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ORIGINAL ARTICLE

Resistance of Georgian Hazelnut (*Corylus* L.) to Brown Marmorated Stink Bug – *Halyomorpha halys* (Stål)

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A B S T R A C T

Brown Marmorated Stink Bug (BMSB), *Halyomorpha halys* (Hemiptera: Pentatomidae) (Stal, 1855) is an invasive, highly polyphagous, severe agricultural pest, that reduces the availability, quality, and value of hazelnut production. The most important method against *H. halys* is the use of resistant hazelnut varieties. The purpose of our work is to investigate the potential of hazelnut resistance to *H. halys*. Research objects are Georgian commercial varieties Berdznula (Corylus avellana) and Tita (*Corylus pontica*) in the condition of West Georgia. Hazelnut fruit shell thickness, qualitative and quantitative lignin contents and *H. halys* stylet length were determined by microscopic and biochemical methods, also *H. halys* and nut phenologies, and the degree of nut damage were evaluated according to the number of healthy kernels. It was demonstrated that overwintered insects and individuals of all ages of the first- and second-generation damage hazelnut fruit before the shell ripens, which is synchronized with the early stages of hazelnut fruit development. H. *halys* cannot damage the fruit after lignification of the pericarp. Lignin content and damage intensity are inversely proportional to each other according to Pearson's correlation. Hazelnut resistance depends on the synchronous development of *H. halys* and hazelnut phenopases, and also on the time and quantity of lignin biosynthesis in hazelnut pericarp. According to the above-mentioned resistance markers, Berdznula is more resistant than Tita. Research results contribute to maintaining a healthy environment, sustainable crop production, and food security.

Introduction

Many of temperate fruits are growing commercially in Georgia (Maghlakelidze *et al.*, 2017). Among them, hazelnut (*Corylus* L., Betulaceae)(Holstein *et al.*,2018) is widely spread in Georgia naturally and has great economic importance for the country. In *Georgian phytocoenosis*, there are 6 species of hazelnuts: *C. iberica* Witten et Kem.Nath., *C. colurna* L., *C. avellana* L., *C. imeretina* Kem.Nath., *C. cachetina* Kem.Nath., *C. colchica* Albov., (Gagnidze*et al.*, 2005). The ancient remains of the genus *Corylus* were found in Eocene

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sediments existing in the country, and those of *C. avellana* L. and *C. colchica* Albov. were found in Pleistocene sediments (Shatilova *et al.,* 2011). The major phenotypic characteristics of hazelnut (growth intensity, duration of vegetative period, nutshell thickness, oil content) are genetically determined (Khomizurashvili, 1978; Sichinava, 2005; Mirotadze, 2011; Kutateladze *et al.*, 2013; Frary *et al*., 2019). Nowadays, there are approximately 15 Georgian cultivars, (Khomizurashvili, 1978; Sichinava, 2005), among them are Berdznula (*C. avellana*) and Tita (Dedoflis titi) (*C. colchica*) (Information about Georgian hazelnut, 2014).

Since 2014 a polyphagous invasive insect The Brown Marmorated Stink Bug (BMSB), *Halyomorpha halys* (Hemiptera: Pentatomidae) (Stal, 1855) (Hoebeke and Carter, 2003) has been invaded by Russia (Neimirovets, 2018), distributed in west Georgia and damages the hazelnut. The insect caused the loss of 70-90% of the major industrial hazelnut harvest (Lee *et al*., 2013; Meskhi, 2017; Murvanidze *et al*., 2018, EPPO Global Database, 2019; CABI, 2019; Leskey *et al*., 2012a; Hedstrom *et al*., 2014; Bergmann *et al*., 2015; International Nut and Dried Fruit Council, 2019; Kharabadze, 2019).

In Georgia, insecticides containing Bifenthrin, synthetic pyrethroid, and oil form of deltamethrin have been used against the *H. halys* since 2017 (Meskhi, 2019). There are numerous scientific publications about Bifenthrin's side effects (Yang *et al*., 2018; Ullah *et al*., 2019). Maximal reduction of chemicals and their replacement with alternative control methods are required to develop sustainable agriculture (Sathe *et al*., 2000; Peterson *et al*., 2016). The selection of resistant hazelnut cultivars will reduce ecological and economic problems caused by *H. halys*. (Peterson *et al*., 2016). Some passive and active mechanisms determine plant resistance. The existence of structural peculiarities of epidermal tissue is important at the first stage of the pathological process. Moreover, the insects choose host plants by epidermal tissue (Dowd and Sattler, 2015, Bernays, 1992). Phenology plays an important role in plant resistance against insect damage. Most cases of insect damage are connected with the certain development stage of the host plants (Bernays, 1992). Insect mouthpart structure and it's biological needs are the causing reasons for plant damage characteristics.

H. halys has piercing-sucking mouthparts. Insect inserts its stylet into the plant tissues (mechanical damage) (Joseph *et al*., 2015; Leskey *et al.*, 2012b) injects saliva, and causes tissue necrosis (chemical damage)(Giacometti *et al*., 2020, McPherson and McPherson, 2000; Peiffer and Felton, 2014). Some kinds of secondary infections can be developed on the damaged parts of the plants (Jones and Lambdin, 2009). For the feeding process, the insect chooses soft parts (such as leaves and watery fruits) as well as actively growing fruits of almond (Rijal and Sudan, 2018), pistachio (Jesús *et al*., 2017), green walnut (Karpun and Protsenko, 2016) and hazelnut (Murvanidze *et al*., 2018; Hedstrom *et al*., 2014).

Hazelnut fruit development starts from double fertilization and needs 5-6 months according to its variety (Khomizurashvili, 1978; Solar and Stampar, 2011). Ripe hazelnut seed is covered with a rough layer (pericarp). The pericarp is developed from the ovary wall and is composed of a three zone: exocarp (out layer), mesocarp (middle layer), and endocarp (inner layer). During the ripening process, parenchymal cell walls of mesocarp are getting lignified and sclerified, cells are losing content, getting highly thickened (Evert and Esau, 2006). Pericarp protects hazelnut seeds from abiotic and biotic stressors. Hazelnut shell thickness, lignin content rate, and resistance of Georgian variates to *H. halys* are not studied.

Despite the huge loss in hazelnut production, there is no information about the interaction of phenological phases between *H. halys* and hazelnut plants. Interaction between the ripening process of hazelnut pericarp and insect stylet growth has not been studied either.

The purpose of our work is to investigate these abovementioned issues to study the potential for hazelnut resistance to *H. halys*.

Material and Methods

Plants and insects

Plant and insect materials were collected from an experimental hazelnut orchard (2 ha) in the area of Ninoshvili village, Guria (42°001’58” N, 41°056’28” E, altitude: 73 m), one of the major regions for commercial hazelnut cultivation in western Georgia. The two most commonly grown native hazelnut cultivars Berdznula (*C. avellana*) and Tita (*C. colchica*) were used in the experiments. Twenty-five individual trees of each variety were selected and 50 clusters of nuts were taken from the south-exposed side of the plants. The number of nuts in each cluster was 2.2 ± 0.2 for Berdznula and 2.4 ± 0.4 for Tita. Immediately after sampling, nut damage categories were determined. Additionally, we measured the number of healthy hazelnut kernels, shell thickness, and lignin content from visually healthy hazelnuts (II category) (Fig.1). Nut clusters were collected in 2019-2020 (from June till August) in the growth stages “kernel fully expanded” and “kernel mature” (phenology according to Hedstrom *et al*., 2014; Khomizurashvili, 1978; Sichinava, 2005; Mirotadze, 2011). Individuals of *H. halys* were attracted by using, attract-and-kill” traps baited with the insect’s aggregation pheromone and collected by hand or with an insect net, placed in cotton sacks, and taken to the laboratory for research.

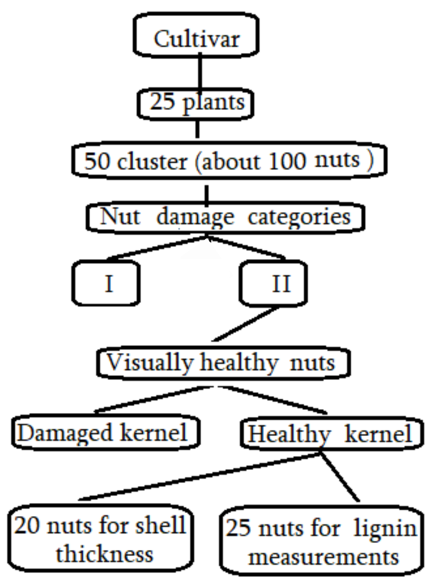

Fig 1. Sampling design.

Information on precipitation and air temperature conditions was obtained from the Agrometeorological Bulletin of the Georgian National Environment Agency (nea.gov.ge 2021). Agrometeorological data were measured at Poti meteorological station (42°09′0″N 41°40′0″E), located 16 km from the experimental field site. (Appendix A).

Phenotyping of hazelnut feeding damage

External signs of hazelnut damage caused by *H. halys* were assessed and divided into two damage categories. Category I: numerous perforations are visible on the surface of a nutshell, the nut is strongly withered and the nutshell has longitudinal splits, fungal infection is occasionally observed. Category II: no perforations of the nutshell surface are visible, the nut appears healthy from the outside. Category II nuts were further assessed by cracking the shell open and determining the health status of the kernel inside.

Hazelnut shell thickness and lignin content

Hazelnut shells of both cultivars were assessed for thickness and lignin content. Nuts were collected in the developmental stages of "kernel fully expanded" and "kernel mature" (Hedstrom *et al*., 2014).

The thickness of each shell was measured (Khomizurashvili, 1978) at its widest dimension around the nut's center. The thickness of the unripe nutshell was measured by using a light microscope (МБР-1) with a micrometer (MOB-1-15X OMO USSR).

Lignin in hazelnut shells was assessed microscopically (in the growth stage "kernel expression begins") and gravimetrically (in phases "kernel fully expressed", Mature) measuring. For assessing the lignin formation in shells from immature hazelnuts, thin slices (15 μm) of the shell were prepared with a microtome in the stage of "kernel express begins" (Microtome Leitz Germany 661) and then dyed. The slices were stained with pale pink safranin solution (1 g l^{-1} w/v) for 24 h. The staining intensity corresponded to the degree of lignification; tissues lacking lignin remained unstained. Following the staining treatment, shell slices were washed with 50% (v/v) ethanol, acidified with a few drops of 1% (v/v) acetic acid (Japaridze, 1958), and studied under a light microscope (МБР-1). Acid-insoluble lignin content was quantified using the Klason method (Ayeni *et al*., 2015, Lin and Dence, 1992). For the complete hydrolysis, hazelnut shells were ground with a coffee grinder (VitekVT 1540) to a particle size of 1-4 mm. Samples of hazelnut shell powder (1 g) were treated with 10 ml of 72% sulfuric acid for 24h. The samples were diluted with 140 ml portions of water and autoclaved at 125°C, 1.5 atmospheres for 40 min. Mixtures were filtered with a glass fiber filter (SS GF 52 × 47 mm) and washed with distilled water. The filters with the acid-insoluble lignin (Klason lignin) were dried at 103°C, cooled in the desiccator, and weighed.

Measuring insect stylet

The length of the stylet was measured in second and third instars nymphs, and in adults of *H. halys*, according to Rahman & Lim (2017). Ten individuals of each life stage were assessed.

Statistics

For statistical analysis of the obtained results, we used the following tests: T-test to determine the difference between the shell thicknesses in different varieties. An F-test was performed to determine the variation in shell thickness of the Berdznula and Tita varieties. A Chi-square test was performed to determine the relationships between hazelnut varieties and damage categories, as well as between shell thickness and damage categories.

Results

Meteorological conditions and phenology

The vegetation periods from April until September in 2019 and 2020 differed in average air temperature and precipitation. In particular, 2019 was characterized by lower rainfall and higher temperatures compared to 2020 (Appendix A). Starting by the end of April, adult *H. halys* emerge from overwintering sites, and after maturation feeding, produce the first generation at the end of May-beginning of June). The second decade of April 2020 was 0.5°C warmer, and the third decade was 1.5°C colder and rainy compared to the corresponding periods in 2019. In spring 2019, the first insects were observed in the 2nd decade of May, and in 2020 in the 3rd decade of May. In spring 2020, the number of overwintering insects was greatly reduced - we caught five individuals. At the end of April, the nut is in the embryonic phase, after it goes through the following phenological phases: c. the presence of an embryo, d. kernel expression begins, pericarp formation, e. kernel fully expressed, end of sclerification, f. kernel matures, and harvests. Variety Berdznula undergoes phenophases 7-10 days earlier than Tita.

Hazelnut fruit damages

Hazelnut fruit feeding damages were observed in both cultivars Tita and Bedznula, with the number of injuries in 2019 exceeding those of 2020. The visually healthy fruit of the Berdznula was 60%, while in the Tita was only 43% (Fig.2). Some of the visually healthy fruits were also found to be damaged by the insect. The symptoms of the damages were the same as in general: more damage was reported in both varieties in 2019, although the percent of kernel damage in the Berdznula was less than one year in both species (Fig. 3). Typical cases of hazelnut kernel injury caused by *H. halys* are shown in Fig. 4. In the kernel of a healthy, ripe nut (Fig. 4. A) the embryo (1) and its nutrition tissue in the cotyledon (2) are clearly visible. As a result of *H. halys* feeding, both the embryo and the reserve tissue (Fig. 4. B.C.) were necrotic.

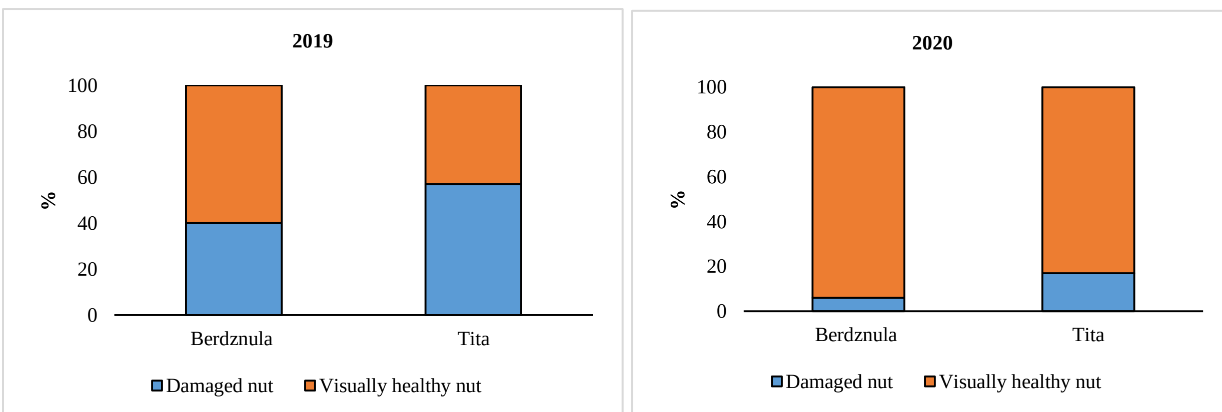

Fig. 2. Proportion of visually healthy and damaged nuts in Berdznula and Tita variates according 2019-2020 years.

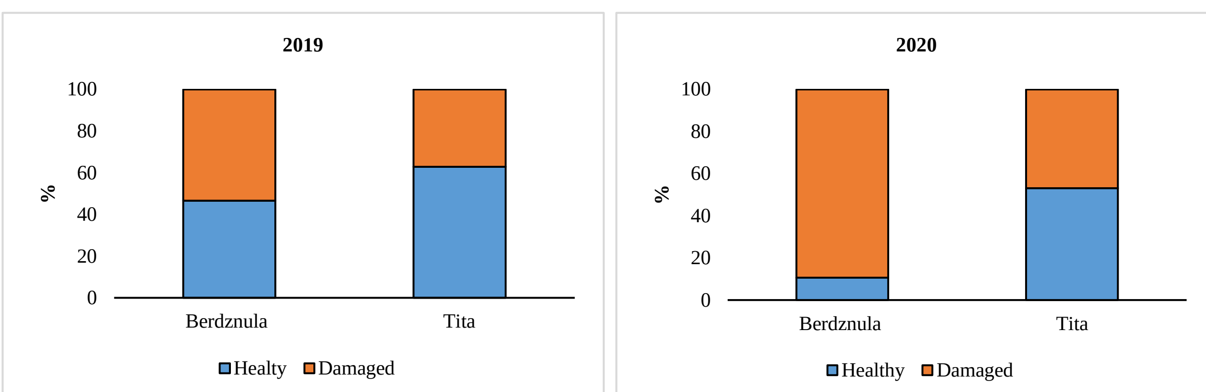

Fig. 3. Proportion of damaged kennels in Berdznula and Tita varieties healthy looking nuts after split open 2019-2020 years.

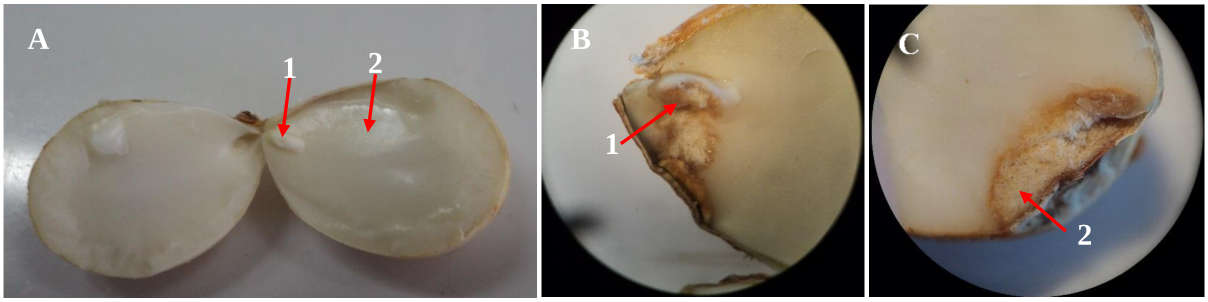

Fig. 4. Healthy (A; natural size, 1. embryo 2. nutrition tissue in the cotyledon) and corking damage symptoms (B-embryo, C- lowest part of the kernel; x20) of hazelnut.

After splitting open visually healthy nuts a proportion of the kernels were found to be damaged by the insect (Fig.3). In 2019, on average 60% of all nuts were healthy looking, of which 32.0 ± 1.16% showed healthy kernels in cultivar Berdznula. In 2020, 94±1.16% visually healthy kernels were observed, of which healthy kernels were 84 ± 2.08%. In the case of Tita in 2019 visually healthy fruits reached 43.3 ± 0.88%, of which only 16.3±0.88 had healthy kernels. In 2020 83±0.58% were visually healthy, of which 39±1.73% had a healthy kernels (Fig.3). Chi-square test shows that there is a dependency between hazelnut variates (Berdznula and Tita) and damage categories (p-value = 0.048464 < 0.05).

Nutshell thickness

The thickness of the shell depended on the hazelnut variety and its developmental stage. In the first ten days of June when the first and second instar nymphs of the first *H. halys* generation were observed, most nutshell growth was completed and shells had reached their maximum thickness. Thicker nutshells were observed in the cultivar Berdznula than in Tita (Fig. 5).

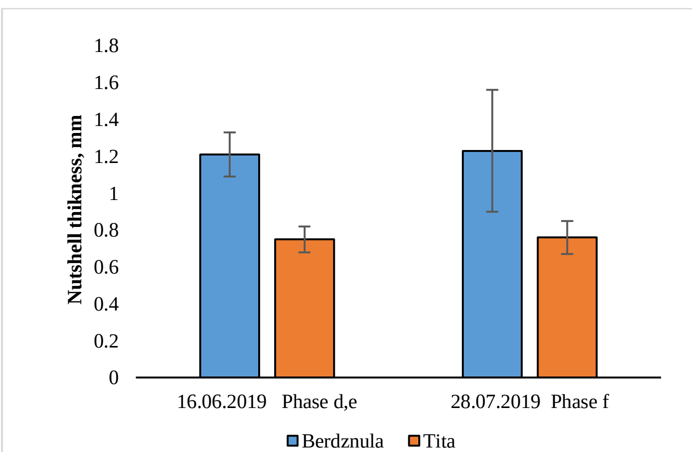

Fig. 5. Changes in the thickness of the hazelnut nutshells during ontogenesis.

Bedznula nuts in the stage ”kernel expression begins“(16.06.19) had a shell thickness of 1.21 ± 0.04 mm (mean ± SE) while the shell thickness of Tita nuts was 0.75 ± 0.02 mm (mean ± SE). At the second measurement 70 days later, hazelnuts were in the growth stage “kernel mature “(27.07.19). At this time point, the thickness of Berdznula shells had reached 1.23 ± 0.11 mm (mean ± SE), while the thickness of the Tita shells was 0.76 ± 0.03 mm (mean ± SE).

A comparison of nutshell thickness between varieties Tita and Berdznula assumes that variances for the two cultivars are significantly different (*p-value* = 0.008).

The t-statistic is 4.281 with 18 degrees of freedom. Also, corresponding two-tailed *p-value* is less than 0.05.

Lignin formation in a nutshell

In the first days of June, the pericarp cells in the nutshell started to lignify. Enhanced thickening of the cell walls, the breakdown of cell content, and the formation of dead sclerotized tissue were found. Microscopic observation showed that cultivar Berdznula had a thicker lignified zone than Tita (Fig. 6, arrow 3). In the pericarp of Berdznula lignification was father advanced and clearly expressed as dark Bordeaux-red coloring (Fig. 6 A), while in Tita lignification was still in an early stage (light pink coloration, Fig. 6 B).

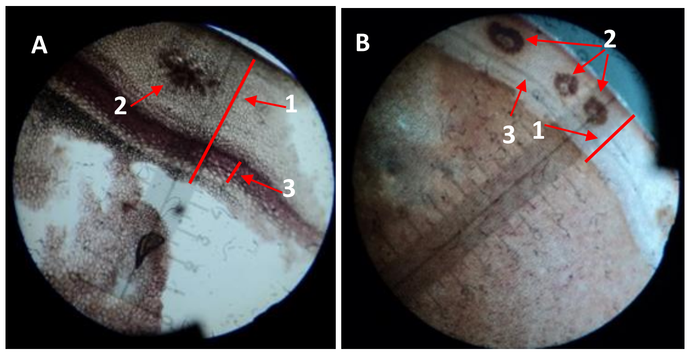

Fig. 6. Micrographs of safranin stained hazelnut shells (A. Berdznula, B. Tita). 1. pericarp, 2. conductive vein in pericarp, 3. lignified zone. x 24. (10.06.19)

A quantitative analysis of the lignin concentration showed that in 2019, hazelnut shells in Berdznula had accumulated significantly more lignin than shells in Tita (Mann-Whitney U-test, U = 50.5, p < 0.001, n = 19). However, no difference in nutshell lignin concentration was found in 2020 (Mann-Whitney U-test, U = 116.0, p = 0.063, n = 18-20). Differences between lignin quantity Berdznula and Tita 8% were observed in 2019.

The lignin quantity of the cultivar Berdznula in 2019 reached 51.79±0.001% (mean± Std. error) and in 2020 was less than the previous year 39.21±0.001%. 2019 Tita consisted of 43.95±0.003% of lignin and 40.13±0.0003% in 2020 were accumulated (Fig.7). No significant difference was observed between both cultivars in 2020.

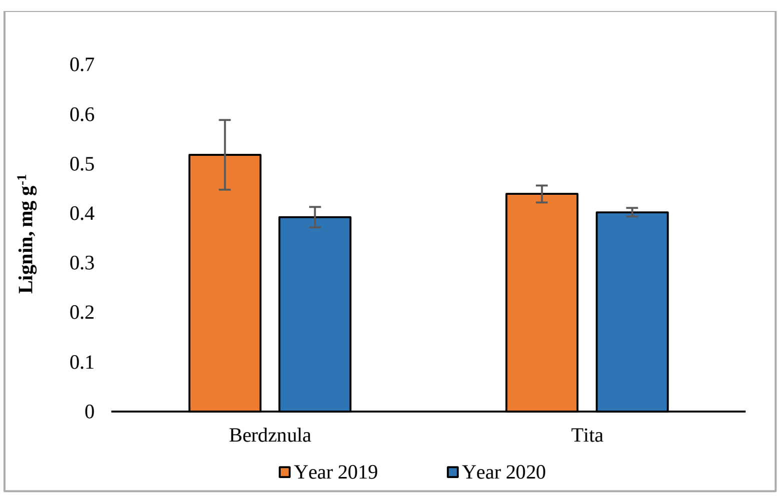

Fig. 7. Lignin content in the nutshell of different hazelnut cultivars in 2019-2020 from II category.

Tita begins to accumulate lignin later than Berdznula (Fig. 6). Analogical results were obtained according to kernel damage percentage in 2019-2020 (Fig. 3). In particular, during both years Tita showed higher levels of damage by *H. halys*. In 2019, 46.7% of the fruit of Berdznula and 63% of the Tita were damaged. In 2020, both the number of *H. halys and* damaged nuts decreased, but the damage in Tita (53%) was more severe than in Berdznula (10.6%) (Fig. 2).

Changes in length of H. halys stylet according to the stage

Stylet length of *H. halys* differs significantly between developmental stages (one-way ANOVA, F = 367.054, df = 2, P < 0.05 followed by Tukey HSD test) In the second instar, the stylet length of *H. halys* is approximately 2 mm, it's the time when it begins feeding with plant tissues, the stylet grows up and reaches 7-8 mm at the adult stage (Fig. 8).

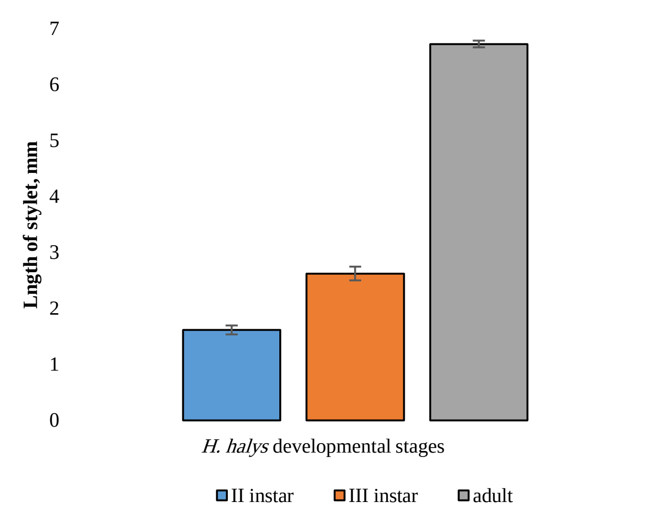

Fig. 8. Length of *H. halys* stylets of different nymph stages.

Discussion

H. halys selects more accessible host plants for feeding and egg laying, which are different by geographical and ecological peculiarities of the insect spread area (Niva and Takeda, 2003, Bakken *et al*., 2015). On some plant species (*Ailanthus altissima* Mill. Swingle, *Catalpa speciosa* Warder, *Paulownia tomentosa* Thunb., Prunus sp.), which grow in West Georgia as well, the insect may go through a complete life cycle (Lee *et al*., 2013). In the Black sea subtropical areas of Georgia (Guria, Imereti, Samegrelo, Abkhazeti), *H. halys* uses cultural, wild, and ornamental plants for feeding (Kharabadze, 2019), but from those, the insect prefers a hazelnut (Meskhi, 2017). Hazelnut leaves and fruit are rich in fat and other organic compounds (Mirotadze, 2011) which completely satisfy the vital requirements of *H. halys*.

Oviposition of *H. halys* depends on photoperiod. The ovary developmental threshold is 14 hours (Lee *et al*., 2013). In Georgia, such daylight starts in the first 10 days of April and finishes in the first 10 days of August (Javakhishvili, 1988). Photoperiod, temperature, air relative humidity (Funayama, 2006; Javakhishvili, 1988; Acebes, 2016), and amount of food can provide three generations of *H. halys* during one vegetation period (Fig. 9) in the subtropical zone of Western Georgia (our data, Karpun and Protsenko, 2016). The number of generations of *H. halys* during one vegetation period (Fig. 9) in the subtropical zone of Western Georgia is a controversial subject. According to Karpun & Perchenko (2016), on the east coast of the Black Sea basin (Krasnodar, Sochi, Abkhazia, Adjara), with a subtropical climate, *H. halys* develops three generations. Mamedov (2018) reported, that in the subtropical zone of Azerbaijan (Lenkoran, Absheron, and Zakatala) three generations occurs as well. By the dates of M. Murvanidze *et al*. (2018) and Jakeli & Nikolaishvili (2019), *H. halys* develops two generations in hazelnut orchards of Western Georgia.

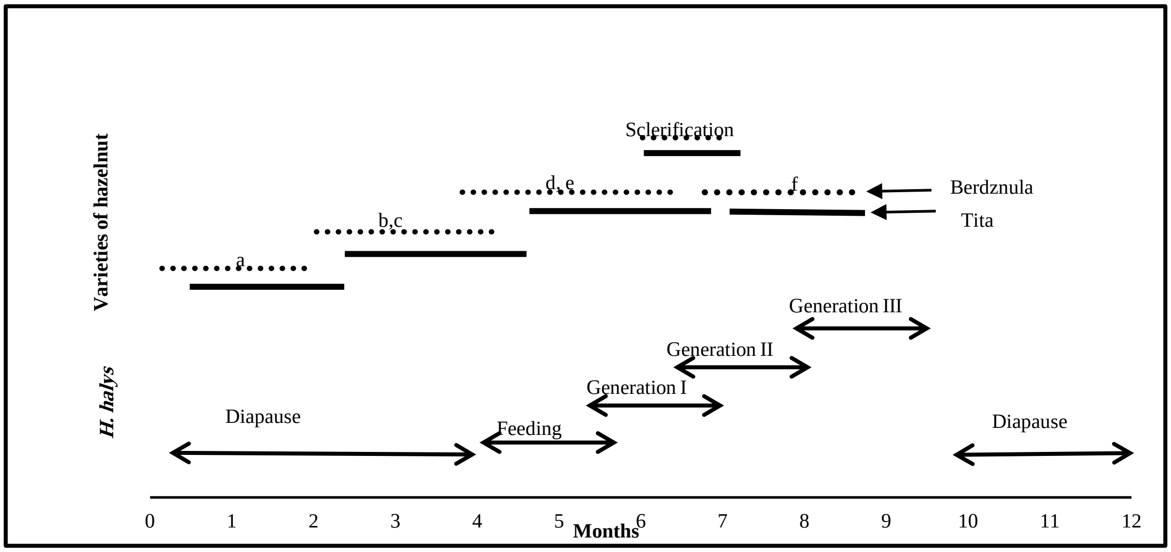

Fig. 9. Synchrony of development hazelnut fruits and *H. halys.* a. flowering, b. terminal bud set, c. embryo present, d. kernel expression begins, formation of the pericarp, e. kernel fully expressed, The finish of sclerification, f. kernel nature, harvest

To estimate the number of generations that *H. halys* can produce under the climatic conditions of the Black Sea region 1. The climatic data of the humid subtropical zone of Georgia with the ecological requirements of *H. halys* was Compared. 2. The dates of egg collection obtained under natural conditions were analyzed. It was concluded that the insect can develop three generations. The third generation is the overwintering generation. Additionally, we monitored not only hazelnut orchards but maize fields, citrus orchards, ornamental trees, other crops, and wild plants as well. In August and September of 2019-2020 oviposition on hazelnut and aggregation were not observed. They were detected on the *Zea maize* L., *Diospyros kaki* L., *Vitis labruska* L., variety Isabela (Adesa), Citrus, Actinidia, *Sambucus ebulus* L.

In the Guria area hazelnut starts blooming from the first 10 days of December and completes it in the second 10 days (early-ripening cultivars) or in the third 10 days (late-ripening cultivars) of February (Sichinava, 2005). In March, hazelnut fruits are set (Fig. 9). *H. halys* diapause lasts from the third decade of October to the third decade of April. Coming out from diapause starts at the end of April or at the beginning of May. After wintering, the insect needs additional feeding for reproduction which lasts nearly a month (Skillman *et al*., 2018). *H. halys* uses hazelnut fruits for feeding when they are in the intensive growth and development stage (Fig. 9). During this period, the green husk is not removed from the fruit, also many trichomes are developing, and the nutshell is not formed yet. From the end of May till the beginning of June, females lay eggs on the leaves of hazelnut fruitful branches. In the first decade of June, the first-generation insects of I-II ages occur. Thus, from the end of April until the end of May hazelnut is damaged by overwintered *H. halys*. After egg laying the wintering forms die, however, the first-generation insects continue damaging the plant. The newly hatched *H. halys* nymphs are in the group for 2-3 days and do not feed on the plant (Funayama, 2006), then disseminate and begin feeding on the leaves and unripe fruits (Funayama, 2006). In mid-June, nutshell lignification starts intensively, the degree of which depends on the hazelnut cultivar (Fig. 9). In the middle of June, unlike Berdznula, the nutshell of cultivar Tita has not been lignified yet. We have observed that the lignification of the nutshell ends after 1.5 months of completion of the nutshell thickness. *H. halys* stylet length exceeds the thickness of pericarp in all ages (Fig.8, Rahman and Lim 2017). The immature nutshell cannot create a barrier for *H. halys* of any age, especially for wintering forms. Therefore, damage categories of unripe hazelnut cultivars are not different (Hedstrom *et al*., 2014). Hazelnut fruits are matured gradually, so the most damage to fruit is caused by the overwintering forms and the 1st generation of *H. halys*. The 2nd generation insects damage only less lignified fruits. The probability of damage caused by the 3rd generation of insects is very low due to the complete lignification of a nutshell (Fig. 9). No lignin-degrading enzymes were found in the saliva of *H. halys* (Peiffer and Felton, 2014; Will *et al*., 2012; Giacometti *et al*., 2020). The insect is unable to damage lignified shells of the almond and pistachios as well (Jesús *et al*., 2017; Rijal and Sudan, 2018).

Hazelnut is monocarpic, one-seeded (rarely two-seeded) plant; the pericarp of the fruit is strongly sclerified and fragile and it is easily removed from cotyledons. The petrous cell walls are impregnated with Ca or Si salts. The pericarp is formed from ovary walls and protects the embryo and cotyledons from the negative impact of abiotic and biotic factors. Pericarp layers (exocarp, mesocarp, and endocarp) are not distinguished in the nutshell of matured hazelnut. The thickness of hazelnut nutshell depends on a cultivar (Fig. 5, Sichinava, 2005; Hedstrom *et al*., 2014) and may vary within a certain range by year (Hedstrom *et al*., 2014). Hardening of a nutshell is accompanied by accumulation of lignin in parenchymal cell walls and formation of mechanical tissue (sclerenchyma) (Evert and Esau, 2006).

According to our experiments, the starting time and duration of the lignification process are very important for the hazelnut resistance to *H. halys.* More damaged hazelnut variety Tita consists of less lignin and lignification starts later, than the less damaged Berdznula. The amount of lignin in the shell is genetically determined (Osakabe *et al*., 1999) and modified by various biotic and abiotic factors, especially by drought during the vegetation period (Boudet, 2000; Moura *et al*., 2010; Moura-Sobczak *et al*., 2011; Lauvergeat *et al*., 2001; Hano *et al*., 2005; Liu *et al*., 2018). The amount of lignin in the shell of both varieties of hazelnuts was less during the precipitation-rich 2020 (Appendix A) vegetation period (Fig. 7). In the more droughty 2019, both varieties accumulated more lignin than in 2020, but the amount of lignin in Berdznula was still more than Tita (Fig. 7). We think this explains why the Berdznula was less damaged than Tita in 2019. The Berdznula has a better expression of lignin accumulation potential and resistance than Tita. In relatively rainy 2020, the lignin content decreased in the nutshells of both varieties, although the damage percentage of Tita was found to be 5.4 times higher than Berdznula. This once again proves that the insect prefers Tita due to its thinner shell, its late lignification, and less lignin content (Fig. 5, 6,7). Thus, based on the analysis of obtained materials and literature data, we suppose that hazelnut fruit is suitable for feeding *H. halys* before the lignification of the nutshell. In the early-ripening cultivar Berdznula, the lignification process starts earlier.

Conclusions

Comparative resistance of hazelnut to *H. halys* depends on several factors: (1) Which category the cultivar belongs to, early or late ripening; (2) How much time and amount of lignin is formed in a fruit pericarp; (3) synchronous development of *H. halys* and hazelnuts phenophases and (4) amount of precipitation. According to these factors, Berdznula is more resistant to feeding damage by *H. halys* than Tita. Research results contribute to maintaining a healthy environment, sustainable crop production, and food security.

Author Contributions

Natalia Kharabadze: performed experiments, collected the data, and prepared figures and tables, the original draft version. Nino Tsiklauri: provided methodology of lignin analyses and supported the experiment. Medea Burjanadze: provided methodology of statistical analyses, supported with final analysis of the data, preparing the manuscript. Nona Chkhaidze: conceptualization, performed experiments, provided methodology and supervised the research, translated and corrected terms, supported with final analysis of the data, preparing the manuscript.

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Conflicts of interest

The authors declare no conflict of interest.

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