Research Article

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Assessment of the Damages on Maize Crop by the Invasive Stink Bugs *Halyomorpha halys* (Stål, 1855) and *Nezara viridula* (Linnaeus, 1758) (Hemiptera: Pentatomidae)

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Abstract: Recently, the brown marmorated stink bug, *Halyomorpha halys,* and the southern green stink bug, *Nezara viridula*, two invasive, highly polyphagous species, have caused huge damages on many crops, including fruits, vegetables, soybean, wheat and field maize, both in the USA and Europe. The two stink bugs rest on the maize ears and feed through the husk, by penetrating with their rostrum each kernel and sucking its content, leaving the maize kernels discoloured and shrunken, with mottled appearance. Early attacks lead to deformations of the maize cobs. Observations made on six maize hybrids in maize fields in southern Romania, in 2016, revealed 23-29% attacked kernels in non-irrigated fields and almost 37% attacked kernels in irrigated fields, under the pressure of maximum 16 adults/plant. The starch content decreased by 20-22%, with no significant differences between hybrids. The statistical analysis of protein content reduction indicated that the hybrids may have a different reaction to stink bugs feeding.

Key words: Invasive pests, *Halyomorpha halys*, *Nezara viridula*, maize, morpho-anatomic changes, population structure

Introduction

In the last decade, the brown marmorated stink bug, *Halyomorpha halys* (Stål, 1855) and the southern green stink bug *Nezara viridula* (Linnaeus, 1758) (Hemiptera: Pentatomidae) have caused damages of billion Euros to agricultural crops all around the world (Rice et al. 2014). Both species are highly polyphagous, with more than 300 host species reported until now for *H. halys* (Nielsen et al. 2008, Kriticos et al. 2017) and more than 150 host species for *N. viridula* (Panizzi 2008). They attack mainly fruit trees and vegetables, but also field crops as soybean and maize or some ornamentals and weeds. Paula et al. (2016) considered *H. halys* a devastating invasive species in the USA, as the direct losses produced in 2010, in the mid-Atlantic apple orchards alone, were about $37 millions. In Europe, the only loss estimation was done in Italy, and the amount for 2016 was 1 billion Euro (Fontana 2016). *Halyomorpha halys* has been present in Europe since 2004 and reported from 15 countries (Wermelinger et al. 2008, Heckmann 2012, EPPO 2013a, b, Milonas & Partsinevelos 2014, Vétek

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et al. 2014, EPPO 2016a, b, c), while *N. viridula* has been present in Europe for almost 40 years (Redei & Torma 2003). *Halyomorpha halys* is also a major nuisance pest due to its overwintering aggregation behaviour, which leads to home invasions (Inkley 2012). Human contact dermatitis has also been documented (Anderson et al. 2012, Uhara et al. 2016).

The symptoms of the stink bugs attack on maize were described by many researchers, especially from the USA extension services (Pinero & Miller 2017), and also by the main maize seed producers. During the early stages, before the grain fill, severe infestations and feeding may deform the maize cob, making it look like a cow horn or boomerang, and expose the kernels to bird and insect damages because the cob shuck also stops developing. At later stages, feeding is associated with a 'mottled' appearance, due to kernels scarring or bruising, especially near the tip of the ear, and shrunken and missing kernels (Leskey et al. 2012). The shrivelled kernels are more susceptible to fungal diseases (Tooker 2012). Some descriptions of damages on maize were published by Opoku et al. (2016), who stated that the percentage of injured maize kernels is around 23% on the edge plants and only 3% in the middle plants of a sweet maize field. Leskey et al. (2012) mentioned that sweet maize is a highly preferred host, with reported losses of 100%. In maize as in soybeans, stink bugs tend to be 'edge' species, meaning the highest density of individuals is found on the edge of fields, without spreading toward the interior (Tooker 2012). Based on qPCR analysis, Opoku et al. (2016) demonstrated the ability of *H. halys* to increase the *Fusarium* spp. infections and the fumonisin contaminations in maize. Regarding the economic threshold, Cissel et al. (2015) considered that a density of one *H. halys* per ear is enough to cause significant quality reductions in maize. Hunt et al. (2017) published the first indications for maize growers, recommending one stink bug on four or two plants as the economic threshold, regardless of the species.

In Romania, Macavei et al. (2015) described for the first time *H. halys*, found in Bucharest in September 2014. However, the authors stated that its presence could date back to at least 1-2 years, due to the fact that individuals of different stages were found on a range of few kilometres. *Nezara viridula* was first mentioned as a crop damaging insect in Romania by Grozea et al. (2012). It was reported that the species produced qualitative and quantitative depreciations on tomatoes in Timisoara area (western part of Romania) and its damaging behaviour was further confirmed by the same authors, in 2015 (Grozea et al. 2015). Kurzeluk et al. (2015) reported on the presence of the pest in Muntenia area (southern part of the country) and also stated that in Bucharest area *N. viridula* has been identified on a goji experimental field since 2011. The two stink bugs damaged maize and goji crops in 2016, in the testing fields of the University of Agronomic Sciences and Veterinary Medicine of Bucharest (USAMV Bucharest). The estimated population density of *H. halys* was 3.4 insects/plant, or 25.5 insects/m^2, in the testing field border area, and 1.3 insects/plant, or 9.75 insects/m^2, in the testing field centre (Ciceoi et al. 2016).

The aim of our study was to assess the damages of the invasive stink bugs *H. halys* and *N. viridula* on maize crops in southern Romania by studying the feeding preferences of stink bugs on six maize hybrids and by estimating the qualitative and quantitative variations in the dried maize kernels under the attacks of the stink bugs.

Materials and Methods

The experimental field, with six different maize hybrids was set up in the testing fields of the USAMV Bucharest location, in the Faculty of Agriculture testing fields (N 44°28'13.9"; E 26°03'48.7"). Five maize hybrids: Bonito (FAO 300), ES FERIA (FAO500), PR 35 T06 (FAO 400), Mikado (FAO400), and LG3409 (FAO400), were placed in the non-irrigated plots, while one hybrid: P9911 (FAO410 AQ) was used for the irrigated trial.

The crop technology applied to maize in the non-irrigated experimental field was as follows: the previous crop was maize, ploughing at depth of 30 cm, disking at depth of 8 cm, seedbed depth of 5 cm, sowing density of 60,000 plants/ha, NKP fertilisation with 100 kg active substance 15-15-15, weeding with postemergence products Universal Buctril 0.8L/ha and Equip 2L/ha. The irrigated plot crop technology was as follows: no previous crop, ploughing at depth of 30 cm, disking at depth of 8 cm, seedbed depth of 5 cm, and sowing density of 75,000 plants/ha, irrigated by drip irrigation system.

The weekly field observations started at the beginning of July 2016, in the period of kernel development and continued until the end of September. During each visit, around 50-70 plants were visually analysed. The population density of *H. halys* was assessed on 29 July 2016, in the irrigated field, by visual population evaluation (naked-eye counting). We counted both adults and larvae of *H. halys*, on 100 maize plants, randomly chosen in the border area (4 m wide, all around the maize field)

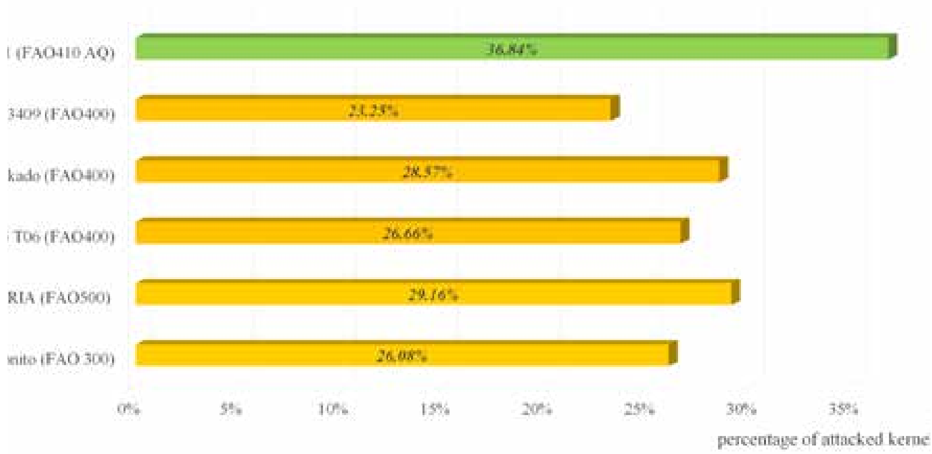

Fig. 1. The percentage of maize kernels attacked by the stink bugs *Halyomorpha halys* and *Nezara viridula* in six maize hybrids: P9911 – in an irrigated plot; LG3409, Mikado, PR 35 T06, ES FERIA, and Bonito – in non-irrigated plots

Fig. 2. The decrease (%) in starch content of the six maize hybrids under the feeding of the stink bugs *Halyomorpha halys* and *Nezara viridula*. P9911 – in an irrigated plot; LG3409, Mikado, PR 35 T06, ES FERIA, and Bonito – in non-irrigated plots

and in the central zone of the field. Only occasional counts were performed for *N. viridula.*

The laboratory observations were done in February 2017, four months after the maize harvest, on dried maize cobs, 6% kernels moisture for all hybrids, in 10 replications. We analysed the variation in the maize kernel size, the percentage of affected kernels, and the weight variation with a Partner WPS 1100/C/10 precision balance. The visual changes occurring on the kernel pericarp were analysed with a Leika stereomicroscope, while the starch and protein content variation in the attacked kernels were studied using the Perten Inframatic NIR Grain Analyzer. To determine the percentage of attacked kernels, all grains with at least one feeding mark were counted as altered.

In order to test the influence of the maize hybrids on the stink bug feeding activity, we used chi-square test to compare the binomial distribution (attacked kernel – non-attacked kernel) in each hybrid (the differences in distribution were significant at p-values <0.05). The Spearman rank correlation coefficient was used to determine the differences in the influence of the stink bug attacks on starch, proteins and thousand kernel weight (TKW) between different hybrids.

Results and Discussion

The feeding marks produced by both stink bug species were still visible on the dried maize kernels at the fourth month after harvest, a high percentage of kernels having the pericarp seriously altered (Fig. 1). For non-irrigated variants, LG3409 had the lowest percentage of attacked kernels (23.25%), while ES Feria had the highest percentage of damaged kernels (29.16%), which may suggest that *'dentiformis'* maize hybrids are more susceptible than *'indurate'* hybrids.

The differences between the five non-irrigated hybrids showed that the hybrid may influence the feeding behaviour of the stink bugs on maize, and at the same time, indicated that different hybrids had a different reaction to the stink bug feeding (Table 1).

Table 1. The significance of influence of the maize hybrids on feeding behaviour of the stink bugs *Halyomorpha halys* and *Nezara viridula*; * p – 0.01-0.05; ** p – 0.001-0.01; *** p – lower than 0.001

	Bonito (FAO 300)	PR 35 T06 (FAO400)	Mikado (FAO400)	ES FERIA (FAO500)
LG3409 (FAO400)	0.07	0.03*	0.00 ***	0.00 ***
Bonito (FAO 300)		0.74	0.15	0.04*
PR 35 T06 (FAO400)			0.26	0.09
Mikado (FAO400)				0.69

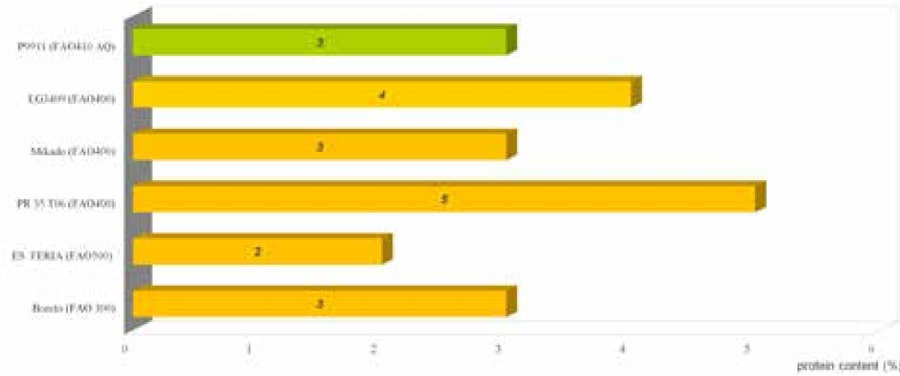

Fig. 3. The decrease (%) in protein content of the six maize hybrids under the feeding of the stink bugs *Halyomorpha halys* and *Nezara viridula*. P9911 – in an irrigated plot; LG3409, Mikado, PR 35 T06, ES FERIA, and Bonito – in non-irrigated plots

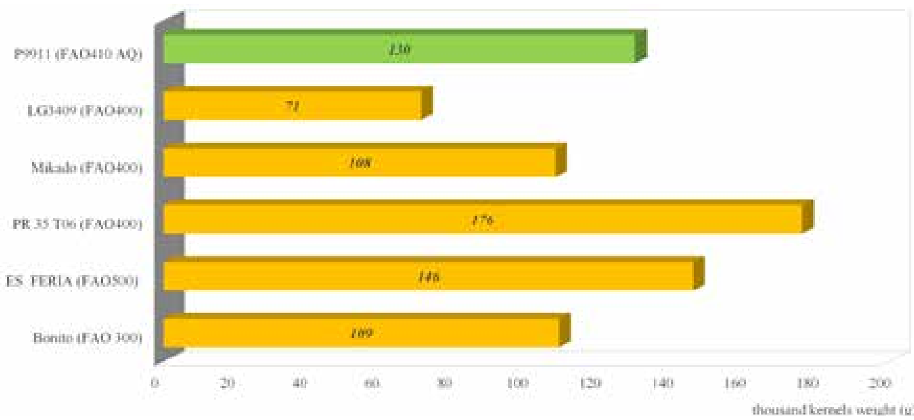

Fig. 4. The decrease (g) in thousand kernel weight (TKW) of the six maize hybrids under the attack of the stink bugs *Halyomorpha halys* and *Nezara viridula*. P9911 – in an irrigated plot; LG3409, Mikado, PR 35 T06, ES FERIA, and Bonito – in non-irrigated plots

In respect to starch content, there was no significant variation between the rank of hybrids in the two groups (Fig. 2), as confirmed by the Spearman rank correlation coefficient (ρ=0.92; p=0.03; <0.05). The starch content had a decrease of 20-22% in all variants. The hybrids had no different reaction under the stink bug feeding process.

The protein content was the lowest in PR 35 T06, with a decrease of 41.67%. At the same time, ES FERIA had a decrease in protein of only 20%. The protein content of unaffected kernels was between 10% and 12%. As the Spearman rank correlation coefficient was 0.15 (p=0.81; >0.05), we can conclude that the hybrids had a different reaction to the stink bug feeding, as illustrated in Fig. 3.

The thousand kernel weight (TKW) was seriously affected in all six maize hybrids. In PR 35T06 hybrid, the TKW of affected kernels was with 53.4% lower than the TKW of unaffected kernels. The lowest TKW difference was registered in LG3409, 71 g, which represented a reduction with 33.02% when compared with unaffected kernels.

Fig. 5. The presence of the stink bugs *Halyomorpha halys* and *Nezara viridula* on the upper third of maize cobs in 2016

The weakness of the Spearman rank correlation coefficient (ρ=0.70; p=0.19; >0.05) showed no rank correlation between data of the two groups, which means that the hybrids reacted differently to the stink bug attacks, as illustrated in Fig. 4.

Qualitative losses

The weekly observations revealed the presence of a maximum of seven stink bugs adults/cob and 16 adults/plant (12 *H. halys* and 4 *N. viridula*). Both adults and larvae of *H. halys* and *N. viridula* were located mostly on the upper third of the ear, starting from the silk emergence phases, during all cob formation and grain filling phases until the wax maturity (Fig. 5).

In all hybrids, the kernels were smaller, shrivelled, and cracked. These could ease the development of fungal agents. The irrigated P9911 hybrid had a significantly higher percentage of attacks (36.84%). This fact may be justified by the following: the higher kernel attractiveness for a longer period of time, as the kernels were juicier due to water availability; by the preference of *H. halys* for places with a higher relative humidity or lower light intensity, as the plant density was higher in the irrigated plot; or by other elements of the crop technology. Our results also showed that *N. viridula* dominated in the non-irrigated maize plots, approximately 90% of the total number of stink bugs, while *H. halys* preferred the irrigated maize, with 75% of the total number of stink bugs. However, because there was no non-irrigated trial with P9911, we could not determine if there is any influence of the humidity on feeding behaviour of the stink bugs. It might be also possible that *H. halys* just preferred the P9911 (FAO410 AQ) hybrid, which is missing in non-irrigated trials. Regarding diseases, the climatic conditions of 2016, along with the cracked kernels, favoured the development of *Fusarium* spp., *Cephalosporium* spp., and *Cladosporium* spp.

Quantitative losses

The high weight reductions, more than a half for PR 35T06, followed a very high infestation of maize cobs with stink bugs (16 adults/plant). This result should be considered in the context of the specific conditions of 2016 and needs to be confirmed in the following years. Cissel et al. (2015) suggested that one stink bug per plant is enough to cause maize damages, while Koch et al. (2017) indicated 1 stink bug/2 plants as economic threshold.

Biological observations

Although the literature states that the overwintering adults become active in April, (Haye et al. 2014), in 2017, the first flying *H. halys* adult was spotted on 8 March near a building in the university campus, followed by other interceptions in the days afterwards, in other areas of Bucharest. This shows the high adaptability of this species. Despite the fact that no population density was estimated for *N. viridula* in 2016, our visual observations suggest that *H. halys* preferred the irrigated plots. The distance between the irrigated and non-irrigated fields was 300 m, a distance that can be easily flown by the adults of both stink bugs.

Regarding the edge phenomena, we compared the mean number of stink bugs inside the field maize (m=1.3 stink bugs/plant) with the mean of insects on the field edge (m=3.4 stink bugs/plant), and the results indicated a significant differences (t=4.34; df=67.65; p=0.00).

Conclusions

The invasive stink bugs proved to be a serious threat to the maize crop in the specific climatic conditions of 2016 and should be carefully studied in the next years. The percentage of affected kernels, below 30% in the non-irrigated fields and almost 37% in the irrigated fields, may indicate a serious yield reduction. The weight of a thousand kernels was decreased by 66.98% for LG3409 and only by 47.61% for PR 35T06, under the overwhelming infestations of a maximum of 16 adults/plant.

The large stink bug populations reduced the maize yields and affected the quality of the crop. The starch content of the affected kernels decreased by 26-28% while the protein content was reduced by 20-42%. The presence of fungal agents and the contamination they produce, as well as the quantity of mycotoxins present in the dried maize used as animal feed, needs to be further investigated. Further studies are required in order to establish the most appropriate sampling method and the economic threshold for both stink bug species, for the main maize growth stages.

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