An overview on wheat bug damage & characterization of wheat bug enzymes

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LACC IGW

4th ICC Latin American Cereals Conference

13th International Gluten Workshop

Pre-harvest damage caused by *Heteropterous* insects

- *Eurygaster spp.* } Middle East, North Africa
- *Aelia spp.* } East & South Europe

- *Nysius huttoni* } New Zealand

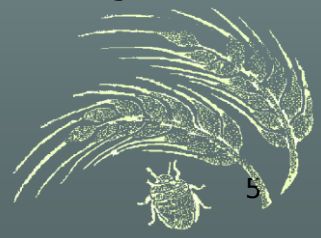
* Significant quantities of wheat → **unusable**

PREHARVEST DAMAGE TO WHEAT

Suni-bug (*Eurygaster* spp) feeding on a wheat spike

Wheat-bug / Suni bug / Sunn pest

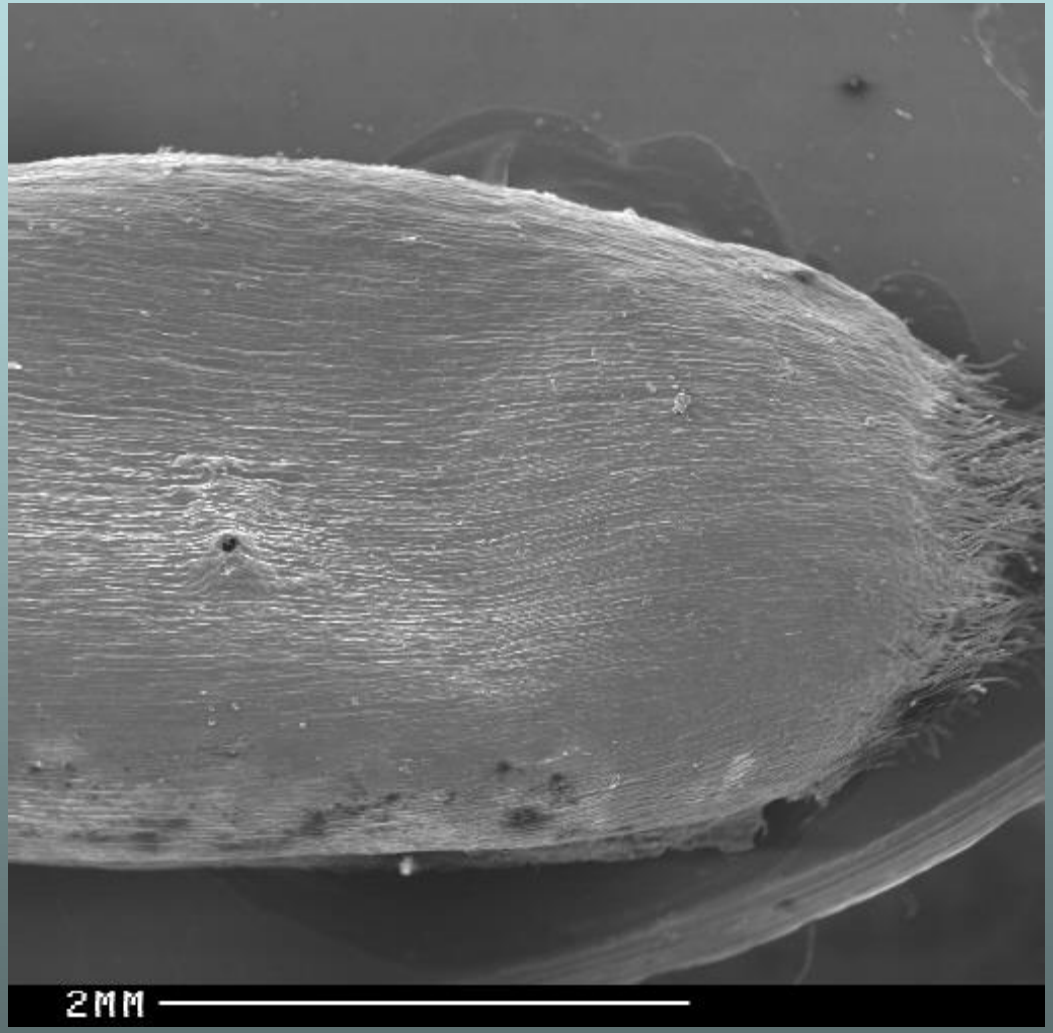
- Like many other Heteropteran insects, suni bug ingests plant liquids by inserting sucking mouthparts into plant tissues.
- Extra-oral digestion is facilitated by the secretion of digestive enzymes including proteases from the salivary glands.
- The protease remains in flour produced from damaged wheat and the resulting dough has weak rheological properties and produces unsatisfactory bread with poor volume and texture.



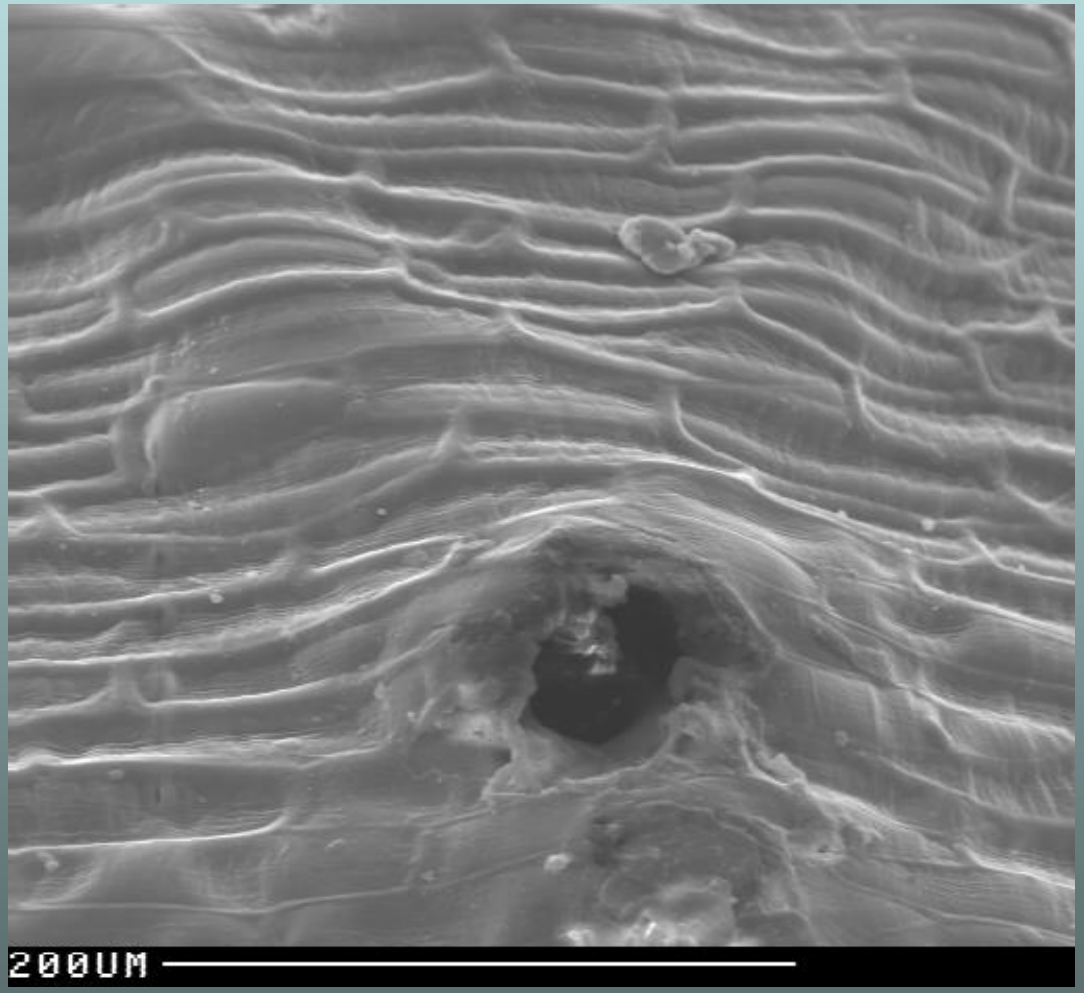
Suni Bug Damage

- Early stages of kernel development (milk ripe stage)
  - smaller, lighter and shrivelled kernels (easy to remove)
  - yield ↓
- Later stages of kernel development (full ripe stage)
  - kernels with normal size and shape (difficult to remove)
  - breadmaking quality ↓

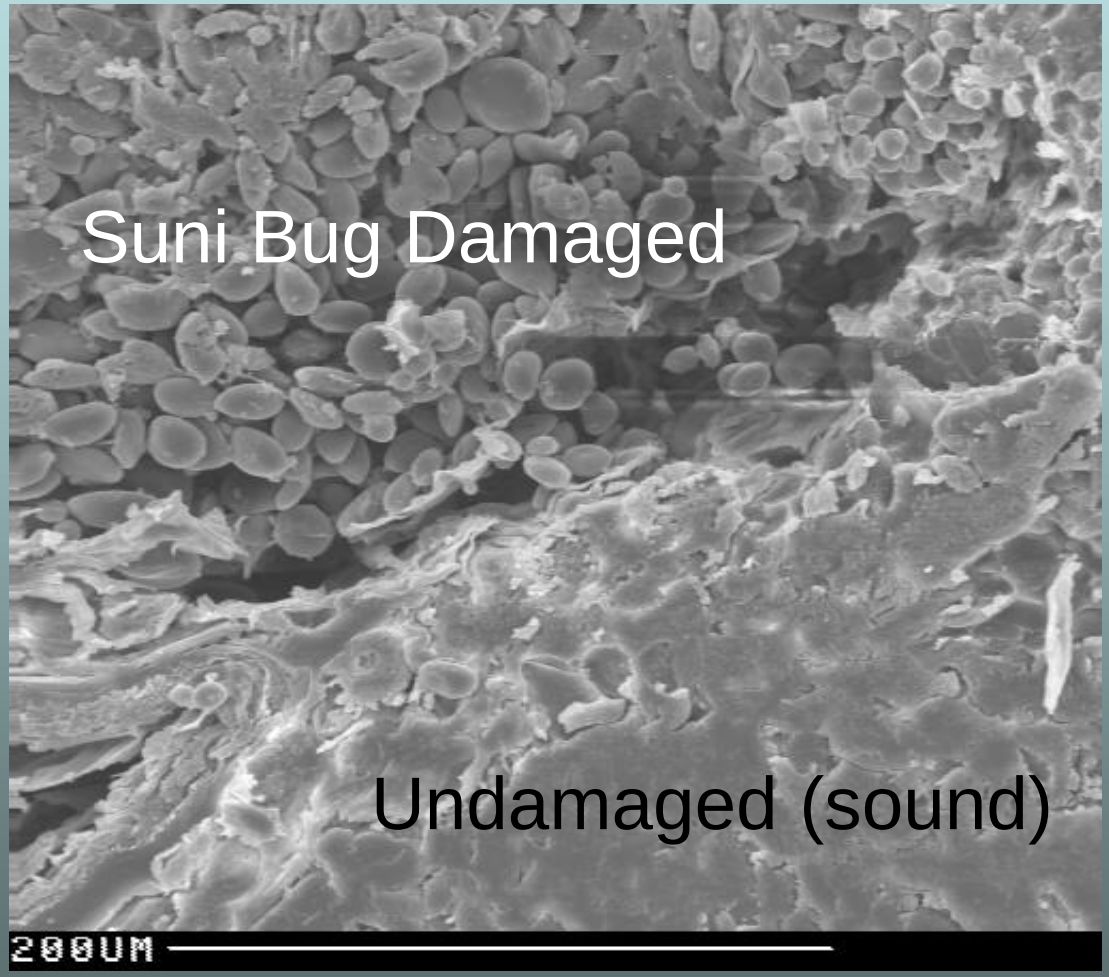
(SEM-I)



(SEM-II)

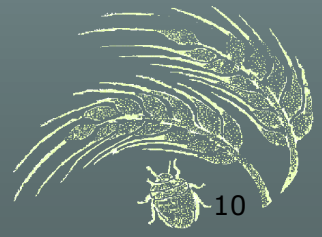


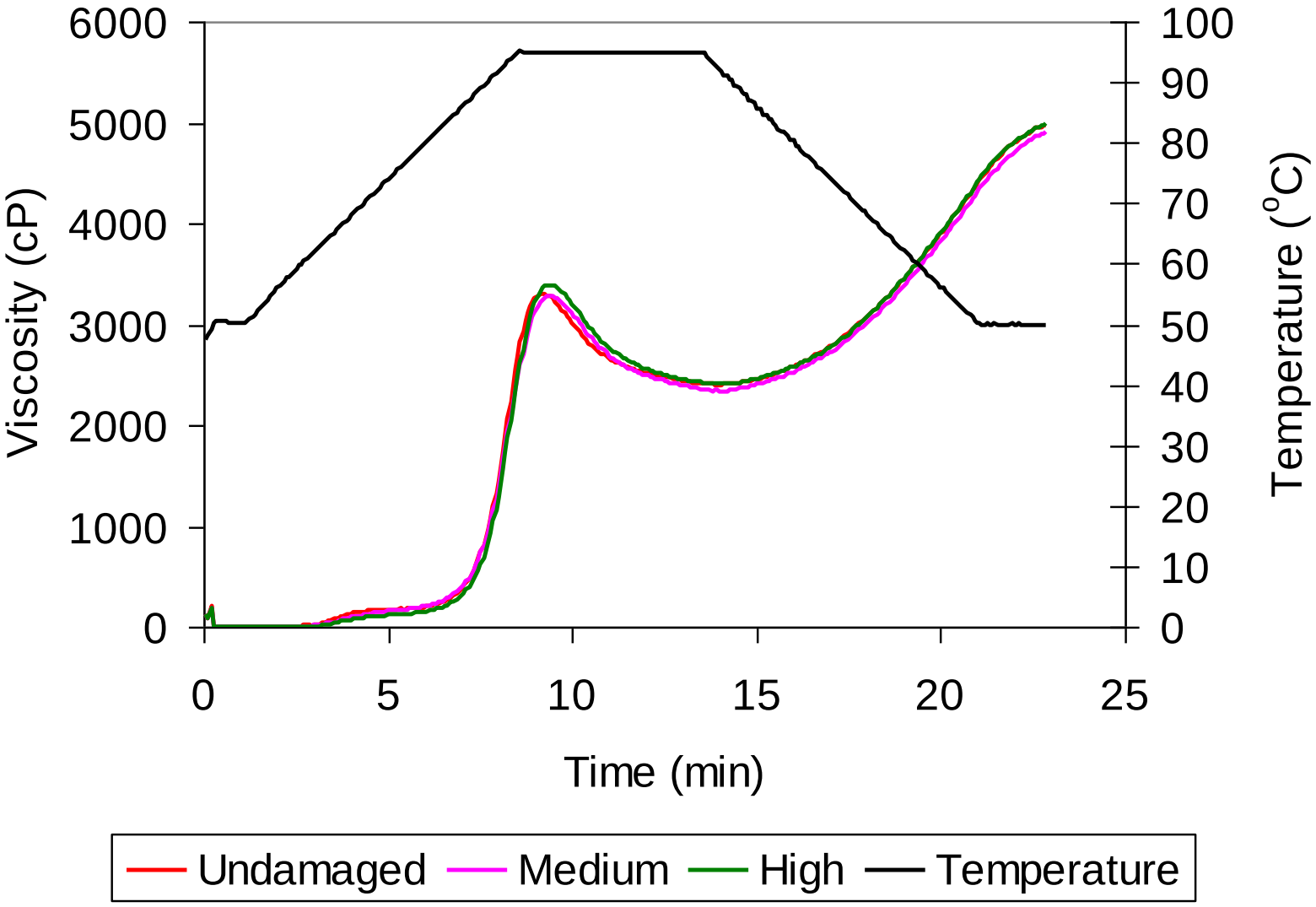
(SEM-III)



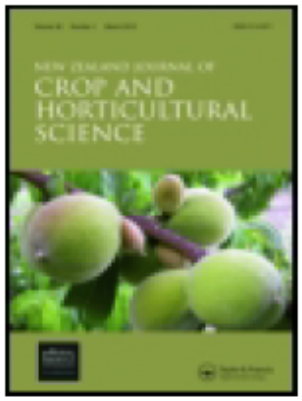
Suni Bug Damage

- When immature grain is attacked in the field by the insects, the resulting dough is weak due to progressive degradation of the gluten proteins by the bug protease secreted by the insect.
- The bug protease hydrolyses both glutenin and gliadin proteins.





RVA graphs (standard profile) of Diyarbakir semolina sample



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Effects of wheat bug (Eurygaster maura) proteolytic enzymes on electrophoretic properties of gluten proteins

D. Sivri , H. Köksel & W. Bushuk

Electrophoresis: 6 bread wheat cultivars

- Bezostaya, Lancer & Gun

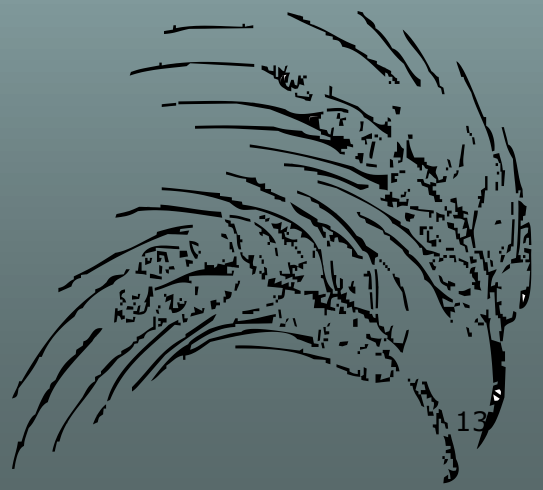
  (Strong gluten, hard-red)

- Ankara & Kirkpinar

  (Medium gluten quality, semi hard - white)

- Kirac

  (Weak gluten quality, soft-white)



Electrophoresis: Sample preparation

Damaged kernels

↓

Grinding

↓

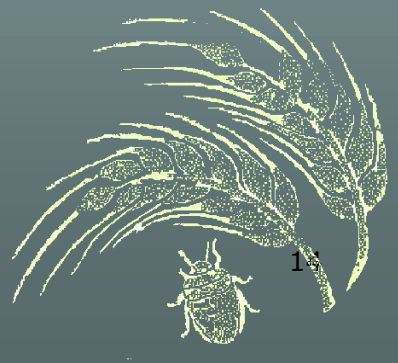
Ground sample (40 mg) + DD water (200 µl)

↓

Incubation (37°C, 0, 15, 30, 60, 120 & 240 min)

↓

Lyophilization



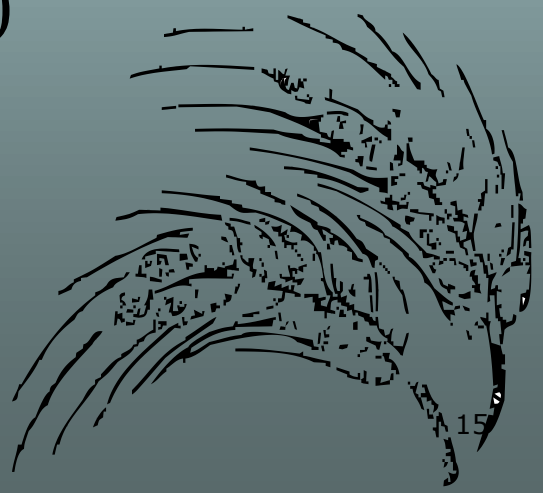
Electrophoresis: Methods

- **GLIADIN**

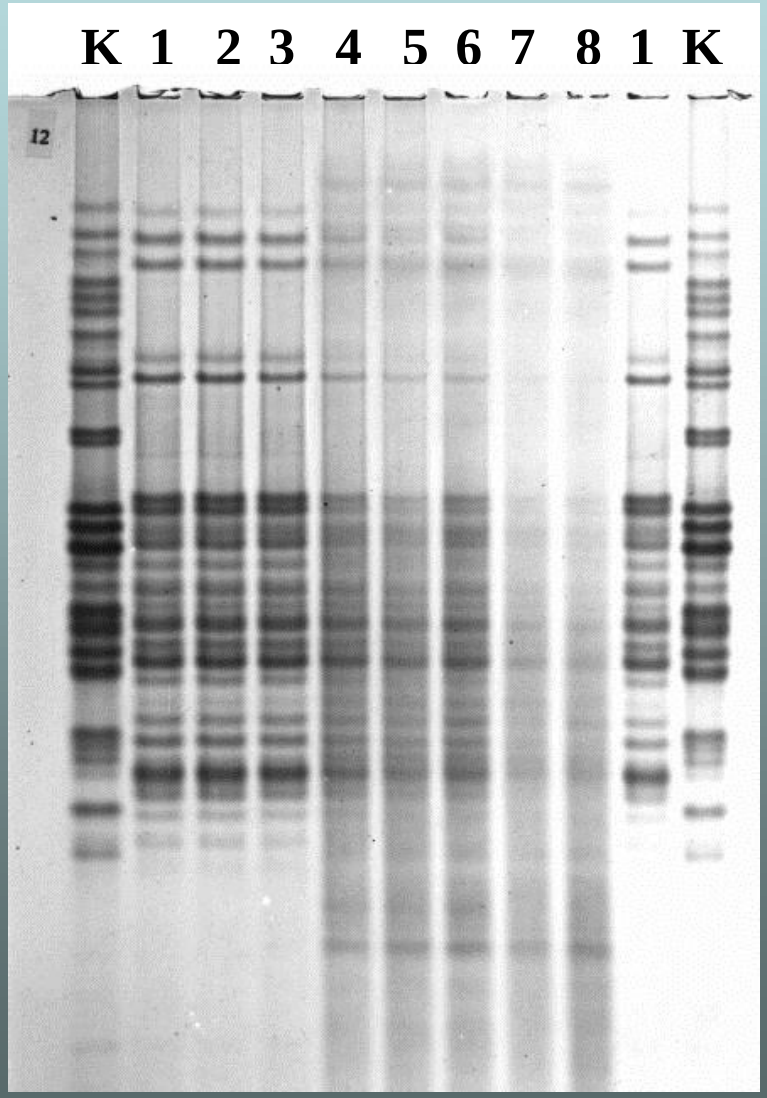
  Acid Poliacrylamide Gel Electrophoresis (A- PAGE) (Bushuk ve Zillman, 1978)

- **GLUTENIN**

  SDS-PAGE (Fu ve Sapirstein, 1996)



Effects of suni-bug protease on gliadin proteins of wheat cv. Bezostaya (A- PAGE)



(K) Katepwa (Standard)

- **Sound**

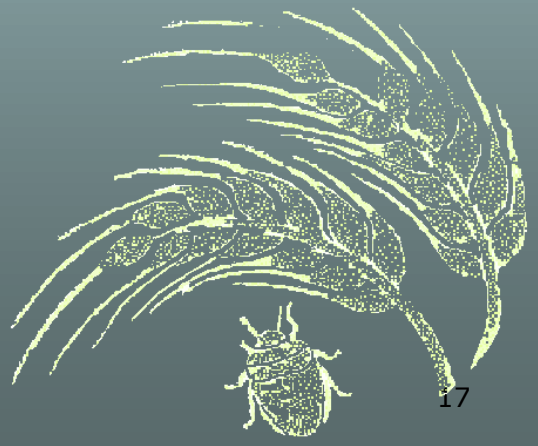
(1) Control
(2) Incubated, 240 min

- **Damaged**

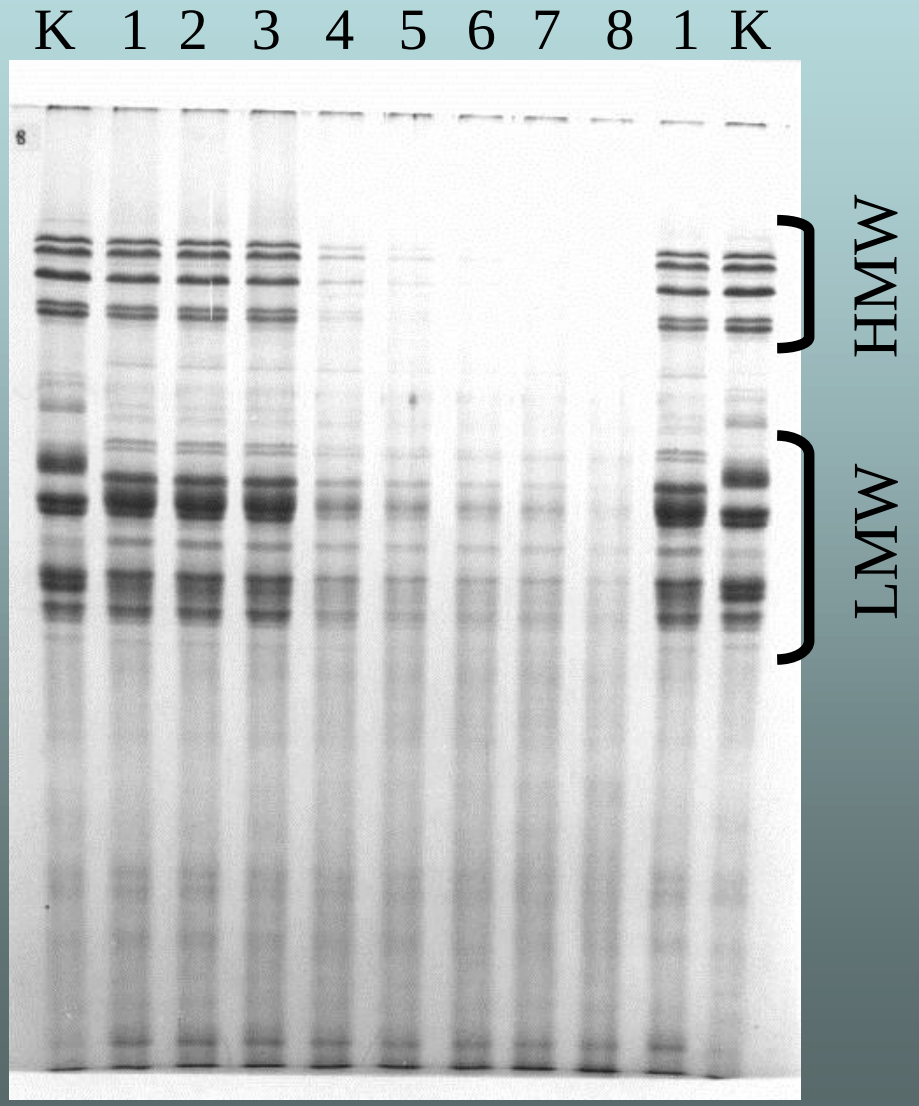
(3) Not incubated
(4) Incubated, 15 min
(5) Incubated, 30 min
(6) Incubated, 60 min
(7) Incubated, 120 min
(8) Incubated, 240 min

A-PAGE RESULTS (Sivri et al. 1998)

- No detectable effect on gliadins before incubation
- Significant changes on gliadins after incubation
- More obvious changes with increasing incubation time
- New bands appeared in A-PAGE patterns
- Most of the original bands disappeared after 120 min and longer incubation



Effects of suni-bug protease on glutenin proteins of wheat cv. Bezostaya (SDS- PAGE)



(K) Katepwa (Standard)

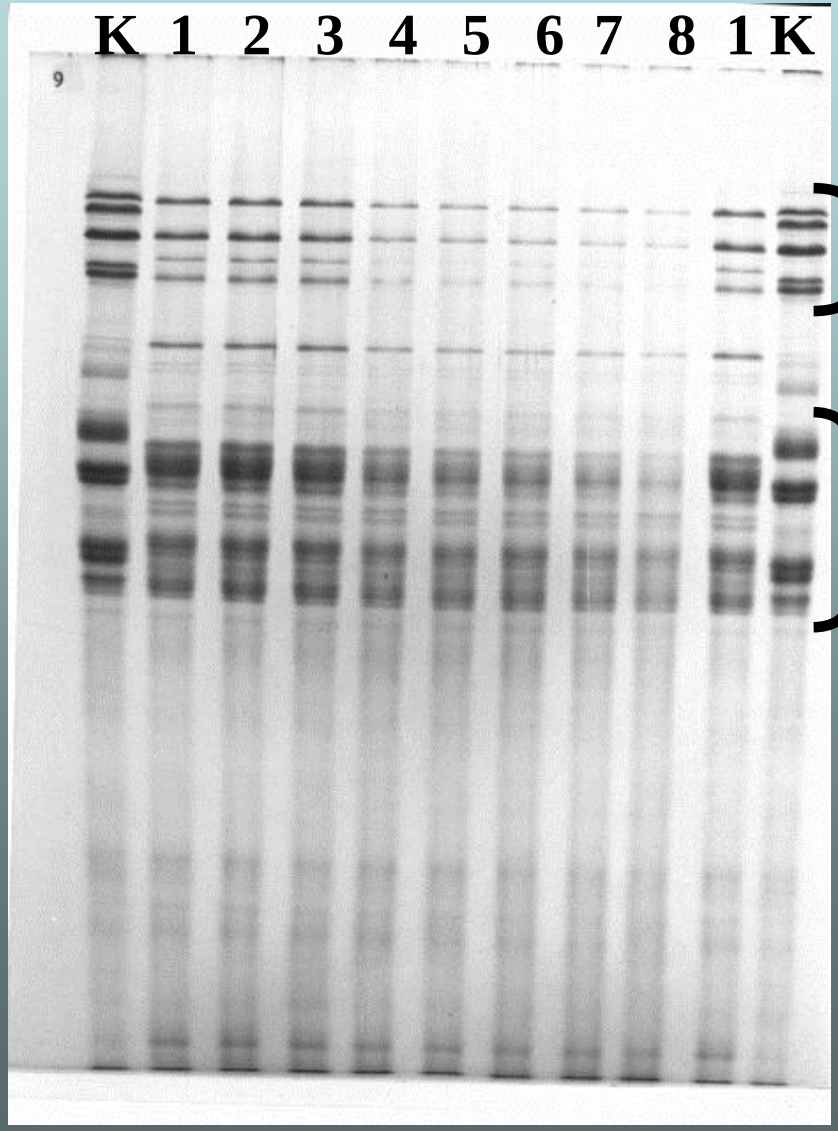
- **Sound**

(1) Control
(2) Incubated, 240 min

- **Damaged**

(3) Not incubated
(4) Incubated, 15 min
(5) Incubated, 30 min
(6) Incubated, 60 min
(7) Incubated, 120 min
(8) Incubated, 240 min

Effects of suni-bug protease on glutenin proteins of wheat cv. Ankara (SDS- PAGE)



(K) Katepwa (Standard)

- **Sound**

(1) Control
(2) Incubated, 240 dak

- **Damaged**

(3) Not incubated
(4) Incubated, 15 dak
(5) Incubated, 30 dak
(6) Incubated, 60 dak
(7) Incubated, 120 dak
(8) Incubated, 240 dak

SDS-PAGE RESULTS (Sivri et al. 1998)

- Similar results to the those of A-PAGE
- No new bands observed
- HMW-GS disappeared or drastically decreased after 120 min of incubation
- LMW-GS decreased substantially after 15 min of incubation and decreased slightly beyond this incubation period

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Wheat Intercultivar Differences in Susceptibility of Glutenin Protein to Effects of Bug (*Eurygaster integriceps*) Protease

D. Sivri, H. D. Sapirstein ✉, W. Bushuk, H. Köksel

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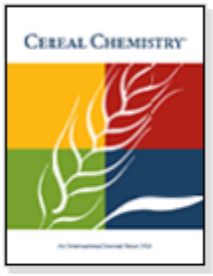

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ABSTRACT

Preharvest bug damage to wheat can cause significant losses in bread-making quality. One of the most prevalent forms of bug damage which frequently occurs in most countries of the Middle East, Eastern Europe and North Africa can be attributed to *Heteropterous* insects, particularly *Eurygaster* spp. Intercultivar differences in the susceptibility of glutenin

Effects of Wheat Bug (*Eurygaster maura*) Protease on Glutenin Proteins

September 1999 , Volume 76, Number 5
Pages 816 - 820

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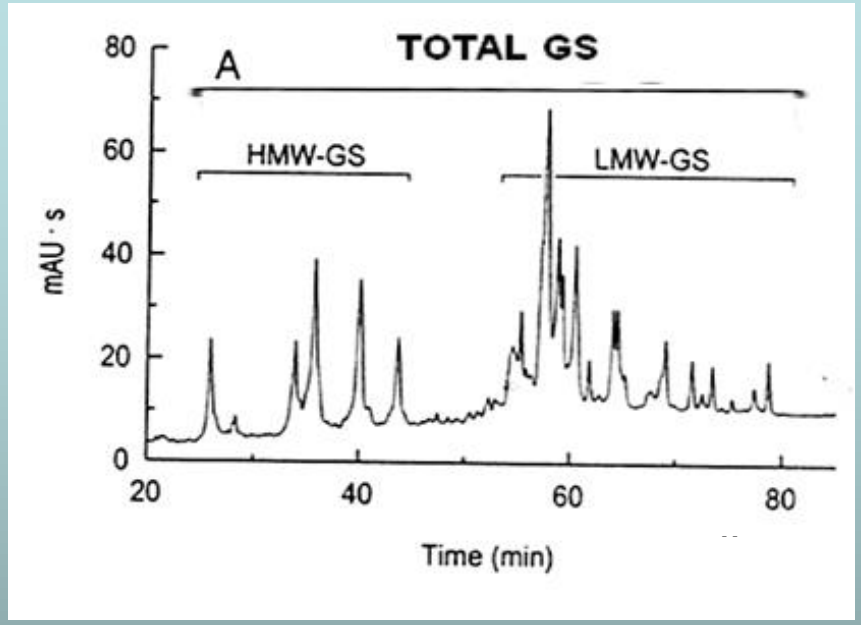
Go to article: http://dx.doi.org/10.1094/CCHEM.1999.76.5.816

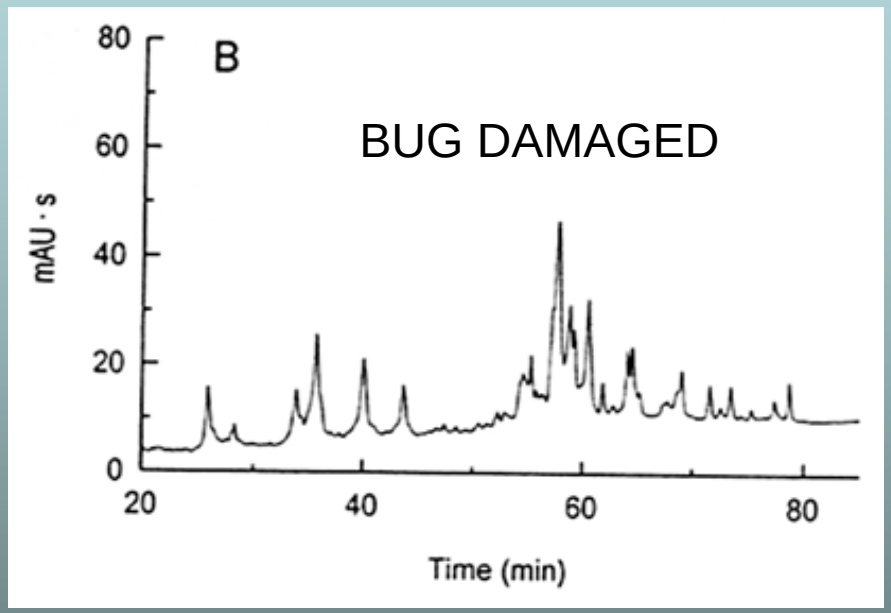
Accepted March 15, 1999.

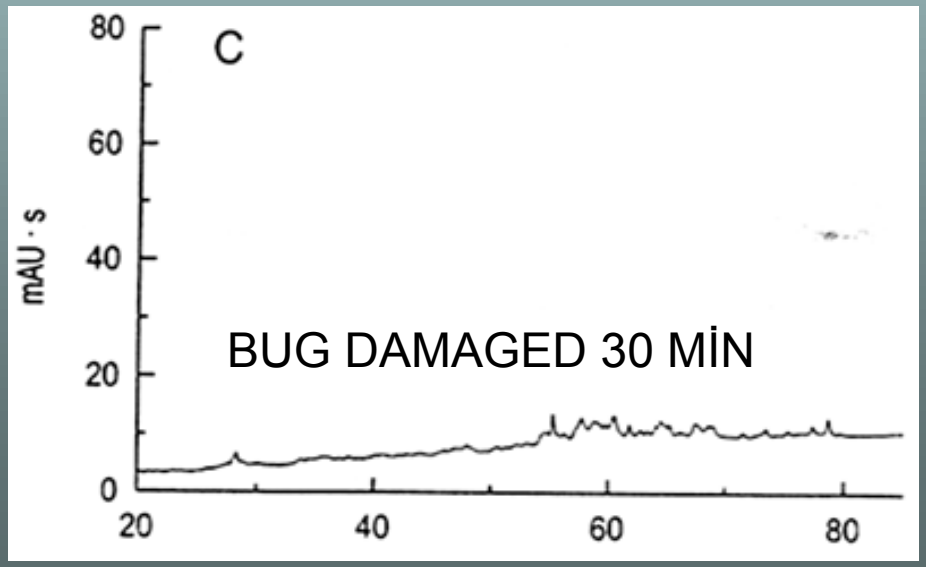
Proteolytic degradation of 50% 1-propanol insoluble (50PI) glutenin of six common wheat cultivars by wheat bug (*Eurygaster maura*) protease was investigated using reversed-phase HPLC. Wheat at the milk-ripe stage was manually infested with adult bugs. After harvest, bug-damaged kernels were blended (2:1, kernel basis) with undamaged grain of the same cultivar. Samples of ground wheat were incubated in distilled water for different times (0, 30, 60, and 120 min). The incubated whole meal samples were subsequently freeze-dried and stored until analysis. The degree of proteolytic degradation of 50PI glutenin was determined based on the quantity of total glutenin subunits (GS), high molecular weight GS (HMW-GS), and low molecular weight GS (LMW-GS). For ground wheat samples incubated for ≥30 min, 50PI glutenin was substantially degraded as evidenced by a >80% decrease on average in total GS, HMW-GS, and LMW-GS. Some cultivars showed different patterns of glutenin proteolysis as revealed by differences in the ratios of HMW-GS to LMW-GS between sound and bug-damaged samples; a significant decrease in this ratio was found for four cultivars. This evidence, combined with other observations, indicated that there were intercultivar differences in polymeric glutenin resistance to the protease of the wheat bug *Eurygaster maura*. While the nature of this resistance is unknown, it should be possible to select and develop wheat cultivars with improved tolerance for wheat bug damage. Propanol insoluble glutenin, which corresponds to relatively large glutenin polymers, appears to be an excellent quantitative marker for this purpose.

CONTROL



RP-HPLC





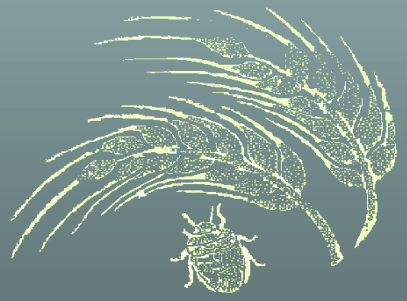
RP-HPLC RESULTS (Sivri et al. 1999)

- Little reduction in the amounts of total-GS, HMW-GS and LMW-GS before incubation
- Total-GS substantially decreased (more than 80%) after 15 min of incubation
- Wheat cultivars showed different patterns of glutenin degradation (Intercultivar differences may exist in gluten resistance to the bug protease)

Determination of suitable substrates

Substrate (50mg) + H_2O (500 µL) + EE → incubation (37°C, 16 h) → SDS-PAGE

- Glutenin ***
- Gelatin **
- Azocasein *
- Hemoglobin
- Elastin
- Collagen
- Fibrin
- Hide powder



Determination of suitable substrates

SDS-PAGE

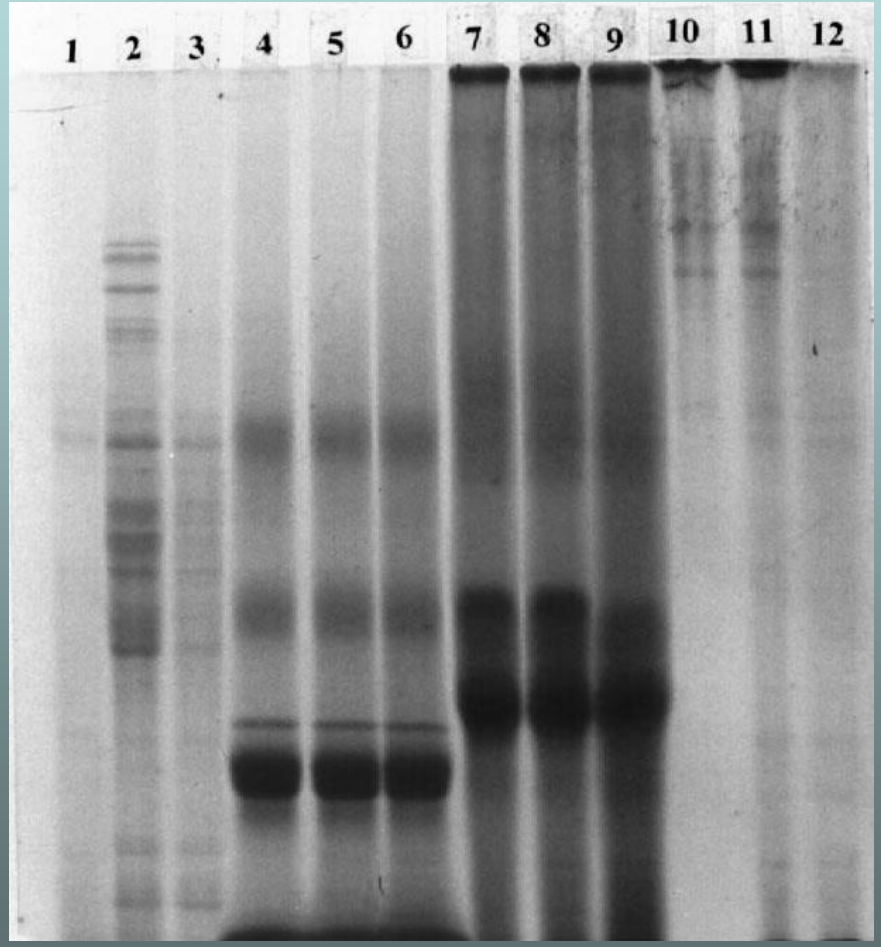
1: EE

2, 3: Glutenin
(cv.Katepwa)

4, 5, 6: Hemoglobin

7, 8, 9: Azocasein

10, 11, 12: Gelatin



Water & SDS soluble gluten proteins

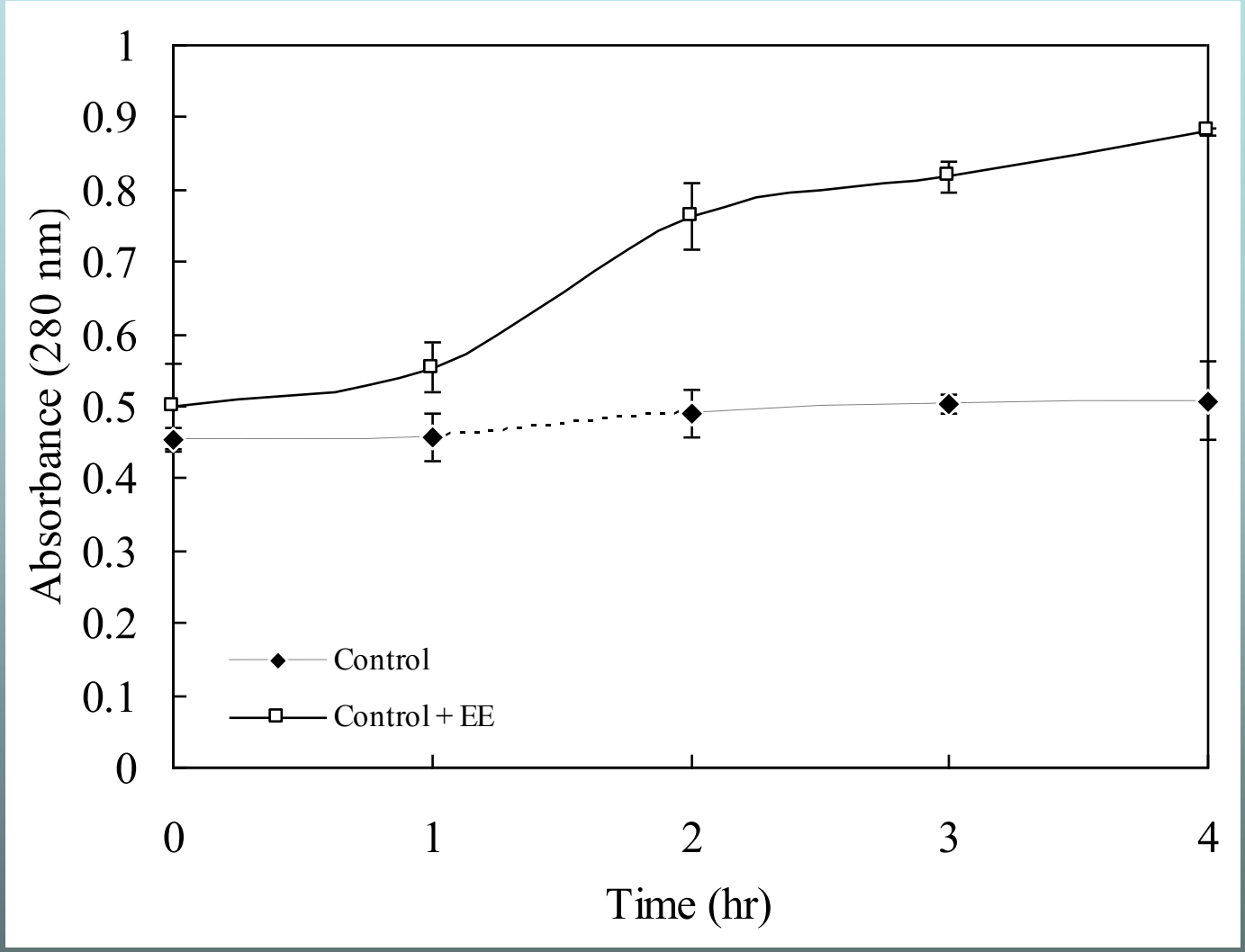
Vital wheat gluten + EE → incubation

25 mg/mL (100 μL) (37°C; 1, 2, 3, 4 h)

Centrifuge → supernatant → dilution → Abs. (280 nm) (S1)

precipitate → mix with water → centrifuge → dilution → Abs (280 nm) (S2)

Total Abs. = S1 + S2


Absorbance (280 nm)
1
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
0
1
2
3
4
Time (hr)
Control
Control + EE

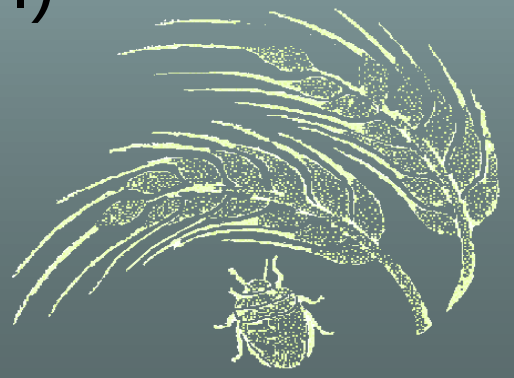
Polymeric glutenin (not soluble in 50% 1-propanol)

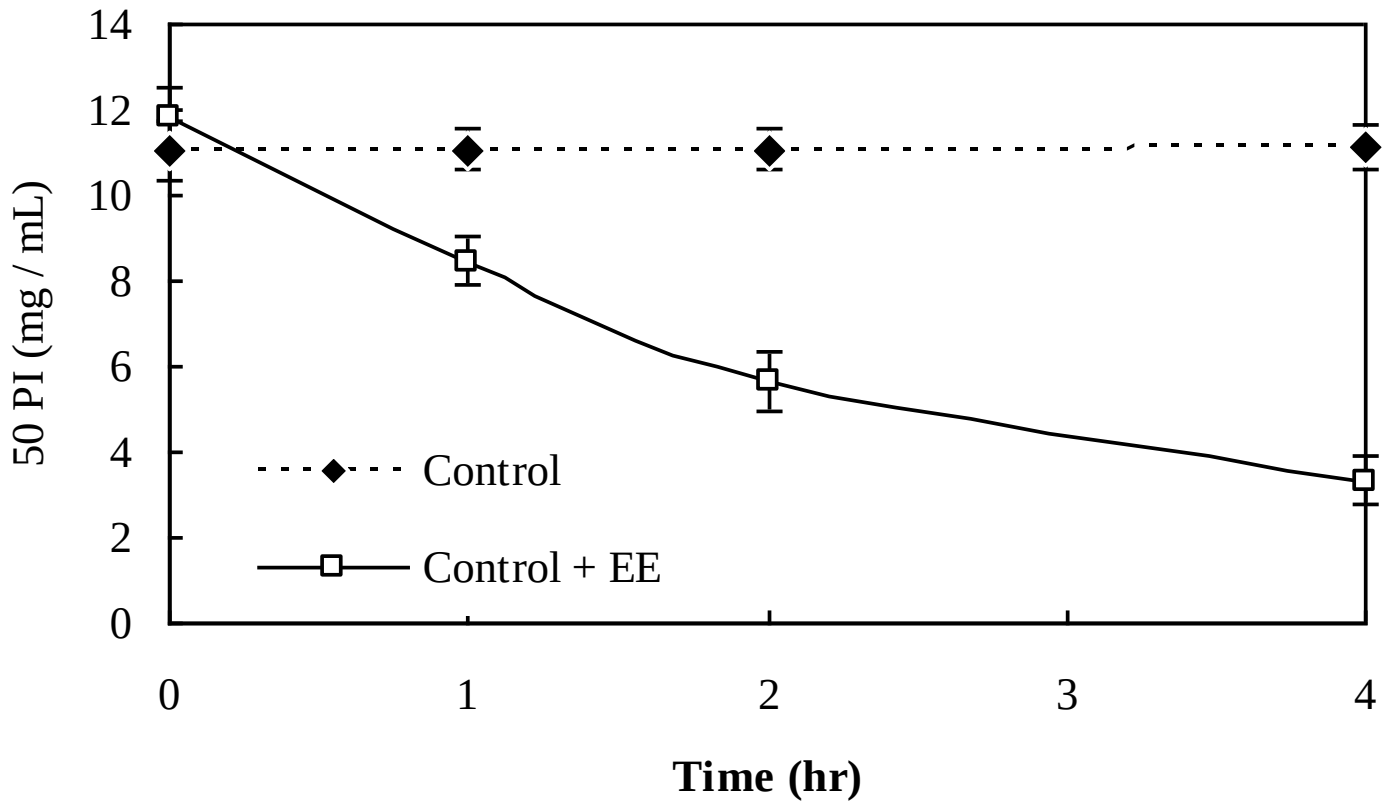
Katepwa flour + EE + H_2O $\rightarrow$ incubation

(250mg) (50mg) (200 μL) (37°C; 1-4 h)

$\rightarrow$Extraction $\rightarrow$ unsoluble glutenin (mg/mL)

(%50 1-propanol) content (50 PI)

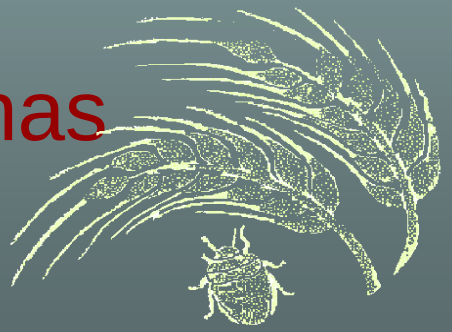



14
12
10
8
6
4
2
0
50 PI (mg / mL)
Control
Control + EE
0
1
2
3
4
Time (hr)

Determination of Suni-bug Protease Acitivities

- Conventional methods → not suitable
- Specific activity of suni-bug enzymes on gluten proteins (especially HMW-GS)
- Two spectrophotometric method

  (gluten substrate)
- 50 PI glutenin based method → has advantages



SUNI BUG PROTEASE CHARACTERIZATION

PURIFICATION

- Ammonium Sulphate Precipitation
- Gel permeation Chromatography (Sephadex G-75)
- Ion exchange Chromatography **(**QAE-Sephadex A-50)

AMINO ACID ANALYSIS

Characterization

- pH 3.5-11
- Optimum pH 8.5,
- Optimum T 35°C,
- pI 8 (Pharmalyte 3-10, IEF)
- Subgroup of Serine proteases including an –SH group

Amino Acid Analysis

- Phe (x 10)
- Pro, Tyr, Lue (x 4)

- HMW-GS repititive domain (β-spiral)

Pro : most abundant aa

Tyr, Lue : many

Sivri, D. and H. Köksel " Wheat bug protease: A protease enzyme with specific activity for gluten proteins" *Wheat Quality Elucidation: The Bushuk Legacy*, Eds. P. K. W. Ng and C. W. Wrigley, American Association of Cereal Chemists (AACC), Chapter 7, s: 113-126, ISBN 1-891127-28-4, St Paul, MN.

JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

ARTICLE

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Characterization of a Glutenin-Specific Serine Proteinase of Sunn Bug *Eurygaster integricepts* Put.

Alexander V. Konarev,[†] Frédéric Beaudoin,[§] Justin Marsh,[§] Nina A. Vilkova,[†] Ludmila I. Nefedova,[†] Dilek Sivri,[‡] Hamit Köksel,[‡] Peter R. Shewry,[*,§] and Alison Lovegrove[§]

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Supporting Information

ABSTRACT: Glutenin hydrolyzing proteinases (GHPs) have been purified, by affinity chromatography, from wheat seeds damaged by the Sunn bug *Eurygaster integriceps* (Hemiptera, Scutelleridae). A 28 kDa protein was partially sequenced by mass spectrometry and Edman degradation which showed homology to serine proteases from various insects. Three full length clones were obtained from cDNA isolated from Sunn bug salivary glands using degenerate PCR based on the sequences obtained. The cleavage site of the protease was determined using recombinant and synthetic peptides and shown to be between the consensus hexapeptide and nonapeptide repeat motifs present in the high molecular weight subunits of wheat glutenin (PGQGQQ^GYYPTSLQQ). Homology models were generated for the three proteinases identified in this study using the high resolution X-ray structure of a crayfish (*Pontastacus leptodactylus*) trypsin complexed with a peptide inhibitor as template (PDB accession 2F91). The novel specificity of this protease may find applications in both fundamental and applied studies.

KEYWORDS: Gluten hydrolyzing protease, *Eurygaster intrigriceps*, cleavage site specificity, molecular modeling

SE-HPLC & 2D SDS PAGE

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www.publish.csiro.au/journals/ajar *Australian Journal of Agricultural Research*, 2004, 55, 477–483

Changes in the composition and size distribution of endosperm proteins from bug-damaged wheats

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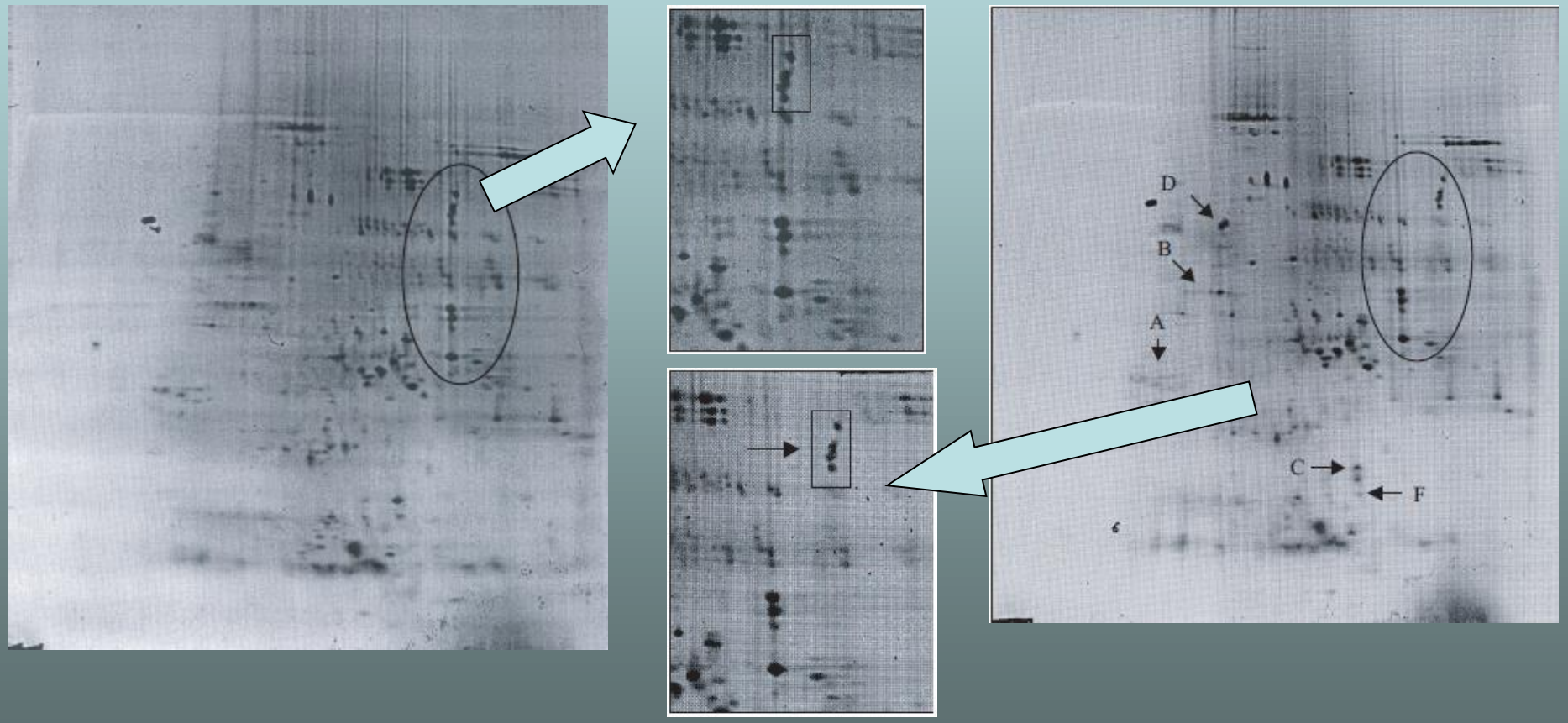
Abstract. In this study, grain that had been damaged by the bug *Eurygaster* spp. and/or *Aelia* spp., plus some undamaged grain, was selected from hard red winter (HRW) wheat. The changes in endosperm proteins were determined by 2-dimensional (2-D) electrophoresis and size-exclusion high-performance liquid chromatography (SE-HPLC). Although some new protein spots and a slight decrease in the staining intensities of some polypeptides were observed in the 2-D map of the bug-damaged sample, other parts of the gels were similar to the sound (control) sample in terms of relative mobilities and intensities of the polypeptide spots. The major difference between bug-damaged and control samples was that a group of polypeptides, presumably HMW-glutenins, shifted to a more basic region of the map. The SE-HPLC patterns of the total proteins extracted from control and bug-damaged samples in SDS-buffer showed that they differed in the size distribution of the polymeric glutenin protein and in their glutenin/gliadin ratios. The solubility of proteins in SDS buffer was greater in the bug-damaged sample. The 'unextractable' polymeric protein (only extractable in SDS-buffer after sonication) (UPP %) was significantly lower in the bug-damaged sample than in the control. The results of 2-D analysis and the decline in the quantity of

2-D MAP



Control

Bug Damaged

REMEDIES

- Modifications of milling
- Hydrothermal processing
- Radiation
- Microwave
- Transglutaminase
- Additives
- Natural inhibitors

LACC IGW

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13th International Gluten Workshop

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THANK YOU