

Polygalacturonase gene expression
in wild collected *Lygus lineolaris*
(Palisot de Beauvois) (Hemiptera:
Miridae)



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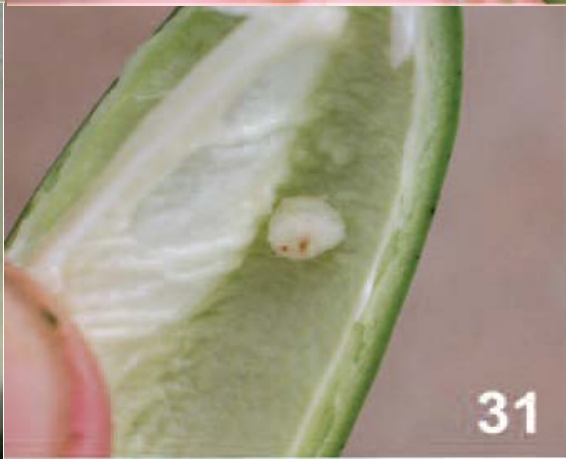
Lygus lineolaris

- Tarnished plant bug
- Highly polyphagous
- Similar to *L. hesperus* in western US
- Major cotton pest in SE US
 - #1 cotton pest in MS
 - Cost MS growers \$28 million per year for insecticides and still caused an average of \$8 million damage per year (2009-2011)
 - \$194/ha in Delta vs. \$46/ha in Hills
 - Resistant to many insecticides



Damage

- Cause damage by inserting stylet into plant tissue and inject salivary enzymes to breakdown plant pectins



Polygalacturonase

- Salivary enzymes used by plant bugs to breakdown plant pectins
- Shown to degrade plant tissue, associated with *Lygus* type damage (Strong and Kruitwagen 1968, Shackel 2005)
- Three different PG genes in *L. lineolaris* (Allen and Mertens, 2008)
- Genes may be up and down regulated based on current host (Walker and Allen 2010)
- PG may be used in oviposition site preparation (Cooper and Spurgeon 2011)
- Anti-PG genes have been discovered

My research

PG expression in wild *L. lineolaris*
populations collected from cotton, pigweed,
and horseweed in the Delta and Hills
regions of MS

Materials and Methods

- Field

- Collected 10 adult TPBs per location per host
- 5 locations
- Stored in plastic bags in -80°C freezer

- Preparation for molecular analysis

- Surface sterilized in 70% EtOH
- Salivary glands removed (head removed from body)
- Stored in DNase and RNase free microcentrifuge tubes in -80°C freezer

Materials and Methods

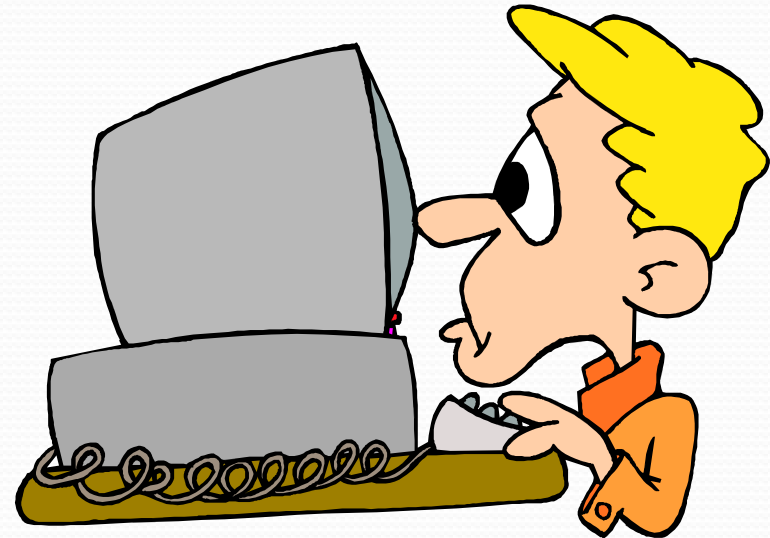
- Molecular analysis
 - Homogenized as 10 insect samples in Trizol (Sigma)
 - RNA isolated
 - Genomic DNA removed (rDNase I-Takara)
 - RNA purified and quantity estimated by Take 3 nonodrop in BioTek HiM reader
 - cDNA synthesized using Iscript cDNA synthesis kit (Biorad)
 - Gene expression measured by qRT-PCR in Eppendorf realplex² Master Cycler

Materials and Methods

- Statistical analysis
 - Raw data converted to fold change
 - Ct mean - housekeeper = ΔCt
 - Housekeeper rpl6
 - ΔCt - normalizer = $\Delta\Delta\text{Ct}$
 - Normalizer ΔCt values are from a colony reared on diet
 - Fold change = $2^{-\Delta\Delta\text{Ct}}$
 - Log transformed
 - Analyzed using Statistica Statsoft software GLM
 - Comparisons made of
 - Delta vs. Hills
 - PG expression by host (ex. PG1 in cotton vs. horseweed vs. pigweed)

Results (Overall)

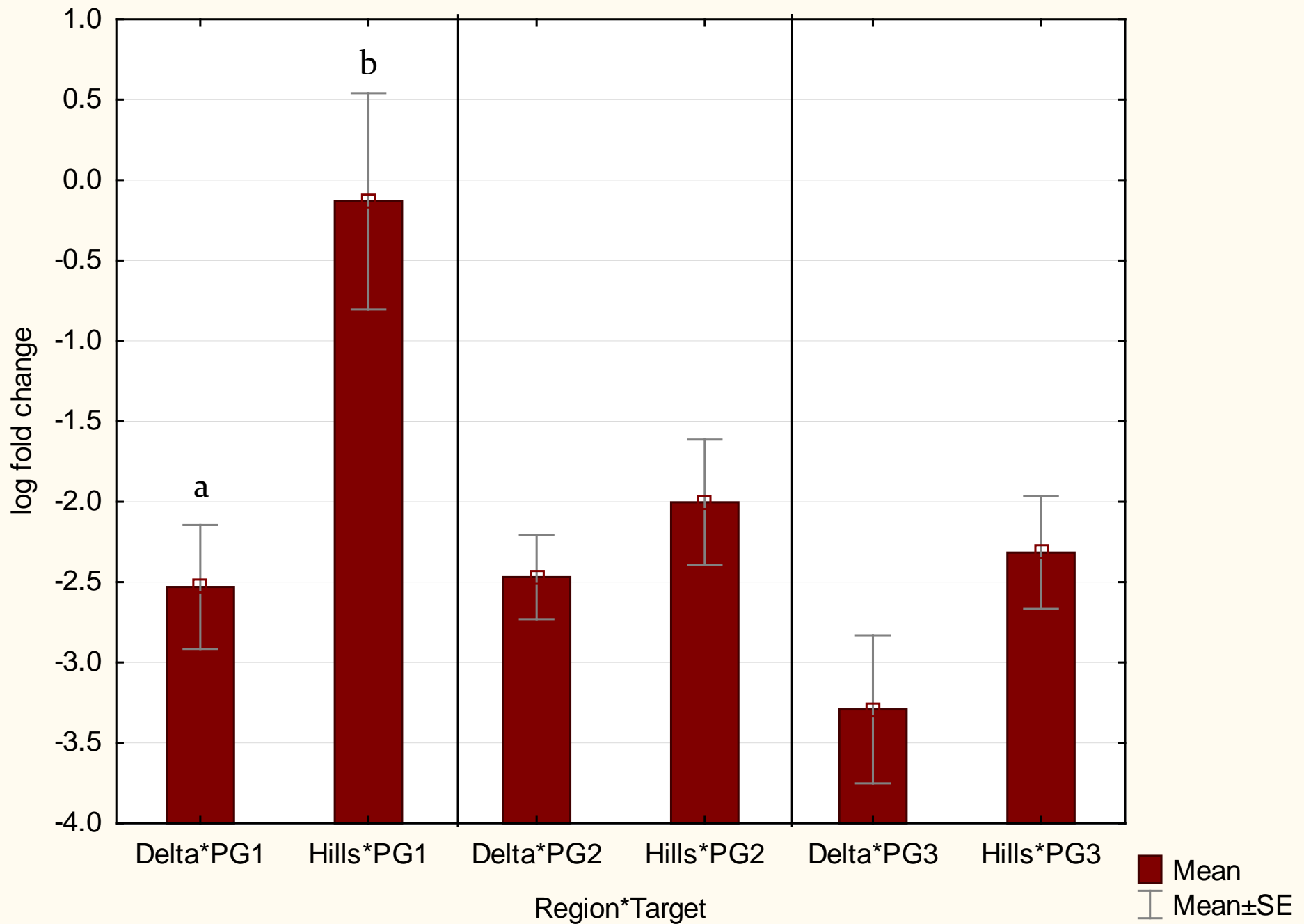
- Region by Host interactions
 - PG₁ (df=2, 17, F=5.70, p=0.01)
 - PG₂ (df=2, 17, F=2.60, p=0.10)
 - PG₃ (df=2, 17, F=3.20, p=0.07)
- Main effects
 - Region
 - PG₂ (df=1, 19, F= 2.16, p=0.16)
 - PG₃ (df=1, 19, F=0.12, p=0.73)
 - Host
 - PG₂ (df=1, 19, F=8.67, p<0.01)
 - PG₃ (df=2, 19, F=3.83, p=0.04)



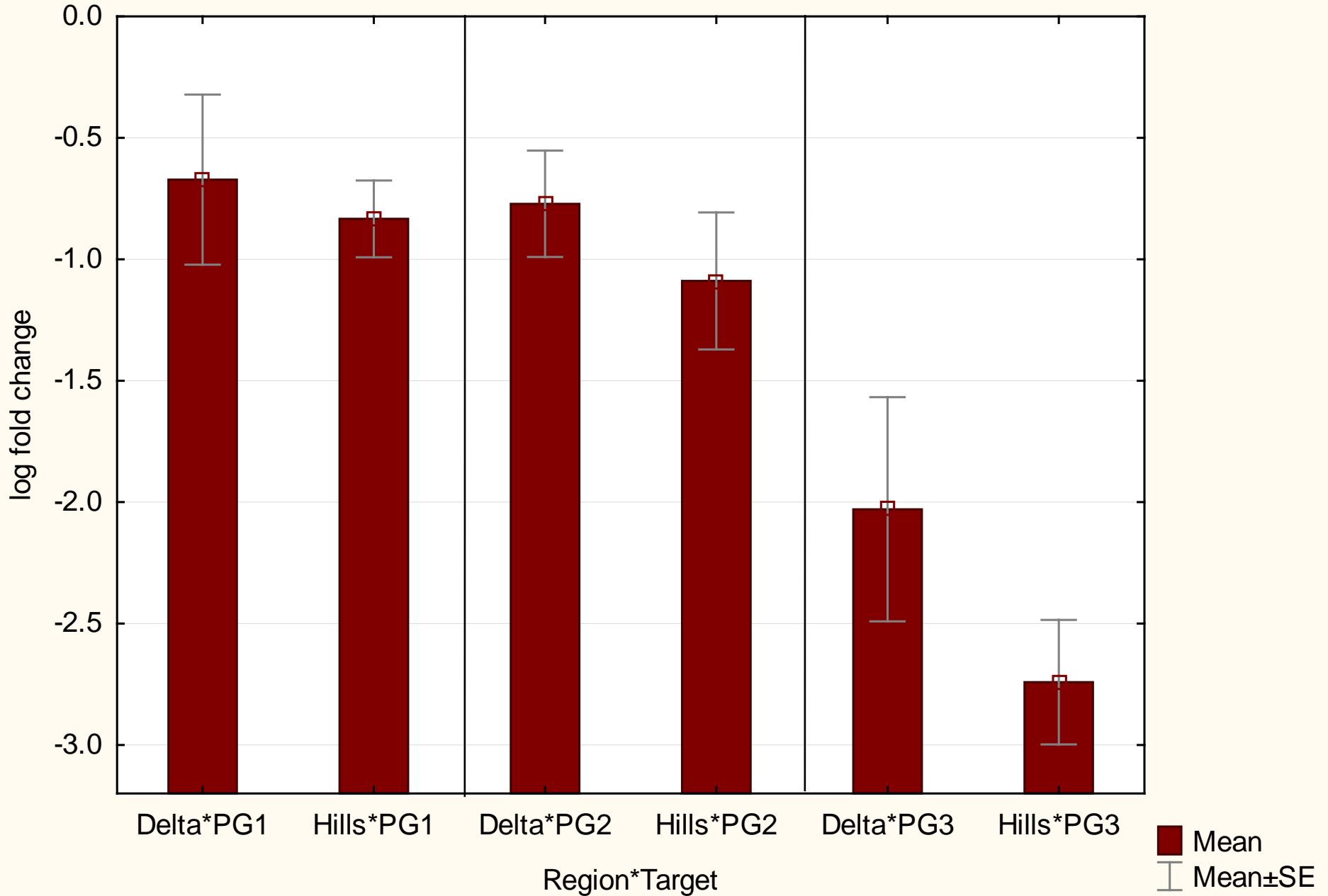
Results (Delta vs. Hills)

- Cotton
 - PG₁
 - More expression in Hills TPBs
 - (df=1, 6, F=9.55, p=0.02)
- Horseweed
 - No significant differences for any PG
- Pigweed
 - PG₂
 - More expression in Hills TPBs
 - (df=1, 6, F=9.00, p=0.02)

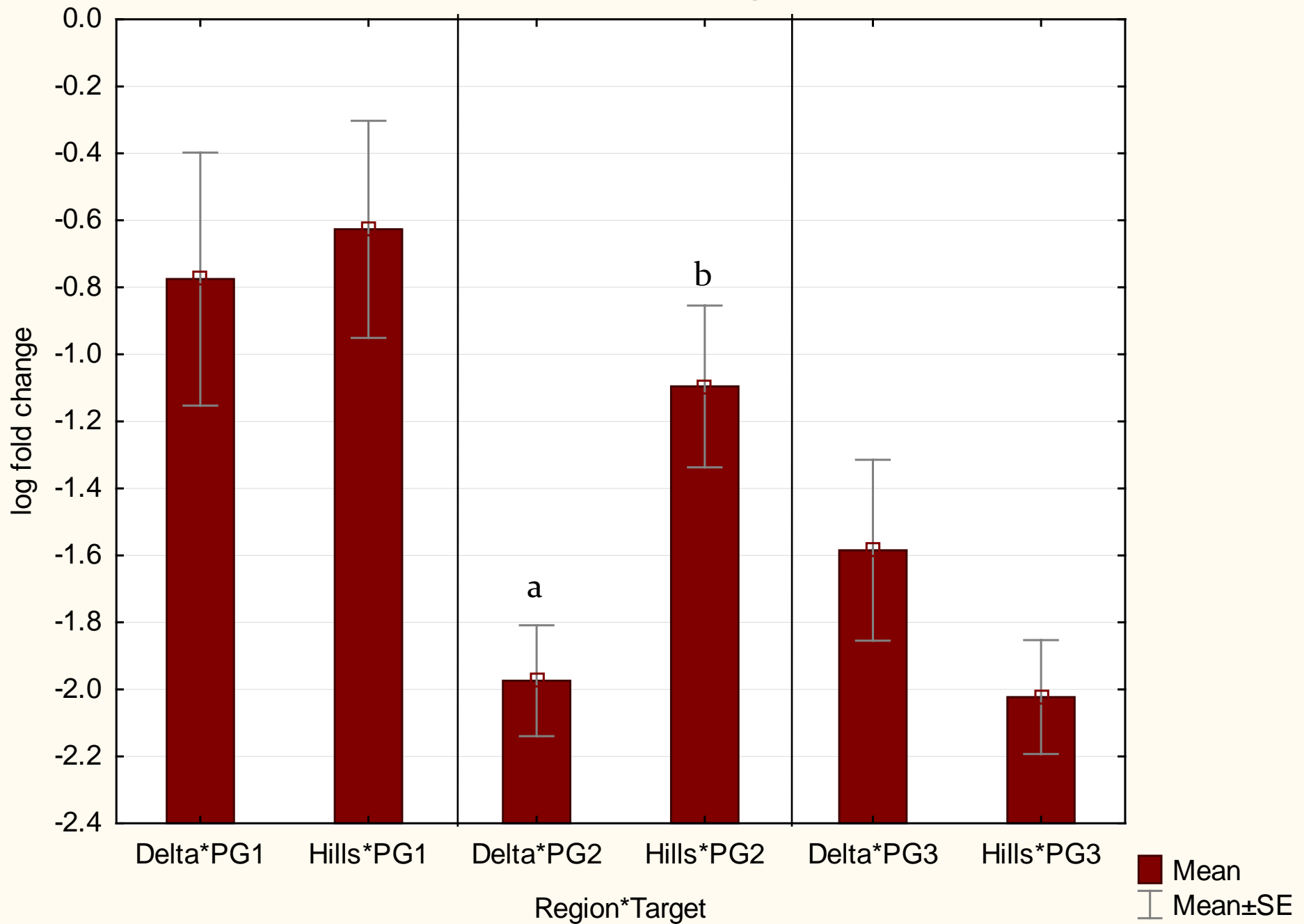
Delta vs. Hills Cotton



Delta vs. Hills Horseweed



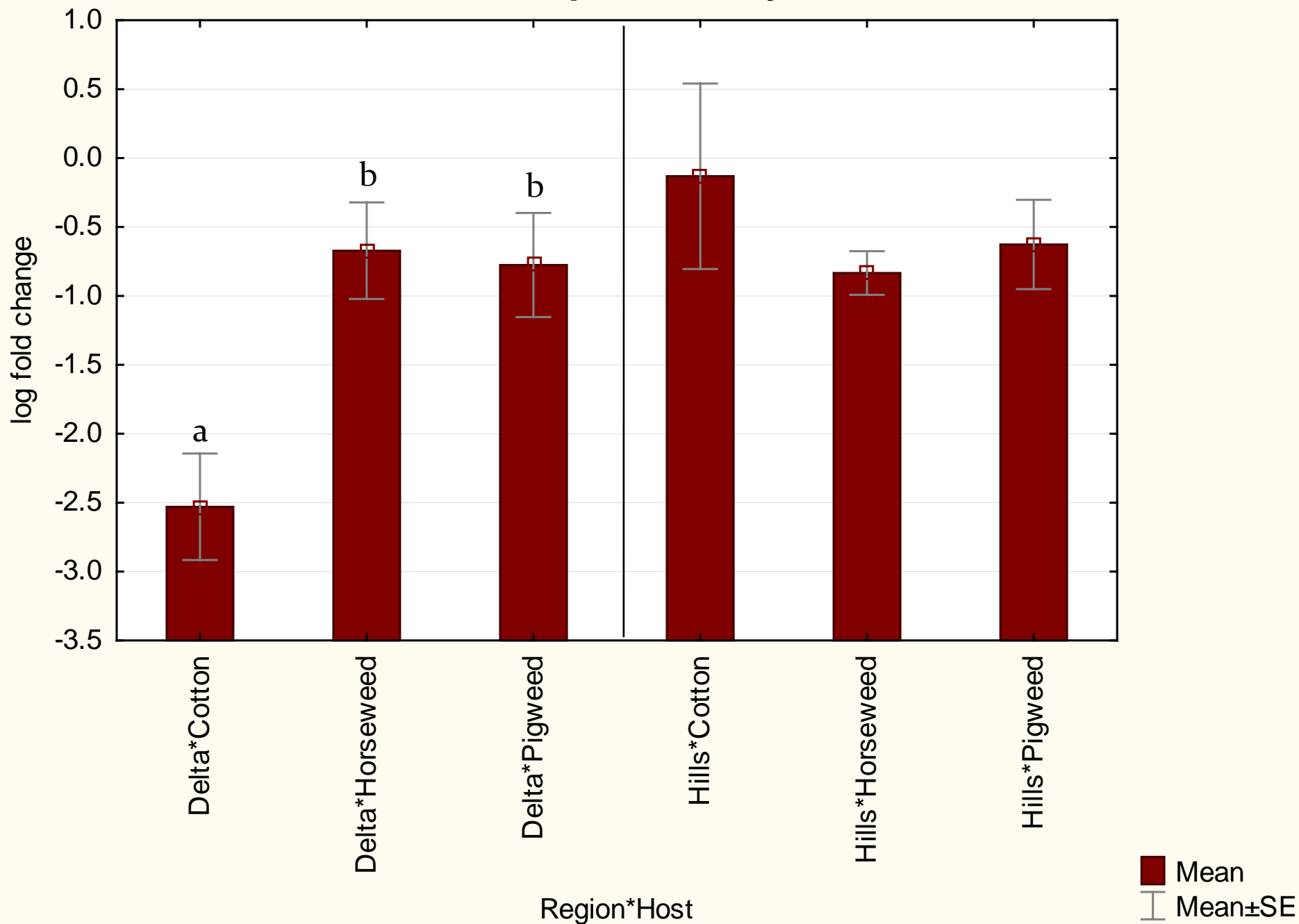
Delta vs. Hills Pigweed



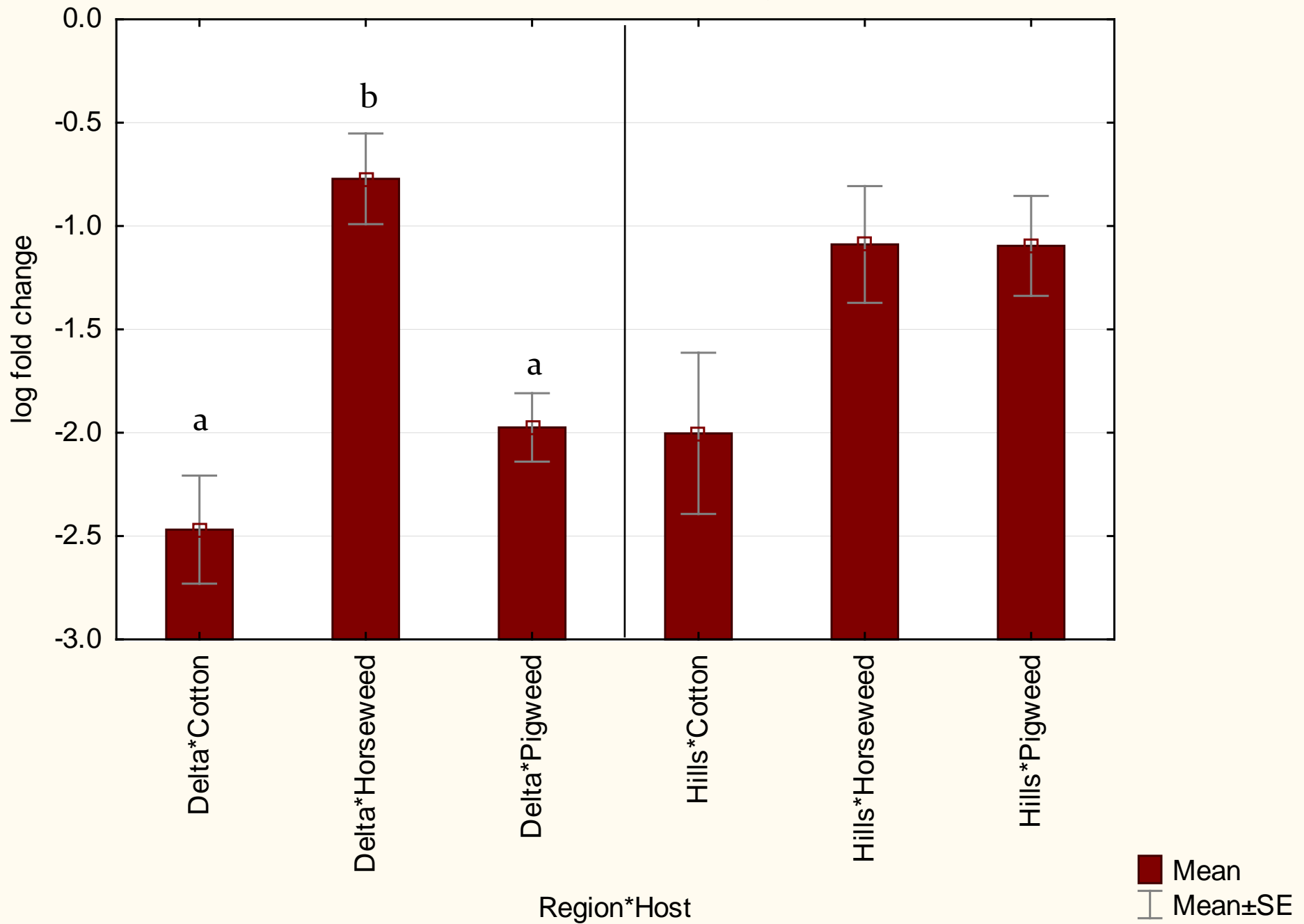
PG expression by host

- Delta
 - PG₁
 - Less expression in cotton than horseweed or pigweed
 - (df=2, 8, F=7.80, p=0.01)
 - PG₂
 - More expression in horseweed than in cotton or pigweed
 - (df=2, 8, F=14.16, p<0.01)
 - PG₃
 - Less expression in cotton than in horseweed or pigweed
 - (df=2, 9, F=4.72, p=0.04)
- Hills
 - No significant differences

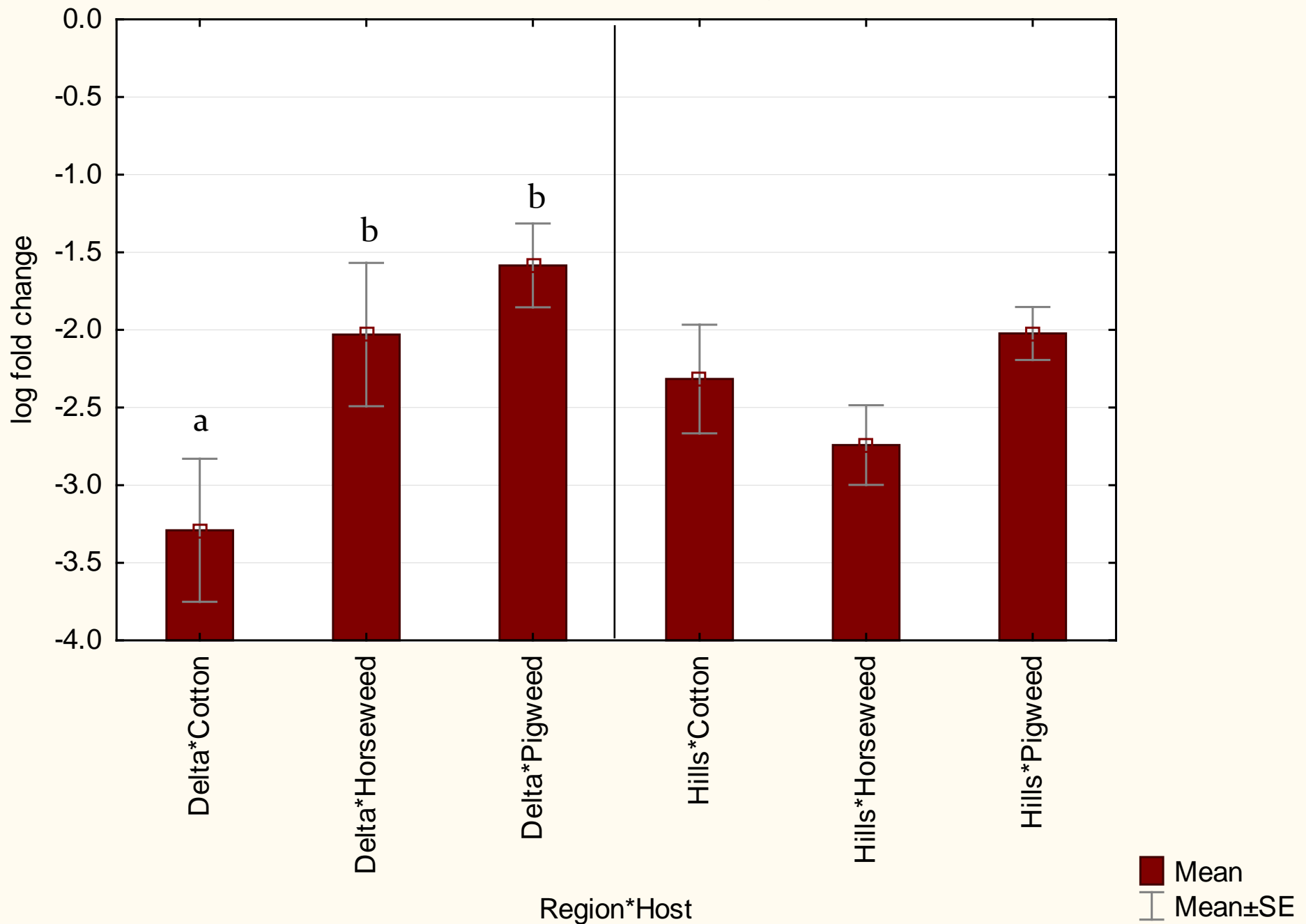
PG1 expression by host



PG2 expression by host



PG3 expression by host



Summary

- All samples show a general down regulation of PG expression compared to the normalizer (artificial diet)
- Few differences between Delta vs. Hills and Hosts
- At this point it is difficult to determine what may be a real effect vs. random effect
 - Movement between hosts
 - Time on current host
 - Age and health of the plants
 - Age and health of the insects

Future Work

- Male vs. Female
- Age
- Nymphs vs. Adults
- Changes from artificial diet to host tissue

Questions and suggestions???

Have any PG
antibodies
been
developed?

