Significance of Flavonoids in Plant Resistance and Enhancement of Their Biosynthesis

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Abstract: The roles of flavonoids in plant defence against pathogens, herbivores, and environmental stress are reviewed and their significant contribution to plant resistance is discussed. The induction of flavonoids is of particular interest for gathering evidence of their roles. Tools are mentioned which may enhance flavonoid biosynthesis and accumulation. These include metabolic engineering and UV light. The induction of defence-related flavonoids is modified by other determining factors and competition between growth and secondary metabolism may exist. In an evolutionary context, stress-related oxidative pressure may have been a major trigger for the distribution and abundance of flavonoids. UV protection is one of their most significant, or even the most significant, functional role for flavonoids. The multi-functionality of these compounds, however, often complicates the interpretation of experimental results but, overall, it supports the importance of flavonoids.

Key words: Secondary metabolism, host-pathogen interaction, induced resistance, phytoalexin.

1 Introduction

Flavonoids occur widely in plants and are a biologically important and chemically diverse group of secondary metabolites that can be divided into subgroups including: anthocyanidins, flavonols, flavones, flavanols, flavanones, chalcones, dihydrochalcones, and dihydroflavonols. Isoflavonoids and pterocarpanes may also be included but are not considered in this paper. Among the large number of phenolic phytoalexins, the isoflavonoids, phenylpropanoids, and simple phenolics are well elucidated (Harborne, 1994), whereas the role of flavonoids in defence is less well known, except for the catechins and proanthocyanidins (Feucht and Treutter, 1999). In contrast to former times, all these compounds are no longer considered to be waste products, or evolutionary remnants without current function, nor merely metabolic endproducts that are toxic to the plant and are therefore stored away in vacuoles. Moreover, they possess a wide range of biological activities. They are also beneficial for the plant itself as physiologically active compounds, stress protecting agents, attractants, or feeding deterrents and, in general, play a significant role in plant resistance.

2 Flavonoids as Protectants Against Environmental Stress

Since plants are immobile, they must have mechanisms which make them resistant to adverse environments and which retain their fitness. Frost hardiness (Chalker-Scott and Krahmer, 1989) and drought resistance (Pizzi and Cameron, 1986; Moore et al., 2004) are sometimes attributed to flavonoids or other phenolic compounds with respect to functions in the cell wall and in membranes. Tattini et al. (2004) hypothesised that antioxidant flavonoids provide protective functions during drought stress. Flavonoids may also help plants to live on soils which are rich in toxic metals such as aluminium (Barceló and Poschenrieder 2002). Photoprotection is a predominant role of flavonoids afforded by high quercetin/kaempferol ratio, for example, in *Petunia* leaves (Ryan et al., 2002).

Evidence for a UV-protectant role of flavonoids were listed by Reuber et al. (1996), Shirley (1996), and Rozema et al. (1997): epidermal flavonoids which absorb UV radiation protect the internal tissues of leaves and stems; sensitivity of flavonoid-deficient mutants of maize and *Arabidopsis thaliana* to UV-B radiation is increased; flavonoids are potent scavengers of reactive oxygen species and thus prevent peroxidation of lipids. It is assumed by several authors that flavonoids may also provide antioxidant functions in response to excess light (Gould et al., 2002; Rozema et al., 2002; Tattini et al., 2004).

Flavonoids often accumulate in specialized cells. There have been few studies on how these stored flavonoids meet the pathogens against which they should “fight” or how these compounds may protect the plant from environmental stress. With respect to their roles as UV screens and antioxidants, flavonoids are very well placed in epidermal cell layers (Merzylak et al., 2002; Pietrini et al., 2002; Tattini et al., 2004) or in the cuticle of leaves and fruits (Alcerito et al., 2002; Ju and Bramlage, 1999).

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3 Flavonoids as Plant Chemical Defence Compounds

The defence-related flavonoids can be divided into two groups: "preformed" and "induced" compounds. The "induced" compounds are synthesized by plants in response to physical injury, infection, or stress. They may also be constitutively synthesized but, additionally, their biosynthesis is often enhanced under the influence of several types of stress. They may also occur only after infection or following several types of stress, as the so-called phytoalexins.

The "preformed" flavonoids are innate compounds which are synthesised during the normal development of plant tissue. The involvement of preformed flavonoids in several host-pathogen interactions was reviewed by Schlösser (1994). These preformed compounds are often stored at strategically important sites where they may play a signalling and/or a direct role in defence. The relation between the localised accumulation of flavonoids and their activity is plausible with regard to the accumulation of UV-absorbing flavonoids in epidermal tissues (details are discussed below). The site of accumulation seems to clearly indicate a beneficial function in other cases. Proof, however, is much more difficult to find. The occurrence of flavanols in the nuclei of several tree species is still highly debated (Feucht et al., 2004, 2005). Microscopic studies using DMACA staining revealed flavanols in *Camellia sinensis* anthers and in shoot tip cells of *Tsuga canadensis* during the early anaphase and in prophase nuclei. Chromosomes could be stained for flavanols and artefacts could be excluded to the greatest possible extent, but the function of flavanols at this site remains obscure.

3.1 Plant-microbe interactions

Most plants live in close companionship with microbial partners or symbionts that provide nutrients and thereby assist their host to grow on poor soils. The roots of many legumes are colonised by N_2-fixing bacteria which are beneficial for plant growth. Several flavonoids are exuded from plant roots and act as signals that induce the transcription of bacterial genes, where protein products are required for the infection process (reviewed by Hungria and Stacey, 1997; Broughton et al., 2003; Mathesius, 2003; Kobayashi et al., 2004; Cooper, 2004). This is a significant action of flavonoids in improving plant growth and fitness. A plant-microbe partnership has also been found for some non-leguminous plants and in these too flavonoids are important signals. It was reported that the flavanone naringenin can stimulate the colonisation of wheat roots by diazotrophic bacteria, via lateral root crack invasion by *Azorhizobium caulinodans,* and leading to an increased number of lateral roots per plant (Webster et al., 1998). The expression of a symbiosis-related gene is also induced in a cyanobacterium by naringin (Cohen and Yamaseki, 2000). The nodulation of red alder *(Alnus rubra)* by *Frankia (Actinomycetales)* is suggested to be influenced by flavonoids (Benoit and Berry, 1997). The role of flavonoids in symbiosis of plants with arbuscular mycorrhizal fungi is not yet clear. However, there seems to be a relationship, since an increased biosynthesis of flavonoids in mycorrhizae was sometimes found (Ponce et al., 2004). In root cells of *Medicago trunculata* colonised by *Glomus versiforme* elevated levels of PAL and CHS transcripts were detected (Harrison and Dixon, 1994). The flavonol rutin is assumed to be one of the signalling molecules in *Eucalyptus globulus* ssp. *Bicostata,* promoting the hyphal growth of the ectomycorrhizal fungus *Pisolithus* sp. (Lagrange et al., 2001).

Table 1 Flavonoids in the testa of barley genotypes and resistance to *Fusarium* (Skadhauge et al., 1997)

Genotype	Flavonoids in the testa	Inhibition of *Fusarium* infection
Triumph	anth., cat., pa	++
Alf	anth., cat., pa	++
Grit	anth., cat., pa	++
*ant*18-159 (DFR mutant)	dihydroquercetin	+++
*ant*17-148 (FHT mutant)	homoeriodictyol, chrysoeriol	–
*ant*13-152	–	–

anth. = anthocyanins, cat. = catechins, pa = proanthocyanidins

Ironically, flavonoids can also act as attractant molecules for pathogenic micro-organisms such as *Agrobacterium tumefaciens* or *Pseudomonas syringae* and as inducers of their virulence genes (Mo et al., 1995). Stimulation of oviposition by flavonoids was observed for the monarch butterfly *Danaus plexippus* (Baur et al., 1998). Some phagostimulatory activities are attributed to a diverse range of flavonoids (VanLoon et al., 2002), though their contribution to dietary preferences could not always be verified (Lavola et al., 1998).

3.2 Plant-plant interactions

Antimicrobial effects of flavonoids have been described to participate in allelopathic plant-plant interactions (reviewed by Chou, 1999; Inderjit and Gross, 2000). Their roles and mode of action are not yet fully understood. The growth reduction found in gramineous plants (maize, rice, and *Echinochloa oryzicola*) caused by the flavanone naringenin is attributed to the inhibition of 4-coumarate CoA ligase and therefore of lignification (Deng et al., 2004). Recently, (-)-catechin was identified as a phytotoxic allelochemical (Bais et al., 2003). *Centaurea maculosa* exudes (-)-catechin from its roots and inhibits growth and seed germination of *Centaurea diffusa* and *Arabidopsis thaliana.* The flavanol triggers a wave of reactive oxygen species which leads to a Ca^{2+} signalling cascade and to the death of the root system.

3.3 Plant-pathogen interactions

A survey of antifungal phenolic compounds from higher plants was provided by Grayer and Harborne (1994) and many flavonoids are among these compounds. A study of barley mutants showed that proanthocyanidins, and even small amounts of dihydroquercetin, are involved in defence against *Fusarium* species (Skadhauge et al., 1997; Table **1**). The role of barley flavonoids in resistance against *Fusarium* may be related to the following mechanisms (Skadhauge et al., 1997): cross-linking of microbial enzymes; inhibition of microbial cellulases, xylanases, and pectinases; chelation of metals necessary for enzyme activity; and/or formation of a hard, almost crystalline, structure as a physical barrier against pathogen attack.

In a time-space model of host-parasite interactions, the possible role of preformed phenolics in periderm formation in wilt disease resistance was reviewed by Beckman (2000). He described some examples where phenolic compounds, among them flavonoids, are stored in specialised cells from where they can be infused into attacked tissue, such as xylem vessels. Such leaching is probably involved in the hypersensitive response and in programmed cell death, which are common mechanisms of pathogen defence. Beckman (2000) pointed out that phenolic infusion often occurs early after infection. He also discussed the modulating effect on the action of IAA by flavonoids which may lead to changes in tissue differentiation and promote the formation of callus and tylose, thus closing vessels and locking out aggressive invaders.

Some *in vitro* studies reveal a structure-activity relationship with respect to the anti-fungal effect of flavonoids. The fungal blast pathogen *Pyricularia oryzae* shows a differential sensitivity to growth inhibition by naringenin, kaempferol, quercetin, and dihydroquercetin, in decreasing order (Padmavati et al., 1997). Naringenin also inhibited spore germination of the bacterial blight pathogen *Xanthomonas oryzae* pv. *Oryzae,* whereas the other flavonoids tested were ineffective. *Rhizoctonia solani*, the cause of the fungal sheath blight of rice, was unaffected (Padmavati et al., 1997). Quercetin and its derivatives inhibited the growth of *Arabidopsis thaliana* but also of the fungus *Neurospora crassa* (Tomita-Yokotani et al., 2003; Parvez et al., 2004).

The induced formation of flavonoids after injury by pathogens or pests is a well known phenomenon (Barry et al., 2002; Gallet et al., 2004). In cotton leaves, epidermal anthocyanin production is an indicator of resistance to the bacterial blight *Xanthomonas campestris* pv. *malvacearum* (Kangatharalingam et al., 2002). Wound periderm formation as a response of *Eucalyptus globulus* to infection by *Cytonaema* sp. (Eyles et al., 2003) is accompanied by the accumulation of flavanols in the lesion margins. The accumulation of catechins and proanthocyanidins induced during wound healing has been reviewed by Feucht and Treutter (1999). Several promising techniques for resistance induction using benzylaminopurine and "Brotomax" have been described for olive, grape, and *Citrus* (Del Rio et al., 2000, 2001, 2003). Chitosan treatment of rice plants elicited the production of anti-fungal sakuranetin, as well as other phytoalexins (Agrawal et al., 2002).

Flavonoids also play a major role in post-harvest resistance of fruits and vegetables (Lattanzio et al., 1994; Lattanzio, 2003). High concentrations of flavonoids in fruits are often found in parallel with low incidence of pathogens, thus unripe fruits are usually more resistant to fungal decay. Some industrial methods of induced resistance have been described for postharvest diseases. Accumulation of flavonoids in citrus peel, for instance, can be modified by light, UV radiation, temperature, humidity, and phytoregulators (Arcas et al., 2000). UV irradiation of *Citrus aurantium* fruits increased the concentrations of following the flavonoids in citrus peel: naringin, tangeretin, sinensetin, and nobiletin (Arcas et al., 2000). The treatment also reduced decay caused by *Penicillium digitatum*. An *in vitro* assay showed the growth inhibiting effect of tangeretin. Chitosan coating of litchi fruits increased their content of flavonoids and resistance to browning, and post-harvest decay (Zhang and Quantick, 1997). Milsana®, a plant extract, elicited the synthesis of flavonoids at the infection site of cucumber leaves and reduced incidence of powdery mildew (Fofana et al., 2002; McNally et al., 2003). The flavonoids were found to be localised within the haustorial complex of the pathogen *Podosphaera xanthii,* where they may contribute to the collapse of the pathogen.

3.4 *Plant-insect interactions*

The role of flavonoids in plant-insect interactions is widely accepted. Moreover, a popular concept assumes that plant secondary metabolites, among them flavonoids, evolved for defence against herbivores (Feeny, 1976). However, it is not a simple equation as to which of the partners may gain most benefit (Simmonds, 2003). There are insects which can sequester plant flavonoids in their body cuticle as a defence against predators, or into their wings to attract mates. Flavonoids can also have negative effects on non-adopted insects or may reduce the nutritive value of the food. They may behave as feeding deterrants, as digestibility reducers, and as toxins. Several insects are sensitive to flavonoids or are deterred by flavonoids in feeding tests (Brignolas et al., 1998; Berhow and Vaughn, 1999; Hoffmann-Campo et al., 2001; Widstrom and Snook, 2001; Haribal and Feeny, 2003; Thoison et al., 2004; Chen et al., 2004). However, flavonoids do not simply act as broad spectrum defensive mechanisms (Forkner et al., 2004; Nykänen and Koricheva, 2004).

An inheritance study with groundnut provided good evidence that the flavonols quercetin and its glycoside rutin are related to larval mortality of the tobacco armyworm *Spodoptera liture* (Mallikarjuna et al., 2004). *Arachis hypogaea* is a non-resistant host species whereas the wild species *A. kempff-mercadoi* is resistant due to its flavonols. Feeding experiments with interspecific hybrids revealed a positive correlation between the flavonol content of the plants and larval mortality. The biochemical basis for nematode resistance of banana is formed by flavan-3,4-diols and condensed tannins (Collingborn et al., 2000). A physiological mechanism by which flavonoid exudates can elicit an avoidance reaction in herbivores was recently described (Sosa et al., 2004). The exudate of *Cistus ladanifer* L. contains apigenin and 3,7-di-O-methylkaempferol, which inhibit the skeletal muscle sarcoplasmic reticulum (Ca^{2+}-ATPase), thus impairing mouth skeletal relaxation.

4 Tools for Enhancing Flavonoid Accumulation

With the consciousness of the beneficial effects of flavonoids in protecting plants from pests and pathogens, one may consider the idea to actively stimulate their biosynthesis and accumulation in order to support plant defence mechanisms at the time and site of attack. Simmonds (2003) stated that at least "in theory we could be creating a world of plants richer in flavonoids". It has often been shown that pathogens induce the biosynthesis of resistance-related metabolites, but also non-pathogenic strains are capable of eliciting secondary metabolism (Yamamoto et al., 2000). Furthermore, flavonoid synthesis varies and can be induced by ecological factors such as UV light, hydric stress, temperature (reviewed by Chaves and Escudero, 1999), or ozone, which can mimic biotic stressors (Sandermann et al., 1998).

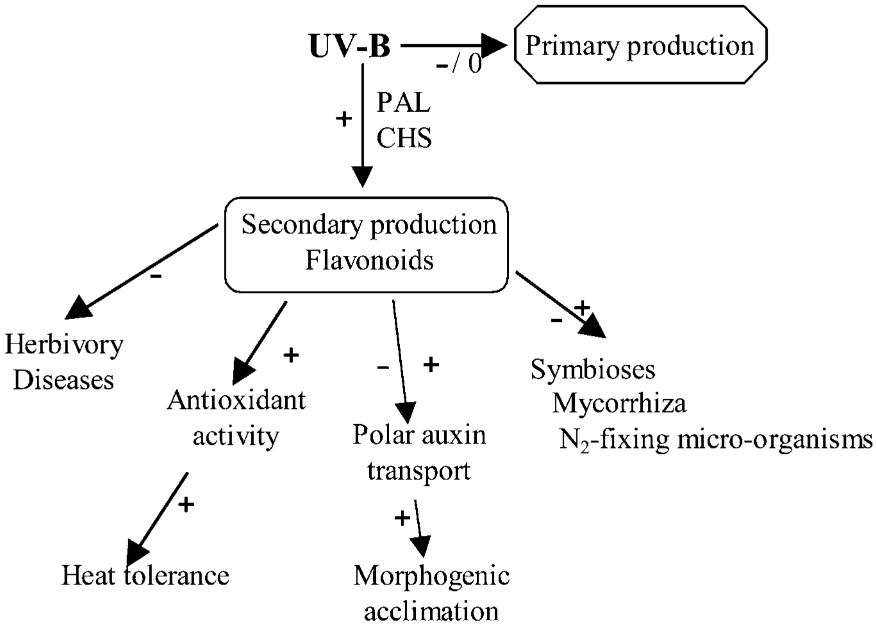

Fig. 1 Flavonoids – mediators of UV-B action? (Modified after Rozema et al., 1997; Jansen, 2002; Teklemariam and Blake, 2003.)

4.1 *UV-induced accumulation of multifunctional flavonoids*

It was reported for Norway spruce (*Picea abies*) that even near-ambient UV-B irradiation enhanced the concentration of flavonols when compared to close-to-zero treatments (Fischbach et al., 1999). UV-B was also shown to induce the production of flavonols in silver birch and grape leaves (Tegelberg et al., 2004; Kolb et al., 2001). Near UV (300–400 nm) also increased flavonoids in roots of pea plants (Shiozaki et al., 1999). Such treatment also promoted net photosynthesis and enhanced growth (shoot length, fresh weight), which is presumably due to enhanced nodulation and symbiotic N_2 fixation induced by flavonoids.

Beside the induced accumulation of flavonoids, other UV-B induced changes in plant architecture have been often found and were discussed as acclimation responses (reviewed by Jansen, 2002) including: leaf thickening, cotyledon curling, inhibition of stem elongation, axillary branching, shifts in the root-shoot ratio, and increased flower number. In all these morphogenic effects, auxins may be especially implicated (Jansen, 2002). Regulation of polar auxin transport by flavonoids has been found (Murphy et al., 2000; Mathesius et al., 1998). Reduced plant growth under enhanced UV-B must also be discussed in relation to the costs involved in the production of secondary metabolites, in the repair of DNA damage or membrane damage, or in scavenging of radicals (Rozema, 1997). However, up to now, there are no quantitative data available.

UV-B radiation can increase PAL and CHS activity, as well as accumulation of multi-functionally active flavonoids in the respective tissue (Fig. **1**). This may improve the symbiotic relationships between plants and micro-organisms. An accumulation of certain flavonoids, such as tannins, may affect herbivory. The antioxidant activity of flavonoids may increase heat tolerance, which was shown for cucumber seedlings (*Cucumis sativus* L.) by Teklemariam and Blake (2003). The UV-induced flavonols reduced the oxidative degradation of membrane lipids.

Besides the numerous beneficial effects of induced flavonoids as UV screens, an adverse effect may occur when the concentration of light-absorbing flavonoids is excessive. A growth retardation of a purple rice genotype *(T-65 Pl)* by UV-B was reported, in spite of high amounts of anthocyanins in the tissue (Hada et al., 2003). It was shown that, in these plants, photorepair by the enzyme photolyase was ineffective. This is due to blue light absorbance by anthocyanins, since the photolyase requires blue light (350–450 nm) as energy to effectively cleave DNA damage products. The accumulation of these compounds blocks transcription and replication and thus may induce growth retardation. On the other hand, optical masking of chlorophyll in red-senescing leaves (absorption 495–644 nm) is supposed to reduce the risk of photo-oxidative damage to leaf cells and to maintain nutrient retrieval (Feild et al., 2001).

4.2 *Metabolic engineering*

Among the tools which may improve our understanding of the role of flavonoids in defence and which may be helpful for the use of their beneficial effects is, of course, metabolic engineering. Prospects for genetic manipulation of flavonoid biosynthesis were outlined by Dixon and Steel (1999) with respect to potential contributions to a better appreciation of the role of flavonoids in plant-micro-organism interactions. Future goals may also include developing and enhancing allelopathic properties of agronomic species by genetic engineering (Chou, 1999). Transgenic plants that over-produce phytoalexins or do not produce them must be tested to understand the role of putative defensive compounds. With respect to transformation of flavonoid genes and their effects, much can be learned from the application of manipulation of flower colours by gene transfer (Nielsen et al., 2002; Davies et al., 2003; Rosati et al., 2003; Schijlen et al., 2004).

A prerequisite for the beneficial use of metabolic engineering in plants with respect to resistance is complete understanding of flavonoid biosynthesis. This is discussed by Winkel-Shirley (1999), on the basis of enzyme complexes which make it more difficult to redirect metabolic flux into the desired end-products by introducing a particular transgene. A more complete knowledge is needed of subcellular organisation of pathways, interaction of enzymes with other enzymes, and interaction with structural components of the cell.

A co-ordinated control system between hydroxycinnamate and flavonoid pathways was suspected to operate in *Ligustrum vulgare* when exposed to excess light (Tattini et al., 2004). A promising example is the transfer of structural and regulatory genes from maize into rice, which activated the anthocyanin pathway and increased the resistance of the transgenic plants against rice blast (Gandikota et al., 2001). However, when using transgenic plants, it must be ensured that the introduced changes in metabolism do not result in the accumulation of other compounds that may interfere with the defence reaction (Hammerschmidt, 1999).

5 "Trade-Off" between Growth and Defence-Related Metabolism

Beside the tools mentioned above (stress factors, metabolic engineering), it is the general supply of nutrients which can modify secondary metabolism. This may be indirectly via controlling growth and differentiation processes (Herms and Mattson, 1992). The directed induction of both flavonoid biosynthesis and accumulation with a defence-promoting goal may be limited by substrate availability, i.e., carbon supply and energy.

Several hypotheses have been formulated which attempt to explain carbon allocation within the plant: "Growth-differentiation balance hypothesis" (Loomis, 1953; Herms and Mattson, 1992), "Carbon-nutrient balance hypothesis" (Bryant et al., 1983), and "Resource availability hypothesis" (Coley, 1985; Bazzaz, 1987). These hypotheses generally assume that the synthesis of carbon-rich secondary chemicals is limited by the availability of photosynthates and that growth processes dominate over differentiation and/or production of defence-related secondary metabolites, as long as conditions are favourable for growth. When growth is limited more than photosynthesis, then allocation towards defence will increase. The causal relationship, however, is not definitively clear. It is still an open question of what comes first. Is it restricted growth which reduces the availability of carbon for flavonoids (Herms and Mattson, 1992)? Or is it the investment of carbon in defensive flavonoids which reduces plant growth (Matyssek et al., 2002)? Or are there independent regulatory mechanisms (Riipi et al., 2001)?

The results of Estiarte et al. (1999), who found higher flavonoid concentrations in wheat after CO_2 enrichment, are in accordance with the hypothesis that higher carbon availability can be invested in flavonoids. Furthermore, there are numerous reports about the increase in flavonoids in N-deficient plants where, simultaneously, growth is reduced (Stewart et al., 2001; Awad et al., 2002; Keller et al., 2003 a; Mattson et al., 2004). Zinc and sulfur deficiencies can also up-regulate flavonoid biosynthetic pathways (Manthey et al., 2000; Nikiforova et al., 2003). The susceptibility of grapevine to powdery mildew *Uncinula necator* is modified by UV radiation and nitrogen (Keller et al., 2003 a). The observed improved resistance is assessed as a by-product of grapevine acclimatisation to high UV radiation and to low nutrient availability.

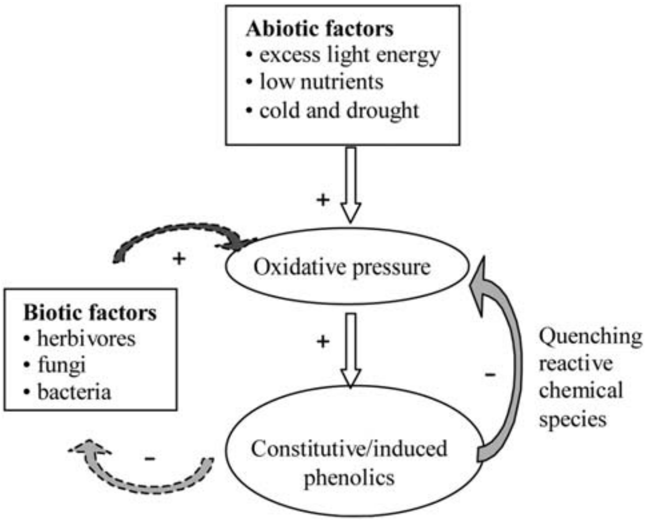

Fig. 2 "Oxidative pressure hypothesis" as an evolutionary concept for flavonoid distribution and abundance (after Close and McArthur, 2002).

The effect of UV-B irradiation on growth and surface flavonols of *Gnaphalium luteo-album* was studied by Cuadra et al. (1997). Their experiments showed that high irradiance treatment decreased biomass (plant height, leaf fresh weight) and increased flavonols. This would be in line with the "Growth-differentiation balance hypothesis". Low irradiance treatment, however, increased biomass and, at the same time, increased flavonols. This observation does not fit with the "Growth-differentiation balance hypothesis". The findings of Riipi et al. (2001) are also contradictory to this theory. Their studies on seasonal changes in birch leaf chemistry failed to find proof that the accumulation of phenolics is hindered by leaf or shoot growth. Studies on ant-plant relationships also did not support the "Growth-differentiation balance hypothesis" (Heil et al., 2002).

Reviewing the existing hypotheses in order to explain the evolution of flavonoids Close and McArthur (2002) formulated the "Oxidative pressure hypothesis" (Fig. **2**): "The distribution and abundance of many phenolics may be explained as a response by plants to prevent or minimise photodamage, *not* as a trade-off in resource allocation in resource-limited environments, *not* as a response to herbivory".

6 Required Evidence and Examples

Plants and pathogens coexist and both have evolved dynamic strategies for their survival. In this context, we must be aware that phytoalexins and induced resistance participate in co-evolution (Kuc, 1994). The mere existence of a metabolite with plant protecting capacity does not necessarily mean that the respective plant is resistant. Also, the intensified biosynthesis of defensive compounds before or after pathogen attack may

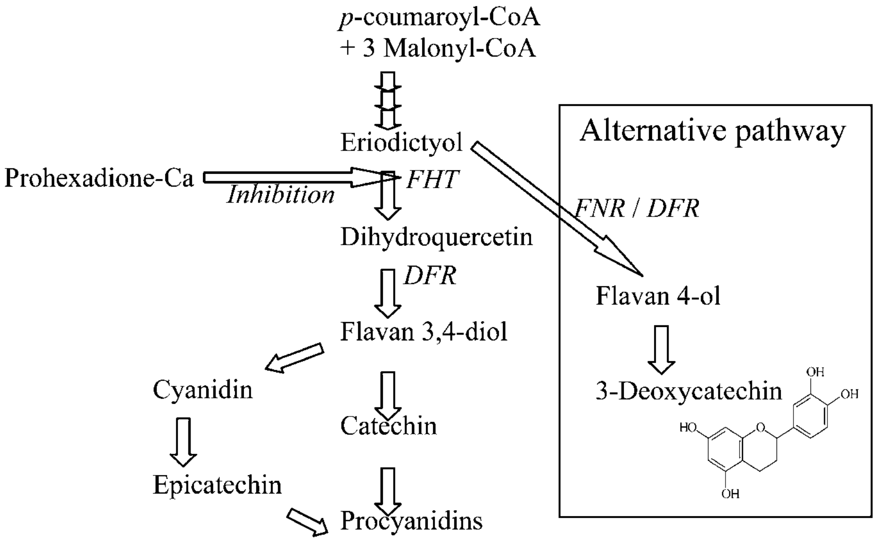

Fig. 3 The defence-related alternative pathway in apple leaves after transient inhibition of the enzyme flavanone 3-hydroxylase (FHT) (modified after Römmelt et al., 2003).

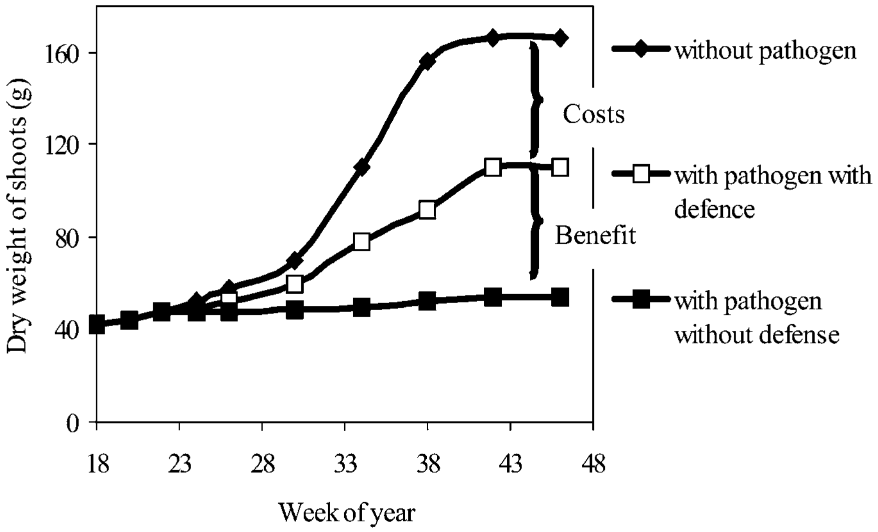

Fig. 4 Costs and benefits of allocation to defensive compounds in young apple trees (modified after Gayler et al., 2004).

not be sufficient for acquired resistance, since many micro-organisms are capable of metabolising flavonoids (Farooq and Tahara, 1999). Pathogens may also modulate defence responses of the host by suppressing the expression of flavonoid genes (Venisse et al., 2002).

The requirements supporting the role of flavonoids in plant resistance were discussed in great detail by Hammerschmidt (1999) in his review "What have we learned about phytoalexins?" According to Hammerschmidt's list, the evidences for a role of flavonoids in resistance must include data that document:

1. localisation and timing of flavonoid accumulation in infected/stressed tissue in relation to pathogen/stress development;
2. strong positive correlation for rapid flavonoid production/accumulation with incompatible plant-pathogen interaction or with stress tolerance;
3. association of rapid flavonoid biosynthesis/accumulation with expression of resistance genes;
4. use of metabolic inhibitors that enhance susceptibility and block flavonoid production;
5. an increase in plant tissue resistance by stimulation of flavonoid production prior to inoculation/stress;
6. the mechanism of flavonoid action in defence; and
7. the defensive role should have a measurable benefit for the plant.

6.1 *Phytoalexins in* Sorghum bicolor

Prominent phytoalexins are the 3-deoxyanthocyanidins of *Sorghum* (Lo et al., 1999). The line of evidence for a defensive role of these phytoalexins has nearly been completed through detailed observations in sorghum seedlings after fungal infection (Snyder and Nicholson, 1990; Snyder et al., 1991; Nicholson and Wood, 2001; Nicholson and Hammerschmidt, 1992; Lo et al., 1999; Aguero et al., 2002):

1. inclusion bodies appear in epidermal cells beneath fungal appressoria;
2. these migrate toward the infection peg;

3. phytoalexins (3-deoxyanthocyanidins) accumulate in the inclusions at fungitoxic concentrations (0.5 – 1.2 ng luteolinidin, 0.2 – 0.9 ng apigeninidin); and
4. a faster defence response was found in a resistant cultivar with earlier induction of defence-related genes (CHS).

The mode of action, however, has still to be clarified.

6.2 *Defensive role of flavonoids in apple* (Malus domestica)

The presence of preformed flavanols in apple leaves may account for efficient defence against the fungus *Venturia inaequalis* (Picinelli et al., 1995; Treutter and Feucht, 1990). However, it is not yet clear if the constitutive level of flavanols is really involved in defence since, after scab infection, a few cells surrounding the infection site accumulate flavanols (Mayr and Treutter, 1998). It was, furthermore, shown that the inhibition of the enzyme phenylalanine ammonia-lyase led to severe scab symptoms on leaves of a resistant cultivar (Mayr et al., 1997). Environmental conditions (N fertilisation) favouring the growth of apple trees inhibited their flavonoid biosynthesis and increased the susceptibility to the pathogen (Rühmann et al., 2002; Rühmann and Treutter, 2003; Leser and Treutter, 2005). A transient inhibition of the flavanone 3-hydroxylase (FHT) in apple leaves by treatment with the dioxygenase inhibitor *Prohexadione-Ca®* induced several changes in their flavonoid composition (Fig. **3**). The most pronounced change is an accumulation of flavanones, which are further metabolised through an unusual pathway towards the 3-deoxycatechins (Römmelt et al., 1999, 2003; Halbwirth et al., 2003). Concomitant with this altered flavonoid pathway, the susceptibility of apple leaves to scab *(Venturia inaequalis),* as well as to the pathogenic bacterium *Erwinia amylovora,* decreased (Bazzi et al., 2003).

Evidence for the significance of flavanols in resistance of apple to scab:
1. accumulation at the infection site (Treutter and Feucht, 1990);
2. flavanol concentration is associated with field resistance (Mayr et al., 1997);
3. transient inhibition of PAL activity in a resistant cultivar led to strong infection (Mayr et al., 1997); and
4. enhanced biosynthesis (N, bioregulator) improves resistance (Leser and Treutter 2005; Römmelt et al., 1999; Bazzi et al., 2003).

Again, the mode of action at the infection site remains an open question.

The question whether the investment in defensive compounds is beneficial for the apple is not easy to answer. Recently, in a model calculation, Gayler et al. (2004) compared costs and benefits of allocation to defensive compounds of young apple trees (Fig. **4**). They showed that defensive flavonoids are expensive and that their accumulation may take place at the expense of plant growth.

7 Concluding Remarks

This literature review shows, again, that there are many studies indicating a role for flavonoids in plant resistance, while almost no firm evidence exists regarding this particular function. This evidence also cannot explain why the biosynthesis and accumulation of flavonoids has been developed during evolution. The benefit of flavonoids for plants is obvious but it is not yet quantified. It remains ambiguous whether the contribution of flavonoids to defence is the dominant function of flavonoids. This aspect is not easy to clarify since the multifunctional roles of flavonoids influence plant physiology in a complex manner.

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