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Induction of Glutathione S-Transferase in *Helicoverpa zea* Fed Cashew Flour

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ABSTRACT

Helicoverpa zea and other insects have evolved strategies to counteract the plant protective proteins and defensive compounds they may encounter during feeding. We sought to take advantage of this phenomenon by identifying proteins up-regulated in H. zea in response to the inclusion of cashew nut flour in their diet. Tree nut and peanut seed storage proteins can act as defensive compounds protecting the tissue from insect damage by inhibiting digestion. When ingested by humans, these proteins can act as potent food allergens that may cause harmful physiological responses in food allergy affected individuals. Cashew allergens, such as the 2S albumin Ana o 3, have homology to protease or amylase inhibitors that inhibit digestion. Insects such as the corn earworm, H. zea, feed on various plant species that contain similar protease or amylase inhibitors and additional defensive compounds. Here, we compared the intestinal protein profile of H. zea fed on media containing pinto bean, cashew, or peanut. Through comparative feeding studies, mass-spectrometry, and glutathione binding assays we demonstrate that Glutathione S-transferases (GST) are specifically induced in the larvae of H. zea when cashew protein is included in their diet. We assessed the ability of purified H. zea GST proteins to break the disulfide bonds of cashew extract proteins and the purified Ana o 3 allergen. Continued research in this area could identify insect enzymes that may be useful in enzymatic processing steps to reduce or eliminate nut allergens and may have application in the food industry or health fields.

Keywords: *Helicoverpa zea*, cashew, allergen, glutathione S-transferase

INTRODUCTION

Over the last 400 million years, insects have evolved to effectively use plant seeds or nuts for sustenance and have developed adaptive measures to compete effectively against plant defenses. Insects alter the gene expression of specialized enzymes for digestion and as countermeasures for plant defenses. These include a number of specific xenobiotic defense enzymes including cytochrome P450s [37], salivary enzymes [40, 42], and digestive enzymes [50, 41]. Up-regulated enzymes may detoxify defensive compounds, inactivate or degrade defensive proteins, or replace the function of insect digestive enzymes that are being inhibited by defensive compounds.

Many insect species are able to feed on a variety of different plant species that contain an array of different plant defensive compounds that must be counteracted by the feeding insect. Among these polyphagous insects is the corn

earworm, *Helicoverpa zea*, which feeds on many plant species in the Graminae, Solanacea, Leguminaceae and other families [39].

Food allergy is a serious medical condition and the only currently accepted treatment is strict avoidance of the allergy inducing food. Food allergy is a Type I hypersensitivity mediated by immunoglobulin E (IgE) binding of specific food proteins. IgE bound to food allergens results in degranulation of basophils and mast cells and the release of inflammatory mediators. Allergy symptoms vary and patients must constantly be on guard against accidental exposure. Peanuts and tree nuts are foods that commonly cause allergic reactions and the frequency of reported nut allergies appears to be on the rise [44]. Allergy to peanuts and tree nuts is rarely outgrown [9] and exposure often results in serious reactions [45]. In particular, studies have indicated that reactions to cashew are frequently severe [3, 25].

Cashew nuts are a healthy snack and contain approximately 15-25 percent protein. Seed storage proteins are an essential store of amino acids during germination and outgrowth and can also have roles in plant defense and protection [36]. Three IgE binding cashew nut seed storage proteins have been characterized as allergens including Ana o 1 [14], Ana o 2 [15], and Ana o 3 [22]. Ana o 1 is a minor component of total protein, Ana o 3 makes up approximately 10-15 percent, and Ana o 2 is estimated to be approximately 50 percent [47]. Ana o 2 and Ana o 3 contain disulfide bonds, and each of the cashew allergens are resistant to physical and thermal processing methods [29, 31]. In particular, the 2S albumin Ana o 3 is a member of the prolamin superfamily of plant allergens [8] and harbors 8 conserved cysteine residues that form 4 disulfide bonds within the protein [11]. The network of disulfide bonds are thought to contribute to the stability of the 2S albumins to processing and digestion.

Plant allergens may also act as plant defensive compounds [18]. Research with 2S albumins suggests that they may function as trypsin or amylase inhibitors and act as plant protective proteins. For example, a mustard 2S albumin was isolated as a trypsin inhibitor [33], and castor bean 2S albumins have been shown to inhibit insect larvae α-amylases and growth [49]. These and other studies suggest 2S albumins may function to protect plants from consumption by inhibiting digestive enzymes. Similarly, 2S albumins have been shown to survive digestion by human protease [20, 46, 21, 7, 12, 17, 28, 30, 24], and this is thought to contribute to their ability to sensitize individuals to specific food allergens.

This study sought to identify proteins up-regulated by *H. zea* in response to the inclusion of cashew nut proteins in their diet. This type of research could identify novel insect enzymes that may be useful in new tree nut and peanut processing methods able to reduce or eliminate nut allergens. We compared the protein profile of *H. zea* fed on media containing pinto bean, cashew, or peanut. Through these comparative feeding studies we demonstrate that Glutathione S-transferase is induced in the larvae of *H. zea* when cashew protein is included in their diet.

MATERIALS AND METHODS

Materials

Cashew flour was purchased from Nuts Online (http://www.nuts.com, Cranford, NJ) and peanut flour was generously donated from the Golden Peanut Company (Alpharetta, GA USA). Soy bean trypsin inhibitor (SBTI) was purchased from Sigma Aldrich (St. Louis, MS, USA). Cashew extracts and purified Ana o 3 were generated as described previously [7]. The rabbit anti-GST antibody was purchased from Sigma Aldrich.

Helicoverpa zea larvae

The *H. zea* larvae were obtained from a colony that has been in culture for several years and was originally collected from corn, *Zea mays*. Larvae were reared on pinto bean based diet at 27$\pm$ 1° C, 40 $\pm$ 10% relative humidity, and a 14:10 light: dark photoperiod, as described previously [10]. Larvae were reared on the pinto bean diet to the fourth instar, and then transferred to test diet once they ceased feeding and the head capsule had slipped just prior to molting. Larvae were allowed to feed on the test diet for two days, and then a portion of the midgut (which contains most of the digestive and defensive enzymes) just anterior to the most forward attachment of the malpighian tubules was removed by dissecting in pH 7.4, 0.1 M sodium phosphate buffer. The gut contents were removed, and each gut was frozen individually in 1.5 ml tubes. Each test diet treatment included 7 replicates. For the nut protein diet, 1 gm of either defatted peanut or cashew powder was mixed into 4 ml of 3% agar held as a liquid at 60° using a vortex mixer. Purified soybean trypsin inhibitor was added to pinto bean diet in an analogous manner, to yield a

concentration of 1000 ppm. No larval mortality or developmental delay was observed among the different diets during the 2 day feeding period.

Protein extraction

H. zea gut proteins were extracted by the addition of 1000 µL of ice cold lysis buffer (50 mM Tris pH 8.3, 150 mM sodium chloride, and 1 mM PMSF) to a single gut. The samples were resuspended using a polypropylene pestle and then passed through a 22 gauge needle 10 times. The supernatant was collected following centrifugation at 17,000 x g for 30 minutes and protein concentration determined with a NanoDrop spectrophotometer (ThermoFisher Scientific, USA) normalized to a set of BSA standards (BioRad, Hercules, CA, USA).

SDS-PAGE

An aliquot equivalent to 50 µg of *H. zea* gut extracted proteins was added to NuPAGE LDS Sample Buffer (Invitrogen, USA) containing reducing agent and samples were heated to 65°C for 15 minutes. Proteins were resolved on Mini-Protean TGX 'Any kD' precast tris-glycine gels (Bio-Rad, Hercules, CA, USA) using a Mini Protean system II (BioRad, Hercules, CA, USA). Prestained Precision Plus molecular weight markers (BioRad, Hercules, CA, USA) were used as protein standards. Following electrophoresis, protein bands were visualized with Safe Stain (Invitrogen, Grand Island, NY, USA) and images were captured with the 680 nM channel on an Odyssey CLX infrared imaging system (LI-COR, Lincoln, NE, USA). Protein signal intensity in each sample lane or band region was quantified using the IRdye 680 signal channel to ensure equal loading. Sample protein content was normalized using Excel software (Microsoft, Redmond, WA, USA) to ensure equal loading.

Trypsin digestion

Samples were excised from gels and cut into small 1 mm cubes. Gel cubes were rinsed sequentially in water, 100 mM ammonium bicarbonate, and 50% acetonitrile. After drying and reduction with DTT, gel cubes were alkylated with iodoacetamide and incubated with sequencing-grade modified trypsin (Promega, Madison, WI, USA) for 16 hours at 37°C with gentle agitation. The supernatant was collected and gel slices were extracted once with 25 mM ammonium bicarbonate and then with 5% formic acid. The supernatants were pooled and dried under vacuum.

LC-MS/MS Mass-Spectrometry

Dried samples were resuspended in 5% formic acid and analyzed via liquid chromatography with tandem mass spectrometry (LC/MS/MS) using an Agilent 1200 LC system, an Agilent Chip Cube interface and an Agilent 6520 Q-TOF tandem mass spectrometer (Agilent Technologies, Santa Clara, CA, USA). Chromatographic separation was accomplished using a chip consisting of a 160 nL enrichment column and a 150 mm analytical column packed with C18, 5 µm beads with 300 Å pores. One microliter aliquots of the sample were transferred to the enrichment column via the capillary pump operating at a flow rate of 4 µl/min. The nano pump was operated at a flow rate of 600 nL/min. An initial gradient (Solvent A: 100% H2O, 0.1% Formic Acid; Solvent B: 90% acetonitrile [ACN], 10% H2O and 0.1% Formic Acid) of 97% solvent A was changed to 60% solvent A at 20 min, 20% at 22 min and held until 25 min. A post-run time of 4 min was employed for column equilibration between samples. The MS source was operated at 300°C with 5 L/min N_2 flow and a fragmentor voltage of 175 V. Nitrogen was used as the collision gas, and the collision energy varied as a function of mass and charge using a slope of 3.7 V/100 Da and an offset of 2.5 V. Both the quad and time of flight (TOF) were operated in positive ion mode. Reference compounds of 322.048121 Da and 1,221.990637 Da were continually leaked into the source for mass calibration. An initial MS scan was performed from m/z 300 to 1,600 and up to three multiply charged ions were automatically selected for MS/MS analysis. Following the initial run, a second injection was made excluding ions previously targeted in the MS/MS analysis. LC chromatograms and mass spectra were analyzed using Mass-Hunter software (Version B.0301; Agilent Technologies). Data files were transferred to an Agilent workstation equipped with Spectrum Mill software (Agilent Technologies) for peptide sequencing and protein identification using a *Helicoverpa*-specific library constructed from the NCBI database.

Glutathione binding

Samples of *H. zea* gut extracts containing approximately 200 µg protein were added to 20 µL of pre-washed Pierce Glutathione (GSH) Agarose beads (ThermoScientific, Waltham, MA, USA) in lysis buffer and incubated overnight at 4°C with gentle mixing. The following morning the beads were collected by centrifugation and washed 4 times with 500 µL of ice cold lysis buffer. Bead bound material was released by the addition of 40 µl of 2X SDS-PAGE sample buffer and samples were evaluated by SDS-PAGE and LC MS/MS Mass-Spectrometry.

H. zea GST cashew extract assay

GSH bead bound *H. zea* GST was eluted with reduced glutathione according to manufacturer's instructions, and excess glutathione was removed by centrifugation with a 3 kDa spin filters (Millipore, Billerica, MA, USA). After buffer exchange and concentration with spin filters, approximately 1 µg of eluted *H. zea* GST was added to either 16 µg of cashew extract or 1 µg of Ana o 3 in 100 mM Tris (pH varied from 7.4-8.5) and 1 mM reduced glutathione in a final volume of 10 µl. The samples were incubated at 37°C for 1 hr with gently agitation. After the 1 hr incubation, 4 µl of NuPAGE (Life Technologies, Grand Island, NY, USA) 4X LDS sample buffer (lacking reducing agent) was added to each sample, heated at 65°C for 15 minutes, and then resolved by SDS-PAGE.

RESULTS AND DISCUSSION

Protein profile of soluble H. zea gut tissue

Soluble protein profiles extracted from the guts of *H. zea* larvae fed on media containing pinto bean, soybean protease inhibitor, defatted cashew protein, or defatted peanut protein were compared by SDS-PAGE. The intensity of the resolved proteins was compared in each lane and the intensity of one band in particular migrating at approximately 23kDa, was increased in the guts of *H. zea* grown on diets containing cashew protein (Figure 1). Comparison of the IRdye 680 intensity of this band on the scanned gels indicated it was approximately 40 percent more intense specifically in the cashew containing diet sample. A second protein band migrating at approximately 37kDa was also increased in the cashew containing diet sample relative to the other proteins. The 37kDa band was only slightly (5-10%) more intense than the corresponding region from the other sample lanes (data not shown).

Protein identification by mass-spectrometry

To identify the proteins corresponding to the 23kDa and 37kDa bands of increased intensity from the cashew lane, these bands were excised and the proteins digested with trypsin and analyzed by LC-MS/MS. We identified 13 distinct peptides matching glutathione S-transferase (GST) sequences from *H. armigera* (GenBank ADD17089) (Figure 2) using the 23kDa sample. This represents 64 percent of the predicted protein sequence of 220 amino acids. The carboxy terminal domain of this GST suggests it is a member of the Delta and Epsilon subfamily. The matching peptides we observed included residues predicted to be in the glutathione and substrate binding pockets. A second isoform of *H. armigera* GST (Genbank ADI32888) belonging to the Sigma class was also identified in the LC-MS/MS analysis from the cashew containing diet gut sample (Figure 2). Only 6 peptides were matched to this second isoform corresponding to 26 percent of the predicted 206 amino acid protein, and the same peptide sequences were identified. Consistent with the band intensities from gel pictures, we detected only 3 GST peptides (GenBank ADD17089) from a corresponding position in the gel excised from the pinto bean diet sample lane. We tested whether the putative GST proteins we identified could react with a commercially available anti-GST antibody from *Schistosoma japonicum*. While this antibody was able to recognize a positive control protein containing the *S. japonicum* GST, the antibody was not able to recognize the putative *H. zea* GST proteins on a western blot (data not shown). This finding is not surprising given that GST family members are comprised of very diverse amino acid sequences.

We also analyzed the 37kDA protein band from the cashew containing diet sample using the same techniques just described, and 13 peptides were identified matching the sequence of *H. armigera* arginine kinase (GenBank ADD22718), representing 39 percent of the protein (data not shown). *Arginine kinases* are important in the storage of cellular energy and regulation of cellular ATP levels, and they catalyze the formation of omega-phospho-L-arginine from ATP and L-arginine in invertebrates [48]. Arginine kinase is an important pan-allergen and the shellfish and cockroach Arginine kinases, among others, bind to IgE in allergic individuals [19, 2, 34, 6]. Arginine kinase has previously been identified as an abundant component of the *H. armigera* midgut [51].

Glutathione S-transferase purification

To confirm that the 23kDa protein was indeed a GST, we incubated lysates from the pinto bean and cashew nut diet gut samples with glutathione agarose beads. When bead-bound material was evaluated by SDS-PAGE we observed an intense band migrating at approximately 23kDA in the cashew diet lane (Figure 3). A similar band was present in the pinto bean diet sample, however the intensity of this band was reduced. Importantly, the 23kDA band was greatly reduced in the remaining unbound material in both the cashew nut and pinto bean diet sample lanes. Trypsin digestion and LC-MS/MS analysis of the bead bound material confirmed the identity of the bound protein to be the same GST proteins we identified from the gel slices, GenBank ADD17089 and Genbank ADI32888. These

observations strongly support the conclusion that the 23kDa band represents at least one member of the GST protein family.

GST bioassay with cashew proteins

Our results suggested the possibility that GST may target disulfide bonds within proteins of the cashew extracts to aid metabolism of these proteins. Both the Ana o 2 and Ana o 3 proteins contain disulfide bonds, and we speculated that they might be GST targets. We tested this by incubating cashew extracts with purified *H. zea* GST eluted from GSH beads to determine if they could catalyze the conjugation of GSH to cashew proteins (Figure 4). Changes in Ana o 2 and Ana o 3 migration pattern in SDS-PAGE gels containing reducing agents gels are easily observed [7]. The breaking and conjugation of GSH to disulfide bonds in Ana o 2 or Ana o 3 would be expected to generate a similar pattern. We varied the pH (7.5-8.3) as well as the GSH and salt concentrations, but could not identify conditions where we could observe changes in the migration pattern of cashew proteins that would suggest GSH had been conjugated to any of the proteins in the extract. To simplify the test we used purified Ana o 3, whose migration on SDS-PAGE has previously been shown to be sensitive to reduction with DTT [7, 22]. However, we could not observe any changes in the purified Ana o 3 protein's migration (Figure 4). We increased the pH in our reactions as there are reports that the midgut pH of lepidopteran species could be as high as 9.5 [23]. However, the reaction of Ana o 3 with GSH was pH sensitive, and at pH 8.3 and concentrations as low as 0.3mM, glutathione was able to reduce Ana o 3 disulfide bonds by itself, and change the migration of the protein on SDS-PAGE was observed (data not shown).

We sought to identify *H. zea* gut enzymes whose level was increased in response to the inclusion of cashew nut proteins in their diet. Cashew nut and other nut seed storage proteins may be resistant to digestion and can act as potent food allergens [3, 25, 7, 22, 14, 15]. Insects have evolved unique enzymatic strategies to counteract plant defense mechanisms meant to prevent or inhibit plant seed consumption and digestion. These enzymes may be able to better digest the seed storage proteins found in tree nuts and peanuts that can inhibit metabolic activity and also act as food allergens in humans. Identification and characterization of these unique enzymes could eventually lead to their incorporation into food processing or medical therapy steps to reduce the number or intensity of food allergy reactions.

Our results identified GST family members that were specifically elevated in response to the presence of cashew flour in the *H. zea* diet. It is not clear why the increased level of GST proteins was only observed specifically when cashew nut flour was included in the diet. Cashew, peanut, and pinto beans contain some of the same conserved seed storage proteins however the level of specific proteins varies within each of these different plant seeds. In addition, there are numerous other differences within these seeds that may account for the increase in GST level we observed in this project. Regardless, we used several methods including mass-spectrometry and a glutathione binding assay to confirm that the proteins we observed at an elevated level were GST family members.

Members of the GST family are diverse their sequence, but they share a conserved function to conjugate reduced glutathione to various substrates [35, 26]. Their role is important in the metabolism and detoxification of cells during growth and response to stress. Here we identified at least two *H. zea* GST proteins whose amounts were increased when larvae are fed on a diet containing cashew flour. Some cashew allergens are hard to digest with human protease, but can be made easier to digest after disruption of disulfide bonds within the proteins. Although we did not identify conditions to demonstrate it, it is possible that the *H. zea* GST may function to open up the structure of hard-to-digest proteins, such as the cashew 2S albumin Ana o 3. This would increase the nutritional value of the cashew flour that was included in the diet. The 11S legumin and 2S albumin are conserved seed storage proteins, and although the amount of these proteins varies, they are commonly identified as food allergens from plant foods [11]. The soy 2S albumin, Gly m 2S, is similar to the cashew 2S albumin allergen and may be useful as a diagnostic marker for soy allergy [27, 38, 21]. The castor bean 2S albumins, Ric c 1 and 3, which are also allergens, play a role in insect resistance, and can act as α-amylase inhibitors and reduce larval growth [49]. A directed blast search with the Ana o 3 sequence also identified segments of homology to alpha-amylase inhibitors from bread wheat (GenBank ABO46001.1) and hordoindoline b-1 from barley (Gen Bank AAV37632.1). Thus the ability of insects to counteract enzymatic inhibitory activity of 2S albumins and related proteins and utilize their nutritional value would provide a competitive advantage. Conjugation of GSH on disulfide bonds would presumably provide a mechanism to both reduce enzymatic activity and destabilize any protein dependent upon disulfide bonds for their structure.

Enzymatic reduction of the disulfide bonds within 2S albumins has been previously demonstrated. For example, the 2S albumin from castor seed has been reduced using a reaction with thioredoxin [43]. The thioredoxin gene family encodes proteins that can reversibly alter the oxidation state of substrates using a catalytically active disulfide site [5]. Several hundred proteins have been shown to be targets of thioredoxins [13]. For example, wheat and milk allergens have been shown to be acted upon by thioredoxin, resulting in increased digestibility and reduced allergen potential [4, 16]. Thioredoxin proteins are considered cross-reactive pan-allergens for asthma and food allergy [1, 32].

Figure 1

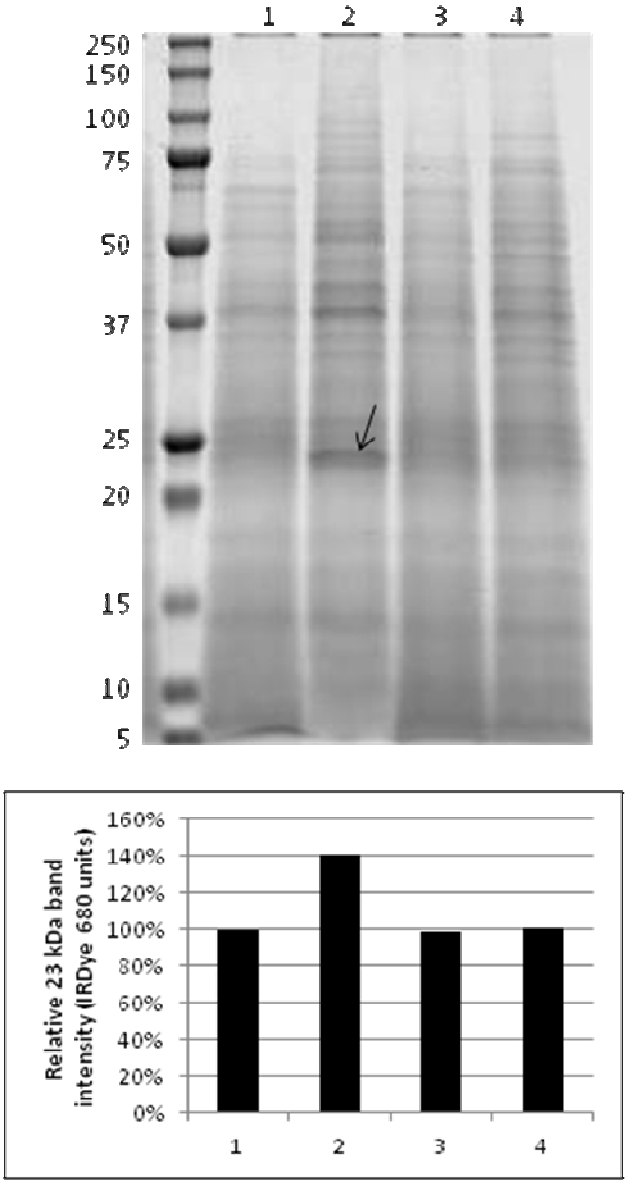

Fig. 1. Protein samples extracted from *H. zea* guts were analyzed by SDS-PAGE (top panel) and protein signal intensity of a 23kDa band (bottom panel) was quantified with images captured on a Li-Cor Odyssey CLx using the 680 nm infrared channel. Signals from individual bands were normalized to total protein signal within each lane. Arrow indicates 23 kDa band of increased intensity from cashew flour containing diet. Lane 1 – soybean diet, lane 2 – cashew flour diet, lane 3 – peanut flour diet, and lane 4 – soy bean trypsin inhibitor diet.

Figure 2

Glutathione S-transferase (GenBank ADDI7089)

```
1   MSLDLYYAPG SAPCRVVLLV AAALDVHFNP RILNLRNGEH LTEEFLKINP CHTVPTLVDG DFSLWESRAI GKYLVNKYGG 80
81  EMNDLYPSDP KARAIVDQRL DFDLGTLYPR FGNYIYPQIF GGAKADEALI KKLEEALCFL NTFLEGQKYA AGDELTLADL 160
161 SLVATVSTID AVDISLKEYP NVEKWFELVK ATAPGYQEAN EAGLKAFRAM VAQLKARTEL   220
```

Glutathione S-transferase (GenBank ADDI32888)

```
1   MPKAVIYYIK MKGLGEPCRL LLAYSGVDFE DIRFERHGQS WQDFKPKTPF GCVPVLEMDG RTYAQSYSIA RFLGREFGLC 80
81  GDNIQSSFII DQIVDLIDDL RKRASSVDYE PEPDLKEKRH AIYAKTVYPD LLQRINDIIV KNNGYMALGK LTWGDFILAG 160
161 LIDYLKYMLR MPDLSKQYPA PKQVVDKVFA FPKVKAYVDA APEALF    206
```

Fig. 2. Sequences of *H. armigera* glutathione S-transferase (GST) proteins (top, GenBank ADD17089 and bottom, Genbank ADI32888). Peptide fragments observed by mass-spectrometry in samples from larvae fed on the cashew flour containing test diet are underlined.

Figure 3

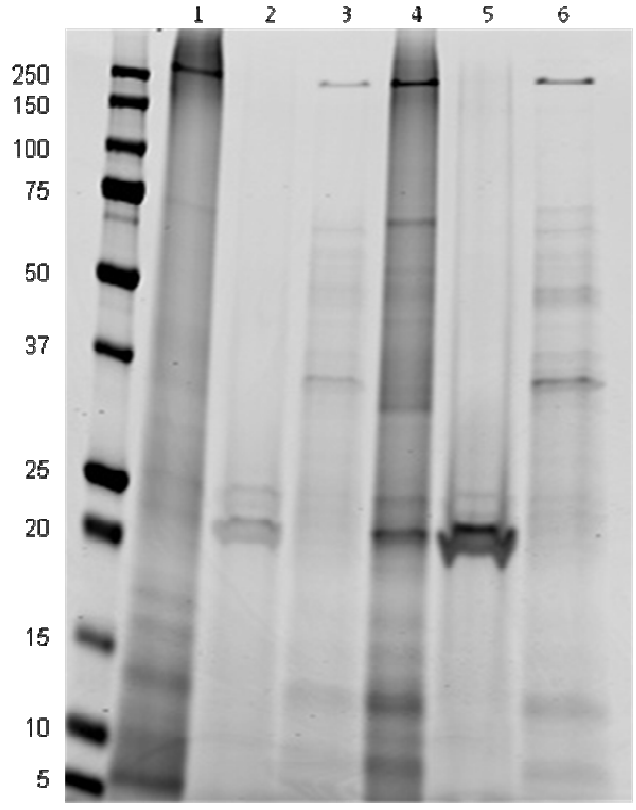

Fig. 3. Protein samples from gut contents of *H. zea* fed on soybean or cashew flour diet samples prior to and after incubation with GSH agarose beads separated by SDS-PAGE. Lane 1 – soybean diet, lane 2 – GSH bead bound material from soybean diet, lane 3 – unbound material from soybean diet, lane 4 – cashew flour diet, lane 5 - GSH bead bound material from cashew flour diet, lane 6 - unbound material from cashew flour diet.

It is unclear what purpose the increased GST served when *H. zea* was fed the cashew flour diet. It may be that there are other plant defensive compounds that were retained in the cashew flour used in our experimentation that caused the induction of GST. However, our findings suggest that continued testing with this type of research may identify additional enzymes specifically up-regulated in response to seed storage proteins from cashew or other tree nuts and peanuts. Seed storage proteins can be food allergens with serious consequences for those with food allergy. We tested *H. zea* because its genome has been sequenced and there is considerable information already available on this insect due to its impact on common agricultural crops. Subsequent projects tailoring insect and natural plant food sources may increase the odds of identifying useful enzymes that may be able to degrade or modify allergenic plant proteins. For example, a similar project identifying the digestive enzymes induced in the cashew apple and nut borer, *Thylocoptila panrosema*, when fed cashew flour might lead to enzymes that have been evolutionarily targeted specifically for breakdown of cashew proteins. New enzymatic methods for the reduction or elimination of these proteins from nuts would be useful to the food allergen field. Examining insect pests which specifically target allergen containing foods can therefore yield a new spectrum of enzymes useful in reducing or eliminating food allergy related issues.

Figure 4

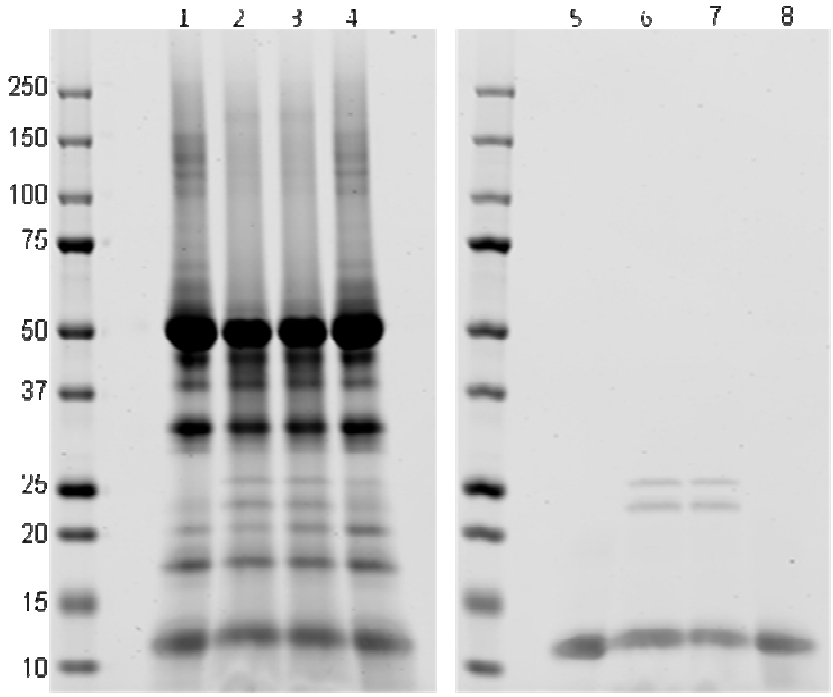

Fig. 4. Cashew flour or purified Ana o 3 was incubated with isolated *H. zea* GST and samples were analyzed by SDS-PAGE with sample buffer lacking reducing agent. Lane 1 - cashew flour extract, lane 2- cashew flour extract with *H. zea* GST, lane 3 - cashew flour extract with *H. zea* GST and 1 mM reduced GSH, lane 4 - cashew flour extract with 1 mM reduced GSH, lane 5 – purified Ana o 3, lane 6 – purified Ana o 3 with *H. zea* GST, lane 7 - purified Ana o 3 with *H. zea* GST and 1 mM reduced GSH, lane 8 - purified Ana o 3 with 1 mM reduced GSH.

CONCLUSION

Insects have developed specific strategies to counter plant defenses over millions of year and our work attempts to take advantage of this phenomenon to identify proteins that could be used to break down tree nut and peanut seed storage proteins. These proteins can act as allergens in humans that may cause harmful physiological responses in those affected by allergy to nuts. Some nut allergens, such as 2S albumins, have been shown to inhibit protease activity. Our comparative feeding studies indicate that Glutathione S-transferases (GST) are specifically up-regulated in *H. zea* larvae in response to the inclusion of cashew protein in their diet. Continued research using our strategy may identify additional insect enzymes that could be incorporated into tree nut and peanut processing steps to reduce or eliminate the ability of nuts to cause allergy in affected individuals.

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