Functional diversifications of cyanogenic glucosides
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Cyanogenic glucosides are present in many plants and their ability to liberate toxic HCN offers an immediate chemical defense response to herbivores and pathogens causing damage of the plant tissue. Countermeasures have evolved to overcome this type of defense and in some cases herbivores and pathogens are able to exploit the presence of cyanogenic glucosides to their own advantage. In plants, cyanogenic glucosides have gained additional functionalities as transporters of nitrogen and operation of an endogenous turnover pathway may enable plants to withdraw the nitrogen and glucose deposited in cyanogenic glucosides for use in primary metabolism. The aim of this review is to provide an overview of the new knowledge on these diverse functionalities of cyanogenic glucosides.

Introduction

Plants, herbivores, and pathogens have coevolved in a constant chemical warfare for about 430 million years. Plants are known to produce more than 200,000 different bio-active natural products (also denoted secondary metabolites, specialty compounds) including cyanogenic glucosides [1,2,3]. Cyanogenic glucosides (α-hydroxynitrile glucosides) are derived from the five protein amino acids Val, Ile, Leu, Phe and Tyr and from the nonproteinogenic amino acid cyclopentenyl glycine. Although derived from six different building blocks, they constitute a very small class with around 50 different known structures [2*]. In some plants, β-hydroxyalk(en)yl and γ-hydroxyalk(en)yl glucosides co-occur with cyanogenic glucosides derived from either Leu or Ile. Key enzyme classes involved in the formation of α-hydroxynitrile, β-hydroxynitrile, and γ-hydroxynitrile glucosides include cytochrome P450s and glucosyltransferases [4–11]. Recent reviews have outlined the biosynthetic schemes for several of these compounds and revealed unique features of the cytochrome P450s and glucosyltransferases involved [1,2*,12–14]. The focus of this review is on recently discovered and putative new functionalities of cyanogenic glucosides in plants and in plant, herbivore and pathogen interactions.

Functionalities as phytoanticipins

Cyanogenic glucosides are widely distributed in the plant kingdom and more than 2500 different plant species have been reported to contain cyanogenic glucosides including ferns, gymnosperms and angiosperms [1]. Pteridophytes and Gymnosperms contain cyanogenic glucosides derived from aromatic amino acids whereas angiosperms may contain cyanogenic glucosides derived from either aliphatic or aromatic amino acids [1]. Cyanogenic glucosides are classified as phytoanticipins [15]. Their general function in plants is dependent on activation by β-glucosidases to release toxic volatile HCN as well as a ketones or aldehydes to fend off herbivore and pathogen attack [16]. The β-glucosidase is brought into contact with the cyanogenic glucoside following disruption of the plant tissue, for example, by a chewing insect. This process is denoted cyanogenesis (Figure 1). In textbooks, this binary system is often listed as the classical example of how bio-active natural products provide plants with an immediate chemical defense response to herbivores and pathogens causing tissue damage. This notion has permeated the literature in spite of the inability of several studies to establish such an effect [17]. Gleadow and Woodrow have outlined main reasons for the many exceptions to this rule [18]. Some studies did not recognize: first, the importance of cyanogenic glucoside concentration and its large variability between individual plants in a population, second, the importance of avoidance of cyanogenic glucoside containing plants when alternative feed sources are available, third, importance of feeding style to avoid release of toxic HCN, fourth, the fact that plant material containing cyanogenic glucosides may not be cyanogenic due to lack of β-glucosidase activity required for HCN release [19] and fifth, the fact that specialized herbivores and pathogens have coevolved to circumvent cyanogenesis based plant defense strategies.

It is apparent that the ability of cyanogenic glucosides to release HCN renders consumption of plant derived foods containing high amounts of these compounds and β-glucosidase activity toxic to humans and most animals [20]. Goats have been used as an experimental model to study the general effects of chronic cyanide exposure [21] but the effects of chronic exposure to cyanide is also known from humans exposed to improperly processed...
The golden bamboo lemur (Hapalemur aureus) primarily feeds on an endemic highly cyanogenic giant bamboo (Cephalostachium viguieri). The animal has been estimated to eat about 500 g of bamboo every day — equivalent to 12 times the lethal dose of HCN in most other animals [23]. The mechanism enabling the golden bamboo lemur to tolerate the consumption of these high amounts of cyanogenic glucosides without intoxication remains unknown.

Circumvention of cyanogenesis based plant defenses

The toxicity of HCN to aerobic organisms implies a positive correlation between cyanide potential and plant defense. Thus the presence of cyanogenic glucosides would be expected to combat fungal attack if the incipient attack causes HCN release or if the intact cyanogenic glucoside would inhibit fungal growth. However, cyanogenesis is an estimated 430 million years old plant defense system [1] and fungi have coevolved to counteract and exploit cyanogenesis-based defenses. Fungal pathogens of cyanogenic plants are generally tolerant to HCN [24] as mediated by their ability to convert HCN into formamide in a reaction catalyzed by formamide hydrolyase [25] (Figure 2). Hydrolysis of formamide provides the fungus with ammonia as an excellent source of reduced nitrogen. Within some plant species, highly cyanogenic varieties show increased susceptibility to fungal infection in comparison to varieties with reduced cyanide potential. This applies to the rubber tree (Hevea brasiliensis)—Microcyclus ulei interactions [26]. H. brasiliensis produces the aliphatic cyanogenic glucoside linamarin. M. ulei is the causative agent of rubber tree leaf blight. M. ulei is highly tolerant to HCN released during disease development whereas the host plant is not. In the highly cyanogenic varieties, the accumulated levels of free HCN in the leaf following infection impair plant defense by inhibiting the formation of the phytoalexin scopoletin and most likely also by inhibiting the activity of peroxidases and polyphenoloxidases [26]. A recent study in lima bean (Phaseolus lunatus) [27] provides further results documenting that plant defenses do not always act synergistically but may be trade-offs against each other due to negative interactions at the biochemical level. The high cyanogenic glucoside content in the lima bean was found to provide effective protection toward herbivores. In contrast, the fungal pathogen Colletotrichum gloeosporioides profited from the presence of the cyanogenic glucosides because of its ability to effectively detoxify HCN [28] and because the concentration of gaseous HCN liberated from the damaged plant cells was high enough to inhibit the plant polyphenoloxidase based defense system of the plant. Gaseous HCN diffuses through a leaf within 30 s [26]. Barley (Hordeum vulgare) contains five hydroxynitrilealk(en)yl glucosides epidermin, sutherlandin, osmaronin and dihydroosmaronin, of which only epidermin is an α-hydroxynitrile glucoside [19] (Figure 3). The hydroxynitrilealk(en)yl glucosides are restricted to the leaf tissues and 99% are confined to the epidermal cell layers of the leaf blade [19]. The hydroxynitrilealk(en)yl glucosides account for 90% of the soluble carbohydrates within the epidermal cells with the remaining 10% occurring as glucose, fructose and sucrose [29]. All five hydroxynitrilealk(en)yl glucosides are derived from leucine [19,30]. It has been proposed that the aglycons of all five hydroxynitrilealk(en)yl glucosides are formed by the initial action of a single CYP79 enzyme, whereas the subsequent conversion of the oxime into the corresponding nitrile and subsequent hydrations at the α-carbon, β-carbon or γ-carbon atoms and dehydrations resulting in the formation of hydroxynitrilealkenyl glucosides may be catalyzed by a single or
multiple CYP71 or CYP83 class enzymes and by different glucosyltransferases [2,19,29,30]. The barley powdery mildew (Blumeria graminis f.sp. hordei) is specialized to infect only the epidermal cell layer of the barley leaf [19,31,32], that is, the cell type where the hydroxynitrilealk(en)yln glucosides are stored. No clear relationship between susceptibility to barley powdery mildew and cyanide potential of the leaf tissue has been established [19,29]. As an obligate biotroph, the powdery mildew fungus nourishes from the living host cells suggesting that the fungus is able to utilize the hydroxynitrilealk(en)yln glucosides as a source of glucose and nitrogen. No free HCN was liberated during infection and barley leaves do not contain a β-glucosidase able to cleave epiheterodendrin. Cleavage of the other hydroxynitrilealk(en)yln glucosides most likely requires the action of other β-glucosidases although the identity of these remain unknown. When cyanogenesis was reconstituted in the barley leaf by single cell expression of dhurrinase-2 from sorghum, powdery mildew colonization was severely reduced. In contrast, epiheterodendrin was shown to stimulate appressoria and appresorial hook formation in vitro. In combination, these studies indicate that the barley powdery mildew may use the presence of epiheterodendrin as a controlling element in host recognition and that the fungus is able to utilize epiheterodendrin as well as the non-cyanogenic nitrilealk(en)yln glucosides as nutrients according to a metabolic scheme not involving release of HCN [32] (Figure 2).

Bacteria also have various options to cope with the presence of cyanogenic glucosides and their toxic degradation products [33]. β-Cyanoalanine synthase catalyzes the reaction between free HCN and cysteine to form β-cyanoalanine (Figure 2). Pseudomonas fluorescens is a plant growth promoting bacterium that expresses a NIT4-type nitrilase (pinA) when exposed to β-cyanoalanine. This enzyme catalyzes the conversion of the toxic β-cyanoalanine into aspartic acid and thereby enables P. fluorescens to use β-cyanoalanine as a nitrogen source [33]. Expression of Pf-pinA in wild-type Arabidopsis thaliana plants resulted in their increased growth in the presence

Figure 2

Cyanide detoxification pathways catalyzed by (panel A) formamide hydrolyase and (panel B) β-cyanoalanine synthase and a NIT4-type nitrilase.
of β-cyanoalanine and increased root elongation. Cyanide metabolism and nitrilase activity thus affects root physiology and root development and may also be an important factor in removing toxic metabolites and thereby facilitate bacterial colonization of plant roots.

Legumes are able to carry out symbiotic nitrogen fixation. In pea (Pisum sativum), proper development of the bacteroids has been reported to depend on the supply of Val, Leu and Ile from the plant [34]. This conclusion was based on studies of mutated amino acid transporters thought to be localized in the peribacteroid membrane and an observed transcriptional downregulation of the biosynthesis of these three branched amino acids in the bacteroids. It was suggested that this enables plants to control nodule development and persistence through controlled supply or deprivation of branched amino acids. Some legumes are able to synthesize Val and Ile derived cyanogenic glucosides [35,36]. Synthesis of cyanogenic glucosides might serve as yet another controlling element in the establishment and maintenance of the legume–rhizobium interaction required for symbiotic nitrogen fixation. The site of cyanogenic glucoside synthesis could, for example, be the meristematic cells, initially giving rise to the formation of the nodule. In the studies with the rhizobium transporter mutants, cyanogenic glucoside synthesis might be abolished due to an altered flux and availability of Val and Ile into the bacteroids. The content of cyanogenic glucosides in legume roots is generally very low which might indicate synthesis in few specialized cells or specific induction [36]. In Lotus japonicus, two differentially expressed CYP79D paralogs, CYP79D3 and CYP79D4, have been found, of which CYP79D4 is specifically expressed in roots and wound inducible whereas CYP79D3 is expressed in the aerial parts. This suggested the operation of two independently regulated pathways for cyanogenic glucoside synthesis in the aerial parts of the plant and in roots [36].

**Endogenous turnover of cyanogenic glucosides**

An endogenous turnover pathway for cyanogenic glucosides not involving the formation of free cyanide has recently been proposed based on studies in species of Poaceae (grasses) [37**] (Figure 4). This proposed pathway neither involves formation of a cyanohydrin nor of free HCN as intermediates. The experimental focus of these studies was on sorghum containing the tyrosine derived cyanogenic glucoside, dhurrin [37**]. Dhurrin constitutes up to 6% of the dry weight of young sorghum seedlings. The high amount of dhurrin is synthesized upon germination and peaks after four days, after which catabolism prevails [38,39]. The glucoside of 4-hydroxyphenylacetonitrile was found to accumulate coconitantly with dhurrin degradation [37**]. 4-Hydroxyphenylacetonitrile may subsequently be converted into ammonia and 4-hydroxyphenylacetic acid in a reaction catalyzed by a heteromeric nitrilase complex \( \delta/\delta \)NIT4A/B2 [37**]. Sorghum bicolor contains three NIT4 isoforms: \( \delta/\delta \)NIT4A, \( \delta/\delta \)NIT4B1, and \( \delta/\delta \)NIT4B2. Individually, each isoform does not possess catalytic activity, whereas the heteromeric complexes \( \delta/\delta \)NIT4A/B1 and \( \delta/\delta \)NIT4A/B2 hydrolyze β-cyanoalanine with high activity. The ability to efficiently metabolize 4-hydroxyphenylacetonitrile is a specific property of the \( \delta/\delta \)NIT4A/B2 complex. The substrate specificity of the small nitrilase family is thus defined and extended by combinatorial biochemistry. The identity of the catalytic active subunit within the heteromeric complexes shifts in dependence of the substrate being metabolized. This was determined by site-specific mutagenesis of the essential catalytic cysteine residue within each of the different nitrilase subunits and investigation of the substrate specificity of the mutated heteromeric complexes [37**]. The enzyme system putatively involved in the conversion of dhurrin into β-hydroxyphenylacetonitrile currently remains elusive. The proposed key role of the \( \delta/\delta \)NIT4A/B2 heterocomplex in endogenous turnover of the cyanogenic glucoside dhurrin proceeding via 4-hydroxyphenylacetonitrile, would be parallel to the envisioned role of NIT1 homologs in glucosinolate metabolism. Different NIT1 homologs have evolved high specificity toward nitriles produced from the glucosinolates present in their host plants and are envisioned to function in glucosinolate metabolism [40]. Biosynthesis of cyanogenic glucosides has been documented to be regulated at the transcriptional level in sorghum [39]. It is not known how endogenous turnover of cyanogenic glucosides is regulated but it may involve interacting proteins like BGAF (β-glucosidase aggregating factor) [41] and share reminiscence to the glucosinolate/myrosinase/epithiospecifier system in the Brassicaceae [42] although the main role of these systems is currently thought to be related to the optimization of defense reactions.

The unusual nitrilase complement found within the Poaceae species and consisting of one or more NIT4A and NIT4B homologs reflects an early gene duplication. It remains to be investigated whether non-Poaceae species like lotus, flax, and cassava, which also may produce high amounts of cyanogenic glucosides, harbor a similar endogenous turnover pathway catalyzed by a nitrilase-based activity. Through further neofunctionalization, nitrilases may also be involved in the endogenous turnover of the different non-cyanogenic β-hydroxynitrile and γ-hydroxynitrile glucosides, for example, occurring in L. japonicus [36]. The ancestral nitrilase present in all plant species belongs to the NIT4 family and the primordial physiological function of this enzyme was to detoxify HCN. The first step in this process is catalyzed by β-cyanoalanine synthase which, in the presence of HCN, converts cysteine into β-cyanoalanine. NIT4 then converts β-cyanoalanine into a mixture of asparagin, aspartic acid and ammonia (Figure 2).
Several plant species containing cyanogenic glucosides also contain primary amide glucosides with structures corresponding to the cyanogenic glucosides present. Chemically, this conversion may be achievable in the presence of hydrogen peroxide (Radziszewski process [43]) (Figure 4). Analysis of chlorotic leaves of, for example, *Olinia ventosa* producing large amounts of reactive oxygen species showed conversion of the cyanogenic glucoside prunasin into the corresponding amide (prunasinamide) and also subsequent cleavage of the amide to form the free carboxylic acid [44]. Fresh green leaves subjected to strong light irradiation also showed amide accumulation. These results parallel studies in the rubber tree and sorghum. In the rubber tree, it was shown that the content of the cyanogenic glucoside lotaustralin in leaves exhibited a diurnal pattern. The levels were highest at dawn but decreased rapidly upon exposure to sunlight and were re-established after sunset and during the night [45]. This was speculated to reflect the combined action of reduced biosynthetic activity and unaltered translocation from the leaves to other parts of *H. brasiliensis*. In sorghum seedlings, synthesis and accumulation have been reported to proceed at a higher rate in the dark [46]. As exposure to high doses of light may be associated with the production of higher amounts of reactive oxygen species (ROS), the reduction of cyanogenic glucoside content in the light could also reflect a function of cyanogenic glucosides as scavengers of, for example, hydrogen peroxide, resulting in their conversion into amides by the Radziszewski process. This property may help the plant tissue to reduce damage caused by excessive light. In addition it may also serve to quench the formation of hydrogen peroxide produced as a result of pathogen or insect attack. This may be an advantage for the plant if the quenching serves to protect noninfected plant cells from damage but would be a disadvantage to plant defense if the ROS species produced to block further attack were neutralized by the presence of cyanogenic glucosides (Figure 4).

**Transporters of nitrogen**

Cyanogenic glucosides may serve an important function in primary metabolism as transporters of nitrogen and glucose. This was first demonstrated in studies with *H. brasiliensis* [35]. In the seed, 90% of the cyanogenic glucoside content is stored in the endosperm as the monoglucoside linamarin. Upon germination, linamarin is converted into the diglucoside linustatin which is then transported over long distances to the seedling. In the seedling, an α-diglucosidase has been proposed to convert linustatin into gentiobiose and acetone cyanohydrin. The latter compound is labile and HCN released is assimilated into asparagine and aspartic acid in a reaction catalyzed by β-cyanoalanine synthase [35] (Figure 2). A high content of cyanogenic glucoside in the seed may thus serve to provide aspartate or asparagine for transamination reactions required to balance amino acid supply to the
developing seedling. A similar function of cyanogenic glucosides to buffer nitrogen and glucose supply in *H. brasiliensis* during latex formation has recently been reported [45]. A positive correlation between cyanogenic glucoside content in leaves and latex yield was observed. Likewise in virgin *H. brasiliensis* trees, the cyanogenic glucoside content in the inner trunk bark and leaves was proportional and decreased on tapping [45]. This suggests that cyanogenic glucosides serve to supply renewable nitrogen and carbon for latex regeneration and thus for rubber production. A similar role of cyanogenic glucosides has been demonstrated in almonds [47,48].

In the developing fruit, the tegument was identified as the specific site of *de novo* synthesis of the cyanogenic monoglucoside prunasin. In the bitter genotypes studied, prunasin synthesized in the tegument was transported into the developing cotyledons and converted into the diglucoside amygdalin. In the sweet genotypes, this transfer was prevented due to the presence of a cell layer rich in cytoplasmic and vacuolar localized β-glucosidases at the inner epidermis of the tegument facing the nucleus. The resulting hydrolysis of prunasin and subsequent detoxification of the HCN released by β-cyanoalanine synthase offer an additional supply of ammonia, Asp and Asn. The sweet almond varieties may therefore profit by having a more balanced and alternative direct supply of reduced nitrogen and free amino acids for protein synthesis in the developing cotyledons. The bitter varieties accumulating amygdalin in the cotyledons may profit from the protection offered by this bio-active natural product toward herbivores and pathogens. In the bitter genotype, this opportunity of using the bio-active natural product as a buffer for primary metabolism has been retained, and is exploited when the amygdalin-containing seed germinates and the amygdalin stored in the cotyledons is turned over quickly. The UDP-glucosyltransferase UGT85A19 that stereo-selectively catalyzes the conversion of *R*-mandelonitrile into prunasin shows higher activity in bitter varieties compared to sweet varieties and has also been associated with bitterness in almonds [49]. Accordingly, cyanogenic glucoside production and accumulation in almonds constitute yet another example where formation of a secondary plant product serves to balance processes in primary metabolism by providing a metabolic buffer capacity.

**Coevolution**

Cyanogenic glucosides have been reported as deterrents of generalist insects. However, some specialized insects preferentially feed on cyanogenic plants [1]. Such insects have acquired the ability to metabolize cyanogenic glucosides or to sequester them for use in their own defense against predators. A few species of arthropods are able to sequester cyanogenic glucosides from their feed plant and to supplement the supply by *de novo* biosynthesis of the very same cyanogenic glucosides [50,51,52]. This applies to larvae of *Zygaena* (*Zygaenidae*). *Lotus corniculatus* contains the two aliphatic cyanogenic glucosides, linamarin and lotaustralin, although this is a polymorphic trait and some lines are completely acyanogenic. *Zygaena filipendulae* larvae reared on acyanogenic plants showed decelerated development [50] and the larvae strive to maintain a certain threshold content and specific ratios between the two cyanogenic glucosides, regardless of the content in their food plants [50]. Detailed analyses showed that the cyanogenic glucosides play several important roles in addition to defense in the life cycle of *Zygaena* [51]. When ready for mating, the perching females emit plumes of HCN that may serve to attract flying males.

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*Figure 4*

Proposed pathways for (panel A) *in vivo* turnover of dhurrin in *Sorghum bicolor* avoiding concomitant release of hydrogen cyanide and with the NIT4B2/A heterocomplex as a key enzyme and for (panel B) the ability of dhurrin to quench the formation of hydrogen peroxide by being converted into primary amides in a Radziszewski reaction.
The females prefer males carrying a high content of cyanogenic glucosides and during mating, the male transfers a nuptial gift containing cyanogenic glucosides [51]. In other insect species, males are also known to transfer seminal gifts containing bio-active natural products during mating [53]. It remains to be investigated whether the cyanogenic glucoside content of the nuptial gift donated by the Z. filipendulae male is used to imbue her eggs with cyanogenic glucosides to make them less attractive to predators or serve to reduce the female’s fitness to accomplish re-mating with the overall goal of the male to increase paternity.

Zygaenae larvae may also be reared on L. japonicus plants but exhibit reduced growth [51]. L. japonicus contains linamarin and lotaustralin although the linamarin content is strongly reduced in comparison to L. corniculatus. In addition, L. japonicus contains the two isoleucine-derived hydroxynitrilealk(en)yl glucosides rhodiocyanoside A and D [30]. It is not known whether the presence of rhodiocyanosides or the low linamarin content reduces larval growth. The rhodiocyanosides are β-hydroxynitrile and γ-hydroxynitrile alk(en)yl glucosides and do not liberate HCN upon cleavage [2]. Like lotaustralin, they are made from Ile [30]. Several plant species producing either isoleucine-derived or leucine-derived cyanogenic glucosides contain a suite of structurally related β-hydroxynitrile and γ-hydroxynitrile alk(en)yl glucosides representing one or more hydroxylations of all possible carbon atoms of the parent amino acid and subsequent dehydrations [2] (Figure 3). Because of their high frequency of co-occurrence and striking structural similarities, it has been proposed that the compounds are related biosynthetically. All characterized members of the CYP79 family convert amino acids to the corresponding Z-oximes [2]. This functionality is thus conserved across the biosynthetic pathways for cyanogenic glucosides and glucosinolates [3]. In contrast, the CYP71 family is functionally diverse and enzymes within this family play key roles in emergence and evolution of several different classes of natural products. It is difficult to imagine that the pathway for synthesis of β-hydroxynitrile and γ-hydroxynitrile glucosides should not have evolved at least in part from CYP71 paralogs providing nitrile substrates for subsequent hydroxylation and dehydrating enzyme(s). However, single CYP71 enzymes able to catalyze hydroxylations at a multiplicity of carbon atoms may also be envisioned [2]. A large structural variation is also observed within the cyclopentenylglycine-derived structures of hydroxynitrile glucosides and appears to be the result of diversification at several levels of the biosynthetic pathway [54]. One type of variation is caused by secondary modifications of the core structures by sulfurylation. D-Allose has also been found as the sugar component of natural cyanogenic glycosides [55].

The formation of a complex suite of hydroxynitrilealk(en)yl glucosides within the same plant species may be the result of the coevolutionary arms race between plants, herbivores and pathogens and provide plants with defense against specialist herbivores and pathogens that have evolved to circumvent a primordial defense mechanism based on HCN release. The synthesis and possible biological functions of hydroxynitrilealk(en)yl glucosides as deterrents of generalist herbivores have recently been reviewed [2].

Most cyanogenic glucosides and the β-hydroxynitrilealk(en)yl and γ-hydroxynitrilealk(en)yl glucosides are monoglycosides but diglycosides are found both as transport and storage forms [35,48]. The Phe derived diglucoside amygdalin accumulates in high amounts in bitter almonds with gentobiose as the attached disaccharide [48]. In other plant species, the second glucose residue is attached at different positions [56,57]. This changes the sensitivity of the compounds to hydrolysis by β-glucosidases [57]. Secondary modifications like malonylation and acylation may guide further differentiation in sensitivity to cleavage by β-glucosidases, transport and cell specific expression and enable a suite of yet undiscovered functionalities. Detailed engineering studies to analyze the effect of individual α-hydroxynitrile, β-hydroxynitrile or γ-hydroxynitrile glucosides in vivo have to await identification of the genes involved in their synthesis.

**Model versus crop plants**

Cyanogenic glucosides are present in a large number of important crop plants and trees [58]. Biochemical and molecular knowledge of their formation and turnover and of their role in plant herbivore and pathogen interactions offers opportunities for targeted crop amelioration as documented in cassava by depleting the content of cyanogenic glucosides to limit the risk of cyanide intoxication [52] and by the approaches to control bitterness in almonds to increase the yield of sweet almonds for use in foods [47,48,59]. In rosaceous and euphorbiaceous plants, cyanogenic glucosides appear to serve an important function as long distance transporters of reduced nitrogen and glucose at specific developmental stages and may reduce the demand for nitrogen supply at specific growth stages [5,22,35,48]. Among the genus Eucalyptus about 30 of more than 600 species are cyanogenic [60]. In parallel with white clover [61,62], Eucalypts are now being developed as an excellent experimental system to study cyanogenesis as a polymorphic trait. Eucalypts offer additional opportunities to study variability in cyanide potential at the population level and ontogenetic regulation [60,63]. L. japonicus is being introduced as a genetically well defined model plant to dissect the effect of the formation and accumulation of specific cyanogenic glucosides or β-hydroxynitrilealk(en)yl and γ-hydroxynitrilealk(en)yl glucosides at the molecular level [36,62,64]. The successful transfer of the entire pathway for dhurrin synthesis to the genetic model plant Arabidopsis thaliana and the resulting effect
on insect resistance serve to document the usefulness of such approaches [65].

Conclusion
Cyanogenic glucosides have been shown to carry out a number of different functions in plants in addition to their well known ability to give rise to release of toxic HCN as an immediate chemical defence response to herbivores and pathogens causing tissue damage. Application of new technologies like laser capture dissection microscopy enabling analysis of the metabolite, proteome and transcript profile of individual cells offers the possibility to further dissect the classical plant defense process of cyanogenesis and to elucidate the putative pathway resulting in endogenous turnover of cyanogenic glucosides without release of HCN. Elucidation of the biological significance of structural diversification of cyanogenic glucosides into β-hydroxynitrilealk(en)yl and γ-hydroxynitrilealk(en)yl glucosides will add a new dimension to our understanding of plant herbivore and pathogen interactions. The use of L. japonicus as a well defined genetic model system offers good possibilities to elucidate many of these biological issues because this plant contains cyanogenic glucosides as well as β-hydroxynitrilealk(en)yl and γ-hydroxynitrilealk(en)yl glucosides and is the host of interesting herbivores and pathogens. Except for a very few plant species, the occurrence of cyanogenic glucosides and glucosinolates appears mutually exclusive. The mechanistic reason for the bifurcation of the two pathways needs to be resolved. A clue to this may be to study the evolution of the CYP79 and CYP71/CYP83 enzymes from pteridophytes to core eudicots. There are lots of important biological issues to address!

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References and recommended reading
Papers of particular interest, published within the period of review, have been highlighted as:

- of special interest
- • of outstanding interest


Typically, plants are simultaneously attacked by multiple herbivores and pathogens. The authors document that some defenses may trade-off against each other due to direct negative biochemical interactions.


The authors report on the specific catalytic functions of different nitratase 4 homomers and proposes an endogenous turn-over pathway for cyanogenic glucosides not involving release of HCN.


45. The authors demonstrate that cyanogenic glucosides may be able to quench reactive oxygen species and in the process be converted into amides.


The authors provide good evidence that cyanogenic glucosides in the rubber tree constitute a source of carbon and nitrogen that may be recruited to increase the biosynthetic machinery required for rubber production.


The authors report a versatile histochemical method that can be used for localization of any β-glycosidase that upon incubation with its specific substrate releases a reducing sugar.


The authors report that cyanogenic glucosides may be part of nuptial gifts in insects and play roles in addition to those related to defense.


60. Neilson EH, Goodger JQD, Woodrow IE: Novel aspects of cyanogenesis in Eucalyptus camphora subsp humeana. Funct Plant Biol 2006, 33:487-496. The authors further develop Eucalypts as an excellent model system for ontogenetic studies of cyanogenesis and demonstrate the presence of five different cyanogenic glucosides in leaves.


