Coping with multiple enemies: an integration of molecular and ecological perspectives

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How plants respond to attack by the range of herbivores and pathogens that confront them in the field is the subject of considerable research by both molecular biologists and ecologists. However, in spite of the shared focus of these two bodies of research, there has been little integration between them. We consider the scope for such integration, and how greater dialogue between molecular biologists and ecologists could advance understanding of plant responses to multiple enemies.

Recent research has highlighted how plants respond to the diversity of organisms that attack them in the field. Much of this current interest derives from cellular studies of plant defence pathways. These studies take a reductionist approach and attempt to identify individual signalling pathways that link wounding or attack by a pathogen or herbivore to specific gene expression events. For example, in Arabidopsis this approach has been successful in identifying key signalling molecules and their relative position in particular pathways. It has also identified possible points of cross-talk between pathways and synergistic or antagonistic events. Recently, there has been increasing interest in the possible interactions between plant signalling pathways, including those involved in responses to disease and herbivory.

Induced defence is also an important area of ecological study. In contrast with molecular studies, the ecological view of induced defence is broad, and is seen within the context of the overall ‘defence strategy’ of the host plant. This wider strategy includes constitutive defences, the ‘quality’ of host tissues as a resource for consumers, and tolerance (i.e. the capacity of the plant to adjust its physiology to buffer the effects of herbivory or disease). With few exceptions, ecologists have paid little attention to plant responses to multiple enemies, and studies of non-crop hosts have concentrated largely on defence against herbivores.

Here we consider induced resistances from the contrasting perspectives of molecular biology and ecology, and whether integration of these different standpoints offers any novel insights into plant defence against multiple enemies. We will concentrate on three broad issues: cross-talk between pathways, specificity of induced resistances and the evolution of induced resistances in the context of attack by multiple enemies.

Cellular perspective on systemic induced resistance

Of the several pathways of systemic induced defence in plants the best characterized are systemic acquired resistance (SAR), which provides enhanced resistance to pathogen infection, and the wound response pathway, which may provide constitutive resistance. Both pathways have been shown to induce the expression of distinct sets of genes. SAR has been shown to be induced by salicylic acid and, in at least some systems, salicylic acid appears to be both necessary and sufficient for inducing SAR. By contrast, the wound response pathway appears to be induced by jasmonic acid and ethylene without salicylic acid. Although it has been widely accepted that the pathways responsive to pathogen or herbivore attack are distinct, some recent evidence suggests that jasmonic acid also mediates induced resistance to some pathogens. Thus, the strict division of induced responses might be incorrect. In addition, the salicylic acid and jasmonic acid signalling pathways can interact.

Although the nature of the cross-talk is not yet fully defined, recent data suggest that induction of the salicylic acid pathway often inhibits the systemic induction of the jasmonic acid pathway by later herbivory. Conversely, the induction of the jasmonic acid pathway can reduce induction of the salicylic acid pathway following subsequent pathogen attack.

Ecological perspective on systemic induced resistance

The molecular biology of induced resistances has been investigated mostly in herbaceous species. More than 100 species are known to express induced resistance following herbivory, but two-thirds of these are trees or shrubs. The vast majority of ecological research into induced resistance deals with herbivores, ranging from mites to large mammals, and it is clear that induced systemic resistances are widespread and of sufficient magnitude to significantly reduce herbivore populations.

In long-lived plants, induced changes can persist to reduce herbivore populations in the years following induction, and in several forest ecosystems the decay of induced resistances can be over several herbivore generations.

Where the mechanisms underlying these ecologically significant induced defences have been defined, it is clear that different plants species use a great diversity of responses. As well as induced chemical and morphological changes, responses include elements that are more closely linked to

Box 1. Rumex –Gastrophysa–Uromyces: an ecological model for host-herbivore-pathogen interactions

The beetle Gastrophysa viridula and the biotrophic rust fungus Uromyces rumicis (Fig. 1) both attack the leaves of Rumex species (docks). The physiology and ecology of this system have been intensively studied. Rust infection of dock reduces the growth, survivorship and fecundity of Gastrophysa (Fig. 2), which is associated with changes in the nutritional status of host tissues following infection. Conversely, G. viridula feeding induces resistances to the rust fungus, both locally and systemically (Fig. 2). These effects are not confined to controlled environments: rust infection is significantly and substantially reduced in the field by both beetle grazing and artificial wounding. Thus, the combined effect of the beetle and the rust fungus on the host might be expected to be inhibited (i.e. the effect less than that of one of the agents alone). However, the effect is usually either equivalent (in Rumex obtusifolius, damage is equivalent to that of the agents) or additive (in R. crispus, damage is equivalent to the sum of the effects of each agent alone; Fig. 2). Illustrating the difficulty in extrapolating from the effects of the agents on each other to their effect on the host. This effect is robust even when plants are grown with differing levels of nitrogen fertilization. Tripartite interactions in dock are not confined to Gastrophysa and Uromyces, because herbivory also significantly reduced field infection by two other fungal pathogens of dock, the necrotroph Ramularia rubella, and the hemibiotroph Venturia rumicis. We are currently applying molecular techniques to the Rumex system to try to identify genes that are up-regulated in response to attack by either the pathogen or the herbivore, and then to examine their spatial and temporal expression patterns in response to multiple attack. Our aim is to apply these techniques to investigate the mechanisms of interactions between Rumex, Gastrophysa and Uromyces in the field.
In this sense, current or past attack or disease is correlated with the risk of future attack. This in turn leads to questions concerning the population genetics and evolution of induced defense. Current theories are based on the concept that current herbivory or disease is correlated with the risk of future attack. In this sense, current or past attack can be seen as having the potential to provide information about the future environment. On this basis, induced resistances will be selected for only if (i) current attack is a reliable predictor of future attack, and (ii) if attack reduces plant fitness.

This body of ecological information on induced defense against herbivores is in stark contrast with the limited knowledge of comparable responses to pathogens. Given the limited understanding of plant pathogens in natural systems it is not surprising that responses to multiple enemies are also poorly understood. One of the few examples of multiple defense to have been studied in a non-crop system—Rumex spp. attacked by the beetle Gastrophysa viridula and the rust fungus Uromyces rumicis—highlights the potential complexity of such interactions (Figs 1 and 2). Several whole plant studies using crops also highlight the diversity of herbivore-pathogen interactions: with examples of attack by one organism inducing resistance to attack by another, but there are also many examples of exceptions.

Defence against multiple enemies

Cross-talk

Molecular biologists have considered “defence on multiple fronts” in terms of interactions between different pathways of induced resistance. For example, whether induction of the salicylic acid pathway influences subsequent induction of the jasmonic acid pathway and vice versa. In this respect there is evidence that resistance induced via one pathway can suppress induced responses mediated via another pathway. However, this is only part of the story, and there are examples of induction by one agent resulting in increased resistance to another. It is also increasingly clear that the concept of specific pathogen and wounding (herbivore)-induced pathways mediated by salicylic acid and jasmonic acid, respectively, is simplistic. Jasmonic acid also mediates resistance to certain pathogens, and both the salicylic acid and jasmonic acid pathways induce a spectrum of
We echo the recent suggestion that the value of research that could benefit from closer liaison between molecular biologists and ecologists. There has been considerable debate over the costs of induced resistances, which should be considered when determining the selective advantage of 'fine-tuned' resistances. There are many questions here, such as:

- Is the cost of a fine-tuned resistance less than that of a broad-spectrum resistance?
- Is induced resistance finely tuned to a particular attacker likely to confer more or less benefit than a broad-spectrum induced resistance?

Reviews of the molecular biology of induced resistance suggest that plants might 'fine-tune' induced resistances to specific attackers12. These arguments seem consistent with current evolutionary theory. Induced resistance is selected for when past or current attack is correlated with the risk of future attack. Such correlation is likely to be greatest when considering current and future attack by the same organism, favouring selection of a 'fine-tuned' induced response. However, selection reflects the interplay of benefits and costs of induced resistances, which should be considered when determining the selective advantage of 'fine-tuned' resistances. There are many questions here, such as:

- Is the cost of a fine-tuned resistance less than that of a broad-spectrum resistance?
- Is induced resistance finely tuned to a particular attacker likely to confer more or less benefit than a broad-spectrum induced resistance?

Although these questions remain unanswered it is notable that the suggestion of fine-tuning appears to be at odds with much of the literature that shows that resistance induced by one organism is often effective against a range of potential attackers. This might be explained partly by a wider range of induced changes than are often considered as resistance mechanisms. Systemic changes in the nutritional quality of host tissues (e.g. nitrogen chemistry) following pathogen infection might have rather non-specific effects on herbivores, and potentially modify responses to induced allelochemicals. There is less evidence that herbivore-induced changes in 'non-defence' chemistry have systemic effects on pathogens. However, these broader changes in host chemistry should not be overlooked when considering the biology of plant responses to multiple enemies.

**Evolution of induced resistances in the context of attack by multiple enemies**

There has been considerable debate over whether a characteristic of the host affecting a particular attacker has necessarily resulted from selection pressure imposed by that attacker. Of course, this also applies to characteristics acting against multiple enemies.
Further, because the products of induced resistance(s) might be active against a wide range of organisms, it is possible that broad-spectrum induced resistance is the result entirely of selection by a single attacker, rather than integrated selection by diverse enemies. However, given that attack by multiple and diverse enemies is commonplace in the field, can current models for the selection of induced resistances be applied to this situation? Such models are based on ‘information’, defined as the ability of current attack to predict future attack. Selection for induced resistance is predicted when past or current attack provides ‘information’ concerning (i.e. is well correlated with) the risk of future attack. Such correlation has been shown experimentally in a few systems and might be expected for many others. However, is it likely that attack by a herbivore contains any information about future attack by pathogens, or vice versa? Arguably, herbivory, by creating wounds that might be vulnerable to invasion by micromicroorganisms predicts an increased risk of future pathogen attack. However, this is a localized response, consistent with the induction of resistance in the vicinity of wounds. Is it likely that herbivory predicts the risk of pathogen attack in ungrazed tissues? Equally, on a whole-plant basis, is past or current pathogen attack likely to be closely correlated to future herbivory? If such correlations between current and future attack are unlikely across multiple enemies, this suggests that selection of broad-spectrum induced resistance is unlikely. Selection based on ‘information’ is also difficult to relate to negative interactions between induced responses, such that the response to one attacker might constrain the plant’s ability to respond to another. Indeed, recent arguments suggest that such interactions reflect the manipulation of pathways by attackers, although this appears to be based on the concept of specific herbivore (jasmonic acid) and pathogen (salicylic acid) pathways, which is at odds with some recent evidence.

An alternative view of selection arises if we develop the suggestion that induced resistance should be considered in the context of the overall response of a plant to attack. This includes what have been called ‘civilian’ responses in host physiology. For example, following foliar attack, civilian responses include remobilization of reserves from storage tissues, increased partitioning of assimilate to leaves rather than roots and up-regulation of photosynthesis in undamaged leaves. Mechanisms of tolerance appear common to attack by both leaf-feeding herbivores and foliar pathogens. These physiological

Fig. 4. ‘Defence’ and ‘civilian’ elements of induced responses and the possible interactions between them. The range of responses in host metabolism and physiology that result from attack by herbivores and attack by pathogens can be divided into ‘defence’ and ‘civilian’ reactions. Induced defence includes responses mediated via the salicylic acid and jasmonic acid pathways, which are well defined. Civilian responses reflect the interplay of damage and tolerance – physiological and metabolic adjustments made in response to damage. The nature of damage varies markedly with the attacking organism, but adjustment at the whole-plant level shares a degree of commonality between, for example, the range of organisms that attack the foliage. The regulation of the balance between damage and tolerance remains poorly defined. Even less defined are the interactions between the civilian and defence responses. Possible interactions include the effects of salicylic acid and jasmonic acid on civilian responses independent of defence, and the effects of metabolic shifts necessary for induced resistance on civilian responses. Equally, the shifts in metabolism and physiology inherent in civilian responses might influence the expression of induced resistances (at the level of the production of resistance compounds and/or the responses of attackers to those compounds). Host growth, development and, ultimately, fitness cannot be seen as a function of defence or civilian responses, but as the outcome of the interaction between both sets of responses.
responses to attack buffer the host from the effects of an initial attack but can increase vulnerability to the effects of future attack. Taking up this theme, a more phytoecentric viewpoint of selection for induced resistance might be that current attack (regardless of the organisms doing the attacking) conveys information about the vulnerability of the plant to the physiological effects of any future attack. We hypothesize that attack by any (fictile) attacker is a reliable predictor of the physiological effect of future attack by any (other) foliar attacker(s). This is also consistent with the suggestion that induced resistances allow maximum expression of the plant’s potential to tolerate herbivory or disease, because the spatial distribution of attack over the plant is altered, maintaining undamaged tissues capable of compensatory physiological changes.

Viewing current attack as providing information about the physiological effects of future attacks requires integration between induced resistance and changes in the primary physiology of the plant following attack. Physiological responses to salicylic acid and jasmonic acid are known to extend far beyond induced resistance, but their effects on ‘civilian’ responses are poorly understood. However, it is notable that exogenous applications of both salicylic acid and jasmonic acid have been shown to inhibit photosynthesis, reducing levels of Rubisco and chlorophylls. Exogenous applications of salicylic acid or jasmonic acid lack the temporal and spatial organization of responses to attack by consumer organisms. However, these effects of exogenous jasmonic acid and salicylic acid are in marked contrast with the increase in photosynthesis in undamaged leaves that is a common trait in the few model systems with field data was the relative scarcity of whole plant investigations of induced responses to herbivory and/or disease. There is a risk that quantifying the effects of induced responses on the herbivores or on the pathogens rather than on the host plant has falsely emphasized mechanisms that induce resistance, but fail to benefit plants, while underestimating the significance of tolerance and other induced ‘civilian’ responses.

Consideration of this ‘middle ground’ has highlighted the need to integrate the available information from this ‘phytoecentric’ viewpoint. We echo the views that defence and civilian aspects of induced responses to attack should be considered together (Fig. 4). By analogy with the term used in the context of abiotic stress, induced resistance and tolerance can be treated as elements of acclimation to attack by herbivores and/or pathogens. Although tolerance and (constitutive) resistance might be alternative adaptive strategies, tolerance and induced resistance can be considered as complementary elements of phenotypic plasticity. Thus, we would argue that it would be foolish for molecular biologists, ecologists and plant physiologists to consider the functional links between tolerance and induced resistance.

Clearly, progress in this area will benefit from the use of appropriate model systems. We agree with the recent suggestion that resolving uncertainties about signalling conflicts and synergies in induced resistance will benefit from the use of systems that are convenient for genetic, molecular, biochemical and physiological studies. However, the use of standard laboratory models can provide little information about the ecology of plant responses to multiple enemies. We would argue that molecular biologists should not shy away from applying well-established techniques to native plants whose responses to multiple attackers have been defined in the field.

Conclusions

We have approached the issue of defence against multiple enemies from the contrasting backgrounds of molecular biology and plant ecology. Once the initial hurdle of the lack of a common terminology was overcome, it became clear that the major limitation to integrating these viewpoints was the gap between the level of organization we normally deal with. A key constraint on the integration of the details of signalling pathways and specific gene products in the few model systems with field data was the relative scarcity of whole plant investigations of induced responses to herbivory and/or disease. There is a risk that quantifying the effects of induced responses on the herbivores or on the pathogens rather than on the host plant has falsely emphasized mechanisms that induce resistance, but fail to benefit plants, while underestimating the significance of tolerance and other induced ‘civilian’ responses.

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References

Does the plant mitochondrion integrate cellular stress and regulate programmed cell death?

Alan Jones

Research on programmed cell death in plants is providing insight into the primordial mechanism of programmed cell death in all eukaryotes. Much of the attention in studies on animal programmed cell death has focused on determining the importance of signal proteases termed caspases. However, it has recently been shown that cell death can still occur even when the caspase cascade is blocked, revealing that there is an underlying oncotic default pathway. Many programmed plant cell deaths also appear to be oncotic. Shared features of plant and animal programmed cell death can be used to deduce the primordial components of eukaryotic programmed cell death. From this perspective, we must ask whether the mitochondrion is a common factor that can serve in plant and animal cell death as a stress sensor and as a dispatcher of programmed cell death.

Until recently, research in programmed cell death (PCD) in animals focused more on the role of the proteolytic cascade of caspases than on the morphotype of death. Caspases play a pivotal role in animal cell death, serving both as the amplifiers of extrinsic death signals and the means by which specific cellular components are dismantled by apoptosis. However, PCD can also occur independently of caspases, this finding led to a re-examination of the role played by these proteases in relation to cell death. In studies where the activity of caspases was blocked with general caspase inhibitors, apoptotic death was either prevented or only partially executed. Genetic knockouts of apoptotic factors of the caspase cascade [including Apaf-1 (Ref. 4), caspase 3 (Ref. 5) and caspase 8 (Ref. 6)] did not prevent death even though some or all the apoptotic features were blocked. In spite of this apoptotic inhibition, the cells died – they exhibited a type of death termed oncotic, which is generally considered to be unprogrammed. The observation that cells can indubitably die, even when another programmed cell death is blocked, provokes the question: what is the basis of this oncotic-like death and can it be described at the molecular level? What is emerging from more critical analyses of cell death is that caspase-driven death might not be the central execution pathway, but instead operates on top of an underlying oncotic-like pathway. When caspase-driven death is blocked, the underlying death pathway is revealed as being oncotic. PCD occurs in other organisms, including plants, but without the apoptotic morphotype where corpse morphology is the result of caspase action. Thus, it is not altogether surprising that there is neither sequence similarity to caspase genes in yeast and plant sequence data bases nor unequivocal evidence for caspase activity in these organisms. Is there a primordial death mechanism that is universal in all eukaryotes, but upon which the caspase proteolytic cascade operates in animal cells? Do caspases represent an evolved sophistication of cell death control and corpse management in animals? Analyses of death control and corpse management in organisms such as plants and yeast should answer these questions.

What features are shared among different cells?

Programmed cell death, which occurs independently of a central role for caspases, has retained...