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*Mini-Review*

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## CONSTRAINTS ON EFFECTIVENESS OF CYANOGENIC GLYCOSIDES IN HERBIVORE DEFENSE

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**Abstract**—Cyanogenesis is the process by which hydrogen cyanide is released from endogenous cyanide containing compounds. Many cyanogenic plants release HCN in sufficient quantities to be toxic and, as a result, tend to be avoided by herbivores. However, there are many exceptions with some herbivores either immune to the cyanogenic status of the plant, or in some cases attracted to cyanogenic plants. This has led to a certain degree of scepticism regarding the role of cyanogenic glycosides as defense compounds. In this review, we examine evidence showing that differences in the effectiveness of cyanogenic glycosides in deterring herbivory can usually be reconciled when the morphology, physiology, and behavior of the animals, together with the concentration of cyanogenic glycosides in the host plant, are taken into account. Cyanogenic glycosides are not effective against all herbivores, and not all cyanogenic plants release enough cyanide to be considered toxic. Nevertheless, they do form part of the broad spectrum of toxic and distasteful compounds that herbivores must accommodate if they are to feed on cyanogenic plants.

**Key Words**—Cyanide, herbivory, defense, toxic compounds, diet selection, plant secondary metabolites, cyanogenesis.

### INTRODUCTION

Over 2500 plant species have the capacity to release hydrogen cyanide from endogenous cyanide-containing compounds (Conn, 1981; Jones, 1988; Seigler, 1998). Many of these cyanogenic plants are capable of releasing HCN in sufficient

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quantities to be toxic and, as a result, are often avoided by herbivores (Nahrstedt, 1985; Jones, 1998). Nevertheless, there has been a certain amount of controversy over the effectiveness of cyanogenesis as a herbivore defense system (e.g., Hruska, 1988), which has become all the more important to resolve with the recent "engineering" of cyanogenesis into the otherwise acyanogenic *Arabidopsis thaliana* (Tattersall et al., 2001). In this review, we examine studies of cyanogenesis in relation to herbivory and argue that conflicting data can generally be reconciled when the morphology, physiology, and behavior of the herbivores, together with the concentration of cyanogenic glycosides in the host plant, are taken into account.

### CYANOGENESIS

The most common cyanogens found in plants are glycosides of  $\alpha$ -hydroxynitriles (i.e., cyanohydrins) (Figure 1) (Møller and Poulton, 1993; Lechtenberg and Nahrstedt, 1999; Møller and Seigler, 1999). In cyanogenesis, the sugar moiety is cleaved from the cyanogenic glycoside in a process catalyzed by one or more  $\beta$ -glucosidases (Figure 2). The resulting cyanohydrin is relatively unstable and degrades, either spontaneously or enzymatically, to produce HCN and an aldehyde or a ketone (Conn, 1981). Autotoxicity is prevented by spatial separation—either at the subcellular or tissue level—of the degradative enzymes and the cyanogenic glycoside (e.g., Saunders et al., 1977; Frehner and Conn, 1987; Poulton, 1988; Swain et al., 1992). Tissue disruption, as might happen when tissue is chewed by a herbivore, is necessary for HCN to be released (Nahrstedt, 1985).

Cyanogenic compounds also function in the metabolism and transport of nitrogen (Lieberei et al., 1985; Selmar et al., 1988; Selmar, 1993; Gleadow et al., 1998) and the control of germination (e.g., Esashi et al., 1996). These roles do not, however, need to be seen as an alternative to their role in herbivore defense. Just as some plants store nitrogen as inactive Rubisco (Woodrow, 1994), cyanogenic plants may store nitrogen in a toxic form for the dual purpose of defense.

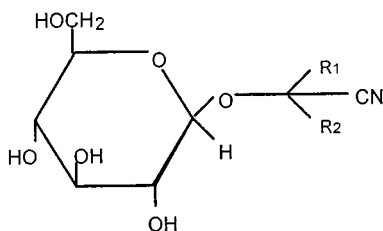


FIG. 1. General structure of cyanogenic glycosides. The aglycone is typically derived from one of the hydrophobic protein amino acids: tyrosine, phenylalanine, valine, leucine, or isoleucine. The glycoside is usually a monoglycoside, although there are a number of diglycosides and one triglycoside (Lechtenberg and Nahrstedt, 1999).

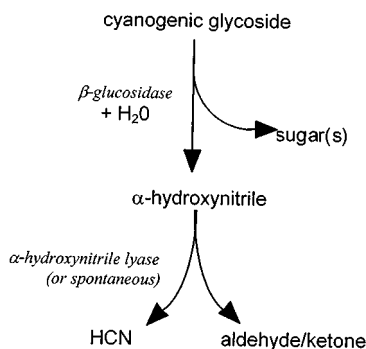


FIG. 2. Degradation of cyanogenic glycosides is a two-step process. First, the molecule is cleaved, resulting in the production of a sugar and a cyanohydrin. The cyanohydrin is unstable and is further degraded, either spontaneously or in the presence of an  $\alpha$ -hydroxynitrile lyase, to produce hydrogen cyanide and a carbonyl group (Poulton, 1988).

#### ASSOCIATION BETWEEN CYANOGENIC PLANTS AND POISONING

The association between certain plants and acute poisoning in humans and domestic animals is well known. There are many well-documented examples of deaths and serious illness from consumption of cyanogenic plants in humans (e.g., Cock, 1982; Cardoso et al., 1998; Banea-Mayambu et al., 2000), cattle (e.g., Robinson, 1930; Finnemore et al., 1935; Boyd et al., 1938; Hopkins, 1995; Zentek, 1997), sheep (e.g., Robinson, 1930; Cooper-Driver et al., 1977; Crush and Caradus, 1995), koalas (e.g., Pratt, 1937), goats (e.g., Webber et al., 1985), and other grazing mammals (e.g., Harborne, 1982; Saucy et al., 1999). Moreover, grazing mammals do not usually eat large quantities of cyanogenic food when alternatives are available (e.g., Jones, 1962; Cooper-Driver et al., 1977; Jones, 1988; Crush and Caradus, 1995; Caradus and Forde, 1996). Many insects and mollusks also tend to avoid cyanogenic plants (e.g., Cooper-Driver and Swain, 1976; Keymer and Ellis, 1978; Dritschilo et al., 1979; Dirzo and Harper, 1982; Foulds, 1982; Schreiner et al., 1984; Nahrstedt, 1985, 1988; Jones, 1988; Kakes, 1989; Patton et al., 1997; Schappert and Shore, 1999b,c; Gleadow and Woodrow, 2000a,b; Magalhaes et al., 2000).

Many of the examples involve studies that are not necessarily conclusive (see, for example, the discussion of Dritschilo et al., 1979, in Hruska, 1988). Nevertheless, there are many cases where it is quite reasonable to attribute feeding patterns to the cyanogenic glycoside content of the plant. Patton et al. (1997), for example, fed leaves from a range of species of *Prunus* to Japanese beetles (*Popillia japonica*) in a fully replicated, no-choice experiment. They found a strong negative correlation between the concentration of the cyanogenic glycoside prunasin in the

leaves and the feeding intensity of the beetles. Moreover, in a parallel study, they incorporated the cyanogenic diglycoside, amygdalin, into artificial diets and found a similar relationship.

Despite evidence documenting the deterrent effect of cyanogenic glycosides, there are a number of studies in which glycosides are shown to have little or no effect on herbivores (e.g., Scriber, 1978; Ferreira et al., 1997; Glander et al., 1989). Indeed, in some cases, plant cyanide production actually acts as a phagostimulant rather than an inhibitor (e.g., Calatayud et al., 1994; Calatayud and Le Rü, 1996; Mowat and Clawson, 1996).

Nearly all of the variability in the effectiveness of cyanogenic glycosides in defense can be explained by four confounding factors. First, the concentration of the cyanogenic glycosides may be below the threshold toxicity. Second, the animal feeding on the species under examination may be a specialist that has evolved mechanisms to cope with high levels of HCN in the diet. Third, the cyanogenic plant might normally be consumed as part of a mixed diet and, therefore, might not be toxic. Fourth, the mode of feeding may be such that the animal does minimal damage to the leaf, thereby limiting the mixing of the cyanogenic glycoside with the degradative  $\beta$ -glucosidases.

#### CONCENTRATION EFFECTS

Many studies of the effectiveness of cyanogenic compounds in herbivore defense only tested for the presence or absence of cyanogenic glycosides. Natural populations of plants, however, vary widely in cyanogenic glycoside concentration (e.g., Nass, 1972; Hughes, 1991; Thomsen and Brimer, 1997; Schappert and Shore, 1999a,b; Buhrmester et al., 2000; Gleadow and Woodrow, 2000b, Goodger et al., 2002), which in turn, impacts on the degree of herbivory (Feeny, 1976). Gleadow and Woodrow (2000a), for example, detected a significant inverse correlation between the concentration of cyanogenic glycosides in the young leaves of *Eucalyptus cladocalyx* and the amount of damage by herbivores. Similarly, Schappert and Shore (1999b) found that the concentration of cyanogenic glycosides was important not only in the selection of *Turnera ulmifolia* as a source of food, but also as an oviposition site for *Euptoieta hegesia*.

Cyanogenic glycoside concentration also varies with leaf age and environmental conditions (e.g., Patel and Wright, 1958; Bernays et al., 1977; Caradus et al., 1990; Gleadow et al., 1998; Gleadow and Woodrow, 2000a). A particular plant may be innocuous under one set of conditions, but lethal under another (see Bernays et al., 1977). Studies that show little or no influence of cyanogenic glycosides on feeding preferences could be testing plants that are essentially nontoxic to the herbivore. This has been particularly well documented in *Sorghum*, which

is highly toxic to grazing stock when young, but becomes suitable for pasture as plants mature (Martin et al., 1938; McBee and Miller, 1980; Haskins et al., 1987).

#### SPECIALISTS VERSUS GENERALISTS

Cyanogenesis appears to be an effective inhibitor of feeding by infrequent or opportunistic herbivores rather than by specialists (Compton and Jones, 1985; Nahrstedt, 1985; Hughes, 1992; Schappert and Shore, 1999c, 2000). For example, the feeding and reproductive behavior of the specialist clover weevil (*Sitona lepidus*) is the same on both cyanogenic and acyanogenic *Trifolium repens* (Mowat and Clawson, 1996). *Phenacoccue manihot*, which feeds on *Manihot esculenta*, actually uses the presence of linamarin as a phagostimulant (Calatayud and Le Rü, 1996). By contrast, the generalist-feeding insects *Schistocerca gregaria* and *Sodpτερα littoralis* avoid feeding on cyanogenic plants (Cooper-Driver and Swain, 1976; Schwarz et al., 1996).

Schappert and Shore (1999c) studied the abundance and diversity of herbivores on populations of *Turnera ulmifolia* in detail on Jamaica. They found that the number of herbivore taxa in any particular population was inversely proportional to the cyanogenic glycoside content of the leaves. However, the amount of herbivory was similar in all populations (1–9% leaf area lost), and most of the damage to cyanogenic plants came from a relatively small suite of insects. Thus, in *T. ulmifolia*, cyanogenesis appears to deter generalist herbivores, but not specialists (Schappert and Shore, 1999c).

Specialist herbivores appear to have evolved mechanisms to tolerate cyanogenic glycosides (e.g., Feeny, 1976; Provenza et al., 1992). Some even use them as a source of nitrogen, as do some fungi (e.g., Birk et al., 1996; Osbourne, 1996). Larvae of the neotropical butterfly, *Heliconius sara*, do this by sequestering the cyanogens from the leaves of the host plant (*Passiflora auriculata*), preventing the release of cyanide. The cyanogen is then metabolized within the insect, and the nitrogen is recovered and used in protein synthesis (Engler et al., 2000). Other specialist herbivores retain the sequestered cyanogenic glycosides and employ them in defense against predators (Nahrstedt, 1985, 1988; Hughes, 1991; Engler et al., 2000). For example, larvae of *Eupitoiea hegesia* take up cyanogenic glycosides from their host plant (*Turnera ulmifolia*) and become distasteful to common predators such as *Anolis* lizards (Schappert and Shore, 1999b). A more complex story is seen in *Lotus corniculatus* (Nahrstedt, 1988). While some snails (e.g., *Helix aspersa*) are deterred from feeding on cyanogenic *L. corniculatus*, larvae of *Zygaena trifolii* are not. These larvae both synthesize and sequester cyanogenic glycosides from *L. corniculatus*, increasing their resistance to predators, similar to *Eupitoiea hegesia*. However, a specialist predator on *Z. trifolii*, *Apanteles*

*zygaenarum*, is itself able to tolerate HCN, circumventing the complex defense mechanism employed by the *Z. trifolii* larvae (Nahrstedt, 1988).

Specialist herbivores gain a competitive advantage by feeding on plants that are unpalatable to other animals (Edwards, 1989; Bryant et al., 1992). This is well illustrated by the feeding preferences of three sympatric species of bamboo lemur in Madagascar (Glander et al., 1989). The golden bamboo lemur (*Haplemur aurens*) feeds heavily on the cyanogenic shoots of *Cephalostachym* cv. *viguieri*, consuming up to 12 times the calculated lethal dose of HCN per day. The distribution of the golden bamboo lemur overlaps with that of two other bamboo lemurs (*H. griseus* and *H. sium*), which preferentially feed on the acyanogenic *C. perrieri* and acyanogenic parts of *C. viguieri* (Glander et al., 1989). While the mechanism by which the golden bamboo lemur tolerates such high concentrations remains unknown, it is clear that food preferences have contributed to the sympatric distribution of these primate species.

Most omnivorous mammals are generalists and, in general, are susceptible to cyanide poisoning. The dose required to cause acute poisoning varies with body size and species. The rate of ingestion is also important, as animals can detoxify the cyanide over time (Cock, 1982). Humans are generalists and can only tolerate about 1.4 mg HCN/kg body weight, or about 50–200 mg/day (Harborne, 1982), compared with sheep, which can tolerate about 2.5 mg/kg (Jones, 1972). Unlike other generalists, humans consume many highly cyanogenic plants—two thirds of all crop plants have at least some cyanogenic parts (Jones, 1998)—but because foods are processed before consumption, we do not consume large quantities of the cyanogenic glycosides themselves. Processed food has the added benefit of becoming sweeter through the production of glucose (Jones, 1998; Cardoso et al., 1998).

#### DIET SELECTION

In practice, animals are unlikely to overingest toxins if they are given a choice in their diet (Provenza et al., 1992). Cyanogenic plants frequently have a bitter taste that is known to deter herbivores (Nahrstedt, 1985). Humans, too, can detect the bitter taste of intact cyanogenic glycosides such as linamarin and lotaustralin, (King and Bradbury, 1995; Jones, 1998). Farmers in Malawi use the degree of bitterness as an indicator of the degree of toxicity of *Manihot esculenta* (Chiwona-Karlton et al., 1998, 2000), although the flavor may be modified by the presence of various organic acids (King and Bradbury, 1995).

Hungry animals are generally less discriminating in their choice of food (e.g., Bernays et al., 1977; Crush and Caradus, 1995; Caradus and Forde, 1996). Webber et al. (1985), for example, documented a case where a herd of 50 goats in western Victoria, Australia, was exposed to large amounts of *Eucalyptus cladocalyx* leaves when a tree was felled. With little alternative pasture due to a drought, the goats

fed ravenously on the young leaves resulting in the death of over half the herd, even after veterinary treatment (Webber et al., 1985).

Results of no-choice feeding experiments may, therefore, seriously underestimate the degree of avoidance that occurs in the field. Young nymphs of *Zonocerus variegatus*, for example, will eat *Manihot esculenta* leaves in captivity when there is no alternative, but do not feed on them at all in the field (Bernays et al., 1977). Even the older, more cyanide-tolerant instars of *Z. variegatus* only feed extensively on *M. esculenta* when they are deprived of other food, resulting in reduced growth rates (Bernays et al., 1977). In binary choice experiments, animals usually choose to eat the acyanogenic plants, thereby avoiding cyanide poisoning. For example, when voles (*Arvicola terrestris*) were offered a choice between cyanogenic and acyanogenic *Trifolium repens*, they consistently preferred the acyanogenic morph. When they were offered only cyanogenic plants (no-choice), they consumed them, but their overall food intake was reduced (Saucy et al., 1999; Viette et al., 2000).

History of exposure to cyanogenic glycosides can also affect feeding patterns (Jones, 1988; Viette et al., 2000). Increased tolerance to amygdalin develops over time in the insect *Spodoptera frugiperda* and is related to improved detoxification of the aglycone (Ferreira et al., 1997). By contrast, habituation to the cyanogenic glycosides amygdalin and salicin by another insect, *Diatraea saccharalis*, has been attributed to the decreased synthesis of endogenous  $\beta$ -glucosidases in the midgut (Ferreira et al., 1997). Similarly, in a controlled experiment, Keymer and Ellis (1978) observed that the mollusks *Helicella itala* and *Cochlicella acuta* ate more leaves on their second exposure to cyanogenic *Lotus corniculatus* than in the first phase of the experiment. The mechanism for this is not clear, particularly as mollusks are known to produce a range of endogenous cyanogenic  $\beta$ -glucosidases (Freeland and Janzen, 1974). Nevertheless, it does illustrate a degree of habituation by the herbivore.

In most animals, gaseous HCN combines with thiosulfate to form less toxic thiocyanate in a process catalyzed by rhodanese (thiosulfate cyanide sulfur transferase, EC 2.8.1.1; Figure 3). Humans and other mammals that regularly consume highly cyanogenic foods increase the production of the rhodanese, and can, therefore, tolerate higher concentrations of cyanide (McMahon et al., 1995). The amount of protein in the diet affects this degree of tolerance. This is particularly true for proteins high in cysteine, as they provide the sulfur essential for the thiosulfate production (Conn, 1991). Problems do arise, however, in humans from long-term ingestion of HCN including hyperthyroidism, goiter, cretinism, tropical ataxic neuropathy, konzo, and tropical diabetes (Cock, 1982; McMahon et al., 1995; King and Bradbury, 1995; Banea-Mayambu et al., 1997). Similar disorders have also been noted in sheep grazing on highly cyanogenic *Trifolium repens* in New Zealand (Crush and Caradus, 1995). As a result of diet selection, food processing and detoxification, acute poisoning from food in humans is rare, even in Africa where consumption rates of *Manihot esculenta* are high (Cock, 1982).

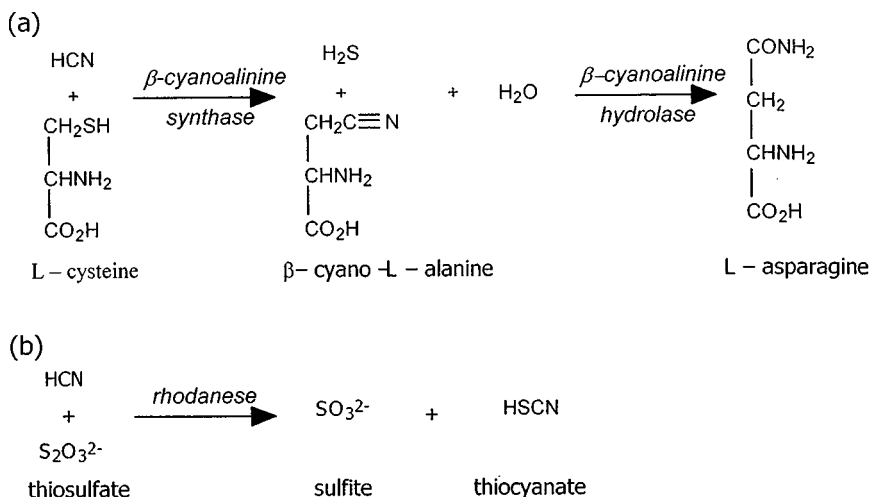


FIG. 3. Detoxification of cyanide by (a)  $\beta$ -cyanoalanine synthase and (b) rhodanese. The former is common in plant tissue and also occurs in some insects. The latter pathway is most commonly found in mammals and microorganisms, but it is also found, to a smaller extent, in plant tissues (Conn, 1981; Manning, 1988).

#### MODE OF FEEDING

In natural systems, cyanogenesis only occurs when a cyanogenic glycoside is mixed with the appropriate degradative enzymes, for example, when the tissue is chewed. Mammals tend to consume whole leaves (Cork, 1996) and almost inevitably consume  $\beta$ -glucosidases along with the cyanogen. Different orders of insects, however, have different types of mouthparts, which affect their feeding style. Generally, they have either sucking mouthparts (e.g., Hemiptera, Homoptera) or chewing mouth parts (e.g., Coleoptera, Hymenoptera, Orthoptera). If the insect herbivore has sucking mouthparts, then there is minimal tissue disruption, thereby avoiding the defense process. On the other hand, insects that chew leaves tend to mix the cyanogenic glycoside with the degradative enzymes, which are separated at either the cellular or tissue level in the plant (Frehner and Conn, 1987; Poulton, 1988). This difference in mouthparts could explain the difference between, for example, aphids (*Macrosiphum euphorbiae*, Hemiptera) and sawflies (*Strongylo-gaster impressata*, Hymenoptera), which show neutral and discriminatory feeding behavior, respectively, on polymorphic *Pteridium aquilinum* (Schreiner et al., 1984). One notable exception to this hypothesis is the field study by Dritschilo et al. (1979), who found that aphids fed preferentially on acyanogenic *Trifolium repens*.



The length of the stylus of sucking insects may also determine feeding patterns. Fully mature cassava burrowing bugs (*Cyrtomenus bergi*, Hemiptera) are able to feed effectively by penetrating beyond the outer layers of the root, which are particularly high in linamarin, while the young insects, bearing a shorter stylus, are deterred from feeding (McMahon et al., 1995).

### CONCLUSION

The effectiveness of cyanogenesis as an herbivore deterrent is not universal, and this has led to some degree of scepticism. However, variation in the ability to deter herbivores can usually be explained in terms of the concentration of the cyanogenic glycosides themselves, the availability of alternative foods, whether or not the herbivore is a specialist, and the mode of feeding. Cyanogenic glycosides are not effective against all herbivores, and not all cyanogenic plants release enough cyanide to be considered toxic. Nevertheless, they do form part of the broad spectrum of toxic and distasteful compounds that herbivores must overcome if they are to feed on cyanogenic plants.

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