Comparing two strategies of counter-defence against plant toxins: A modeling study on plant-herbivore interactions

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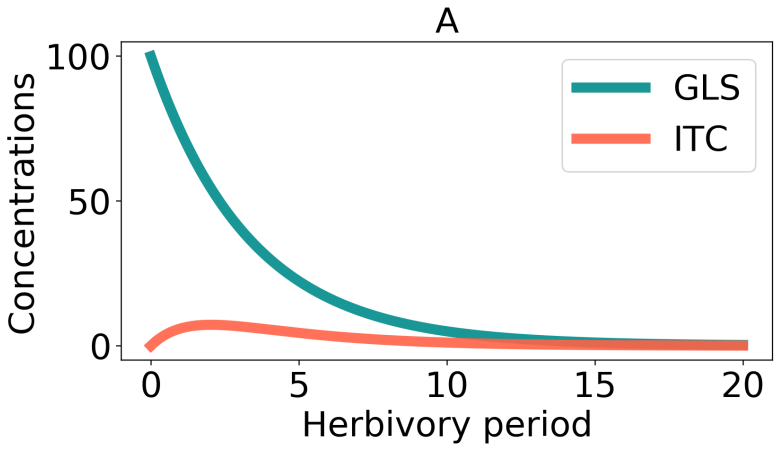
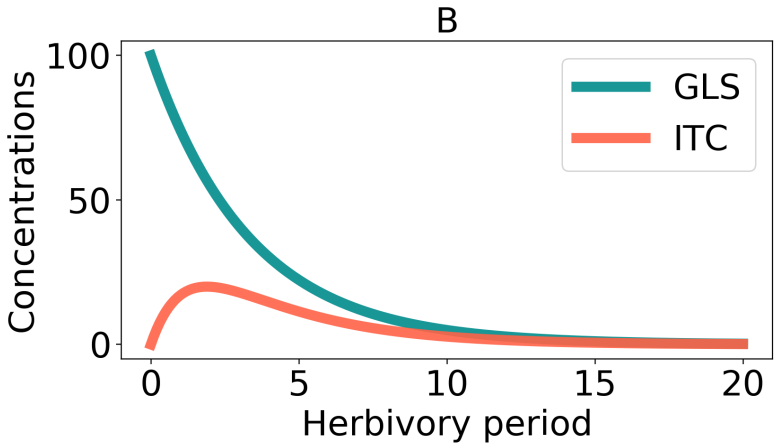
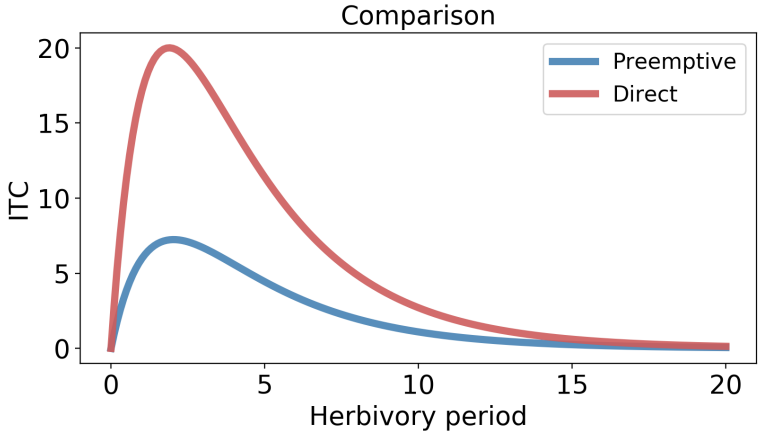
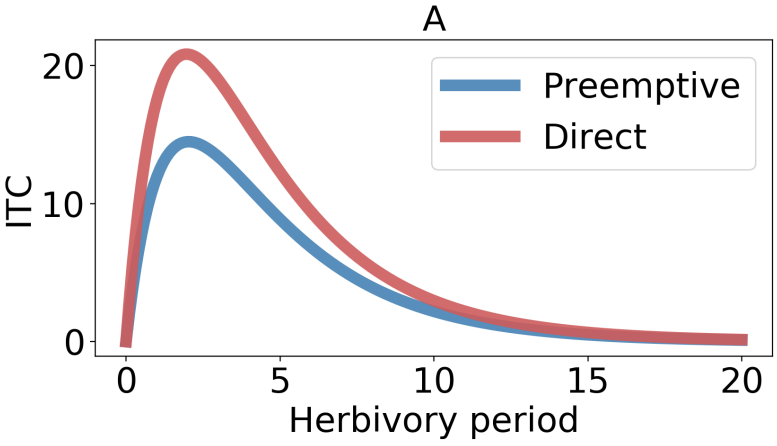
Abstract

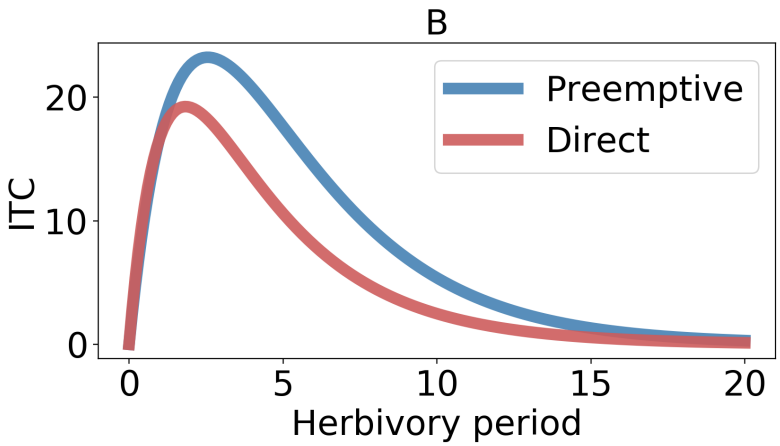
Various herbivorous insects prefer plants of the Brassicaceae family as their hosts, although they are toxic. The two-component chemical defence system of the Brassicaceae against herbivores consists of glucosinolates (GLS) and the activating enzyme myrosinase. GLS hydrolysis by myrosinase leads to isothiocyanate (ITC) products, which are toxic and deterrent to many insect herbivores. Some insects that feed on Brassicaceae, however, have evolved specific adaptations (called counter-defences) against GLS. Two different types of counter-defences can be distinguished: a preemptive counter-defence that prevents the GLS from being hydrolysed to ITC due to metabolic redirection and direct counter-defence, where the ITC is formed, but then metabolized to a non-toxic conjugate. Preemptive counter-defence is believed to be more efficient due to the lower exposure to ITC, but this has not been well demonstrated experimentally. Here, we prove on theoretical grounds that preemptive counter-defence reduces exposure to ITC compared to direct counter-defence by studying the dynamics of GLS defence and counter-defence with two separate ordinary differential equation models. By quantifying the specific ITC concentrations that herbivores are exposed to during feeding with the two types of counter-defences, we show that herbivores with a preemptory detoxification system are less exposed to ITC. In addition, our models explain how the decline in the level of ITC is achieved by both counter-defences, which helps to understand the overall mechanisms and benefits of these techniques.











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Abstract

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Preemptive counter-defence is believed to be more efficient due to the lower exposure to ITC, but this has not been well-demonstrated experimentally. Here, we prove on theoretical grounds that preemptive counter-defence reduces exposure to ITC compared to direct counter-defence by studying the dynamics of GLS defence and counter-defence with two separate ordinary differential equation models. By quantifying the specific ITC concentrations that herbivores are exposed to during feeding with the two types of counter-defences, we show that herbivores with a preemptory detoxification system are less exposed to ITC. In addition, our models explain how the decline in the level of ITC is achieved by both counter-defences, which helps to understand the overall mechanisms and benefits of these techniques.

Keywords. *Preemptive counter-defence, direct counter-defence, glucosinolate (GLS), isothiocyanate (ITC), mathematical model, ITC exposure.*

1 Introduction

One of the best studied plant chemical defences are the glucosinolates (GLS), found principally in the Brassicaceae and related families. GLS are accompanied by a glucohydrolase called myrosinase that upon herbivory converts GLS into active forms, which are toxic and deterrent to herbivores (Halkier *et al.* 2006; Wittstock *et al.* 2003). The most widespread active forms of GLS are isothiocyanates (ITC), which have been demonstrated to be toxic to many insect herbivores (Wittstock *et al.* 2010; Sun *et al.* 2019). Despite the GLS-myrosinase system, some insects are observed to feed on GLS-containing plants. In several cases, these insects have been demonstrated to possess different types of detoxification enzymes (Jeschke *et al.* 2016; Zou *et al.* 2016; Schramm *et al.* 2012).

Specialist feeding insects that feed exclusively on GLS-containing plants often convert GLS prior to myrosinase activation to a metabolite that is not activated by myrosinase. This detoxification scheme can be referred to as a preemptive counter-defence, because it avoids the formation of toxic ITC. For example,

larvae of the large cabbage white (*Pieris rapae*) redirect GLS hydrolysis to form less toxic nitriles by using a nitrile-specifier protein (NSP) (Wittstock *et al.* 2004). Another example is provided by the larvae of the diamondback moth (*Plutella xylostella*) that desulfate GLS before they can be hydrolyzed (Ratzka *et al.* 2002). However, a portion of GLS can escape being metabolized by these preemptive mechanisms and produce ITC products via myrosinase-catalysed hydrolysis (Jeschke *et al.* 2017).

Another adaption of some specialist feeders is to absorb or accumulate GLS in their bodies for their own defence (Petschenka *et al.* 2016; Beran *et al.* 2019; Yang *et al.* 2020; Sporer *et al.* 2021). For example, larvae of the turnip sawfly (*Athalia rosae L.*) store the GLS of their host plants in their haemolymph (Müller *et al.* 2001), while larvae and also the adults of horseradish flea beetles (*Phyllotreta armoraciae*) absorb GLS (Sporer *et al.* 2021). Hydrolysis of GLS by myrosinase is avoided by rapid adsorption after ingestion and by partial inhibition of myrosinase activity (Sporer *et al.* 2021). This adaptation can also be considered a type of preemptive counter-defence. However, a portion of GLS can escape the sequestration process and produce ITC through myrosinase-catalyzed hydrolysis (Yang *et al.* 2020; Sporer *et al.* 2021).

In contrast to specialist feeders, generalists feed only occasionally on GLS-containing plants and typically do not possess preemptive detoxification systems. Once ITC has been formed, part of it is detoxified directly via conjugation to the tripeptide glutathione (GSH) (Yu 1987; Wadleigh *et al.* 1988; Schramm *et al.* 2012). Therefore, we call this adaptation direct counter-defence. Experimental studies have reported that lepidopteran generalists (e.g. *Spodoptera littoralis, S. exigua, Trichoplusia ni, Mamestra brassicae and Helicoverpa armigera*) employ this detoxification strategy. In this case, a major portion of the ITC is not conjugated to GSH, but is released in the faeces (Schramm *et al.* 2012; Jeschke *et al.* 2017).

Experimental studies show that specialist feeders generally perform significantly better on GLS-containing plants than generalists (Li *et al.* 2000; Hopkins *et al.* 2009; Sarosh *et al.* 2010; Rohr *et al.* 2011) presumably due to lower exposure to ITC. For example, when the preemptive desulfation detoxification system of *P. xylostella* was knocked-down by interference RNA, the level of ITC present in the gut increased by over ten-fold (Sun *et al.* 2019). Thus, preemptive counter-defence appears to be superior to direct counter-defence. However, it is not clear if preemptive detoxification actually involves less ITC exposure than direct detoxification, and this is difficult to measure experimentally at short intervals in a time course.

Here, we attempt to model the metabolism of GLS in specialist and generalist feeders to determine the theoretical exposure of insects to ITC during preemptive vs. direct detoxification. Mathematical modelling helps to understand the change in substrate concentration (plant defence compounds in our case) over time (Johnson *et al.* 2011; Srinivasan 2022; Knoke *et al.* 2009). By developing two different ordinary differential equation models, we simulate the dynamics of ITC concentrations in these two cases. Our results show less ITC exposure for insects with a preemptive counter-defence than for those relying on direct counter-defence, where the overall exposures to ITC (for specialists and generalists, respectively) are obtained from the area under the ITC curves (Wagner *et al.* 1985; Schuster *et al.* 2019). Our models also help to explain how both counter-defences may entirely degrade the host plant defence.

2 Models and results

We develop two different deterministic models, one for preemptive counter-defence and the other for direct counter-defence. For the model formulation, we assume herbivory and plant GLS degradation are simultaneous processes. Therefore, plant GLS degradation (either by myrosinase or the preemptive detoxification by specialists) starts as soon as herbivory begins. On the other hand, ITC detoxification (direct detoxification by generalists) starts as soon as the ITC contact detoxification enzymes. For simplicity, we assume that GLS are only a constitutive plant defence (Dicke 1998), i.e. that they are present in plants in a fixed amount, and their accumulation is not induced by herbivory.

2.1 Preemptive counter-defence

In case of insects with a preemptive detoxification system, let α be the rate constant of plant GLS degradation by the preemptive detoxification enzyme, whereas β be the rate constant of ITC formation by the hydrolysis of GLS that escape preemptive detoxification. Further, the free ITC in the insect gut are released in the faeces with a rate constant, γ. Based on mass-action kinetics, the rate equations are:

$$\frac{dS_P}{dt} = -(\alpha + \beta)S_P \tag{1a}$$

$$\frac{dT_P}{dt} = \beta S_P - \gamma T_P \tag{1b}$$

where S_P is the plant GLS concentration and T_P is the ITC concentration at time t for insects with preemptive counter-defence. The model (1) has an equilibrium point $(0, 0)$, which is asymptotically stable. So, without doubt, the preemptive counter-defence can degrade the ITC concentration to 0. Since the model (1) is a simple linear ODE system, the equations can be solved analytically:

$$S_P = S_{P_0} e^{-(\alpha+\beta)t} \tag{2a}$$

$$T_P = \frac{\beta S_{P_0}}{\gamma - (\alpha + \beta)} \left(e^{-(\alpha+\beta)t} - e^{-\gamma t} \right) \tag{2b}$$

where S_{P_0} is the initial plant GLS concentration that insects with a preemptive detoxification system are exposed to. The time-course of model (1) is shown in Figure 1 (A).

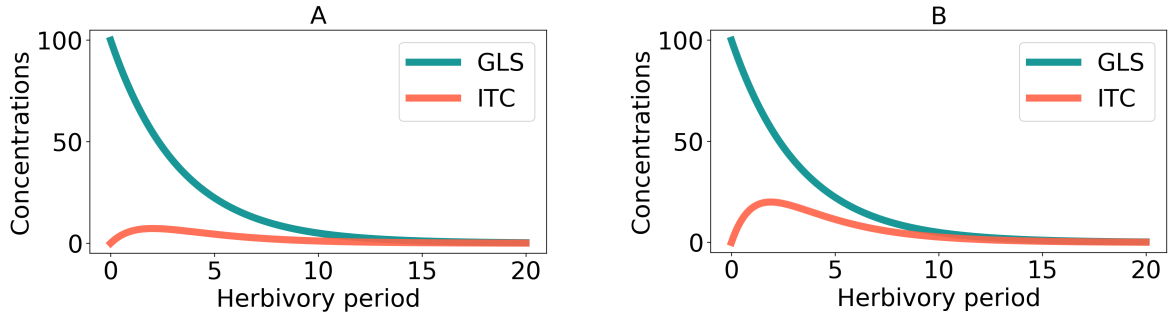

Figure 1: A) Degradation of plant GLS and ITC exposure during preemptive counter-defence from model (1), parameters: $S_{P_0} = 100$, $\alpha = 0.2$, $\beta = 0.1$ and $\gamma = 0.75$. B) Degradation of plant GLS and ITC exposure during direct counter-defence from model (3), parameters: $S_{D_0} = 100$, $\delta = 0.3$, $\mu = 0.1$ and $\eta = 0.75$.

2.2 Direct counter-defence

In the case of insects with a direct detoxification system, let δ be the rate constant at which plant GLS are hydrolysed to ITC by myrosinase, μ be the rate constant at which ITC is reacted to produce ITC-conjugates, whereas with a rate constant η, the unmetabolized ITC gets released in the faeces. Eventually, the active portion of ITC is decreased with an overall rate constant $\mu + \eta$. The rate equations are:

$$\frac{dS_D}{dt} = -\delta S_D \tag{3a}$$

$$\frac{dT_D}{dt} = \delta S_D - (\mu + \eta)T_D \tag{3b}$$

where the subscript D refers to direct counter-defence. The only equilibrium point of model (3) is $(0, 0)$, which is also asymptotically stable. Similar to the preemptive counter-defence, direct counter-defence can also degrade the ITC concentration to 0. The time-course of model (3) is shown in Figure 1 (B). Due to its simplicity, model (3) can also be solved analytically:

$$S_D = S_{D_0} e^{-\delta t} \tag{4a}$$

$$T_D = \frac{\delta S_{D_0}}{\mu + \eta - \delta} \left(e^{-\delta t} - e^{-(\mu+\eta)t} \right) \tag{4b}$$

where S_{D_0} is the initial plant GLS concentration that insects with a direct detoxification are exposed to.

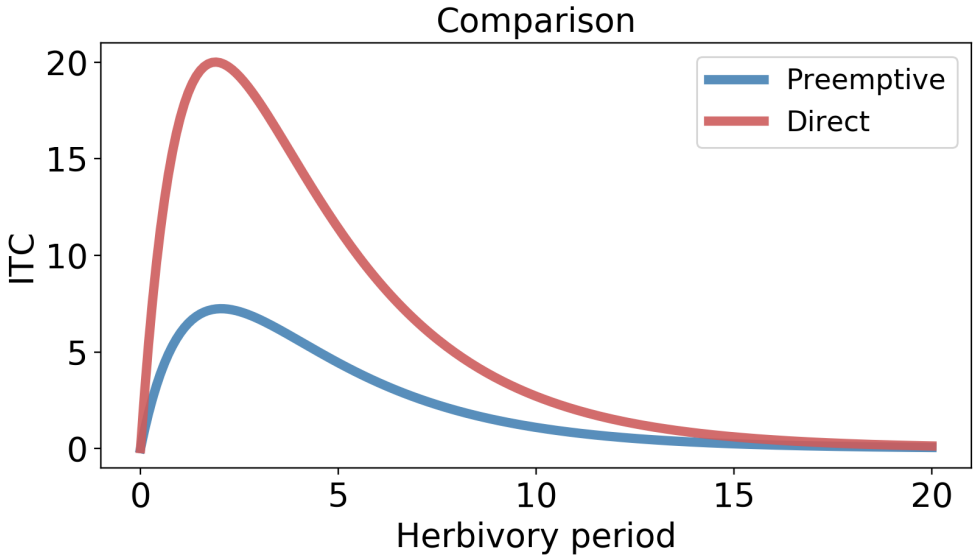

Figure 2: Area enclosed by ITC concentrations during the herbivory period, obtained from model eq. (1) and model eq. (3), respectively. Parameter values, same as in Figure 1.

2.3 Quantifying the ITC exposure

Using Haber's rule in both models (1) and (3), we integrate T_P and T_D with respect to time (t) within the time range 0 to ∞. That gives the area enclosed by the ITC curves, called area under the curve, (AUC) (Wagner *et al.* 1985; Lappin *et al.* 2006; Connell *et al.* 2016; Schuster *et al.* 2019). Literally, AUC gives the entire amount of ITC that the feeding insects are exposed to during the period of herbivory, shown in Figure 2. Let AUC_P and AUC_D be the ITC exposure of the insect population with preemptive and direct counter-defence, respectively. Integrating Eqs. (2b) and (4b), we obtain:

$$AUC_P = \int_0^\infty T_P \, dT_P = \frac{\beta S_{P_0}}{(\alpha + \beta)\gamma} \tag{5a}$$

$$AUC_D = \int_0^\infty T_D \, dT_D = \frac{S_{D_0}}{\mu + \eta} \tag{5b}$$

It is worth noting that the parameter δ does not appear in the formula for AUC_D. Moreover, note that S_{P_0} and S_{D_0} are not necessarily equal to each other. The feeding capacity of insects with preemptive counter-defence may differ from the insects with direct counter-defence, if they feed on plants of different size, or they stop feeding in the middle and move to a different patch of plants.

2.4 Comparison

To make a comparison under equal conditions, we assume that insects with preemptive and direct detoxification systems feed on plants or patches of plants that are identical in GLS concentration. Therefore, insects with the two types of detoxification are initially exposed to an equal volume of plant GLS, i.e. $S_{P_0} = S_{D_0} = S_0$. By comparing the ITC exposure eqs. (5a) and (5b), proving $AUC_P < AUC_D$ is enough to explain why the negative effects of ITC are higher in insects with direct rather than preemptory detoxification. Hence, to prove:

$$\frac{\beta}{\alpha+\beta} < \frac{\gamma}{\mu+\eta} \tag{6}$$

From the available experimental results, we can establish some relationships among the parameters of the inequality (6).

Property 1. *For an insect with a preemptive detoxification system, only a small amount of GLS escape to form ITC, whereas most of the GLS is detoxified, determined by GC-MS analysis (Wittstock et al. 2004), LC-MS analysis and direct radioactivity measurement (Jeschke et al. 2017). Thus, we obtain $\beta < \alpha$.*

Property 2. *In direct detoxification, the major portion of free ITC is excreted unmetabolized, whereas a smaller portion is converted to non-toxic conjugates, measured by LC-MS analysis and flux measurements with radioactive labelling (Schramm et al. 2012; Jeschke et al. 2017). Hence, μ is very small and $\mu < \eta$.*

Property 3. *Without loss of generality, we consider $\gamma \approx \eta$ (but not equal) by assuming that the excretion mechanism is more or less the same for all insects. Therefore, $\mu < \gamma$, following prop. (2).*

Theorem 1. *$AUC_P < AUC_D$ or inequality (6) is always true for $\beta \leq \alpha$.*

Proof. Since μ might be rather small, there is no obvious relation between γ and $\mu + \eta$. Therefore, we distinguish the two cases $\gamma \geq \mu + \eta$ and $\gamma < \mu + \eta$, to prove the theorem. The first case is relevant, in particular, if $\mu \to 0$ because $\gamma \approx \eta$, following prop. (3).

In the second case, the inequality (6) can be transformed into:

$$\frac{\beta}{\alpha} < \frac{\gamma}{\mu+\eta-\gamma}, \quad \text{where } \mu+\eta > \gamma \tag{7}$$

Case 1. $\gamma \geq \mu + \eta$

The l.h.s. of inequality (6) is < 1 for any α and β, while the r.h.s. of inequality (6) is ≥ 1. This implies inequality (6).

Case 2. *At $\gamma < \mu + \eta$, inequality (7) is true.*

From prop. (3), $(\gamma - \eta) \to 0$. Therefore, the r.h.s of inequality (7) turns into:

$$\frac{\gamma}{\mu}, \quad \text{where } \mu < \gamma, \text{ explained in props. (2) and (3)}$$

Obviously $\frac{\gamma}{\mu}$ is a finite value > 1 for $\mu \neq 0$, while the l.h.s of the inequality (7) is ≤ 1 at $\beta \leq \alpha$. This entails inequality (7). Note that at $\mu \to 0$, inequality (7) holds more strongly, because its r.h.s is close to ∞. This completes the proof.

□

Theorem 1 explains that if $\beta \leq \alpha$ is satisfied, preemptive counter-defence is stronger than direct counter-defence, shown in Figure 2. However, it does not mean that $\beta > \alpha$ makes preemptive counter-defence inferior, see Figure 3 (A). On the contrary, it can be proved that preemptive counter-defence remains superior under the conditions stated in the following theorem (below). Moreover, it is justified to assume that $\beta \not\gg \alpha$ because if preemptive counter-defence is observed in plant-insect interactions, it is always found to be efficient enough that not almost the entire plant GLS is hydrolysed to ITC.

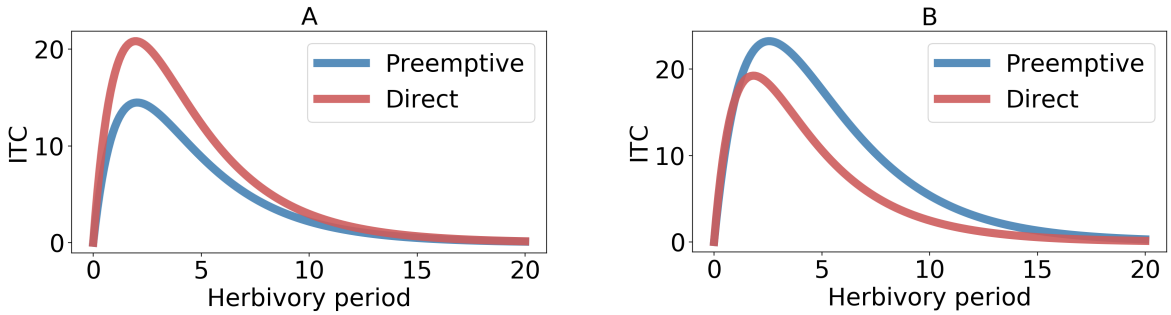

Figure 3: A) Preemptive counter-defence remains superior at $\beta > \alpha$ too, parameters: $\alpha = 0.1$, $\beta = 0.2$, $\gamma = 0.75$, $\delta = 0.3$, $\mu = 0.05$ and $\eta = 0.75$. B) Direct counter defence may perform better at $\beta > \alpha$, $\eta > \gamma$ and $\mu \not\to 0$, parameters: $\alpha = 0.05$, $\beta = 0.25$, $\gamma = 0.5$, $\delta = 0.3$, $\mu = 0.25$ and $\eta = 0.65$.

Theorem 2. *At $\mu \to 0$, $AUC_P < AUC_D$ or inequality (6) is true for $\beta > \alpha$, too.*

Proof. In case of $\gamma \geq \mu + \eta$, the proof is similar to case (1) of Theorem (1).

In case of $\gamma < \mu + \eta$ in inequality (7), the l.h.s is > 1 (as $\beta > \alpha$) and a finite value can be achieved (assuming $\beta \not\gg \alpha$). However, the r.h.s of this inequality is close to ∞, because $\mu \to 0$, $\gamma \approx \eta$. Therefore, inequality (7) is true.

This case is of special interest because the superiority of preemptive counter-defence is then less intuitive. □

Remark 1. *Theoretically, a direct counter-defence may perform better if $\beta > \alpha$, $\mu \not\to 0$ and η being significantly greater than γ, shown in Figure 3 (B). However, that is an unrealistic case, because γ and η should not differ much and μ is expected to be much lower than γ and η, explained in props. (1), (2) and (3).*

Remark 2. *We did not make a direct comparison between the dynamic ITC concentrations T_P and T_D, because to verify whether or not $T_D - T_P > 0$, we need to establish relations among the parameters α, β and δ. It can be assumed that $\delta < \alpha + \beta$, because insects with direct counter-defence, feed slowly on toxic hosts (Jeschke et al. 2021; Zalucki et al. 2021). However, we have to be more specific to make such parameter comparisons. Fortunately, AUC_D in eq. (5b) is free of δ. Therefore, we do not require a relation between α, β and δ to compare between the quantified toxin exposures AUC_P and AUC_D (eq. (6)).*

3 Discussion

Insect herbivores employ two different strategies to detoxify activated plant defences like GLS. There is no a priori reason why herbivores could not possess both preemptory and direct counter-defences, except the potentially high metabolic costs. Our work shows that

1. A preemptive counter-defence always outcompetes a direct counter-defence, as explained by Theorems 1 and 2.

2. Although the ITC exposure is comparatively low when a preemptive counter-defence is operating, it is not negligible, because AUC_P is a positive value. A negligible exposure to ITC is possible if $AUC_P \to 0$, which can only be attained through $\beta \ll \alpha$.

The universal superiority of preemptive vs. direct counter-defence guarantees that herbivores possessing this strategy have an advantage over other herbivores on toxic host plants because they minimize contact with toxins. The toxic effects of ITC on feeding insects exposed to this toxin (AUC_P or AUC_D), cause reductions in feeding rate, growth and survival (Sun *et al.* 2019; Jeschke *et al.* 2021; Zalucki *et al.* 2021). Thus, a low ITC exposure (AUC_P) obviously implies only minor effects on insect feeding behavior, growth and mortality (Li *et al.* 2000; Hopkins *et al.* 2009; Rohr *et al.* 2011), whereas a high AUC_D value leads to poor feeding behaviour, slow growth and a high mortality rate (Jeschke *et al.* 2021; Zalucki *et al.* 2021).

The lower exposure to ITC in preemptive detoxification (AUC_P) versus direct detoxification (AUC_D) may have an empirical basis due to the location of these reactions in the insect. The preemptive detoxification reactions of GLS, such as desulfation, are known to occur extracellularly in the insect gut lumen by acting on GLS in the plant tissue being digested (Sun *et al.* 2019). In contrast, once ITC are formed by GLS breakdown in the gut, the direct detoxification reaction, conjugation with glutathione, occurs intracellularly. The ITC formed thus need to cross through a membrane and enter a cell before being detoxified (Jeschke *et al.* 2016). This longer path to the site of detoxification in direct counter-defence, allows more opportunities for the ITC to react with target sites than in preemptory detoxification.

The effectiveness of preemptive detoxification does not necessarily mean that insects employing this strategy completely escape the adverse effect of ITC. As described in point (3) above, negative effects occur as long as $\beta \ll \alpha$ does not hold. That could explain why some experimental studies report that insect species known to be preemptive detoxifiers of GLS are affected by ITC (Mewis *et al.* 2005, 2006; Gols *et al.* 2007, 2008). For the preemptively detoxifying *P. xylostella*, larvae feeding on plants without any GLS at all perform significantly better than those on GLS-containing plants, suggesting that some exposure to ITC occurs despite an effective detoxification strategy (Sun *et al.* 2019). However, preemptory detoxification has also been documented to be very effective, with many studies reporting that species with this strategy are only marginally affected by the GLS-myrosinase defence system of their host plants (Slansky *et al.* 1977; Blau *et al.* 1978; Broadway 1995; Li *et al.* 2000; Sarosh *et al.* 2010; Rohr *et al.* 2011). In such cases, β is likely to be much less than α.

Our results may also apply to insects that sequester GLS in their own defence, as these are also reported avoiding the negative effects of ITC (Müller 2009; Müller *et al.* 2006; Beran *et al.* 2019; Sporer *et al.* 2021). This phenomenon is explainable from the model (1) by assuming α to be the absorption or sequestration rate of GLS, where β remains the rate of GLS hydrolysis. In fact, quick sequestration certainly leads to the situation $\beta \ll \alpha$, a conclusion supported experimentally by the rapid absorption of GLS measured in insect guts of sequestering herbivores (Petschenka *et al.* 2016; Abdalsamee *et al.* 2014; Sporer *et al.* 2021).

In natural systems, many plants of the Brassicaceae that produce GLS constitutively have also been found to accumulate higher concentrations after herbivore damage (Textor *et al.* 2009; van Dam *et al.* 1993; Agrawal 1998). Experimental studies report that such GLS induction has noticeable adverse effects on insect herbivores (Agrawal 2000; Agrawal *et al.* 2003; van Dam *et al.* 2000). Therefore, accommodating the induction of GLS in model (1) or (3) could be of interest in future studies of defence vs. counter-defence paradigms during plant-herbivore interactions. Intuitively, we can say that the induction of GLS

may drastically increase the ITC exposure (i.e. AUC_P and AUC_D). As a result, the toxic effect of ITC can be raised.

Our study adds to experimental results indicating that herbivore feeding on GLS-containing plants can be costly, even for preemptory detoxification systems. Thus, it may seem puzzling that specialist herbivores with such detoxification systems use plant GLS or ITC content as a cue for their oviposition and feeding preference (Mewis *et al.* 2002; Renwick 2002; Miles *et al.* 2005; Badenes-Perez *et al.* 2020), and thus prefer GLC-containing plants compared to plants without GLS despite the costs. A possible explanation is the reduced competition enjoyed on GLS-containing plants because of their generally toxic nature to most herbivores. From an evolutionary perspective, feeding on plants with GLS or other toxins must benefit herbivores. Otherwise, the evolutionary origin of detoxification traits (Dobzhansky 1968; Darwin 1859) is hard to understand. Comparative fitness studies on toxic vs. non-toxic plants, both with and without competition, may help explain the shift to toxic plants.

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Author Contributions

S.C. established the mathematical model and performed the simulations. J.G. and S.S. coordinated and supervised the study. J.G. contributed expertise in chemical ecology. S.S. contributed expertise in mathematical modelling. All authors verified the results and wrote this manuscript.

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