Evolutionarily relevant costs are simply those occurring under natural growing conditions [16•], and thus can be affected by a plant's ecological interactions.

The small number of studies reporting ecological costs most likely results from the limited number of studies that have been conducted under natural conditions rather than from a low frequency of this type of costs. Promising research on this topic will require a three-step approach to

integrate field observations with laboratory experiments and further experiments under natural conditions. Descriptive work under natural conditions is required to identify sources of putative ecological costs. Although producing results of high ecological and evolutionary relevance, observational field studies often suffer from limited reproducibility and the lack of defined chains of causation. Experiments conducted under controlled laboratory conditions that make use of recent physiological and molecular techniques are therefore required to define intra-plant mechanisms that give rise to costs [16•,52••,53••]. The evolutionary relevance of their results should then be ensured by further field experiments. Fitness is defined as an organism's genetic contribution to the next generation, and suitable fitness measures (such as seed set and quality, pollen number, and success of asexual reproduction in vegetatively propagating plants) must be applied to assess the frequency and relative importance of the ecological costs of induced resistance.

Acknowledgements

I thank Wilhelm Boland, Ian T Baldwin, Thomas Mitchell-Olds and Jonathan Gershenzon (all of the Max Planck Institute of Chemical Ecology, Jena) for their critical reading of earlier versions of this manuscript, and Karsten Mody (Zoologie III, University of Würzburg) for sharing unpublished data. Financial support from the German Research Foundation (DFG, grant He 3169/1-4) and the Max-Planck-Gesellschaft is gratefully acknowledged.

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