

First Record of a Microsporidium in the Population of Brown Marmorated Stink Bug *Halyomorpha halys* (Stål, 1855) (Heteroptera: Pentatomidae) in the Republic of Georgia

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Abstract: The first record of a microsporidium from the population of the brown marmorated stink bug *Halyomorpha halys* (Stål, 1855) (Heteroptera: Pentatomidae) in West Georgia is reported and information on its occurrence and distribution is given. This entomopathogen was found in bugs from the Guria Region. Different stages of *H. halys* were inspected during the summer and autumn of 2018. Infection caused by the microsporidium was observed in the fat body of the first generation adults and nymph instar, as well as in the second generation of overwintering adults. Data on the occurrence and pathogen prevalence in bugs of different generations, collected at the same sample sites in different seasons of the year are also presented. The highest prevalence (62.9%) was documented in October.

Key words: *Halyomorpha halys*, hazelnut orchard, pathogens, microsporidium, Guria Region, Georgia

Introduction

The brown marmorated stink bug (BMSB) *Halyomorpha halys* (Stål, 1855) (Heteroptera: Pentatomidae) (Fig. 1) is native to China, Japan, Korea and Taiwan (HOEBEKE & CARTER 2003, LEE et al. 2013). It is one of the most economically important, polyphagous insect species in agriculture and forest and has more than 100 host plant species of fruits, vegetables, flowering and forest plants (LEE et al. 2013). In addition, *H. halys* has become a serious nuisance due to its behaviour of overwintering in homes and other human-made structures (HAMILTON 2009, INKLEY 2012).

Halyomorpha halys was also introduced into North America and first recorded in Allentown,

Pennsylvania in 1996 (HOEBEKE & CARTER 2003) and has spread to at least 43 states in the USA. Since the mid 2000s, pest populations have become established in Europe (WERMELINGER et al. 2008, LESKEY et al. 2012, CALLOT & BRUA 2013, HAYE et al. 2015). Few individuals of BMSB were first observed in the Republic of Georgia in 2015 in the Samegrelo Region, West Georgia. Later, the pest spread to the Guria and Imereti Regions. BMSB established itself in 2016 with the first significant damages to hazelnuts being reported during 2016-2017 in Western Georgia, resulting in losses for individual farmers as well as to the country's overall economy (<http://www.moa.gov.ge/En/>).

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Since 2017, high infestation levels of the pest were observed on many agricultural crops, such as stone fruit, vegetables (tomato and pepper), ornamentals and field crops (mainly on maize). BMSB can potentially cause losses in crop value of fruit and vegetables of up to 70% (LESKEY et al. 2012). In Georgia, hazelnut production and export has sharply decreased in the last two years. Ninety percent of hazelnut-growing areas are located within the household plots of the local population, where the use of chemical insecticides by mechanical equipment is limited. Furthermore, it is very important to restrict the use of chemicals because of their detrimental effect on the environment and negative impacts on the production of honey in Samegrelo, Guria and Imereti Regions. On the other hand, natural enemies of BMSB were found in hazelnut orchards in the Samegrelo Region (KERESOLIDZE et al. 2018), which may also suffer population reductions caused by the use of chemical insecticides. As the use of chemical pesticides is also a social issue, the objectives of nutrition, health and environmental problems must be solved by the implementation of integrated pest management techniques (IPM, see NORSGARD 1976). Biological control of pests is an alternative control method to chemical pesticides and has the potential to minimise pest damage to agricultural and forest plant species by reducing the BMSB population to an acceptable level.

Several studies have sought a safe and effective natural control agents for the biological control of hazelnut and other plant pests (YAMAN et al. 1999, YAMAN & DEMIRBAG 2000). Entomopathogenic organisms are the most promising biocontrol agents. Therefore, efforts have been made to study the potential of different entomopathogens against insect pests. It is believed that entomopathogenic microorganisms can decrease insect population densities

and reduce the duration of outbreaks (MYERS 1988). Among entomopathogens, the microsporidia are common pathogens of insects and are considered important regulators of the population dynamics (LINDE et al. 2000, SOLTER et al. 2012). Most microsporidian species are very host-specific, avoiding non-target effects on other insect species (YAMAN et al. 2016, HOCH & SOLTER 2017). Although *H. halys* is one of the most important pest species, there are no records of naturally-occurring pathogens in BMSB from Georgia. There is, however, one report of a microsporidium, *Nosema maddoxi*, detected in this pest in the USA (HAJEK et al. 2018).

We present the first report of a microsporidium detected in the population of *H. halys* in the Guria Region, West Georgia. Infection rates of different generations of this insect pest in different seasons and spore measurements are also presented.

Materials and Methods

A total of 975 individuals of *H. halys* were collected in different seasons from four different sampling plots in the Guria Region, in the villages: Naruja – plot 1, Anaseuli – plot 2 and plot 3, Makvaneti – plot 4, where an outbreak of BMSB was observed and serious damage to agricultural crops occurred. In June, July and August of 2018, adults and nymphs of BMSB were collected by hand from foliage of hazelnut trees and in household orchards, which were located adjacent to farmers' houses. In October, only overwintering adults were collected inside of buildings and in closed human-made structures (Table 1).

The collected material was brought to the laboratory and stored at 3°C in a refrigerator. Individuals of *H. halys* were dissected on microscopical slides. Samples of the fat body and midgut were removed and wet smears of tissues were prepared and ex-



Fig. 1. Adults and newly-hatched nymphs of *Halyomorpha halys* on hazelnut leaves.

Table 1. Sampling plots of *Halyomorpha halys* collection in Guria Region, Georgia, in 2018.

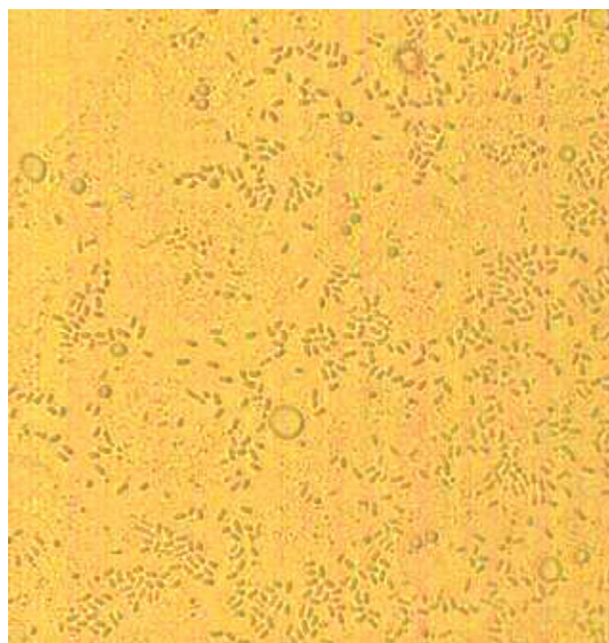
Locality	Naruja (Plot 1)			Anaseuli (Plot 2)		Anaseuli (Plot 3)	Makvaneti (Plot 4)	
	Month of collection	June	July	October	August	October	October	August
Place of collection	Hazelnut orchard	Hazelnut orchard	Inside building	Hazelnut orchard	Inside building	Inside building	Hazelnut orchard	Inside building
GPS coordinates	41.972988E 41.908831N			41.984622E 41.901865N		41.97939E 41.908070N	41.972334E 41.911640N	

amined under a phase contrast microscopy at a magnification of 200-400× for the detection of entomopathogens. When infection caused by a microsporidium was observed, a fragment of infected host tissue was lacerated and spread on a slide. The smear was air-dried and then fixed with methanol for 5 minutes. Afterwards, the slides were stained in a freshly prepared 5% solution of Giemsa stain for approximately 40 min (BECNEL, 2012). Slides were then washed, air dried and once more examined under a phase contrast microscope. Detected microsporidian spores were measured and photographed using a digital camera and an image analysis system (NIKON ECLIPSE Ci-L).

Results

Our microscopic study revealed the presence of a naturally occurring microsporidium. The spores were ovoid in shape and the size of fresh spores was $3.76 \pm 0.56 \mu\text{m}$ (mean \pm SD) \times $1.72 \pm 0.28 \mu\text{m}$ (mean \pm SD; $n=50$). The size of methanol fixed and Giemsa stained spores was $3.46 \pm 0.57 \mu\text{m}$ \times $1.70 \pm 0.40 \mu\text{m}$ ($n=50$). Infection was observed in adults, as well as in nymphal stages of *H. halys*. High spore loads were found in the fat body tissue (Figs 2, 3).

Pathogen prevalence in insects collected during the seasons was highly variable. Spores of the pathogen were observed in the fat body of the first generation adults and nymph instar and in the second generation of overwintering adults (Table 2). It must be mentioned that from the first sampling plot (Naruja), neither adults nor nymphs collected in June were infected, while in July at the same plot one nymph was infected from a total of 90 individuals (72 nymphs, 4 males and 14 females). In October, however, as *H. halys* started overwintering, only adult specimens were collected and inspected, showing high infection rates of 62.9% (39 infected from total 62 examined insects). On the second sampling plot (Anaseuli), *H. halys* was collected in August and October. The total number of examined insects from this site in August is 572, of which 51 individuals (8.91%) were infected. As in Naruja, infection was higher in October and reached 50% in the adults. Collections from the

**Fig. 2.** Fresh spores of the microsporidium found in *Halyomorpha halys* wet tissue smear from fat body, 900x

third plot (Anaseuli) were conducted only in October and exclusively from inside buildings. The infection rate was 36% (in total 9 of 25 adults). From plot 4 in Makvaneti, 93 bugs were collected in August, with 9.6% prevalence. In October, the infection rate in adults was also higher and reached 25.5%. In total, 146 of 975 dissected specimens were infected (15%; Table 2).

Our dissections of *H. halys* from different sites and seasons in the Guria Region showed variability in the prevalence of infection with the microsporidium. The pathogen was recorded from all sampling plots, except in individuals collected in June in Naruja. The prevalence of infection was relatively low in adults and nymphs collected in June, July and August but always higher in individuals collected in October at every sampling site.

Discussion

The pathogen found in the Georgian population of BMSB is without any doubt a microsporidium. So far, only one microsporidium, *Nosema maddoxi*, was

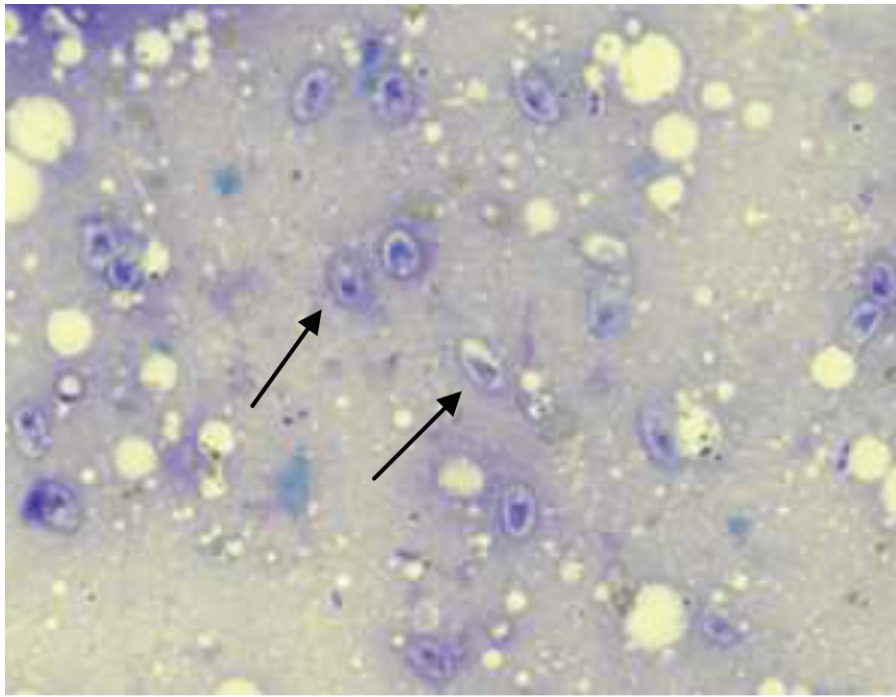


Fig. 3. Giemsa stained spores of the microsporidium from *Halyomorpha halys*, 2700x

Table 2. Occurrence and distribution of the microsporidium from *Halyomorpha halys* from various sampling plots in Guria Region, Georgia in 2018. No. = number.

Sampling plot	Month of collection	No. of adults (males)		No. of adults (females)		No. of nymphs		No. of examined insects	No. of infected/%
		Dissected	Infected/%	Dissected	Infected/%	Dissected	Infected/%		
Naruja (Plot 1)	June	2	0	1	0	12	0	15	0
	July	4	0	14	0	72	1/1.3	90	1/1.1
	October	40	25/62.5	22	14/63.6	0	0	62	39/62.9
Anaseuli (Plot 2)	August	68	16/23.5	58	8/13.8	446	27/6	572	51/8.91
	October	25	13/52.0	3	1/33.3	0	0	28	14/50
Anaseuli (Plot 3)	October	12	2/16.7	13	7/53.8	0	0	25	9/36
Makvaneti (Plot 4)	August	9	1/11.1	10	2/20.0	74	6/8	93	9/9.6
	October	54	17/31.5	36	6/16.6	0	0	90	23/25.5
Total								975	146/15

described in *H. halys* (HAJEK et al. 2018) in populations in the USA. The spore size described by Hajek et al. (2018) differs from our findings, as spores of *Nosema maddoxi* are larger compared to spores of the Georgian microsporidium: $4.72 \pm 0.05 \times 2.19 \pm 0.03 \mu\text{m}$ (mean \pm SE, fresh, $n = 30$) and $4.06 \pm 0.05 \times 2.07 \pm 2.07 \mu\text{m}$ (mean \pm SE, fixed, $n = 30$). It is well known, however, that spore size and even the nuclear arrangement (diplocaryotic/uninuclear) in microsporidia can vary greatly, in particular in non-lepidopteran hosts (HAJEK et al. 2018). In their first description of the microsporidium found in BMSB, HAJEK et al. (2018) justify the classifica-

tion as *Nosema maddoxi* with the results of molecular studies and suppose that morphological characters like spore size and shape and number of nuclei are of lesser importance. This is in accordance with FRANZEN (2008), who states that “microsporidian taxonomy has obviously reached a breaking point where classical morphological-based methods are losing importance and will be supplemented or even replaced by molecular-based methods”.

As we do not have ultrastructural nor molecular data at this time, we cannot yet determine the identity of the microsporidium found in the Georgian population of BMSB. To confirm whether it is iden-

tical with *Nosema maddoxi*, we plan further research including ultrastructural and molecular studies of this pathogen.

It must be mentioned that *N. maddoxi* was detected throughout the eastern Mid-Atlantic States in the U.S. (HAJEK et al. 2018). The authors hypothesise that *N. maddoxi* may be native to other stink bug species in the U.S. and, therefore, may be found throughout the distribution of established *H. halys* populations but could also be introduced or re-introduced along with its host, *H. halys*. *Nosema maddoxi* was also recovered from *H. halys* collected in Asia, confirming that it had a Holarctic distribution before the introduction of *H. halys* to North America (HAJEK et al. 2018).

It is therefore possible that *N. maddoxi* is also present in native stink bug populations in Georgia and uses the newly introduced BMSB as host or that the invading stink bugs were already carrying the microsporidium. When overwintering, the bugs gather in high numbers and high densities in small places inside the houses. Thus, the probability of pathogen transmission is higher. BMSB is also known to cannibalise on other stink bugs: this is considered the main route for horizontal transmission of the pathogen (HAJEK et al., 2018). This behaviour resulted in higher infection levels in October.

Invasive species may be susceptible to pathogens that are endemic in native host species. However, it is not always a simple matter to determine whether a pathogen attacking a newly invasive species was introduced with the host or is endemic to the invaded area. In particular, when invasive species are not major pests in their areas of origin, associated pathogens in the area of endemism might not be known (HAJEK et al., 2018). At this stage, we do not know whether the microsporidium was introduced with BMSB or already present in the Republic of Georgia.

Conclusion

The brown marmorated stink bug *H. halys* is an invasive insect pest in Georgia causing significant economic damage to agricultural crops since 2017. With the relatively recent invasion of this pest into Georgia, there has been interest in identifying and testing natural control agents for this bug. As a result of our study, a microsporidium was recorded for the first time in *H. halys* in Georgia in the Guria Region. Data about the prevalence of the pathogen in various locations and seasons are reported. For the exact determination of the microsporidian species and its potential in the biological control of BMSB, further studies are needed.

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