

# Tansley Review No. 72

## Secondary metabolites in plant defence mechanisms

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(Received 4 April 1994)

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### SUMMARY

Many secondary metabolites found in plants have a role in defence against herbivores, pests and pathogens. In this review, a few examples are described and discussed, and some of the problems in determining the precise role(s) of such metabolites highlighted. The role of secondary metabolites in defence may involve deterrence/anti-feedant activity, toxicity or acting as precursors to physical defence systems. Many specialist herbivores and pathogens do not merely circumvent the deterrent or toxic effects of secondary metabolites but actually utilize these compounds as either host recognition cues or nutrients (or both). This is true of both cyanogenic glucosides and glucosinolates, which are discussed in detail as examples of defensive compounds. Their biochemistry is compared and contrasted. An enormous variety of secondary metabolites are derived from shikimic acid or aromatic amino acids, many of which have important roles in defence mechanisms. Several classes of secondary products are 'induced' by infection, wounding or herbivory, and examples of these are given. Genetic variation in the speed and extent of such induction may account, at least in part, for the difference between resistant and susceptible varieties. Both salicylates and jasmonates have been implicated as signals in such responses and in many other physiological processes, though their precise roles and interactions in signalling and development are not fully understood.

Key words: Cyanogenic glucosides; glucosinolates, alkaloids, phenolics, phytoalexins, salicylic acid, methyl jasmonate.

### I. INTRODUCTION

Vascular plants contain an enormous variety of chemical compounds, distinct from the inter-

mediates and products of primary metabolism, which vary according to family and species. The restricted distribution of many such compounds enables them to be used as taxonomic markers, and

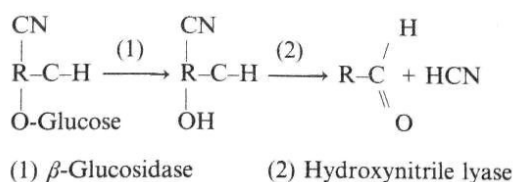
the so-called 'secondary metabolites' make a major contribution to the specific odours, tastes and colours of plants. The term 'secondary metabolite' is rather unsatisfactory, as it covers somewhat indiscriminately a very wide variety of unrelated compounds and implies a secondary, unimportant role for them. In the past such secondary metabolites have been viewed as waste products resulting from 'mistakes' of primary metabolism, and therefore of little importance to plant metabolism and growth. It has become clear that such views are largely inaccurate and misguided, and that many secondary products are key components of active and potent defence mechanisms – part of the age-long 'chemical warfare' fought between plants and their pests and pathogens. In this review, we intend to discuss some examples of such defence mechanisms and cover some aspects of the biology and biochemistry of the secondary metabolites used in this way.

The literature on plant secondary metabolites is extensive, and the chemistry and biology of most major classes have been reviewed many times. This review will therefore be deliberately selective and limited, taking a few classes of secondary metabolites as examples of defensive mechanisms. For comprehensive reviews of many aspects of plant-herbivore interactions, consult the two volumes edited by Rosenthal & Berenbaum (1991) and, for details of the chemistry and biochemistry of plant secondary metabolites in general, consult Stumpf & Conn (1981) and Lea (1993).

## II. CYANOGENIC GLUCOSIDES

### 1. Taxonomic distribution and location

Perhaps the most apparently obvious defence-related secondary metabolites are the cyanogenic glucosides, whose general structure is shown in Figure 1. The R-CH-CN core of the molecule derives from an amino acid, cyanogenic glucosides derived from aromatic or branched-chain amino acids being commonly found. These compounds are widely distributed in nature, being found in more than 2000 species covering all vascular plant groups including angiosperms, both monocots (e.g. sorghum and barley) and dicots (e.g. cassava and clover) (see Seigler, 1991 for a recent general review). Tissue breakdown exposes the vacuole-located glucosides to  $\beta$ -glucosidases and hydroxynitrile lyases with subsequent hydrolysis leading to the release of cyanide



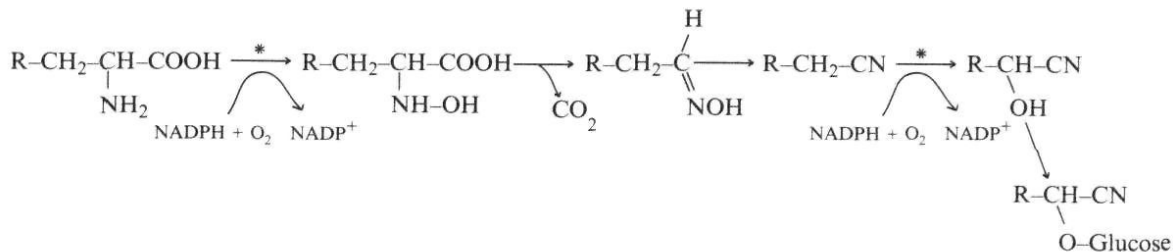
**Figure 1.** The structure of cyanogenic glucosides, and their metabolism to release cyanide.

(Fig. 1), which is a universal respiratory poison. The presence of cyanogenic glucosides in crop plants can cause severe problems, for both animals and humans. In the case of cassava, a staple starch crop for many in the Third World, preparation methods for the tubers have been developed to reduce exposure to cyanide (see Rosling, 1988). Whilst breeding programmes have produced cassava lines with lowered cyanogenic glucoside content, to date no totally acyanogenic lines have been obtained. Such lines may well be undesirable in practice: one of the major advantages of cassava as a subsistence crop is that the tubers can be stored for very long periods without being attacked by pests or diseases, almost certainly because of the cyanogenesis.

### 2. Cyanogenesis and plant protection

Both plants and animals possess the ability to detoxify cyanide, either through 3-cyanoalanine synthesis or the action of rhodanese, which converts cyanide and thiosulphate to sulphate and thiocyanate (though the latter is itself poisonous). So the presence of cyanogenic glucosides in plant material is not necessarily inimical to herbivory. Larvae of the southern armyworm, *Spodoptera eridania*, for example, prefer to graze on cyanogenic glucoside-containing plants and indeed grow better when cyanide is present in their diet (Brattsen *et al.*, 1983). Studies on insect behaviour have shown that cyanogenic glucosides can act as either feeding deterrents or phagostimulants, depending on the insect species. Seigler (1991) concludes that there are few statistically sound data to prove that cyanogenic glucosides are effective in deterring herbivores (but no distinction is made between generalist and specialist herbivores). Berenbaum & Zangerl (1991) quote research that both does and does not implicate the presence or absence of cyanogenesis with feeding preference and behaviour in molluscs! However, clear examples of the protective effect of cyanogenesis do exist, even against apparently specialist pests. For example, the study by Bellotti & Arias (1993) indicated that the presence of cyanogenic glucosides improved the resistance of cassava tubers to the cassava root borer. In contrast, other workers have suggested that cyanogenesis may be harmful to the plant, impairing the action of other defence mechanisms (Lieberei, 1986; Lieberei *et al.*, 1989).

There is a significant metabolic cost to the production of cyanogenic glucosides, as with any secondary metabolite. Kakes (1989) reported that cyanogenic members of a segregating population of *Trifolium repens* produced significantly fewer flower heads than acyanogenic individuals, but were protected as both seedlings and adult plants against feeding by snails. From an analysis of these plants, it was concluded that maintaining cyanogenic glucoside content imposed a significant metabolic



**Figure 2.** The biosynthesis of cyanogenic glucosides (\*, cytochrome P<sub>450</sub>-linked enzyme).

cost, more than could be accounted for simply by the initial biosynthesis. Presumably this extra cost is balanced by enhanced survival of cyanogenic individuals, either through better protection from herbivores or some other (unidentified) mechanism.

### 3. Biosynthesis and metabolism

The biochemistry of cyanogenic glucoside synthesis and metabolism has been extensively studied (enzymology reviewed by Moller & Poulton, 1993). The biosynthetic pathway as currently understood is shown in Figure 2. A precursor amino acid (aromatic and branched-chain amino acids are precursors of many common cyanogenic glucosides) is N-hydroxylated via a cytochrome P450 enzyme (Halkier & Moller, 1991), with subsequent decarboxylation to produce an aldoxime. Another cytochrome P450 enzyme is involved in the C-hydroxylation of the acetonitrile intermediate (Halkier & Moller, 1991) prior to glycosylation to the final cyanogenic glucoside. The entire pathway, bar the final step, appears to be a closely linked and channelled membrane-bound complex, as microsomal preparations will catalyse the whole process (Saunders *et al.*, 1977; Conn, 1988; Halkier & Moller, 1989).

Cyanogenic glucosides may be transported through the plant from the site of synthesis. In the emerging cassava seedling, biosynthetic activity is confined to the cotyledon, yet cyanogenic glucosides are distributed throughout the seedling (Koch *et al.*, 1992). In *Hevea brasiliensis* (rubber), cyanogenic glucoside transport is as the diglucoside, which is not hydrolysed by the same enzyme as the simple glucoside (Selmar, Lieberei & Biehl, 1988). Halkier & Moller (1989) suggest that in sorghum the cyanogenic glucosides are stored at the site of synthesis. There is also evidence to suggest that cyanogenic glucosides stored in the seed are metabolized during germination to release N for seedling growth (Selmar *et al.*, 1988), demonstrating that like many other secondary metabolites the cyanogenic glucosides have multiple roles in plant metabolism.

The tissue content of cyanogenic glucosides varies with age and development, though a detailed study of developmental changes in glucoside content does not appear to have been reported for any species (see

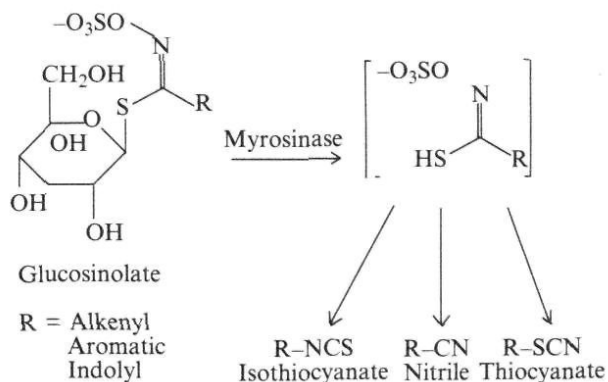
Seigler, 1991). Likewise, there is as yet no indication of the 'induction' of cyanogenic glucoside synthesis and accumulation in response to herbivory or infection, in contrast to several other secondary metabolites (see below).

## III. GLUCOSINOLATES

### 1. Chemistry and location

The glucosinolates are sulphur- and nitrogen-containing compounds found in plants of the order Capparales and a few other unrelated taxa. Their general structure is shown in Figure 3. The R-side chain is derived from an amino acid, and at least 100 different structures have been identified (see for example Daxenbichler *et al.*, 1991, and review of taxonomic distribution by Rodman, 1981). In the intact plant, glucosinolates are located in a separate compartment (probably the vacuole—Grob & Matile, 1979) away from a specific thioglucosidase, 'myrosinase' (EC 3.2.3.1), which may be sequestered in specialized 'myrosin' cells. When plant tissues are disrupted (by physical damage, herbivory, pathogen attack), the enzyme hydrolyses the glucosinolates to produce a variety of products (Fig. 3). The biological effects of glucosinolates and their breakdown products have been reviewed by Chew (1988).

These breakdown products, collectively described as 'mustard oils', are responsible for the flavours of many food plants such as mustards, radishes and cabbages. For this reason the glucosinolate/



**Figure 3.** The structure of glucosinolates, and their myrosinase-catalysed breakdown.

myrosinase system has been extensively studied, though, as will become clear, we are only now beginning to understand the biochemistry of glucosinolate synthesis and the interaction of glucosinolate-containing plants with their pests and pathogens.

## 2. Glucosinolate interactions with pests and pathogens

Much of the work on glucosinolates and pest/pathogen attack has focused on oilseed rape (*Brassica napus* L.), a major oil crop in Europe and North America. Breeders have sought to reduce the glucosinolate content of harvested seed, because of the problems associated with using the protein-rich seed meal obtained after oil extraction as a feed stuff for farm animals. Early efforts along these lines produced cultivars low in both seed and vegetative tissue glucosinolates, but these were unable to survive in the field because of heavy fungal infection (Anon., 1985). Modern 'double low' (00) lines, low in both erucic acid and seed glucosinolates, are almost as resistant as their high glucosinolate predecessors (Mithen, 1992). In these 00 lines the glucosinolate content of vegetative tissues is very little different from the 'high glucosinolate' 0 lines (Porter *et al.*, 1991; Mithen, 1992; Fieldsend & Milford, 1994), and no significant differences could be found in the activity of some biosynthetic enzymes compared to 0 varieties (Wallsgrove *et al.*, 1993).

Some clear correlations have been reported between the glucosinolate content of plants and insect feeding or herbivore damage. Malik, Anand & Srinvasahar (1983) reported a significant negative correlation between the glucosinolate content of 27 species and the fecundity of aphids (*Lipaphis erysimi*) feeding on them. Glen, Jones & Fieldsend (1990) demonstrated that a slug species (*Deroceras reticulatum*) was deterred from feeding on the emerging cotyledons of oilseed rape in direct proportion to the glucosinolate content of the seedlings. Other studies have failed to find any such correlation – feeding by rabbits and woodpigeons on rape was

not apparently influenced by glucosinolate content (Inglis *et al.*, 1992).

Several insect species feed preferentially on Brassicaceae, and studies on host plant selection have demonstrated that it is the presence of glucosinolates which induces feeding by such species (Feeny *et al.*, 1970; Nielsen, 1989). Indeed studies of feeding preference of cabbage stem flea beetles (*Psylliodes chrysocephala*) found that they would only feed on glucosinolate-containing plants, both Brassicas and glucosinolate-containing non-Brassicaceae such as nasturtium (*Tropaeolum majus*) (Bartlett & Williams, 1991), and that addition of glucosinolates to agar significantly enhanced feeding by the beetles (Williams & Bartlett, 1993). Electrophysiological studies on both the flea beetle and the cabbage seed weevil (*Ceutorhynchus assimilis*) found antennal receptors for certain isothiocyanates derived from glucosinolate breakdown (Blight *et al.*, 1989), and these same isothiocyanates positively attracted both insects in behavioural tests (Bartlett *et al.*, 1992, 1993). Isothiocyanates are also effective baits in field traps for the cabbage stem flea beetle (Bartlett *et al.*, 1992). These specialist feeders have clearly turned the glucosinolate defence mechanism against the plant, using the presence of the defence compounds to identify and locate the host. They are not the only insects to exploit the system, however, as there is some evidence to suggest that parasitoids of such specialist feeders may also be attracted by the volatile isothiocyanates (see Pickett *et al.*, 1991). The ecology of the system is obviously complex, so perhaps we should not be looking for simplistic correlations between secondary metabolite content and resistance, particularly in field situations.

## 3. Biosynthesis

In purely chemical terms, there are strong similarities between the biosynthetic pathways for glucosinolates (Fig. 4) and cyanogenic glucosides. Based on this, some workers have assumed that the biochemistry is also common between these two secondary metabolite classes (see Halkier, Lykkesfeldt & Moller, 1991; Poulton & Moller,

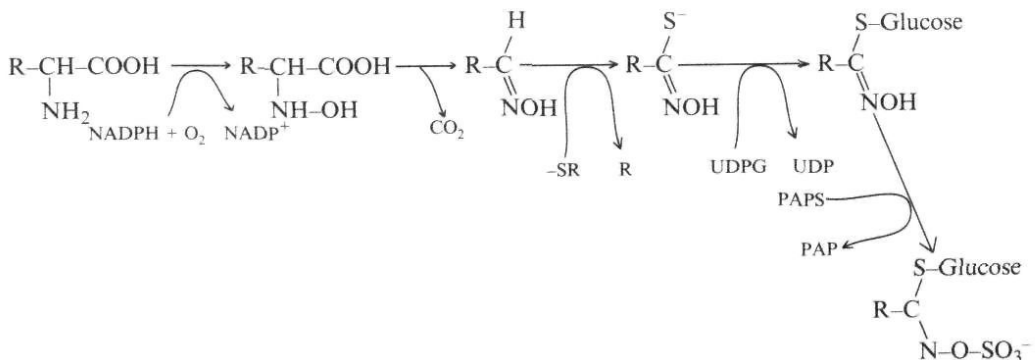


Figure 4. The biosynthesis of glucosinolates

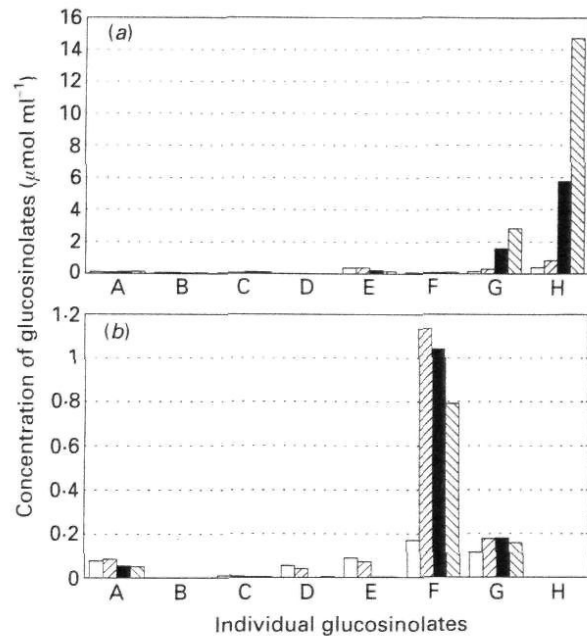


1993, for example). However, recent work in our laboratory has established that the biochemical pathway for the common initial steps (amino acid-hydroxyamino acid-aldoxime) is quite different for glucosinolates from that described for cyanogenic glucoside biosynthesis. In particular, the initial hydroxylation is catalysed by enzymes which closely resemble the flavin monooxygenases found in animal tissues, not by cytochrome P450-type enzymes (Bennett *et al.*, 1993). A coordinated and channelled enzyme complex as found in sorghum (see earlier section) does not appear to operate in glucosinolate biosynthesis, even though the initial steps are catalysed by microsomal enzymes (Bennett *et al.*, 1993; Dawson *et al.*, 1993). On this basis it would appear that glucosinolates and cyanogenic glucosides have evolved independently, and great care should be taken in interpreting taxonomic links between families which contain either of these secondary products. (Despite one report to the contrary, the two classes of compound are apparently never found in the same plant – Larsen, 1981.) What is known of the later steps in glucosinolate biosynthesis is reviewed by Poulton & Moller (1993), though much of the pathway is still poorly understood in biochemical terms.

#### 4. Developmental and induced changes in glucosinolate content

The glucosinolate system in oilseed rape is dynamic – both the amounts and types of glucosinolates present vary with plant tissue, age and developmental status (see for example Sang *et al.*, 1984; Milford *et al.*, 1989*a*; Fieldsend & Milford, 1994*a*). In leaves the glucosinolate content is high in young developing tissues, reaching a maximum at full leaf expansion and then declining (Porter *et al.*, 1991). This turnover and metabolism of glucosinolates (via pathways yet to be established), points to a potential storage role for the compounds, though in rape the vegetative tissue glucosinolates do not seem to provide a significant proportion of the glucosinolate sulphur later found in seeds (Fieldsend & Milford, 1994*b*).

In addition, a great many studies have found that glucosinolate accumulation can be 'induced' by a variety of factors, such as insect attack (Lammerink, MacGibbon & Wallace, 1984; Koritsas, Lewis & Fenwick, 1989; Birch, Griffiths & Smith, 1990), mechanical damage (Bodnaryk, 1992) and fungal infection (Doughty *et al.*, 1991). Such induction does not lead to similar increases in the content of all glucosinolates in a tissue, but rather to more discreet increases in certain compounds, most studies reporting particular increases in indolyl glucosinolates. A detailed time-course of glucosinolate induction in leaves of rape infected with a fungus indicated that all classes of glucosinolate responded in young



**Figure 5.** The induction of glucosinolates in fully expanded leaves of oilseed rape following treatment with (a) methyl jasmonate vapour ( $\square$ , control;  $\boxtimes$ , 1 ppm;  $\blacksquare$ , 10 ppm;  $\boxplus$ , 100 ppm), or (b) salicylic acid soil drench ( $\square$ , control;  $\boxtimes$ , 5 mM;  $\blacksquare$ , 10 mM;  $\boxplus$ , 20 mM). Glucosinolates: A, 2-hydroxy-3-butenyl (R); B, 2-hydroxy-3-butenyl (S); C, 2-hydroxy-4-pentenyl; D, 3-butenyl; E, 4-pentenyl; F, 2-phenylethyl; G, 3-indolylmethyl; H, 1-methoxy-3-indolylmethyl. [From Kiddle *et al.* (1994) & Doughty *et al.* (1994).]

tissues, but at different times after infection, and that there was no increase in aliphatic glucosinolates in older leaves (Doughty *et al.*, 1991). Recent work has shown that treatment with salicylic acid (Kiddle, Doughty & Wallsgrave, 1994) or methyl jasmonate (Bodnaryk, 1994; Doughty *et al.*, 1994) strongly induces glucosinolate accumulation in rape leaves, but that the effects of the two elicitors are different and each induces a very limited range of glucosinolates (Fig. 5). Neither elicitor fully mimics the effects of infection or insect feeding, so what role (if any) they have in natural defence response signalling is unclear. The physiology and biochemistry of the 'induction' of glucosinolates requires considerable further study – differences in the speed and extent of 'induction' (Doughty *et al.*, 1991) may account for some of the observed differences in disease and pest resistance between varieties (Milford *et al.*, 1989*b*; Rawlinson *et al.*, 1989).

#### IV. NON-PROTEIN AMINO ACIDS

An enormous variety of non-protein amino acids is found in plants, with the legumes in particular having a diverse range of compounds and high concentrations. The toxicity of many of these compounds and their role in plant defence is well established (see Rosenthal, 1991; D'Mello, 1994 and references therein). Detailed studies with some

particular non-protein amino acids clearly demonstrate their deterrent effect on non-specialist herbivores, and the mechanisms whereby certain pest organisms can overcome the toxicity.

For example, canavanine is a widely distributed analogue of arginine, found in both tree and forage legumes. Both canavanine and its breakdown product, canaline (an analogue of ornithine), are effective substrates for enzymes which utilize arginine or ornithine, and therein lies their toxic effect. In particular, the arginyl-tRNA synthetase of most organisms cannot distinguish between arginine and canavanine and so canavanine is incorporated into proteins with markedly deleterious effects (reviewed in Rosenthal, 1991). Some insects (such as *Caryedes brasiliensis* and *Sternechus tuberculatus*) which feed on canavanine-containing seeds have an arginyl-tRNA synthetase which *does* discriminate between the protein and non-protein amino acids, leading to a very much reduced incorporation of canavanine into protein compared with a sensitive insect (see Rosenthal, 1991).

Another example is provided by mimosine, an aromatic amino acid found in the tropical forage legume *Leucaena leucocephala*. Mimosine has a variety of deleterious and toxic effects, some caused by its breakdown by gut bacteria to hydroxypyridone (DHP). In areas where *L. leucocephala* is native or well established (e.g. Central America), local ruminants possess gut bacteria capable of fully metabolizing mimosine and DHP, and are able to graze safely on the plant. Transfer of the appropriate bacteria to non-adapted ruminants greatly improves their ability to graze safely on *Leucaena* (Quirk *et al.*, 1988).

Other non-protein amino acids have been shown to be effective against pest and disease organisms. For example  $\beta$ -isoxazolinonyl-alanine from pea roots was shown to be a potent growth inhibitor of phytopathogenic fungi (Schenk, Lambein & Werner, 1991). No effect could be found on bacteria; in particular the compatible symbiotic bacterium *Rhizobium meliloti* could tolerate high concentrations of the amino acid. A survey of *Vicia* species and varieties found a strong correlation between non-protein amino acid content in the plant tissues and resistance to three species of aphid (Holt & Birch, 1984).

These are just a few examples of non-protein amino acids which apparently function as defence chemicals in plants. This field has also provided some clear and striking examples of biochemical and behavioural adaptations by pests and herbivores to overcome the deleterious and toxic effects of such amino acids. As with most of the chemical defence mechanisms we are discussing, no such strategy provides 'perfect' defence, but non-protein amino acids can nonetheless be potent and effective protective agents.

## V. ALKALOIDS

The alkaloids have been divided into three major classes depending on the precursors and the final structure (for reviews on structure, biosynthesis and roles see Culvenor, 1973; Petterson, Harris & Allen, 1991; Waterman, 1993). The true alkaloids are derived from amino acids, are basic and contain nitrogen in a heterocyclic ring, e.g. nicotine and atropine. Common alkaloid ring structures include the pyridines, pyrroles, indoles, pyrrolidines, isoquinolines and piperidines. The pseudoalkaloids are basic but are not derived from amino acids, e.g. caffeine and solanidine. The protoalkaloids are derived from amino acids, are basic but the nitrogen is not in a heterocycle, e.g. the phenylethylamine-derived alkaloids such as mescaline. The alkaloids are often unevenly distributed in plant families. One exception to this are plants from the Papaveraceae; all genera studied contained at least one alkaloid. Common alkaloid containing plants can be found in the Leguminosae, the Liliaceae, the Solanaceae and the Amaryllidaceae. As well as the large number of useful pharmacological properties of the alkaloids utilized by man, alkaloids have been shown to be important resistance factors against herbivorous pests. The evidence for this role is increasing and there are several good examples. It has been shown that a group of quinolizidine alkaloids were effective feeding deterrents against a number of herbivores including insects, molluscs and mammals (Petterson *et al.*, 1991). Furthermore these alkaloids were also toxic against some fungi and bacteria. However, it has yet to be demonstrated if the concentrations used in the bioassays occur in the plants. In cultivars of *Locustra angustifolius* grown in Western Australia a reduction in the concentration of alkaloids below 5 mmol kg<sup>-1</sup> led to a significant loss of the crop by increased aphid feeding (Petterson *et al.*, 1991).

The role of the alkaloids in potato in relation to insect-resistance has been assessed by several groups; especially resistance against the Colorado beetle (*Leptinotarsa decemlineata*) and the potato leafhopper (*Empoasca fabae*). The common potato (*Solanum tuberosum*) contains the alkaloid solanine, which has no effect on Colorado beetle. Potato species (*Solanum demissum*) resistant to this pest were found in South America (Harborne, 1988). The resistance appeared to be dependent on the concentrations of the alkaloid demissine. This alkaloid is structurally related to solanine but the beetles were unable to detoxify it. The importance of *Solanum* alkaloids, particularly those related to solanidine, was demonstrated in resistance to the leafhopper (Sanford *et al.*, 1990). Increasing concentrations of the alkaloids in various cultivars were positively correlated with a reduction in leaf hopper infestation.

In barley the concentration of the alkaloid gramine in the leaves was shown to be associated with

resistance to the aphid *Schizaphis graminum* (Zuniga, Salgado & Corcuera, 1985). As barley plants mature the foliar concentration of gramine decreases and the rate of feeding and survival of the aphid increases. If the aphids are given diets supplemented with gramine, similar to concentrations found in the young plants, feeding and development are severely retarded.

When tall fescue (*Festuca arundinacea*) was colonized by the fungal endophyte *Acremonium coenophialum*, there was a significant reduction in feeding and survival of two aphid species; *Rhopalosiphum padi* and *Schizaphis graminum* (Johnson *et al.*, 1985). Analysis of various extracts from control and endophyte-colonized plants demonstrated a role for the pyrrolizidine alkaloids. In the colonized plants, the concentrations of the alkaloids were greatly elevated. Various feeding experiments using extracts from control and endophyte-colonized plants showed that the alkaloids were very potent antifeedants and were also extremely toxic to *R. padi* and *Oncopeltus fasciatus* (a milkweed bug).

The number of examples of insect-resistance in plants related to alkaloid concentrations are increasing. There are a number of elegant examples of coevolution of the host plant and the insect pests, especially with regard to specialist and non-specialist feeders (Harborne, 1988).

## VI. PLANT PHENOLICS

### 1. Introduction

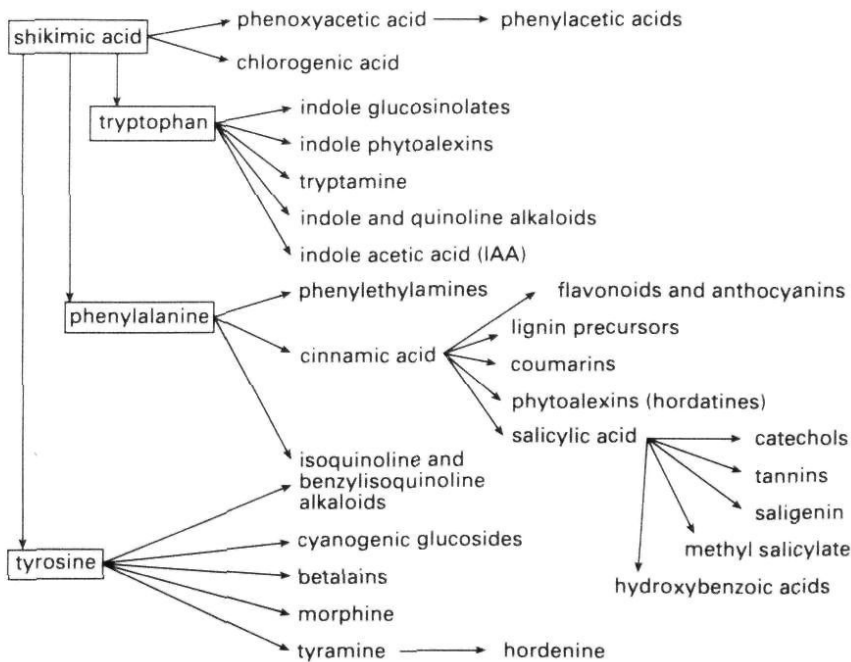
The term, phenolics, has been used to describe a group of structurally diverse plant secondary

metabolites (Wong, 1973). This group includes metabolites derived from the condensation of acetate units (e.g. terpenoids), those produced by the modification of aromatic amino acids (e.g. phenylpropanoids; cinnamic acids, lignin precursors, hydroxybenzoic acids, catechols and coumarins), flavanoids, isoflavanoids and tannins (dihydroxyphenols and flavanols polymerized by the action of peroxidases and polyphenoloxidases). The phenolics derived from aromatic amino acids and their precursors are merely some of the very wide range of compounds derived from shikimic acid (Fig. 6), including many other secondary metabolites discussed in this review.

There are several examples of constitutive phenolics acting as feeding deterrents for herbivores and inhibitors of enzymes (Cheeke, 1989). The evidence for their role in resistance against fungi, bacteria and nematodes is more circumstantial (Friend, 1981, 1985; Stoessl, 1983; Harborne, 1988; Kuc, 1990; Siqueira *et al.*, 1991). In these interactions, it appears to be the speed and duration of *de novo* biosynthesis of phenolics that is more important for resistance than the constitutive concentrations. Two modes of action appear to operate, direct toxic effects (phytoalexins and free radicals formed from lignin precursors) and the active and rapid deposition of barriers such as lignin.

### 2. Plant phenolics and resistance to insects

Wheat cultivars with high constitutive concentrations of soluble and cell wall-bound phenolics are much less attractive to the cereal aphid (*Rhopalosiphum padi*).



**Figure 6.** Some of the secondary products and other plant metabolites derived from shikimic acid and the aromatic amino acids, and their biosynthetic relationships.

*siphum padi*) than cultivars with low phenolic concentrations (Leszczynski, Warchol & Niraz, 1985). When presented with four wheat cultivars containing different concentrations of phenolics the aphids preferentially fed on the cultivar with the lowest concentration. Hydroponic uptake of various phenolics into an aphid-susceptible wheat cultivar demonstrated that pyrocatechol (a dihydroxyphenol) and ferulic acid (a lignin precursor) were the most effective feeding deterrents (Leszczynski *et al.*, 1985).

Young willow plants (*Salix dasyclados*) that had been light- and nutrient-stressed (low carbon supply) contained concentrations of phenolics three times lower than either control or low nitrogen-fed plants (Larsson *et al.*, 1986). These plants low in phenolics were significantly more attractive to the leaf beetle (*Galerucella lineola* F.) than the control or low-N plants. It was clear that nitrogen limitation was not a major factor in the defence against the leaf beetle. These results provide evidence that poor carbon supply and reduced photosynthetic ability, leading to low phenolic concentrations, may be important in increased susceptibility to insect pests. In the Silicaceae three major phenolic glycosides have been identified, and these have been implicated in resistance against herbivores including insect pests (Zucker, 1972; Rowell-Rahier, 1984*a, b*; Tahvaneinen *et al.* 1985). The glycosides have been identified as salicin, salicertin and fragilin; all derived from salicylic acid. When tissue is damaged they are hydrolysed and all three release salicyl alcohol (Tahvaneinen *et al.*, 1985).

There is a strong correlation between the constitutive concentrations of catechol-based phenolics in strawberry leaves and resistance to the two-spotted spider mite (*Tetranychus urticae*) (Luczynski, Isman & Raworth, 1990). The development of the mite on cultivars containing high concentrations of phenolics was clearly suppressed; especially in cultivars with high catechol concentrations. The delayed development of the mites may be due to the phenolics covalently binding to mite digestive enzymes and inactivating them. It has also been shown that mite damage will induce the *de novo* synthesis of phenolics in plants (Kielkiewicz & Van de Vrie, 1982; Inoe *et al.*, 1985). Other examples of resistance to the two-spotted spider mite involving phenolics include their interaction with peppermint (monoterpenes and phenolics: Larson & Berry, 1984) and with chrysanthemum (phenolics: Kielkiewicz & Van de Vrie, 1990).

Gossypol, the cotton phenolic pigment, has indirectly been shown to be an important deterrent against numerous insect pests (Abou-Donia, 1989). The highest concentrations of gossypol and greatest levels of biosynthesis are associated with specific glands on the cotton plants. When the glands were removed from the cotton plants they became susceptible to several insect pests not normally

associated with cotton (Bottger, Sheehan & Lukefahr, 1964; Maxwell, Lafever & Jenkins, 1965). Inclusion of gossypol in artificial diets was toxic to the tobacco bollworm (*Heliothis virescens*), the bollworm (*Heliothis zea*) and several other insect pests (Maxwell *et al.*, 1965). This is strong evidence for the role of gossypol in resistance against non-specialist insect pests; specialist feeders have developed strategies to overcome the gossypol toxicity.

A more complex interaction between insect pests, insect predators and phenolics in the host plant was reported in two species of Mimosoideae (Koptur, 1985). In *Inga densiflora* and *Inga punctata* growing at low altitudes the phenolic content was low in both species. The plants have extrafloral nectaries that attract nectar-drinking ants. Any insects that the ants encounter on the plants, especially caterpillars, are killed. At higher altitudes the prevalence of nectar-drinking ants is much lower and two strategies are thought to have evolved. The populations of the two *Inga* species at higher altitudes contain significantly higher phenolic concentrations in both young and old leaves, compared with the populations at lower altitudes. However, there is also a much higher incidence of insect parasitic wasps and insect-parasitoids attracted to the high altitude plants. This may be due to the lower numbers of ants feeding on the high altitude plants (more nectar available and less chance of attack by the ants). There did not appear to be a direct correlation between phenolic concentrations and the deterrence of the insect pests. However, in such a complex interaction the contribution of individual components was difficult to accurately assess. The high phenolic concentrations are also possibly acting against microorganisms or may be functioning as a block against increased u.v. light at the higher altitudes. This is a dynamic and complex ecosystem involving not only the host plant and its secondary metabolites but the pests and their predators.

The role of tannins and resistance to the larvae of the Oak moth (*Opherophthera brumata*) was reported by Feeny (1970). The moth larvae feed on the young leaves of the trees in the spring but by mid-June they suddenly stop. No environmental factors or the levels of predators could account for this change in feeding. Feeny (1970) found that the levels of tannins in the leaves significantly increased prior to cessation of feeding by the larvae. The deterrence was thought to be because of the complexation of the tannins with the host proteins (making them indigestible) and the reaction of the tannins with digestive enzymes in the gut of the larvae.

### 3. Plant phenolics and resistance to other herbivores

Resistance to attack by birds has been recognized in high tannin-containing sorghum plants (Butler, 1989). It has been demonstrated in field trials that



the 'bird-resistant' cultivars were high in tannins compared with low tannin cultivars that were severely damaged. It is thought that the astringency and poor digestibility of these high tannin plants makes them less appealing to the birds.

Slugs are a major pest on a number of economically important crops, attacking both seedlings and mature plants. The major slug pest of potato, *Deroceras reticulatum*, causes extensive damage to the crop (Storey, 1985). It was found that certain potato cultivars were significantly less attractive to the slugs (Atkin, 1979; Storey, 1985). A factor of this resistance, particularly in the tubers, appears to be due to high levels of phenolics and polyphenoloxidase activities (Johnson, Kershaw & Pearce, 1989). Generally, plants expressing high levels of phenolics are much less palatable to herbivores and polyphenolics such as tannins are strongly implicated as general anti-feedants (reviewed in detail by Fahey & Hans-Joachim, 1989; Mole, 1989).

#### 4. Plant phenolics and resistance to fungi

The role of plant phenolics in resistance against fungi is more dynamic than their role against insects. In most cases the interaction between fungi and plants is very intimate and the association occurs over a longer time period. Distinct structures and barriers develop in the host in response to infection. The definitions that have been used to describe the secondary metabolites involved in the interactions are often inaccurate (Ingham, 1973; Stoessl, 1983; Harborne, 1988, 1991). The terms pre-infectious (encountered on the leaf surface and considered to be constitutive, e.g. waxes and phenolics) and post-infectious (encountered in the host cells) chemicals are misleading. It has been shown that most of the pre-infectious secondary metabolites are significantly induced, e.g., phenolics, and the divisions between constitutive and induced defence metabolites are not clear. The examples in this section show some of the roles of plant phenolic barriers, particularly lignin, and resistance to fungi. The role of phytoalexins in resistance will be covered in a later section.

The formation of lignin as a defence mechanism against fungal pathogens has long been recognized (Vance, Kirk & Sherwood, 1980; Aist, 1983). There is a strong correlation between the rapid induction of phenylalanine ammonia-lyase and other phenylpropanoid biosynthetic enzymes in hypersensitive plants (including cinnamate alcohol dehydrogenase and peroxidases leading to a large increase in phenylpropanoids and lignin precursors) and resistance to fungi (Ralton *et al.*, 1988; Chuahe & Zhou Zhongming, 1989; Haberer *et al.*, 1989; Shiraishi, Yamaoka & Kunoh, 1989; Southerton & Deverall, 1990). There is also a strong correlation between resistance and the rapid deposition of other wound plugs derived from phenylpropanoids, such

as papillae, that prevent fungal ingress (Aist, 1976, 1983; Sahashi & Shishiyama, 1986; Aist & Gold, 1987). Other examples of phenolic structural barriers include the cross-linking of cell wall polysaccharides by ferulic acid dimers catalyzed by peroxidases; the cross-linking may make the cell walls resistant to fungal cell wall degrading enzymes (Fry, 1986). Resistance in pea to powdery mildew (*Erysiphe polygoni*) is strongly correlated with the concentrations of total and ortho-dihydroxyphenols in the leaves (Kalia & Sharma, 1988). The resistant cultivars expressed much higher levels of both phenolics and oxidative enzymes (peroxidases and polyphenoloxidases) and this may create a very toxic environment in and around infected tissue, comprising of very reactive phenylpropanoid free-radicals and the actual process of lignification.

In American beech trees (*Fagus grandifolia*) resistant to infection by *Nectaria coccinea* var. *faginata*, the concentration of phenolics in the bark remained high 6 months after attack (Ostrowsky, Shortle & Blanchard, 1984). In the initial phases of infection there was no apparent difference between susceptible and resistant trees, but after 6 months the levels had significantly declined in the susceptible trees. In apple fruits there appeared to be a correlation between high levels of epicatechin and 4'-caffeoylquinic acid and resistance to scab (Amiot, 1990; Macheix, Fleuriet & Billot, 1990).

#### 5. Plant phenolics and resistance to nematodes

The colonization of plants by nematodes is similar in some respects to fungal invasion. As with fungal infection, distinct structures form in the host in response to the various developmental stages of the nematode. The hypersensitive response (HR) and elevated phenolic levels, leading to barrier deposition (e.g. lignin), have been shown to be important in resistance to the root-knot nematode *Meloidogyne incognita* (Paulson & Webster, 1972; Melillo *et al.*, 1989). In susceptible tomato plants there is no HR induced after nematode infection. In the resistant tomato plants the nematode-induced HR, including induction of phenolics, was preceded by an elevation in the activity of carboxylesterases associated with the cell walls; these enzymes have been implicated in priming the plant before oxidation and deposition of phenolics (Keplan & Keen, 1980; Giebel, 1982; Melillo *et al.* 1989). In sweet potato cultivars resistant to root-knot nematode (*Meloidogyne incognita*) there was also a significant increase in the concentrations of soluble and wall-bound phenolics after infection (Gapasin, Valdez & Mendoza, 1987).

#### VII. PLANT TERPENES, SESQUITERPENES AND STEROLS

Terpenes and related plant secondary metabolites

(sesquiterpenoids and sterols) have been shown to be important factors in resistance to several insect pests and pathogens (Harborne, 1988). There are more examples of plant terpenes involved in resistance to insects than against microorganisms. The insecticidal activity of the terpenes is either due to their action as antifeedants (or deterrents), toxins, or as modifiers of insect development, e.g. sterols such as the phytoecdysones (Harborne, 1988). A terpenoid (limonene) deters *Atta cephalotes* (a leaf-cutting ant) from citrus plants (Cherrett, 1972). Other important terpenoid deterrents and toxins include gossypol, polygodial, glaucolide-A and the cucurbitacins (Harborne, 1988). In desert plants a number of terpenoids and sesquiterpenoids have been found to be good insect deterrents (Rodriguez, 1983).

In cotton that was resistant to infection by *Verticillium dahliae* there was a strong induction of four sesquiterpenoid phytoalexins: desoxyhemigossypol, hemigossypol, desoxy-6-methoxygossypol and 6-methoxygossypol (Garas & Weiss, 1986). The concentrations of the two methoxy-phytoalexins, detected 3 d after inoculation, were high enough to inhibit the fungus completely and were positively correlated with resistance to the fungus. In the susceptible cultivars the phytoalexin induction was poor, whilst in tolerant cultivars it was between that of the resistant and susceptible cultivars. Infection of resistant cultivars of tobacco with *Phytophthora nicotianae* var. *nicotianae* caused a rapid induction of sesquiterpenoid phytoalexins (capsidiol, phytotuberin, phytotuberol and rishitin) and phenylalanine ammonia lyase (PAL) activity (Nemestothy & Guest, 1990). The induction of these responses in a susceptible cultivar was much slower, as was lignin deposition. In both of these examples there was a positive correlation between rapid induction of the sesquiterpenoid phytoalexins and resistance to the fungal pathogens.

#### VIII. PHYTOALEXINS

The term phytoalexins was first defined by Muller & Borger (1941) to describe the fungitoxic and fungistatic plant secondary metabolites produced after infection of potato by *Phytophthora infestans*. Since then the definition has been expanded to include the induction of secondary metabolites that inhibit or kill bacteria. The phytoalexins, as with phenolics, are structurally diverse and their role and biosynthesis have been extensively reviewed (Bailey & Mansfield, 1982; Stroessl, 1983; Darvill & Albersheim, 1984; Ersek & Kiraly, 1986; Collinge & Slusarenko, 1987; Harborne, 1988; Barz *et al.*, 1990; Siqueira *et al.*, 1991). An important aspect of determining the role of phytoalexins in resistance is their temporal and spatial distribution after pathogen attack. In most cases the phytoalexins are localized in the tissue beneath, or very close to, the site of fungal

or bacterial infection. The difference between susceptible and resistance plants appears to be related to the rapidity of the host response including phytoalexin induction. The following examples show a strong correlation between rapid phytoalexin biosynthesis and resistance to the pathogen involved. There are several other plant-pathogen interactions where the role of phytoalexins in resistance is not so obvious.

When roots of a resistant cultivar of soybean (*Glycine max*) were infected with the fungus *Phytophthora megasperma* f. sp. *glycinea* the phytoalexin glyceollin I was induced within 2 h (Hahn, Bonhoff & Grisebach, 1985). Eight hours after infection the concentration of glyceollin I had exceeded the  $EC_{90}$  *in vitro* of the fungus. The phytoalexin in the resistant cultivar was associated with the epidermal cells but it was also detected in other parts of the root in inhibitory concentrations. The development of the fungus was stopped soon after penetration of the epidermal layer. In the susceptible cultivars, infected with the fungus, significant levels of glyceollin I were only found in the epidermal cells. In the susceptible cultivar these concentrations were reached 14 h after infection, by which time the fungus had grown through this cell layer and extensively colonized the root cortex. It was also demonstrated that daidzein, the precursor of glyceollin I, was important in resistance to *P. megasperma* f. sp. *glycinea* (Graham, Kim & Graham, 1990). Upon fungal attack the daidzein was enzymatically cleaved from stored glycosides. In the resistant cultivars the release was rapid and high concentrations of daidzein accumulated. In the susceptible cultivars the enzymatic cleavage was slow and final daidzein concentrations were low.

The relationship between phytoalexins and host versus non-host resistance to bacteria has also been assessed in soybean (Fett & Jones, 1984). Non-pathogens such as *Corynebacterium flaccumfaciens* pv. *flaccumfaciens*, *Bacillus cereus*, and *Erwinia carotovora* subsp. *atroseptica* did not induce an HR, or the soybean phytoalexins (including glyceollin I and its precursor daidzein) or isoflavone glycosides. Two non-host *Pseudomonas syringae* strains induced HR and the phytoalexins and glycosides, as did an incompatible (avirulent) *P. syringae* pv. *glycinea*. A compatible (virulent) strain of *P. syringae* pv. *glycinea* did not induce the phytoalexins or the glycosides. There appear to be two resistance mechanisms in operation: one against non-hosts and one against HR-inducing races of *P. syringae* involving the phytoalexins. In these interactions there was a strong correlation between phytoalexin induction and resistance to the bacteria.

Infection of sorghum (*Sorghum bicolor* L.) by *Colletotrichum graminicola* leads to the induction of 3-deoxyanthocyanidin flavanoids (Snyder & Nicholson, 1990). These are highly pigmented

**Table 1.** Phytoalexin induction and resistance to plant pathogens

Plant	Pathogen	Phytoalexin	Reference
Grapevine ( <i>Vitis</i> spp.)	<i>Plasmopara viticola</i>	Viniferins	Derks & Creasy, 1989
Grapevine ( <i>Vitis</i> spp.)	<i>Botrytis cinerea</i>	Viniferins	Langcake & McCarthy, 1979
Carnation ( <i>Dianthus</i> spp.)	<i>Fusarium oxysporum</i>	Dianthalexin	Baayen <i>et al.</i> , 1991
Pea ( <i>Pisum sativum</i> )	<i>Pseudomonas syringae</i>	Methoxydianthramide S	Hadwiger & Webster, 1984
Chickpea ( <i>Cicer arietinum</i> )	<i>Ascochyta rabiei</i>	Pisatin	Weigand <i>et al.</i> , 1986
		Medicarpin	
		Maakiain	
Citrus spp.	<i>Phytophthora citrophthora</i>	Scoparone	Afek & Szetejnberg, 1988
Oat ( <i>Avena sativa</i> )	<i>Puccinia coronata</i>	Avenalumins	Mayama, 1983

phytoalexins clearly visible in the leaf tissue in distinct inclusions. The site of synthesis is restricted to epidermal cells beneath the invading fungus. The phytoalexins in this interaction must have a distinct role; if they were general stress metabolites they would have been induced throughout the whole plant. Other examples of correlations between phytoalexin induction and resistance are presented in Table 1.

The evidence for correlations between phytoalexins and resistance to plant pathogens is strong. There are several clear examples of resistant cultivars accumulating far greater concentrations of phytoalexins compared with susceptible cultivars. Although most of the examples involve pathogens attacking dicotyledons, the relationship between phytoalexin induction in monocotyledons and resistance is also being assessed, particularly in oats and wheat (Deverall, 1989).

## IX. SALICYLIC ACID AND METHYL JASMONATE

### 1. Secondary or primary metabolites?

There has been considerable interest recently in the roles of the secondary metabolites salicylic acid and methyl jasmonate (and jasmonic acids) in plants, not only with respect to their potential roles as plant signals but also their production and biosynthetic regulation. Both salicylic acid and methyl jasmonate modulate and induce many plant genes; they have recently been found to regulate the biosynthesis of other secondary metabolites. There has consequently been some question as to whether they should still be considered as secondary metabolites because of the vital role they play in plants. In the following sections, a brief summary of the biosynthesis and roles of salicylic acid and methyl jasmonate in plant resistance to pathogens will be given; where possible, reviews will be referred to for further information.

### 2. Salicylic acid biosynthesis and its role in plant resistance

Salicylic acid (SA) can be synthesized in plants via two pathways, but in both cases the main precursor is phenylalanine. The first step is the conversion of

phenylalanine to trans-cinnamic acid catalyzed by PAL. This is a key step in several biosynthetic pathways including those for phytoalexins, polyphenols and phenylpropanoid lignin precursors (see Fig. 6). It has been shown that the activity of some PAL isoenzymes may be allosterically controlled by phenolics including SA (Boudet, Ranjeva & Gadal, 1971). The biosynthesis of SA can proceed via the conversion of trans-cinnamic acid to benzoic acid (followed by hydroxylation) or via *o*-coumaric acid (followed by a  $\beta$ -elimination reaction) depending on the species of plant and the tissue involved (El-Basyouni *et al.*, 1964; Billek & Schmook, 1966, 1967; Raskin, 1992).

The role of SA as a systemic signal for the induction of pathogenesis-related proteins (PR-proteins) and resistance to pathogens has been demonstrated by numerous groups (Malamy *et al.*, 1990; Mettraux *et al.*, 1990; Rasmussen, Hamerschmidt & Zook, 1991; Ward *et al.*, 1991; Yalpani *et al.*, 1991; Malamy & Klessig, 1992). It has also been found that SA will selectively induce glucosinolates in oilseed rape (*Brassica napus*) when applied as a spray or as a soil drench treatment (Kiddle *et al.*, 1994); there was a selective induction of phenylethyl glucosinolate (gluconasturtin) 7 d after SA treatment (see Fig. 5). Recently the possible role of SA-glucosides in resistance has been demonstrated (Henning *et al.*, 1993). There has also been a more detailed study on changes in the SA biosynthetic enzymes in healthy and virus-infected plants (Yalpani *et al.*, 1993); induction of benzoic acid 2-hydroxylase has been measured after virus infection (Leon *et al.*, 1993).

Salicylic acid has been shown to be an important signal in plant resistance to pathogens but there are still many unanswered questions regarding its transport and sites of action within the plant. For a recent review of salicylic acid in plants and its role(s), see Pierpoint (1994).

### 3. Methyl jasmonate biosynthesis and its role in plant resistance

Methyl jasmonate (MJ) and jasmonic acids have been found in a large number of plant species



(Anderson, 1989). They are synthesized in plants from the fatty acid linolenic acid. The first step in the pathway is catalysed by a lipoxygenase to form a hydroperoxide (Anderson, 1989). The second enzyme in the pathway has been identified as a cytochrome P450 that converts the hydroperoxide into an allene oxide (Song & Brash, 1991). The allene oxide can then be cyclized to form a precursor common to both the prostaglandins and the jasmonates.

Methyl jasmonate and jasmonic acids are known to regulate a number of physiological processes in plants including the induction of senescence, vegetative storage proteins, proteinase inhibitors (herbivore antifeedants), meristematic growth and interplant signals (Farmer & Ryan, 1990; Koda, 1992). They also have a role in signal transduction especially in relation to defence gene induction, e.g., PAL (Gundlach *et al.*, 1992). When oilseed rape plants were either sprayed or exposed to volatile MJ there was a selective induction of the indole glucosinolates in the leaves (Fig. 5) (Bodnaryk, 1994; Doughty *et al.*, 1994). Lipoxygenases are induced in hypersensitively reacting plants (Croft, Voisey & Slusarenko, 1990) and furthermore a lipoxygenase in *Arabidopsis* has been found to be induced by MJ and bacterial attack (Melan *et al.*, 1993). Some wounding responses in plants also appear to be partly regulated by MJ (Hildmann *et al.*, 1992). All of these responses that are regulated or induced by MJ provide evidence for a key role for this compound in plant development and defence, especially the induction of responses directly involved in resistance, i.e. glucosinolates, PAL and lipoxygenases. How the regulation of pathways is coordinated by SA, MJ and other signals in healthy and infected plants (susceptible or resistant) needs to be determined.

## X. CONCLUSIONS

This review has dealt with a small selection of plant secondary metabolites and their potential roles in defence mechanisms. In addition to the topics we have covered, there is an enormous range of other compounds present in the plant kingdom, with a very varied distribution. Some compounds are species-specific, whilst others have almost universal distribution (and in some cases perhaps should not be considered 'secondary products' at all – such as the salicylates and jasmonates). Secondary metabolites have a great variety of roles, not all of which we yet understand or appreciate. In addition to pest and pathogen defence, these compounds may be involved in storage, protection from UV, protection against osmotic and other environmental stresses, attraction of pollinating organisms, allelopathic interactions with other plants, and probably many others. Any given compound may well have several such roles.

With regard to defence, many studies have concluded that particular secondary products were not effective in protection against particular pests or pathogens. Should we conclude from such studies that these compounds are not defence-related? No, because one must make a distinction between generalist and specialist attacking organisms – where a pest or pathogen has become adapted to feeding on or infecting a particular plant, it must by definition have developed a way to overcome the defence mechanisms present in that plant! In such an interaction, one would not expect to find a simple relationship between secondary metabolite content and degree of predation/infection. That same metabolite may nonetheless be acting very effectively to deter a wide range of other organisms. Other 'defence' aspects of secondary metabolites exist, not necessarily to the plant's advantage – many herbivorous insects make use of plant-derived compounds to protect themselves from predators and parasitoids (see review by Pickett *et al.*, 1991; Rowell-Rahier & Pasteels, 1992).

In addition, a single metabolite or class of metabolites present in a plant will not comprise the only defence system. A wide variety of defence-related compounds may be present – in particular tannins, polyphenols, proteases and chitinases are very widely distributed even in species which contain other major secondary metabolites such as cyanogenic glucosides, glucosinolates, alkaloids, etc. There are also physical defence mechanisms, secondary thickening, thorns and barbs, cuticular waxes, leaf hairs, and other structural factors known to protect plants (see reviews by Royle, 1976; Kollatakudy & Koller, 1983). The interaction between a plant species and its pests and non-pests is complex, and many factors are involved. Only in very few cases do we have a clear understanding of the ecological interactions, even in the relatively simple situation of a monocultured crop plant such as oilseed rape. Such interactions in natural ecosystems, with a wide variety of both plants and potential pests and pathogens, are likely to be much more complicated. Secondary metabolites very often have a role (or roles) in plant/environment interactions, sometimes a major or dominant role, but they are not the only factors involved.

The distribution of a secondary metabolite within a plant, both between tissues and during growth and development, is rarely uniform. Many compounds are synthesized by, and accumulate in, young developing tissues, particularly leaves, or in reproductive tissues such as flowers and seeds. There appear to be many examples of secondary metabolites providing protection for young tissues, becoming less abundant and important as the tissue ages (when other, non-chemical, protective mechanisms may develop). In recent years it has become clear that some secondary metabolite systems are



dynamic, responding to attack, infection or stress, and that enhanced synthesis/accumulation of secondary metabolites is part of an integrated defence mechanisms. Other components of the system include the PR proteins with their various functions (see Bol, Linthorst & Cornelissen, 1990; Bowles, 1990). The same signalling mechanisms trigger the whole range of responses, but we are a long way from understanding these signalling systems and their interconnections. Chemical, electrical, and hydraulic signals have been described in plants, but their relative significance in a given system and their inter-relationships are far from clear. Whatever the signal, some secondary metabolites are under the same defence-related control systems. Few have been investigated in this respect – doubtless many more examples will come to light.

Many plant secondary metabolites play a role in plant defence (in its widest sense). We only partly understand their role(s), and in only a limited range of plants. Considerably more work is required, in all aspects of secondary metabolite biology – physiology, biochemistry, ecology – if we are to appreciate fully their significance in plants and function in ecological systems in general. The prospects for exploiting plant secondary metabolites for agricultural crop protection are enormous (see for example Wink, 1988; Hallahan *et al.*, 1992), but we need to know much more before we can exploit them effectively and safely.

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