

## Assessing the susceptibility levels of chestnut cultivars/genotypes to Asian chestnut gall wasp (*Dryocosmus kuriphilus* Yasumatsu)

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### Abstract

The Asian chestnut gall wasp *Dryocosmus kuriphilus* is a major insect pest affecting chestnut trees worldwide. Due to the galls that it creates on shoots and leaves, this pest prevents shoot development and blooming and decreases the fruit yield. In Türkiye, this pest was first seen in Gacık village of Yalova province in the year 2014. Today, it is seen in orchards and forests in different provinces of Marmara, Aegean, and Black Sea regions. The medium- and long-term control of this pest can be improved by making use of resistant or less-susceptible cultivars and hybrid clones. The susceptibility levels of our cultivars to this pest are not known. For this purpose, by using three infestation indicators, the susceptibility to Asian chestnut gall wasp (ACGW) of 3 hybrids (*Castanea sativa* x *Castanea crenate*) and 20 cultivars/genotypes (*C. sativa*), which came to the forefront via selection studies, were assessed for five years (2017-2021). The susceptibility to ACGW varied between the tested genotypes/cultivars and, since no gall development was observed in 'YD-1', 'Tülü', and 'Ertan' cultivars of *C. sativa* species, they were determined to be completely resistant to ACGW. Furthermore, the numbers of ACGW eggs on the buds of cultivars were recorded and it was found that ACGW laid eggs on the buds of all the cultivars. In microscopic analyses, it was revealed that the resistance originated from the prevention of larva development within the bud. The finding that resistant cultivars belong to *C. sativa* species is thought to be important for future genetic breeding programs.

**Keywords:** *Castanea sativa*; cultivars; *Dryocosmus kuriphilus*; level of infestation; resistance

### Introduction

Türkiye is an important country considering the global chestnut production. The main chestnut production areas are the Black Sea, Aegean, and Marmara Regions of Türkiye. Particularly Marmara and Aegean regions have many domestic cultivars bred in previous centuries. On the other hand, grafted chestnut trees are rarely seen in the Black Sea region. Chestnut trees are useful for their fruits, woods, and so on. Unfortunately, diseases and pests have damaged trees severely, such that they may have caused decreases in the yield and sometimes killed the trees completely. In recent years, an insect named Asian chestnut gall wasp (ACGW) *Dryocosmus kuriphilus* Yasumatsu (Hymenoptera: Cynipidae), which caused decreases in chestnut

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yield, has been discussed. This insect is native to China and harmful to all species belonging to *Castanea* genus (Fagaceae) (Nugnes *et al.*, 2018). This insect prevents shoot growth and flower formation via gall formation on shoots and leaves and can decrease the fruit yield by 80%. If the insect population increases to a specific level, then it would kill young chestnut trees (Battisti *et al.*, 2014).

ACGW is a major insect pest affecting chestnut trees worldwide. It is an invasive species, which became a source of concern in many countries where chestnut is grown. This pest was detected first time in Türkiye in April 2014 in Yalova and Bursa provinces (Çetin *et al.*, 2014). Since the first detection, this pest was reported to spread in orchards and forests in different provinces of Marmara, Aegean, and Black Sea regions (Yıldız *et al.*, 2020; Mıcık *et al.*, 2021). It is very difficult to control this pest. Although some cultural procedures are implemented in specific orchards, there mainly are two effective control methods for this problem at this moment. The first one is the biological control using the parasitoid *Torymus sinensis* Kamijo (Hymenoptera: Torymidae) and the establishment of orchards with resistant or less-susceptible chestnut cultivars. In Yalova, the biological control activities using *T. sinensis* Kamijo were initiated in the year 2015. However, it takes time for biological control to be effective.

Chestnut gall wasp is a univoltine specie; it reproduces once a year. Reproduction occurs without mating (parthenogenesis). Its life cycle consists of the egg, three larva episodes, pupa, and adult periods. Adult females emerge early summer and lay eggs on the new buds on chestnut trees during this period (Cho and Lee, 1963). Multiple adults might lay eggs on a single bud. Its infestation creates simple or complex galls, which can be mono- or multilocular and mainly develop on shoots (stem gall) and leaves. Medium- and long-term control of this insect pest can be improved by using resistant or less-susceptible cultivars and hybrid clones.

The existence of chestnut cultivars or genotypes that are resistant to *D. kuriphilus* was first reported in Japan by Shiraga (1948). In Italy, breeding studies aiming to select resistant individuals were started after the infestation by this pest. Susceptibilities of 62 different cultivars to this insect pest were assessed and 7 resistant cultivars were detected. One of them belongs to *C. crenata* ('Idea'), 4 belong to Europe x Japan hybrid ('Bouche de Bétizac', 'Marlhac', 'Maridonne', and 'Vignols'), and 2 were *C. sativa* cultivar 'Pugnenge' (Italian cultivar) and 'Savoie' (French cultivar). Moreover, significant differences were found between susceptibility levels of 64 cultivars (Sartor *et al.*, 2015) and other researchers stated that there were differences between susceptibilities of chestnut cultivars/genotypes (Panzavolta *et al.*, 2012; Míguez-Soto *et al.*, 2018; Castedo-Dorado *et al.*, 2021).

*Castanea sativa* Mill. has spread out in European countries and Anatolia. Despite the sensitivity of this species against the pest, it has been known that the genotypes of this species have shown great variation in this respect. This species has been grown in Türkiye under different ecological conditions and, considering the rich sources of the genotypic variation taken, it can be thought that there might be different genotypes having different sensitivity levels in the populations. Türkiye has many cultivars/genotypes with good organoleptic characteristics. Superior chestnut genotypes within the seedling populations found in naturally grown chestnut forests were selected by selection studies (Ayfer *et al.*, 1977; Ozkarakas *et al.*, 1995; Serdar, 1999; Serdar and Soylu, 1999; Serdar, 2002; Ertan *et al.*, 2007; Omerci *et al.*, 2016). However, the sensitivity levels of the genotypes have not been known. Preliminary observations showed that there was a difference among the genotypes in relation to the gall intensity in the contagious areas.

As a criterion for the level of infestation, susceptibility to chestnut gall wasp is assessed by making use of the effects such as the number of galls per bud or the ratio of infected buds (Kotobuki *et al.*, 1985; Sartor *et al.*, 2009). Moreover, since it is an important factor while analyzing the effects of insect pests, it is important to also consider the ratios and locations of galls forming on the plant organ during the assessment of susceptibility (Castedo-Dorado *et al.*, 2021).

A chestnut collection orchard incorporating cultivars/genotypes selected from different regions of Bursa province was established in the year 2013 in order to carry out scientific studies and preserve the genetic

material. In that chestnut collection orchard also includes 19 cultivars/genotypes of *Castanea sativa* species, which are grown in different regions and come to the forefront via selections studies, and ‘Bouche de Betizac’ and ‘Marigoule’ hybrid clones (*C. sativa* x *C. crenata*). Since the orchard is located in an area that is highly contaminated by ACGW, it led us to investigate the ACGW resistance of these cultivars, which have good organoleptic properties and are grown in different regions. In the present study, by conducting observations and counting on trees from 23 cultivars/genotypes located in the chestnut collection orchard and a farmer’s orchard in the same region for 5 years (2017-2021), ACGW susceptibilities were determined using different infestation indicators. At the same time, the number of eggs laid on a bud was counted for 3 years (2018 to 2020) for each cultivar and the reason for the resistance was examined by analyzing buds, which were taken at different time points of the dormant period, under a stereo microscope.

## Materials and Methods

In the present study, 23 chestnut cultivars/genotypes, 21 in the Chestnut Collection Orchard located in Bursa-Cumalıkızık and 2 (‘Maraval’ and ‘Tülü’) in a farmer’s orchard in the same region, were examined. The cultivars/genotypes examined in the present study and their origins are presented in Table 1. The chestnut collection orchard was established in the year 2012. There are 2 hybrid clones and also cultivars/genotypes, which were selected in natural distribution areas in Marmara, Aegean, and Black Sea regions. The altitude of the orchard is 345m. Trees were planted at 8x8m distances. It is assumed that the insect pest came in the summer of 2014 because the fresh galls were observed first in the growth season of 2015. The population of this pest rapidly increased in the following years. The study was started 2 years after the infestation by gall wasps in the area.

**Table 1.** List of 23 chestnut cultivar/genotypes evaluated in this study and their geographic origin

Cultivar/genotype	Species	Origin
‘Alimolla’ (G)	<i>Castanea sativa</i>	Bursa/Cumalıkızık
‘Derekızık’ (G)	<i>Castanea sativa</i>	Bursa/Fidyekızık
‘Dursun’	<i>Castanea sativa</i>	İnegöl/Esenköy
‘Gavuraşı’ (G)	<i>Castanea sativa</i>	Bursa/Fidyekızık
‘Hacıömer’	<i>Castanea sativa</i>	Yalova/Gacık
‘Mahmutmolla’	<i>Castanea sativa</i>	Bursa/Cumalıkızık
‘Öküzgüzü’ (G)	<i>Castanea sativa</i>	Bursa
‘Sarıaşlama’	<i>Castanea sativa</i>	Bursa
‘Sarıkeşane’ (G)	<i>Castanea sativa</i>	Yalova-Şenköy
‘Seyrekdiken’	<i>Castanea sativa</i>	İzmit-Yenice-Balaban
‘Tülü’ (G)	<i>Castanea sativa</i>	Bursa/Cumalıkızık
‘Karamehmet’	<i>Castanea sativa</i>	Yalova/Karamürsel
‘Firdola’	<i>Castanea sativa</i>	Yalova/Karamürsel
‘YD-1’ (G)	<i>Castanea sativa</i>	Yalova/Karamürsel
‘Erfelek’	<i>Castanea sativa</i>	Sinop
‘Serdar’	<i>Castanea sativa</i>	Samsun
‘Ersinop’	<i>Castanea sativa</i>	Sinop
‘Ertan’ (N-7-3)	<i>Castanea sativa</i>	Aydın
‘N-2-5’ (G)	<i>Castanea sativa</i>	Aydın
‘N-23-1’ (G)	<i>Castanea sativa</i>	Aydın
‘Marigoule’	<i>Castanea crenata</i> x <i>Castanea sativa</i>	Fransa
‘Maraval’	<i>Castanea crenata</i> x <i>Castanea sativa</i>	Fransa
‘Bouche de Bétizac’	<i>Castanea crenata</i> x <i>Castanea sativa</i>	Fransa

G: genotype

*Evaluation of susceptibility*

Galls on trees were counted in June for 5 years (2017 to 2021). The buds were considered infested if a gall was observed in any organ of shoots forming as a result of the elongation of buds. Moreover, galls were recorded by classifying them as located on a shoot, leaf, or stipule (Gençer and Mert, 2019). For each cultivar, at least 200 newly developed shoots selected from all directions on the trees were assessed. These data were used in calculating the susceptibility index values of cultivars.

Three susceptibility indices focusing on assessing the damage caused by the ACGW attack were calculated and interpreted. The first one is the ratio of the number of infected buds (aB) (the buds with galls) to the total number of buds (tB) (Kotobuki *et al.*, 1985). The second one is the ratio of the number of galls on shoots (sG) to the total number of buds (tB). And the third one is the ratio of the number of galls on shoots (sG) to the total number of galls (tG) (Castedo-Dorado *et al.*, 2021). Moreover, the ratios of galls observed on shoots, leaves, and petioles were calculated for each cultivar/genotype (Gençer and Mert, 2019).

*Counting the eggs on buds and microscopic analyses*

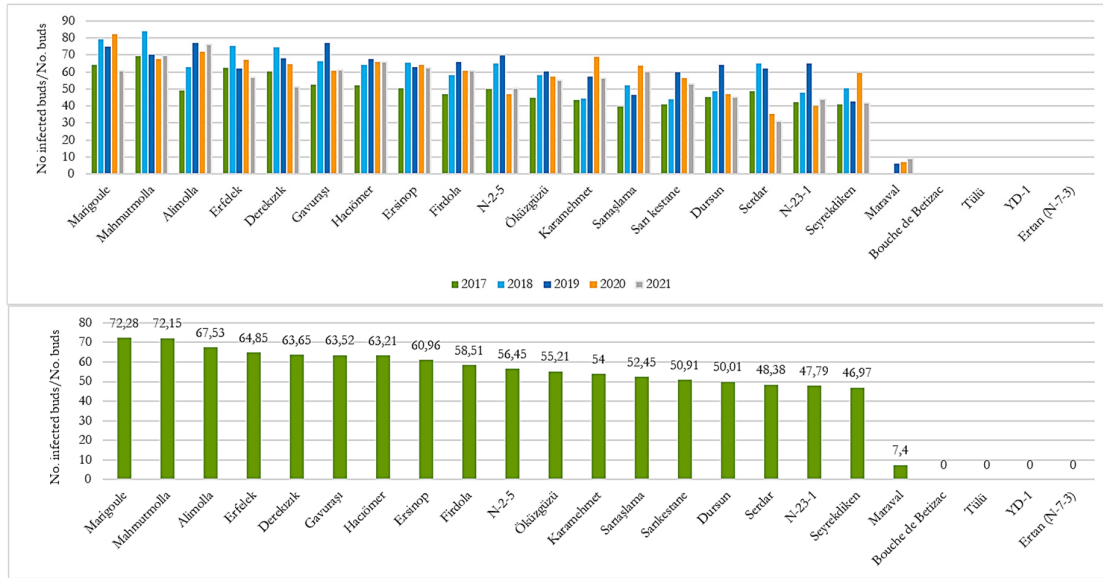
The number of ACGW eggs per bud was recorded for three years (2019 to 2021). First, to determine the time of taking the bud samples, yellow adhesive traps were hung in the orchard and the emergence of ACGW was followed. During the period when ACGW flights decreased (end of July), fresh shoots (5-6 pieces) were taken from different directions on the trees, and then taken to the laboratory in a cold container. By using the switching-on method, the number of eggs of this pest per bud was counted and recorded using a stereo microscope. By counting the eggs in at least 25 buds for each cultivar/genotype, the mean number of eggs per bud was determined.

To investigate the reason for resistance, the buds taken at different time points during the dormant period (November, December, and January) were examined using a stereo microscope. The photos of the areas, which were considered to be suitable, were taken using a DP-20 camera system. Analyses were conducted on at least 10 buds for each cultivar/genotype.

**Results and Discussion**

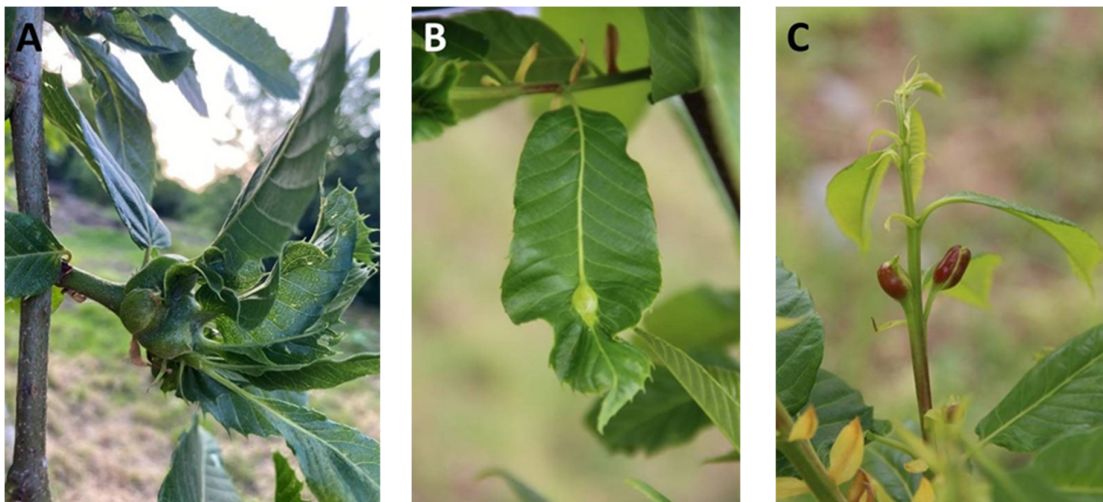
Susceptibility to *D. kuriphilus* was examined for a 5-year period (2017-2021) in 23 cultivars/genotypes that are largely grown. The susceptibility index was calculated for each cultivar by computing the ratio of the number of attacked buds (i.e., buds with galls) to the total number of buds (aB) (Kotobuki *et al.*, 1985). Susceptibility indices of cultivars/genotypes by years are illustrated in Figure 1. In general, an increase was observed in the ratio of buds, on which gall formed, by years and then followed by a partial decrease. ACGW susceptibility of cultivars/genotypes varied. Given the mean value of the 5-year period, it was determined that the ratio of gall-containing buds of cultivars/genotypes ranged between 7.2% and 72.28% (Figure 1). The lowest value was observed in 'Maraval' and the highest one in 'Marigoule' (72.28%), followed by 'Mahmutmolla' (72.15%) and 'Alimolla' (67.53%). The ratio of gall-containing buds was found to be lower than 50% in 'Serdar' (48.38%), 'N-23-1' (47.79%), 'Seyrekdiken' (46.97%), and 'Maraval' (7.4%) cultivars, whereas it was higher than 50% in others. During these analyses for 5 years, it was revealed that most of the chestnut cultivars were susceptible to ACGW attack. Besides that, no gall formation was observed in 4 cultivars in any of the five years and these cultivars were considered to be fully resistant to ACGW. One of them was the hybrid clone 'Bouche de Betizac', whereas others were 'YD-1', 'Tülü', and 'Ertan' cultivars, the resistance of which was determined for the first time, from *C. sativa* species (Figure 1). In some studies resistance was reported for 'Bouche de Betizac' cultivar before (Dini *et al.*, 2012; Sartor *et al.* 2015). To date, all the ACGW-resistance cultivars were from different species of European chestnut (*C. sativa* Mill.). They were interbred with Chinese chestnut (*C. mollissima* Blume), Chinquapin, or *C. pumila* (L.) Mill.) or *C. crenata*. Resistance

was reported in only two cultivars of *C. sativa* ('Pugnenga' and 'Savoie') (Sartor *et al.*, 2015), but couple of less-susceptible cultivars or genotypes were identified (Sartor *et al.*, 2015; Bombi *et al.*, 2018; Miguez-Soto *et al.*, 2018; Nugnes *et al.*, 2018; Castedo-Dorado *et al.*, 2021). Considering the resistance mechanism and genetic studies, the full resistance to ACGW in three cultivars from *C. sativa* being grown and having good organoleptic properties was considered to be important.



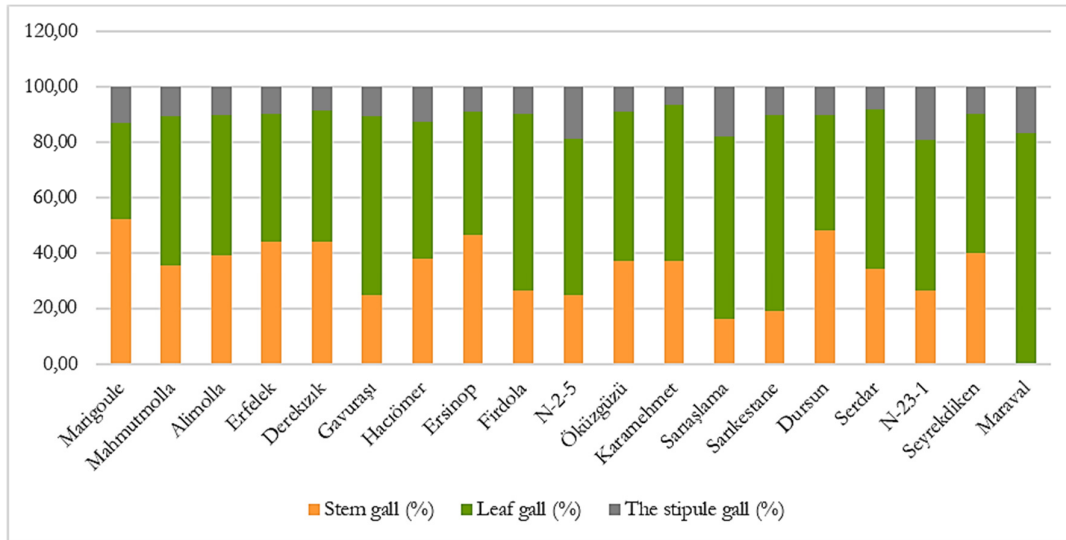
**Figure 1.** Values of indicator used for assessing the susceptibility level (no infected buds/no buds) of 23 chestnut cultivars/genotypes (first row) by years (2017-2021) and susceptibility levels of cultivars/genotypes by 5-year averages (second row)

Gall formations observed in shoots (Figure 2A), leaves (Figure 2B), and stipules (Figure 2C) as a result of the elongation of buds infected by the eggs of the insect pest during the vegetation period (Gençer and Mert, 2019). From this aspect, the gall types observed in each cultivar during the 5-year period (2017-2021) were recorded by classifying them as gall on shoot (stem), leaf, and stipule.

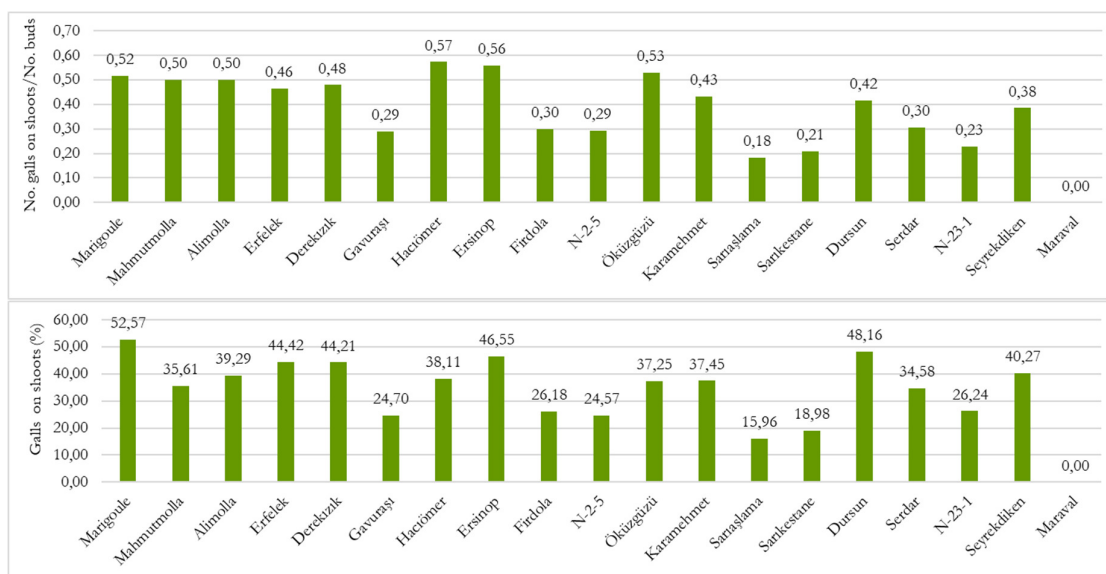


**Figure 2.** The galls formed in the chestnuts by *Dryocosmus kuriphilus*, gall on shoot (A), leaf gall (B) and stipule gall (C)

The ratios of stipule, leaf, and stem gall by cultivars presented in Figure 3. Considering the cultivars/genotypes, the ratio of gall formation was found to range between 6.58% and 19.01% for stipules, between 34.55% and 83.33% for leaves, and between 15.96% and 52.57% for stems. Stem gall was observed to be at 40% or higher levels in ‘Marigoule’ (52.57%), ‘Dursun’ (48.16%), ‘Ersinop’ (46.55%), ‘Erfelek’ (44.42%), ‘Derekızık’ (44.21%), ‘Alimolla’ (41.98%), and ‘Seyrekdiiken’ (40.27%) cultivars, whereas it was lower than 20% in ‘Sarışlama’ (15.96%) and ‘Sarıkestane’ (18.98%) cultivars. No gall formation was observed on shoots in ‘Maraval’ cultivar. Ratios of gall types provide information about to what extent the cultivar was affected by the pest. The damage of the pest is at a higher level for the cultivars having higher stem gall ratios because the presence of galls on shoots prevents stem elongation and green biomass formation, therefore, galls are responsible for the high proportion of dead shoots and the most significant reduction in leaf area (Kato and Hijii, 1997; Gehring *et al.*, 2020). Some researcher recommended the use of the ratio of galls on shoots since they are more useful in assessing the real damage in chestnut trees (Gençer and Mert 2019; Castedo-Dorado *et al.*, 2021) To better interpret the ACGW damage, the cultivars/genotypes were evaluated using two different susceptibility indices suggested by Castedo-Dorado *et al.* (2021) (Figure 4). Considering the ratio of the number of galls on shoots to the total number of buds, ‘Sarışlama’ (0.18), ‘Sarıkestane’ (0.21), ‘N-23-1’ (0.23), ‘N-2-5’ (0.29), ‘Gavuraşı’ (0.29), ‘Serdar’ (0.30), and ‘Firdola’ (0.30) cultivars were found to be 0.30 and lower, whereas ‘Hacıömer’ (0.57), ‘Ersinop’ (0.56), ‘Marigoule’ (0.52), ‘Öküzgözü’ (0.53), ‘Alimolla’ (0.50), and ‘Mahmutmolla’ (0.50) cultivars were found to be 0.50 and higher. Considering the ratio of the number of galls on shoot to the total number of galls, the lowest levels of gall formation on shoots were found in ‘Sarışlama’ (15.96%), ‘Sarıkestane’ (18.98%), ‘N-2-5’ (24.57%), ‘Gavuraşı’ (24.70%), ‘Firdola’ (26.18%), and ‘N-23-1’ (26.24%) cultivars, whereas the highest levels were found in ‘Marigoule’ (52.57%), ‘Ersinop’ (46.55%), ‘Erfelek’ (44.42%), ‘Derekızık’ (44.21%), ‘Dursun’ (48.16%), and ‘Seyrekdiiken’ (40.27%). These ratios show the differences between ACGW susceptibility levels of cultivars/genotypes. The ranking of cultivars/genotypes varied depending on the two indices used in assessing the level of infestation, although the lowest values were observed in ‘Maraval’, ‘Sarışlama’, and ‘Sarıkestane’.

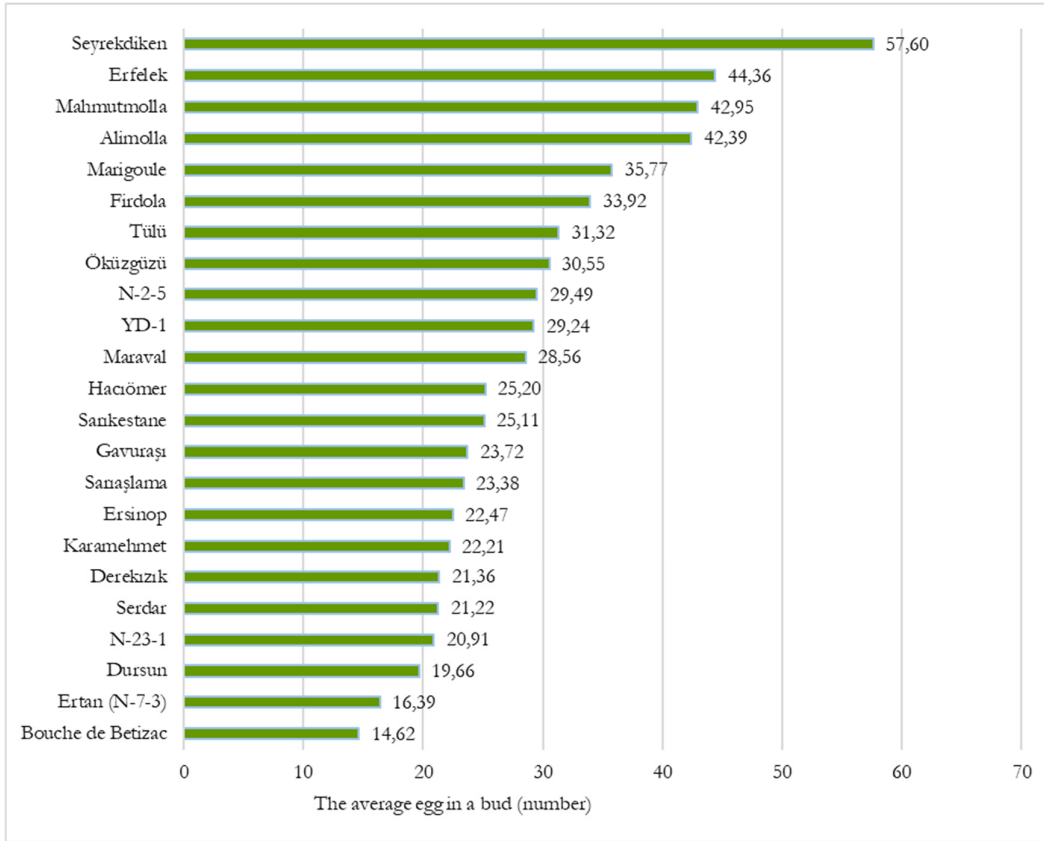


**Figure 3.** Ratios of stem, leaf and leaf stipule galls of chestnut cultivar/genotypes



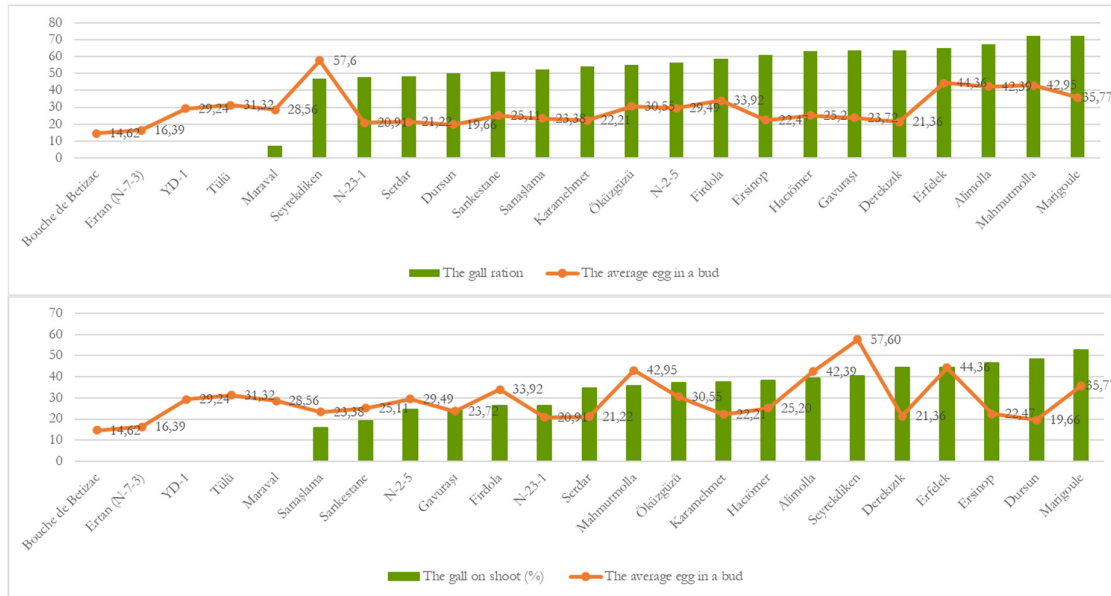
**Figure 4.** Values of indicator used for assessing the susceptibility level. The ratio between the number of galls on shoots and the number of buds (first row), the ratio of the number of galls on shoot (second row) of cultivars/genotypes

The number of eggs on each bud was determined for each cultivar/genotype for 3 years (2019 to 2021) and the mean values of the 3-year period presented in Figure 5. By the cultivars/genotypes, the mean number of eggs ranged between 14.62 and 57.60. It was found that ACGW laid eggs also on cultivars considered to be fully resistant. It was observed that ACGW's egg-laying intensity differed between both susceptible and resistant cultivars. It was found that they laid eggs more intensely on resistant 'Tülü' and 'YD-1' cultivars/genotypes and fewer eggs on 'Ertan' and 'Bouche de Betizac' cultivars/genotypes. The results achieved here are consistent with those reported by Fukuda and Okudai (1951), who concluded that egg-laying selectivity didn't differ between susceptible and resistant chestnut cultivars. The cultivars/genotypes chosen for laying eggs the most are 'Seyrekdikeni' (57.60 eggs), 'Erfelek' (44.36 eggs), 'Alimolla' (42.39 eggs), and 'Mahmutmolla' (42.39 eggs), whereas those preferred the least were 'Dursun' (19.66 eggs), 'Ertan' (16.39 eggs), and 'Bouche de Betizac' (14.62 eggs). Several studies were carried out on the cultivar preference of this insect pest for laying eggs (Kato and Hijii, 2001; Panzavolta *et al.*, 2012; Guyot *et al.*, 2015; Karaoğlu, 2018; Castedo-Dorado *et al.*, 2021). While *D. kuriphilus* was reported to prefer larger buds to lay eggs on (Kato and Hijii, 2001; Panzavolta *et al.*, 2012), some studies stated that the size of bud didn't play an effective role in ACGW's preference for laying eggs (Karaoğlu, 2018; Castedo-Dorado *et al.*, 2021). It was emphasized that a remarkable portion of ACGW's lay eggs on the same tree, on which they came into the world, and the rest colonized in nearby trees (Castedo-Dorado *et al.*, 2021). The results achieved here suggested that studies should be carried out in order to examine which plant characteristics affect the egg-laying preference of ACGW.



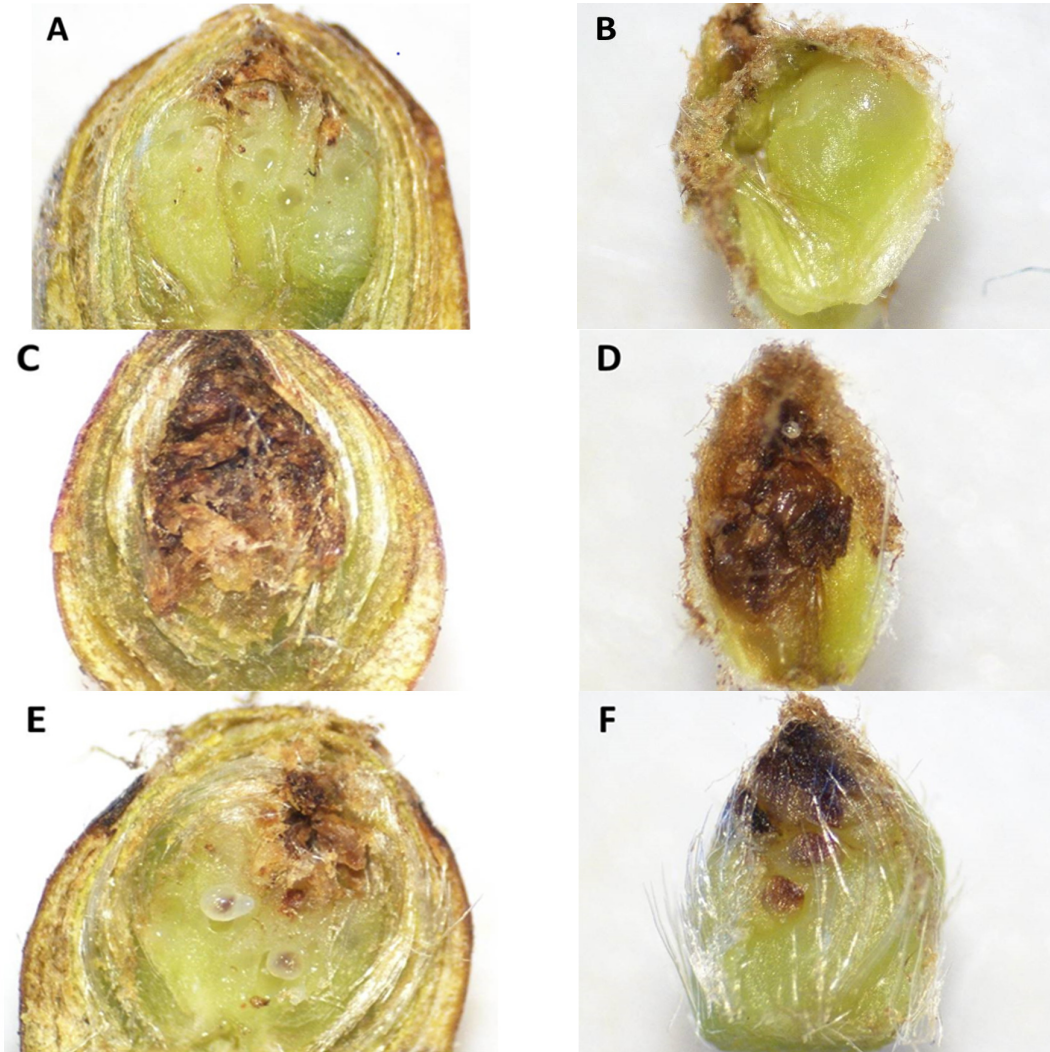
**Figure 5.** Mean number of eggs on each bud for the chestnut genotypes/cultivars assessed

The ratio of the mean number of eggs per bud to the gall ratios ( $G/tB$ ) and the ratio of the number of galls on shoot ( $sG/tG$ ) were comparatively assessed (Figure 6). It was determined that the differences between the levels of ACGW infestation by cultivars/genotypes couldn't be related to the number of eggs on buds. Similarly, the ratio of galls on shoots was found to not be related with the number of eggs on buds. Thus, by taking bud samples from different cultivars/genotypes at different times (November-January), they were examined under a stereo microscope. In observations conducted on all cultivars/genotypes, it was found that epidermal cells surrounded the young larva by proliferating and the tissue developments creating a larva room were observed (Figures 7A-F).



**Figure 6.** Comparison between the ratio of the mean number of eggs on a bud to the gall ratios (G/tB) (first row) and the ratio of the mean number of eggs on a bud to the number of galls on a shoot (sG/tG) for chestnut cultivars/genotypes assessed

The fully-resistant cultivars/genotypes seen that there was necrosis on the tissues surrounding of larva room and they prevented gall development (Figures 7C and 7D). It is thought to be caused by induced resistance. It was observed that, in less-susceptibility cultivars/genotypes, there were some necrotic spots (death) in developing gall tissues, i.e., some gall tissues could not develop (Figures 7E and 7F). These microscopic examinations suggest that different levels of resistance to ACGW seen in cultivars/genotypes originated from the prevention of larva development within buds. As a result of microscopic examinations on resistant cultivars, Japanese researchers observed that the tissue surrounding the living chamber of the larva induced necrosis and pest death occurred at level of the larval chamber in the resistant genotypes (Shimura, 1972). It was reported that buds of resistant ‘Bouche de Bétizac’ cultivar contained larvae or eggs and no larva development was observed after the bud burst (Dini *et al.*, 2012).



**Figure 7.** Stereo microscope micrographs of the gall tissues in bud. (A) Larvae and larval chambers in gall tissues in longitudinal section of bud; (B) Developing gall on leaf primordia; (C) The necrosis on gall tissues and dead larvae in longitudinal section of bud; (D) The necrosis on gall tissues; (E) The larvae and larval chambers in gall tissues and some gall tissue necrosis in longitudinal section of bud; (F) Developing galls and some necrotic spots on the tissues surrounding the galls

Necrotic lesions seen in gall tissues were interpreted as a larva induced hypersensitive response (HR) (Figures 7C-F). The induction and development of galls expose plant tissues to high oxidative stress. In response to these gall-forming insects activities, plants change their biochemical status, eliciting a HR. Hypersensitive response is one of the best known resistance reactions (Höglund *et al.*, 2005). It is effective in different biochemical mechanisms. Little is known about the defensive patterns of chestnuts against ACGW. Therefore, biochemical and molecular mechanisms should be investigated in cultivars resistant to ACGW.

## Conclusions

In conclusion, ACGW susceptibility levels of 23 cultivars/genotypes were examined. It was found that 'YD-1', 'Tülü', and 'Ertan' cultivars/genotypes of *C. sativa* species were fully resistant to ACGW, while 'Maraval', 'Sarıağlama', and 'Sarıkestane' cultivars had lower susceptibility levels. It was observed that ACGW laid eggs on all cultivars/genotypes and the differences between the levels of ACGW infestation in cultivars/genotypes were not related with the number of eggs on buds. During the microscopic examinations on buds, it was revealed that necrotic spots developed on tissues, where larva rooms formed, in fully resistant and less-susceptible cultivars and there were larva deaths. It was thought that those deaths might be caused by induced resistance and, at this moment, this resistance mechanism is examined with the secondary metabolite, RNA-Seq, and histological approaches. Moreover, study results provided important conclusions about the plant material selection for new orchard establishment in areas contaminated by ACGW and contributed to the existing knowledge on ACGW susceptibility.

## Authors' Contributions

Conceptualization: CM; Data curation and Formal analysis; Methodology; Visualization: CM and BM, Writing - original draft: CM; Writing - review and editing: BM and NSG. All authors read and approved the final manuscript

## Ethical approval (for researches involving animals or humans)

Not applicable.

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## Conflict of Interests

The authors declare that there are no conflicts of interest related to this article.

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