Susceptibility of Red Flour Beetle Life Stages to Elevated Temperatures

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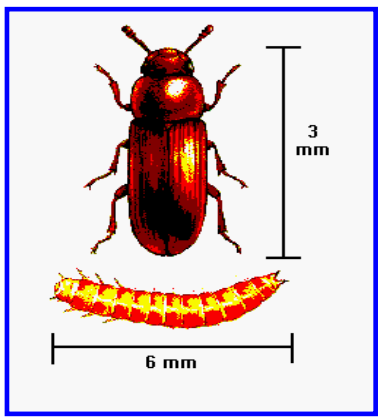
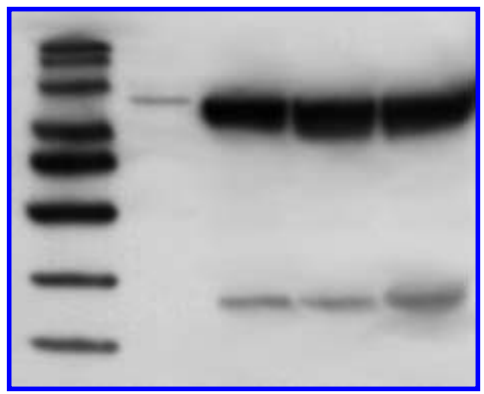




Overview

- Introduction
- Objectives
- Methods
- Results
- Conclusions
- Future work

What is the Rationale for Using Heat to Kill Insects?

- Resistance in insects to chemicals, including fumigants
- Facility not suitable for fumigation
- Companies do not want to use chemicals
- Environmental problems associated with chemical use

Thermal Death Effects of Heat on Insects

- Heat paralysis / anesthesia
- Asphyxiation (production of excess CO_2)
- Coagulation of protoplasm
- Coagulation of proteins
- Destruction of enzymes essential to nerve conduction
- Decrease of hemolymph pH
  - Desiccation and cellular abnormalities cause death

Susceptibility of Insects to Elevated Temperatures

- Mortality depends on many factors
  - Environmental conditions (Temperature, RH)
  - Species
  - Stage and age of insects
  - Temperature history
  - Heat tolerance

How Heat Tolerance is Acquired ?

- Thermal acclimation
- Through selection
- Synthesis of Stress Proteins/Heat Shock Proteins (HSP)
  - Refolding of denatured proteins
  - Stabilizing proteins by removal of coagulated proteins
  - Folding and translocation of polypeptides
  - Assembly and disassembly of oligomeric protein complex
  - Roles in immune responses
  - HSP 73 is constitutive and HSP 72 stress-inducible

Test Insects

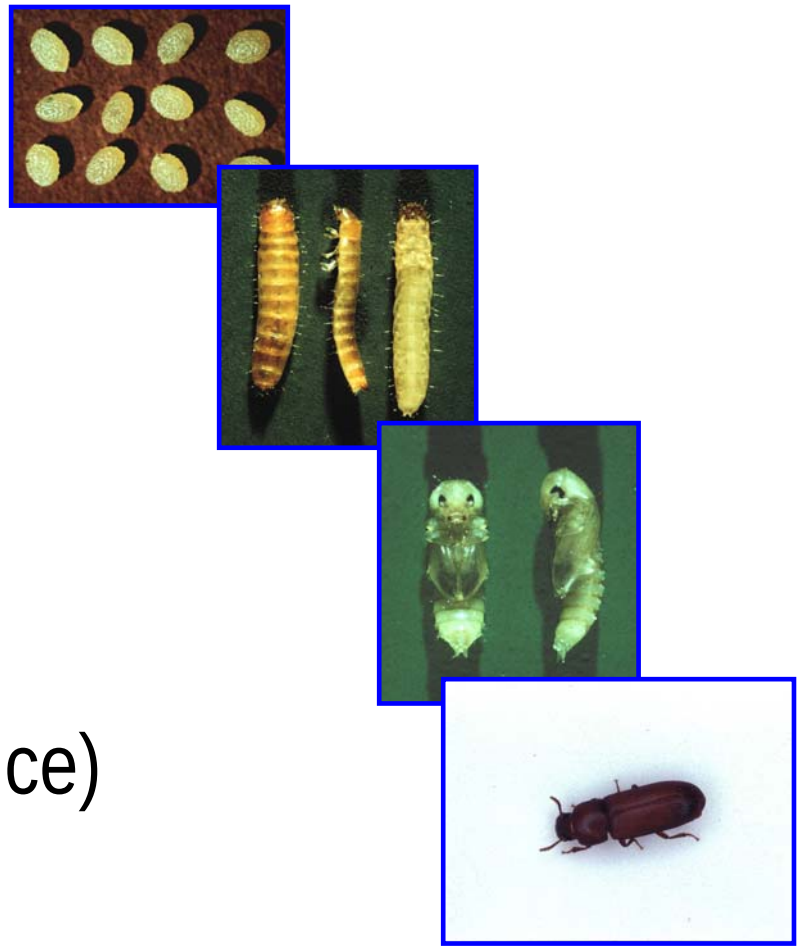
Tribolium castaneum (Herbst), Red Flour Beetle
(Tenebrionidae: Coleoptera)

Objective 1

To identify the most heat tolerant stage of *T. castaneum ?*

Materials and Methods

- *T. castaneum* life stages
  - Eggs (2-d-old)
  - Young larvae (6-d-old)
  - Old larvae (22-d-old)
  - Pupae (26-d-old)
  - Adults (2-wk-after emergence)



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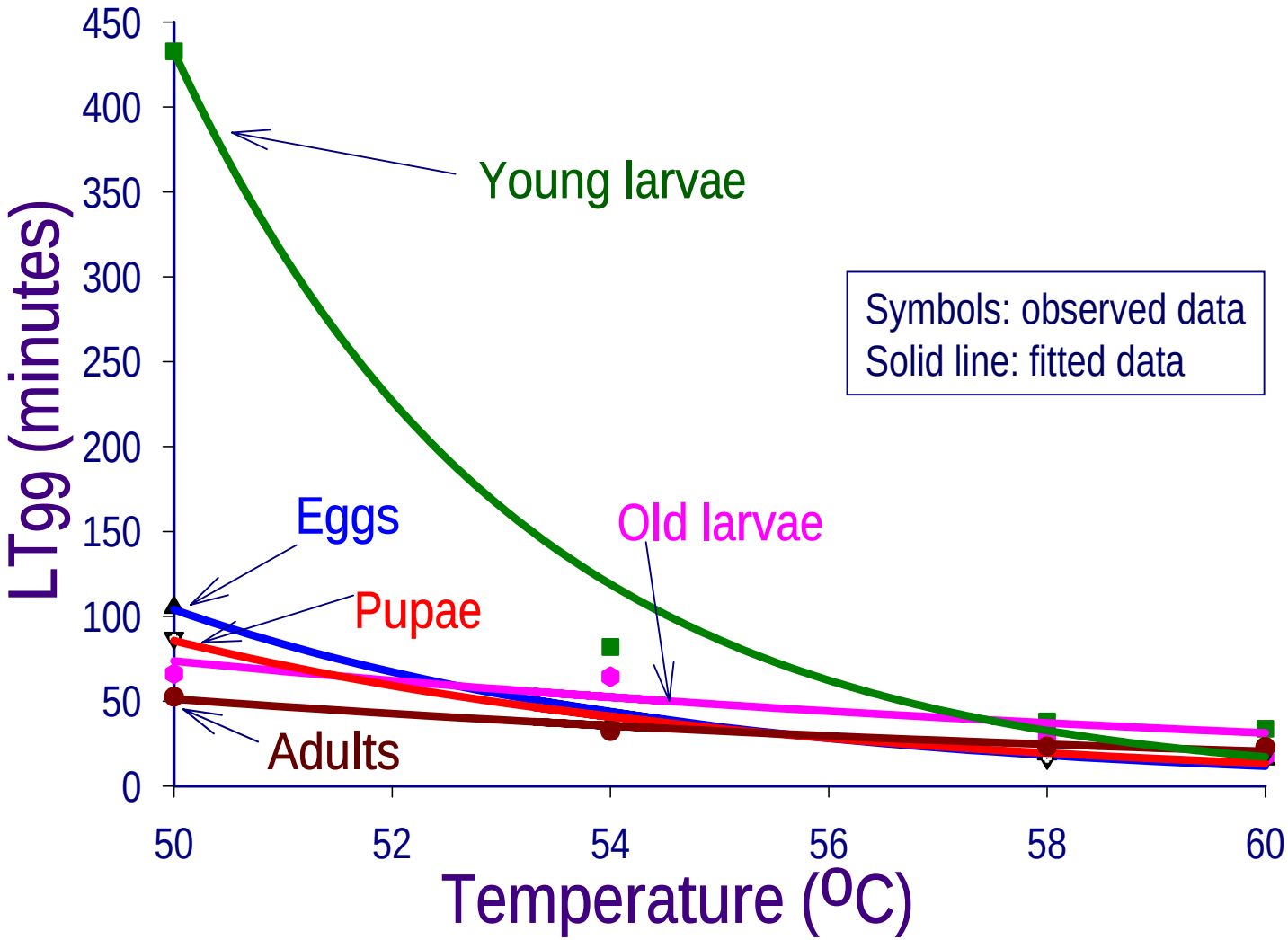
Life stages exposed to high temperatures

- 42, 46, 50, 54, 58 and 60°C
- For varying lengths of time
- 5 boxes containing 20 individuals per box for each time interval

- Reared at 28°C and 65% RH
- Mortality recorded

Figure describing LT_{99} of *T. castaneum* life stages as a function of temperature



Objective 2

Does heat shock protein (HSP 70) mediate heat tolerance in *T. castaneum*?

Test Insects

T. castaneum

- Eggs
- Young larvae
- Old larvae
- Pupae
- Adults

Heat Shock Protein Analysis

- Heat shock
  - 28 and 40^{o}C (1 hour) and 23^{o}C control
- Total protein concentration
  - BCA assay with kinetic microplate reader
  - Standard protein curve
  - Standard amount of protein loaded: 80 µg / well

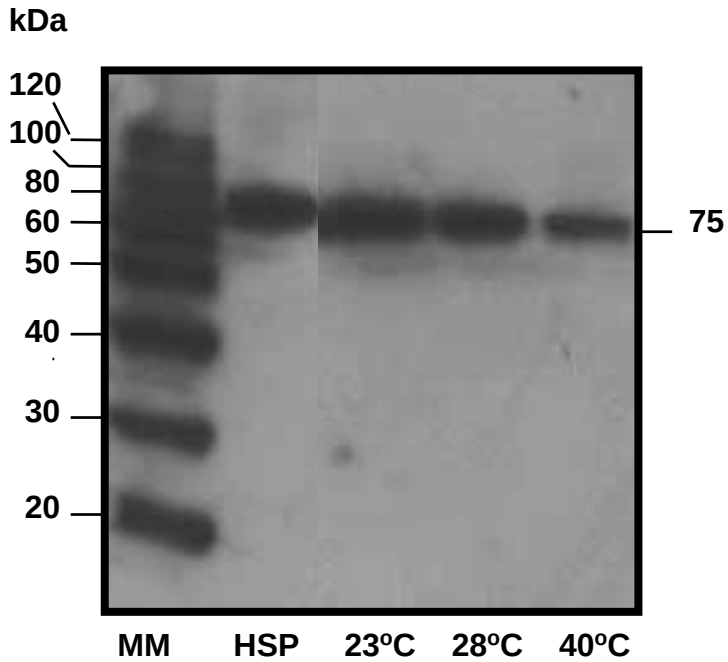
Heat Shock Protein Analysis (Cont.…)

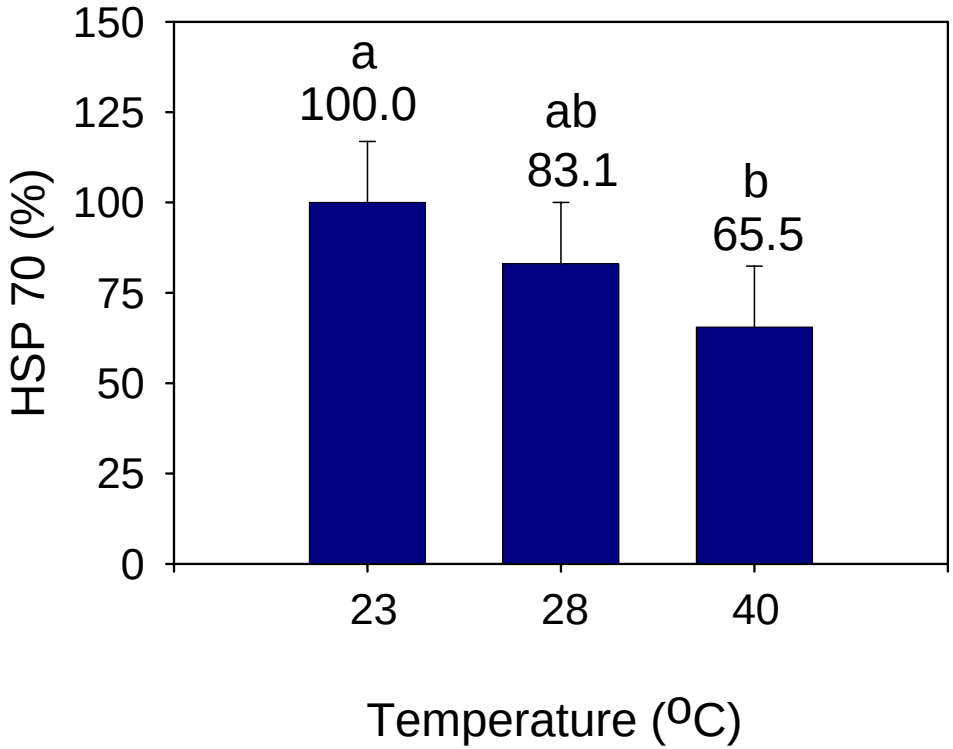
- Western Blot Technique
  - 4-20% Tris-glycine pre-cast gels (1.3% bis-acrylamide; Invitrogen)
  - Standard HSP - HSP 70 from bovine brain (Sigma)
  - Molecular size marker – The MagicMark™ Western Standard (Invitrogen)
  - Primary antibody – Monoclonal anti HSP 70 from mouse immunized with bovine brain HSP70 (clone BRM 22, Sigma)
  - Secondary antibody - Anti-mouse IgG conjugated with alkaline phosphatase (Invitrogen)
  - Immunodetection performed using the chemiluminescent method and the membrane exposed to X-Ray film for 2 mins.

Gray Value Quantification of Protein Bands

- Bands quantified using densitometric image analysis system (Ambis Imaging System and GelExpert Analysis System; Nucleotech Corporation).
- Absolute gray values quantified after background subtraction
- The gray value of the 23^{o}C samples (control) set arbitrarily to 100% as standard reference.
- The relative percentage of gray values for 28 and 40^{o}C calculated based on standard reference.
- Data analyzed using Proc GLM and mean separation done using Fisher's least significant difference (LSD).
- Results given as percentage relative HSP 70 values ± standard error

HSP Detected in Eggs Exposed to Different Temperatures

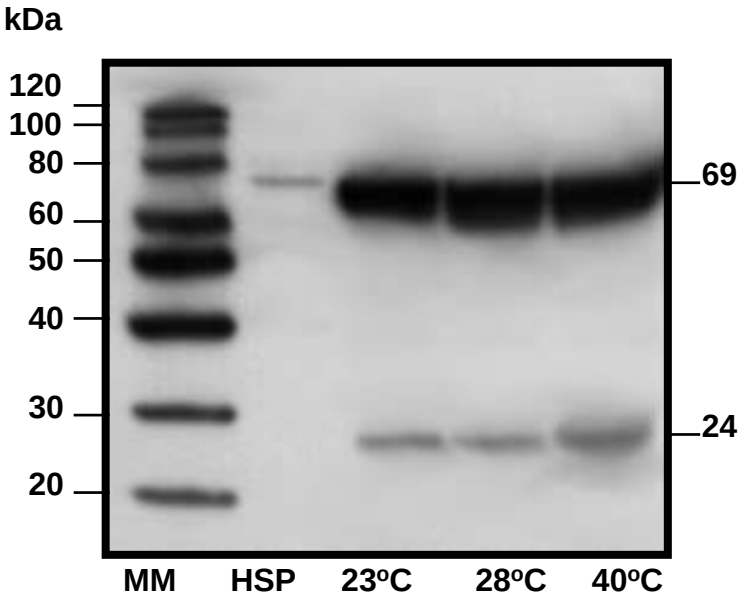


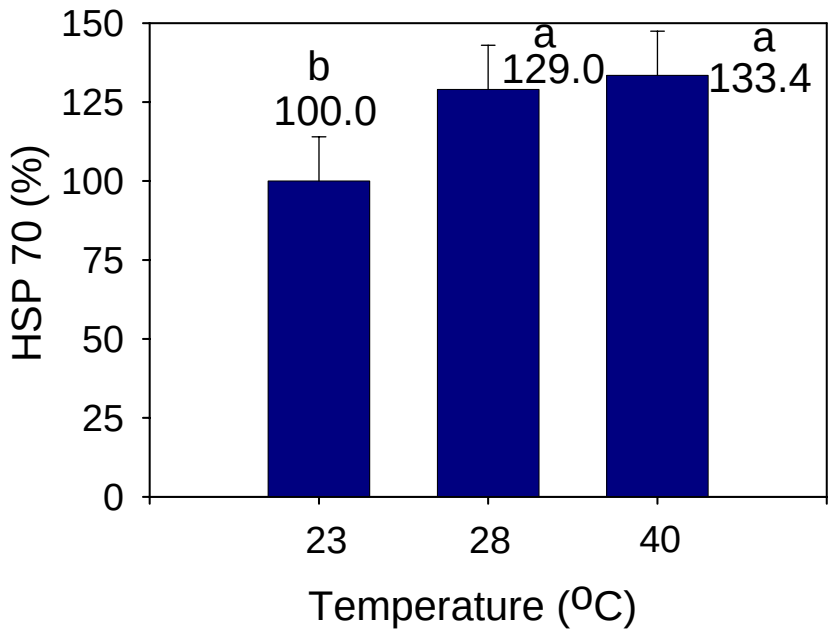


Calculated molecular mass of HSP (70) is 75 kDa

F = 0.4.17; *d.f.* = 2; *P* = 0.05; *n* = 4 (Proc GLM, LSD)

HSP Detected in Young Larvae Exposed to Different Temperatures (Larger MM Bands)

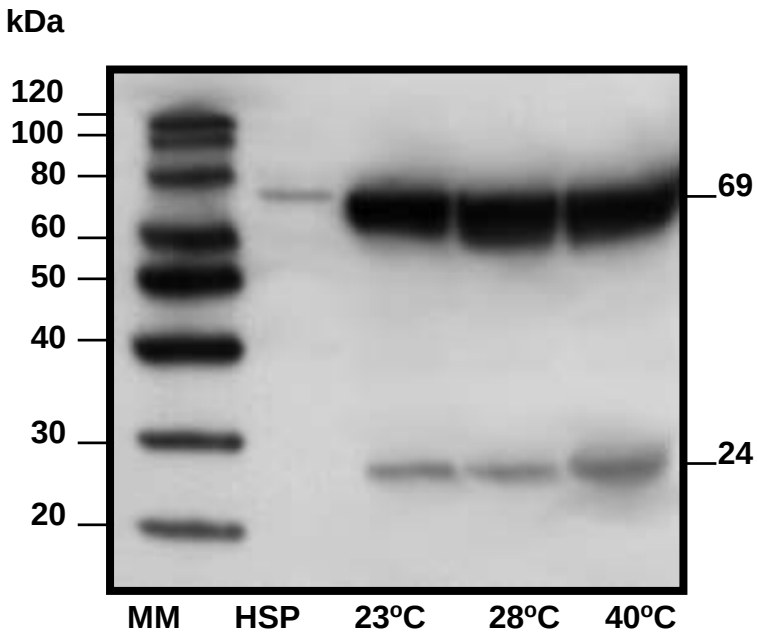


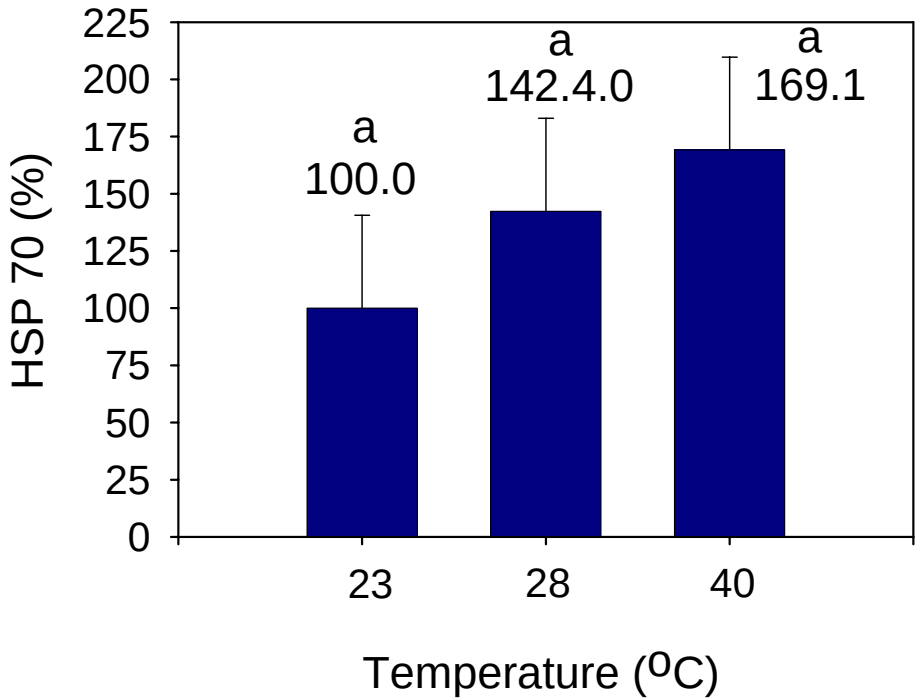


Calculated molecular mass of HSP (70) is 75 kDa

F = 6.72; *d.f.* = 2; *P* = 0.01; *n* = 4 (Proc GLM, LSD)

HSP Detected in Young Larvae Exposed to Different Temperatures (Smaller MM Bands)

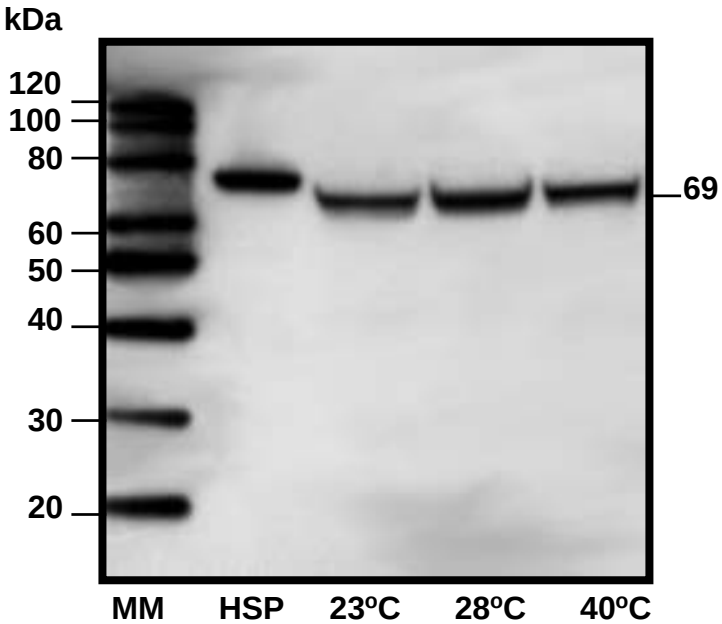


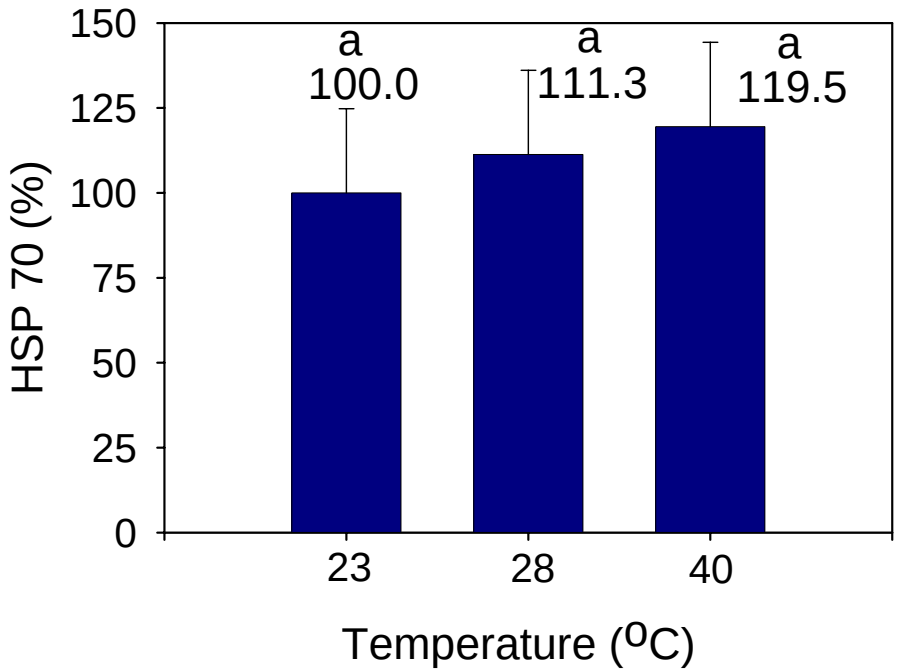


Calculated molecular mass of HSP (70) is 75 kDa

F = 2.59; *d.f.* = 2; *P* = > 0.05; *n* = 4 (Proc GLM, LSD)

HSP Detected in Old Larvae Exposed to Different Temperatures

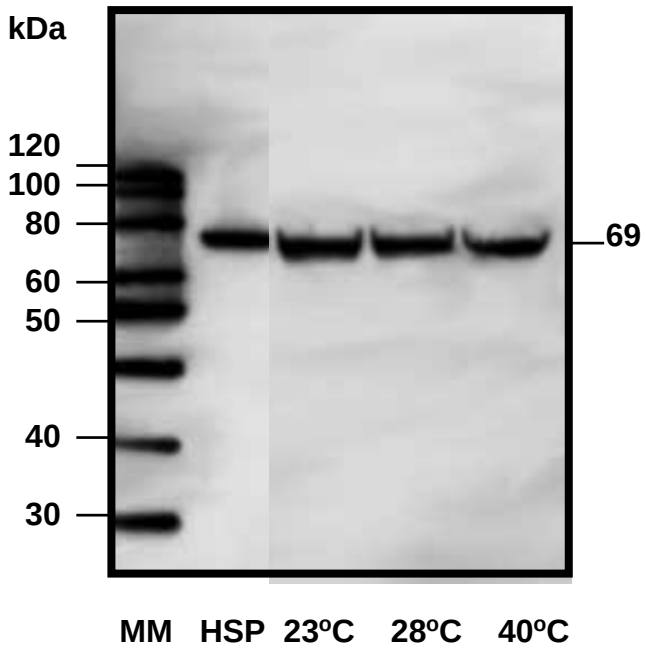


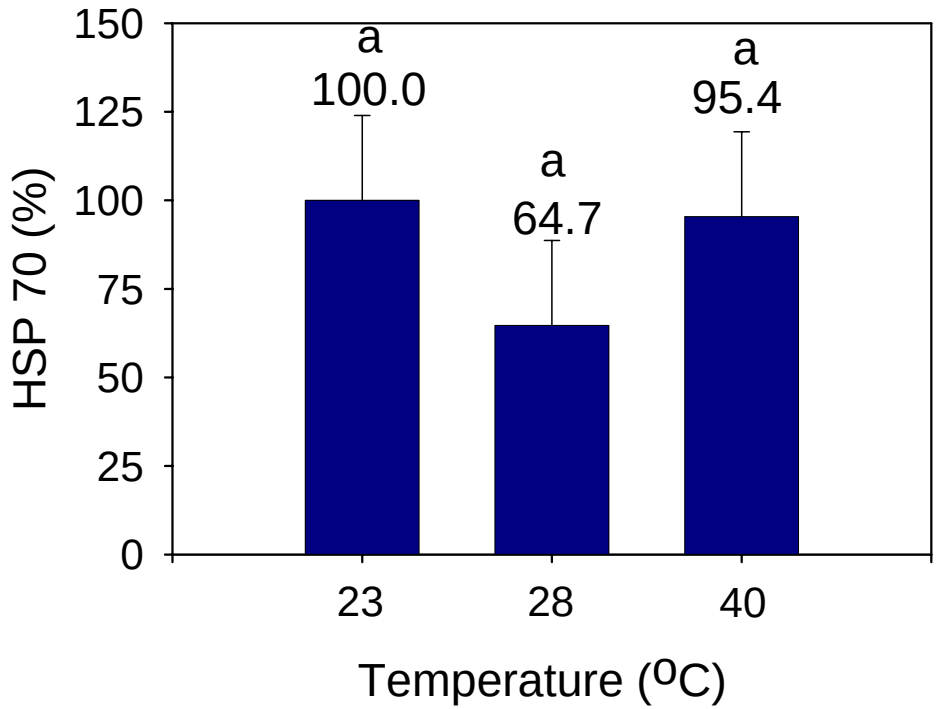


Calculated molecular mass of HSP (70) is 75 kDa

F = 0.62; *d.f.* = 2; *P* = > 0.05; *n* = 4 (Proc GLM, LSD)

HSP Detected in Pupae Exposed to Different Temperatures

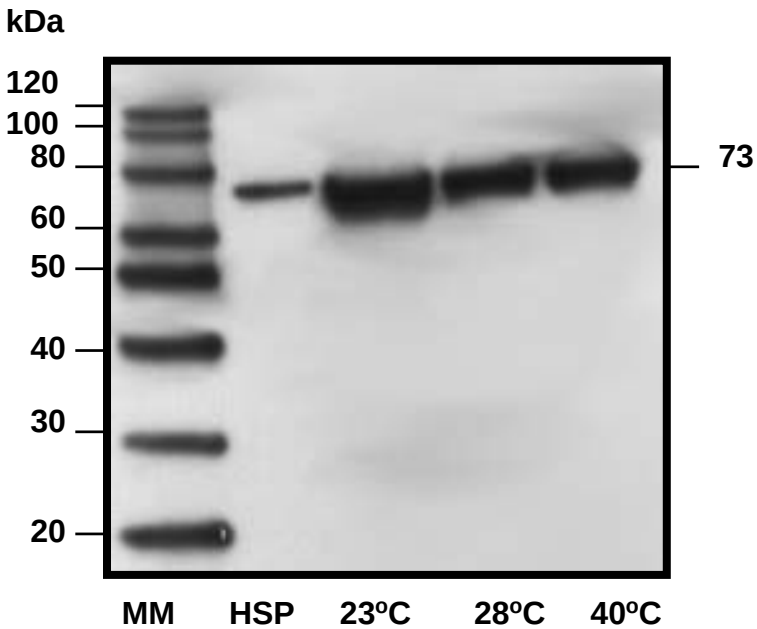


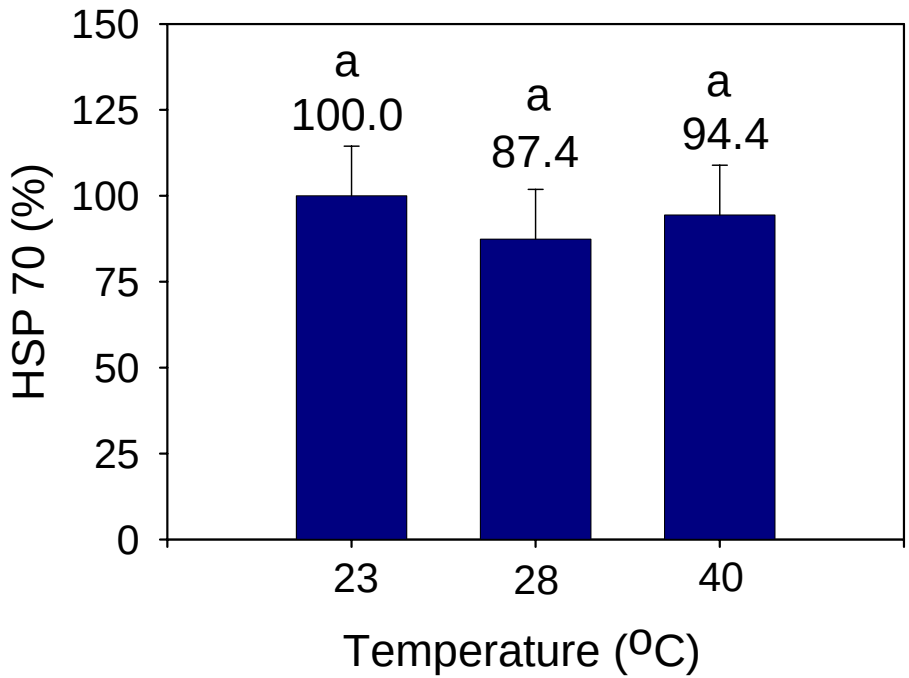


Calculated molecular mass of HSP (70) is 75 kDa

F = 2.57; *d.f.* = 2; *P* = > 0.05; *n* = 4 (Proc GLM, LSD)

HSP Detected in Adults Exposed to Different Temperatures





Calculated molecular mass of HSP (70) is 75 kDa

F = 0.76; *d.f.* = 2; *P* = > 0.05; *n* = 4 (Proc GLM, LSD)

Summary

- Young larvae were the most heat tolerant stage while eggs were the least heat tolerant.
- The expression of HSP 70 in young larvae was significantly increased by about 33% when temperature increased from 23 to 40^{o}C. However, for eggs the expression of HSP 70 decreased by about 35%.
- For old larvae, pupae and adults the expression of HSP 70 did not vary significantly.
- Increased thermotolernace in young larvae could be due to either increased expression of HSP70 at higher temperatures and / or the additional HSP with lower molecular mass of 24 kDa.
- Reduced thermotolerance in eggs may be due to the denaturation of HSP70 with increasing temperatures.

Future Research

- To study the stability of HSP from young larvae exposed to 40^{o}C for different time periods.
- To study the stability of HSP from young larvae exposed to 40-60^{o}C.
- Molecular characterization of different HSP in relation to different temperature-time treatments.

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