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The interplay between toxin-releasing β-glucosidase and plant iridoid glycosides impairs larval development in a generalist caterpillar, *Grammia incorrupta* (Arctiidae)

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ABSTRACT

Herbivores with polyphagous feeding habits must cope with a diet that varies in quality. One of the most important sources of this variation in host plant suitability is plant secondary chemistry. We examined how feeding on plants containing one such group of compounds, the iridoid glycosides, might affect the growth and enzymatic activity in a polyphagous caterpillar that feeds on over 80 plant species in 50 different families. Larvae of the polyphagous arctiid, *Grammia incorrupta*, were reared exclusively on one of two plant species, one of which contains iridoid glycosides (*Plantago lanceolata*, Plantaginaceae) while the other does not (*Taraxacum officinale*, Asteraceae). Larval weight was measured on the two host plants, and midgut homogenates of last instar larvae were then assayed for activity and kinetic properties of β-glucosidases, using both a standard substrate, 4-nitrophenyl-β-D-glucose (NPβGlc), and the iridoid glycoside aucubin, one of the two main iridoid glycosides in *P. lanceolata*. Larvae feeding on *P. lanceolata* weighed significantly less and developed more slowly compared to larvae on *T. officinale*. While the larval midgut β-glucosidase activity determined with NPβGlc was significantly decreased when fed on *P. lanceolata*, aucubin was substantially hydrolyzed and the larval β-glucosidase activity towards both substrates correlated negatively with larval weight. Our results demonstrate that host plants containing high concentrations of iridoid glycosides have a negative impact on larval development of this generalist insect herbivore. This is most likely due to the hydrolysis of plant glycosides in the larval midgut which results in the release of toxic aglycones. Linking the reduced larval weight to the toxin-releasing action of an iridoid glycoside cleaving β-glucosidase, our results thus support the detoxification limitation hypothesis, suggesting fitness costs for the larvae feeding solely on *P. lanceolata*. Thus, in addition to the adaptive regulation of midgut β-glucosidase activity, host plant switching as a behavioral adaptation might be a prerequisite for generalist herbivores that allows them to circumvent the negative effects of plant secondary compounds.

1. Introduction

Insect herbivores are faced with the dual challenge of finding appropriate food and evading natural enemies. For polyphagous insect herbivores, there is an additional challenge: different host plant species can vary widely in their quality (Singer, 2001). For example, plant species may differ in nutritional adequacy; the presence of physical defenses such as thorns, trichomes or tough leaves; and the presence or absence of particular types of secondary metabolites. Variation in any of these features may affect the suitability of a plant as food. Plant secondary metabolites are particularly important as they are often crucial in determining insect diet breadth and may be sequestered by the insect or may require metabolic detoxification, either of which can incur physiological costs (Camara, 1997; Pankoke et al., 2010).

The iridoid glycosides are plant secondary metabolites found in over 50 plant families (Albach et al., 2001; Jensen, 1991). These compounds are cyclopentanoid monoterpene-derived compounds consisting of 8-, 9- or 10-carbon skeletons with an attached monosaccharide at C-1, normally β-D-glucose (Boros and Stermitz, 1990). They can be deterrent to non-adapted insects (Bernays and De Luca, 1981; Puttick and Bowers, 1988) and when eaten, can cause post ingestive effects leading to a lower digestive efficiency,

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lower growth rate, reduced larval weight, and a higher larval mortality (Bowers and Puttick, 1988; Puttick and Bowers, 1988; Stamp and Meyerhoefer, 2004). These toxic physiological effects are dose-dependent and differ among individual iridoid glycosides depending on their chemical structure and the presence or absence of functional groups (Bernays and De Luca, 1981; Bowers and Puttick, 1988; Puttick and Bowers, 1988).

In general, to exert toxic effects, iridoid glycosides require activation by enzymes or by acid hydrolysis. To date, only the hydrolytic β-glucosidases (EC 3.2.1.21) have been shown to convert the non-reactive iridoid glycosides into highly reactive aglycones (Kim et al., 2000; Konno et al., 1999). The aglycone's mechanism of toxicity is hypothesized to resemble that of alkylating agents that bind covalently to nucleophilic side chains (e.g., $-NH_2$ of lysine) via imine formation (Bartholomaeus and Ahokas, 1995; Kim et al., 2000; Konno et al., 1997, 1999). Several studies showed that, after enzymatic hydrolysis, the iridoid aglycones cross-link proteins and also act as enzyme inhibitors (Bartholomaeus and Ahokas, 1995; Konno et al., 1999; Ling et al., 2003; Park et al., 2010).

β-Glucosidases are ubiquitous enzymes found in bacteria, fungi, plants and animals. In insects, β-glucosidases are mainly found in the gut where they act as digestive enzymes (Terra and Ferreira, 1994, 2005). Based on their relative catalytic efficiency towards several substrates, insect β-glucosidases are divided into two classes, A and B, according to Terra and Ferreira (2005), where the Class B β-glucosidases are active on substrates with hydrophobic aglycones, such as 4-nitrophenyl-glycosides and plant glycosides (Yu, 1989; Terra and Ferreira, 1994, 2005). As β-glucosidases may release toxic aglycones from digested plant glycosides potentially leading to self-intoxication in the insect, they have become focal enzymes in the study of plant–insect interactions. Several herbivorous insect species have been shown to adaptively decrease their β-glucosidase activity in response to feeding on diets containing toxic glycosides (Desroches et al., 1997; Ferreira et al., 1997; Lindroth, 1988; Pankoke et al., 2010).

In this study, we focused on the polyphagous arctiid caterpillar *Grammia incorrupta* Edwards (Lepidoptera, Arctiidae) (formerly known as *Grammia geneura*) (Fig. 1). This species feeds on over 80 different species in about 50 different families (Singer, 2001). In addition, however, it has the ability to sequester two very different groups of plant secondary metabolites, pyrrolizidine alkaloids and iridoid glycosides, that are used as anti-parasitoid or anti-predator defenses by many other arctiids too (Bernays and Singer, 2005; Bowers and Stamp, 1997; Hristov and Conner, 2005; Rothschild et al., 1979; Singer et al., 2004, 2009; Smilanich et al., 2011; von Nickisch-Rosenegk and Wink, 1993; Weller et al., 1999). *G. incorrupta* is a true generalist and an individual caterpillar may feed on a dozen or more different species during its lifetime. In its natural environment, preferred species include *Plantago patagonica* (Plantaginaceae) (Singer and Stireman, 2001), a species containing iridoid glycosides (Bowers, 1996). Larvae of *G. incorrupta* can sequester iridoid glycosides, although at relatively low concentrations, typically less than 0.5% dry weight (Smilanich et al., 2011). These low levels of sequestered iridoid glycosides suggest that most of the iridoid glycosides are eliminated or metabolized (Smilanich et al., 2011).

Fig. 1. Larva of *Grammia incorrupta* (Lepidoptera, Arctiidae).

To investigate the effects of larval diet on β-glucosidase activity, *G. incorrupta* larvae were reared on either *Taraxacum officinale* (Asteraceae) (dandelion) or on *Plantago lanceolata* (Plantaginaceae) (narrow-leaved or ribwort plantain), both of which are readily consumed by these caterpillars. These two plant species differ in their composition of secondary compounds. *T. officinale* contains sesquiterpene lactones and phenolic compounds (Schütz et al., 2006, 2005; Williams et al., 1996) but does not contain iridoid glycosides. In contrast, *P. lanceolata* contains iridoid glycosides, primarily aucubin and catalpol, in amounts ranging from less than 1% to as high 12% dry weight (Barton and Bowers, 2006; Rønsted et al., 2003, 2000; Willinger and Dobler, 2001). This study addressed four questions: 1) How do larvae perform on these two host plants, one with and the other without iridoid glycosides? 2) How does host plant species affect the larval midgut β-glucosidase activity? 3) What is the substrate affinity of larval β-glucosidases towards a model substrate, 4-nitrophenyl β-D-glucoside (NPβGlc) and the iridoid glycoside aucubin? 4) What are the underlying mechanisms that could enable a generalist insect herbivore to tolerate iridoid glycosides in their diet?

2. Materials and methods

2.1. Insect feeding experiment

Eggs of *G. incorrupta* were obtained from a laboratory culture from Michael Singer (Wesleyan University, Middletown, Connecticut, USA). The resulting larvae were reared at Hamburg University on an artificial diet (Bergomaz and Boppré, 1986) in a climate chamber at 26 °C until pupation. Eggs from several females from this culture were mixed randomly and the resulting offspring was used for the feeding experiment. After hatching, all the larvae were fed on artificial diet (Bergomaz and Boppré, 1986) for five days. Then, we divided the larvae randomly into two groups of similar size. To allow habituation to the plant diet, from days five to eight, larvae were given leaves of either *T. officinale* (Asteraceae) or *P. lanceolata* (Plantaginaceae) along with some artificial diet. From day eight on, artificial diet was removed and larvae were exclusively reared on the appropriate food plant. Food was always provided ad libitum. Larvae were reared in transparent plastic containers (114 mm × 114 mm × 58 mm, corresponding to 500 ml) at 26 °C and a 16:8 h light:dark photoperiod. Host plants were collected at least twice a week from local populations and stored in a climate chamber at 16 °C until use. Larval weight was determined on days 21, 28, 42 and 49 after being first offered plant material. To test the effects of host plant species on larval development time, once pupation began, we recorded the number of larvae that had pupated within seven days after the first pupa was found.

2.2. Enzyme preparation

Because larval development rate differed between the two plant species, we standardized for duration of larval development when determining the midgut β-glucosidase activity; final instar larvae reared on either of the two host plants were dissected on two consecutive days, day 47 (*T. officinale*) and day 48 (*P. lanceolata*). Before dissection, larvae were weighed to the nearest of 0.1 mg. Then, larvae were dissected in cold 125 mM NaCl on ice (Ferreira et al., 1994). Midguts were isolated, freed from the peritrophic membrane and the food within and rinsed with 125 mM NaCl to completely remove the midgut contents. Using a glass Potter–Elvehjem tissue grinder, the gut sections were homogenized in double-distilled water while kept on ice. Then, midgut samples were frozen at −20 °C. To extract most of the β-glucosidase activity, which is mainly attached to the glycocalyx (Terra and