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Original scientific article

SUSCEPTIBILITY LEVELS OF SOME CHESTNUT CULTIVARS AND GENOTYPES TO THE CHESTNUT GALL WASP IN TURKEY

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The Asian chestnut gall wasp (ACGW) is one of the most important pests threatening most *Castanea* species. The best management strategy against this pest can be establishing new orchards with resistant cultivars along with biocontrol (parasitoid *Torymus sinensis* Kamijo (Hymenoptera: Torymidae)). In Turkey, ACGW was first detected in 2014 at Gacık village of Yalova province. Bursa, İstanbul, Sakarya, Kocaeli, Balıkesir, Bilecik, Düzce, Giresun, Bartın, Zonguldak, Sinop and İzmir provinces are also infected with ACGW. This study was carried out to determine the levels of susceptibility of chestnut cultivars/genotypes to ACGW. The study was carried out in Yalova province between 2016 and 2018. In total, 15 cultivars/genotypes were involved in the study, including European (*C. sativa*) chestnuts (Albayrak, Altınay, 'Erfelek', 'Osmanoğlu', Salıpazarı, 'Serdar', 'Ünal'), interspecific hybrids ('Marigoule' and BDB-L) and complex hybrids ('Akyüz', 'Macit 55', 'Ali Nihat', A9, A55, A56) were tested in the study. In April 2016, five- to seven-year-old seedlings were "bark" grafted, and susceptibility to the ACGW was evaluated by recording the ratio of infected buds (%), visual assessment of the damage (severity), and calculations of an infestation index. Among the cultivars and genotypes tested in the study, the 'Akyüz' cultivar was determined to exhibit the highest resistance against the ACGW. To fully understand the resistance mechanism of the 'Akyüz' cultivar, molecular and biochemical studies should be done and compared with other results.

Key words: Castanea sativa, complex hybrid, infestation index, *Torymus sinensis*

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INTRODUCTION

Species of the genus *Castanea* along with *Fagus* L. and *Quercus* L. are members of the Fagaceae family. In the Northern hemisphere, there are 13 identified chestnut species (SOYLU, 2004). However, chestnut cultivation is mainly done with three species (*C. sativa* Mill, *C. crenata* Siebold & Zucc. and *C. mollissima* Blume) and with hybrids of four species (*C. sativa*, *C. dentata* Borkh, *C. crenata*, and *C. mollissima*). In 2020, the world's chestnut production was estimated at 2.240.898 tons, where Turkey was recognized as the second country in Europe and the third in the world with a production volume of 76.045 tons (FAOSTAT, 2022).

Invasive species are important factors disturbing natural and managed ecosystems. Damage caused by these species can change the stability and productivity of ecosystems that provide vital resources for human well-being and biodiversity (PAINI *et al*., 2016; LIEBHOLD *et al*., 2017). Since the late 1980s, Turkey's chestnut production has been experiencing a decline mainly due to epidemics of chestnut blight (*Cryphonectria parasitica* (Murill) M.E.Barr and chestnut root rot (*Phytophthora* spp.) disease. For example, compared with 1987, when the production of chestnuts in the country amounted to 90,000 tons, in 2002, production decreased by almost 50% (FAOSTAT, 2022). From 2002 to the present day, the production of chestnut has increased due to the effective management of chestnut blight and chestnut root rot diseases, which were a severe reason for the decline in production. Nowadays, the Asian chestnut gall wasp (ACGW) (*Dryocosmus kuriphilus* Yasumatsu, 1951) is another successful invasive pest negatively affecting chestnut production in Turkey and around the world. The chestnut gall wasp is a univoltine species and reproduces parthenogenetically (EPPO, 2005). The life cycle consists of an egg, three larval stages, pupae, and adult stages (NUGNES *et al*., 2018). Adults are seen before the beginning of summer (EPPO, 2005; SARTOR *et al*., 2009). The larvae feed in the gall for 30-40 days, pass the second and third larval stages and become pupae (VIGGIANI and NUGNES, 2010). Adult females emerge from the galls from mid-June to mid-August (about 2.5 months) and lay their eggs in 3-5 pieces inside the various parts of the buds (SARTOR *et al*., 2009). The ACGW is a pest native to China (ZHANG *et al*., 2009) and spread from China to Japan (1941) (MORIYA *et al*., 2003), South Korea (1950) (MURAKAMI *et al*., 1995), United States of America (1974) (RIESKE, 2007), and Italy (2002) (BRUSSINO *et al*., 2002). By 2005, the ACGW had spread all over Italy and expanded to France (EPPO, 2007). In China, the first record of the yield drop due to ACGW was reported in 1929 (ZHANG *et al*., 2009). Later, chestnut production decreased by up to 50-75% in some countries due to ACGW infection (ANAGNOSTAKIS, 2014; BOROWIEC *et al*., 2014). In Turkey, the ACGW was first detected in 2014 in Gacık village of Yalova province (ÇETIN *et al*., 2014). It spread into the provinces of Bursa, İstanbul, Sakarya, Kocaeli, Balıkesir, Bilecik, Düzce, Giresun, Bartın, Zonguldak, Sinop and İzmir (MICIK and IPEKDAL, 2021; MICIK *et al*., 2021; YILDIZ *et al*., 2020). It is estimated that the average spreading rate of the ACGW is 25 km per year (RIESKE, 2007; GRAZIOSI and SANTI, 2008).

The management methods for the ACGW are complicated due to its life cycle inside the chestnut buds and galls (NUGNES *et al*., 2018). The most effective way is to use a parasitoid named *Torymus sinensis* Kamijo, 1982 (Hymenoptera: Torymidae) (QUACCHIA *et al*., 2014), a biocontrol agent of the ACGW. The establishment of *T. sinensis* and its effectiveness requires a long time, from 6 to 18 years (MURAKAMI *et al*., 2001; QUACCHIA *et al*., 2014). Thus,

establishing new orchards with resistant cultivars can be a management strategy along with biocontrol (SARTOR *et al*., 2009; but see MORIYA *et al*., 2003 for how the strategy using resistant cultivars can fail). After the first report of the ACGW in Turkey, in 2015 and 2016, *T. sinensis* was imported from Italy and released into affected orchards in these provinces (IPEKDAL *et al*., 2014).

The tolerance levels against the ACGW amongst *Castanea* species vary widely. The ACGW attacks *C. crenata* (Japanese chestnut), *C. dentata* (American chestnut), *C. mollissima* (Chinese chestnut), and *C. sativa* (European chestnut) and their hybrids, however, no galls were observed on *C. pumila* (L.) Mill. (Syn.: *C. alnifolia* Nutt.) (ANAGNOSTAKIS *et al*., 2011). Also, *C. henryi* Rehder & E.H.Wilson, *C. ozarkensis* Ashe, and some Japanese chestnuts (*C. crenata*) were either resistant to infestation or tolerant to the ACGW (ANAGNOSTAKIS, 2014). According to PANZAVOLTA *et al.* (2012) and BERNARDO *et al*. (2013), cultivars of *C. sativa* show considerable variation in susceptibility to the ACGW.

The high genetic diversity of the chestnut in Turkey (VILLANI *et al*., 1991) can be an advantage for searching resistant germplasms to multiple pathogens and pests. To our knowledge, to mitigate the effect of pathogens and pests on production, different *Castanea* species, cultivars, and hybrids were imported into Turkey from other countries (SERDAR and MACIT, 2010; MACIT *et al*., 2018). In the present study, we investigated susceptibility levels of cultivars/genotypes that grow in Turkey (both native and introduced) to the ACGW.

MATERIALS AND METHODS

Study sites and chestnut cultivars/genotypes

The Gacık village of Yalova is the first place the ACGW was detected. Three sites in Gacık village of Yalova/ were selected. These sites are natural stands where wild European chestnut plants are grown (40°36'17"N, 29°20'05"E). In the study, 15 well-known cultivars/genotypes that grow in Turkey were tested (Table 1).

Table 1. The chestnut cultivars/genotypes tested in the study.

Species	Cultivar	Genotype
C. sativa	'Erfelek', 'Osmanoğlu', 'Serdar', 'Ünal'	Altınay, Albayrak, Salıpazarı
C. sativa x *C. crenata*	'Marigoule'	
'King Arthur' (*C. mollissima* x *C. seguine*) X 'Lockwood' (*C. crenata* x *C. sativa* x *C. dentata*)	'Akyüz', 'Macit 55', ' Ali Nihat'	A9, A55, A56
Castanea spp*		BDB-L

*Seedling of 'Bouche de Betizac'.

'Erfelek', 'Osmanoğlu', 'Serdar', 'Ünal' cultivars and Altınay (SA 5-1), Albayrak, Salıpazarı (554-14) genotypes are selected from different places of the country due to their superior properties (SERDAR, 1999; SERDAR and SOYLU, 1999; SOYLU, 2004; SERDAR et al., 2011; SERDAR *et al*., 2013a). 'Marigoule' cultivar is a hybrid of Japanese x European chestnuts (BARRENECHE *et al*., 2019). It is a popular cultivar in Turkey due to its resistance to *Phytophthora* spp. and tolerance to the chestnut blight (*C. parasitica*). 'Akyüz', 'Macit 55', 'Ali Nihat' cultivars and A9, A55, A56 genotypes are the hybrid of 'King Arthur' (*C. mollissima* x *C. seguine*) and 'Lockwood' (*C. crenata* x *C. sativa* x *C. dentata*) cultivars. The hybridization study was done in the Connecticut Agricultural Research Station in the USA in 2004, and 50 seeds were obtained from this study in 2005. These genotypes were tested for their yield, pomological, and growth characteristics between 2006 and 2014, and superior ones were selected. The BDB-L genotype was imported from Lebanon as a 'Bouche de Betizac' cultivar. However, phenological analyses showed that it is not a 'Bouche de Betizac' cultivar but similar.

Grafting material and procedure

This study was conducted between 2016 and 2018. The scion woods were collected from Ondokuz Mayıs University Faculty of Agriculture Ali Nihat Gökyiğit Chestnut Research Station in February 2016. The research station was monitored for the ACGW, and no damage was seen. Every piece of scion wood was surface sterilized by dipping it into 1% NaClO solution for 2 minutes and allowed to dry at room temperature (approximately 22°C) over a paper towel for 20 minutes. Sterilized pieces of scion wood were wrapped with cling wrap to prevent moisture loss and stored in a cold room (+4°C) until the grafting date.

On 27 April 2016, grafting was performed following a bark grafting method previously described by SERDAR *et al.* (2013b) and ER *et al*. (2017). At each site, seven to ten-year-old European chestnuts were used as rootstocks. Five rootstocks were grafted for each cultivar/genotype in each site (5 x 15), and 225 grafts were made. For each graft, three scions were used.

As all the grafts were not successful, three grafted plants were selected for each cultivar/genotype from each site. From three parcels, 135 (3 site x15 genotype/cultivar x 3 rootstocks) grafts were examined for ACGW damage. Rootstocks' tops were cut 1 m above soil level for grafting using a hand-held chainsaw (HUSQVARNA 120). Harmed tissue on each rootstock due to the chainsaw was removed with a grafting knife. The graft area was wrapped with a grafting band, and wax was applied to the cutting surfaces of the rootstock and the top of the scion wood.

On 14 May 2017 and 17 May 2018, the number of healthy and infected buds on the annual branches of each graft was recorded and used to calculate the ratio of infected buds and expressed as a percentage. This study recorded and presented visual appraisals of the damage caused by the ACGW on each annual branch as severity following a rating scale ranging from 0 to 5, where 0 corresponds to no damage (Figure 1).

In 2018 (three branches of two-year-old branches from each tree), each branch's total number of galls (a whole structure was counted as one) was recorded and compared against the total number of buds registered the previous year. This comparison was used to calculate the infestation index (SARTOR *et al*., 2015).

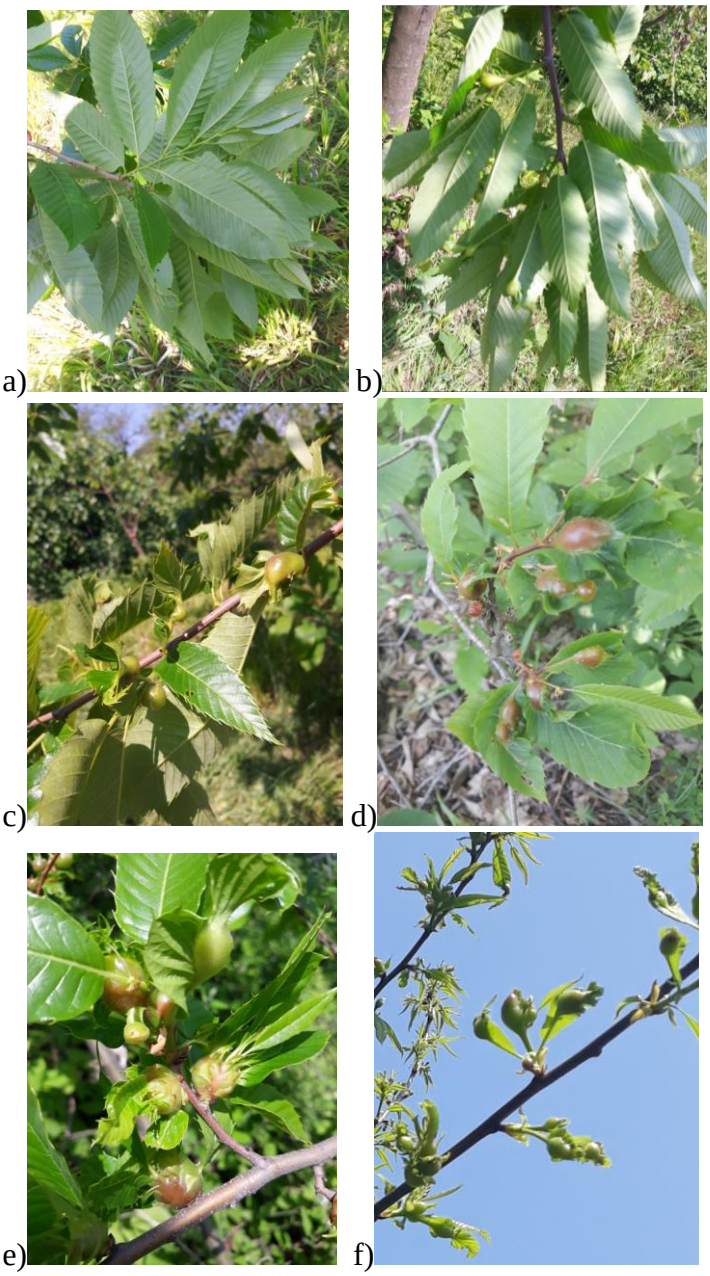

Figure 1. Symptoms and infestation percentage of chestnut cultivars: a) 0 (100% of the leaves are healthy), b) 1 (99-80% of the leaves are healthy), c) 2 (79-60% of the leaves are healthy), d) 3 (59-40% of the leaves are healthy), e) 4 (39-20% of the leaves are healthy, f) 5 (19-0% of the leaves are healthy)

Microscopic observation

In resistant cultivars, it is crucial to determine if the ACGW lays its eggs into the buds. For this aim, eight branches with 11-16 buds were collected in resistant cultivars in August 2018. Buds were examined under a stereoscopic microscope (Leica, Germany) for the eggs of the ACGW. Firstly, a thin cut was made in the buds with the help of a scalpel, and then the bud layers were carefully opened by a needle, and the eggs were counted. Only resistant cultivars to the ACGW were examined under the microscope because infected cultivars or genotypes already had the eggs of the ACGW.

Statistical analysis

The study followed a randomized design with three replications (sites). For each cultivar/genotype, three successfully grafted rootstocks were selected in each site. Values of the infected bud ratio were transformed using the following equation: $\sqrt{(x + 1)}$ due to zero presence in the data set (TOSUN, 1991). ANOVA comparisons were calculated and analyzed SPSS statistical package program, and the differences between the means were determined by Duncan Multiple Comparison Test (SPSS 21.0; SPSS Inc., Chicago, IL, USA).

RESULTS AND DISCUSSION

Damage severity and infestation index

The ACGW is a vital pest that threatens chestnut cultivation worldwide. The present study examined the chestnut cultivars' susceptibility level and genotypes to the ACGW, infected bud ratio, and visual damage severity (0-5 scale) (Table 2). According to the observation in 2017, the infected bud ratio ranged from 0.0 to 72.4%, where the 'Akyüz' cultivar had the lowest infected bud ratio, 0%, whereas the 'Marigoule' cultivar had the highest, 72.4%. The second lowest score was 16.1% on the 'Macit 55' cultivar. On the other hand, the Salıpazarı genotype was found to be as sensitive (67.1%) as 'Marigoule'. Similar results were observed from the visual damage severity scores. The best scores were obtained from the 'Akyüz' and 'Macit 55' cultivars with 0 and 1.31, respectively (Table 2). According to the first-year results, the 'Akyüz' and 'Macit 55' cultivars showed better results than other cultivars tested in the study.

Graft compatibility can be observed in chestnut, categorized into two forms: early and late graft incompatibility. Early failure starts after grafting for up to 19 months (ORAGUZIE *et al*., 1998). In 2018, 'Serdar' and 'Ali Nihat' cultivars failed to survive due to possible graft incompatibility. In 2018, the infected bud ratio ranged from 0.0 to 100.0%. Most of the cultivars tested in the study had more infected bud ratio than in 2017. However, as in 2017, in 2018, no galls were observed in the 'Akyüz' cultivar with an infected bud ratio of 0%. On the other hand, contrary to expectations high ratio was observed for the 'Macit 55' cultivar, with a 79.0% infected bud ratio. Statistically, no differences were found among the remaining genotypes and cultivars tested. Visual damage severity scores were in concordance with the infected bud ratio. Severity scores ranged between 0.00 and 5.00. The best score was obtained from the 'Akyüz' cultivar, and the worst was observed for the 'Marigoule' cultivar and Altınay genotype.

SARTOR *et al*. (2015) reported that the infestation index is a suitable parameter for evaluating cultivars and genotypes against the ACGW, and it has values between 0.00 and 1.00. According to SARTOR *et al*. (2015), an infestation index lower than 0.30 causes no significant

losses, and values between 0.30-0.60 result in a moderate decrease in productivity. However, in the study, except for the 'Akyüz' cultivar, all cultivars and genotypes had a higher infestation index than 0.60. Based on two years' results and SARTOR *et al.* (2015) index, the 'Akyüz' cultivar is the only cultivar that can be used as a resistant cultivar to the ACGW.

Table 2. Infected bud ratio, visual damage severity, and infestation index values for the chestnut cultivars and genotypes evaluated in this study.

Cultivar/Genotype	2017		2018		
	Infected bud ratio (%)	Visual damage severity	Infected bud ratio (%)	Visual damage severity	Infestation index
Albayrak	53.7 c-f*	3.67 cd	94.6 c	4.91 cd	0.75 c-e
Altınay	42.5 c-e	3.40 b-d	97.9 c	5.00 d	1.00 e
Erfelek	47.0 c-f	3.67 cd	94.3 c	4.83 bd	0.79 de
Osmanoğlu	50.5 c-f	3.50 b-d	93.7 c	4.44 bd	0.64 bd
Salıpazarı	67.1 ef	4.40 d	100.0 c	5.00 d	1.00 e
Serdar	45.4 c-e	3.33 b-d	**	**	**
Ünal	50.6 c-f	3.72 cd	98.3 c	4.50 bd	0.62 bd
Marigoule	72.4 f	4.33 d	95.7 c	5.00 d	0.96 de
Akyüz	0.00 a	0.00 a	0.00 a	0.00 a	0.00 a
Macit 55	16.1 b	1.31 a	79.0 b	4.08 b	0.66 bd
Ali Nihat	34.1 c	2.83 ab	**	**	**
A9	44.4 c-e	3.55 b-d	88.8 bc	4.10 bc	0.76 c-e
A55	63.0 d-f	3.67 cd	91.0 c	4.33 bd	0.85 de
A56	33.7 c	2.53 b	94.7 c	4.58 bd	0.86 de
BDB-L	38.1 cd	2.90 ab	93.3 c	4.66 bd	0.86 de
P	≤0.01	≤0.01	≤0.01	≤0.01	≤0.01

* There is no difference between the means indicated by the same letter in the same column.
** Data not available.

In Japan, 'Tanzawa', 'Otumune', and 'Tsukuba' cultivars were selected as resistant to the ACGW (SAITO *et al.*, 2018). In Italy, SARTOR *et al.* (2009) tested 41 chestnut cultivars and hybrids for their resistance to the ACGW. In three years of observations, complete resistance to the ACGW was only found in the 'Bouche de Bètizac' cultivar (*C. sativa* x *C. crenata*) with no gall formation. Registration of the 'Bouche de Bètizac' cultivar was also done by the Republic of Turkey Ministry of Agriculture and Forestry Variety Registration and Seed Certification Center. It can be used for the establishment of ACGW resistant orchards. However, 'Bouche de Bètizac' is a pollen-sterile cultivar (METAXAS, 2013). 'Akyüz' is not pollen sterile and can be used as a resistant cultivar to the ACGW.

In a previous study by GENCER *et al.* (2017), chestnut trees growing in Yalova and Bursa demonstrated various levels of susceptibility to the ACGW. The infected bud ratio of 'Bursakestanesi', 'Hacıömer', 'Osmanoğlu', 'Marigoule', 'Maraval' and 'Alimolla' cultivars and wild chestnut genotypes ranged between 2-100%. The lowest infected bud ratio observed was on

'Tülü' (2%) and 'Maraval' (7%) cultivars. On the other hand, the highest infected bud ratio observed was on the 'Marigoule' cultivar (84%) and seedling trees (100%). Similar results for 'Mariguole" were obtained in our study, which indicates that 'Marigoule' is sensitive to the ACGW. Also, SARTOR et al. (2015) indicated that 'Marigoule' is sensitive to the ACGW.

Microscopic observations

The 'Akyüz' cultivar showed the best results among the evaluated cultivars and genotypes in this study. No galls were observed in the 'Akyüz' cultivar. As a result of the microscopic observations conducted through eight 'Akyüz' branches with a total of 107 buds, we found 971 eggs of the ACGW. The average number of eggs per branch was 121.37 (22-227) and 9.07 (1-40) per bud. It is reported that *D. kuriphilus* lays 20-30 eggs in buds (EPPO, 2005) which is similar to our results. Also, in our study, the ACGW laid eggs into buds of all cultivars tested without exception. In Southern Italy, NUGNES *et al*. (2018) state that the 'Red Salernitan' ecotype (RSE) of *C. sativa* has moderate resistance, and there were few eggs in the buds. Also, in the buds of the resistant cultivar, 'Tsukuba' and 'Daap', 15 or more and one or fewer eggs were found, respectively (KIM *et al*., 2005). These results were consistent with our results. It is understood that the ACGW lays eggs into the chestnut buds. However, due to some reasons, larvae do not develop. Microscopic observations showed that in the buds of the 'Akyüz' cultivar, necrosis was observed in the cell where the larva was found.

The other cultivars and genotypes ('Macit 55', 'Ali Nihat', A9, A55, A56) genetic backgrounds, which are similar, were not resistant to the ACGW as 'Akyüz'. GENG *et al*. (2015) worked on the resistance mechanism based on candidate genes of *C. mollissima* 'Shuhe-WYL' race to *D. kuriphilus* at the molecular level. As a result, the resistance mechanism could be explained as the high expression of the OsCDPK2, receptor-like protein, OsNAC6 protein, and bHLH genes and PAL. ZHU *et al*. (2019) annotated 21306 genes using by BLAST database to investigate the dynamic changes in the genes of a *C. mollissima*. 2410, 7373, 6294, and 9412 genes were differentially expressed in four gall-formation stages; according to annotation analysis, tested genes could be essential for *D. kuriphilus* gall formation. Nevertheless, many more studies must be done to understand the resistance mechanism to the ACGW fully.

CONCLUSION

Among the cultivars and genotypes tested in the study, the 'Akyüz' cultivar was determined to exhibit the highest resistance against the ACGW. However, the mechanism for resistance is still unclear. On the other hand, the ACGW lays eggs on the buds of the 'Akyüz' cultivar. Therefore, the underlying resistance mechanism is probably not a result of this cultivar's repellent (or less attractive) character. Also, the 'Akyüz' cultivar was screened in the 2019 and 2020 growing seasons. Still, no galls were observed in the 'Akyüz' cultivar. (Registered on 25.10.2019), but currently there is a limited number of plants to distribute. Thus, efforts are being made to produce the 'Akyüz' cultivar in mass efficiently and in a timely fashion. Also, the 'Akyüz' cultivar can soon be used for breeding studies to obtain other resistant cultivars to the ACGW. To fully understand the resistance mechanism of the 'Akyüz' cultivar, molecular and biochemical studies should be done and compared with other results. As for further research, the 'King Arthur' and 'Lockwood' plants will be tested for ACGW in the USA.

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NIVOI OSETLJIVOSTI NEKIH KULTIVARA I GENOTIPOVA KESTENA NA KESTENOVU OSU U TURSKOJ

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Izvod

Azijska kestenova žučna osa (ACGV) je jedan od najvažnijih štetočina koji ugrožava većinu vrsta *Castanea*. Najbolja strategija upravljanja protiv ove štetočine može biti uspostavljanje novih voćnjaka sa otpornim sortama uz biokontrolu (parazit *Torymus sinensis* Kamijo (Himenoptera: Torimidae)). U Turskoj, ACGV je prvi put otkriven 2014. godine u selu Gacik u provinciji Jalova. Provincije Bursa, Istanbul, Sakaria, Kocaeli, Balıkesir, Bilečik, Duzce, Giresun, Bartin, Zonguldak, Sinop i Izmir su takođe zaražene ACGV. Ovo istraživanje je sprovedeno da bi se utvrdili nivoi osetljivosti sorti/genotipova kestena na ACGV. Studija je sprovedena u provinciji Jalova između 2016. i 2018. godine. Ukupno, 15 sorti/genotipova je bilo uključeno u studiju, uključujući evropski (C. sativa) kesten (Albairak, Altınai, 'Erfelek', 'Osmanoglu', Salıpazarı,' U studiji su testirani Serdar', 'Unal'), interspecifični hibridi ('Marigoule' i BDB-L) i kompleksni hibridi ('Akiuz', 'Macit 55', 'Ali Nihat', A9, A55, A56). U aprilu 2016. kalemljene su sadnice stare pet do sedam godina, a osetljivost na ACGV je procenjivana beleženjem odnosa zaraženih pupoljaka (%), vizuelnom procenom oštećenja (ozbiljnosti) i proračunom indeks zaraze. Među sortama i genotipovima testiranim u studiji, utvrđeno je da sorta 'Akiuz' pokazuje najveću otpornost na ACGV. Da bi se u potpunosti razumeo mehanizam otpornosti sorte 'Akiuz', treba uraditi molekularne i biohemijske studije i uporediti ih sa drugim rezultatima.

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