Adverse Effects of Raw Soybean Extract in Artificial Diet on Survival and Growth of *Lygus hesperus* Knight[1]

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J. Entomol. Sci. 40(4): 390-400 (October 2005)

Abstract Bioassays were conducted to examine the effects of raw soybean extract in artificial diet on the development, survival, adult weight and biomass accumulation of *Lygus hesperus* Knight. The diet containing raw soybean extract significantly reduced survival of *L. hesperus*. Total biomass per rearing unit and survival from eggs to adults were significantly less for *L. hesperus* fed diet containing raw soybean extract than it was for those fed heat-treated extract, diet with extraction buffer but no extract, or control (standard) diet. Development period, weight of individual adults, and egg production were not significantly different among the four treatments. However, development time was greater for the *L. hesperus* exposed to raw soy extract, and egg production, as well as individual adult weights, were consistently lower than those fed on the other treatments. Raw and autoclaved soy extracts were analyzed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE), revealing differences in protein banding patterns, including those from total protein and glycoprotein profiles. Activity of soy trypsin inhibitor (STI) also was measured in raw and heated samples, and it was determined that autoclaving greatly decreased the inhibition activity of this protein. The heat-based deactivation of STI, and the disappearance of most protein bands are most likely associated with the denaturation of proteins and formation of large aggregates that fail to migrate in an electrophoretic field.

Key Words Miridae, biomass, survival, egg production, adult weight, soy protein profiles, insect diets

Insect rearing systems based on artificial diets are essential tools that serve as the foundation for numerous research efforts and for most programs in biologically-based pest management (Cohen 2001, 2003). The development of artificial diet-based rearing systems and the quality and reliability of those systems depend upon the incorporation of appropriate diet materials and diet processing technology. However, problems arise in the development and maintenance of rearing systems, such as reduction in rates of insect production or in quality of the target insects (Cohen 2003). The etiology of these problems frequently goes undiagnosed, mainly because of gaps in our understanding of many of the fundamentals of rearing systems, especially in

[1]Received 20 January, 2004; accepted for publication 20 June 2005.

Mention of a proprietary product does not constitute an endorsement or recommendation for its use by the USDA/ARS.

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regard to artificial diets, which are centerpieces of most rearing programs. Sometimes, materials that are most commonly used in diets and ones assumed to be most reliable can cause serious production problems (Cohen 2003). Such problems often stem from improper substitution of ingredients or from poorly planned changes in diet processing steps. An excellent documentation of how seemingly simple changes in a rearing protocol can have profound ramifications is to be found in the investigation of the gypsy moth "abnormal performance syndrome" (Keena et al. 1998, ODell 1992, ODell et al. 1997).

An example of a mass rearing program based on artificial diet is the system for rearing plant bugs at the Gast Rearing Facility, USDA, ARS, MS State, MS, where two species of plant bugs are reared, tarnished plant bugs (*Lygus lineolaris* Palisot de Beauvois, Miridae: Hemiptera) and western tarnished plant bugs (*Lygus hesperus* Knight). Western tarnished plant bugs are serious pests of cotton, cereals, fruits, vegetables, alfalfa, and other economically important crops (Tingey and Pillemer 1977, Hedlund and Graham, 1987, Butts and Lamb 1991). Systems of rearing the parasitoids and predators of *L. hesperus* have been devised for using laboratory-reared hosts for production of these natural enemies. To succeed in production of substantial numbers of parasitoids and predators, a system of mass production of the host must be in place (Cohen et al. 1999, Leppla and King 1996). Such systems have been devised for rearing parasitoids and predators of several pests, including western tarnished plant bugs (Hedlund and Graham 1987, Debolt 1987). Efforts to increase the scale of rearing and to reduce costs are a continual operation in mass rearing facilities. Such efforts are directed at reducing the costs of components, modifying diet and insect production systems to reduce costs of labor, space, and utilities. However, any modification requires documentation of the retention of diet and insect quality. An example of such reduction in costs is the improved diet for *Lygus* spp. described by Cohen (2000) where replacement of many of the chemically defined and very expensive ingredients of the Debolt (1982) Lygus diet were replaced by inexpensive, nondefined components, including soy flour. However, the replacement of components with soy flour is not a risk-free action, considering the antinutrient components found in soybeans (*Glycine max* (L.) Merrill) and their products.

Henry and Lattin (1987) commented on the peculiarity of the fact that although *Lygus* spp. are known to damage to a variety of legumes, "they have not transferred to soybeans". These comments, the sparseness of reports on *Lygus* damage to soy, and an incident in our facility where inadvertent undercooking of an artificial diet that contains soy flour caused appreciable damage to our *L. hesperus* colony caused us to hypothesize that some heat labile component in soy is deleterious to these mirids. We hypothesized that if raw soybean had adverse effects on *L. hesperus*, insects fed diet containing raw soybean extract would have low rate of development, low survival percentage, lower adult weight and egg production compared with insects fed artificial diet without raw soybean extract. This study was conducted to determine the effects of raw soybean extract on *L. hesperus* biological characteristics including development, survival, adult weight, biomass accumulation and egg production. In conjunction with this goal, we also sought to examine the direct effects of cooking soy proteins to shed some light on the adverse actions of poorly cooked soy in our preliminary observations.

Soy products contain a wide array of nutrients such as storage proteins (glycinins and conglycinins), lipids such as lecithin and phytosterols, and a small amount of carbohydrates, vitamins, and minerals (Fukushima 1991). The excellent nutritional

characteristics of soy have made such products as soy flour, tofu, soy milk, misu, yuba, texturized soy proteins highly nutritious foods for humans. The high nutritional value of soy, its versatility, and its relatively low cost support use of soy for use in insect diets. In fact the addition of soy has helped make a diet for *L. hesperus*, *L. lineolaris*, and several other species of phytophagous insects highly nutritious and economical (Cohen 2000, 2001). However, in experiments directed at optimizing use of the *Lygus* diet, it was discovered (Cohen, unpubl. data) that provision of slightly undercooked diet resulted in decreases in the fitness of the target insects, including decreases in the yield of adults per cage, decreases in body weight of adults, and increases in the duration of the developmental stages. This finding prompted the investigation to test whether some factor in the undercooked soy flour in the diet was the cause of the decreases in fitness of the *L. hesperus* culture.

Materials and Methods

Lygus hesperus used in this study were obtained from a colony from Biotactics, Inc. (Riverside, CA) and had been provided with standard diet (Cohen 2000) for about 10 generations prior to this study at USDA/ARS, Biological Control and Mass Rearing Research Unit, MS. Voucher specimens were placed in the Entomological Museum, MS State University, MS.

Insects used in the assays were maintained in plastic storage boxes (Rubbermaid®, 1.2 L) with openings cut in the top and replaced with 0.4 mm organdy cloth at 27°C (±1°C) with a 16 h light/8 h dark cycle and RH about 60%. Rearing cages were placed on metal racks to allow air circulation and light to reach each rearing cage. Five hundred *L. hesperus* eggs were used for each treatment in each cage. Egg packets were placed inside the cages with shredded papers. The feeding packets were placed on the organdy cloth on the top to feed insects; these were changed every other day after eggs hatched. The 2% carrageenan gel (Cohen 2000) was used for oviposition, and an egg packet was placed on top of each cage as was a feeding packet. Oviposition packets were collected so that eggs could be counted every day after adults began oviposition.

Mature dry soybeans were ground in a Miracle mill, MC 200 (Open Chute, Inc., Glendale, AZ), and each 100 g of ground soybean was extracted with 500 ml of phosphate buffered saline on a mechanical shaker for 1 h at room temperature. After 1 h shaking, the extraction was continued at 4°C in a refrigerator overnight. Then, the solution was filtered through 0.4-mm organdy cloth. The supernatant containing raw soy extract was used as added in aliquots of 100 ml per 2700 ml of diet in the treatments designated as TR (diet with raw soy extract) and TA (diet with autoclaved soy extract). The average protein concentration of extracts was 2 mg/ml. This resulted in an additional 200 mg of soy protein per 2700 ml of diet, with this additional amount of protein totaling less than 0.1% of the total diet mass.

There were four treatments plus a control in this experiment. They were standard plant bug diet (Cohen 2000) used as control, TR (standard diet with raw soy extract), TA (standard diet with raw soy extract that was autoclaved for 20 min at 121°C), and BF (standard diet with extract buffer, phosphate buffered saline). The extracts and controls were prepared identically, except that an aliquot of 100 ml of the 700 ml of water routinely added to the diet slurry was replaced with phosphate buffered saline (BF treatment), raw extract (TR treatment), autoclaved extract (TA treatment). Experiments were a completely randomized block design with three equal replications.

To evaluate the effects of raw soybean extract on *L. hesperus*, the following data were collected for each treatment or control on the tenth day after the adults' eclosion in the control cages: (1) the survival of nymphs and adults in each rearing cage; (2) the mean weight of sexually mature adults; (3) the mean biomass accumulated per rearing container, including live adults and dead nymphs and adults; (4) the mean number of eggs produced in each rearing container; (5) the developmental period in days from eggs to adults. Adult weights were measured by weighing samples of 10 adults of each sex from each rearing cage (30 females and 30 males per treatment or control) to determine the fresh weights. Egg production was determined over a 5-d period after the adults began to lay eggs in the control rearing cages. Data were analyzed by analysis of variance (ANOVA), and means were compared by Fisher's Protected Least Significant Difference (FPLSD) (SAS Institute 1988) at $P = 0.05$.

Sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) was performed in a Mini-PROTEAN II electrophoresis cell (BioRad Laboratories, Hercules, CA) powered with 200 V for 45 m (Laemmli 1970). Each soy sample was diluted 1:2 with SDS-PAGE Sample Buffer. The samples were mixed and held in boiling water for 5 min. SDS-PAGE analyses were performed on 4 to15% polyacrylamide Ready Gels (Sigma), and following electrophoresis the gels were washed 3 times with dH_2O and stained with GelCode Blue for 2 h at room temperature. High and low molecular weight standards (BioRad) were run in the same gels. Glycosylated proteins were detected with a glycoprotein detection kit (Sigma Chemical Co., St. Louis, MO). Gels were destained with dH_2O for 1 h. This step was repeated 2 times with fresh dH_2O. After the final wash, gels were scanned using the GS-700 Densitometer (BioRad). Extracts that were unheated and aliquots that had been autoclaved similarly to those used in bioassays were tested for trypsin inhibition activity by incubating aliquots of 50 µL of extract with an equal volume of porcine pancreas trypsin (Type II-S, Sigma # T 7,409) diluted to 5,000 U for 10 min then adding 200 µL of substrate mixture and further incubating at 22°C for 1 h. The substrate mixture was that described by Stewart (1973) and modified by Zeng et al. (2002) and consisted of *N*-benzoyl-L-arginine-*p*-nitroanilide (L-BA*p*NA) (B 3,133, Sigma, St. Louis, MO) in 5 ml dimethyl sulfoxide (DMSO) before adding 95 ml of 0.05 M Tris-HCL buffer (pH 8.2). Samples that contained extract-free buffer were tested simultaneously to allow comparison of the effects of the putative inhibitors from the soy extracts. All standards and samples were measured in a plate reader (Spectra Max® Plus, Molecular Devices Corporation, Sunnyvale, CA) at 420 nm.

Results

The mean percentages (±SE) of *L. hesperus* survived from eggs to adults were 23.9 (±3.7) on TR, 46.7 (±3.4) on BF, 48.5 (±4.5) on TA and 51.2 (±2.8) on standard diet. The percentage of survival per rearing cage for *L. hesperus* on TR diet was significantly lower than that of *L. hesperus* on other treatments (standard diet, TA and BF) ($F = 11.81$; df = 3, 8; $P < 0.05$).

There were no significant differences for development time (d) from eggs to adults among different treatments ($F = 1.3$; df = 3, 8; $P > 0.05$). Whereas not significantly different, appearance of adults from cages provided with raw soy extract was consistently 1-2 d behind emergence of those in cages provided the other three treatments.

Mean fresh weight of each adult eclosion was 12.6-13.1 mg for females, and 8.9-9.5 mg for males in the four treatments (Table 1). Mean female adult weights of *L. hesperus* were numerically lower in the TR treatment than those from other diet treatments; however, these differences were not statistically different (F = 0.98; df = 3, 8; $P > 0.45$). Similarly, mean adult weight was lower for males from the TR treatment than those on other treatments, but these differences were not statistically significant (F = 1.42; df = 3, 8; $P > 0.31$).

Mean biomass was significantly lower for *L. hesperus* fed artificial diet with raw soybean extract (TR) than those from the other TA, BF and standard plant bug diets (F = 7.74; df = 3, 8; $P < 0.05$) (Table 1). During the testing period, mean biomass accumulated per rearing cage for *L. hesperus* fed TR diet was only 43%, 46% and 50% of that fed standard, TA and BF diet.

The mean total egg production over the test period was only 1896 for *L. hesperus* fed TR diet (Fig. 1). The total egg production in the test period for *L. hesperus* fed on TR was 41%, 28% and 32% of that *L. hesperus* fed BF, TA and standard diets, respectively. However, these differences were not statistically significant (F =1.78; df = 3, 8; $P > 0.05$).

Banding patterns of unheated extracts and autoclaved extracts are shown in Figs. 2 and 3. In Fig. 2, proteins stained with Gel Code Blue, a general stain similar to Coomassie blue, can be seen to have separated into numerous distinct bands in the subsamples that were not heat-treated (lanes 3, 4, and 5); however, the protein extracts that had been subjected to autoclave treatment lost the distinct appearance of multiple bands (lanes 6, 7, and 8). In Fig. 3, which shows glycoproteins were selectively stained, it is also clear that most of the proteins that formed distinct bands in the SDS-PAGE analyses (bands 3,4, and 5) were altered so that they appeared blurred or dissipated (lanes 6, 7, and 8). It is likely that the apparent disappearance of the proteins that were heated is attributable to aggregation along with the denaturation process (Damodaran 1996, Fukushima 1991).

Trypsin activity was almost totally inhibited by the raw soy extract, and more than 95% of the activity was restored after autoclave treatment of the soy extract prior to incubation with porcine pancreas trypsin.

Table 1. Effects of different treatments on *Lygus hesperus* weights of sexually mature adults and biomass

Treatment	Adult weight of females (mg)*	Adult weight of males (mg)*	Biomass (g)**
Standard diet	12.62 ± 0.54	9.49 ± 0.13	3.26 ± 0.29
TR (standard diet + raw soy extract)	12.33 ± 0.18	8.91 ± 0.40	1.41 ± 0.22
BF (standard diet + buffer only)	13.09 ± 0.21	8.87 ± 0.20	2.81 ± 0.28
TA (standard diet + autoclaved soy extract)	12.73 ± 0.18	9.16 ± 0.14	3.07 ± 0.39

* Mean (adults) ± SE, n = 10.

** Mean (total biomass for rearing containers) ± SE, n = 3.

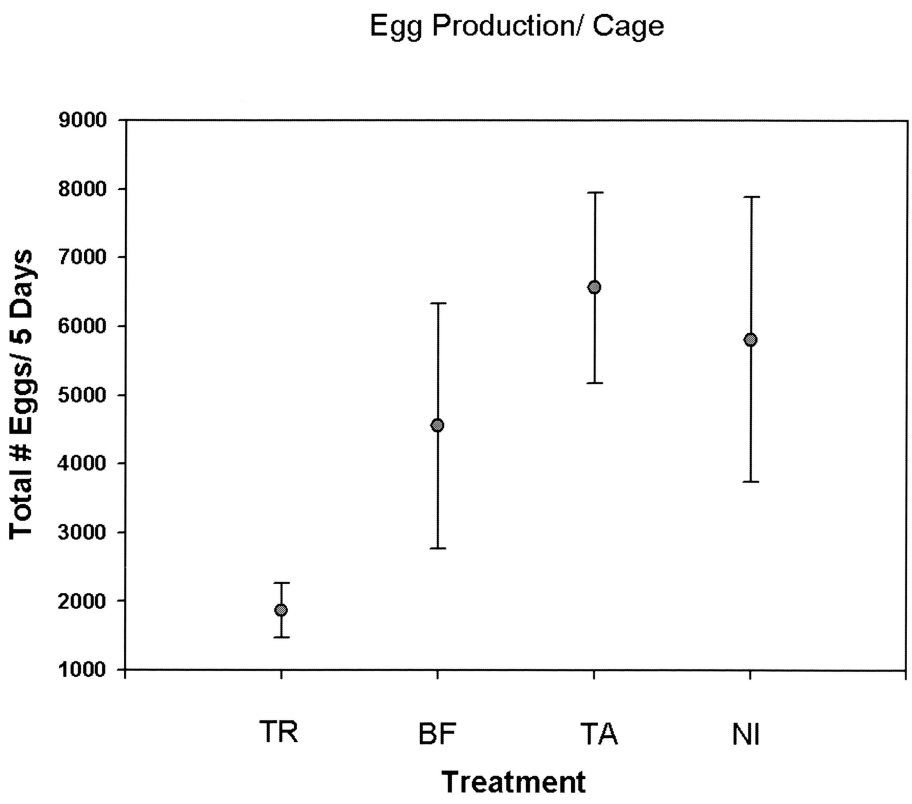

Fig. 1. Numbers of eggs produced by *Lygus hesperus* fed diets with added raw soy extract (TR), extraction buffer only (BF), autoclaved soy extract (TA), or standard plant bug diet. Eggs were counted for a total of five days, post onset of oviposition.

Discussion

The results from this study support the hypothesis that there are adverse effects of raw soybean extract on the growth, survival, and reproductive capability of *L. hesperus*. The *L. hesperus* fed diet containing raw soybean extract had lower survival and biomass accumulation than the *L. hesperus* fed on control and other treatments including diet with autoclaved soybean extract. There are several precedents where plant extracts added to artificial diets caused adverse effects on the growth or metabolism of various insect species. For example, Broadway and Duffey (1988) found that soybean trypsin inhibitor adversely affected growth of larval beet armyworm *Spodoptera exigua* Hübner larvae. Harper et al. (1998) also reported that wheat germ in the diet greatly reduced the larval body weight of European corn borer (*Ostrinia nubilalis* Hübner). Powell et al. (1998) found that snowdrop lectin in artificial diet can decrease survival and reduce the growth rate of the brown plant hopper *Nilaparvata lugens* (Stal). Sundari (1998) also reported that *Catharanthus roseus* (L.) alkaloids

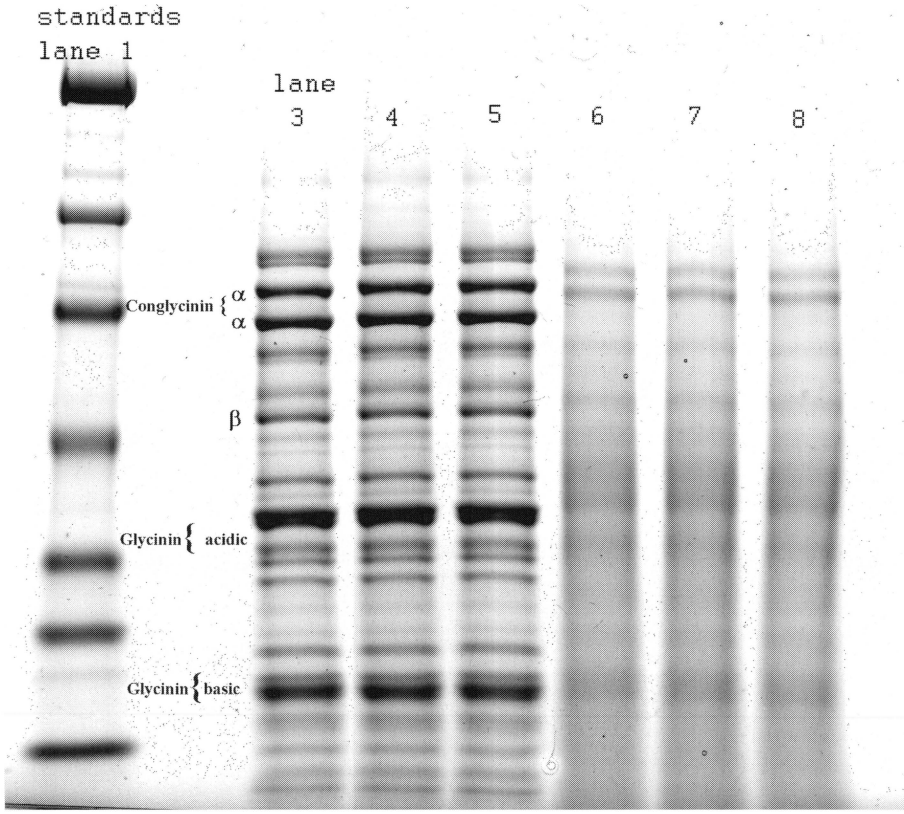

Fig. 2. SDS-PAGE gel containing proteins from raw soy extract (lanes 3, 4, and 5) and autoclaved soy extract (lanes 6, 7, and 8). Proteins were stained with Gel Code Blue.

reduce protein and carbohydrate content in treated *Euproctis fraterna* (Denis & Schiffermüller) larvae, and egg production of adults. Houseman and Morrison (1985) reported that soybean trypsin and other inhibitors inhibited cysteine protease activity in the ambush bug *Phymata wolffii* Stal.

Valencia et al. (2000) found that the activity of α-amylases in the coffee berry borer (*Hypothenemus hampei* Ferrari;Coleoptera: Curculionidae: Scolytinae) was inhibited 80% by plant inhibitor from the common bean. Jouanin et al. (1998) reviewed the literature on utilization of toxins that were active in genetically transformed plants. Pechan et al. (2002) reported a unique protein, a cysteine protease that whose mode of action against target insects was destruction of the peritrophic matrix. By contrast to this study, Bolter and Latoszek-Green (1997) described the actions of a cysteine protease inhibitor against Colorado potato beetles, *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae). Huesing et al. (1991) demonstrated that α-amylase inhibitor, not phytohemagglutinin, explains resistance of common bean seeds to cowpea weevil. Similarly, Zeng and Cohen (2000) reported that α-amylase inhibitor from wheat significantly inhibited the amylase activity in *L. hesperus* and *L. lineolaris*.

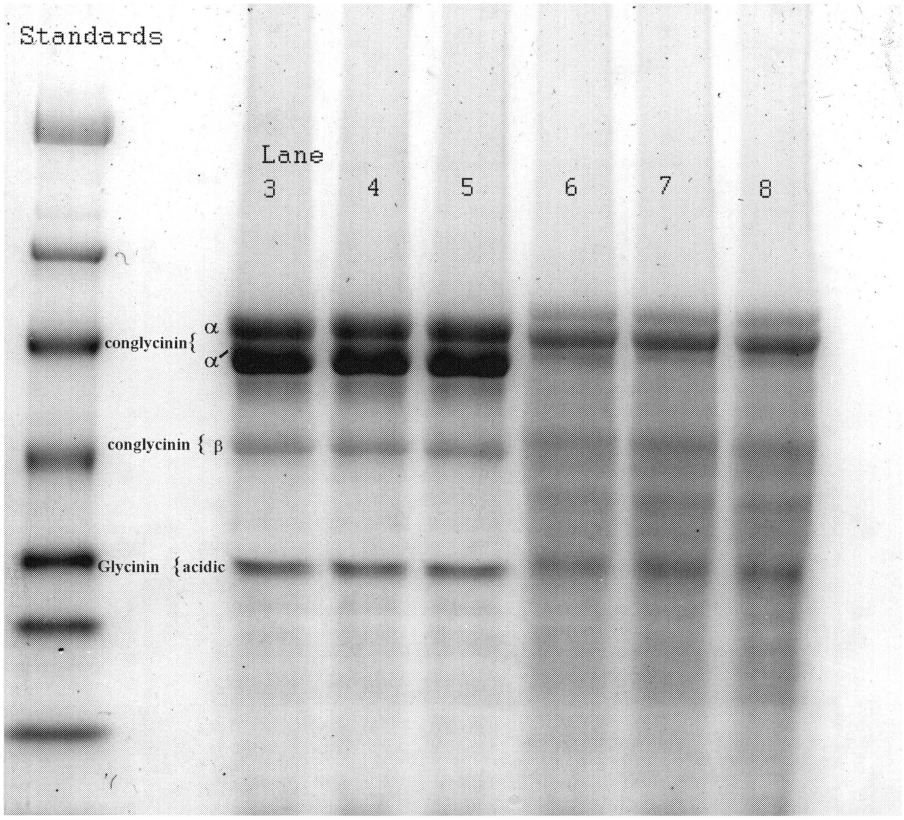

Fig. 3. SDS-PAGE gel containing proteins from raw soy extract (lanes 3, 4, and 5) and autoclaved soy extract (lanes 6, 7, and 8). Glycoproteins were stained with a glycoprotein staining kit.

Cohen (2000) discussed various indicators of quality of insects in mass rearing systems, including survival %, collective biomass accumulation, rate of development, individual weight gain, and fecundity. The data from this study show that raw soybean extract has adverse effects on these important indicators. *Lygus hesperus* fed diet with raw soybean extract had numerically lesser body weights, lower biomass accumulation, and reduction in numbers of eggs produced. It should be noted that although statistically significant differences were found in survival % and collective biomass accumulation, the treatments containing raw soybean extract were consistently inferior with respect to all biological parameters. It was evidently the very high variability among replications that obscured the biological differences resulting from the different diet additives. Figure 1 illustrates this point, showing that the standard error of the mean often exceeded 1/3 of the mean. This high degree of variability was found despite the efforts used to assure uniformity in setting up and conducting these experiments.

This poor performance on raw soybean components may help explain why *Lygus*

bugs have never become serious pests of soybeans and why they cause little or no damage to soybeans in the field. *Lygus hesperus* fed on diet containing autoclaved soybean extract had the highest female adult weight, egg production and shortest development time. This could indicate that the extra protein, with its inhibitors denatured by heating, may have provided useful nutrients to *L. hesperus.*

Cohen (2003) discussed the complexity of insect diets and how seemingly subtle changes in processing or ingredients could impart huge changes in diet quality. This point was illustrated by the alarming decline in the *L. hesperus* colony observed at the Gast Mass Rearing Facility in 1999 - 2000. In efforts to detect the factor(s) responsible for a near loss of the entire colony, the most likely cause of the problem was tentatively associated with an effort to increase diet quantity from ~2 L batches to ~6 L batches. Evidently, the autoclaving process used routinely for the smaller batches with great success served to under-heat the diet in the larger batches (Cohen, unpubl data). This result was surprising to the rearing personnel because earlier efforts to scale up from ~2 L to ~4 L batches were accomplished with no evident losses in colony quality (Cohen, unpubl data). Retrospectively, it seems evident that the increases in batch size introduced toxins from the soy and possibly from other components such as the lima bean meal, which is a prominent component of the standard (plant bug) diet.

These toxins include the soy trypsin inhibitor documented here as being detoxified by heating and probably a host of other plant-derived toxins. The process of autoclaving may also have increased the bioavailability of the other soy proteins such as glycinins and conglycinins, considering the fact that heat denaturation of otherwise palatable and digestible proteins further enhances their nutritional qualities (Cohen 2003). However, the most important point of this study is that a seemingly innocuous change in diet processing, in this case, undercooking the larger batch of diet was responsible for a precipitous decline in the quality of an otherwise highly effective diet. It was especially impressive that a very small amount of raw soy protein (<0.1%) caused a decline in the biological fitness of the target species.

Acknowledgments

The authors thank Gay McCain and Brenda Woods and for rearing the insects used in this study and Chiou Ling Chang and Eric Villavaso for reviewing an earlier version of this manuscript.

References Cited

Butts, R. A. and R. J. Lamb. 1991. Seasonal abundance of three *Lygus* species (Heteroptera: Miridae) in oilseed rape and alfalfa in Alberta. J. Econ. Entomol. 84: 450-456.

Cohen, A. C. 2000. New oligidic production diet for *Lygus hesperus* and *L. lineolaris.* J. Entomol. Sci. 35: 301-310.

2001. Artificial diets for arthropods. US Patent 6,235,528.

2003. Insect Diets: Science and Technology. CRC Press. Boca Raton, FL.

Cohen, A. C., D. N. Nordlund and R. A. Smith. 1999. Mass rearing of entomophagous insects and predaceous mites: are the bottlenecks biological, engineering, economic, or cultural? Biocont News Info. 20: 85-90.

Bolter, C. J. and M. Latoszek-Green. 1997. Effect of chronic ingestion of the cysteine proteinase inhibitor, E-64, on Colorado potato beetle gut proteinases. Entomol Exp et Applic. 83: 295-303.

Broadway, R. M. and S. S. Duffey. 1988. The effect of plant protein quality on insect digestive physiology and the toxicity of plant proteinase inhibitors. J. Insect Physiol. 34: 1111-1117.

Damodaran, S. 1996. Amino acids, peptides, and proteins, Pp. 321-429. *In* O. R. Fennema (ed.), Food Chemistry. 3rd Edition. Marcel Dekker, Inc. New York.

Debolt, J. W. 1982. Meridic diet for rearing successive generations of *Lygus hesperus.* Ann. Entomol. Soc. Am. 75: 119-122.

1987. Augmentation: rearing, release and evaluation. *In* R. C. Hedlund & H. M. Graham [eds.], Economic importance and biological control of *Lygus* and *Adelphocoris* in North America. USDA-ARS-64.

Fukushima, D. 1991. Recent progress of soybean foods: chemistry, technology, and nutrition. Food Rev. Int. 7: 323-351.

Harper, M. S., T. L. Hopkins and T. H. Czapla. 1998. Effect of wheat germ agglutinin on formation and structure of the peritrophic membrane in European corn borer (*Ostrinia nubilalis* Hübner) larvae. Tissue Cell 30: 167-176.

Hedlund, R. C. and H. M. Graham. 1987. Economic importance and biological control of *Lygus* and *Adelphocoris* in North America. USDA, Tech. Bull. ARS-64.95.

Henry, T. J. and J. D. Lattin. 1987. Economic importance and biological control of *Lygus* and *Adelphocoris* in North America, Pp. 54-65. *In* R.C. Hedlund and H.M. Graham, (eds.), USDA, Tech. Bull. ARS-64.

Houseman, J. G. and P. E. Morrison. 1985. Cathepsin B and aminopeptidase in the posterior midgut of *Phymata wolffii* Stal (Hemiptera: Phymatidae). Can. J. Zool. 63: 1288-1291.

Huesing, J. E., R. E. Shade, M. J. Chrispeels and L. L. Murdock. 1991. α-Amylase inhibitor, not phytohemagglutinin, explains resistance of common bean seeds to cowpea weevil. Plant Physiol. 96: 993-996.

Jouanin, L., M. Bonade-Bottino, C. Girard, F. Morrot and M. Giband. 1998. Transgenic plants for insect resistance. Plant Sci. 131: 1-11.

Keena, M. A., T. M. ODell and J. A. Tanner. 1998. Environmentally based maternal effects are the primary factor in determining the developmental response of gypsy moth (Lepidoptera: Lymantriidae) to dietary iron deficiency. Ann. Entomol. Soc. Am. 91: 710-718.

Laemmli, U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 277: 680-685.

Leppla, N. C. and E. G. King. 1996. The role of parasitoid and predator production in technology transfer of field crop biological control. Entomophaga 41: 343-360.

ODell, T. M. 1992. Straggling in gypsy moth production strains: a problem analysis for developing research priorities, Pp. 325-350. *In* T. A. Anderson and N. C. Leppla [eds.], Advances in insect rearing for research and pest management. Westview, Boulder, CO.

ODell, T. M., M. A. Keena and R. B. Willis. 1997. Dietary influence of iron formulation on the development of gypsy moth (Lepidoptera: Lymantriidae) in laboratory colonies. Ann. Entomol. Soc. Am. 90: 149-154.

Pechan, T., A. C. Cohen, W. P. Williams and D. S. Luthe. 2002. Insect feeding mobilizes a unique plant defense protease that disrupts the peritrophic matrix of caterpillars. Proc. Natl. Acad. Sci. USA 99: 13313-13323.

Powell, K. S., J. Spence, M. Bharathi, J. A. Gatehouse and A. M. R. Gatehouse. 1998. Immunohistochemical and developmental studies to elucidate the mechanism of action of the nowdrop lectin on the rice brown plant hopper, *Nilaparvata lugens* (Stal). J. Insect Physiol. 4: 529-539.

S. A. S. Institute. 1988. SAS/STAT User's Guide, Release 6.0 ed. SAS Institute, Cary, NC.

Stewart, K. K. 1973. A method for automated analyses of the activities of trypsin, chymotrypsin and their inhibitors. Anal. Biochem. 51: 11-18.

Sundari, M. S. N. 1998. Inhibitory activity of *Catharanthus roseus* (L.) alkaloids on enzyme activity and reproduction in *Euproctis fraterna* (Denis & Schiffermüller) (Lepidoptera: Lymantridae). Ann. Appl. Biol. 133: 149-154.

Tingey, W. M. and E. A. Pillemer. 1977. Lygus bugs: crop resistance and physiological nature of feeding injury. Bull. Entomol. Soc. Am. 23: 277-287.

Valencia, A., A. E. Bustillo, G. E. Ossa and M. J. Chrispeels. 2000. α-amylases of the coffee berry borer (*Hypothenemus hampei* Ferrari) and their inhibition by two plant amylase inhibitors. Insect Biochem. Mol. Biol. 30: 207-213.

Zeng, F. and A. Cohen. 2000. Partial characterization of α-amylase in the salivary glands of *Lygus hesperus* and *L lineolaris*. Comp. Biochem. Physiol. B 126: 9-16.

Zeng, F., Y. Zhu and A. Cohen. 2002. Partial characterization of trypsin-like protease and molecular cloning of a trypsin-like precursor cDNA in salivary glands of *Lygus lineolaris.* Comp. Biochem. Physiol. B 131: 453-463.