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# Detection of damage due to bug feeding on hazelnut and wheat by biochemical techniques

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## Abstract

*Gonocerus acuteangulatus* (Goeze) (Hemiptera Coreidae) and *Eurygaster maura* (L.) (Hemiptera Scutelleridae) can cause detrimental effects on the yield quality of hazelnut and wheat, respectively, because of proteolytic enzymes injected into kernels via their saliva during the feeding activity. The research was carried out to develop an alternative rapid diagnostic method able to detect damage through the presence of bug proteinase in hazelnut and wheat kernels using biochemical markers; in particular, polyclonal antibodies against salivary glands of *Leptoglossus occidentalis* Heidemann (Hemiptera Coreidae) and of *E. maura* were assayed to detect saliva in bug-damaged nut and wheat kernels. The promising results achieved are showed and discussed.

**Key words:** *Gonocerus acuteangulatus*, *Eurygaster maura*, salivary glands, polyclonal antibody, SDS-PAGE.

## Introduction

Some bug species (Hemiptera Heteroptera) are responsible for serious damage to different crops worldwide; in particular in Italy *Gonocerus acuteangulatus* (Goeze) (Hemiptera Coreidae) on hazelnut (Tavella *et al.*, 2003) and *Eurygaster maura* (L.) (Hemiptera Scutelleridae) on wheat (Vaccino *et al.*, 2006) can cause detrimental effects on quality because of proteolytic enzymes injected into the kernels via their saliva during the feeding activity. At harvest, damage is currently detected on hazelnut by a visual inspection of the kernels, whereas on wheat the visual inspection, often not fully reliable, must be associated with technological analyses of grain and flour. Recently an electrophoretical method (SDS-PAGE) of protein extracts of nuts and grains was developed to separate healthy and bug-damaged samples; however this method is complex and not suggestable for a rapid evaluation of the yield quality at harvest since it needs well-equipped laboratories and highly-trained staff.

The present research was carried out with the aim to develop an alternative rapid diagnostic method able to detect damage through the presence of bug proteinase in hazelnut and wheat kernels using biochemical markers.

## Materials and methods

Hazelnut proteins were extracted following Gifford *et al.* (1982). Samples (1.6 g) of healthy and bug-damaged nuts were homogenized in 200 µl of solution A ($NaPO_4$ 0.05M; $NaH_2PO$ 0.05; pH 7.5) and centrifuged. Then a solution composed of Tris-HCl (62.5 mM, pH 6.8), SDS 2%$_{w/v}$, glycerol 10%$_{w/v}$ was added to pellets and centrifuged at 13000 rpm at 4 °C for 15 min. After washing twice with solution A and removal of the lipidic residual, pellets were suspended in 75 µl of solution A and 25 µl of cracking buffer (2-Mercaptoethanol 15%, glycerol 29%, SDS 6%, Tris 138 mM, pH 6.8, bromophenol blue 0.01%) and analyzed by means of 12.5% discontinuous polyacrylamide gel.

Salivary glands of *G. acuteangulatus*, *Leptoglossus occidentalis* Heidemann (Hemiptera Coreidae) and *E. maura* were dissected, while the saliva of *L. occidentalis* and *E. maura* was extracted using pilocarpine (Binnington and Schotz, 1973). The polyclonal antibodies against salivary glands of *L. occidentalis* were kindly provided by C.G. Lait (Simon Fraser University, BC Canada), whereas the polyclonal antibodies against salivary glands of *E. maura* were directly produced in rabbit.

Western analyses were performed on homogenized salivary glands (10 µl), saliva (5 µl), and protein extracts from healthy and bug-damaged hazelnuts (10 µl). The primary antibodies against *L. occidentalis* and against *E. maura* were used at a dilution of 1:2000 and 1:100, respectively. The membranes were incubated overnight with the primary antibody, then for 2 h with the secondary antibody diluted 1:5000 (conjugated with alkaline phosphatase for *L. occidentalis* and with peroxidase for *E. maura*).

## Results and discussion

The SDS-PAGE of protein extracts from samples of healthy and bug-damaged hazelnuts (figure 1) revealed an obvious reduction of some components (➢) in the damaged samples, associated with the appearance of some fragments of different molecular weight (★) that could represent the product of protease degradation as a consequence of bug feeding.

The electrophoresis of salivary gland extracts of *G. acuteangulatus* and *L. occidentalis* showed a pattern of polypeptides characterized by molecular weights varying from 7 to 100 kDa for *G. acuteangulatus* and from 7 to 160 kDa for *L. occidentalis*. The polyclonal antibody produced against salivary glands of *L. occidentalis* showed an adsorption affinity with various proteins of

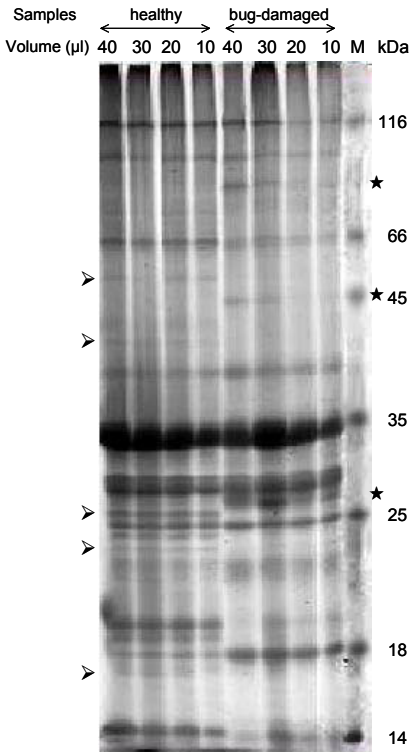


**Figure 1**. SDS-PAGE of protein extracts from healthy and bug -damaged hazelnuts.

the salivary glands of *G. acuteangulatus*, particularly with a protein band of a molecular weight of about 25 kDa, very obvious also in the protein profile of the saliva of *L. occidentalis*. In the protein extracts from hazelnut samples, the antibody showed a high affinity with some bands of about 40, 50 and, less clearly, 80 kDa present in the bug-damaged kernels but absent in the healthy kernels.

In the saliva and salivary glands of *E. maura*, the Western analysis detected three common protein components of about 8, 20 and 32 kDa. Moreover, the antibody produced against *E. maura* salivary glands revealed a band of about 35 kDa, much more obvious in the saliva together with several other minor bands. On the contrary, the patterns of *G. acuteangulatus* and *L. occidentalis* were different from each other, and from *E. maura* pattern.

On the basis of the promising results so far achieved, the research will be worth carrying on with the aim to implement a rapid and reliable method to detect damage by bugs in hazelnut and wheat.

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