Detecting bug damage on hazelnut

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Abstract

Insect species that belong to *Coreidae* and *Pentatomidae* families in the *Hemiptera* order are responsible for severe damage to hazelnut, causing alterations in qualitative traits of kernels during their feeding. Currently, the most harmful species on hazelnut in Italy are the invasive brown marmorated stink bug, *Halyomorpha halys*, which has recently colonized many hazelnut production areas, and the native box bug *Gonocerus acuteangulatus*. However, studies on the correlation between numbers of *G. acuteangulatus* sampled in the hazelnut orchards and numbers of corked kernels, as well as on characteristics of corked kernels, are still scarce. Therefore, the injury of *G. acuteangulatus*, in terms of damaged kernels per individual and alterations of kernel characteristics, was assessed by caging single *G. acuteangulatus* on hazelnut branches. The potential suitability of an electronic nose, texture analysis and sensory analysis for discerning between undamaged and damaged kernels was evaluated. The electronic nose was also tested to distinguish kernels damaged by different bug species. The damage rate caused by *G. acuteangulatus* was confirmed to be still unpredictable, due to the high variability between individuals, some of which damaged all the nuts inside the cage and others not even one. This variability may be due to behavioral or physiological factors (e.g., salivary enzyme characteristics) which deserve further investigation. Damaged and undamaged hazelnuts can be discriminated with all the techniques proposed. In particular, the roasting process was very important in the sensory analysis, and after roasting, damaged hazelnut kernels differed greatly from undamaged ones. In the texture analysis, the hardness and Joung module values were higher for undamaged hazelnuts. The electronic nose was able to discern between undamaged and damaged kernels, and between kernels damaged by different species. Therefore, after an appropriate training, it could be used both in the field to monitor the presence of bug species and in the storehouse to detect damaged hazelnuts.

Keywords: *Gonocerus acuteangulatus*, *Halyomorpha halys*, corked kernels, electronic nose, sensory analysis, texture analysis

INTRODUCTION

Insect species belonging to *Coreidae* and *Pentatomidae* families in *Hemiptera* order are responsible for severe economic losses on numerous crops worldwide, including hazelnut. By feeding on hazelnuts, such bug species can cause alterations in quality traits of the nuts (Hamidi et al., 2022; Memoli et al., 2017; Singldinger et al., 2018; Turan, 2021), due to the proteolytic enzymes injected into the kernels via their saliva (Vaccino et al., 2008). In Europe and Turkey, the box bug *Gonocerus acuteangulatus* (Goeze) (*Hemiptera: Coreidae*), the green shield bug *Palomena prasina* (L.), and the southern green stink bug *Nezara viridula* (L.) (*Hemiptera: Pentatomidae*) are the main bugs that colonize hazelnut orchards and damage kernels (Bosco et al., 2018; Hamidi et al., 2022; Moraglio et al., 2014; Tuncer et al., 2014). In Italy, *G. acuteangulatus* was the most damaging species (Tavella et al., 2001, 2003) up until the accidental introduction of the brown marmorated stink bug *Halyomorpha halys* (Stål) (*Hemiptera: Pentatomidae*). *Halyomorpha halys* has now colonized many hazelnut production areas and has become the most harmful species in hazelnut orchards (Ak et al., 2019; Bosco et al., 2018; Japoshvili et al., 2022).

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Despite the detrimental injury caused by bugs, limited reports are available on the correlation between bug population levels in the hazelnut orchards and the rate of corked kernels, as well as on characteristics of corked kernels. Therefore, the first aim of this study was to assess the actual damage level by the native species *G. acuteangulatus*, in terms of both damaged kernels per individual and alterations of kernel characteristics. The potential of texture analysis and sensory analysis to distinguish between undamaged and damaged kernels, on fresh and stored hazelnuts, as well as on raw and roasted ones, was evaluated.

The separation of healthy from damaged kernels is essential in the processing industry to increase product quality and food safety. Automatic detection of damage before kernels pass to the production line could reduce wasted resources and economic losses due to storage and transport of low-quality products. However, detecting bug damage on harvested hazelnuts is difficult, due to the need for shelling the nuts and often dissecting the kernels. Therefore, the second purpose of this study was to evaluate the potential suitability of an electronic nose (E-nose) for the automatic detection and selection of hazelnuts in the food industry by discerning between healthy and damaged kernels, as already tested for *N. viridula* and *Chinavia hilaris* (Say) (*Hemiptera: Pentatomidae*) on cotton balls (Henderson et al., 2010; Lan et al., 2008).

MATERIALS AND METHODS

Field trials

To assess the damage by *G. acuteangulatus*, a three-year trial was carried out by caging single *G. acuteangulatus* on branches of the cultivar 'Tonda Gentile delle Langhe' (TGL) in an organic hazel orchard. Annually from 2008 to 2010, in the second half of May, 250 hazelnut branches with at least four clusters of nuts were isolated with polythene net sleeve cages, as previously reported (Bosco et al., 2018), to avoid possible damage by bugs already present in the field. Then, when nuts had reached their final volume and kernels were at an advanced stage of development (Tavella et al., 2001, 2003; Hedstrom et al., 2014; Valentini et al., 2015; Hamidi et al., 2022), *G. acuteangulatus* coming from mass rearing were individually released into the sleeve cages, and kept until early September, to obtain as many damaged kernels as possible. Each year, at least 50 cages were left empty to obtain undamaged hazelnuts for comparison with damaged ones. Overall, 530 *G. acuteangulatus* (259 females, 186 males, and 258 nymphs) were placed in as many cages over the three years. At the end of the trials, all *G. acuteangulatus* individuals were checked for survival.

Damage assessment

After harvest in September each year, all cages were removed, and nuts were collected and stored in paper bags separately from each cage. In the laboratory, all nuts were shelled, and the kernels were examined, and if necessary, cut in four parts, for symptoms of bug damage. The kernels were divided into groups as follows: i) damaged ("corked"), ii) undamaged from cages with bugs ("without symptoms"), and iii) undamaged from cages without bugs ("healthy"). In 2008 and 2009, part of the kernels was stored for eight months in a refrigerated room at 4°C and 55% RH. All other kernels were vacuum packed and stored at 5°C until their use for sensory tests. In 2010, the kernels were all kept intact and roasted at 160°C for 20 min, and then only external symptoms of damage were identified. The kernels were therefore divided into "corked" (i.e., showing external symptoms) from cages with bugs, and "healthy" from cages without bugs.

Sensory analysis

Sensory analysis was performed on kernels obtained from the three-year field trial. A panel of tasters performed a Duo-Trio Test (UNI EN ISO 10399:2018) to compare the three groups of hazelnuts: "corked", "without symptoms" and "healthy". Tests were performed on hazelnuts collected in the year, namely raw kernels in 2008 and 2009, roasted kernels in 2010, and stored kernels in 2009 and 2010. In 2010, a ranking test (ISO 8587:2006) was also performed on kernels stored in the refrigerated room for eight months.

Texture analysis

Hardness measurements of the kernels were carried out using a Universal Testing Machine TAxT2i® Texture Analyzer (Stable Micro System, Godalming, Surrey, UK). The machine was equipped with a perforated HDP/90 platform, flat face probe (P/35) and load cell of 50 kg. Hardness is represented by the breaking force (N) relative to the first fracture point, as this parameter correlates most closely with the sensory properties of crispness and crunchiness. The energy required (mJ) for breaking the kernels and the Young's modulus (N mm^{-1}) were also evaluated. The kernels were subjected to compression at a constant speed (1 mm s^{-1}) along the compression axis parallel to the suture line. All acquisitions were performed at 400 Hz using the dedicated Texture Expert Exceed® (Stable Micro System, Godalming, Surrey, UK, Version 2.54) software supplied with the instrument. The measurements were performed on 30 hazelnut kernels.

Electronic nose analysis

Kernels damaged by the main detrimental hazelnut bugs, namely *G. acuteangulatus*, *H. halys*, *N. viridula* and *P. prasina*, and undamaged kernels, both obtained from branches caged with or without the bugs in a field trial carried out in 2015 (Bosco et al., 2018), were stored at 4°C until analysis. A commercial portable E-nose (PEN 3 model, WMA Airsense Analytics, Inc., Germany), equipped with 10 different metal oxide semiconductors, was used to quantify the sensor responses for the volatiles of kernels undamaged or damaged by different bug species. The response data collected by the E-nose is defined as the conductance or ratio G/GO where G represents the resistance of each sensor after the exposition of headspace volatiles and GO represents the resistance of the sensor exposed to reference air. The E-nose analysis was performed according to Pastorelli et al. (2007) with slight modifications. Two g of nut powder was placed in a 20-mL vial, sealed with a PTFE/silicone septum and a cap with a hole. The vial was equilibrated at 30°C for 30 min to homogenize the headspace phase and analyzed at the same temperature. Nine replicate vials for each sample were analyzed. The sampling time for each sample was 60 s which was enough for each sensor to reach a stable value. The rinsing time between two following samples was 90 s. The conductance of each sensor between the 40^{th} and 50^{th} seconds of the sampling time was extracted as the static feature for further analysis.

Statistical analysis

For the statistical analysis, only cages in which *G. acuteangulatus* individuals were alive at the end of each trial were considered in order to compare damage caused during an equal period of exposure of the nuts to bug feeding. Pearson product-moment correlation coefficients were calculated between numbers of available and damaged nuts for each *G. acuteangulatus*. The mean percentages of nuts damaged by females, males and nymphs were compared via GLM with a binomial distribution and a logit link function. The data obtained by texture analysis were subjected to one-way ANOVA, and in the case of significant differences, the means comparisons were calculated with the Tukey posthoc test. A principal component analysis was performed on data obtained by the E-nose. All analyses were performed with the software IBM SPSS® Statistics (IBM Corp., NY, USA, Version 13).

RESULTS AND DISCUSSION

Damage by *Gonocerus acuteangulatus*

In the three-year field trial, an average of 13 kernels (range 1 to 56), were harvested per cage. An average of 3.5 kernels (range 1 to 26), were damaged in each cage, and only 12 individuals managed to damage all available kernels in the cage. Furthermore, some *G. acuteangulatus* did not cause any symptoms on the kernels. No significant correlation was found between numbers of kernels available within each cage and those damaged by *G. acuteangulatus* ($r=0.116$, $P=0.112$), nor were there any significant differences between mean percentages of nuts damaged by females (33.96±2.54%), males (31.94±2.01%) or nymphs (36.05±4.44%) ($\chi^2=484.942$; df=1; p=0.776).

Sensory analysis

In duo-trio tests on fresh and stored raw kernels, samples from the three groups, i.e., “corked”, “without symptoms” and “healthy”, were not always correctly matched. On the contrary, on roasted kernels both “corked” and “healthy” samples were significantly correctly paired (corked: $\chi^2=6.250$; dl=1; p=0.012; healthy: $\chi^2=4.000$; dl=1; p=0.046). Moreover, in the ranking test on stored kernels, the tasters showed a preference for the “healthy” sample over the “corked” one.

Texture analysis

Breaking force (N) and breaking work (mJ) were not significantly different for corked and healthy fresh kernels (corked: N=95.6; mJ=160.5; healthy: N=92.2; mJ=138.9), while the Young’s modulus (N mm^{-1}) was significantly higher for healthy kernels (corked: 31.8 N mm^{-1}; healthy: 35.5 N mm^{-1}). Healthy kernels were therefore more resistant and crunchier, while corked ones were more elastic and deformable. After eight months of storage, no differences were found in breaking force, breaking work, or Young’s modulus, between corked or healthy kernels.

Electronic nose

The principal component analysis (PCA) showed a discrimination between kernels damaged by different bug species, and in particular between the profiles of *H. halys* and *N. viridula*, placed at opposite extremities (Figure 1). On the contrary, the differences between the other species and the control were less evident in the PCA (Figure 1). The discrimination between kernels damaged by *H. halys* and by *N. viridula* was evident on the response curves of almost all of the 10 employed sensors against sampling time. In particular, the volatile profiles of sensor number 2 (broad range), 7 (inorganic sulfur compounds) and 9 (aromatic compounds, inorganic sulfur and organic compounds) showed a different response for the two species, *H. halys* and *N. viridula* (Figure 2).

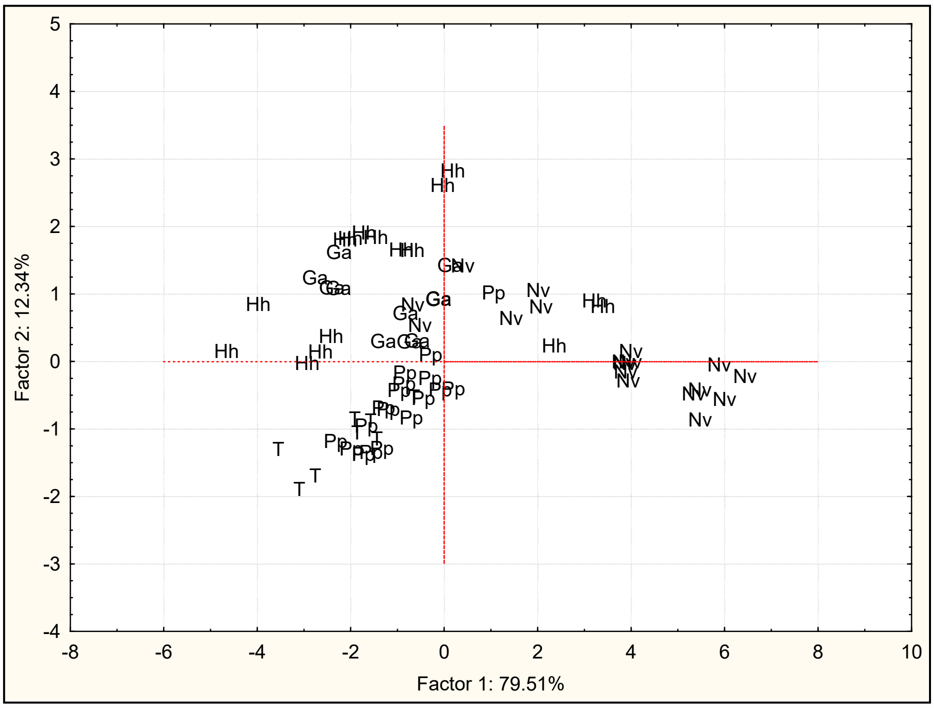

Figure 1. Results of principal component analysis (PCA) based on data obtained with a PEN3 E-nose (Ga: *Gonocerus acuteangulatus*; Hh: *Halyomorpha halys*; Nv: *Nezara viridula*; Pp: *Palomena prasina*; T: control).

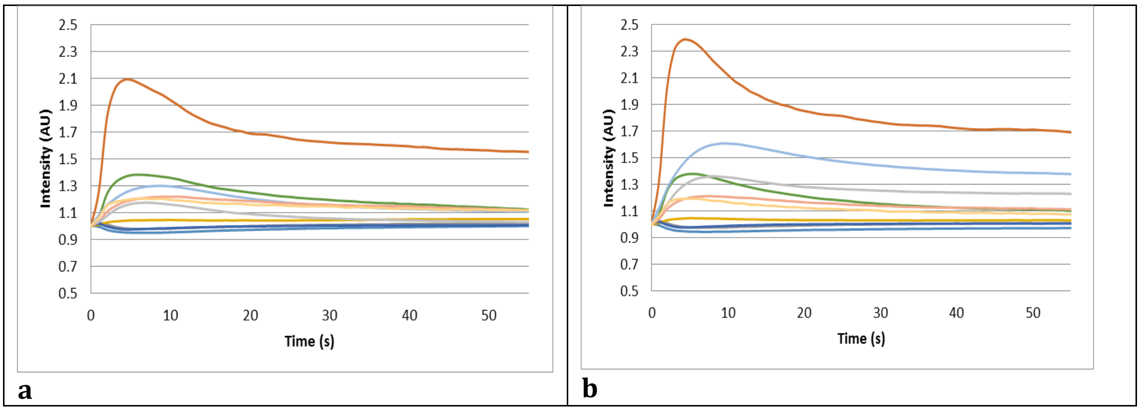

Figure 2. Comparison of the response curves obtained by the 10 sensors of a PEN3 electronic nose to (a) *Nezara viridula* and (b) *Halyomorpha halys.*

CONCLUSIONS

The damage rate caused by *G. acuteangulatus* was confirmed to still be unpredictable, due to the high individual variability. This variability may be due to behavioral or physiological factors (e.g., feeding on other plant parts, characteristics of salivary enzymes), which can also explain why some *G. acuteangulatus* did not damage any kernel throughout the trial. Therefore, it will be worth investigating further to define how and on which components the saliva injected by bugs interacts, similar to studies conducted to assess the effect of bug nutrition on gluten proteins (Vaccino et al., 2017). Damaged and undamaged hazelnuts can be discriminated by all proposed techniques: texture analysis, sensory analysis, and E-nose. In particular, the roasting process was very important in the sensory analysis, because the effects of bugs appeared amplified in the roasted product. Therefore, the implementation of roasting methods, in terms of times and temperatures, capable of counteracting the quality decay of hazelnuts caused by the trophic activity of bugs, could be of great interest. The E-nose was able to discern between damaged and undamaged kernels, as assessed for *N. viridula* and *C. hilaris* on cotton balls (Henderson et al., 2010; Lan et al., 2008), consequently showing its potential suitability for automatic detection and selection of hazelnuts in food industry. Furthermore, the E-nose even showed a higher potential since it was able to recognize kernels damaged by different bug species. Therefore, after an appropriate training, the E-nose could be a useful tool in the processing industry, for example to estimate the percentage of damaged hazelnuts within a batch. Moreover, the E-nose could allow a better approach to pest management in hazelnut orchards, by easily revealing the main damaging pests in the field, especially considering that bug monitoring is costly in terms of time and energy, and not always reliable, as damage often occurs without the bugs being effectively detected.

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