

PLANT–MICROBE–INSECT INTERACTIONS: Cytokinins as key regulators in plant–microbe–insect interactions: connecting plant growth and defence

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Summary

1. Plant hormones play important roles in regulating plant growth and defence by mediating developmental processes and signalling networks involved in plant responses to a wide range of parasitic and mutualistic biotic interactions.

2. Plants are known to rapidly respond to pathogen and herbivore attack by reconfiguring their metabolism to reduce pathogen/herbivore food acquisition. This involves the production of defensive plant secondary compounds, but also an alteration of the plant primary metabolism to fuel the energetic requirements of the direct defence.

3. Cytokinins are plant hormones that play a key role in plant morphology, plant defence, leaf senescence and source–sink relationships. They are involved in numerous plant–biotic interactions.

4. These phytohormones may have been the target of arthropods and pathogens over the course of the evolutionary arms race between plants and their biotic partners to hijack the plant metabolism, control its physiology and/or morphology and successfully invade the plant. In the case of arthropods, cytokinin-induced phenotypes can be mediated by their bacterial symbionts, giving rise to intricate plant–microbe–insect interactions.

5. Cytokinin-mediated effects strongly impact not only plant growth and defence but also the whole community of insect and pathogen species sharing the same plant by facilitating or preventing plant invasion. This suggests that cytokinins (CKs) are key regulators of the plant growth–defence trade-off and highlights the complexity of the finely balanced responses that plants use while facing both invaders and mutualists.

Key-words: cytokinins, defence modulation, growth–defence trade-off, nutritional resources allocation, plant signalling, symbiosis

Introduction

Plants constitute key nutritional resources for many organisms on Earth and therefore interact with multiple biotic partners ranging from antagonists to mutualists. Plants are under selection pressure to enhance interactions with mutualists while reducing interactions with antagonists. As allocation strategies are constrained by a limiting pool of nutrients, plants are faced with a trade-off between allocation of resources to growth versus defence (Herms & Mattson 1992). In this context, it is interesting to see that plant

growth and defence can be regulated by similar phytohormones (Fig. 1).

Various community members can contribute to plant growth, while others attack plants and need to be countered by effective defences that may be induced by the attackers. Interactions of plants with members of their ecological community, once perceived by the plant, can lead to a profound metabolic reconfiguration of the plant physiology, which favours beneficial organisms and deters antagonists like pathogens or herbivores (e.g. Kessler & Baldwin 2002; Pieterse & Dicke 2007; Schwachtje & Baldwin 2008). For instance, under pathogen infection and

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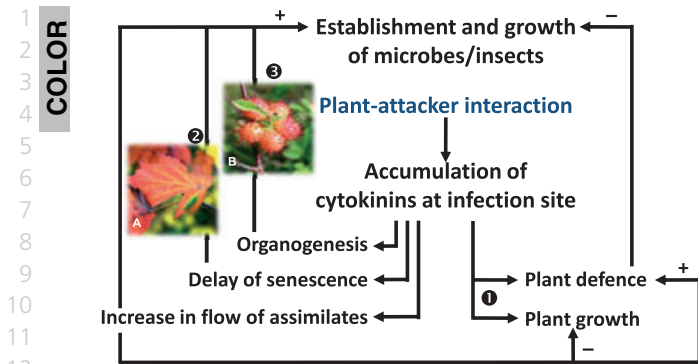


Fig. 1. The cytokinin-mediated growth/defence trade-off. Insect or pathogen attack is usually followed by an increase of cytokinins (CKs) at the infection site. These can lead to a profound reconfiguration of the plant primary and secondary metabolism associated with plant-induced defence. As allocation strategies are constrained by a limited pool of nutrients, plants are faced with a growth-defence trade-off (1). For example, modification of resource allocation can decrease energetic supply for plant growth but can fuel the plant defence machinery. Cytokinin-mediated effects can increase plant defence against biotic invaders but also facilitate the establishment and growth of microbes and insects by delaying leaf senescence (2) and providing them with nutritional supply or by inducing the creation of new organs (such as galls (3) and nodules) used as shelter against biotic and abiotic factors. Small captions: (a) Insect-induced green island. (b) insect-induced gall. © D. Giron. + stimulating effects/– reducing effects.

herbivore attack, plants mount a defensive response, which targets specifically the biotic invader. A key step in this defence process is the recognition of the biotic partner and the activation of a signalling network that will regulate, both locally and systemically, the biochemical reconfiguration of the plant (e.g. Pieterse *et al.* 2009). In recent years, the improvement of molecular techniques and the use of key model plants such as *Arabidopsis* or *Nicotiana* have allowed for a tremendous increase in our understanding of the signalling cascades involved. The plant hormones jasmonic acid (JA), salicylic acid (SA) and ethylene (ET) have rapidly emerged as key response regulators leading to specific defence gene activation (Kessler & Baldwin 2002; Pieterse & Dicke 2007; Erb *et al.* 2008; Schwachtje & Baldwin 2008; Pieterse *et al.* 2012). Their involvement in plant defences and their consequences for plant survival and fitness has been investigated under laboratory conditions as well as field conditions where plants were exposed to natural ecological communities (Baldwin 1998; Thaler 1999; Kessler, Halitschke & Baldwin 2004). Thus, there is a thorough understanding of the role of the three main phytohormones involved in the regulation of plants defences, plant physiology and plant ecology. Linking research on subcellular mechanisms with studies on the ecological functions provides profound understanding of ecological interactions (Dicke & Baldwin 2010).

Other phytohormones, such as abscisic acid (ABA), gibberellins (GBs), auxins and cytokinins (hereafter CKs) have more recently emerged as important defence regulators as well (Robert-Seilaniantz *et al.* 2011). Because the

role of CKs in the modulation of plant defences is relatively anonymous to date, the aim of the current review is (i) to highlight CKs as key regulatory molecules inducing profound morphological and/or biochemical reconfiguration of plants when challenged by various biotic partners. We will also seek (ii) to emphasize that such regulators can be the target of both insects and microbes to disrupt the plant defensive response and/or to withdraw plant resources for their own benefit. (iii) The evolutionary origin of CKs in plants and their associated biotic partners as well as the ecological implications of these interactions will also be addressed. It is now clear that CKs can be synthesised by both microbes and plants, and emerging data strongly suggest that insects may indirectly produce such regulators thanks to their association with endosymbiotic bacteria (Table 1). This could give rise to intricate plant–microbe–insect interactions. The ability to perceive, interpret and manipulate plant signals likely provides insect herbivores and plant pathogens with novel adaptive capacities and allows them to expand their ecological niche. But, CK-mediated effects on plant physiology can have dramatic consequences for plant resource allocation which can impact not only plant growth and defence but also the whole community of species sharing the same plant.

A pivotal role for cytokinins in plant defence against pathogens and insects

Cytokinins are a group of plant hormones that promote cell division and play a major role in the regulation of various biological processes associated with active growth, metabolism and plant development (Mok & Mok 2001; Sakakibara 2006). They are also known to play a role in the synthesis and maintenance of chlorophyll and are known to influence chloroplast development and metabolism. As such, CKs have long been known to delay senescence (Mothes & Engelbrecht 1963; Gan & Amasino 1995). CKs also impact plant nutrient translocation by converting source tissues into active sinks (Mok & Mok 2001). Finally, they are also known to play a role in integrating diverse environmental stress responses (Hare, Cress & Van Staden 1997). For instance, an increase in CK concentration is commonly observed after insect or pathogen attack, suggesting that these molecules play a pivotal role in the profound reconfiguration of the plant primary and secondary metabolism associated with plant-induced defence (Fig. 1).

CYTOKININS AS KEY FACTORS DELAYING PLANT SENESCENCE AND MEDIATING SOURCE–SINK RELATIONSHIPS

The observation that radioactively labelled nutrients are preferentially transported and accumulated in CK-treated tissues suggests that the hormone creates a new source–sink relationship, thus causing nutrient mobilization (Mothes & Engelbrecht 1961). Further support for a role

of CKs in nutrient translocation comes from data that link CK and invertase activity. Indeed, several studies showed that extracellular invertases were induced in various plants by biologically relevant concentrations of CKs (Ehness & Roitsch 1997; Godt & Roitsch 1997). The latter work also found that hexose transporters were co-induced with extracellular invertase by CKs. The coordinated upregulation by CKs of the two functionally linked key enzymes of an apoplasmic phloem unloading pathway may account for the transport of nutrients to CK-treated tissue and the accumulation of nutrients at infection sites first highlighted by Mothes and coworkers in the 1960s. Lara *et al.* (2004) found that extracellular invertase is also an essential component of the CK-mediated delay in senescence, suggesting that CK accumulation results in nutrient mobilization and delayed senescence by increasing the activity of extracellular invertase (Walters & McRoberts 2006; Walters, McRoberts & Fitt 2008).

Such accumulation of nutrients at the infection site can provide the increased demand for energy and carbon skeletons to sustain the plant defence machinery and/or to directly act as plant defensive compounds (Schwachtje & Baldwin 2008; Kerchev *et al.* 2012).

CYTOKININS AS KEY REGULATORS PRIMING PLANTS AND INDUCING THE PRODUCTION OF PLANT SECONDARY METABOLITES

Mechanical wounding and herbivory have been shown to increase CK concentration that increases plant resistance to insects by stimulating wound-inducible gene expression and by inducing the accumulation of insecticidal compounds (Smigocki *et al.* 1993; Hui *et al.* 2003; Dervinis *et al.* 2010). Elevated CK concentrations also cause higher inducibility of a plant endogenous cytochrome P450 gene involved in the synthesis of a variety of secondary plant metabolites (Dervinis *et al.* 2010). It was also suggested that increased CK concentration may contribute to tissue repair by stimulating cell division (Crane & Ross 1986).

More recently, it has been shown that CKs prime plant responses to wounding by conditioning CK-treated plants for a more rapid or higher-magnitude response to a subsequent insect attack (Dervinis *et al.* 2010). Priming is a physiological process by which a plant displays either faster or stronger, or both, activation of the various cellular defence responses that are induced following attack by either pathogens or insects or in response to abiotic stress

Table 1. Examples of organisms known to influence plant cytokinin levels through direct and/or indirect synthesis

Species	Interaction with cytokinins	References
Plant-pathogenic bacteria Examples: <i>Agrobacterium rhizogenes</i> , <i>Agrobacterium tumefaciens</i> , <i>Agrobacterium vitis</i> , <i>Erwinia herbicola</i> , <i>Pseudomonas Solanacearum</i> , <i>Pseudomonas syringae</i> and <i>Rhodococcus fascians</i> .	Direct synthesis (genes for CK synthesis in plasmids)	Jameson 2000; Kakimoto 2001; Frébert <i>et al.</i> 2011;
Plant-mutualistic bacteria Examples: <i>Sinorhizobium meliloti</i> and <i>Mesorhizobium loti</i> .	Direct synthesis	Lohar <i>et al.</i> 2004; Frugier <i>et al.</i> 2008;
Plant-phytopathogenic viruses Examples: Geminiviruses, Begomoviruses (Cabbage leaf curl virus and Tomato golden mosaic virus), Curtoviruses (Spinach curly top virus) and Potexviruses (White clover mosaic potexvirus).	Indirect synthesis (modulation of plant-derived CKs)	Jameson 2000; Baliji, Lacatus & Sunter 2010;
Plant-pathogenic fungi Examples: Hemibiotrophic fungi (<i>Helminthosporium teres</i> , <i>Plasmidiophora brassicae</i> , <i>Pyrenopeziza brassicae</i> , <i>Rhodococcus fascians</i> , and <i>Venturia inaequalis</i>) and biotrophic fungi (<i>Blumeria graminis</i> , <i>Cladosporium fulvum</i> , <i>Fusarium moniliforme</i> , <i>Puccinia tritica</i> and <i>Uromyces fabae</i>).	Direct synthesis + eventually indirect synthesis (modulation of plant-derived CKs)	Cooper & Ashby 1998; Jameson 2000; Walters & McRoberts 2006; Walters, McRoberts & Fitt 2008; Pertry <i>et al.</i> 2009;
Plant-mutualistic fungi Examples: Arbuscular mycorrhiza (<i>Glomus intraradices</i>) and ectomycorrhiza (<i>Lactarius piperatus</i> , <i>Rhizopogon roseolus</i> and <i>Thelephora terrestris</i>).	Direct synthesis and/or indirect synthesis (modulation of plant-derived CKs)	Barker & Tagu 2000; Jameson 2000; Walters, McRoberts & Fitt 2008;
Plant-gallinginsects/Plant-leaf-mining insects: Examples: <i>Ectodemia argentipedula</i> , <i>E. argyropeza</i> , <i>Stigmella argyropeza</i> , <i>Stigmella argentipedula</i> (Lepidoptera: Nepticulidae), <i>Phyllonorycter blancardella</i> (Lepidoptera: Gracillariidae), <i>Pontania proxima</i> (Hymenoptera: Tenthredinidae), <i>Hartigola annulipes</i> (Diptera: Cecidomyiidae), <i>Eurosta solidaginis</i> (Diptera: Tephritidae) and <i>Pachypsylla</i> spp. (Homoptera: Psyllidae).	Direct synthesis potentially mediated by bacterial endosymbionts	Engelbrecht, Orban & Heese 1969; Engelbrecht 1971; Elzen 1983; Mapes & Davies 2001; Giron <i>et al.</i> 2007; Kaiser <i>et al.</i> 2010

(Conrath *et al.* 2006; Frost *et al.* 2008a). The majority of previous work on defence priming in plant–insect interactions has focussed on volatile compounds as priming signals highlighting plant-to-plant communication (Engelberth *et al.* 2004; Frost *et al.* 2008b). However, it was also suggested that nonvolatile molecules transported through the plant vasculature are used to prime plants systemically (Frost *et al.* 2007; Heil & Silva Bueno 2007; Erb *et al.* 2008).

Studies indicate that CKs also enhance plant resistance to pathogens (more specifically pathogens that do not secrete CKs). Indeed, elevated CK levels can modulate SA signalling and enhance resistance against viruses and bacteria through an increased expression of SA-related defence genes (Jameson 2000).

Cytokinins as a tool for invading the host plant

Besides their role in plant defence against pathogens and insects, CKs have also been shown to play a central role in plant colonization and exploitation by various plant-associated organisms including both antagonists and mutualists. In such interactions, CKs can be the specific target of biotic invaders to withdraw plant resources for their own benefit. A clear example of this is the implication of CKs in the formation, after pathogen or insect attack, of often spectacular green and metabolically active areas in otherwise yellow senescent leaves known as ‘green islands’. In these islands, nutrients are redirected towards the infection site where host cell death is delayed. As important molecules implicated in plant defence, CKs can also be used to disrupt defensive responses. There has been considerable debate about the likely origin of CKs in infected leaves, as it is not yet clear whether they are produced by the pathogen/insect or by the plant. Indeed, pathogens and herbivorous insects potentially influence the levels of phytohormones by inducing plant genes involved in CKs biosynthesis, degradation or response, but they can also produce and secrete relevant phytohormones themselves (Jameson 2000; Farnsworth 2004; Robert-Seilanianitz *et al.* 2007; Walters, McRoberts & Fitt 2008; see also Table 1).

PLANTS AND ANTAGONIST MICROBES

Phytopathogenic bacteria

Recent investigations provide support for the role of CKs as key regulators of plant defence response against non-CK-producing plant-pathogenic bacteria such as *Pseudomonas syringae* by suppressing bacterially induced hypersensitive response symptoms and by increasing antioxidative enzyme levels (Barna, Smigocki & Baker 2008; Choi *et al.* 2010). However, the synthesis of CKs as a mechanism for successful invasion of plant tissues seems to be widespread in phytopathogenic bacteria, which commonly carry genes for CK synthesis in plasmids. CK production is exclusive to pathogenic strains with examples

among gram-negative and gram-positive phytopathogenic bacteria (Jameson 2000). The best characterized system is the crown gall formation induced by *Agrobacterium tumefaciens*. In this bacterium, biosynthetic genes for CK [*isopentenyl transferase (ipt)*] and auxin production are carried by bacterial plasmids, which have been integrated into the plant genome (Jameson 2000). Similar *ipt* genes have been found in other bacteria of the same genus (i.e. *A. vitis* and *A. rhizogenes*), but also in other plant-pathogenic bacteria (reviewed in Kakimoto 2001; Frébort *et al.* 2011). For example, pathogenicity of *Rhodococcus fascians* that is responsible for leafy gall diseases of numerous host plants is also associated with the presence of a large linear plasmid on which a gene with homology to the *ipt* gene (known as *fasI*) has been localized (Jameson 2000). This bacterium secretes six CKs that synergistically redirect the developmental programme of the plant to stimulate proliferation of young shoot tissue, which expands the niche the bacteria can colonize and live in (Frébort *et al.* 2011). CK biosynthetic genes located on plasmids have also been reported in other gall-inducing bacteria such as *P. syringae* and *Erwinia herbicola* (Jameson 2000; Frébort *et al.* 2011).

Phytopathogenic viruses

Viruses also have the ability to interfere with endogenous plant hormone levels and can induce a wide range of symptoms including abnormal growth forms such as the formation of galls and general tissue distortions or alterations of photosynthesis like chlorosis or the formation of green islands (Jameson 2000; Walters, McRoberts & Fitt 2008). As hormones involved in plant defence, CKs can interfere with virus proliferation, but the relationship between CK content and virus replication remains often unclear and the underlying mechanisms poorly understood. CKs enhance the resistance of *Arabidopsis* against the *tobacco mosaic virus*, of *Phaseolus vulgaris* against the *White clover mosaic virus* and of tobacco against a broad spectrum of plant viruses through an increased activity of a pathogenesis-related protein (Sano *et al.* 1996; Synkova, Semoradova & Burketova 2004; Choi *et al.* 2010). In contrast, a clear example of the role of CKs in viral phytopathogenicity comes from studies on Gemini-, Begomo- and Curto-viruses. These viruses have evolved multiple strategies to provide a cellular environment favourable to viral amplification, including an increase in CK levels and an increase in the pool of bioactive CKs (by preventing their phosphorylation) (Baliji, Lacatus & Sunter 2010). Both factors promote cell proliferation that viruses rely on for replication of their DNA genome. Additional support for a role of CKs in viral pathogenesis is provided by observations that exogenous application of CKs results in an increase in the kinetics of viral DNA accumulation and reduces the mean latent period for symptom appearance of both *Tomato golden mosaic virus* and *Spinach curly top virus* (Baliji, Lacatus & Sunter 2010). Viral infection can also down-regulate plant

defences through decreasing the proportion of CKs in active form prior to virus replication (Jameson 2000). In addition, application of exogenous CKs or transgenic plants over-expressing CKs has been shown to delay senescence, which would be advantageous to virus survival as senescence eventually leads to leaf death and mobilization of nutrients to other parts of the plant (Li, Hagen & Guilfoyle 1992).

Phytopathogenic fungi

Infection by fungi is often associated with delayed senescence, nutrient accumulation at infection sites and growth malformations, suggesting specific interactions with the host plant cell cycle, photosynthesis and nutrient allocation (Walters & McRoberts 2006). It is now clearly established that ectomycorrhizal and arbuscular mycorrhizal, biotrophic and hemibiotrophic fungi have the ability to produce CKs, while necrotrophic fungi lack this capacity (Jameson 2000; Walters & McRoberts 2006; Walters, McRoberts & Fitt 2008). This suggests a correlation between obligate parasitism and CK production.

Evidence for the role of CKs in symptoms induced by fungi comes from data showing (i) increased CK concentrations in infected tissues; (ii) the ability of infected tissues to grow *in vitro* on hormone-free medium, whereas healthy tissues require CKs (but also auxins); (iii) the fact that CKs are found in spores, mycelial extracts and culture filtrates in several fungal species; and (iv) direct evidence for biosynthesis of CKs by phytopathogenic fungi based on radioactive isotope studies (Jameson 2000; Walters & McRoberts 2006). Additionally, (as with some bacteria) the hemibiotrophic actinomycete *R. fascians* has been shown to have a functional *ipt* gene and to produce CKs, which are recognized by CK receptors in *Arabidopsis* (Pertry *et al.* 2009). As with some viruses, plant infection by some phytopathogenic fungi is also associated with spectacular green islands, which have been found to correlate with the ability to produce CKs (Walters & McRoberts 2006). There is, however, conflicting evidence about the ability of different fungi to produce CKs. For example, green islands have been commonly observed in obligate biotrophic and some species of hemibiotrophic fungal pathogens as a strategy to redirect plant nutrients towards the infection site and to suppress plant cell death. By contrast, obligate necrotrophic and other species of hemibiotrophic fungal pathogens induce localized green areas, in which the pathogen cells are alive but the host plant cells are dead or dying. These green islands are induced by toxins produced by these fungal pathogens (Walters, McRoberts & Fitt 2008). Another question that still needs to be clarified is whether fungi could manipulate plant physiology to the extent that the plant releases its own CKs. Cooper & Ashby (1998), for instance, found that β -glucosidases produced by several fungal pathogens release active CKs from stored plant CK-glucosides.

PLANTS AND MUTUALISTIC MICROBES

Mutualistic bacteria

Not only bacterial pathogens but also bacterial mutualists form galls and evidence suggests that CKs are produced by nodulating rhizobia (Frugier *et al.* 2008). The Nod factor signalling pathway is known to be directly involved in nodule formation in these species, but CKs are also essential and likely act downstream of this pathway. Indeed, (i) CKs can mimic some of the morphogenetic effects of the *Nod* factor (Cooper & Long 1994), (ii) positive correlations between plant CK concentration and nodulation have been reported in several legume species (Lorteau, Ferguson & Guinel 2001), (iii) CKs rapidly appear at the infection site (Lohar *et al.* 2004), (iv) CK signalling genes are up-regulated after rhizobial inoculation (Frugier *et al.* 2008), (v) reduced CK accumulation and/or perception blocks nodulation (Murray *et al.* 2007), and finally (vi) gain-of-function mutants at the *LHK1* CK receptor develop nodules spontaneously in the absence of rhizobia demonstrating that CK signalling is necessary and sufficient to induce cortical cell divisions and nodule organogenesis (Tirichine *et al.* 2007). An interesting feature is that, despite a lack of direct evidence linking rhizobial CK production and nodulation, bacterial CK and/or CK-like compounds seem to be important for *Nod* factor-independent nodulation in some legumes, suggesting a combination of plant and bacterial-mediated processes in the formation of nodules. All together, these results point to CKs as the key differentiation signal for nodule organogenesis and genes homologous to the *A. tumefaciens ipt* gene have been recorded in the rhizobial bacteria *Sinorhizobium meliloti* and *Mesorhizobium loti*.

Mutualistic fungi

Mycorrhizal symbioses are mutualistic interactions between plant roots and fungi where the microbe and the plant exchange water and mineral nutrients for photosynthates. Mycorrhizae can also benefit plants by increasing resistance against biotic (pathogens and herbivores) and abiotic stresses (Pineda *et al.* 2010; Kiers *et al.* 2011; Zamioudis & Pieterse 2012). Phytohormones play a primary role in physiological and morphological alterations of roots and CK accumulation throughout the plant is specifically enhanced by symbiosis (Barker & Tagu 2000). Although clear examples of CK production by mycorrhizae are known (Jameson 2000), it is not always clear whether the CKs are of fungal or plant origin, or both. Irrespective of their origins, hosts and fungal symbionts exchange several rhizospheric signals that are responsible for important morphological and physiological changes in both hyphae and roots and CKs appear as multifunctional regulators (Barker & Tagu 2000). As a result of CK accumulation, we can observe increased root growth (reduced apical growth, elongation of the colonized section and

increased lateral root induction), which is likely to enhance colonization, and altered expression of plant defence response genes. The altered hormone balance was also suggested to be directly responsible for suppressed expression of PR protein, chitinase, endoglucanase and chalcone isomerase defence genes, but underlying mechanisms remain unclear (Barker & Tagu 2000).

PLANTS AND ANTAGONIST HERBIVORES

Gall insects and leaf-mining insects

Cytokinins have been found to mediate plant resistance against insects most likely through the production of secondary metabolites. This has been reported in different species like the tobacco hornworm *Manduca sexta*, the gypsy moth *Lymantria dispar* or the green peach aphid *Myzus persicae* whereby these responses might deter insect feeding, delay larval development or reduce weight gain by insect larvae (Dervinis *et al.* 2010). Endophagous arthropods (i.e. living within plant tissues), such as galling or leaf-mining insects, are characterized by a very intimate association with their host plant, leading to an active manipulation of the plant morphology and/or physiology. This includes the differentiation of additional tissues to feed on, the upregulation of protein and/or sugar synthesis *in situ*, the induction of green islands, and/or the modification of source-sink relationships leading to nutrient translocation towards the insect's feeding site (Stone *et al.* 2002; Giron *et al.* 2007; Schwachtje & Baldwin 2008). Already in the 1970's, several authors suggested that CKs could be the underlying cause for green island formation in insects based on increased levels of CKs in infected tissues. This was first described in mining microlepidopterans (Engelbrecht, Orban & Heese 1969; 1971) and later in the green islands surrounding the galls of phytophagous hymenopterans, psyllid homopterans and cecidomyiids as well as tephritid dipterans (Engelbrecht 1971; Elzen 1983; Mapes & Davies 2001). In the *Malus domestica/Phyllonorycter blancardella* leaf-mining system, for example, increased levels of several CKs (zeatin, isopentenyladenine and isopentenyl adenosine) are found in infected tissues. This could explain the 'stay-green' phenotype of mined areas, while other regions of the same leaf are turning yellow, as well as the net accumulation of nutrients in the mined tissues in a specific pattern matching the energetic requirements of the developing larvae (Giron *et al.* 2007; D. Giron unpublished data). A similar increase in isopentenyladenine was found in galls induced by larvae of *E. solidaginis*, but also in plant-bacteria interactions (Jameson 2000; Mapes & Davies 2001; Sakakibara 2006).

Given the evidence for CK production by gall-forming bacteria and green island inducing fungi, various workers suggested that localized delay of senescence and other phenomena associated with green islands on insect-infected leaves might be the result of release of CKs by the insects (Walters, McRoberts & Fitt 2008; Kaiser *et al.* 2010).

Engelbrecht, Orban & Heese (1969) already suggested that larvae were able to produce CKs because high CK levels were found in larval frass and in gastrointestinal tracts and labial glands of leaf-mining larvae (Engelbrecht 1971). Plant growth hormones such as CKs have also been found in a wide range of galling insect species (reviewed by Elzen 1983), primarily in insect secretions or glands associated with oviposition and initiation of galls, suggesting again that insect larvae may act as a source of CKs (Elzen 1983). The finding of CKs inside larvae that had been otherwise emptied from vegetal remains – and in similar proportions to those found in mined tissues – strongly suggests that some (if not all) extra CKs in the *Malus domestica* leaves infected by *P. blancardella* larvae could originate from the insect (D. Giron unpublished data).

Origin of cytokinins: phylogenetic espionage, manipulation and insect endosymbionts

Due to the regulatory role of CKs on plant morphology, plant defence, leaf senescence and source-sink relationships (causing nutrient mobilization towards the infection site), it is not surprising that these phytohormones have been a privileged target of arthropods and pathogens over the course of the evolutionary arms race between plants and their biotic partners. The ability to perceive, interpret and manipulate plant signals is likely to provide insect herbivores or plant pathogens with novel adaptive capacities enabling them to invade new ecological niches (Schultz 2002; Schultz & Appel 2004; Kaiser *et al.* 2010). Indeed, many signalling molecules involved in plant response to insects and pathogens are phytohormones and a high number of them are similar across kingdoms. Such similarities set the ground for possible exploitation of signalling pathways by one participant for its own benefit. Cyanobacteria, for example, are able to synthesize several plant hormones including CKs, auxins, GBs, ABA, ET and JA. In the particular case of CKs, genome-wide studies suggest that their metabolism in plants has evolved from these bacteria that contain the minimal genetic apparatus needed for the biosynthesis and metabolic control of the hormone but with a different signal-transduction machinery (Frébort *et al.* 2011). It is plausible that CK-producing plant-associated partners manipulate CK signalling to their own benefit by promoting the host division cycle (potentially leading to the formation of new plant organs such as galls/nodules), nutrient mobilization and leaf longevity (leading, for example, to green-island phenotypes), processes that are prerequisite for their successful development on plants (Jameson 2000; Choi *et al.* 2010). As mentioned above, this is well known in plant-associated bacteria especially in *Agrobacterium*, which induces spectacular galls through CK signalling (Robert-Seilaniantz *et al.* 2007). Similarly, phytophagous insects and other plant-exploiting organisms may have taken advantage from their close association with endosymbiotic bacteria to hijack the

1 plant metabolism by acquiring the ability to control the
2 regulation of plant CK levels.

3 4 CYTOKININ-INDUCED PHENOTYPES CAN BE MEDIATED 5 BY INSECT BACTERIAL SYMBIANTS 6

7 Many invertebrates are known to have intimate relation-
8 ships with bacterial symbionts and molecular studies on
9 the evolution and ecological function of these symbionts
10 are revealing diverse ecological and evolutionary effects on
11 insect hosts (Frago, Dicke & Godfray 2012). As research
12 progresses, it has become apparent that many phenotypes
13 traditionally associated with insects are now attributed to
14 their symbionts living in tight association. This might
15 explain why insects represent over 75% of all animal spe-
16 cies and have successfully invaded very diverse habitats,
17 illustrating their remarkably adaptive capacities. These
18 insect symbionts have contributed to insect adaptation
19 mainly by providing novel metabolic capacity, enabling
20 better exploitation of nutritional resources but also provid-
21 ing novel defence mechanisms and affecting reproductive
22 strategies (Janson *et al.* 2008). Nutritional provisioning
23 has been proposed to be one of the major routes leading
24 to symbiosis between micro-organisms and many insects.
25 These symbionts can enable their hosts to develop on lim-
26 ited nutrient sources such as blood in the case of blood-
27 feeding insects, or plant sap and wood in the case of
28 herbivore–plant interactions.

29 Given that bacteria are key partners of many plant–
30 insect interactions and that microbes can produce CKs, it
31 was recently investigated whether insect symbionts could
32 be implicated in host plant manipulation and CK-induced
33 phenotypes (Barr *et al.* 2010; Kaiser *et al.* 2010). The dis-
34 covery that *Wolbachia* is involved in the formation of
35 green islands induced by the caterpillars of *P. blancardella*
36 suggests that insect bacterial symbionts can be involved in
37 insect herbivore–plant interactions by a different mecha-
38 nism than nutrient synthesis. This might involve, for exam-
39 ple, manipulation of host plant physiology through
40 manipulation of CK levels. Interestingly, a decrease of
41 plant defences in mined and galled tissues is also fre-
42 quently observed and recent data also suggest a primary
43 role of insect microbial symbionts in the down-regulation
44 of maize defence against the root-feeding insect *Diabrotica*
45 *virgifera virgifera* (Barr *et al.* 2010). Whether or not CKs
46 are involved in this interaction and in the reduction of
47 plant defences remains to be elucidated.

48 For leaf miners, the full molecular mechanisms at the
49 basis of green-island induction and in particular the origin
50 of CKs within the green mine are still unknown, but sev-
51 eral hypotheses can be advanced to explain the implication
52 of symbiotic bacteria in the green-island phenotype. Sym-
53 biotic bacteria might (i) directly synthesize CKs or (ii)
54 enable the insect to synthesize/secrete CKs, or even (iii)
55 produce regulators of plant CK gene expression. The first
56 two hypotheses are supported by data on insect CK com-
57 position (D. Giron unpublished data) and previously

obtained results by Engelbrecht, Orban & Heese (1969)
who show that large quantities of CKs are present in the
labial glands of several leaf miners. Furthermore, the
ability of bacteria to produce CKs has already been dem-
onstrated in a variety of plant–bacteria interactions such as
the bacterial pathogens *Agrobacterium* spp. or the symbi-
otic plant interaction involving nodulating bacteria. Addi-
tionally, forms of CKs involved in plant–insect
interactions closely match those used by bacteria to manip-
ulate the plant physiology (Jameson 2000; Sakakibara
2006; Giron *et al.* 2007; Kaiser *et al.* 2010).

Plant tissues offer ample opportunity for herbivores and
microbes to interact. One possible first step for the estab-
lishment of these associations could be through insects vec-
toring plant pathogens which might become insect
mutualists when the insect benefits from feeding on a dis-
eased plant (Frago, Dicke & Godfray 2012). In the case of
CK-producing microbes, the benefits for the vector might
not only arise through localized increase in plant nutri-
tional quality as in the case of the green islands or galls
but also through systemic manipulation of plant defence.
The origin of CKs, as shared ancestral traits among plants
and their biotic partners, still remains poorly understood.
Apart from being acquired through symbiotic associations,
CKs might also have evolved as the result of adaptive con-
vergence or they can even have been acquired through
horizontal gene transfer. Using antibiotic curing or
genome-wide sequencing techniques, it would be very
interesting to explore in depth through which route specific
taxons of plant invaders have acquired these traits.

Ecological implications of cytokinin-mediated effects

CASCADING EFFECTS IN THE FOOD WEB

The importance of CKs for plant physiological alterations
(such as green island formation) and for regulation of
altered organogenesis (such as rhizobial nodulation and
gall formation) have recently gained much interest. Data
collected on numerous biological models suggest poten-
tially converging mechanisms for plant–microbe and plant
–endophagous insect symbioses and point towards a key
regulatory role of CKs in many biotic interactions. As stat-
ed earlier in this review, CKs might benefit insect herbi-
vores and plant pathogens via their effects on extracellular
invertase which can stimulate the cell cycle and therefore
delay senescence, induce sink metabolism and provide
nutrients. Additionally, the implication of CKs in signal-
ling processes underlying plant growth and development,
nutrient translocation and plant defences can positively
impact the plants (through endogenous synthesis but also
indirectly *via* their associated symbionts), but also some-
times their pathogens and herbivores. These interactions
can have ecological consequences still to be unravelled and
that can scale up to plant-associated communities (Fig. 2).
The well-studied case of the green islands, for example,

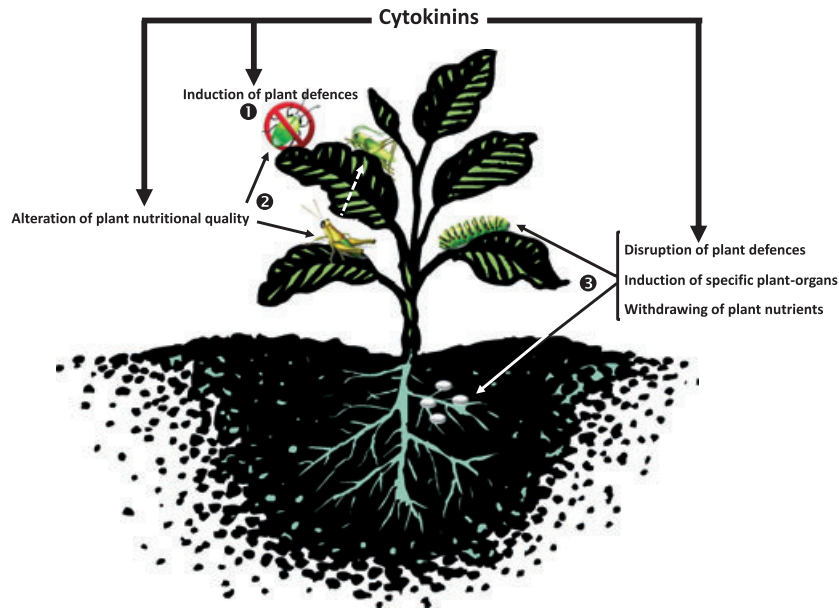


Fig. 2. The cytokinin-mediated facilitation/defence trade-off. Cytokinins are involved in plant defence against pathogens and herbivores (1) and as such can positively impact plant fitness. They can also alter plant nutrient allocation leading to strong modifications of plant quality for plant biotic invaders (2). This can impact directly or indirectly the whole community of herbivores/pathogens and either increase or decrease plant fitness. Finally, cytokinins (CKs) can also be used by these biotic invaders to disrupt the plant defence machinery, to induce specific plant organs that could be used as shelter, but also to withdraw plant nutrients for their own benefits (3). This can facilitate the plant colonization by plant mutualists such as nodulating rhizobacteria but also by plant antagonists such as herbivorous insects and plant pathogens. As key regulatory molecules in plants, cytokinins can impact the whole community of organisms associated with plants.

suggests that the use of CKs to manipulate plant physiology might be widespread. Green islands appear as islands only because the leaf is turning yellow, but they might represent events, which also occur unnoticed earlier in the season. Green islands can therefore be the only visible part of a more extensive phenomenon among insects as well as plant pathogens. As in the case of the leaf miner *P. blancardella*, the green island provides a precious food supply for the insect (Giron *et al.* 2007; Kaiser *et al.* 2010), which enables the insect to win a race against time and hence potentially enable the completion of a supplementary generation (Engelbrecht, Orban & Heese 1969; Giron *et al.* 2007). This can offer competitive advantages over other species but also affect interactions with natural enemies if it provides a window of low natural enemy pressure (Godfray 1994). Additionally, recent studies have revealed how organisms sharing the same plant can be interconnected through indirect interactions mediated by changes in plant quality (Ohgushi 2005; Utsumi, Ando & Miki 2010). After colonization by phytopathogenic microbes, for example, induced changes in plant defensive chemistry can affect herbivores feeding on the same plant, and vice-versa (Stout, Thaler & Thomma 2006). Through changes in plant quality, early season herbivores can also affect the whole community of insects feeding on these same plants later in the season (Poelman *et al.* 2008). In the context of plant-mediated indirect effects, a deeper understanding of CKs and their role in the regulation of complex source-sink relationships will help to understand the structure and function of plant-based food webs.

Although considered as separated modules, plant roots and shoots share the limited plant resources available. Plant-associated insects and microbes might benefit from the use of CKs to increase local abundance of resources on the tissues they inhabit. These interactions, however, might be asymmetric depending on whether they occur below- or above-ground given that biochemical signals transmitted from roots may serve as active regulators of many physiological processes affecting growth and development of both shoots and roots (Aiken & Smucker 1996; Pineda *et al.* 2010). In the particular case of CKs, their synthesis in the roots and translocation to other plant modules can be used by plants to integrate environmental signals contributing to the development of a coordinated plant defensive and growth response both below- and above-ground (van Dam 2009). As such, CKs may play a central role in plant-mediated interactions between above- and below-ground plant-associated communities Van Dam & Heil (2011). In this context, a recent example suggests that the endosymbiotic bacterium *Wolbachia*, which is associated with the western corn rootworm, *Diabrotica virgifera*, plays an important role in down-regulating beetle-induced plant responses in maize (Barr *et al.* 2010). Although these changes were found not to affect beetle fitness the manipulation of induced plant defence using CKs cannot be ruled out as endosymbiotic bacteria in the same genus are known to be capable of synthesizing CKs (Kaiser *et al.* 2010). If this is the case, the *Diabrotica virgifera* – *Wolbachia* system would be an ideal system to test belowground-aboveground interactions mediated by plant-associated partners

using CKs to manipulate plant defences. Although the local effects of CK production by insect herbivores or gall-forming organisms is partially understood in some systems, it remains to be uncovered how these effects influence nearby tissues, and the whole plant physiology.

THE GROWTH-DEFENCE TRADE-OFF IN PLANTS

Plants must grow fast enough to compete against other plants but also need to allocate enough resources to chemical and structural defences necessary for their survival in the presence of herbivores and phytopathogens. As primary and secondary metabolic pathways share common precursors, allocation strategies are constrained by a limiting available pool of nutrients which underlies the evolutionary trade-off between growth and defence in plants (Hermes & Mattson 1992). CKs, by affecting cell division, growth, nutrient translocation, retardation of senescence, and plant defence undoubtedly play an important role in the growth-defence trade-off (Fig. 1). The dual role of CKs in plant growth on the one hand and on induced plant defence on the other hand suggests that an appropriate plant adaptive response to environmental stresses relies on a finely tuned and complex regulation of CKs (Hare, Cress & Van Staden 1997). In this context, not only CK quantities in various plant tissues are important but also the type of CKs (which may differ if they are originating from the plant or from the biotic invaders), the nature of the target cells and the developmental state of the plant, and the hormonal balance between CKs and other phytohormones (Hare, Cress & Van Staden 1997; Farnsworth 2004; Frébert *et al.* 2011).

THE FACILITATION/DEFENCE TRADE-OFF IN PLANTS

Additionally, while CKs are actively involved in plant defence against pathogens and herbivores, they can also be used by these biotic invaders to disrupt the plant defence machinery, to induce specific plant organs that could be used as shelters and protection against biotic and abiotic factors, but also to withdraw plant nutrients for their own benefits (Figs 1 and 2). These contrasting effects of CKs in plants are well illustrated, for example, by conflicting data on viruses where viral infection success can be facilitated either by an increase or a decrease of CK activity and quantity in infected tissues (Hare, Cress & Van Staden 1997; Jameson 2000). This CK-mediated facilitation of plant exploitation by biotic invaders can have profound cascading effects both in plants and through the entire plant-associated community. For instance, plant association with beneficial organisms such as nodule-inducing bacteria and symbiotic mycorrhizae is highly dependent on CKs. The main advantage of these plant symbionts is in improving plant nutrient uptake, especially P and N, in exchange for carbon (Kiers *et al.* 2011). If these costs are low relative to the benefits gained from improved nutrient uptake, then the symbiosis is advantageous for the host

plant, the fungi/bacteria can be considered as mutualistic symbionts, and this results in higher plant growth and competitive ability. By promoting or preventing the development of biotic invaders (e.g. through their action on plant defence or on plant nutrient allocation), CKs can impact photosynthetic capacities, and nutrient levels in plants which, in turn, can shift the balance between costs and benefits in the plant-symbiont relationship. For example, if CKs facilitate the development of herbivores consuming photosynthetic organs and depleting plant reserves of photosynthates, this can erase the selective advantage of plant symbionts and is expected to lead to their elimination.

Future perspectives

Understanding the cellular and molecular dialogue between plants, microbes and insects, which involves shared hormonal signals, and studying ecological and evolutionary implications can greatly improve our understanding of the interactions of plants with microbes and insects. Such information is important to understand the options for each partner to adopt an adaptive response to its biotic environment and the possible implication and origin of key universal regulatory molecules shared by many plant-biotic interactions.

It is now becoming clear that signalling networks that are activated by plants in response to parasitic, herbivorous and beneficial organisms interact either positively or negatively. The crosstalk mediating these signalling network interactions may explain the specificity of the responses but also highlight the complexity of the finely balanced responses that plants use while facing both invaders and mutualists. The *interplay* between CKs and other signalling phytohormones and their dynamics in plant-biotic interactions remains to be investigated, especially regarding the fact that CKs might act upstream of JA biosynthesis and expression of wound- and herbivory-induced genes, and that auxin and CKs have been known for a long time to act either synergistically or antagonistically to control several significant plant developmental processes. Such investigations may help to unravel the different successive steps of interactions and to understand whether CKs can really be considered as key regulatory molecules acting universally in plant-biotic interactions. The *origin* of CKs in these interactions remains also to be clearly investigated and the possible role of endosymbionts for insects deserves in depth exploration. The *dynamics* of the CK signal also remain poorly understood. Whether this is a transient or a continuous signal or a feed-forward mechanism involving secondary signalling molecules such as sugars, and how this signal evolves over the course of the infection and/or pest development (regarding successive larval stages with different requirements and/or feeding habit for example) needs further investigations and may open new avenues for the control of plant pathogens and pests. Moreover, in addition to mechanical and chemical

processes, various electric and hydraulic perturbations of the plant may also contribute to the plant's response. They usually are early events occurring before herbivore attack and pathogen attack-related gene expression that are responsible for cascades of events and signal transductions, eventually leading to indirect and direct plant responses. How these perturbations impact production and action of CKs (and other phytohormones) in plants remains to be established. Finally, CKs are a large group of different molecules that can act not only for the benefits of the plant (e.g. stimulation of plant defences) but also for the benefits of the invader (e.g. reduction of the plant defences). An accurate *characterization* of CKs involved in plant-biotic interactions may not only help to better understand their plant or pathogen/herbivore origin but also to clearly identify their function in the plant, their role in the interaction and their ecological consequences for the whole plant-associated communities.

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References

- Aiken, R.M. & Smucker, A.J.M. (1996) Root system regulation of whole plant growth. *Annual Review of Phytopathology*, **34**, 325–346.
- Baldwin, I.T. (1998) Jasmonate-induced responses are costly but benefit plants under attack in native populations. *Proceedings of the National Academy of Sciences of the USA*, **95**, 8113–8118.
- Baliji, S., Lacatus, G. & Sunter, G. (2010) The interaction between geminivirus pathogenicity proteins and adenosine kinase leads to increased expression of primary cytokinin-responsive genes. *Virology*, **402**, 238–247.
- Barker, S.J. & Tagu, D. (2000) The Roles of auxins and cytokinins in mycorrhizal symbioses. *Journal of Plant Growth Regulation*, **19**, 144–154.
- Barna, B., Smigocki, A.C. & Baker, J.C. (2008) Transgenic production of cytokinin suppresses bacterially induced hypersensitive response symptoms and increases antioxidative enzyme levels in *Nicotina* spp. *Phytopathology*, **98**, 1242–1247.
- Barr, K.L., Hearne, L.B., Briesacher, S., Clark, T.L. & Davis, G.E. (2010) Microbial symbionts in insects influence down-regulation of defense genes in maize. *PLoS ONE*, **5**, e11339.
- Choi, J., Huh, S.U., Kojima, M., Sakakibara, H., Paek, K.H. & Hwang, I. (2010) The cytokinin-activated transcription factor ARR2 promotes plant immunity via TGA3/NPR1-dependent salicylic acid signaling in *Arabidopsis*. *Developmental Cell*, **19**, 284–295.
- Conrath, U., Beckers, G.J.M., Flors, V., Garcia-Agustin, P., Jakab, G., Mauch, F., Poinssot, B., Pozo, M.J., Pugin, A., Schaffrath, U., Ton, J., Wendehenne, D., Zimmerli, L. & Mauch-Mani, B. (2006) Priming: getting ready for battle. *Molecular Plant-Microbe Interactions*, **19**, 1062–1071.
- Cooper, S.J. & Ashby, A.M. (1998) Comparison of cytokinin and cytokinin-O-glucoside cleaving beta-glucosidase production *in vitro* by *Venturia inaequalis* and other phytopathogenic fungi with differing modes of nutrition *in planta*. *Physiological and Molecular Plant Pathology*, **53**, 61–72.
- Cooper, J.B. & Long, S.R. (1994) Morphogenetic rescue of *Rhizobium meliloti* nodulation mutants by *trans*-zeatin secretion. *Plant Cell*, **6**, 215–225.
- Crane, K.E. & Ross, C.W. (1986) Effects of wounding on cytokinin activity in cucumber cotyledons. *Plant Physiology*, **113**, 219–226.
- Dervinis, C., Frost, C.J., Lawrence, S.D., Novak, N.G. & Davis, J.M. (2010) Cytokinin primes plant responses to wounding and reduces insect performance. *Journal of Plant Growth Regulation*, **29**, 289–296.
- Dicke, M. & Baldwin, I.T. (2010) The evolutionary context for herbivore-induced plant volatiles: beyond the 'cry for help'. *Trends in Plant Science*, **15**, 167–175.
- Ehness, R. & Roitsch, T. (1997) Co-ordinated induction of mRNAs for extracellular invertase and a glucose transporter in *Chenopodium rubrum* by cytokinins. *Plant Journal*, **11**, 539–548.
- Elzen, G.W. (1983) Cytokinins and insect galls. *Comparative Biochemistry and Physiology A*, **76**, 17–19.
- Engelberth, J., Alborn, H.T., Schmelz, E.A. & Tumlinson, J.H. (2004) Airborne signals prime plants against insect herbivore attack. *Proceedings of the National Academy of Sciences USA*, **101**, 1781–1785.
- Engelbrecht, L. (1971) Cytokinin activity in larval infected leaves. *Biochemie und Physiologie der Pflanzen*, **162**, 9–27.
- Engelbrecht, L., Orban, U. & Heese, W. (1969) Leaf-miner caterpillars and cytokinins in the green islands of autumn leaves. *Nature*, **223**, 319–321.
- Erb, M., Ton, J., Degenhardt, J. & Turlings, T.C.J. (2008) Interactions between arthropod-induced aboveground and below ground defenses in plants. *Plant Physiology*, **146**, 867–874.
- Farnsworth, E. (2004) Hormones and shifting ecology throughout plant development. *Ecology*, **85**, 5–15.
- Frago, E., Dicke, M. & Godfray, H.C.J. (2012) Insect symbionts as hidden players in insect-plant interactions. *Trends in Ecology and Evolution* (under review), ???, ???–???.
- Frébort, I., Kowalska, M., Hluska, T., Frébortová, J. & Galuszka, P. (2011) Evolution of cytokinin biosynthesis and degradation. *Journal of Experimental Botany*, **62**, 2431–2452.
- Frost, C.J., Appel, M., Carlson, J.E., De Moraes, C.M., Mescher, M.C. & Schultz, J.C. (2007) Within-plant signalling via volatiles overcomes vascular constraints on systemic signalling and primes responses against herbivores. *Ecology Letters*, **10**, 490–498.
- Frost, C.J., Mescher, M.C., Carlson, J.E. & De Moraes, C.M. (2008a) Plant defense priming against herbivores: getting ready for a different battle. *Plant Physiology*, **146**, 818–824.
- Frost, C.J., Mescher, M.C., Dervinis, C., Davis, J.M., Carlson, J.E. & De Moraes, C.M. (2008b) Priming defense genes and metabolites in hybrid poplar by the green leaf volatile cis-3-hexenyl acetate. *New Phytologist*, **180**, 722–733.
- Frugier, F., Kosuta, S., Murray, J.D., Crespi, M. & Szczygłowski, K. (2008) Cytokinin: secret agent of symbiosis. *Trends in Plant Science*, **13**, 115–120.
- Gan, S.S. & Amasino, R.M. (1995) Inhibition of leaf senescence by auto-regulated production of cytokinin. *Science*, **270**, 1986–1988.
- Giron, D., Kaiser, W., Imbault, N. & Casas, J. (2007) Cytokinin-mediated leaf manipulation by a leafminer caterpillar. *Biology Letters*, **3**, 340–343.
- Godfray, H. (1994) *Parasitoids: Behavioral and Evolutionary Ecology*. Princeton University Press, ????????
- Godt, D.E. & Roitsch, T. (1997) Regulation and tissue-specific distribution of mRNAs for three extracellular invertase isoenzymes of tomato suggests an important function in establishing and maintaining sink metabolism. *Plant Physiology*, **115**, 273–282.
- Hare, P.D., Cress, W.A. & Van Staden, J. (1997) The involvement of cytokinins in plant responses to environmental stress. *Plant Growth Regulation*, **23**, 79–103.
- Heil, M. & Silva Bueno, J.C. (2007) Within-plant signaling by volatiles leads to induction and priming of an indirect plant defense in nature. *Proceedings of the National Academy of Sciences of the USA*, **104**, 5467–5472.
- Hermes, D.A. & Mattson, W.J. (1992) The dilemma of plants – To grow or defend. *Quarterly Review of Biology*, **67**, 283–335.
- Hui, D., Iqbal, J., Lehmann, K., Gase, K., Saluz, H.P. & Baldwin, I.T. (2003) Molecular interactions between the specialist herbivore *Manduca sexta* (Lepidoptera, Sphingidae) and its natural host *Nicotiana attenuata*: V. Microarray analysis and further characterization of large scale changes in herbivore-induced mRNAs. *Plant Physiology*, **131**, 1877–1893.
- Jameson, P. (2000) Cytokinins and auxins in plant pathogen interactions – an overview. *Plant Growth Regulation*, **32**, 369–380.
- Janson, E.M., Stireman, J.O., Singer, M.S. & Abbot, P. (2008) Phytophagous insect-microbe mutualisms and adaptive evolutionary diversification. *Evolution*, **62**, 997–1012.
- Kaiser, W., Huguet, E., Casas, J., Commin, C. & Giron, D. (2010) Plant green-island phenotype induced by leaf-miners is mediated by bacterial symbionts. *Proceedings of the Royal Society of London, series B*, **227**, 2311–2319.
- Kakimoto, T. (2001) Biosynthesis of cytokinins. *Journal of Plant Research*, **116**, 233–239.

- Kerchev, P.I., Fenton, B., Foyer, C.H. & Hancock, R.D. (2012) Plant responses to insect herbivory: interactions between photosynthesis, reactive oxygen species and hormonal signaling pathways. *Plant, Cell and Environment*, **35**, 441–453.
- Kessler, A. & Baldwin, I.T. (2002) Plant responses to insect herbivory: the emerging molecular analysis. *Annual Review of Plant Biology*, **53**, 299–328.
- Kessler, A., Halitschke, R. & Baldwin, I.T. (2004) Silencing the jasmonate cascade: induced plant defense and insect populations. *Science*, **305**, 665–668.
- Kiers, E.T., Duhamel, M., Beesetty, Y., Mensah, J.A., Franken, O., Verbruggen, E., Fellbaum, C.R., Kowalchuk, G.A., Hart, M.M., Bago, A., Palmer, T.M., West, S.A., Vandenkoornhuysse, P., Jansa, J. & Buecking, H. (2011) Reciprocal Rewards Stabilize Cooperation in the Mycorrhizal Symbiosis. *Science*, **333**, 880–882.
- Lara, M.E.B., Garcia, M.C.G., Fatima, T., Ehness, R., Lee, T.K., Proels, R., Tanner, W. & Roitsch, T. (2004) Extracellular invertase is an essential component of cytokinin-mediated delay of senescence. *Plant Cell*, **16**, 1276–1287.
- Li, Y., Hagen, G. & Guilfoyle, T.J. (1992) Altered morphology in transgenic tobacco plants that overproduce cytokinins in specific tissues and organs. *Developmental Biology*, **153**, 386–395.
- Lohar, D.P., Schaff, J.E., Laskey, J.G., Kieber, J.J., Bilyeu, K.D. & Bird, D.M. (2004) Cytokinins play opposite roles in lateral root formation, and nematode and Rhizobial symbioses. *Plant Journal*, **38**, 203–214.
- Lorteau, M.A., Ferguson, B.J. & Guinel, F.C. (2001) Effects of cytokinin on ethylene production and nodulation in pea (*Pisum sativum*) cv. Sparke. *Physiologia Plantarum*, **112**, 421–428.
- Mapes, C.C. & Davies, P.J. (2001) Cytokinins in the ball gall of *Solidago altissima* and in the gall forming larvae of *Eurosta solidaginis*. *New Phytologist*, **151**, 203–212.
- Mok, D.W.S. & Mok, M.C. (2001) Cytokinin metabolism and action. *Annual Review of Plant Physiology and Plant Molecular Biology*, **52**, 89–118.
- Mothes, K. & Engelbrecht, L. (1961) Kinetin-induced directed transport of substances in excised leaves in the dark. *Phytochemistry*, **1**, 58–62.
- Mothes, K. & Engelbrecht, L. (1963) On the activity of a kinetin-like root factor. *Life Sciences*, **11**, 852–857.
- Murray, J.D., Karas, B.J., Sato, S., Tabata, S., Amyot, L. & Szczyglowski, K. (2007) A cytokinin perception mutant colonized by *Rhizobium* in the absence of nodule organogenesis. *Science*, **315**, 101–104.
- Ohgushi, T. (2005) Indirect interaction webs: herbivore-induced effects through trait change in plants. *Annual Review of Ecology Evolution and Systematics*, **36**, 81–105.
- Pertry, I., Vaclavikova, K., Depuydt, S., Galuszka, P., Spichal, L., Temmerman, W., Stes, E., Schmuelling, T., Kakimoto, T., Van Montagu, M. C.E., Strnad, M., Holsters, M., Tarkowski, P. & Vereecke, D. (2009) Identification of *Rhodococcus fascians* cytokinins and their modus operandi to reshape the plant. *Proceedings of the National Academy of Sciences of the USA*, **106**, 929–934.
- Pieterse, C.M.J. & Dicke, M. (2007) Plant interactions with microbes and insects: from molecular mechanisms to ecology. *Trends in Plant Science*, **12**, 564–569.
- Pieterse, C.M.J., Leon-Reyes, A., Van der Ent, S. & Van Wees, S.C.M. (2009) Networking by small-molecule hormones in plant immunity. *Nature Chemical Biology*, **5**, 308–316.
- Pieterse, C.M.J., Van der Does, D., Zamioudis, C., Leon-Reyes, A. & Van Wees, S.C.M. (2012) Hormonal modulation of plant immunity. *Annual Review of Cell and Developmental Biology*, **28**, ???–???. in press.
- Pineda, A., Zheng, S.J., Van Loon, J.J.A., Pieterse, C.M.J. & Dicke, M. (2010) Helping plants to deal with insects: the role of beneficial soil-borne microbes. *Trends in Plant Science*, **15**, 507–514.
- Poelman, E.H., Broekgaarden, C., Van Loon, J.J.A. & Dicke, M. (2008) Early season herbivore differentially affects plant defence responses to subsequently colonizing herbivores and their abundance in the field. *Molecular Ecology*, **17**, 3352–3365.
- Robert-Seilantantz, A., Navarro, L., Bari, R. & Jones, J.D. (2007) Pathological hormone imbalances. *Current Opinion in Plant Biology*, **10**, 372–379.
- Sakakibara, H. (2006) Cytokinins: activity, biosynthesis, and translocation. *Annual Review of Plant Biology*, **57**, 431–449.
- Sano, H., Seo, S., Koizumi, N., Niki, T., Iwamura, H. & Ohashi, Y. (1996) Regulation by cytokinins of endogenous levels of jasmonic and salicylic acids in mechanically wounded tobacco plants. *Plant Cell Physiology*, **37**, 762–769.
- Schultz, J.C. (2002) Shared signals and the potential for phylogenetic espionage between plants and animals. *Integrative and Comparative Biology*, **42**, 454–462.
- Schultz, J.C. & Appel, H.M. (2004) Cross-kingdom cross-talk: Hormones shared by plants and their insect herbivores. *Ecology*, **85**, 70–77.
- Schwachtje, J. & Baldwin, I.T. (2008) Why does herbivore attack reconfigure primary metabolism? *Plant Physiology*, **146**, 845–851.
- Smigočki, A., Neal, J.W., McCanna, I. & Douglass, L. (1993) Cytokinin-mediated insect resistance in *Nicotiana* plants transformed with the *ipt* gene. *Plant Molecular Biology*, **23**, 325–335.
- Stone, G.N., Schönrogge, K., Atkinson, R.J., Bellido, D. & Pujade-Villar, J. (2002) The population biology of oak gall wasps (Hymenoptera: Cynipidae). *Annual Review of Entomology*, **47**, 633–668.
- Stout, M.J., Thaler, J.S. & Thomma, B.P.H.J. (2006) Plant-mediated interactions between pathogenic microorganisms and herbivorous arthropods. *Annual Review of Entomology*, **51**, 663–689.
- Synkova, H., Semoradova, S. & Burketova, L. (2004) High content of endogenous cytokinins stimulates activity of enzymes and proteins involved in stress response in *Nicotiana tabacum*. *Plant Cell Tissue and Organ Culture*, **79**, 169–179.
- Thaler, J.S. (1999) Jasmonate-inducible plant defenses cause increased parasitism of herbivores. *Nature*, **399**, 686–688.
- Tirichine, L., Sandal, N., Madsen, L.H., Radutoiu, S., Albrechtsen, A.S., Sato, S., Asamizu, E., Tabata, S. & Stougaard, J. (2007) A gain-of-function mutation in a cytokinin receptor triggers spontaneous root nodule organogenesis. *Science*, **315**, 104–107.
- Utsumi, S., Ando, Y. & Miki, T. (2010) Linkages among trait-mediated indirect effects: a new framework for the indirect interaction web. *Population Ecology*, **52**, 485–497.
- Van Dam, N. & Heil, M. (2011) Multitrophic interactions below and above ground: en route to the next level. *Journal of Ecology*, **99**, 77–88.
- Walters, D.R. & McRoberts, N. (2006) Plants and biotrophs: a pivotal role for cytokinins? *Trends in Plant Science*, **11**, 581–586.
- Walters, D.R., McRoberts, N. & Fitt, B.D.L. (2008) Are green islands red herrings? Significance of green islands in plant interactions with pathogens and pests. *Biological Reviews*, **83**, 79–102.
- Zamioudis, C. & Pieterse, C.M.J. (2012) Modulation of host immunity by beneficial microbes. *Molecular Plant-Microbe Interactions*, **25**, 139–150.

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During the copy-editing of your paper, the following queries arose. Please respond to these by marking up your proofs with the necessary changes/additions. Please write your answers on the query sheet if there is insufficient space on the page proofs. Please write clearly and follow the conventions shown on the attached corrections sheet. If returning the proof by fax do not write too close to the paper's edge. Please remember that illegible mark-ups may delay publication.

Many thanks for your assistance.

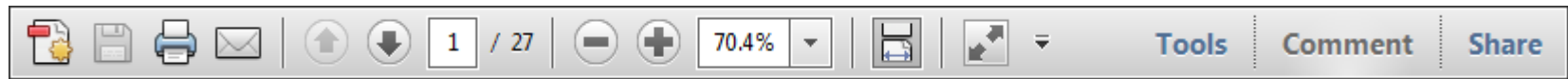
Query reference	Query	Remarks
1	AUTHOR: Please check that authors and their affiliations are correct.	
2	AUTHOR: Robert-Seilaniantz et al. 2011 has not been included in the Reference List, please supply full publication details.	
3	AUTHOR: Kerchev et al. 2011 has been changed to Kerchev et al. 2012 so that this citation matches the Reference List. Please confirm that this is correct.	
4	AUTHOR: Smigocki et al. 1997 has been changed to Smigocki et al. 1993 so that this citation matches the Reference List. Please confirm that this is correct.	
5	AUTHOR: Kakimoto 2003 has been changed to Kakimoto 2001 so that this citation matches the Reference List. Please confirm that this is correct.	
6	AUTHOR: Stone & Schönrogge 2003 has been changed to Stone et al. 2002 so that this citation matches the Reference List. Please confirm that this is correct.	
7	AUTHOR: Please provide the volume number, page range for reference Frago et al. (2012).	
8	AUTHOR: Please provide the publisher location for reference Godfray (1994).	
9	AUTHOR: Please provide the page range for reference Pieterse et al. (2012).	

USING e-ANNOTATION TOOLS FOR ELECTRONIC PROOF CORRECTION

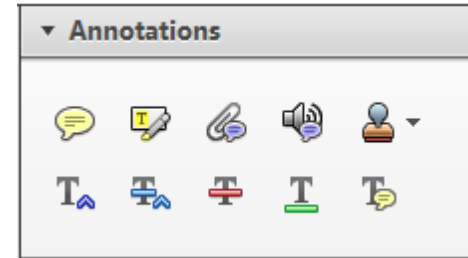
Required software to e-annotate PDFs: Adobe Acrobat Professional or Adobe Reader (version 7.0 or above). (Note that this document uses screenshots from Adobe Reader X)

The latest version of Acrobat Reader can be downloaded for free at: <http://get.adobe.com/uk/reader/>

Once you have Acrobat Reader open on your computer, click on the [Comment](#) tab at the right of the toolbar:



This will open up a panel down the right side of the document. The majority of tools you will use for annotating your proof will be in the [Annotations](#) section, pictured opposite. We've picked out some of these tools below:



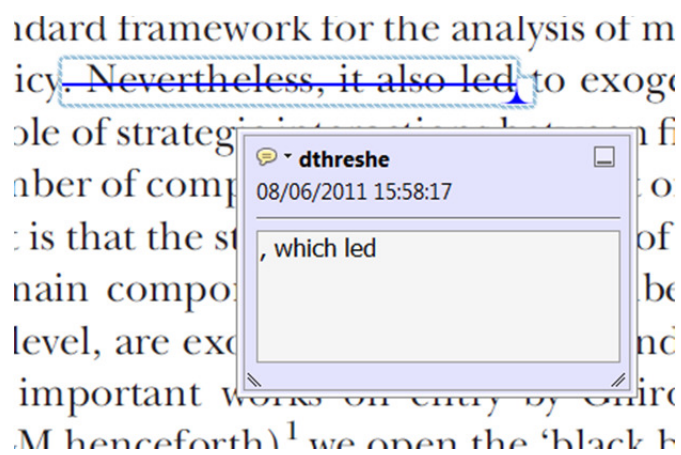
1. Replace (Ins) Tool – for replacing text.



Strikes a line through text and opens up a text box where replacement text can be entered.

How to use it

- Highlight a word or sentence.
- Click on the [Replace \(Ins\)](#) icon in the Annotations section.
- Type the replacement text into the blue box that appears.



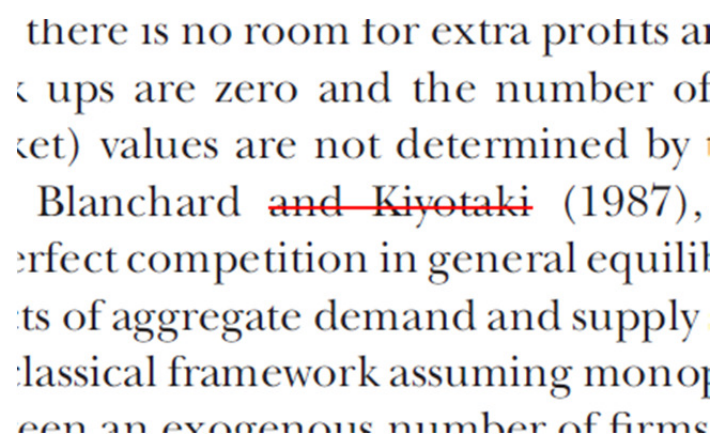
2. Strikethrough (Del) Tool – for deleting text.



Strikes a red line through text that is to be deleted.

How to use it

- Highlight a word or sentence.
- Click on the [Strikethrough \(Del\)](#) icon in the Annotations section.



3. Add note to text Tool – for highlighting a section to be changed to bold or italic.

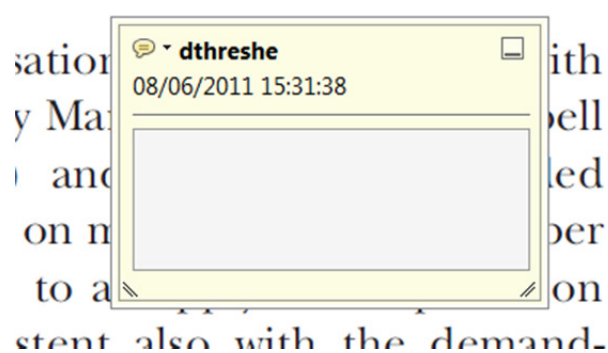


Highlights text in yellow and opens up a text box where comments can be entered.

How to use it

- Highlight the relevant section of text.
- Click on the [Add note to text](#) icon in the Annotations section.
- Type instruction on what should be changed regarding the text into the yellow box that appears.

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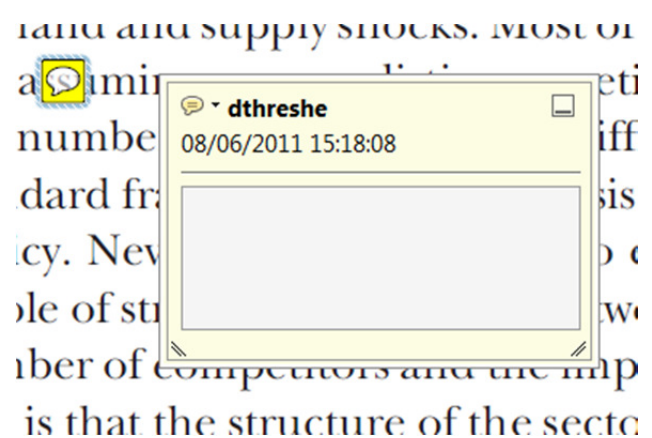
4. Add sticky note Tool – for making notes at specific points in the text.



Marks a point in the proof where a comment needs to be highlighted.

How to use it

- Click on the [Add sticky note](#) icon in the Annotations section.
- Click at the point in the proof where the comment should be inserted.
- Type the comment into the yellow box that appears.



USING e-ANNOTATION TOOLS FOR ELECTRONIC PROOF CORRECTION

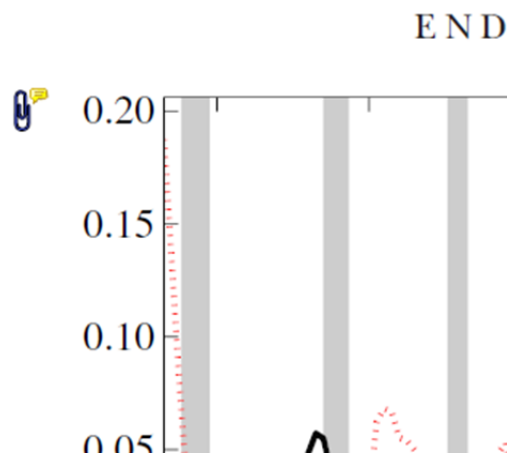
5. Attach File Tool – for inserting large amounts of text or replacement figures.



Inserts an icon linking to the attached file in the appropriate place in the text.

How to use it

- Click on the [Attach File](#) icon in the Annotations section.
- Click on the proof to where you'd like the attached file to be linked.
- Select the file to be attached from your computer or network.
- Select the colour and type of icon that will appear in the proof. Click OK.



6. Add stamp Tool – for approving a proof if no corrections are required.

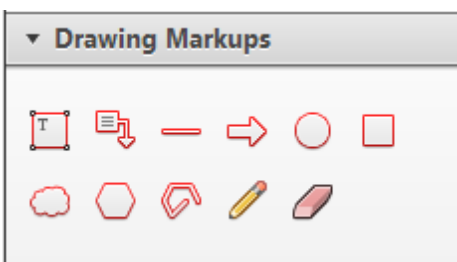


Inserts a selected stamp onto an appropriate place in the proof.

How to use it

- Click on the [Add stamp](#) icon in the Annotations section.
- Select the stamp you want to use. (The [Approved](#) stamp is usually available directly in the menu that appears).
- Click on the proof where you'd like the stamp to appear. (Where a proof is to be approved as it is, this would normally be on the first page).

of the business cycle, starting with the
 on perfect competition, constant return
 production. In this environment goods
 extra profits and the market
 he market. The New-Key
 otaki (1987), has introduced produc
 general equilibrium models with nomin
 ed and supply shocks. Most of this literat

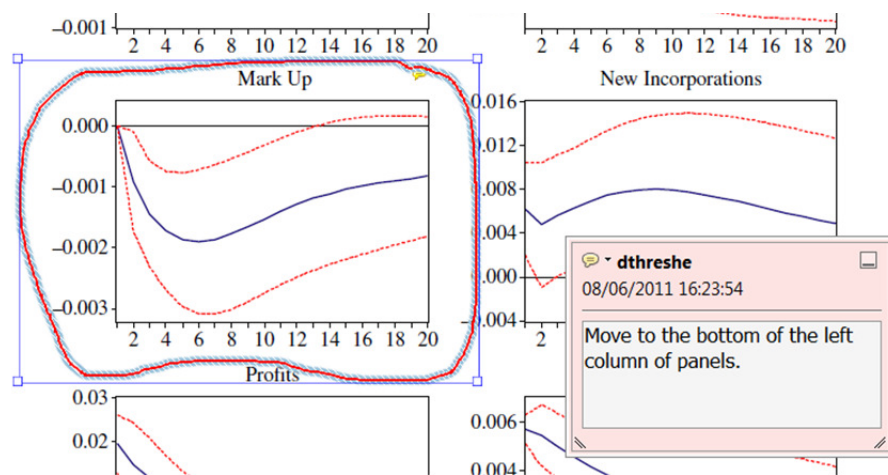


7. Drawing Markups Tools – for drawing shapes, lines and freeform annotations on proofs and commenting on these marks.

Allows shapes, lines and freeform annotations to be drawn on proofs and for comment to be made on these marks..

How to use it

- Click on one of the shapes in the [Drawing Markups](#) section.
- Click on the proof at the relevant point and draw the selected shape with the cursor.
- To add a comment to the drawn shape, move the cursor over the shape until an arrowhead appears.
- Double click on the shape and type any text in the red box that appears.



For further information on how to annotate proofs, click on the [Help](#) menu to reveal a list of further options:

