Effect of diet on *Spodoptera exigua* (Hübner) caterpillar enzyme activity

Jinyuan Ji

Department of Plant Science
McGill University
Montreal, Quebec, Canada

April 2014

A thesis submitted to McGill University in partial
fulfillment of the requirements for the degree of
Master of Science

Table of Contents

List of Figures

List of Appendices

List of Abbreviations

ANOVA	Analysis of variance
APOX	Ascorbate peroxidase
BAEE	N-α-benzoyl-L-arginine ethyl ester
CDNB	1-Chloro-2,4-dinitrobenzene
GOX	Glucose oxidase
GSH	Reduced glutathione
GST	Glutathione *S*-transferase
H_2O_2	Hydrogen peroxide
LSG	Labial salivary glands
NDF	Neutral detergent fiber
OS	Oral secretion
P:C ratio	Protein-to-digestible carbohydrate ratio
PI	Proteinase inhibitor
ROS	Reactive oxygen species
VEG	Ventral eversible gland

Abstract

Insects use enzymes associated with labial salivary glands or guts to detoxify plant defensive compounds or suppress plant induced defenses. Current studies suggest that the activity of these enzymes can be affected by diet due to two main factors: plant secondary metabolites or nutritional quality. How different plant diets affect the enzyme activity of *Spodoptera exigua* (Hübner) caterpillars is not well understood. Therefore, the objective of this research is to understand how plant diet affects the activity of the caterpillar enzymes: glucose oxidase (GOX), ascorbate peroxidase (APOX), glutathione *S*-transferase (GST), trypsin and carboxylesterase. Caterpillars were transferred to plants or artificial diet for 48 hours to compare the effects of diets on enzyme activity. The plant diets are *Arabidopsis thaliana*, *Medicago truncatula* or *Solanum lycopersicum*. As well, starved or artificial diet controls were used.

Caterpillars fed on *Arabidopsis* had higher glutathione *S*-transferase (GST), carboxylesterase and trypsin activity compared to other plant diets. GST activity of caterpillars fed on *Arabidopsis* was more than 7 times or 4 times higher than caterpillars fed on tomato or *Medicago*, respectively. Trypsin activity of caterpillars fed on *Arabidopsis* was almost twice or more than 5 times higher than caterpillars fed on tomato or *Medicago*, respectively. *Arabidopsis*-fed caterpillars had almost 3 times of carboxylesterase activity than that of *Medicago*-fed caterpillars. This result was mimicked by adding extracts of *Arabidopsis* plants to artificial diet, which suggests that it may be plant secondary metabolites that activated these enzymes. As for GOX and trypsin, the nutritional quality (protein-to-digestible carbohydrate ratio, P:C ratio) played an role in determining enzyme activity. The high level of protein in the artificial diet increased GOX activity, while trypsin activity was induced by low protein level. Caterpillars fed on the 24P:17C diet had twice the GOX activity compared to caterpillars fed on the 25P:39C diet. Caterpillars fed on the 25P:39C diet had more than twice the trypsin activity than 24P:17C fed caterpillars. Therefore, the enzyme activity of *S. exigua* caterpillars strongly correlated with the diets that insects feed on.

Résumé

Les insectes utilisent des enzymes associées aux glandes salivaires labiales ou au système digestif pour détoxifier les molécules de défense des plantes ou atténuer leurs défenses induites. Les études récentes suggèrent que l'activité de telles enzymes peut être affectée par l'alimentation à travers deux facteurs principaux : les métabolites secondaires des plantes et la qualité nutritionnelle. La manière dont différents régimes de plantes affectent l'activité enzymatique de chenilles de *Spodoptera exigua* (Hübner) est mal connue. C'est pourquoi l'objectif de cette étude est de comprendre la façon dont différents régimes de plantes affectent l'activité des enzymes suivantes des chenilles : le glucose oxydase (GOX), l'ascorbate peroxydase (APOX), le glutathion S-transférase (GST), la trypsine et le carboxylesterase. Les chenilles furent transférées sur des plantes ou sur une alimentation artificielle pendant 48 heures afin de comparer les effets de l'alimentation sur l'activité enzymatique. Les régimes végétaux sont *Arabidopsis thaliana*, *Medicago truncatula* et *Solanum lycopersicum*. Des contrôles à jeun et d'autres nourris avec une alimentation artificielle furent utilisés.

Les chenilles nourries avec *Arabidopsis* avaient une activité supérieure du glutathion S-transférase (GST), du carboxylesterase et de la trypsine comparativement aux chenilles nourries avec d'autres régimes. L'activité du GST des chenilles nourries avec *Arabidopsis* était respectivement plus de 7 fois et 4 fois supérieure à celle de chenilles nourries sur des plants de tomates ou de *Medicago*. L'activité de la trypsine des chenilles nourries avec *Arabidopsis* était respectivement plus de 2 fois et 5 fois supérieure à celle de chenilles nourries sur des plants de tomates ou de *Medicago*. Les chenilles nourries avec *Arabidopsis* avaient près de 3 fois plus d'activité du carboxylesterase que les chenilles nourries avec *Medicago*. Ce résultat fut imité en ajoutant des extraits d'A*rabidopsis* dans la nourriture artificielle, ce qui suggère que ces enzymes pourraient être activées par des métabolites secondaires. En ce qui a trait au GOX et à la trypsine, la qualité nutritionnelle (ratio de protéines et de glucides assimilables, ratio de P:G) a joué un rôle dans le contrôle de l'activité enzymatique. Le ratio élevé de protéines dans la nourriture artificielle a augmenté l'activité du GOX, tandis que l'activité de la trypsine a été induite par un ratio de protéines faible. Les chenilles nourries avec un mélange à 24P:17G avaient un niveau d'activité du GOX 2 fois supérieur à celui des chenilles nourries à un ratio de 25P:39G. Les chenilles nourries avec un mélange à 25P:39G avaient un niveau d'activité de la

trypsine plus de 2 fois supérieur à celui des chenilles nourries à un ratio de 24P:17G. Ainsi, l'activité enzymatique de chenilles de *S. exigua* est fortement corrélée avec l'alimentation qui leur est fourni.

Acknowledgments

I would like to thank my supervisor, Dr. Jacqueline Bede, for all her intellectual and emotional guidance and support for my project. I would also like to thank my committee members, Dr. Sébastien Faucher and Dr. Philippe Seguin, for their suggestions and assistance.

Thanks to all my lab mates for their friendship and support; Jamuna and Alberto for sharing their knowledge and experience; Tony for helping me take care of the caterpillars; Nicolas for helping me with the résumé of this thesis.

Thanks to all my friends; special thanks to the colleagues from Dr. Jean-Benoit Charron's lab and Dr. Sébastien Faucher's lab for helping me with the *in gel* enzyme assay. Thanks to Dr. Mustafa for plant analysis.

Finally, I would like to thank my parents for their support, love and encouragement.

Contributions of authors

The project presented hereafter was designed by the candidate and Dr. Jacqueline C. Bede, Department of Plant Science, Macdonald Campus of McGill University. The candidate performed all experiments, conducted data analysis and wrote the thesis under the supervision of Dr. Bede.

1. Introduction

1.1. General introduction

During the process of co-evolution, the interaction between insect herbivores and plant hosts have gained much attention. Understanding how insects protect themselves from deleterious effects of the numerous plant defenses can potentially lead to future pest control. Plant defenses, physical or chemical defenses, can be broadly categorized into three strategies: direct defense, indirect defense or tolerance (Karban and Baldwin, 1997). Among chemical defensive strategies, plant nutritional quality and the presence of constitutive or induced secondary metabolites are of key importance (Tallamy and Raupp, 1991). In fact, specific groups of compounds are often associated with plant families (Bennett and Wallsgrove, 1994). Generally, these secondary metabolites can affect insect behavior or their growth, development and reproduction (Harborne, 1993).

At the same time, insects have evolved strategies to overcome these plant defenses. Biochemical resistance, which is of primary importance to insects, includes the increased metabolic capability of detoxifying enzymes, such as carboxylesterases and glutathione *S*-transferases (GSTs) (Brattsten, 1988; Li et al., 2007). These enzymes can make plant chemicals more hydrophilic to increase their excretion or convert them into nontoxic forms (Ahmad and Hopkins, 1993).

Plant defenses can generate oxidative stress caused by reactive oxygen species (ROS), such as hydrogen peroxide (H_2O_2) (Bolwell and Wojtaszek, 1997; Mittler, 2002). High levels of ROS damages cells by reacting with cellular macromolecules (Pardini, 1995). To reduce the negative effects caused by ROS, insects use anti-oxidative enzymes such as ascorbate peroxidase (APOX) to scavenge H_2O_2 by converting it to H_2O (Asada, 1992).

Another common plant defense is proteinase inhibitors (PIs) which act on insect gut-associated proteinases, such as trypsin (Bernays and Chapman, 1994; Felton and Gatehouse, 1996; Stotz et al., 1999; Carlini and Grossi-de-sá, 2002). These PIs impede protein digestion and can lead to a decrease in the availability of amino acids needed by the insect (Broadway and Duffey, 1986a; Broadway, 1995; Koiwa et al., 1998; Zhu-Salzman et al., 2003). If the host plant has this defense, in response, some insect species are able to change their gut proteinases to a PI-tolerant form (Lopes et al., 2004).

In addition to these gut-associated enzymes, insects produce effectors in the

oral secretions (OS) to suppress the induction of plant defenses (Eichenseer et al., 1999; Kahl et al., 2000; Musser et al., 2002; Wu et al., 2012). One of these effectors, glucose oxidase (GOX), is believed to be involved in suppressing the induction of defenses in several plant species (Musser et al., 2002; Zong and Wang, 2004; Bede et al., 2006; Diezel et al., 2009; Tian et al., 2012).

The activity of these enzymes involved in detoxification or suppression of induced plant defence can be affected by the diet that the insect feeds on (Merkx-Jacques and Bede, 2005; Babic et al., 2008; Afshar et al., 2010; Wang et al. 2009; Patankar et al., 2000; Xue et al., 2010; Celorio-Mancera, 2011). This may reflect two dietary factors: either nutritional quality or secondary metabolites (Babic et al., 2008; Hu et al., 2008; Bernays and Chapman, 2000). Diet nutritional composition, such as the protein-to-digestible carbohydrate (P:C) ratio, is a major factor influencing the labial salivary GOX activity of caterpillars of the cotton bollworm, *Helicoverpa armigera* and the beet armyworm, *Spodoptera exigua* (Babic et al., 2008; Hu et al., 2008). Meanwhile, plant secondary metabolites can also affect insect enzymes activities. Often allelochemicals induce detoxification enzyme activity (Snyder and Glendinning, 1996; Bernays and Chapman, 2000).

Spodoptera exigua (Hübner), the beet armyworm, is an important agricultural pest insect. They are generalist pests, which means they can feed on a wide range of host plants including numerous important crop species such as corn, cotton, soybeans, peanuts, cabbage, tomatoes and peppers (Pearson, 1982). The broad host range requires *S. exigua* caterpillars to have mechanisms to protect themselves against diverse plant defenses. There are already some results suggesting that the diets can affect the activity of several *S. exigua* caterpillar enzymes. For example, caterpillars fed on Chinese cabbage had higher carboxylesterase activity compared to those fed on maize, sweet peppers and asparagus lettuce (Zhang et al., 2011).

In this research, *S. exigua* caterpillar responses to the plants *Arabidopsis thaliana*, *Medicago truncatula* or *Solanum lycopersicum* are compared to insects kept on artificial diet or starved. Specifically, the activity of GOX, APOX, GST, carboxylesterase and trypsin were analyzed. For enzymes that showed diet-specific difference, the responsible aspect of the diet, such as the nutritional quality or plant extract, was further investigated.

1.2. Hypotheses

Feeding on different plant diets, generalist *S. exigua* caterpillars face varying qualities and quantities of secondary metabolites and nutritional quality. Therefore, the activity of anti-oxidative, detoxifying and digestive enzymes may change depending on the diet.

1.3. Objectives

A. Identify the effect of plant diet (*A. thaliana*, *M. truncatula* or *S. lycopersicum*), artifical diet or starvation on the glucose oxidase (GOX) or ascorbate peroxidase (APOX) activity in *S. exigua* caterpillar labial salivary glands and the glutathione *S*-transferase (GST), trypsin or carboxylesterase activity in *S. exigua* caterpillar guts.

B. Identify the effect of dietary nutritional quality on enzymes that showed a diet-specific difference.

C. Identify the effect of a *A. thaliana* methanol extract on gut-associated enzymes that showed a diet-specific difference.

2. Literature Review

2.1. Plant-insect interactions

2.1.1. Plant defenses against caterpillar herbivory

Plants have evolved diverse defensive mechanisms against phytophagous insect herbivores. Plant defenses, physical or chemical defenses, can be broadly categorized into two strategies: direct defenses or indirect defenses (Karban and Baldwin, 1997).

2.1.1.1. Direct plant defense

Plants can protect themselves against herbivorous insects by directly negatively affecting their preference, such as the selection of host plants or other behaviors like oviposition and feeding or insect performance, such as growth, development and reproduction (Howe and Schaller, 2008). Direct plant defense against herbivorous insects include morphological features serving as physical defenses, such as thorns and spines or chemical defenses, such as secondary metabolites, digestibility reducing proteins and anti-nutritive enzymes (Howe and Schaller, 2008). All of these traits can be expressed constitutively as preformed resistance factors or induced in response to the attack of insect herbivores (Karban and Baldwin, 1997).

Constitutive defenses do not require insect attack to be produced. There is wide variation in the composition and concentration of constitutive defenses, which can range from mechanical defenses to chemical protection (Karban and Baldwin, 1997).

Induced resistance occurs in response to insect damage and these plant responses can lead to reduced preference and/or performance of the herbivores (Karban and Myers 1989). Like constitutive defenses, these inducible plant defenses include morphological features and chemical composition (Walling, 2000; Gatehouse, 2002; Howe and Schaller, 2008). For example, levels of proteinase inhibitors (PIs) in tomato and potato plants increase under insect attack or mechanical wounding (Green and Ryan 1972). Many of these inducible traits are also present constitutively, which means that these two protective mechanisms are tightly connected (Stahl, 1888; Fraenkel, 1959). For example, the trichomes on the plants' surface can protect the plants by acting as physical barriers to prevent small insects from contacting the leaf

surface or limit their movement (Wilkens et al., 1996; Chu et al., 2003). Glandular trichomes often cantains substances that repel insect herbivores or deter them from feeding (antixenosis) (Wilkens et al., 1996; Chu et al., 2003, Ranger et al., 2004; Wang et al., 2004; Hare, 2005). Generally, they are constitutively produced in plants and are effective at deterring insect herbivory (Myers and Bazely 1991; Schoonhoven et al. 2005). However, trichome density in some plant species can increase in response to insect feeding, therefore, trichomes are also considered as an inducible resistance trait (Bjorkman and Anderson, 1990; Agren and Schemske, 1993; Fernandez, 1994).

Moreover, like increased trichome density, most inducible defensive traits are not only restricted to the site of attack but also expressed in non-infested (systemic) parts of the plant (Green and Ryan 1972; Ryan and Moura 2002). Therefore, a signal must be generated locally as a consequence of insect feeding and transported to distant sites of the plants and induce plant defense throughout the plant (Howe and Schaller, 2008).

2.1.1.2. Indirect plant defense

Unlike direct plant defense that directly affects the preference and performance of the insect herbivores, indirect plant defense protects the plants by attracting natural enemies of the herbivores (Heil, 2008). For example, in response to caterpillar herbivory, many plants release volatile compounds that attract arthropods, birds and predators as well as parasitoids of the herbivore (Heil, 2008). By doing this, the host plant can limit the amount of damage caused by herbivores (Janzen, 1966; van Loon et al., 2000; Fritzsch Hoballah and Turlings, 2001; Tooker and Hanks, 2006). In addition to releasing volatiles, some plant species attract predatory arthropods by providing shelter, such as leaf domatia or offering attractive food sources, such as food bodies or extrafloral nectar (Heil, 2008; Koptur, 1992).

2.1.1.3. Plant secondary metabolites

Among the mechanisms employed by plants to defend themselves against insect herbivory, the presence of constitutive or induced secondary metabolites is of key importance (Tallamy and Raupp, 1991). Unlike primary metabolites, secondary metabolites are organic compounds that are not directly involved in the normal growth, development or reproduction of plants (Fraenkel, 1959; Whittaker and Robert,

1970). By increasing plant fitness, these secondary metabolites play an important role in plant adaptation to their environment (Bourgaud et al., 2001). There are several ways these chemicals increase plant fitness. For example, they can be antibiotic, antifungal or antiviral, and, thus, these chemicals are able to protect plants from pathogens or herbivores (Bourgaud et al., 2001). They may also suppress germination or growth of other plants (allelopathy) (Bourgaud et al., 2001). Therefore, these secondary metabolites are multifunctional compounds that target many biological macromolecules, such as proteins (enzymes, receptors, ion-channels or structural proteins), nucleic acids or membranes (Harborne, 1993).

Plant secondary compounds are usually classified according to their biosynthetic pathways (Harborne, 1999). Generally, there are three large molecule classes: phenolics, terpenes and steroids and alkaloids (Bourgaud et al., 2001). Specific groups of compounds are often associated with plant families (Bennett and Wallsgrove, 1994). For example, *Solanaceous* plants, such as tomatoes, often contain alkaloids in their trichomes (Bennett and Wallsgrove, 1994; Duffey and Stout, 1996). *Arabidopsis* plants use the glucosinolate-myrosinase system to protect themselves, while *Medicago* sp. contain protective saponins (Bennett and Wallsgrove, 1994; Bones and Rossiter, 2006; de Geyter et al., 2007). All of these plant examples use proteinase inhibitors (PIs) as part of their defense (Richardson, 1977). These compounds and proteins can act as feeding deterrents or cause detrimental effects on insect growth, development and reproduction (Tallamy and Raupp, 1991).

2.1.1.4. Plant nutritional quality

Generalist insects are able to feed on diverse plant species that vary in their nutritional quality and secondary metabolites. Plant nutritional quality can affect the preference and performance of herbivorous insects (Awmack and Leather, 2002). In this situation, insect herbivores need to choose the optimal diet for their growth, reproduction and/or fitness (Scriber and Slansky, 1981; Awmack and Leather, 2002). The ratio of dietary protein-to-digestible carbohydrate (P:C) is one component that can be selected by insect herbivores, such as caterpillars and locusts (Simpson and Abisgold, 1985; Lee et al., 2002 and 2003). For example, previous results from our lab show that caterpillars of the beet armyworm, *S. exigua*, prefer a protein-biased diet of 22P:20C (Merkx-Jacques et al., 2008). Dietary proteins are important to insects as they provide the nitrogen needed by insects for growth and reproduction

(Lee, 2007). For example, *S. exigua* caterpillars do not grow well on a diet containing less than 0.6% casein (Broadway and Duffey, 1986b). To prevent insect herbivores from obtaining enough protein, plants often produce PIs that bind to and inhibit digestive proteases in the insect gut (Broadway, 1995). Moreover, some jasmonic acid-inducible plant proteins, such as arginase and threonine deaminase, also disrupt digestive processes in the insect gut by catabolizing essential amino acids (Chen et al., 2005).

As well, certain nutritional factors of host plants affect the amount of food consumed. For example, older and tough leaves, which often contain increased fiber and lignin than younger and tender leaves, can wear down the cutting surface of the mandibles of the imported willow leaf beetle, *Plagiodera versicolora* (Raupp, 1985). Thus, it can reduce the feeding rate of the beetles and, ultimately, result in declined performance (Raupp, 1985).

After the food has been ingested, it needs to be digested and absorbed by the insect. According to Turunen (1985), this process requires dietary water which may be limiting for caterpillars feeding on leaves. After the guts absorbs these nutrients, the insect allocates a portion of them to growth and nutrient accumulation with the remaining portion to be used to supply energy and for metabolic processes (Slansky, 1990). During this process, certain essential nutrients are required such as linolenic acid (C18:3. Bracken 1982; Turunen 1983). The lack of linolenic acid results in poor adult emergence and wing deformities in several Lepidoptera species (Turunen 1983).

2.1.2. Insect resistance against toxic chemicals

To detoxify plant allelochemicals, there are generally three mechanisms used by insect herbivores, namely behavioral adaption, modified physiological processes and biochemical resistance mechanisms (Brattsten, 1988).

Behavioral adaption refers to insect behaviors that reduce their exposure to toxic compounds (Brattsten, 1988; Sparks et al., 1989). Behavioral resistance mechanisms can be further subdivided into stimulus-dependent mechanisms that require contact with the allelochemicals, such as increased repellency and irritancy, and stimulus-independent mechanisms, such as exophily (Georghiou, 1972; Lockwood et al., 1984).

Physiological resistance is when insect herbivores can reduce the toxicity of plant chemicals through insect physiology. Compared to biochemical resistance, in

physiological resistance, chemicals are not broken down, but instead the insects cope with the chemicals by altering one or more physiological functions, such as rapid excretion of ingested deterrents or by sequestrating components in body compartments (Brattsten, 1988; Sparks et al., 1989). Also, insects may have proteins that bind to plant toxins (Brattsten, 1988; Sparks et al., 1989). Another insect physiological mechanism is to strengthen the integument to reduce the absorption of plant compounds (Brattsten, 1988; Sparks et al., 1989).

The third category of insect resistance is biochemical resistance (Brattsten, 1988). This is probably the most important for insects. Biochemical resistance in herbivorous insects occurs by either increasing detoxifying enzymes and/or by reducing target site sensitivity (Li et al., 2007). Target-site insensitivity is a widely occurring counter defense used by insects mainly against plant-derived toxins of the nervous system such as sodium-potassium ATPases (Vaughan and Jungreis, 1977; Moore and Scudder, 1985).

Associated with insect guts and other tissues, a large number of enzymes operate in concert to detoxify plant defensive compounds, such as cytochrome P_{450} monooxygenases, transferases and hydrolases (Brattsten, 1988; Glendining, 2002). Generally, there are two steps that lead to the detoxification of these toxic chemicals by the insect (Ahmad and Hopkins, 1993). Plant compounds are either converted to more hydrophilic products or further conjugated with glutathione, glucose or sulfate before being excreted (Ahmad and Hopkins, 1993). For example, insects detoxify plant phenolics by adding glucose to the phenolic compounds, catalyzed by the enzyme phenol β-glucosyltransferase (Ahmad and Hopkins, 1992, 1993). Conjugation with glucose results in increased water solubility and more rapid excretion (Smith, 1968; Ahmad et al., 1986; Ahmad and Hopkins, 1992, 1993). Insects have many gut-associated enzymes involved in this process such as glucosyltransferase (Yang, 1976).

These enzymes can be regulated by many ways. Insects can increase gene expression. For example, the African malaria mosquito, *Anopheles gambiae* increases *GST* gene expression when they are exposed to hydrogen peroxide (H_2O_2. Ding et al., 2005). Another strategy used by the stable fly, *Stomoxys calcitrans,* is to activate zymogens through catalytic processing (Moffat and Lehane, 1990). *S. calcitrans* store trypsin in a zymogen form to avoid proteolytic damage to their guts. After a blood meal, these flies activate zymogens to digest proteins (Moffat and Lehane, 1990). The signal to initiate the processing may be the ingested proteins (Lehane et al., 1995).

Another level of regulation is through enzyme secretion. When the beet armyworm, *S. exigua* caterpillars are fed on balanced or nutritionally-poor diets, secretion pathways are accelerated compared to those fed a protein-rich diet (Afshar et al., 2013). This may be the reason for the lower LSG GOX activity observed in *S. exigua* caterpillars fed a protein-poor diet (Afshar et al., 2013).

2.1.3. Insects can suppress inducible plant defenses

The major strategy insects have to cope with plant noxious componuds are detoxification enzymes; however, another strategy used by some insect species is to suppress the induction of these chemical defenses. According to Reymond et al. (2000), many genes, such as those involved in the synthesis of aromatic metabolites (e.g., *CCR* and *COMT*) are strongly induced by mechanical damage but lower when *Arabidopsis* was attacked by the caterpillars of the European cabbage butterfly, *Pieris rapae*. In *Nicotiana attenuata,* wound-induced gene expression of proteins involved in defense signaling and accumulation, were repressed by feeding of the tobacco hornworm, *Manduca sexta* compared to mechanical wounding (Samach et al., 1995; Hamberg et al., 1999; Van der Hoeven and Steffens, 2000; Hermsmeier et al., 2001; Schittko et al., 2001).

How feeding of some insect species help to minimize the activation of a subset of defense-related genes is not well understood. However, research points to the involvement of effectors in the oral secretions (OS) of some insect species that can prevent the plant from mounting an inducible response (Eichenseer et al., 1999; Felton and Eichenseer, 1999; Kahl et al., 2000; Musser et al., 2002; Wu et al., 2012). For example, the OS from *M. sexta* applied to wild tobacco, *N. attenuata*, prevents the production of the defensive alkaloid nicotine (Winz and Baldwin, 2001). OS secreted onto the plant during feeding are a combination of gut-derived regurgitant, secretions from the ventral eversible gland (VEG) and salivary secretions from the labial and mandibular salivary glands (Peiffer and Felton, 2009; Zebelo and Maffei, 2012). Proteomic analysis of OS from caterpillars, such as the corn earworm, *Helicoverpa zea* revealed that glucose oxidase (GOX) is the most abundant protein identified in the labial salivary glands and it is believed to play a role in suppressing the induction of defenses in *Nicotiana tabacum*, *N. attenuata*, *Medicago truncatula* and *Arabidopsis thaliana* (Musser et al., 2002; Zong and Wang, 2004; Bede et al., 2006; Diezel et al., 2009; Tian et al., 2012). The mechanism underlying this is unknown but it is believed

that the hydrogen peroxide (H_2O_2) generated by GOX is an upstream signal activating the salicylic acid burst which attenuates jasmonate- and ethylene-induced defenses (Diezel et al., 2009; Weech et al., 2008).

2.2. Caterpillar detoxification enzymes and effectors involved in the suppression of plant induced defenses

Caterpillar enzymes involved in the detoxification of plant defense compounds or in the suppression of their induced biosynthesis predominantly originate from the guts or the labial salivary glands (LSGs).

2.2.1. Caterpillar labial salivary glands and associated enzymes

Caterpillars have two types of salivary glands, the mandibular salivary glands and LSGs. The tubular LSGs found in caterpillars are long, paired tubes, terminating blindly at their posterior ends and joining together at the anterior ends to form a short common duct that opens in the labium near the mouth (Hakim and Kafatos, 1974). The epithelial layer of the labial salivary glands is one cell thick and is covered by a basement membrane (House and Ginsborg, 1985). In these tubular glands, the posterior portion of each gland is responsible for enzyme secretion (Akai, 1998). The short anterior portion, a cuticle-lined duct, conveys the secretion to the common duct, and to the outside releasing the secretions from the spinneret (Akai, 1998).

2.2.1.2. The important enzymes of the labial salivary glands

Enzymes associated with caterpillar LSGs include GOX, which is the most abundant protein among the proteins identified in the LSGs of the corn earworm, *Helicoverpa zea* (Tian et al., 2012). There are also other enzymes such as carboxylesterase, ecdysone oxidase, ascorbate peroxidase and fructosidase (Mathews et al., 1997; Tian et al., 2012). As well, Liu et al. (2004) identified lysozyme.

2.2.1.2.1. Glucose oxidase

GOX is an oxidoreductase that catalyzes the oxidation of β-D-glucose to D-glucono-β-lactone and hydrogen peroxide (Kleppet 1966). This enzyme is highly specific for β-D-glucose and does not act on α-D-glucose (Kleppet 1966). So far, GOX has been detected in the salivary secretions and/or glands of many insect species, such as caterpillars of *S. exigua*, the corn bollworm, *Helicoverpa armigera*,

the tobacco budworm, *Heliothis assulta* and the corn earworm, *Heliothis zea* (Eichenseer et al., 1999; Zong and Wang, 2004; Merkx-Jacques and Bede, 2005). Among these insects, generalists seem to have relatively high GOX activity compared to specialists (Eichenseer et al., 2010).

The reason why caterpillar labial saliva contains GOX is not understood, however, a number of theories have been proposed. Since GOX utilizes oxygen in its reaction, one hypothesis is that it helps to maintain a relatively anaerobic midgut environment which will help lower the reactivity of plant-derived quinones that can react with digested proteins preventing their absorption (Ciucu and Patroescu, 1984; Felton and Duffey, 1991a, b; Felton and Gatehouse, 1996). Another idea is that the antimicrobial hydrogen peroxide (H_2O_2) generated by this reaction can kill insect pathogens present on the plant leaf surface (Eichenseer et al., 1999; Musser et al., 2005). The third theory behind the action of GOX involves the suppression of plant-induced defenses (Felton and Eichenseer, 1999; Musser et al., 2005; Diezel et al., 2009; Musser et al., 2002; Zong and Wang, 2004; Bede et al., 2006). Since generalist insects seem to have relatively high GOX activity compared to specialists, GOX may be also involved in expanding the host plant range by insects (Eichenseer et al., 2010). In addition, by converting glucose to gluconate, a carbohydrate that cannot be utilized by the insect, GOX may allow caterpillars to cope with the detrimental effects of excess carbohydrate consumption since plants often contain sufficient or excess carbohydrates, such as sucrose, with limited quantity and/or quality of proteins (Bede et al., 2006; Matteson, 1980; Scriber and Slansky, 1981; Warbrick-Smith et al., 2006; Felton, 1996; Karowe and Martin, 1989).

Dietary carbohydrate levels can affect transcription of *SeGOX* in the beet armyworm, *Spodoptera exigua*, which encodes GOX. Afshar et al. (2010) shows that the transcript levels of *SeGOX* increased when dietary carbohydrate increased, regardless of protein concentrations. The GOX activity from LSGs of *H. armigera* is significantly increased by higher content of sugar in the diets (Hu et al., 2008). Hu et al. (2008) also found that plant secondary metabolites did not affect GOX activity. They found that some phenolic compounds, such as chlorogenic acid, rutin and quercetin, added to artificial diets had no effect on GOX activity of labial salivary glands after *H. armigera* were fed on these diets. However, GOX activity only increased with increasing dietary carbohydrates when caterpillars were fed protein-rich diets, not on protein-poor diets (Afshar et al., 2010). This may be because when *S.*

exigua caterpillars are fed on nutritionally-poor diets, secretion pathways are accelerated compared to those fed a protein-rich diet (Afshar et al., 2013).

2.2.2. Caterpillar gut and associated enzymes

2.2.2.1. Caterpillar gut

The gut of herbivorous caterpillars is a cylinder that connects the mouth with the anus (Dow, 1986). Caterpillar guts are simple tubular guts with vestigial foreguts and a well-developed midgut needed to process large quantities of plant tissues (Dow, 1986). Gut pH values are important because they can affect enzyme activity involved in digestion or control of solubility and toxicity of ingested poisons, and they can also influence the gut flora (House 1974). Lepidopteran larvae usually have alkaline gut pH values (Berenbaum, 1980). Some plant-derived compounds act by targeting the gut causing either oxidative stress or reduced digestion, lowering the nutritional intake (Krishnan and Kodrík, 2006; Bennett and Wallsgrove, 1994). To cope with this, insects activate digestive enzymes and have antioxidant enzymes (Ahmad, 1986). Gut-associated enzymes, such as glutathione *S*-transferase, can transfer groups onto plant secondary compounds increasing their hydrophilicity to enhance their excretion from the insects (Habig et al., 1974). Therefore, three kinds of gut enzymes are important for insects: detoxifying enzymes, antioxidant enzymes and proteases.

2.2.2.2. Important enzymes of caterpillar guts

2.2.2.2.1. Glutathione *S*-transferase

Glutathione *S*-transferases (GSTs) are a major family of multifunctional detoxification enzymes (Vontas et al., 2001). GSTs catalyze the conjugation of the reduced glutathione (GSH), via its sulfhydryl group, to the electrophilic center of a wide variety of substrates, such as many plant-derived toxins or insecticides (Vontas et al., 2001). The resultant products are more water-soluble metabolites that are more readily excreted (Habig et al., 1974). GSTs can metabolize insecticides by facilitating their reductive dehydrochlorination with reduced glutathione as a cofactor rather than a conjugate (Clark and Shamaan, 1984). Dehydrochlorination is an important mechanism for detoxification of 1,1,1-trichloro-2,2-bis-(*p*-chlorophenyl) ethane (DDT) (Clark and Shamaan, 1984). Since some GSTs can detoxify lipid hydroperoxides, α,β-unsaturated aldehydes, lipid epoxides and may involve in the

repair of radical-damaged DNA, this group of enzyme is critical in protecting insects against oxidative stress (Hayes and Pulford, 1995; Hayes and Strange, 1995; Tew and Ronai, 1999). Therefore, several mechanisms might work together to detoxify xenobiotics. For example, in the case of pyrethroid resistance, GSTs protect insects either by offering a passive protection through binding the insecticide molecules or by detoxifying lipid peroxidation products induced by pyrethroids (Kostaropoulos et al., 2001; Vontas et al., 2001).

The diversity of reactions catalyzed by GSTs is a result of the broad substrate specificities of many individual GST enzymes (Enayati et al., 2005). In insects, GSTs can be categorized into three classes (I, II, and III) (Kostaropoulos et al., 2001; Ranson and Hemingway, 2005; Tu and Akgul, 2005). GSTs diversity in insects may be caused by gene duplications, alternative splicing and genetic rearrangements (Ranson et al., 2002; Ranson et al., 1998; Ding et al., 2003; Zhou and Syvanen, 1997). GST expression and activity also show tissue- and developmental specificity (Enayati et al., 2005).

GST genes and activity can be induced by plant allelochemicals or insecticides. For example, Deng et al. (2009) showed that expression of *Slgste2*, one glutathione *S*-transferase cDNA of the Oriental leafworm moth, *Spodoptera litura*, was up-regulated by some insecticides, such as 1-naphthyl methylcarbamate (carbaryl), DDT, deltamethrin, tebufenozide and *Bacillus thuringiensis* (Bt). GSTs activity increased when the English grain aphid, *Sitobion avenae* larvae were fed on resistant wheat cultivar, which had high concentration of phenolic compounds (Leszczynski et al., 1994). Caterpillars of the gypsy moth, *Lymantria dispar* and forest tent caterpillar, *Malacosoma disstria* fed on aspen leaves supplemented with phenolic glycosides had increased GSTs activity compared to the control group (Hemming and Lindroth, 2000). GST activity of the Oriental tobacco budworm, *Helicoverpa assulta* larvae fed on chili pepper was lower than those fed on tobacco or artificial diet (Wang et al., 2009). The fruit fly, *Bactrocera tau* fed on balsam pear has higher GST activity compared to those fed on cucumber, pumpkin, towel gourd and white gourd (Li and Liu, 2007). Even fed on different cultivars of the same species, the activity of GSTs of insects can vary (Sintim et al., 2012). Sintim et al. (2012) showed that when three insect species, the grasshopper, *Atractomorpha lata*, the green peach aphid, *Myzus persicae* and the diamondback moth, *Plutella xylostella* fed on eight cultivars of sesame, they had different GST activity. This may be due to that

different cultivars produced diversed quantity and quality of chemicals in response to these insect herbivores (Sintim et al., 2012).

2.2.2.2.2. Trypsin

Plants often protect themselves against insect herbivores by proteinase inhibitors (PIs), which act on insect gut-associated proteinases (Bernays and Chapman, 1994; Felton and Gatehouse, 1996; Stotz et al., 1999; Carlini and Grossi-de-sá, 2002). Ingestion of plant-derived PIs by insects will impede protein digestion and lead to a decrease in bioavailability of essential amino acids required by the insect for growth, development and reproduction (Broadway and Duffey, 1986a, b; Broadway, 1995; Koiwa et al., 1998; Zhu-Salzman et al., 2003). For example, using the artificial diet containing soybean trypsin inhibitor to rear the sugarcane borer, *Diatraea saccharalis*, led to a delay of larval development, increasing the length and number of instars and decreasing female longevity (Pompermayer et al., 2001).

One common target of these inhibitors is serine proteinases, such as trypsin. Trypsin preferentially cleaves internal peptide bonds on the carboxyl side of a positively charged amino acid, like lysine or arginine (Evnin et al., 1990).

To overcome trypsin inhibitors in their diet, insects have several mechanisms such as expressing new proteinases that are insensitive to the inhibitor (Mazumdar-Leighton and Broadway, 2001a,b). The corn earworm, *Helicoverpa zea* larvae express two different trypsin isozymes depending on if larvae are fed on control or inhibitor-containing diet (Volpicella et al., 2003). The tobacco budworm, *Heliothis virescens* larvae also vary their complement of trypsin enzymes when fed on control or inhibitor-containing diet (Brito et al., 2001). Herbivorous insects can also regulate midgut trypsins by differential regulation of multiple genes encoding different digestive proteinases (Broadway, 1995; Jongsma and Bolter, 1997; Bown et al., 2004). For example, in response to dietary inhibitors, like soybean Kunitz trypsin inhibitor, there was an initial up-regulation of all proteinases genes in the caterpillars of the cotton bollworm, *Helicoverpa armigera*, which is followed by a down-regulation of genes that encode proteinases sensitive to the inhibitors but sustained expression of genes encoding inhibitor-insensitive proteinases (Bown et al., 1997).

2.2.2.2.3. Carboxylesterase

Carboxylesterases are hydrolases that use water molecules to cleave ester bonds turning target chemicals into corresponding alcohols and acids (Wheelock et al., 2005). In addition, some carboxylesterases also hydrolyze phosphoester and amide bonds (Wheelock et al., 2005). Carboxylesterase is a widely spread group enzyme in the insect kingdom and so far, in the ESTHER database, there are more than 318 nucleotide sequences for genes that encode carboxylesterase (Hotelier et al., 2004). Carboxylesterase can be divided into 14 major clades (Oakeshott et al., 2005). The oldest group is the neuro-developmental class (Oakeshott et al., 2005). The second group is the secreted catalytic class (Oakeshott et al., 2005). The third group is the intracellular catalytic class with dietary detoxification functions (Oakeshott et al., 2005).

In insects, carboxylesterases are important for insecticide resistance. Three different classes of agrochemicals, pyrethroids, organophosphates (OPs) and carbamates, can be detoxified by carboxylesterases (Ahmad, 1986; Casida and Quistad, 1998; Shan and Hammock, 2001; Oakeshott et al., 2005). Midgut carboxylesterase activity increases when caterpillars of the tobacco budworm, *Heliothis virescens,* are exposed to profenofos (Harold and Ottea, 1997). In general, carboxylesterases bind to the substrates and hydrolyze them (Wheelock et al., 2005). In the Australian sheep blowfly, *Lucilia cuprina*, substitution of amino acids at the acyl pocket of the carboxylesterase increased the overall activity compared to the wild type of enzyme (Devonshire et al., 2007).

In insects, carboxylesterases are regulated in many ways, including gene amplification, selection for and expression of mutant carboxylesterases and enhanced transcription of non-amplified, structural genes (Wheelock et al., 2005). For example, overproduction of carboxylesterase *E4* or its paralog *FE4* protein enables the green peach aphid, *Myzus persicae* to degrade diverse insecticides including OPs, carbamates, and pyrethroids (Field and Devonshire, 1998). Carboxylesterases can be induced by plant allelochemicals such as rutin, the indole alkaloid gramine etc. (Ghumare et al., 1989; Gao et al., 1998; Mu et al., 2006; Cai et al., 2009). The increased carboxylesterase activity is probably because some xenobiotics or insecticides can induce the encoding genes. For example, Poupardin et al. (2008) found one gene encoding a carboxylesterase (*CCE*) was significantly induced in fourth stage larvae of the yellow fever mosquito, *Aedes aegypti* larvae following the

exposure to xenobiotics or insecticides (atrazine, copper, fluoranthene, permethrin and temephos).

Plant diet can affect herbivore carboxylesterase activity. For example, in the Oriental tobacco budworm, *Helicoverpa assulta*, larvae fed on chili pepper have lower carboxylesterase activity than those fed on tobacco and artificial diet (Wang et al., 2009). In the beet armyworm, *S. exigua*, carboxylesterase activity was the highest in larvae feeding on Chinese cabbage, but decreased by nearly 60% if caterpillars were fed on maize seedlings (Zhang et al., 2011). In the silverleaf whitefly, *Bemisia tabaci*, populations on cabbage had higher carboxylesterase activity levels compared with garden egg populations (Avicor et al., 2013). Insecticides added into diets can also increase carboxylesterase activity (Gao and Liang, 1993).

2.2.2.2.4. Ascorbate peroxidase

When caterpillars feed on plants, plant allelochemicals can cause oxidative stress (Bolwell and Wojtaszek, 1997; Mittler, 2002). The high levels of reactive oxygen species (ROS), such as superoxide anion radicals, singlet oxygen, hydrogen peroxide and highly reactive hydroxyl radicals can negatively affect insects in several ways (Waris and Ahsan, 2006). They can damage the cells by reacting with the membrane lipids and this will impair the absorption of ingested nutrients in the midgut (Ahmad and Pardini, 1990; Bi and Felton, 1995). Proteins are also vulnerable to oxidative damage. This can lead to protein denaturation (Stadtman, 1990, 1991; Dean, 1991). ROS also react with DNA, which can lead to mutations (Pardini, 1995).

Hydrogen peroxide (H_2O_2) is an important ROS. In addition to direct toxicity toward herbivores, it can also protect plants by contributing to cell wall strengthening, triggering the hypersensitive response in plant protection against pathogens or acting as signal molecule for the induction of defense genes (Wu et al., 1995; Mehdy et al., 1996; Kuźniak and Urbanek, 2000). Ascorbate peroxidase (APOX) can reduce H_2O_2 levels by converting it to H_2O (Asada, 1992). In insects, APOX is more efficient than catalase to catalyze the breakdown of H_2O_2 (Summers and Felton, 1993; Barbehenn et al., 2001). APOX may also scavenge lipid peroxides (Mathews et al., 1997). APOX has been detected in the gut fluid of several species such as the forest tent caterpillar, *M. disstria*, the white-marked tussock moth, *Orgyia leucostigma* and the corn earworm, *H. zea* (Mathews et al., 1997; Barbehenn et al., 2001).

Insect gut APOX activity can be affected when plant allelochemicals like *o*-

dihydroxyphenols and tannic acid are added to the diets (Barbehenn, 2002; Lukasik et al., 2009). Caterpillars of the Egyptian cottonworm, *Spodoptera littoralis* fed on potato leaves rich in allelochemicals, such as chlorogenic acid and tannins, have higher APOX activity in comparison to those reared on semi-artificial diets (Krishnan and Kodrík, 2006). The level of APOX activity was higher in the African maize stalk borer, *Busseola fusca* larvae fed on non-transformed maize plants compared to those fed on *Bt* maize plants (George and Gatehouse, 2013). However, this probably does not reflect ROS levels since control plants do not produce higher levels of H_2O_2 than transgenic plants (George and Gatehouse, 2013).

2.3. Experimental models

2.3.1. Caterpillars

Spodoptera exigua (Hübner), the beet armyworm, is a well-known agricultural pest. The beet armyworm originated in Southeast Asia but has now spread all over the world with a wide range of host plants including numerous important crop species, such as corn, cotton, soybeans, peanuts, cabbage, tomatoes and peppers (Pearson, 1982). The life cycle of this insect can be completed in as few as 24 days (Wilson, 1934). The voracious larvae feed on both plant foliage and fruit. When the caterpillars feed on the host plants, they secret enzymes (Wilson, 1934). Although the whole profile of these enzymes are not well understood, several enzymes have been characterized such as detoxification enzymes like GST (Wang et al., 2006).

2.3.2. Plants

Generally, plants in different families have distinctive phytochemicals to defense themselves (Bennett and Wallsgrove, 1994). Responses of caterpillar to three plant diets were compared: these plants are *Arabidopsis thaliana*, *Medicago truncatula* or *Solanum lycopersicum* that protect themselves with glucosinolates, saponins and alkaloids, respectively.

2.3.2.1. *Arabidopsis thaliana*

A. thaliana is a member of the Brassicaceae family native to Europe and Asia (Mitchell-Olds, 1995). With the relatively short life cycle and small genome, *Arabidopsis* is a popular model organism in plant biology and genetics. *Arabidopsis* contains aliphatic, aromatic and indole glucosinolates as defensive chemicals (Hogge

et al., 1988). Glucosinolates are a class of over 130 organic compounds that contain sulfur and nitrogen and are derived from glucose and amino acids (Halkier and Gershenzon, 2006). They occur as secondary metabolites of plants almost exclusively from the order Capparales, which contains 15 families, including the Brassicaceae, Capparaceae, and Caricaceaein (Rodman et al., 1996). Glucosinolates are sulfur-rich thioglucosides, while myrosinases are thioglucosidases that hydrolyze glucosinolates. Glucosinolates occur throughout the tissues of the plant, whereas myrosinases are only found in scattered myrosin cells (Kelly et al., 1998). Thus, these two components are found in different cells within the same tissue. However, when caterpillars feed on the plant and damage the tissue, these two materials come into contact resulting in the hydrolysis of the thioglucosidic bond of glucosinolates by myrosinase leading to the release of glucose and an unstable aglycone (Chew, 1988; Rask et al., 2000; Bones and Rossiter, 2006). Depending on the plant species, the structure of the glucosinolate side chain, the reaction conditions and the presence of associated proteins, the aglycone rearranges to different biologically active products, including isothiocyanates, oxazolidine-2-thiones, nitriles and epithionitriles (Fenwick et al., 1983; Chew, 1988; Rask et al., 2000; Lambrix et al., 2001). Many of these hydrolysis compounds are toxic to insects, especially the isothiocyanates (Lichtenstein et al. 1962; Zhang et al., 1992; Vaughn and Berhow, 1998; Tierens et al., 2001). For example, Li et al. (2000) showed that allyl-isothiocyanates are toxic upon feeding to the diamondback moth, *Plutella xylostella* and the southern army worm, *Spodoptera eridania*.

2.3.2.2. *Medicago truncatula*

M. truncatula, a member of the Fabaceae family, is distributed mainly around the Mediterranean basin (Frugoli and Harris, 2001). This plant is a close relative of the important forage crop alfalfa, *Medicago sativa* (Frugoli and Harris, 2001). The relatively high level of synteny between legume genomes, together with its self-fertilising nature, short regeneration time and its capacity to be regenerated from cell culture lines make it an ideal legume model (Jorrin et al., 2006).

Medicago sp. contains numerous triterpenoid-derived saponins (Avato et al., 2006). The concentrations of saponins in *Medicago* is about 0.15-0.22% of the dry weight (Tava and Odoardi, 1996; De Geyter et al., 2007). These saponins are a mixture of triterpene glycosides with medicagenic acid, hederagenin, zahnic acid and

soyasapogenols as the main aglycone (Oleszek, 1996). Saponins can increase mortality levels, lower food intake, reduce weight, decrease reproduction and cause retardation and disturbances in development of pest insects (De Geyter et al., 2007). The negative effects of saponins on insects have been demonstrated on a wide variety of species including the flower beetle, *Tenebrio molitor*, the European grape moth, *Lobesia botrana*, the European corn borer, *Ostrinia nubialis* and a number of other insects (Pracros, 1982; Tava et al., 1992; Nozzolillo et al., 1997). The mechanism for this action is probably that saponins could either make the food less attractive to eat (repellent/deterrent activity), cause problems for digestion, cause moulting defects or have toxic effects on cells (De Geyter et al., 2007). Saponins form complexes with proteins and, by this mechanism, they stop and curb digestion in insect guts (Ishaaya and Birk, 1965; Potter et al., 1993). Saponins can form insoluble complexes with sterols, thereby preventing their absorption (Shany et al., 1970). Interference with sterol metabolism may affect the production of insect hormones such as ecdysteroids that control molting (Sehnal, 1989). They can interact with and permeabilize midgut cells, which can lead to a reduction in their ability to transport nutrients (Francis et al., 2002). All these insecticidal activities are exerted only when the sugar component is cleaved off by gut glycosylases to liberate the active aglycone (Adel et al., 2000).

2.3.2.3. *Solanum lycopersicum*

Tomato is an important vegetable crop in the family Solanaceae (Thakur et al., 1996). Tomato contains potentially toxic alkaloids, such as tomatine and solanine, as the main defensive chemicals (Hedin et al., 1974; Schoonhoven, 1972). Alkaloids are low molecular weight, nitrogen-containing organic compounds, usually with a heterocyclic structure (Hedin et al., 1974; Schoonhoven, 1972). Tomatine and solanine are steroid alkaloids and both of them are also glycoalkaloids. Tomatine is the most common alkaloid present in tomatoes. It was first isolated by Fontaine et al. (1948). Two tomatines, α-tomatine and dehydrotomatine, are present in all parts of the tomato plant but levels vary among different plant tissues (Friedman and Levin, 1998; Kozukue et al., 2004). Both of these compounds are found in low levels in tomato leaves compared to other tissues, such as, fruits and flowers, but the percentage of dehydrotomatine in leaves is high among all the tissues measured (Kozukue et al., 2004). Although trichomes generally contain chemical deterrents

against insect herbivores, α-tomatine and dehydrotomatine are not detected in the trichomes of cultivated tomato (Kang et al., 2010).

Tomatines slow growth of the Colorado potato beetle, *Leptinotarsa decemlineata*, delay the appearance and decreased the number of nymphs of the potato aphid, *Macrosiphum euphorbiae*, increase the rate of mortality of the potato leafhopper, *Empoasca fabae*, and decrease the number of hatching eggs of the diamondback moth, *Plutella xylostella* (Kowalski et al., 2000; Guntner et al., 1997; Dahlman and Hibbs, 1967; Lu and Chu, 1993). These alkaloids are believed to inhibit an important enzyme involved in nerve transmission, acetylcholinesterase (Rosenberry, 1975; Zhu and Clark, 1995).

3. Materials and Methods

3.1. Chemicals

Ingredients for the diets and chemicals for assays were all obtained from Sigma, unless otherwise indicated.

3.2. Caterpillar colony

The beet armyworm, *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae) were reared for multiple generations on an artificial wheat germ-based diet (Bio-Serv) in a growth chamber (16:8 light:dark hours; 28.5 °C). There are five instars of caterpillars before pupation under this growth condition. Pupae were collected and put in a glass jar. Adult moths were allowed to mate freely and then the collected eggs were used to maintain the colony.

3.3. Plants maintenance

Plants (*Arabidopsis thaliana*, *Medicago truncatula* and *Solanum lycopersicum*) were grown in a growth cabinet under the following conditions: 16:8 dark to light cycle at 22°C. Plants were fertilized 3 times per week with dilute 20-20-20 NPK (0.15 g per liter). *M. truncatula* was incubated with *Sinorhizobium medicae* (WSM419) when they were 2-weeks-old. At five weeks, these plants were used as a diet for caterpillars.

3.4. Experiment 1. Effect of diet on *Spodoptera exigua* caterpillar enzyme activity

3.4.1. Experimental design

Caterpillars were reared on BioServ artificial diet until early 4th instar. Then they were placed on plants, *A. thaliana* or *M. truncatula* or *S. lycopersicum*, for 48 hours. Five caterpillars were added on every pot containing three individual plants and all of the caterpillars were collected and pooled together for the dissection. As the control, some caterpillars were kept on BioServ artificial diet or starved. Tissues were dissected for enzyme assays. This experiment was repeated three times.

3.4.2. Tissue dissection

Prior to dissection, healthy and actively feeding caterpillars were anesthetized by placing them in a Petri dish on ice. Caterpillars were placed ventral side up. The head was gently removed from the rest of the body. Then the labial salivary glands

attached to the head can be removed. For the guts, a sterile pin was inserted under the head and through the end of the body to secure the insect to the mat. Then the body was cut by sterile scissors to remove the gut. Sterile buffer was used to lift the tissues off the mat and/or remove debris. The inside of the guts were also rinsed with the buffer. Pairs of labial salivary glands from 6 caterpillars or the guts for 2 caterpillars were removed and placed in ice-cold phosphate-buffered saline (pH 7.0, 2.7 mM KCl, 137 mM NaCl, 1.5 mM KH_2PO_4, 8 mM $Na_2HPO_4 \times 7H_2O$). After homogenization, samples were centrifuged at 13,000 g at 4°C for 10 min.

3.4.3. Protein determination

The soluble protein concentration of LSG and gut homogenates were measured by a modified Bradford assay (Zor and Selinger, 1996). A standard curve was prepared using bovine serum albumin (BSA) ranging from 50-1000 μg/ml. As well, a control consisted of reagent and 1 × PBS was used as blank. Protein samples and blanks were prepared in 96-well plate (Costar) and Bradford reagent was added to every well. The absorbance at 590 nm and 450 nm were measured by infinite M200 Pro microplate reader microplate reader. The ratio OD_{590}/OD_{450} was used to calculate the standard curve equation and sample protein concentrations.

3.4.4. Gel electrophoresis

12.5% polyacrylamide gels were used to separate proteins using non-denaturing conditions. For all separations, the ‘‘Mini-Protean II’’ electrophoretic cell (Bio-Rad) were used. Protein electrophoresis were performed at 4°C to prevent protein degradation. After locking the gel into the electrophoresis chamber, protein electrophoresis buffer (1 × Tris-glycine, pH 8.3. 0.192 M glucine, 25 mM Tris-HCl) was added to the tank. To every well, 12 μl sample previously mixed with 3 μl loading dye (Bromophenol Blue) was loaded. After electrophoresis, the gel was rinsed with dH_2O and placed carefully in square Petri dish containing with the appropriate enzyme assay (see below). The gel was gently shaken at room temperature until bands appeared. Then, the gel was rinsed again in dH_2O and photographed. Boiled samples were loaded as negative controls. Below, the substrates for staining different enzymes are outlined.

3.4.4.1. Glucose oxidase

GOX was stained with 0.6 M glucose, 60 U/ml horseradish peroxidase and 0.5 mM o-dianisidine at room temperature (Eichenseer et al., 1999). Fungal GOX (*Aspergillus niger*, Sigma) was used as a positive control.

3.4.4.2. Carboxylesterase

Carboxylesterase was stained with 2.5 mg/mL α-naphthylacetate and 2 mg/mL Fast blue B at room temperature (Lomolino et al., 2001).

3.4.5. Enzyme assays

Enzyme activity was measured spectrophotometrically using an infinite M200 Pro microplate reader. Samples were spotted in triplicate in 96-well plates.

3.4.5.1. Glucose oxidase

GOX activity was assayed by the *o*-dianisidine-horseradish peroxidase assay (Bergmeyer, 1974). The reaction mixture contained 0.1 mM β-D-glucose, 2.1 M *o*-dianisidine and horseradish peroxidase (final concentration 3 U) in 1 × PBS buffer. The glucose was made at least one hour before the assay to allow for complete mutarotation to occur. The homogenate was added to the reaction mixture. Absorbance was spectrophotometrically measured at 460 nm at 35°C for 1 min. Enzyme activity was calculated using the millimolar extinction coefficient of 11.3 cm^{-1} for *o*-dianisidine at 460 nm.

3.4.5.2. Ascorbate peroxidase

APOX activity was assayed by measuring the decrease in absorbance at 290 nm that occurs as ascorbate is oxidized (Asada, 1984). The change of absorbance was measured at 35°C for 2 min. The reaction mixture contained 1 × PBS buffer, 0.5 mM ascorbate, 1.0 M H_2O_2 and homogenate. Enzyme activity was calculated using the millimolar extinction coefficient of 2.8 cm^{-1} for ascorbate at 290 nm.

3.4.5.3. Glutathione *S*-transferase

GST activity was assayed using 1-chloro-2,4-dinitrobenzene (CDNB) as the substrate (Habid et al., 1974) . The reaction mixture contained 1 × PBS buffer, 4 mM GSH, 0.4 mM CDNB (0.1% (v/v) in ethanol). The change of absorbance was

measured at 340 nm at 35°C for 1 min. Enzyme activity was calculated using the millimolar extinction coefficient of 9.6 cm^{-1} for CDNB.

3.4.5.4. Trypsin

Trypsin activity was assayed using N-α-benzoyl-L-arginine ethyl ester (BAEE) as substrate (Shee and Sharma, 2007). The homogenate was mixed with 0.5 mg/ml BAEE in 1 × PBS buffer. Production of N-α-benzoyl-L-arginine was measured spectrophotometrically at 253 nm at 35°C for 1 min. Activity was calculated by using the millimolar extinction coefficient of 0.808 cm^{-1} for BAEE at 253 nm.

3.4.5.5. Carboxylesterase

Carboxylesterase activity was measured spectrophotometrically using *p*-nitrophenyl acetate (Cui et al., 1999). The homogenate was incubated with 1 mM substrate in 1 × PBS buffer, and generation of *p*-nitrophenolate was monitored at 405 nm at 35°C for 1 min. Enzyme activity was calculated using the millimolar extinction coefficient of 13.0 cm^{-1} for *p*-nitrophenolate at 405 nm.

3.5. Experiment 2. Effect of plant extract on *Spodoptera exigua* caterpillar enzyme activity

3.5.1. Experimental design

Caterpillars were reared on BioServ artificial diet until early 4th instar. Then they were placed on weighed blocks of different diets in Petri dishes for 48 hours. Diets were artificial diet, artificial diet with extracts of *Arabidopsis* that were previously fed upon by 4th instar caterpillars for 48 hours when they were 5-week-old or artificial diet with extracts of *Arabidopsis* without previous herbivory. For each diet, three caterpillars were reared. Petri dishes were closed with Parafilm to prevent water loss and placed in a growth chamber (16:8 light:dark hours; 28.5 °C). Tissues were dissected for enzyme assays. This experiment was repeated three times. Enzymes were assayed as described above.

3.5.2. Artificial diet with *Arabidopsis* extracts

Arabidopsis were extracted in methanol according to De Vos et al. (2007). *Arabidopsis* that were previously fed upon by 4th instar caterpillars for 48 hours and *Arabidopsis* without previous herbivory were extracted. The solvent used was 60%

methanol acidified with 0.208% formic acid. The cold extraction solution was added to frozen plant powder (volume/weight ratio of 5). After vortexing each tube for 10 s, samples were sonicated for 15 min at room temperature. After centrifugation at 3600 g at room temperature, samples were lyophilized for 48 hours to allow the methanol to evaporate. The extracts were redissolved in water. The water containing the plant extracts was added to pre-made artificial diets.

3.6. Experiment 3. Effect of nutritional quality (P:C) on *Spodoptera exigua* caterpillar enzyme activity

3.6.1. Diet nutritional quality

3.6.1.1. Plant diet nutritional quality

Plant samples of *A. thaliana* and *M. truncatula*, both of which had been fed upon by 4th instar *S. exigua* caterpillars for 48 hours before collection, were sent to Dr. Mustafa's lab in Department of Animal Science in McGill University to analyze the protein and neutral detergent fiber (NDF) content. Crude protein was analyzed using a Leco Nitrogen Analyzer. NDF was determined using an Ankom Fiber Analyzer. The following equation was used to calculate the protein-to-digestible carbohydrate (P:C) ratio of the plant samples (Sniffen et al., 1992). 100 = Crude Protein + NDF + Digestible Carbohydrates + Ash + Lipids. The content of the lipids is assumed to be 4% of the plant sample (Harwood, 1980). The estimated P:C ratio for *A. thaliana* is 25P:39C and P:C ratio for *M. truncatula* is 24P:17C.

3.6.1.2. Artificial diet nutritional quality

Previous calculations of nutritional content from our lab indicated that the protein-to-carbohydrate (P:C) ratio of the artificial diet (Bio-Serv) is 29P:37C (Merkx-Jacques et al., 2008).

3.6.2. Experimental design

Caterpillars were reared on BioServ artificial diet until early 4th instar. Then they were placed on weighed blocks of different diets in Petri dishes for 48 hours. Diets were artificial diet (Bio-Serv), artificial diet with a P:C ratio of 25P:39C and 24P:17C. Diets were prepared as described in section 3.6.3. Then caterpillars were removed on each diet. Petri dishes were closed with Parafilm to prevent water loss and placed in a growth chamber (16:8 light:dark hours; 28.5 °C). Tissues were

dissected for enzyme assays. This experiment was repeated three times. Enzymes were assayed as described above.

3.6.3. Artificial diet with different protein-to-digestible carbohydrate (P:C) ratios

Based on the estimated P:C ratio of *A. thaliana* and *M. truncatula*, artificial diet with the P:C ratios of 25P:39C and 24P:17C were prepared according to Simpson and Abisgold (1985). The carbohydrate source was glucose and the protein source was the mixture of a 3:1:1 ratio of casein (bovine milk, Bio-Serv), peptone and albumen (egg white, Bio-Serv). Cellulose was used as the non-digestible filler. Except for the carbohydrate and protein source, the rest of the diet constituents were: 0.55% cholesterol, which was solubilized in 0.5% linoleic acid, 2.5% Wesson's salts, 1% ascorbate, 0.5% choline chloride, 0.5% sorbic acid, 0.35% methyl paraben, and 0.06% USDA vitamin premix (F9220B, Bio-Serv). The diet was suspended in a 2% agar solution. The nutritional composition of USDA vitamin premix includes: 43.398% cerelose, 0.092% pantothenie, 0.05% riboflavin, 0.025% folic acid, 0.101% niacin, 0.022% thiamine, 0.021% pyridoxine, 4.18% choline, 50.105% ascorbic acid and 2.004% inositol. In the vitamin premix, there also are 0.002006 mg vitamin B12 and 0.02004 mg biotin.

3.7. Statistical analyses

Statistical analyses were performed by one-way analysis of variance (ANOVA) using SPSS version 21 (SPSS Inc.). Statistical differences were determined by a Tukey post-hoc test at the $p = 0.05$ significance level.

4. Results

4.1. Experiment 1. Effect of diet on *Spodoptera exigua* caterpillar enzyme activity

Caterpillars were reared on BioServ artificial diet until early 4th instar. They were then placed on plants, *A. thaliana* or *M. truncatula* or *S. lycopersicum*, for 48 hours. As controls, some caterpillars were kept on BioServ artificial diet or starved. Pairs of labial salivary glands from 6 caterpillars or the guts of 2 caterpillars were dissected for enzyme assays. There is no difference in the soluble protein concentrations in the LSGs or guts of caterpillars fed on different diets (Figure 1).

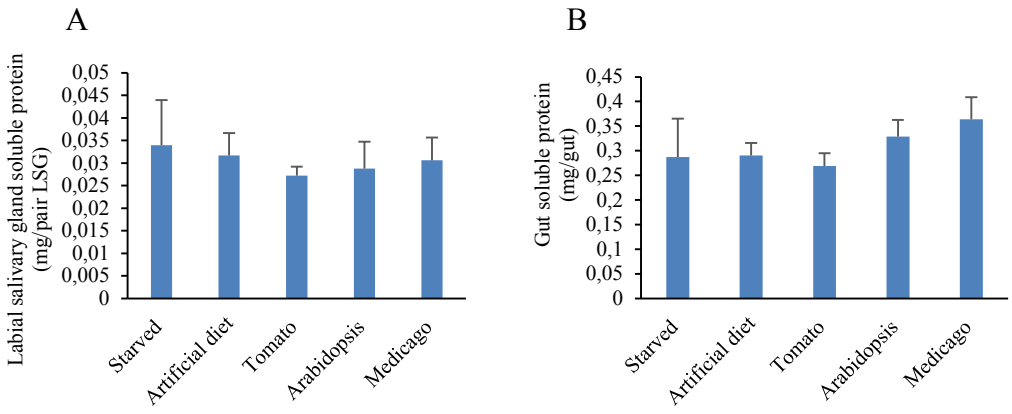


Figure 1. Soluble protein levels in guts or labial salivary glands of *Spodoptera exigua* caterpillars fed on different diets. 4th instar *Spodoptera exigua* caterpillars were transferred from artificial diet to respective diets or starved for 48 hours. Diets were artificial diet, *Arabidopsis thaliana*, *Medicago truncatula* or *Solanum lycopersicum*. The labial salivary glands or guts were dissected and pooled. The soluble protein concentrations were measured by a modified Bradford assay. A. Soluble protein level in the labial salivary glands (LSG. mg/pair LSG). B. Soluble protein level in the guts (mg/gut). Bars represent the means of 3 biological replicates with standard error of means. Soluble protein levels were measured three times with similar results. Differences were compared by one-way ANOVA followed by a Tukey post-hoc test.

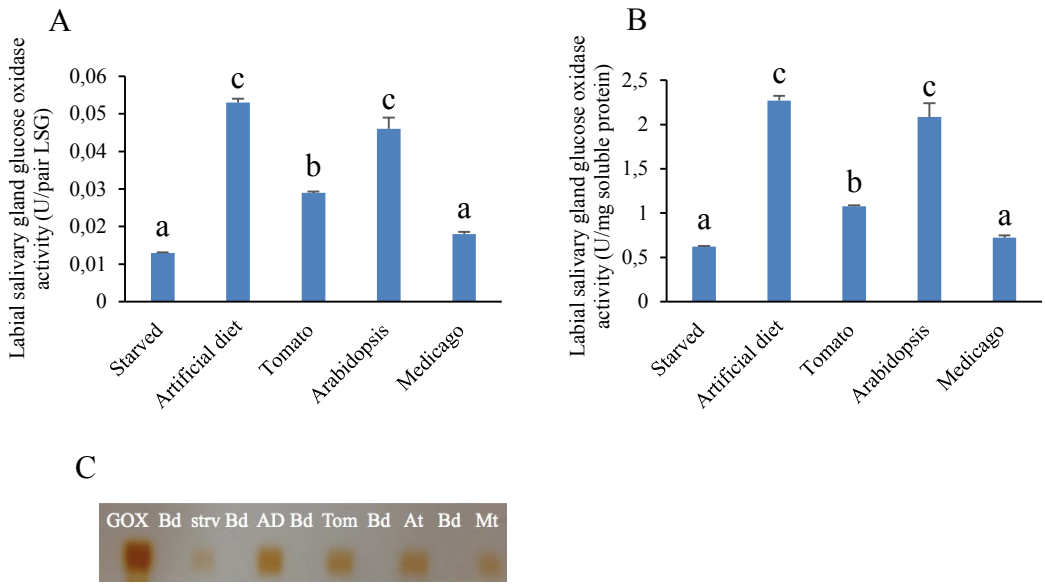


Figure 2. Labial salivary gland glucose oxidase (GOX) activity of *Spodoptera exigua* caterpillars fed on different diets. 4th instar *Spodoptera exigua* caterpillars were transferred from artificial diet to respective diets or starved for 48 hours. Diets were artificial diet, *Arabidopsis thaliana*, *Medicago truncatula* or *Solanum lycopersicum*. The labial salivary glands (LSG) were dissected and pooled. A. Total LSG GOX activity (U/pair LSG). B. GOX activity per mg soluble protein in the LSG. C. *In gel* assay of GOX (strv: starved caterpillars, AD: artificial diet, Tom: Tomato, At: *Arabidopsis* and Mt: *Medicago*. Bd: boiled samples). Bars represent the means of 3 biological replicates with standard error of means. The experiment was repeated three times with similar results. GOX activity was compared by one-way ANOVA followed by a Tukey post-hoc test. Letters indicate significant differences (significant level, $p = 0.05$).

Diet affected caterpillar LSG GOX activity (Figure 2). The activity of GOX from caterpillars fed on *Arabidopsis* and artificial diet was higher than those fed on *Medicago*, tomato or starved. On *Arabidopsis* or artificial diet, caterpillar LSG GOX activity was approximately twice that of caterpillars fed on tomato or *Medicago* and 4 times higher than starved caterpillars. Only one GOX isozyme was detected (Figure 2C).

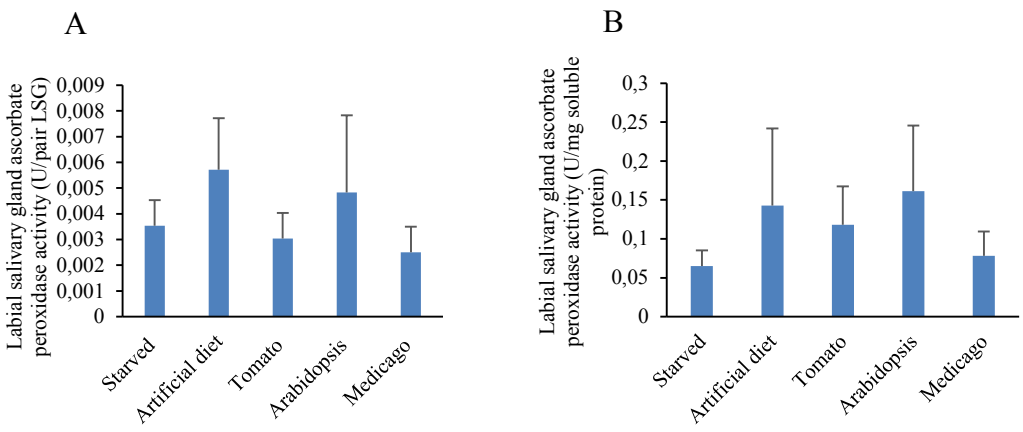


Figure 3. Labial salivary gland ascorbate peroxidase (APOX) activity of *Spodoptera exigua* caterpillars fed on different diets. 4th instar *Spodoptera exigua* caterpillars were transferred from artificial diet to respective diets or starved for 48 hours. Diets were artificial diet, *Arabidopsis thaliana*, *Medicago truncatula* or *Solanum lycopersicum*. The labial salivary glands (LSG) were dissected and pooled. A. Total LSG APOX activity (U/pair LSG). B. APOX activity per mg soluble protein in the LSG. Bars represent the means of 3 biological replicates with standard error of means. The experiment was repeated three times with similar results. APOX activity was compared by one-way ANOVA followed by a Tukey post-hoc test. Letters indicate significant differences (significant level, $p = 0.05$).

Plant or artificial diet did not affect caterpillar LSG APOX activity (Figure 3). Moreover, the activity of LSG APOX was extremely low.

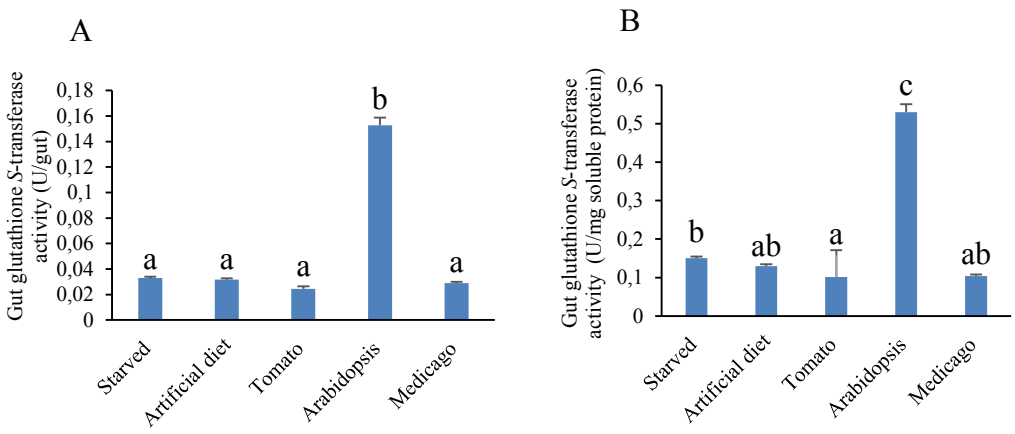


Figure 4. Gut glutathione *S*-transferase (GST) activity of *Spodoptera exigua* caterpillars fed on different diets. 4th instar *Spodoptera exigua* caterpillars were transferred from artificial diet to respective diets or starved for 48 hours. Diets were artificial diet, *Arabidopsis thaliana*, *Medicago truncatula* or *Solanum lycopersicum*. Guts were dissected and pooled. A. Total gut GST activity (U/gut). B. GST activity per mg soluble protein in the gut. Bars represent the means of 3 biological replicates with standard error of means. The experiment was repeated three times with similar results. GST activity was compared by one-way ANOVA followed by a Tukey post-hoc test. Letters indicate significant differences (significant level, $p = 0.05$).

Caterpillar gut-associated GST activity was affected by plant diet (Figure 4). Caterpillars fed on *Arabidopsis* had the highest GST activity compared to those fed on other diets. There was no difference of the total gut GST activity (U/gut) among the caterpillars fed on artificial diet, tomato, *Medicago* and starved (Figure 4A). However, starved caterpillars had a higher GST activity per mg soluble protein compared to tomato-fed caterpillars (Figure 4B).

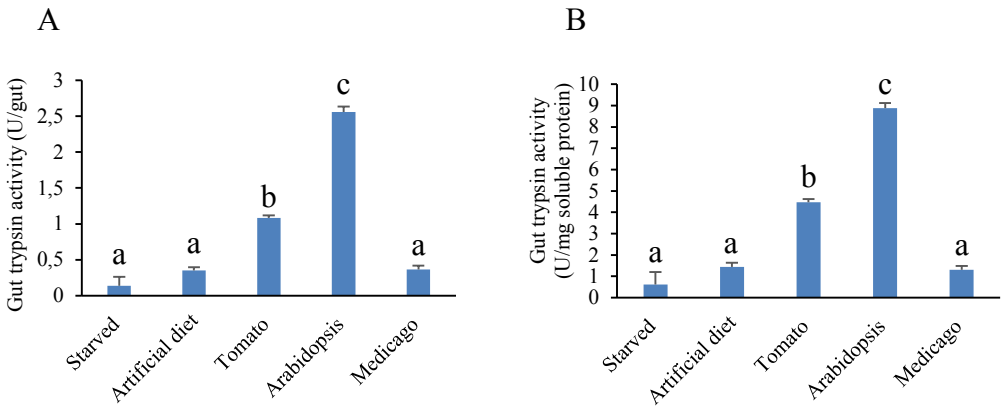


Figure 5. Gut trypsin activity of *Spodoptera exigua* caterpillars fed on different diets. 4th instar *Spodoptera exigua* caterpillars were transferred from artificial diet to respective diets or starved for 48 hours. Diets were artificial diet, *Arabidopsis thaliana*, *Medicago truncatula* or *Solanum lycopersicum*. Guts were dissected and pooled. A. Total gut trypsin activity (U/gut). B. Trypsin activity per mg soluble protein in the guts. Bars represent the means of 3 biological replicates with standard error of means. The experiment was repeated three times with similar results. Trypsin activity was compared by one-way ANOVA followed by a Tukey post-hoc test. Letters indicate significant differences (significant level, $p = 0.05$).

Plant and artificial diet affected *S. exigua* gut-associated trypsin activity (Figure 5). Caterpillars fed on *Arabidopsis* had the highest trypsin activity followed by tomato-fed caterpillars. There was no difference in activity among *Medicago*-, artificial diet-fed or starved caterpillars.

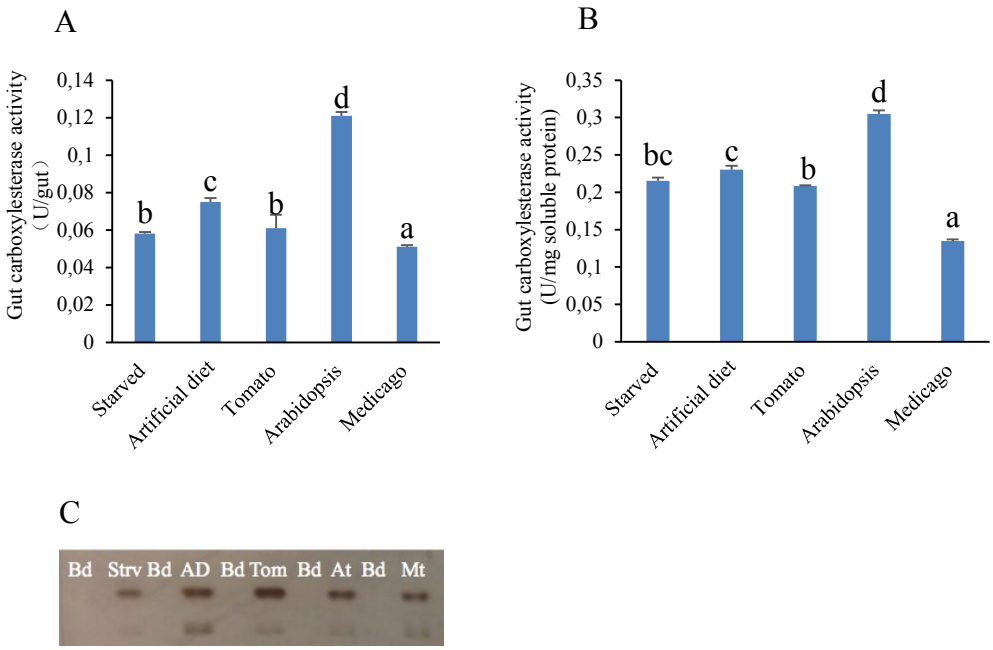


Figure 6. Gut carboxylesterase activity of *Spodoptera exigua* caterpillars fed on different diets. 4th instar *Spodoptera exigua* caterpillars were transferred from artificial diet to respective diets or starved for 48 hours. Diets were artificial diet, *Arabidopsis thaliana*, *Medicago truncatula* or *Solanum lycopersicum*. Guts were dissected and pooled. A. Total gut carboxylesterase activity (U/gut). B. Carboxylesterase activity per mg soluble protein in the guts. C. *In gel* assay of carboxylesterase (strv: starved caterpillars, AD: artificial diet, Tom: Tomato, At: *Arabidopsis* and Mt: *Medicago*. Bd: boiled samples). Bars represent the means of 3 biological replicates with standard error of means. The experiment was repeated three times with similar results. Carboxylesterase activity was compared by one-way ANOVA followed by a Tukey post-hoc test. Letters indicate significant differences (significant level, $p = 0.05$).

Diet affected *S. exigua* gut-associated carboxylesterase activity (Figure 6). *Arabidopsis*-fed caterpillars had the highest activity, approximately twice of that of *Medicago*-fed caterpillars. The *in gel* assay indicated that one carboxylesterase isozyme was constitutively expressed and the other was induced in responsed to the diet the caterpillars were feeding on. This mirrored the total enzyme activity measured spectrophotometrically.

As shown by Experiment 1, diet can affect the activity of some enzymes. To identify the main factors of these effects, the secondary metabolites or nutritional quality, diets with *Arabidopsis* extracts or diets with different protein-to-digestible carbohydrate (P:C) ratios were used to feed 4th instar *Spodoptera exigua* caterpillars for 48 hours.

4.2. Experiment 2. Effect of plant extract on *Spodoptera exigua* caterpillar enzyme activity

Caterpillars were reared on BioServ artificial diet until early 4th instar. Then they were placed on weighed blocks of different diets in Petri dishes for 48 hours. Diets were artificial diet, artificial diet containing the extracts of *Arabidopsis* either with previous 48-hr attack of caterpillars or without caterpillar herbivory. Guts from 2 caterpillars were dissected for enzyme assays. Activity of gut enzymes from *S. exigua* caterpillar were analyzed (Figure 7).

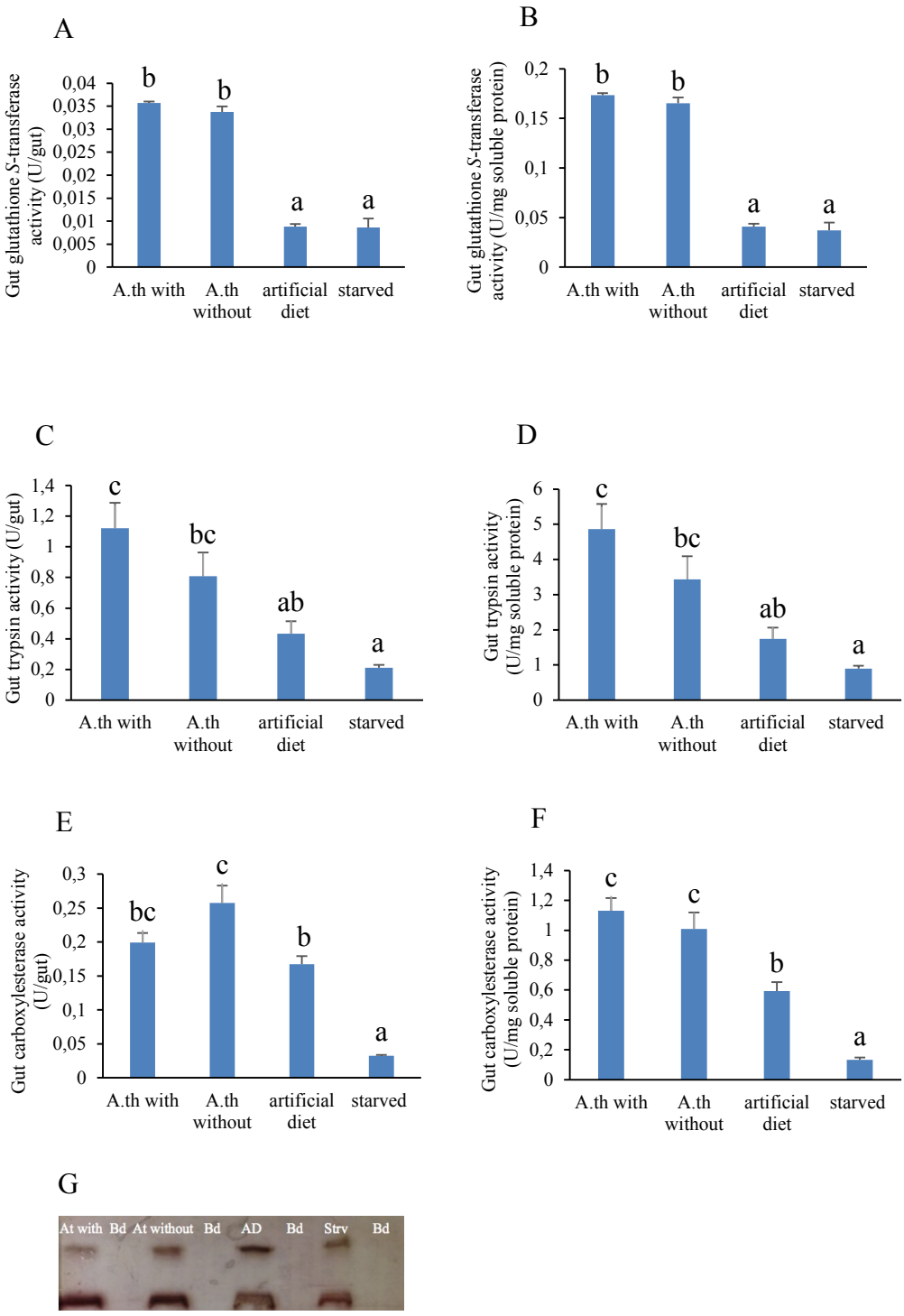

A
Gut glutathione S-transferase activity (U/gut)
0,04
0,035
0,03
0,025
0,02
0,015
0,01
0,005
0
b
b
a
a
A.th with
A.th without
artificial diet
starved
B
Gut glutathione S-transferase activity (U/mg soluble protein)
0,2
0,15
0,1
0,05
0
b
b
a
a
A.th with
A.th without
artificial diet
starved
C
Gut trypsin activity (U/gut)
1,4
1,2
1
0,8
0,6
0,4
0,2
0
c
bc
ab
a
A.th with
A.th without
artificial diet
starved
D
Gut trypsin activity (U/mg soluble protein)
6
5
4
3
2
1
0
c
bc
ab
a
A.th with
A.th without
artificial diet
starved
E
Gut carboxylesterase activity (U/gut)
0,3
0,25
0,2
0,15
0,1
0,05
0
bc
c
b
a
A.th with
A.th without
artificial diet
starved
F
Gut carboxylesterase activity (U/mg soluble protein)
1,4
1,2
1
0,8
0,6
0,4
0,2
0
c
c
b
a
A.th with
A.th without
artificial diet
starved
G
At with
Bd
At without
Bd
AD
Bd
Strv
Bd

Figure 7. Gut glutathione *S*-transferase (GST), trypsin and carboxylesterase activity from *Spodoptera exigua* caterpillars fed on artificial diet containing *Arabidopsis* extract. 4th instar *Spodoptera exigua* caterpillars were transferred from artificial diet to respective diets or starved for 48 hours. Diets were artificial diet, artificial diet containing the extracts of *Arabidopsis* either with previous 48-hr attack of caterpillars (A.th with) or without caterpillar herbivory (A.th without). Guts were dissected and pooled. A. Total gut GST activity (U/gut). B. GST activity per mg soluble protein in the guts. C. Total gut trypsin activity (U/gut). D. Trypsin activity per mg soluble protein in the guts. E. Total gut carboxylesterase activity (U/gut). F. Trypsin activity per mg soluble protein in the guts. G. *In gel* assay of carboxylesterase (At with: artificial diet containing the extracts of *Arabidopsis* with previous 48-hr attack of caterpillars, At without: artificial diet containing the extracts of *Arabidopsis* without caterpillar attack, AD: artificial diet, strv: starved caterpillars. Bd: boiled samples). Bars represent the means of 3 biological replicates with standard error of means. The experiment was repeated three times with similar results. Enzyme activity was compared by one-way ANOVA followed by a Tukey post-hoc test. Letters indicate significant differences (significant level, $p = 0.05$).

Diet with *Arabidopsis* extracts can affect the activity of *S. exigua* enzymes (Figures 7 and 8). Artificial diets containing *Arabidopsis* extract increase the caterpillar gut GST activity compared to the caterpillars fed unadulterated artificial diet or starved (Figures 7A and 8A). When focusing on GST activity of caterpillars fed artificial diets with extract from infested by caterpillars or not, the GST activity did not reflect whether the plant had previous exposure to caterpillar hervivory (Figures 7A and 8A). A difference in GST activity between caterpillars fed artificial diet or starved was not observed (Figures 7A and 8A).

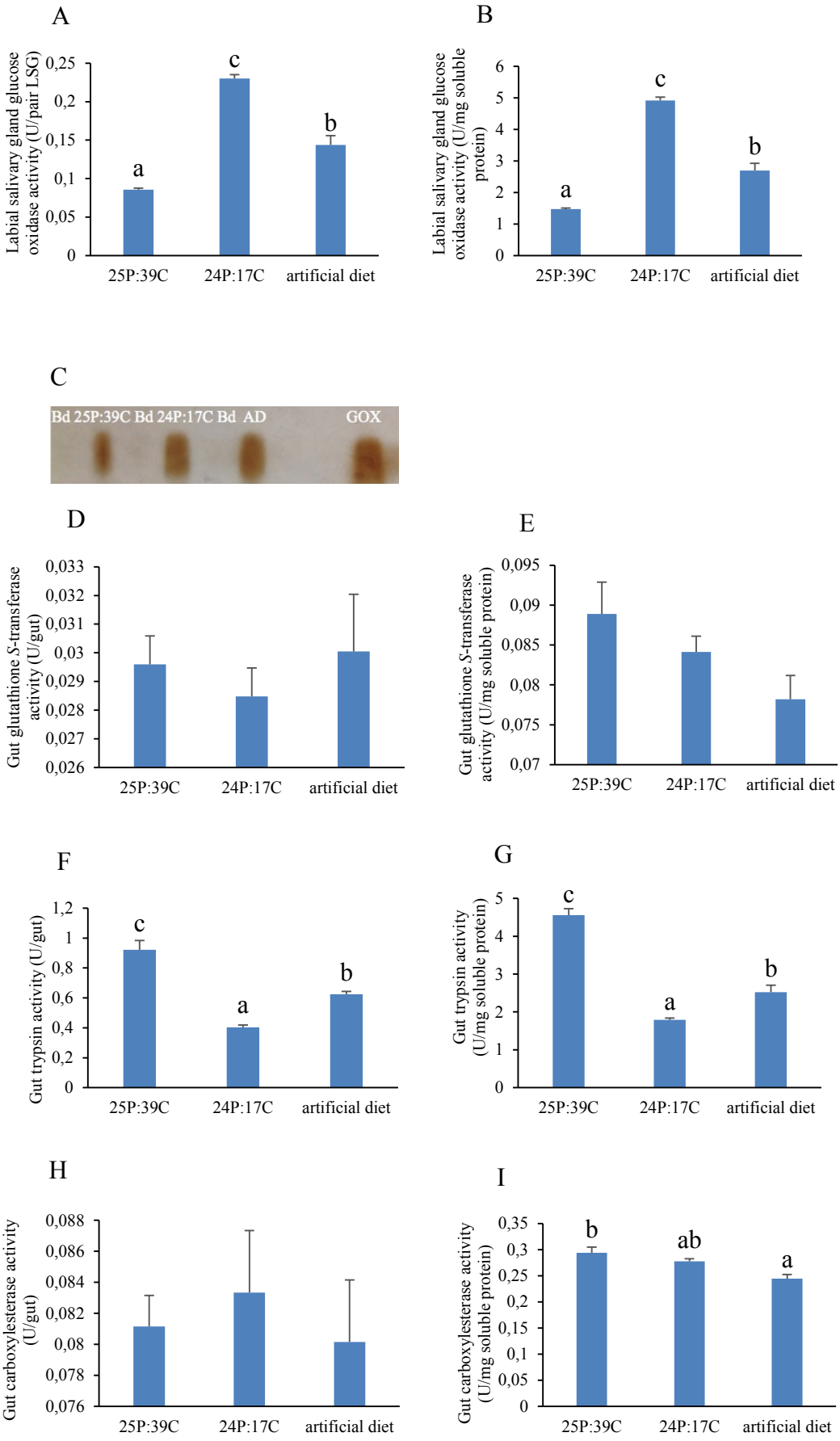
Caterpillars fed on artificial diet containing *Arabidopsis* extracts previously attacked by caterpillars had higher trypsin activity than those fed on unadulterated artificial diet and starved caterpillars (Figures 7B and 8B). A difference in gut trypsin activity between caterpillars fed on artificial diet with two types of *Arabidopsis* extracts was not observed (Figures 7B and 8B). Starved caterpillars had the lowest trypsin activity.

Diets with plant extracts increased the caterpillar gut carboxylesterase activity (Figure 8C). A difference in gut carboxylesterase activity between caterpillars fed on

artificial diet with two types of *Arabidopsis* extracts was not observed (Figures 7C and 8C). Starved caterpillars had the lowest carboxylesterase activity. These results were mirrored in the *in gel* assay (Figure 7D).

4.3. Experiment 3. Effect of nutritional quality (P:C) on *Spodoptera exigua* caterpillar enzyme activity

Caterpillars were reared on BioServ artificial diet until early 4th instar. Then they were placed on weighed blocks of different diets in Petri dishes for 48 hours. Diets were artificial diet (Bio-Serv), artificial diet with the P:C ratio of 25P:39C and 24P:17C. The ratio 25P:39C is approximately the P:C ratio of *Arabidopsis*, whereas the ratio 24P:17C is approximately yhe P:C ratio of *Medicago*. The P:C ratio of the Bio-Serv diet is 29P:37C. Pairs of labial salivary glands from 6 caterpillars or the guts for 2 caterpillars were dissected for enzyme assays. Enzyme activity of *S. exigua* caterpillar fed on different diets is shown as below.


A
Labial salivary gland glucose oxidase activity (U/pair LSG)
0,25
0,2
0,15
0,1
0,05
0
a
c
b
25P:39C
24P:17C
artificial diet
B
Labial salivary gland glucose oxidase activity (U/mg soluble protein)
6
5
4
3
2
1
0
a
c
b
25P:39C
24P:17C
artificial diet
C
Bd 25P:39C
Bd 24P:17C
Bd AD
GOX
D
Gut glutathione S-transferase activity (U/gut)
0,033
0,032
0,031
0,03
0,029
0,028
0,027
0,026
25P:39C
24P:17C
artificial diet
E
Gut glutathione S-transferase activity (U/mg soluble protein)
0,095
0,09
0,085
0,08
0,075
0,07
25P:39C
24P:17C
artificial diet
F
Gut trypsin activity (U/gut)
1,2
1
0,8
0,6
0,4
0,2
0
c
a
b
25P:39C
24P:17C
artificial diet
G
Gut trypsin activity (U/mg soluble protein)
5
4
3
2
1
0
c
a
b
25P:39C
24P:17C
artificial diet
H
Gut carboxylesterase activity (U/gut)
0,088
0,086
0,084
0,082
0,08
0,078
0,076
25P:39C
24P:17C
artificial diet
I
Gut carboxylesterase activity (U/mg soluble protein)
0,35
0,3
0,25
0,2
0,15
0,1
0,05
0
b
ab
a
25P:39C
24P:17C
artificial diet

J

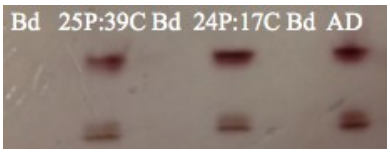


Figure 8. Glucose oxidase (GOX), glutathione *S*-transferase (GST), trypsin and carboxylesterase activity from *Spodoptera exigua* caterpillars fed on artificial diet with different P:C ratios. 4th instar *Spodoptera exigua* caterpillars were transferred from artificial diet to respective diets for 48 hours. Diets were BioServ artificial diet (29P:37C), artificial diet with two different protein to digestible carbohydrate ratios (25P:39C and 24P:17C). Labial salivary glands or guts were dissected and pooled. A. Total LSG GOX activity (U/pair LSG). B. GOX activity per mg soluble protein in the LSG. C. *In gel* assay of GOX (Bd: boiled samples). D. Total gut GST activity (U/gut). E. GST activity per mg soluble protein in the guts. F. Total gut trypsin activity (U/gut). G. Trypsin activity per mg soluble protein in the guts. H. Total gut carboxylesterase activity (U/gut). I. Carboxylesterase activity per mg soluble protein in the guts. J. *In gel* assay of carboxylesterase (Bd: boiled samples). Bars represent the means of 3 biological replicates with standard error of means. The experiment was repeated three times with similar results. Enzyme activity was compared by one-way ANOVA followed by a Tukey post-hoc test. Letters indicate significant differences (significant level, $p = 0.05$).

The nutritional quality (P:C ratio) of the diet affects GOX and trypsin activity, but not GST and carboxylesterase activity (Figures 9 and 10). Caterpillars fed on 24P:17C diet had the highest GOX activity, which was approximately twice of the GOX activity from the caterpillars fed on the 25P:39C diet (Figures 9A and 10A). Similar results were shown by the *in gel* assay (Figure 9E). In comparison, caterpillars fed on the 25P:39C diet had the highest trypsin activity, followed by caterpillars fed the Bio-Serv diet (Figures 9C and 10C).

Caterpillar gut-associated GST and carboxylesterase activity were not affected by P:C ratios (Figures 9C and 9D, Figures 10C and 10D). However, caterpillars fed the 25P:39C diet had higher carboxylesterse activity per mg soluble protein in guts than thosed fed the Bio-Serv diet (Figure 10D). Moreover, Figure 9F shows that the nutritional quality did not affect the carboxylesterase isozymes.

5. Discussion

5.1. Glucose oxidase (GOX)

Caterpillars of the beet armyworm, *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae), are polyphagous herbivores able to feed on a diversity of potential host plants; they feed on more than 130 plants from over 30 different families (Pogue, 2006). As generalists, *S. exigua* have different strategies to cope with plant defences compared to specialist caterpillars (Ali and Agrawal, 2012). As well, the plant elicits distinct compounds to different caterpillars (Ali and Agrawal, 2012). *A. thaliana* increases glucosinolate levels after attacked by *S. exigua* (generalist) caterpillars; this increase was not observed in plants attacked by specialist caterpillars (Mewis et al., 2006). The different responses of the plants to different herbivores may reflect the mechanisms used by some generalist herbivores to suppress plant defenses. One possible mechanism that some caterpillars use is the secretion of GOX in the labial saliva. GOX produced by the Noctuid caterpillar *Helicoverpa zea* is supposed to suppress the induction of plant defences (Musser et al, 1999). Many polyphagous Noctuid species have relatively higher GOX activity levels compared to more specialized species (Eichenseer et al., 2010). Thus, GOX is possibly more important to polyphagous herbivores, such as *S. exigua*, than to specialists.

In some insect species, GOX activity is affected by diet (Merkx-Jacques and Bede, 2005; Babic et al., 2008; Hu et al., 2008). For example, the corn earworm *Helicoverpa zea* larvae that have fed on tobacco had higher labial salivary GOX activity than larvae that fed on cotton (Peiffer and Felton, 2005). The results of the present study show that caterpillars reared on artificial diet had higher labial salivary GOX activity than those fed with tomato and *Medicago*, which is consistent with the previous results (Merkx-Jacques and Bede, 2005; Hu et al., 2008). These studies found that the labial salivary GOX activity was higher from the caterpillars fed on artificial diet than those fed on *M. trunctula* or tobacco leaves. One possible explanation for this is that when caterpillars fed on plant diets, they secret more GOX onto plant surface (Afshar et al., 2013). Thus, the host plant or diet might not only affect the GOX synthesis within the glands, they also affect the secretion onto the plant surface (Peiffer and Felton, 2005; Afshar et al., 2013).

Previous studies either artificial diets or leaf supplements, showed that protein and carbohydrate levels were important in regulating GOX activity (Hu et al., 2008; Afshar et al., 2010). Fifth-instar *H. armigera* caterpillars were fed with tobacco leaves

coated with glucose or sucrose have higher labial salivary GOX activity than those fed with leaves without sugar coating (Hu et al., 2008). The results prove that sugar in diets is probably a major factor influencing the labial salivary GOX activity of *H. armigera* caterpillars (Hu et al., 2008). However, they did not show information about the protein concentrations of their diets. Another study found that when the dietary carbohydrate levels were the same, *S. exigua* caterpillars fed on the protein-rich diet had the highest GOX activity (Babic et al., 2008). This may be because when *S. exigua* caterpillars are fed on nutritionally-poor diets, secretion pathways are accelerated compared to those fed a protein-rich diet (Afshar et al., 2013).

To evaluate the effects of dietary nutritional quality on *S. exigua* caterpillar GOX activity, artificial diet with 25P:39C and 24P:17C were prepared according to P:C ratio of *Arabidopsis* and *M. truncatula*. 4th instar *S. exigua* caterpillars had the highest GOX activity when they fed on the 24P:17C artificial diet. This diet represent the nutritional quality of *M. truncatula* and had the highest ratio of P:C. This is similar to previous findings that high labial salivary GOX activity is assiociated with high protein levels (Babic et al., 2008; Afshar et al., 2010). However, when comparing to the plant feeding experiments, the story is quite different. 4th instar *S. exigua* caterpillars fed on *Arabidopsis* had higher GOX activity than those fed on *M. truncatula*. Therefore, other factors must come into play when caterpillars feed on plants. *M. truncatula* contains high levels of saponins that can can reduce protein digestibility by forming saponin–protein complexes (Potter et al. 1993). Consequently, even though *M. truncatula* possess higher protein levels, the presence of saponins may make it difficult for caterpillars to get enough protein from their plant diets. Therefore, caterpillars may be producing GOX but also rapidly secreting it (Afshar et al., 2013).

The possible effects of secondary metabolites on GOX activity were investigated by Hu et al. (2008). By adding phenolic compounds, such as chlorogenic acid, rutin and quercetin, into artificial diets, they found that these phenolic compounds had no effects on *H. armigera* caterpillars GOX activity. However, the effects of saponins on caterpillar labial salivary GOX activity still need to be characterized.

5.2. Ascorbate peroxidase (APOX)

Insects possess a suite of antioxidant enzymes and small molecular weight

antioxidants to protect them from the onslaughts of dietary and endogenously produced oxidants (Felton and Summers, 1995). Antioxidant enzymes, such as superoxide dismutase, catalase and glutathione reductase, have been well characterized in insects (Felton and Summers, 1995). These enzymes are involved in detoxication of reactive oxygen species (ROS) produced by xenobiotics, such as paraquat, and plant-derived allelochemicals, such as phenolics and quinones (Felton and Summers, 1995).

APOX is an antioxidant enzyme that can scavenge H_2O_2 by using it in a reaction involving in the oxidation of ascorbate (Asada, 1992). It has been indentified in whole body homogenates of *Helicoverpa zea*, with the activity highest in tissues such as the salivary glands and midguts (Mathews et al., 1997).

Other studies focused on the APOX activity from insect midguts (Krishnan and Kodrik, 2006). APOX activity was characterized in the Egyptian armyworm, *Spodoptera littoralis* foregut or midgut tissue when caterpillars were fed on potato plants (*Solanum tuberosum*) or a semi-artificial diet (Manduca Premix–Heliothis Premix) (Krishnan and Kodrik, 2006). In the present study, detectable APOX activity was not found in the midguts of *Spodoptera exigua*. This is in accordance with Mathews et al. (1997), who found that *H. zea* APOX activity was five-times higher in the labial salivary glands than in midguts. Krishnan and Sehnal (2006) found that the diets containing tannic acid significantly increased the activity of APOX in the midguts of the Egyptian armyworm, *S. littoralis* larvae compared to control diets. Besides, the midgut APOX activity of the forest tent caterpillar, *Malacosoma disstria* and the white-marked tussock moth, *Orgyia leucostigma* showed was reduced by ingestion of tannic acid (Barbehenn et al., 2001). These results show that the diet affects APOX activity. In the present study, low APOX activity was detected in *S. exigua* labial salivary glands. A diet-specific difference was not observed. These results suggest that *S. exigua* caterpillars may have other enzymes in other tissues, such as GST in midguts, rather than APOX to act as antioxidants.

5.3. Glutathione *S*-transferases (GSTs)

GSTs are a multifunctional family of enzymes found in all insects (Vontas et al., 2001). They play an important role in the detoxification of both endogenous and

xenobiotic compounds and the protection against oxidative stress by removing toxic oxygen free radical species (Enayati et al., 2005).

The activity of GST can be affected by diets. Wang et al. (2009) found that the chili pepper-fed *H. assulta* larvae had lower GSTs activity than those fed on tobacco or artificial diet. When the fruit fly *Bactrocera tau* fed on cucumber, pumpkin, towel gourd, white gourd and balsam pear, the highest GST activity was observed from the flies fed on balsam pear (Li and Liu, 2007). Our results also indicated that the GST activity of 4th instar *S. exigua* caterpillars can be affected by diets. The caterpillars fed on *Arabidopsis* had the highest GST activity compared to those fed on *Medicago*, tomato, artificial diet or starved.

How diet influences GST activity is not well understood. Most research has focused on the role of plant secondary metabolites as the main factor that determines GST activity. For example, the larvae of the gypsy moth, *Lymantria dispar* and the forest tent caterpillar, *Malacosoma disstria* fed on aspen leaves supplemented with phenolic glycosides had increased GST activity compared to the control group (Hemming and Lindroth, 2000). However, the effects of these allelochemicals may depend on the insect species. The cereal hydroxamic acid, DIMBOA stimulated GST activity in the Asian corn borer, *Ostrinia furnacalis*, while decreased the GST activity in the bird cherry-oat aphid, *Rhopalosiphum padi* (Mukanganyama et al., 2003; Yan et al., 1995). The present results also supports the idea that secondary metabolites can affect the insect GST activity. 4th instar *S. exigua* caterpillars fed on the artificial diets containing *Arabidopsis* extract had significantly higher GST activity compared to the artificial-fed caterpillars or starved caterpillars.

Besides plant secondary metabolites, the nutritional quality of the diets may also affect GST activity. Lindroth and Bloomer (1991) showed that low protein diets reduced GST activity in larvae of the forest tent caterpillar, *M. disstria*. In contrast, according to the present results, the 4th instar *S. exigua* caterpillars fed on artificial diets with varied P:C ratios did not have different GST activity. Therefore, diet nutritional quality does not appear to affect the GST activity in *S. exigua* caterpillars, just as casein supplementation did not change GST activity in *Lymantria dispar* and *M. disstria* (Hemming and Lindroth, 2000).

5.4. Carboxylesterase

Carboxylesterases are also important detoxification enzymes. However,

studies on insect carboxylesterases have been mainly focused on insecticide resistance instead of the mechanisms behind degrading plant allelochemicals (Yu et al., 2009). Carboxylesterases can detoxify many agrochemicals, such as pyrethroids, organophosphates and carbamates, by hydrolyzing these ester-containing compounds (Wheelock et al., 2005).

In insects, diet can affect carboxylesterase activity (Wang et al., 2009; Zhang et al., 2011). For example, the oriental tobacco budworm, *H. assulta* larvae fed on chili pepper had lower carboxylesterase activity than those fed on tobacco or artificial diet (Wang et al., 2009). In *S. exigua*, carboxylesterase activity was higher in larvae fed on Chinese cabbage than those fed on maize seedlings (Zhang et al., 2011). Carboxylesterase activity can be induced by phenolic glycosides in the Eastern tiger swallowtail, *Papilio glaucus*, by plant glycoside rutin in the oriental leaf worm moth, *Spodoptera litura* and by indole alkaloids in the English grain aphid, *Sitobion avenae* (Lindroth, 1989; Ghumare et al., 1989; Cai et al., 2009). In the present study, after feeding on *Arabidopsis*, *Medicago*, tomato and artificial diets, the highest carboxylesterase activity was observed in *Arabidopsis*-fed caterpillars. As well, the data shows that 4th instar *S. exigua* caterpillars adjust midgut carboxylesterase activity to different diets. Two carboxylesterase isoenzymes are detected: one constitutively expressed and the other in response to diet.

To understand how diet affects the carboxylesterase activity, *Arabidopsis* extracts were added to the artificial diet. Diets containing plant extracts increased the activity of 4th instar *S. exigua* caterpillar gut-associated carboxylesterase compared to the controls fed artificial diet or starved caterpillars. In contrast, Rachokarn et al. (2008) found that the extracts of senescent leaves of *Melia azedarach* reduced 2nd instar *S. exigua* caterpillar carboxylesterase activity. Therefore, how *S. exigua* caterpillars change carboxylesterase activity in response to plant extracts might depend on the plant species.

In comparison, diet nutritional quality (P:C ratio) did not affect 4th instar *S. exigua* caterpillars carboxylesterase activity, which is in consistent with Zhang et al. (2011). In their experiment, *S. exigua* caterpillar carboxylesterase activity did not significantly change with artificial diets with different protein and glucose contents. Therefore, secondary metabolites of plants, instead of the nutritional quality, might play major roles in affecting *S. exigua* caterpillar carboxylesterase activity when they fed different diets.

Carboxylesterases and GSTs are important detoxification enzymes in insects for endogenous and exogenous compound metabolism. In the present study, the activity of both of the enzymes were highest in *Arabidopsis*-fed *S. exigua* caterpillars. As well, enzyme activity was correlated with plant extracts containing diet. In the English grain aphid, *Sitobion avenae*, activities of carboxylesterases and GSTs also significantly increased when aphids fed on a wheat cultivar with high hydroxamic acid concentrations (Leszczynski et al., 1993; Loayza-Muro et al., 2000). However, when offered diets containing catechol, gramine and lornithine, carboxylesterases activity in *S. avenae* was more sensitive to gramine and catechol, whereas GST activity was strongly correlated with higher concentrations of gramine and lornithine (Zhang et al., 2013). Unfortunately, we did not separate the compounds from the *Arabidopsis* extracts, so the information about how the enzyme responds to specific secondary metabolites is not available.

5.5. Trypsin

Trypsin is an important serine proteinase in insects. It hydrolyzes dietary protein to peptides, which are further degraded to amino acids (Evnin et al., 1990). These amino acids are required by the insect for growth, development and reproduction. Because of its crucial functions, trypsin is also a common target of protenase inhibitors (PIs) produced by plants.

PIs produced by plants have been regarded as the defensive agents against insect herbivores, but some results show that insects have developed adaptive strategies to overcome the PIs in their host plants (Broadway, 1996). Trypsins secreted by some crucifer specialists (i.e., the small white, *Pieris rapae*; the green-veined white, *Pieris napi*, and the diamondback moth, *Plutella xylostella*) are more resistant to the cabbage PIs than those secreted by some feeding generalists, such as the cabbage looper, *Trichoplusia ni*, the corn earworm, *Helicoverpa zea* and the gypsy moth, *Lymantria dispar* (Broadway, 1996). This may be because those crucifer specialists can reduce the binding capacity of PIs by secreting trypsins with minor changes in the amino acid residues surrounding the active site (Broadway, 1996). Even those generalists who have susceptible trypsins can also overcome the biological activity of PIs by changing susceptible enzymes to inhibitor-resistant types after they consume the same family of PIs in diet (Broadway, 1996). Since the resistance of a trypsin to a single trypsin inhibitor may result in resistance to many trypsin inhibitors

within the same family and the generalists have potentials to access more families of PIs than the specialists (Broadway, 1996). This ability may make those generalists adapted to more PIs even from non-host plants than the specialized insects (Broadway, 1996).

The present results show that 4th instar *S. exigua* caterpillars fed on *Arabidopsis* had highest trypsin activity than those fed on *Medicago*, tomato and artificial diet. So far, studies have identified several serine PIs from tomato, such as potato Inhibitors I and II (Ryan, 1990). The encoding genes and the mechanisms of the systematical regulation of these PIs are also characterized in tomatoes (Koiwa et al., 1997). As well, a wound-inducible trypsin inhibitor had previously been isolated from leaves of alfalfa (*M. sativa*), a close relative of *M. truncatula* and was shown to be the first published example of a wound-inducible Bowman-Birk inhibitor (Brown and Ryan, 1984; Brown et al., 1985; Frugoli and Harris, 2001). Unlike tomato, which is a really good model for PI studies, in *Arabidopsis*, although cDNAs encoding putative proteinase inhibitors have been identified, significant constitutive or wound induced proteinase inhibitor activity has not been successfully demonstrated (McConn et al., 1997). Therefore, although there are some cysteine PIs that have been characterized from *A. thaliana*, little is known about serine PIs (Zhang et al., 2008). The relative absence of serine PIs in *Arabidopsis* may be the main reason for the high trypsin activity in *Arabidopsis*-fed caterpillars. As a generalist, *S. exigua* caterpillars may have the ability to respond to PIs by changing susceptible trypins to inhibitor-resistant ones.

Caterpillars fed on artificial diet containing *Arabidopsis* extract from caterpillar-stressed plants had higher trypsin activity than those fed on unadulterated artificial diet. This might be because that previous caterpillar feeding can trigger the glucosinolate-myrosinase system in *Arabidopsis*, and the hydrolysis products can decreased food efficiency in insects (Anilakumar et al., 2006). Caterpillar feeding can also induce several functional genes including phenylpropanoid pathway genes and it is shown increased biosynthesis of phenylpropanoids can reduced feeding rates of generalist herbivore, the fall armyworm, *Spodoptera frugiperda* (Reymond et al., 2004; Johnson and Dowd, 2004). Without enough ingested proteins, *S. exigua* caterpillars might activate more trypsins to cope with the protein-poor conditions.

Dietary nutritional quality also affects trypsin activity. Both 5th instar *H. zea* and *S. exigua* caterpillars had higher digestive enzyme activity that corresponds to the

increased concentration of dietary casein (Broadway and Duffey, 1986b). Higher protein level in *H. armigera* gut was correlated to increased trypsin activity (Johnston et al., 1993). However, the present results show that *S. exigua* caterpillars fed the artificial diet with low protein ratio have higher trypsin activity in guts. This was unexpected but might reflect the fact that on protein-poor diets, it is more important to have enzymes like trypsin to digest any available proteins.

6. Conclusion

These five enzymes analyzed in this project are important for insects to protect them against the plant defenses, both nutritional deficiency and toxic plant secondary metabolites. We investigated the dietary effects on the activity of these enzymes by feeding the 4th instar *S. exigua* caterpillars on different diets (*Arabidopsis thaliana*, *Medicago truncatula*, *Solanum lycopersicum* and artificial diet or starving them). Our results show that except for APOX, 4th instar *S. exigua* caterpillars adjust the activity of GOX, GST, trypsin and carboxylesterase activity to different diets. Further experiments show that plant extracts affect the activity of GST, trypsin and carboxylesterase, while nutritional quality of the diets played an important role in influencing GOX and trypsin activity.

7. Recommendations for Future Research

Though, the present study found that *Arabidopsis* extracts affected the activity of GST, trypsin and carboxylesterase, the responsible compounds were not identified. For example, caterpillars fed on the artificial diet containing *Arabidopsis* extracts previously acttacted by herbivores had higher trypsin activity than those fed on artificial diet containing *Arabidopsis* extracts without insect-stress. Since the methanol extract did not have PIs in it, the reason for the difference of trypsin activity is not clear.

As well, it would be of interest to understand how low protein levels affect trypsin activity. So far, a lot of studies have focused on how *S. exigua* caterpillars regulate GOX activity when they fed on diets with different nutritional quality, such as P:C ratio, but little is known about how insects react when they fed protein-poor diets.

8. Literature cited

Adel MM, Sehnal F and Jurzysta M. 2000. Effects of alfalfa saponins on the moth *Spodoptera littoralis*. *Journal of Chemical Ecology, 26, 1065-1078.*

Afshar K, Dubea FF, Najafabadi HS, Bonneil E, Thibault P, Salavati R, Bede JC. 2013. Insights into the insect salivary gland proteome: Diet-associated changes in caterpillar labial salivary proteins. *Journal of Insect Physiology, 59, 351-366.*

Afshar K, Dufresne PJ, Pan L, Merkx-Jacques M and Bede JC. 2010. Diet-specific salivary gene expression and glucose oxidase activity in *Spodoptera exigua* (Lepidoptera: Noctuidae) larvae. *Journal of Insect Physiology, 56, 1798-1806.*

Agren J and Schemske DW. 1993. The cost of defense against herbivores an experimental study of trichome production in *Brassica rapa*. *American Naturalist, 141, 338-350.*

Ahamd S. 1986. Enzymatic adaptations of herbivorous insects and mites to phytochemicals. *Journal of Chemical Ecology, 12, 533-560.*

Ahmad S and Pardini RS. 1990. Antioxidant defense of the cabbage looper, *Trichoplusia ni*: Enzymatic responses to the superoxide generating flavonoid, quercetin, and phododynamic furanocoumarin, xanthotoxin. *Photochemistry and Photobiology, 15, 305-311*

Ahmad S, Brattsten LB, Mullin CA and Yu SJ. 1986. Enzymes involved in the metabolism of plant allelochemicals. In: Molecular Aspects of Insect-Plant Associations, pp 7-151 (eds, Brattsten LB and Ahmad S, Plenum Press, New York).

Ahmad S. 1986. Enzymatic adaptations of herbivorous insects and mites to phytochemicals. *Journal of Chemical Ecology, 12, 533-539.*

Ahmad SA and Hopkins TL. 1992. Phenol β-glucosyltransferase and β-glucosidase activities in the tobacco hornworm larva *Manduca sexta* (L.): Properties and tissue localization. *Archives of Insect Biochemistry and Physiology, 21, 207-224.*

Ahmad SA and Hopkins TL. 1993. Phenol β-glucosyltransferases in six species of insects: properties and tissue localization. *Comparative Biochemistry and Physiology, 104, 515-519.*

Akai H. 1998. Silk glands. In Microscopic Anatomy of Invertebrates, pp. 21-53 (ed. Harrison FW, Wiley-Liss, NewYork).

Ali JG and Agrawal AA. 2012. Specialist versus generalist insect herbivores and plant defense. *Trends in Plant Science, 17, 293-302.*

Anilakumar KR, Khanum F and Bawa AS. 2006. Dietary role of glucosinolate derivatives: a review. *Journal of Food Science and Technology, 43, 8-17.*

Asada K. 1984. Chloroplasts: formation of active oxygen and its scavenging. *Methods in Enzymology, 105, 422-429*Asada K. 1992. Ascorbate peroxidase: A hydrogen peroxide-scavenging enzyme in plants. *Plant Physiology, 85, 235-241.*

Avato P, Bucci R, Tava A, Vitali C, Rosato A, Bialy Z and Jurzysta M. 2006. Antimicrobial activity of saponins from *Medicago sp*.: structure–activity relationship. *Phytotherapy Research, 20, 454-457.*

Avicor SW, Eziah VY, Owusu EO and Wajidi MFF. 2013. Host related variation in carboxylesterase activity of the whitefly *Bemisia Tabaci* (Gennadius) populations on cabbage and garden egg. *ARPN Journal of Agricultural and Biological Science, 8, 3.*

Awmack CS and Leather SR. 2002. Host plant quality and fecundity in herbivorous insects. *The Annual Review of Entomology, 47, 817-44.*

Babic B, Poisson A, Darwish S, Lacasse J, Merkx-Jacques M, Despland E and Bede JC. 2008. Influence of dietary nutritional composition on caterpillar salivary enzyme activity. *Journal of Insect Physiology, 54, 286-296.*

Barbehenn RV, Bumgarner SL, Roosen EF and Martin MM. 2001. Antioxidant defenses in caterpillars: role of the ascorbate-recycling system in the midgut lumen. *Journal of Insect Physiology, 47, 349-357.*

Barbehenn RV. 2002. Gut-based antioxidant enzymes in a polyphagous and a graminivorous grasshopper. *Journal of Chemical Ecology, 28, 1329-1347.*

Bede JC, Musser RO, Felton GW and Korth KL. 2006. Caterpillar herbivory and salivary enzymes decrease transcript levels of *Medicago truncatula* genes encoding early enzymes in terpenoid biosynthesis. *Plant Molecular Biology, 60, 519-53.*

Bennett RN and Wallsgrove RM. 1994. Secondary metabolites in plant defence mechanisms. *New Phytologist, 127, 617-633.*

Berenbaum M. 1980. Adaptive significance of midgut pH in larval lepidoptera. *American Naturalist, 115, 138–146.*

Bergmeyer HU. 1974. Methods of Enzymatic Analysis. Verlag Chemie, Weinheim, Germany.

Bernays EA and Chapman RF. 1994. Host-Plant selection by phytophagous insects. Chapman and Hall, NewYork.

Bernays EA and Chapman RF. 2000. Plant secondary compounds and grasshoppers: beyond plant defenses. *Journal of Chemical Ecology, 26, 1773-1794.*

Bi JL and Felton GW. 1995. Foliar oxidative stress and insect herbivory: primary compounds, secondary metabolites and reactive oxygen species as components of induced resistance. *Journal of Chemical Ecology, 21, 1511-1530.*

Bjorkman C and Anderson DB. 1990. Trade-off among anti-herbivore defenses in a South American blackberry (*Rubus bogotensis*). *Oecologia, 85, 247-249.*

Blankemeyer JT, Atherton R and Friedman M. 1995. Effect of potato glycoalkaloids α-chaconine and α-solanine on sodium active transport in frog skin. *Journal of Agricultural and Food Chemistry, 43, 636-639.*

Blankemeyer JT, White JB, Stringer BK and Friedman M. 1997. Effect of α-tomatine and tomatidine on membrane potential of frog embryos and active transport of ions in frog skin. *Food and Chemical Toxicology, 35, 639-646.*

Bolwell GP and Wojtaszek P. 1997. Mechanisms for the generation of reactive oxygen species in plant defence-a broad perspective. *Physiological and Molecular Plant Pathology, 51, 347-366.*

Bones AM and Rossiter JT. 2006. The enzymic and chemically induced decomposition of glucosinolates. *Phytochemistry, 67, 1053-67.*

Bourgaud F, Gravot A, Milesi S and Gontier E. 2001. Production of plant secondary metabolites: a historical perspective. *Plant Science, 161, 839-851.*

Bown DP, Wilkinson H and Gatehouse JA. 2004. Regulation of expression of genes encoding digestive proteases in the gut of polyphagous lepidopteran larva in response to dietary protease inhibitors. *Physiological Entomology, 29, 278-290.*

Bown DP, Wilkinson HS and Gatehouse JA. 1997. Differentially regulated inhibitor-sensitive and insensitive protease genes from the phytophagous insect pest, *Helicoverpa armigera*, are members of complex multigene families. *Insect Biochemistry and Molecular Biology, 27, 625-638.*

Brattsten LB. 1988. Enzymic adaption in leaf-feeding insects to host-plant allelochemicals. *Journal of Chemical Ecology*, *14, 10.*

Brito LO, Lopes AR, Parra JRP, Terra WR, Silva-Filho MC. 2001. Adaptation of tobacco budworm *Heliothis virescens* to proteinase inhibitors may be mediated by the synthesis of new proteinases. *Comparative Biochemistry and Physiology, 128, 365-375.*

Broadway RM and Duffey SS. 1986a. Plant proteinase inhibitors: mechanism of action and effect on the growth and digestive physiology of larval *Heliothis zea* and *Spodoptera exigua*. *Journal of Insect Physiology, 32, 827-833.*

Broadway RM and Duffey SS. 1986b. The effect of dietary protein on the growth and digestive physiology of larval *Heliothis zea* and *Spodoptera exigua*. *Journal of Insect Physiology, 32, 673-680.*

Broadway RM. 1995. Are insects resistant to plant proteinase inhibitors? *Journal of Insect Physiology, 41, 107-116.*

Broadway RM. 1996. Dietary proteinase inhibitors alter compliment of midgut proteases. *Archives of Insect Biochemistry and Physiology, 32, 39-53.*

Broadway RM. 2000. The response of insects to protease inhibitors. In: Recombinant Protease Inhibitors in Plants, pp. 80-88 (ed. Michaud D, Landes Bioscience, Georgetown).

Brown WE and Ryan CA. 1984. Isolation and characterization of a wound-induced trypsin inhibitor from alfalfa leaves. *Biochemistry, 23, 3418-3422.*

Brown WE, Takio K, Titani K and Ryan CA. 1985. Wound-induced trypsin inhibitor in alfalfa leaves: identity as a member of the Bowman-Birk inhibitor family. *Biochemistry, 24, 2105-2108.*

Cai QN, Han Y, Cao YZ, Hu Y, Zhao X and Bi JL. 2009. Detoxification of gramine by the cereal aphid *Sitobion avenae*. *Journal of Chemical Ecology, 35, 320-325.*

Carlini CR and Grossi-de-Sá MF. 2002. Plant toxic proteins with insecticidal properties. A review on their potentialities as bioinsecticides. *Toxicon, 40, 1515-1539.*

Casida JE and Quistad GB. 1998. Golden age of insecticide research: past, present, or future? *Annual Review of Entomology, 43, 1-16.*

Celorio-Mancera MdlP, Courtiade J, Muck A, Heckel DG, Musser RO and Vogel H. 2011. Sialome of a generalist lepidopteran herbivore: identification of transcripts and proteins from *Helicoverpa armigera* labial salivary glands. *Plos One 6, e26676.*

Chen H, Wilkerson CG, Kuchar JA, Phinney BS and Howe GA. 2005. Jasmonate-inducible plant enzymes degrade essential amino acids in the herbivore midgut. *Proceedings of the National Academy of Sciences of the USA, 102, 19237-19242.*

Chew FS. 1988. Biological effects of glucosinolates. In: Biologically Active Natural Products, pp. 155-181 (ed. Cutler HG, American Chemical Society).

Chu CC, Natwick ET, Chen TY and Henneberry TJ. 2003. Analyses of cotton leaf characteristics effect on *Bemisia tabaci* (Homoptera: Aleyrodidae) biotype B colonization on cotton in Arizona and California. *Southwestern Entomologist, 28, 235-240.*

Ciucu A and Patroescu C. 1984. Fast spectrometric method of determining the activity of glucose oxidase. *Analytical Letters, 17, 1417-1427.*

Clark AG and Shamaan NA.1984. Evidence that DDT-dehydrochlorinase from the housefly is a glutathione *S*-transferase, *Pesticide Biochemistry and Physiology, 22, 249-26.1*

Cui W, Winter WT, Tanenbaum SW and Nakas JP. 1999. Purification and characterization of an intracellular carboxylesterase from *Arthrobacter viscosus* NRRL B-1973. *Enzyme and Microbial Technology, 24, 200-208.*

Dahlman DL and Hibbs ET. 1967. Responses of *Empoasca fabae* to tomatine, solanine, leptine I, tomatidine, solanidine and demissidine. *Annals of the Entomological Society of America, 60, 732-740.*

De Geyter E, Lambert E, Geelen D and Smagghe G. 2007. Novel advances with plant saponins as natural insecticides to control pest insects. *Pest Tech 2, 96-105*.

De Vos RC, Moco S, Lommen A, Keurentjes JJB, Bino RJ and Hall RD. 2007. Untargeted large-scale plant metabolomics using liquid chromatography coupled to mass spectrometry. *Nature Protocols, 10, 1038.*

Dean RT. 1991. Protein damage and repair: An overview. In: Oxidative Damage and Repair: Chemical, Biological and Medical Aspects, pp. 341-347 (ed, Davies KJA, Pergamon Press, New York).

Deng HM, Huang YF, Feng QL and Zheng SC. 2009. Two epsilon glutathione *S*-transferase cDNAs from the common cutworm, *Spodoptera litura*: Characterization and developmental and induced expression by insecticides. *Journal of Insect Physiology, 55, 1174-1183.*

Devonshire AL, Heidari R, Huang HZ, Hammock BD, Russell RJ and Oakeshott JG. 2007. Hydrolysis of individual isomers of fluorogenic pyrethroid analogs by mutant carboxylesterases from *Lucilia cuprina*. *Insect Biochemistry and Molecular Biology, 37, 891-902.*

Diezel C, Von Dahl C, Gaquerel E and Baldwin IT. 2009. Different lepidopteran elicitors account for crosstalk in herbivory-induced phytohormone signaling. *Plant Physiology, 150, 1576-1586.*

Ding Y, Hawkes N, Meredith J, Eggleston P, Hemingway J and Ranson H. 2005. Characterisation of the promoters of Epsilon glutathione transferases in the mosquito *Anopheles gambiae* and their response to oxidative stress. *Biochemical Journal, 387, 879-888.*

Ding Y, Ortelli F, Rossiter LC, Hemingway J and Ranson H. 2003. The Anopheles gambiae glutathione transferase supergene family: annotation, phylogeny and expression profiles. *BMC Genomics, 4, 35.*

Dow JAT. 1986. Insect midgut function. *Advances in Insect Physiology, 19, 187-328.*

Duffey SS and Stout MJ. 1996. Antinutritive and toxic components of plant defense against insects. *Archives of Insect Biochemistry and Physiology, 32, 3-37.*

Eichenseer H, Mathews MC, Bi JL, Murphy JB and Felton GW. 1999. Salivary glucose oxidase: multifunctional roles for *Helicoverpa zea*? *Archives of Insect Biochemistry and Physiology, 42, 99-109.*

Eichenseer H, Mathews MC, Powell JS and Felton GW. 2010. Survey of a salivary effector in caterpillars: glucose oxidase variation and correlation with host range. *Journal of Chemical Ecology, 36, 885-897.*

Enayati AA，Ranson H and Hemingway J. 2005. Insect glutathione transferases and insecticide resistance. *Insect Biochemistry and Molecular Biology, 14, 3-8.*

Evnin LB, Vásquez JR and Craik CS. 1990. Substrate specificity of trypsin investigated by using a genetic selection. *Proceedings of the National Academy of Sciences of the United States of America, 87, 6659–63.*

Felton GW and Duffey SS. 1991a. Enzymatic antinutritive defenses of the tomato plant against insects. In: Naturally Occurring Pest Bioregulators, pp. 166-197 (ed. Hedin P, American Chemical Society Symposium Series, Washington, D.C).

Felton GW and Duffey SS. 1991b. Reassessment of the role of gut alkalinity and detergency in insect herbivory. *Journal of Chemical Ecology, 17, 1821-1836.*

Felton GW and Eichenseer H. 1999. Herbivore saliva and its effects on plant defense against herbivores and pathogens. In: Induced Plant Defenses Against Pathogens and Herbivores, pp. 19–36 (eds, Agrawal AA, Tuzun S, and Bent E, APS, St. Paul).

Felton GW and Gatehouse JA. 1996. Antinutritive plant defense mechanisms. In: Biology of the Insect Midgut, pp. 373-416 (eds, Lehane MJ and Billingsley PF, Chapman and Hall, London).

Felton GW and Summers CB. 1995. Antioxidant systems in insects. *Archives of Insect Biochemistry and Physiology, 29, 187-I97.*

Felton GW. 1996. Nutritive quality of plant protein: sources of variation and insect herbivore responses. *Archives of Insect Biochemistry and Physiology, 32, 107-30.*

Fenwick GR, Heaney RK and Mullin WJ. 1983. Glucosinolates and their breakdown products in food and food plants. *Critical Reviews in Food Science and Nutrition, 18, 123-201.*

Fernandes GW. 1994. Plant mechanical defenses against insect herbivory. *Revista Brasileira de Entomologia, 38, 421-433.*

Field LM and Devonshire AL. 1998. Evidence that the E4 and FE4 esterase genes responsible for insecticide resistance in the aphid *Myzus persicae* (Sulzer) are part of a gene family. *Biochemical Journal, 330, 169-73.*

Fontaine TD, Irving GW Jr, Ma RM, Poole JB and Doolittle SP. 1948. Isolation and partial characterization of crystalline tomatine, an antibiotic agent form the tomato plant. *Archives of Biochemistry and Biophysics, 18, 467-475.*

Fraenkel GS. 1959. The raison d'etre of secondary plant substances: these odd chemicals arose as a means of protecting plants from insects and now guide insects to food. *Science 129, 1466-1470.*

Francis G, Kerem Z, Makkar HPS and Becker K. 2002. The biological action of saponins in animal systems: a review. *British Journal of Nutrition, 88, 587-605.*

Friedman M and Levin CE. 1998. Dehydrotomatine content in tomatoes. *Journal of Agricultural and Food Chemistry, 46, 4571-4576.*

Fritzsch Hoballah ME and Turlings TCJ. 2001. Experimental evidence that plants under caterpillar attack may benefit from attracting parasitoids. *Evolutionary Ecology Research, 3, 553-65.*

Frugoli J and Harris J. 2001. *Medicago truncatula* on the move! *Plant Cell, 13, 458-463.*

Gao XW and Liang TT. 1993. Induction of carboxylesterase and glutathione-*S*-transferase activities by atrazine and dichlorvos inlarvae of *Heliothis armigera* and *Muscadomestica vicina. Acta Entomologica Sinica, 36, 167-171.*

Gao XW, Zhao Y, Wang X, Dong XL and Zheng BZ. 1998. Induction of carboxylesterase in *Helicoverpa armigera* by insecticides and plast allelochemicals. *Acta Entomologica Sinica, 41, 5-11.*

Gatehouse JA. 2002. Plant resistance towards insect herbivores: a dynamic interaction. *New Phytologist, 156, 145-169.*

George DGM and Gatehouse AMR. 2013. Oxidative stress enzymes in *Busseola fusca*. *International Journal of Current Microbiology and Applied Sciences, 2, 485-495.*

Georghiou GP. 1972. The evolution of resistance to pesticides. *The Annual Review of Ecology, Evolution, and Systematics, 3, 133-68.*

Ghumare SS, Mukherjee SN and Sharma RN. 1989. Effects of rutin on the neonate sensitivity, dietary utilization and midgut carboxylesterase activity of *Spodoptera litura* (Fabricius) (Lepidoptera: Noctuidae). *Proceedings of the Indian Academy of Sciences, 98, 399-404.*

Glendinning JI. 2002. How do herbivorous insects cope with noxious secondary plant compounds in their diet? *Entomologia Experimentalis et Applicata, 104, 15-25.*

Guntner C, Gonzalez A, Reis RD, Gonzalez G, Vazquez A, Ferreira F and Moyna P. 1997. Effect of Solanum glycoalkaloids on potato aphid, *Macrosiphum euphorbiae*. *Journal of Chemical Ecology, 23, 1651-1659.*

Habig WH, Pabst MJ and Jalkoby WB. 1974. Glutathione *S*-transferase. The first enzymatic step in mercapturic acid formation. *Journal of Chemical Ecology, 249, 7130-7139.*

Hakim RS and Kafatos FC. 1974. The structure and salivary function of the labial gland in adult *Manduca sexta*. *Tissue and Cell, 6, 729-750.*

Halkier BA and Gershenzon J. 2006. Biology and biochemistry of glucosinolates. *Annual Review of Plant Biology, 57, 303-33.*

Hamberg M, Sanz A and Castresana C. 1999. α-Oxidation of fatty acids in higher plants: identification of a pathogen inducible oxygenase (PIOX) as an alpha-dioxygenase and biosynthesis of 2-hydroperoxylinolenic acid. *Journal of Chemical Ecology, 274, 24503-24513.*

Harborne JB. 1993. Introduction to Ecological Biochemistry, fourth ed. Academic Press, London.

Harborne JB. 1999. Classes and functions of secondary products. In: Chemicals from Plants, Perspectives on Secondary Plant Products, pp. 1–25 (eds. Walton NJ and Brown DE, Imperial College Press).

Hare JD. 2005. Biological activity of acyl glucose esters from *Datura wrightii* glandular trichomes against three native insect herbivores. *Journal of Chemical Ecology, 31, 1475-1491.*

Harold JA and Ottea JA. 1997. Toxicological significance of enzyme activities in profenofos-resistant tobacco budworms, *Heliothis virescens* (F.). *Pesticide Biochemistry and Physiology, 58, 23-33.*

Harwood JL. 1980. Plant acyl-lipids: structure, distribution and analysis. In: The Biochemistry of Plants. Vol 4. Lipids: Structure and Function, pp. 1-55 (ed. Stumpf PK, Academic Press, New York).

Hayes JD and Pulford DJ. 1995. The glutathione *S*-transferase supergene family: regulation of GST and the contribution of the isoenzymes to cancer chemoprotection and drug resistance. *Critical Reviews in Biochemistry and Molecular Biology, 30, 44-–600.*

Hayes JD and Strange RC. 1995. Potential contribution of the glutathione *S*-transferase supergene family to resistance to oxidative stress. *Free Radical Research, 22, 193-207.*

Hedin PA, Maxwell FG and Jenkins JN. 1974. Insect plant attractants, feeding stimulants, repellents, deterrents and other related factors affecting insect behavior. In: Proc. Summer Inst. Biol. Control Plant Insects Diseases, pp. 494-527 (eds. Maxwell FG and Harris FA).

Heil M. 2008. Indirect defence via tritrophic interactions. *New Phytologist, 178, 41-61.*

Hemming JDC and Lindroth RL. 2000. Effects of phenolic glycosides and protein on gypsy moth (Lepidoptera: Lymantriidae) and forest tent caterpillar (Lepidoptera: Lasiocampidae) performance and detoxification activities. *Environmental Entomology, 29, 1108-1115.*

Hermsmeier D, Schittko U and Baldwin IT. 2001. Molecular interactions between the specialist herbivore *Manduca sexta* (Lepidoptera, Sphingidae) and its natural host *Nicotiana attenuata*: I. Large-scale changes in the accumulation of growth- and defense-related plant mRNAs. *Plant Physiology, 125, 683-700.*

Hogge LR, Reed DW, Underhill EW and Haughn GW. 1988. HPLC separation of glucosinolates from leaves and seeds of *Arabidopsis thaliana* and their identification using thermospray liquid chromatography–mass spectrometry. *Journal of Chromatographic Science, 26, 551-556.*

Hotelier T, Renault L, Cousin X, Negre V, Marchot P and Chatonnet A. 2004. ESTHER, the database of the α/β- hydrolase fold superfamily of proteins. *Nucleic Acids Research, 32, 145-147.*

House CR and Ginsborg BL. 1985. Salivary gland. In: Comprehensive Insect Physiology, Biochemistry and Pharmacology vol. 11, pp. 195-224 (eds. Kerkut GA and Gilbert LI, Toronto).

House HL. 1974. Digestion. In: Physiology of insecta, pp. 63-117 (ed. Rockstein M, Academic Press, New York).

Howe GA and Schaller A. 2008. Induced Plant Resistance to Herbivory, pp. 349-366

(ed. Schaller A, Springer, New York).

Hu YH, Leung DWM, Kang L and Wang CZ. 2008. Diet factors responsible for the change of the glucose oxidase activity in labial salivary glands of *Helicoverpa armigera*. *Archives of Insect Biochemistry and Physiology, 68, 113–121.*

Ishaaya I and Birk Y. 1965. Soybean saponins IV. The effect of proteins on the inhibitory activity of soybean saponins on certain enzymes. *Journal of Food Science, 30, 118-120.*

Janzen DH. 1966. Coevolution of mutualism between ants and acacias in Central America. *Evolution, 20, 249-75.*

Johnson ET and Dowd PF. 2004. Differentially enhanced insect resistance, at a cost, in *Arabidopsis thaliana* constitutively expressing a transcription factor of defensive metabolites. *Journal of Agricultural and Food Chemistry, 52, 5135-5138.*

Johnston KA, Gatehouse JA and Anstee JH. 1993. Effects of soybean protease inhibitors on the growth and development of larval *Helicoverpa armigera*. *Journal of Insect Physiology, 39, 657-664.*

Jongsma MA and Bolter C. 1997. The adaptation of insects to plant protease inhibitors. *Journal of Insect Physiology, 43, 885-895.*

Jorrin JV, Rubiales D, Dumas-Gaudot E, Recorbet G, Maldonado A, Castillejo MA and Curto M. 2006. Proteomics: a promising approach to study abiotic interaction in legumes. A review. *Euphytica, 147, 37-47.*

Kahl J, Siemens DH, Aerts RJ, Gäbler R, Kühnemann F, Preston CA and Baldwin IT. 2000. Herbivore-induced ethylene suppresses a direct defense but not a putative indirect defense against an adapted herbivore. *Planta, 210, 336-342.*

Kang JH, Shi F, Jones AD, Marks MD and Howe GA. 2010. Distortion of trichome morphology by the hairless mutation of tomato affects leaf surface chemistry. *Journal of Experimental Botany, 61, 1053-1064.*

Karban R and Baldwin IT. 1997. Induced responses to herbivory, pp. 319 (Chicago University Press, Chicago).

Karban R and Myers JH. 1989. Induced plant responses to herbivory. *Annual Review of Ecology, Evolution, and Systematics, 20, 331-348.*

Karowe DN and Martin MM. 1989. The effects of quantity and quality of diet nitrogen on the growth, efficiency of food utilization, nitrogen budget, and metabolic rate of fifth instar *Spodoptera eridania* larvae (Lepidoptera: Noctuidae). *Journal of Insect Physiology, 35, 699-708.*

Kelly PJ, Bones A and Rossiter JT. 1998. Sub-cellular immuno localization of the glucosinolate sinigrin in seedlings of *Brassica juncea*. *Planta, 206, 370-77.*

Kleppet K. 1966. The effect of hydrogen peroxide on glucose oxidase from *Aspergillus niger*. *Biochemistry, 5, 139-143.*

Koiwa H, Bressan RA and Hasegawa PM. 1997. Regulation of protease inhibitors and plant defense. *Trends in Plant Science, 2, 379-384.*

Koiwa H, Shade RE, Zhu-Salzman K, Subramanian L, Murdock LL, Nielsen SS, Bressan RA and Hasegawa PM. 1998. Phage display selection can differentiate insecticidal activity of soybean cystatins. *Plant Journal, 14, 371-379.*

Koptur S. 1992. Extrafloral nectary-mediated interactions between insects and plants, in: Insect–Plant Interactions, vol. IV, pp. 81-129 (ed. Bernays E, CRC Press, Boca Raton).

Kostaropoulos I, Papadopoulos AI, Metaxakis A, Boukouvala E and Papadopoulou-Mourkidou E. 2001. Glutathione *S*-transferase in the defence against pyrethroids in insects. *Insect Biochemistry and Molecular Biology, 31, 313-319.*

Kowalski SP, Domek JM, Sanford LL and Deahl KL. 2000. Effect of α-tomatine and tomatidine on the growth and development of the Colorado potato beetle (Coleoptera: Chrysomelidae): Studies using synthetic diets. *Journal of Entomological Science, 35, 290-300.*

Kozukue N, Han JS, Lee KR and Friedman M. 2004. Dehydrotomatine and α-tomatine content in tomato fruits and vegetative plant tissues. *Journal of Agricultural and Food Chemistry, 52, 2079-2083.*

Krishnan N and Kodrík D. 2006. Antioxidant enzymes in *Spodoptera littoralis* (Boisduval): Are they enhanced to protect gut tissues during oxidative stress? *Journal of Insect Physiology, 52, 11-20.*

Krishnan N and Sehnal F. 2006. Compartmentalization of oxidative stress and antioxidant defense in the larval gut of Spodoptera littoralis. Arch. *Insect Biochemistry and Molecular Biology, 63, 1-10.*

Kuźniak E and Urbanek H. 2000. The involvement of hydrogen peroxide in plant responses to stresses. *Acta Physiologiae Plantarum, 22, 195-203.*

Lambrix V, Reichelt M, Mitchell-Olds T, Kliebenstein DJ and Gershenzon J. 2001. The *Arabidopsis* epithiospecifier protein promotes the hydrolysis of glucosinolates to nitriles and influences *Trichoplusia ni* herbivory. *Plant Cell, 13, 2793-2807.*

Lee KP. 2007. The interactive effects of protein quality and macronutrient imbalance on nutrient balancing in an insect herbivore. *Journal of Experimental Biology, 210, 3236-3244.*

Lehane MJ, Blakemore D, Williams S and Moffatt MR. 1995. Mini-review: Regulation of digestive enzyme levels in insects. *Comparative Biochemistry and Physiology, 110, 285-289.*

Leszczynski B, Matok H and Dixon AFG. 1994. Detoxification of cereal plant allelochemicals by aphids: activity and molecular weights of glutathione *S*-transferase in three species of cereal aphids. *Journal of Chemical Ecology, 20, 387-394.*

Leszczynski B, Urbanska A, Matok H and Dixon AFG. 1993. Detoxifying enzymes of the grain aphid. *Bulletin OILB SROP, 16, 165-172.*

Li Q, Eigenbrode SD, Stringam GR, Thiagarajah MR. 2000. Feeding and growth of *Plutella xylostella* and *Spodoptera eridania* on *Brassica juncea* with varying glucosinolate concentrations and myrosinase activities. *Journal of Chemical Ecology*, *26, 2401-2419.*

Li XC, Schuler MA and Berenbaum MR. 2007. Molecular mechanisms of metabolic resistance to synthetic and natural xenobiotics. *Annual Review of Entomology, 52, 231-253.*

Li XZ and Liu YH. 2007. Diet influences the detoxification enzyme activity of *Bactrocera tau* (Walker) (Diptera: Tephritidae). *Acta Entomologica Sinica, 50, 989-995.*

Lichtenstein EP, Strong FM and Morgan DG. 1962. Identification of phenylethyl isothiocyanate as an insecticide occuring naturally in the edible part of turnips. *Journal of Agricultural and Food Chemistry, 10, 30-33.*

Lindroth RL and Bloomer MS. 1991. Biochemical ecology of the forest tent caterpillar: responses to dietary protein and phenolic glycosides. *Oecologia, 86, 408413.*

Lindroth RL. 1989. Host plant alteration of detoxification activity in *Papilio glaucus* glaucus. *Entomologia Experimentalis et Applicata, 50*, 29-36.

Liu F, Cui L, Cox-Foster D and Felton GW. 2004. Characterization of a salivary lysozyme in larval *Helicoverpa zea*. *Journal of Chemical Ecology, 30, 2439-57.*

Loayza-Muro R, Figueroa CC and Niemeyer HM. 2000. Effect of two wheat cultivars differing in hydroxamic acid concentration on detoxification metabolism in the aphid *Sitobion avenae*. *Journal of Chemical Ecology, 26, 2725-2736.*

Lockwood JA, Sparks TC and Story RN. 1984. Evolution of insect resistance to insecticides: A re-evaluation of the roles of physiology and behaviour. *Bulletin of the Entomological Society of America, 30, 41-50.*

Lomolino G, Lante A, Crapisi A, Spettoli P and Curioni A. 2001. Detection of *Saccharomyces cerevisiae* carboxylesterase activity after native and sodium dodecyl sulfate electrophoresis by using fluorescein diacetate as substrate. *Electrophoresis, 22, 1021-1023.*

Lopes AR, Juliano MA, Juliano L and Terra WR. 2004. Coevolution of insect trypsins and inhibitors. *Archives of Insect Biochemistry and Physiology, 55, 140-152.*

Lu FM and Chu YI. 1993. Influence of tomatine on oviposition preference of diamondback moth *Plutella xylostella* L. *Journal of Entomological Science, 13, 379-384.*

Łukasik I, Goławska S, Wójcicka A (2009) Antioxidant defense mechanisms of cereal aphids based on ascorbate and ascorbate peroxidase. *Biologia, 64, 994-998.*

Mathews MC, Summers C and Felton GW. 1997. Ascorbate peroxidase: a novel antioxidant enzyme in insects. *Archives of Insect Biochemistry and Physiology, 34, 57-68.*

Mattson Jr WJ. 1980. Herbivory in relation to plant nitrogen content. *Annual Review of Ecology, Evolution, and Systematics,11, 119-161.*

Mazumdar-Leighton S and Broadway RM. 2001a. Transcriptional induction of diverse midgut trypsins in larval *Agrotis ipsilon* and *Helicoverpa zea* feeding on the soybean trypsin inhibitor. *Insect Biochemistry and Molecular Biology, 31, 645-657.*

Mazumdar-Leighton S and Broadway RM. 2001b. Identification of six chymotrypsin cDNAs from larval midguts of *Helicoverpa zea* and *Agrotis ipsilon* feeding on the soybean (Kunitz) trypsin inhibitor. *Insect Biochemistry and Molecular Biology, 31, 633-644.*

McConn M, Creelman RA, Bell E, Mullet JE and Browse J. 1997. Jasmonate is essential for insect defense in *Arabidopsis*. *Proceedings of the National Academy of Sciences of the USA, 94, 5473-5477.*

Mehdy MC, Sharma YK, Sathasivan K and Bays NW. 1996. The role of activated oxygen species in plant disease resistance. *Plant Physiology, 98, 356-374.*

Merkx-Jacques M and Bede JC. 2005. Influence of diet on the larval beet armyworm, Spodoptera exigua, glucose oxidase activity. *Journal of Insect Science, 5, 1536-2442.*

Merkx-Jacques M, Despland EMMA and Bede JC. 2008. Nutrient utilization by caterpillars of the generalist beet armyworm, *Spodoptera exigua*. *Physiological Entomology, 33, 51-61.*

Mewis I, Tokuhisa JG, Schultz JC, Appel HM, Ulrichs C and Gershenzon J. 2006. Gene expression and glucosinolate accumulation in *Arabidopsis thaliana* in response to generalist and specialist herbivores of different feeding guilds and the role of defense signaling pathways. *Phytochemistry, 67, 2450-62.*

Mitchell-Olds T. 1995. Interval mapping of viability loci causing heterosis in *Arabidopsis*. *Genetics, 140, 1105-9.*

Mittler R. 2002. Oxidative stress, antioxidants and stress tolerance. *Trends in Plant Science, 7, 405-410.*

Moffat MR and Lehane MJ. 1990. Trypsin is stored as an inactive zymogen in the midgut of *Stomoxys calcitrans. Insect Biochemistry, 20, 719-723.*

Moore LV and Scudder GGE. 1985. Selective sequestration of milkweek (Asclepias sp.) cardenolides in *Oncopeltus fasciatus* (Dallas) (Hemiptera: Lygaeidae). *Journal of Chemical Ecology, 11, 667-687.*

Mu SF, Pei L and Gao XW. 2006. Effects of quercetin on specific activity of carboxylesteras and glutathione *S*-transferase in *Bemisia tabaci*. *Chinese Bulletin of Entomology, 43, 491-495.* In Chinese with English abstract.

Mukanganyama S, Figueroa CC, Hasler JA and Niemeyer HM. 2003. Effects of DIMBOA on detoxification enzymes of the aphid *Rhopalosiphum padi* (Homoptera: aphididae). *Journal of Insect Physiology, 49, 223-229.*

Musser RO, Cipollini DF, Hum-musser SM, Williams SA, Brown JK and Felton GW. 2005. Evidence that the caterpillar salivary enzyme glucose oxidase provides herbivore offense in *Solanaceous* plants. *Archives of Insect Biochemistry and Physiology, 58, 128-137.*

Musser RO, Hum-Musser SM, Eichenseer H, Peiffer M, Ervin G, Murphy JB and Felton GW. 2002. Caterpillar saliva beats plant defences: A new weapon emerges in the coevolutionary arms race between plants and herbivores. *Nature, 416, 599-600.*

Myers JH and Bazely D. 1991. Thorns, spines, prickles, and hairs: are they stimulated by herbivory and do they deter herbivores? In: Phytochemical induction by herbivores, pp. 325-344 (eds. Tallamy DW and Raupp MJ, John Wiley & Sons, New York).

Nozzolill C, Arnason JT, Campos F, Donskov N and Jurzysta M. 1997. Alfalfa leaf saponins and insect resistance. *Journal of Chemical Ecology, 23, 995-1002.*

Oakeshott JG, Claudianos C, Campbell PM, Newcomb RD and Russell RJ. 2005. Biochemical genetics and genomics of insect esterases. In: Comprehensive Molecular Insect Science Pharmacology, vol 5. pp. 309-381 (eds. Gilbert LI, Latrou K and Gill SS, Elsevier, Oxford).

Oleszek W. 1996. Alfalfa saponins: Structure, biological activity, and chemotaxonomy. In: Saponins Used in Food and Agriculture, pp. 155-170 (eds. Waller GR and Yamasaki K, Plenum Press, New York).

Pardini RS. 1995. Toxicity of oxygen from naturally occurring redox-active pro-oxidants. *Archives of Insect Biochemistry and Physiology, 29, 101-118.*

Patankar AG, Giri AP, Harsulkar AM, Sainani MN, Deshpande VV, Ranjekar PK and Gupta VS. 2000. Complexity in specificities and expression of *Helicoverpa armigera* gut proteinases explains polyphagous nature of the insect pest. *Archives of Insect Biochemistry and Physiology, 31, 453-464.*

Pearson AC.1982. Biology, population dynamics, and pest status of the beet armyworm (*Spodopterae exigua*). in the Imperial Valley of California. Ph.D. dissertation, Univ. of California, Riverside Calif.

Peiffer M and Felton GW. 2005. The host plant as a factor in the synthesis and secretion of salivary glucose oxidase in larval *Helicoverpa zea*. *Archives of Insect Biochemistry and Physiology, 58, 106-113.*

Peiffer M and Felton GW. 2009. Do caterpillars secrete “oral secretions”? *Journal of Chemical Ecology, 35, 326-335.*

Pogue MG. 2006. World *Spodoptera Database* (Lepidoptera: Noctuidae) [WWW document]. URL http://www.sel.barc.usda.gov: 591/spod/*Spodoptera*.html.

Pompermayer P, Lopes AR, Terra WR, Parra JRP, Falco MC and Silva-Filho MC. 2001. Effects of soybean proteinase inhibitor on development, survival and reproductive potential of the sugarcane borer, *Diatraea saccharalis*. *Entomologia Experimentalis et Applicata, 99, 79-85.*

Potter SM, Jimenez-flores R and Pollack SM. 1993. Protein-saponin interaction and its influence on blood lipids. *Journal of Agricultural and Food Chemistry, 41, 1287-1291.*

Potter SM, Jimenez-Flores R, Pollack J, LoneTA and Berber-Jimenez MD. 1993. Protein saponin interaction and its influence on blood lipids. *Journal of Agricultural and Food Chemistry, 41, 1287-1291.*

Poupardin R, Reynaud S, Strode C, Ranson H, Vontas J and David JP. 2008. Crossinduction of detoxification genes by environmental xenobiotics and insecticides in the mosquito *Aedes aegypti*: impact on larval tolerance to chemical insecticides. *Insect Biochemistry and Molecular Biology, 38, 540-551.*

Pracros P. 1982. Intéret comparé de l'éstimation biologique de la valeur nutritionnelle des sources protéiniques par des vertébrés (rats et poulets) et par un insecte (Tenebrio molitor L.). *Comptes-rendus des Seances de l'Academie d'Agriculture de France, 1279-1285.*

Rachokarn S, Piyasaengthong N, Bullangpoti V. 2008. Impact of botanical extracts derived from leaf extracts *Melia azedarach* L. (Meliaceae) and Amaranthus viridis L. (Amaranthaceae) on populations of *Spodoptera exigua* (Hubner) (Lepidoptera: Noctuidae) and detoxification enzyme activities. *Communications in Agricultural and Applied Biological Sciences, 73, 451-457.*

Ranger CM, Backus EA, Winter R, Rottinghaus GE, Ellersieck MR and Johnson DW. 2004. Glandular trichome extracts from *Medicago* sativa deter settling by the potato leafhopper *Empoasca fabae*. *Journal of Chemical Ecology, 30, 927-943.*

Ranson H and Hemingway J. 2005. Mosquito glutathione transferases. *Methods in Enzymology, 401, 226-41.*

Ranson H, Claudianos C, Ortelli F, Abgrall C, Hemingway J, Sharakhova MV, Unger MF, Collins FH and Feyereisen R. 2002. Evolution of supergene families associated with insecticide resistance. *Science, 298, 179-181.*

Ranson H, Collins F and Hemingway J. 1998. The role of alternative mRNA splicing in generating heterogeneity within the *Anopheles gambiae* class I glutathione *S*-transferase family. *Proceedings of the National Academy of Sciences of the USA, 95, 14284-14289.*

Rask L, Andreasson E, Ekbom B, Eriksson S, Pontoppidan B and Meijer J. 2000. Myrosinase: gene family evolution and herbivore defense in *Brassicaceae*. *Plant Molecular Biology, 42, 93-113.*

Raupp MJ. 1985. Effects of leaf toughness on mandibular wear of the leaf beetle, *Plagiodera versicolora*. *Ecological Entomology, 10, 73-79.*

Reymond P, Bodenhausen N, Van Poecke RMP, Krishnamurthy V, Dicke M and Farmer EE. 2004. A conserved transcript pattern in response to a specialist and a generalist herbivore. *Plant Cell, 16, 3132-3147.*

Reymond P, Weber H, Damond M and Farmer EE. 2000. Differential gene expression in response to mechanical wounding and insect feeding in Arabidopsis. *Plant Cell, 12, 707-20.*

Richardson M. 1977. The proteinase inhibitors of plants and micro-organisms. *Phytochemistry, 16, 159-169.*

Rodman JE, Karol KG, Price RA and Sytsma KJ. 1996. Molecules, morphology, and Dahlgrens expanded order Capparales. *Systematic Botany, 21, 289-307.*

Rosenberry TL. 1975. Acetylcholinesterase. *Advanced Enzymology, 43,103-218.*

Ryan CA and Moura DS. 2002. Systemic wound signaling in plants: a new perception. *Proceedings of the National Academy of Sciences of the USA, 99, 6519–6520.*

Ryan CA. 1990. Protease inhibitors in plants: genes for improving defenses against insects and pathogens. *The Annual Review of Phytopathology, 28, 425-449.*

Samach A, Broday L, Hareven D and Lifschitz E. 1995. Expression of an amino acid biosynthesis gene in tomato flowers: developmental up-regulation and MeJA response are parenchyma-specific and mutually compatible. *Plant Journal, 8, 391-406.*

Schittko U, Hermsmeier D and Baldwin IT. 2001. Molecular interactions between the specialist herbivore *Manduca sexta* (Lepidoptera, Sphingidae) and its natural host *Nicotiana attenuata*. II. Accumulation of plant mRNA in response to insect-derived cues. *Plant Physiology, 125, 701-710.*

Schoonhoven LM, Van Loon JJA and Dicke M. 2005. Insect-plant biology (Oxford University Press, Oxford).

Schoonhoven LM. 1972. Secondary plant substances and insects. In: Structural and Functional Aspects of Phytochemistry, pp. 197-224 (eds. Runeckles VC and Tso TC, Academic Press, New York).

Scriber JM and Slansky F Jr. 1981. The nutritional ecology of immature insects. *Annual Review of Entomology, 26, 183-211.*

Sehnal F. 1989. Hormonal role of ecdysteroids in insect larvae and during metamorphosis. In: Ecdysone, pp. 271-278 (ed. Koolman J, Georg Thieme-Verlag, Stuttgart).

Shan G and Hammock B. 2001. Development of sensitive esterase assays based on α-cyano-containing esters. *Analytical Biochemistry, 299, 54-62.*

Shany S, Gestetner B, Birk Y and Bondi A. 1970. Lucerne saponins III. Effect of lucerne saponins on larval growth and their detoxification by various sterols. *Journal of the Science of Food and Agriculture, 21, 508-510.*

Shee G and Sharma AK. 2007. Purification and characterization of a trypsin inhibitor from seeds of *Murraya koenigii*. *Journal of Enzyme Inhibition and Medicinal Chemistry, 22, 115-120.*

Simpson SJ and Abisgold JD. 1985. Compensation by locusts for changes in dietary nutrients behavioral mechanisms. *Physiologieal Entomology, 10, 443-452.*

Sintim HO, Tashiro T and Motoyama N. 2012. Effect of sesame leaf diet on detoxification activities of insects with different feeding behavior. *Archives of Insect Biochemistry and Physiology, 3, 148-159.*

Slansky F. 1990. Insect nutritional ecology as a basis for studying host plant resistance. *The Florida Entomologist, 73, 359-378.*

Smith JN. 1968. The comparative metabolism of xenobiotics. In: Advances in Comparative Physiology and Biochemistry, Vol 3, pp. 173-232 (ed. Lowenstein O, Academic Press, New York).

Sniffen CJ, O'Connor JD, Van Soest PJ, Fox DG and Russell JB. 1992. A net carbohydrate and protein system for evaluating cattle diets: II. Carbohydrate and protein availability. *Journal of Animal Science, 70, 3562-3577.*

Snyder MJ and Glendinning JI. 1996. Causal connection between detoxification enzyme activity and consumption of a toxic plant compound. *Journal of Comparative Physiology, 179, 255-261.*

Sparks TC, Lockwood JA, Byford RL, Graves JB and Leonard BR. 1989. The role of behavior in insecticide resistance. *Pesticide Science, 26, 383-399.*

Stadtman ER. 1990. Metal ion-catalyzed oxidation of proteins: Biochemical mechanisms and biological consequences. *Free Radical Biology and Medicine*, *9, 315-325.*

Stadtman ER. 1991. Metal ion-catalyzed oxidation of proteins: Physiological consequences. *Journal of Chemical Ecology*, 266, 2006-2008.

Stahl E. 1888. Pflanzen und Schnecken: Biologische Studie uber die Schutzmittel der Pflanzen gegen Schneckenfrass. *Wissenschaftliche Zeitschrift der Friedrich-Schiller-Universität Jena, 22, 557-684.*

Stotz HU, Kroymann J and Mitchell-Olds T. 1999. Plant–insect interactions. *Current Opinion in Plant Biology, 2, 268-272.*

Summers CB and Felton GW. 1993. Antioxidant role of dehydroascorbic acid reductase in insects. *Biochimica et Biophysica Acta, 1156, 235-238.*

Tallemy DW and Raupp MJ. 1991. Phytochemical Induction by Herbivores. Wiley Interscience, New York.

Tava A and Odoardi M. 1996. Saponins from *Medicago spp*.: Chemical characterization and biological activity against insects. In: Saponins Used in Food and Agriculture, pp. 97-109 (eds. Waller GR and Yamasaki K, Plenum Press, New York).

Tava A, Forti D and Odoardi M. 1992. Alfalfa saponins: Isolation, chemical characterization and biological activity against insects. In: Proceedings, Eucarpia Medicago spp. pp. 283-288 (Group Meeting, Lodi, Italy).

Tew KD and Ronai Z. 1999. GST function in drug and stress response. *Drug Resistance Updates, 2, 143-147.*

Thakur BR, Singh RK and Nelson PE. 1996. Quality attributes of processed tomato products: A review. *Food Reviews International, 12, 375-401.*

Tian D, Peiffer M, Shoemaker E, Tooker J, Haubruge E, Francis F, Luthe1 DS and Felton GW. 2012. Salivary glucose oxidase from caterpillars mediates the induction of rapid and delayed induced defenses in the tomato plant. *PLoS One 7, e36168.*

Tierens K, Thomma BPH, Brouwer M, Schmidt J, Kistner K, Porzel A, Mauch-mani B, Cammue BPA and Broekaery WF. 2001. Study of the role of antimicrobial glucosinolate-derived isothiocyanates in resistance of *Arabidopsis* to microbial pathogens. *Plant Physiology, 125, 1688-1699.*

Tooker JF and Hanks LM. 2006. Tritrophic interactions and reproductive fitness of the prairie perennial *Silphium laciniatum* Gillette (Asteraceae). *Environmental Entomology, 35, 537-545.*

Tu CP and Akgul B. 2005. Drosophila glutathione *S*-transferases. *Methods in Enzymology, 401, 204-26.*

Turunen S. 1983. Absorption and utilization of essential fatty acids in lepidopterous larvae: metabolic implications. In: Metabolic aspects of lipid nutrition in insects, pp. 57-71 (eds. Mittler TE and Dadd RW, Westview, Boulder, Colorado).

Turunen S. 1985. Absorption. In: Comprehensive insect physiology, biochemistry and pharmacology, vol. 4, pp. 241-77 (eds. Kerkut GA and Gilbert LI, Pergamon, Oxford).

Van der Hoeven RS and Steffens JC. 2000. Biosynthesis and elongation of short- and medium-chain-length fatty acids. *Plant Physiology, 122, 275-282.*

Van Loon JJA, de Boer G and Dicke M. 2000. Parasitoid-plant mutualism: parasitoid attack of herbivore increases plant reproduction. *Entomologia Experimentalis et Applicata, 97, 219-227.*

Vaughan GL and Jungreis AM. 1977. Insensitivity of lepidopteran tissues to ouabain: Physiological mechanisms for protection from cardiac glycosides. *Journal of Insect Physiology, 23, 585-589.*

Vaughn SF and Berhow MA. 1998. 1-Cyano-2-hydroxy-3-butene, a phytotoxin from crambe (*Crambe abyssinica*) seedmeal. *Journal of Chemical Ecology, 24, 1117-1126.*

Volpicella M, Ceci LR, Cordewener J, America T, Gallerani R, Bode W, Jongsma MA and Beekwilder J. 2003. Properties of purified gut trypsin from *Helicoverpa zea*, adapted to proteinase inhibitors. *European Journal of Biochemistry, 270, 10-19.*

Vontas JG, Small JG and Hemingway J. 2001. Glutathione *S*-transferases as antioxidant defense agents confer pyrethroid resistance in *Nilaparvata lugens*. *Biochemical Journal, 357, 65-72.*

Walling LL. 2000. The myriad plant responses to herbivores. *Journal of Plant Growth Regulation, 19, 195-216.*

Wang E, Hall JT and Wagner GJ. 2004. Transgenic *Nicotiana tabacum* L. with enhanced trichome exudate cembratrieneols has reduced aphid infestation in the field. *Molecular Breeding, 13, 49-57.*

Wang KY, Zhang Y, Wang HY, Xia XM and Liu TX. 2009. Influence of three diets on susceptibility of selected insecticides and activities of detoxification esterases of *Helicoverpa assulta* (Lepidoptera: Noctuidae). Pestic Biochem Physiol. 96, 51–55.

Wang W, Mo J, Cheng J, Zhuang P and Tang Z. 2006. Selection and characterization of spinosad resistance in *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae). *Pesticide Biochemistry and Physiology, 84, 180-187.*

Warbrick-Smith J, Behmer, ST, Lee, KP, Raubenheimer D and Simpson SJ. 2006. Evolving resistance to obesity in an insect. *Proceedings of the National Academy of Sciences of the USA, 103, 14045-14049.*

Waris G and Ahsan H. 2006. Reactive oxygen species: role in the development of cancer and various chronic conditions. *Journal of Carcinogenesis, 5, 14.*

Weech MH, Chapleau M, Pan L, Ide C and Bede JC. 2008. Caterpillar saliva interferes with induced *Arabidopsis thaliana* defence responses via the systemic acquired resistance pathway. *Journal of Experimental Botony, 59, 2437-48.*

Wheelock CE, Shan G and Ottea J. 2005. Overview of carboxylesterases and their role in the metabolism of insecticides. *Journal of Pest Science, 30, 75-83.*

Whittaker RH and Robert H. 1970. The biochemical ecology of higher plants. In: Chemical ecology, pp. 43-70 (eds. Sondheimer E and Simeone JB, Academic Press, Boston).

Wilkens RT, Shea GO, Halbreich S and Stamp NE. 1996. Resource availability and the trichome defenses of tomato plants. *Oecologia, 106, 181-191.*

Wilson JW. 1934. The asparagus caterpillar: its life history and control. *Florida Agricultural Experiment Station Bulletin, 271, 1-26.*

Winz RA and Baldwin IT. 2001. Molecular interactions between the specialist herbivore *Manduca sexta* (Lepidoptera, Sphingidae) and its natural host *Nicotiana attenuata*. IV. Insect-induced ethylene reduces jasmonate-induced nicotine accumulation by regulating putrescine N-methyltransferase transcripts. *Plant Physiology, 125, 2189-2202.*

Wu G, Shortt BJ, Lawrence EB, Levine EB, Fitzsimmons KC and Shah DM. 1995. Disease resistance conferred by expression of a gene encoding H_2O_2-generating glucose oxidase in transgenic potato plants. Plant Cell 7, 1357–1368.

Wu S, Peiffer M, Luthe DS and Felton GW. 2012. ATP hydrolyzing salivary enzymes of caterpillars suppress plant defenses. *PLoS ONE 7, e41947.*

Xue M, Pang YH, Li QL and Li TX. 2010. Effects of four host plants on susceptibility of *Spodoptera litura* (Lepidoptera: Noctuidae) larvae to five insecticides and activities of detoxification esterases. *Pest Management Science, 66, 1273-1279.*

Yan F, Xu C, Li S, Lin C and Li J. 1995. Effects of DIMBOA on several enzymatic systems in Asian corn borer Ostrinia furnacalis (Guenée). *Journal of Chemical Ecology, 21, 2047-2056.*

Yang RSH. 1976. Enzymatic conjugation and insecticide metabolism. In: Insecticide Biochemistry and Physiology, pp. 177-225 (ed. Wilkinson CF, Plenum, New York).

Yu QY, Lu C, Li WL, Xiang ZH and Zhang Z. 2009. Annotation and expression of carboxylesterase in the silkworm *Bombyx mori*. *BMC Genomics, 10, 553.*

Zebelo SA and Maffei ME. 2012. The Ventral Eversible Gland (VEG) of *Spodoptera littoralis* triggers early responses to herbivory in *Arabidopsis thaliana*. *Arthropod Plant Interact, 6, 543-551.*

Zhang B, Liu H and Hull-Sanders H. 2011. Effect of host plants on development, fecundity and enzyme activity of *Spodoptera exigua* (Hübner) (Lepidoptera: Noctuidae). *Journal of Integrative Agriculture, 10, 1232-1240.*

Zhang MX, Fang TT, Pu GL, Sun XQ, Zhou XG and Cai QN. 2013. Xenobiotic metabolism of plant secondary compounds in the English grain aphid, *Sitobion avenae* (F.) (Hemiptera: Aphididae). *Pesticide Biochemistry and Physiology, 107, 44-49.*

Zhang XX, Liu SK and Takano T. 2008. Two cysteine proteinase inhibitors from *Arabidopsis thaliana*, AtCYSa and AtCYSb, increasing the salt, drought, oxidation and cold tolerance. *Plant Molecular Biology, 68, 131-143.*

Zhang Y, Talalay P, Cho CG and Posner GH. 1992. A major inducer of anticarcinogenic protective enzymes from broccoli: Isolation and elucidation of structure. *Proceedings of the National Academy of Sciences of the USA, 89, 2399-2403.*

Zhou ZH and Syvanen M. 1997. A complex glutathione transferase gene family in the housefly *Musca domestica*. *Molecular Genetics and Genomics, 256, 187-194.*

Zhu KY and Clark JM. 1995. Comparisons of kinetic properties of acetylcholinesterase purified from azinphosmethyl-susceptible and -resistant strains of Colorado potato beetle. *Pesticide Biochemistry and Physiology, 51, 57-67.*

Zhu-Salzman K, Koiwa H, Salzman RA, Shades RE and Ahn JE. 2003. Cowpea bruchid *Callosobruchus maculatus* uses a three-component strategy to overcome a plant defensive cysteine protease inhibitor. *Insect Biochemistry and Molecular Biology, 12, 135-145.*

Zong N and Wang CZ. 2004. Induction of nicotine in tobacco by herbivory and its

relation to glucose oxidase activity in the labial gland of three noctuid caterpillars. *Chinese Science Bulletin, 49, 1596-1601.*

Zor T and Z Selinger. 1996. Linearization of the Bradford protein assay increases its sensitivity: theoretical and experimental studies. *Analytical Biochemistry, 236, 302-308.*

9. Appendices

Table 1. Statistical analysis of effects on enzyme activity from caterpillars fed on different diets. Diets were artificial diet, *Arabidopsis thaliana*, *Medicago truncatula* or *Solanum lycopersicum*. Data were analyzed by one-way ANOVA followed by a Tukey post-hoc test.

Enzyme	Value	Enzyme	Value
GOX (U/pair LSG)	$F_{(4,10)} = 105.5$; $p<0.001$	GOX (U/mg soluble protein)	$F_{(4,10)} = 105.3$; $p<0.001$
APOX (U/pair LSG)	$F_{(4,10)} = 0.573$; $p = 0.689$	APOX (U/mg soluble protein)	$F_{(4,10)} = 0.6$; $p = 0.671$
GST (U/gut)	$F_{(4,10)} = 359.1$; $p<0.001$	GST (U/mg soluble protein)	$F_{(4,10)} = 312.7$; $p<0.001$
Trypsin (U/gut)	$F_{(4,10)} = 178.6$; $p<0.001$	Trypsin (U/mg soluble protein)	$F_{(4,10)} = 118.7$; $p<0.001$
Carboxylesterase (U/gut)	$F_{(4,10)} = 493.6$; $p<0.001$	Carboxylesterase (U/mg soluble protein)	$F_{(4,10)} = 277.8$; $p<0.001$

Table 2. Statistical analysis of effects on enzyme activity from caterpillars fed on artificial diet containing *Arabidopsis* extract or starved. Diets were artificial diet, artificial diet containing the extracts of *Arabidopsis* either with previous 48-hr attack of caterpillars (A.th with) or without caterpillar herbivory (A.th without). Data were analyzed by one-way ANOVA followed by a Tukey post-hoc test.

Enzyme (U/gut)	Value	Enzyme (U/mg soluble protein)	Value
GST	$F_{(3,8)} = 35.70$; $p<0.001$	GST	$F_{(3,8)} = 29.68$; $p<0.001$
Trypsin	$F_{(3,8)} = 11.20$; $p<0.005$	Trypsin	$F_{(3,8)} = 11.85$; $p<0.005$
Carboxylesterase	$F_{(3,8)} = 37.0$; $P<0.001$	Carboxylesterase	$F_{(3,8)} = 16.89$; $p = 0.001$

Table 3. Statistical analysis of effects on enzyme activity from caterpillars fed on artificial diet with different P:C ratios. Diets were artificial diet (Bio-Serv), artificial diet with the P:C ratios of 25P:39C or 24P:17C. Data were analyzed by one-way ANOVA followed by a Tukey post-hoc test.

Enzyme	Value	Enzyme	Value
GOX (U/pair LSG)	$F_{(2,6)} = 87.57$; $p<0.001$	GOX (U/mg soluble protein)	$F_{(2,6)} = 138.8$; $p<0.001$
GST (U/gut)	$F_{(2,6)} = 0.341$; $p = 0.724$	GST (U/mg soluble protein)	$F_{(2,6)} = 3.78$; $p = 0.087$
Trypsin (U/gut)	$F_{(2,6)} = 46.96$; $p<0.001$	Trypsin (U/mg soluble protein)	$F_{(2,6)} = 97.20$; $p<0.001$
Carboxylesterase (U/gut)	$F_{(2,6)} = 0.221$; $p = 0.808$	Carboxylesterase (U/mg soluble protein)	$F_{(2,6)} = 9.158$; $p = 0.015$