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Original Article

Studies on the Gall Characteristics of *Dryocosmus kuriphilus* in Chestnut Genotypes in Yalova and Bursa Provinces of Turkey

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Abstract

The Asian chestnut gall wasp *Dryocosmus kuriphilus* is a global pest of chestnut (*Castanea* spp.). It has been spreading in Turkey's forests and orchards since 2014. This pest imposes a big threat to the Turkish chestnut industry, which is among the top producers in the world. Its gall morphology has been related to pest pressure and host cultivar, thus eventually modulating plant damage with heavy impact on growth and fruit production. We compared gall characters (position on plant organ, ratios, dimensions, volumes, number of larval chambers) in wild *Castanea sativa*, two local cultivars and a Euro Japanese hybrid. Overall, leaf galls were more common (55.36%), followed by the stem (19.6%) and leaf stipule galls (15.29%). The mean number of chamber and volume value of gall types were 1.52-5.93 and 0.43-2.15 cm^3, respectively. The highest values were observed in 'stem gall'. The more gall formation was observed in the wild chestnut trees and 'Marigoule' than the other local varieties.

Keywords: chambers; Cynipidae; gall types; gall wasp; Hymenoptera

Introduction

Turkey is one of the main chestnut producing countries in the world. Annual nut production is about 60,000 tons. In addition chestnut populations are widely spread in the coastal regions e.g. Black Sea, Marmara, and Aegean Sea Regions. These areas have generous populations of *Castanea sativa* Mill., with approximately 2,5 million trees. Therefore, a wide range of genetic variability existed in the populations. Genetic variability exists in nut and tree characteristics, yield, and probably pest and disease resistance, as well as other agricultural traits. However, some lethal diseases of chestnut e.g. ink disease (caused by *Phytophthora* sp.), present since 1940, and later chestnut blight disease (caused by *Cryphonectria parasitica*, present since 1968) had seriously damaged the chestnut grooves and the annual nut production has decreased significantly, especially in Marmara and Black Sea Regions. Damage has been observed recently in the Aegean Region due to the harmful effects of chestnut blight (Soylu and Mert, 2009).

The Asian chestnut gall wasp *Dryocosmus kuriphilus* Yasumatsu (Hymenoptera: Cynipidae) is a serious invasive pest of chestnut globally. The wasp is native to China, but the pest was accidentally introduced first to Japan and Korea and then to the U.S.A. (Shiraga, 1951; Cho and Lee, 1963; Payne *et al.*, 1975; Moriya *et al.*, 1989). In 2002, the wasp was also detected in Europe (Brussino *et al.*, 2002; Seljak, 2006; EPPO, 2007; EPPO, 2010a; EPPO, 2010b; Matošević et *al.*, 2010; Michaelakis *et al.*, 2016). In Turkey, it was first reported in Yalova in 2014 and later in Bursa (Çetin *et al.*, 2014).

Dryocosmus kuriphilus is univoltine and reproduces parthenogenetically. During the oviposition period from June to July, females emerge and lay eggs in the buds of chestnut trees (Cho and Lee, 1963). During spring, at the time of bud burst, galls were seen on new chestnut organs (Cooper and Rieske, 2010; Panzavolta *et al.*, 2012; Morath *et al.*, 2015). Galls are unilocular or multilocular, green- or red-colored, and provide larvae with a nutritive supply (Ôtake, 1980; 1989; Cooper and Rieske, 2011). *D. kuriphilus* gall formation can lead to a marked decrease in fruiting and growth rate, and, in extreme cases, result in plant death (Cho and Lee, 1963; Battisti *et al.*, 2014; Sartor *et al.*, 2015).

The gall is worthy of study for its varying characteristics. It is a product of the interspecific association of the plant with the insect, which develops a specific novel structure in response to the insect stimulus (Weis and Abrahamson,

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1986; Raman, 2007; 2011). Development of a gall can be seen to include three phases: initiation, growth and maturation (Maresquelle and Mayer, 1965). The gall development starts with the wasp laying eggs in the meristematic tissue of the host plants (Rey, 1992). Galls induced by the Cynipidae, with an outer cortical parenchyma and an inner cavity that contains one to many larval chambers (Hernandez-Soto *et al*., 2015). Unilocular and multilocular larval chambers containing one *D. kuriphilus* larva per chamber in buds and stem galls (Warmund, 2013). In some studies, it was reported that there were 3.5 larvae per gall (Radócz *et al*., 2016). Gall size can depend on various factors, such as oviposition strategy, the type of habitat-for example, in highly fragmented habitats (Kato and Hijii, 1993), parasitism pressure (Cooper and Rieske, 2010),the phase of population dynamic (Miyashita *et al*., 1965) and host plant (Nugnes *et al*., 2018). Also, gall inhabitants and histology of gall development were studied in different articles (Warmund, 2013; Reale *et al*., 2016). Additionally, Maltoni *et al.* (2012), classified the damages caused by galls in chestnut trees.

The objective of the present study was to compare *D. kuriphilus* gall characteristics (number of leaf, stem and stipule galls, gall size, number of larval chambers) in two local chestnut cultivars including the wild chestnut trees and an Euro Japanese hybrid.

Materials and Methods

Study site, biological material and procedures

The investigations were carried out in the commercial chestnut orchards at Yalova (Gacık village) (40°.3645' N, 29°.2023' E) and Bursa (Cumalıkızık village) (40°.1021' N, 29°.1016' E) Provinces of Turkey. Two local (cvs. 'Hacıömer' and 'Bursakestanesi'), one hybrid chestnut cultivar (cv. 'Marigoule') and wild chestnut trees were selected for this study. In spring 2016, 20 pieces of two-year-old shoots (30-40 cm length) (a total of 100 shoots) were collected from every chestnut variety between April to June at two-week intervals, and the current generation of galls was counted (Panzavolta *et al*., 2012; Bernardo *et al*., 2013). Galls were classified into three categories: stem galls (occurring on the chestnut shoot with 2-6 attached leaves), leaf galls (occurring along the leaf lamina) and leaf stipule galls (Fig. 1 A, B, C, D). (Cooper and Rieske, 2010; Panzavolta *et al*., 2012).

Galls were examined in the laboratory to measure their lengths (-from the shoot-) and width (-at the widest point-) with an automatic micrometer. In each cultivar, 30-50 specimens were measured for every gall type. The volume of each gall was estimated using the volume formula for a cylinder (Panzavolta *et al*., 2012).

Each gall was then dissected at approximately 2 mm thickness intervals to record the number of unilocular and

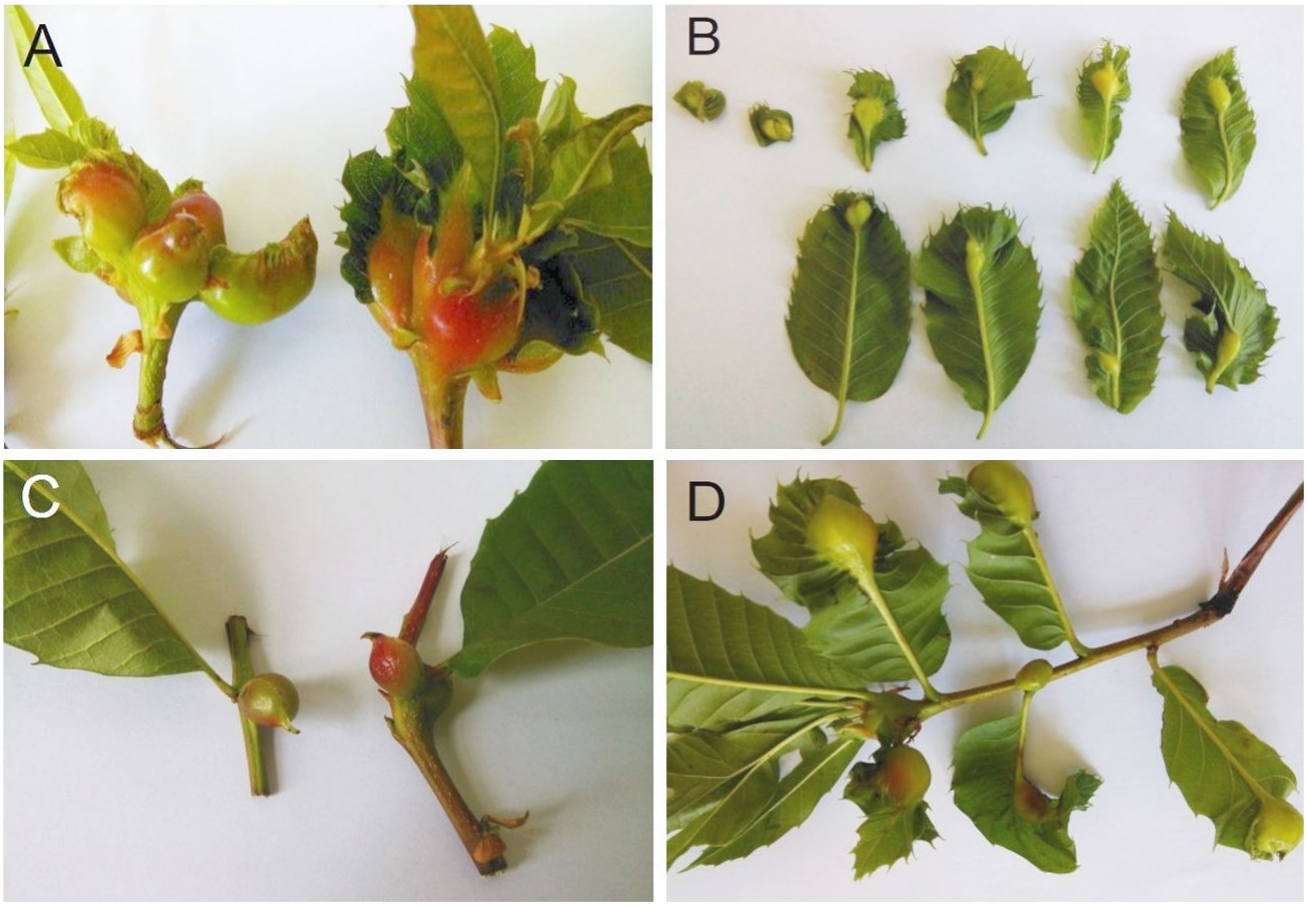

Fig. 1. The galls formed in the chestnuts by *Dryocosmus kuriphilus*, the stem gall (A), the leaf gall (B), the leaf stipule (C) and the leaf galls, stem gall and leaf stipule gall on young shoot (D)

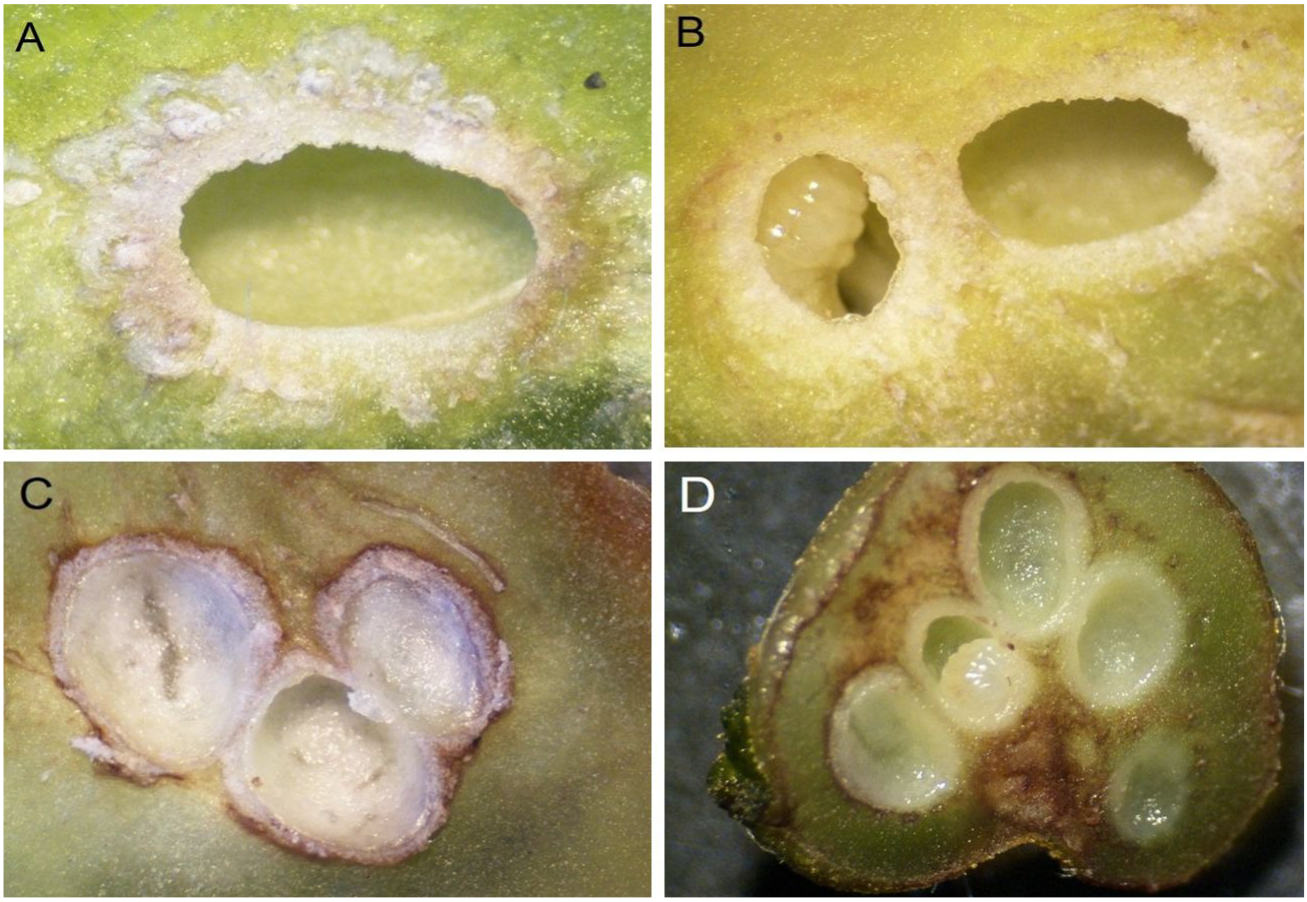

Fig. 2. Unilocular (A) and multilocular (B-D) chambers and *D. kuriphilus* larvae in gall

multilocular chambers present (Fig. 2 A, B, C, D). A total of 100 stem and leaf galls and 50 leaf stipule galls were used for the dissections. Sections were observed with a stereomicroscope. Number of chambers per leaf, stem and leaf stipule gall were counted.

Statistical analysis

Statistical comparisons of the mean values were performed using one-way analysis of variance (ANOVA), followed by Duncan's multiple range test ($p < 0.05$ confidence level) using Minitab 17.0 software.

Results and Discussion

When all genotypes were evaluated, the ratios, average number of chambers, and volume of gall types are given in Table 1. Overall, leaf galls were more common (55.36%), followed by the stem (19.6%) and leaf stipule galls (15.29%). The average number of chamber and volume value of gall types were 1.52-5.93 and 0.43-2.15 cm^3, respectively. The highest values were observed in 'stem gall'.

There were significant differences in gall type ratios in varieties (Fig. 3). Leaf gall ratios in 'Hacıömer' (71.28%) and 'Bursakestanesi' (65.53%) varieties were higher than 'Wild' (65.53%) and 'Marigoule' (23.55%) chestnut varieties (Fig. 3). However, stem gall ratios were high in cv. 'Marigoule' (45.52%) and wild chestnut trees (31.1%). The highest leaf stipule galls were observed in cvs. 'Marigoule' (19.94%), 'Hacıömer' (19.28%) followed by 'Bursakestanesi' (14.13%) (Fig. 3). Nugnes *et al.* (2018), stated that the number of stem galls found on the resistant chestnut trees was very low compared to those found on susceptible trees.

The mean number of leaf, stem and leaf stipule gall chambers varied significantly among varieties ($p < 0.05$) (Fig. 4). Since there were only a few stem galls, measurements were not taken in the 'Hacıömer' and 'Bursakestanesi' cultivars. The stem galls have the highest number of chambers, followed by leaf and leaf stipule galls. The leaf galls contained an average of 2.47, 3.34, 3.56 and 4.08 chambers in cvs. 'Bursakestanesi', 'Hacıömer', 'Marigoule' and wild trees, respectively. The mean number of stem gall chambers were found to be higher than the other gall types in wild chestnut (4.78) and 'Marigoule'

Table 1. Number of chambers, volume and ration of galls in total

Gall type	Gall (%)	Number of chambers/gall	Gall volume (cm^3)
Stem gall	19.6	5.93	2.15
Leaf gall	55.36	3.36	1.29
Leaf stipule gall	15.29	1.52	0.43

Table 2. Stem, leaf and leaf stipule gall dimensions in chestnut varieties (mean ± SD)

Variety	Stem		Leaf		Leaf Stipule	
	Width (mm)	Length (mm)	Width (mm)	Length (mm)	Width (mm)	Length (mm)
'Wild'	13.01 ± 2.56	14.86 ± 2.94	12.15 ± 2.71 a	13.91 ± 3.09 b	8.35 ± 1.94 a	9.46 ± 1.67 b
'Hacıömer'			10.48 ± 2.65 b	12.36 ± 2.87 c	6.21 ± 1.60 c	7.39 ± 1.62 c
'Bursakestanesi'			8.97 ± 2.24 c	11.55 ± 3.68 d	7.02 ± 1.31 bc	8.42 ± 1.65 bc
'Marigoule'	13.96 ± 3.65	12.79 ± 1.63	10.49 ± 1.29 b	14.79 ± 1.35 a	7.96 ± 1.09 ab	10.77 ± 2.09 a

Different letters within the same column indicate significant differences (Duncan test, $p < 0.05$).

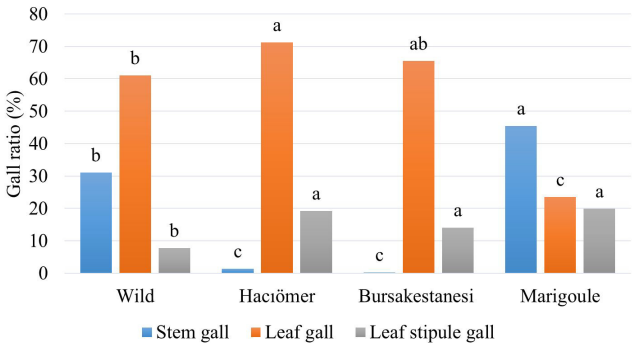

Fig. 3. Ratios of leaf, stem and leaf stipule galls of chestnut varieties

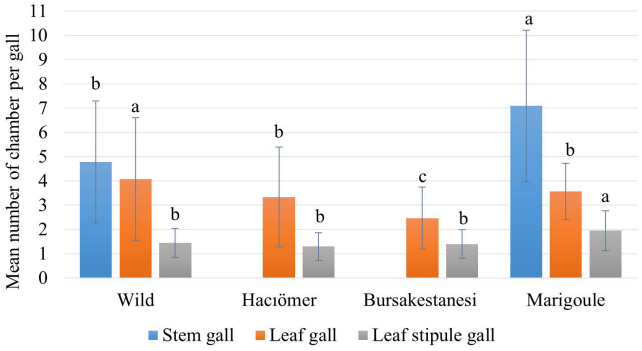

Fig. 4. The mean number of leaf, stem and leaf stipule gall chambers of chestnut varieties

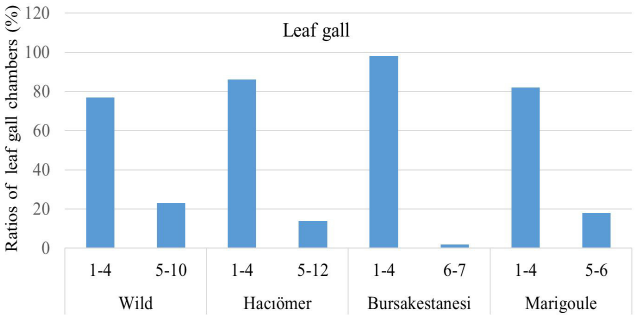

Fig. 5. Ratios of leaf gall chambers of chestnut varieties

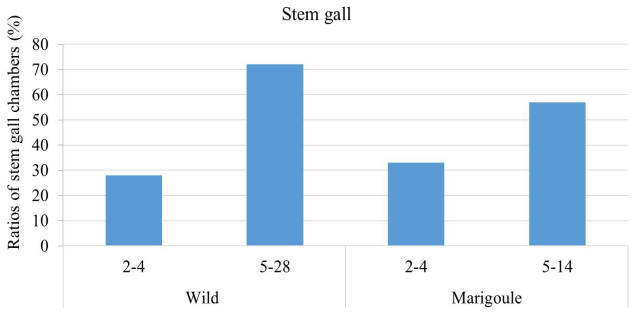

Fig. 6. Ratios of stem gall chambers of chestnut varieties

variety (7.09). The lowest number of chambers were seen in the leaf stipule galls (Fig. 4). But Panzavolta *et al.* (2013), stated that a higher mean number of chambers were found on the cultivated chestnuts (3.29) compared to the wild chestnuts (2.99). When all varieties examined, the ratios of leaf galls with 1-4 chambers were observed to be higher than 5≤ over (Fig. 5). Whereas stem gall ratios with 5≤ over chambers were found to be higher (Fig. 6). There was one *D. kuriphilus* larva per chamber (Warmund, 2013). Therefore, it was understood that the plants with multicellular gall was preferred by gall wasp. Our results were similar to Panzavolta *et al.* (2012), who reported that the number of galls in chestnut varieties may be related to wasp population density.

The stem, leaf and leaf stipule gall dimensions varied significantly among varieties ($p < 0.05$) (Table 2). The largest length (14.86 mm) of stem galls occurred in the wild trees. The leaf gall width and length ranged between 8.97 and 12.15 mm and between 11.55 and 14.79 mm depending on varieties (Table 2). The leaf stipule gall width and length ranged from 6.21 to 8.35 mm and from 7.39 to 10.77 mm depending on varieties. Our findings were higher than those obtained by other researchers. The gall diameters ranged between 5-20 mm in *Castanea crenata* (Ôtake, 1980) and 8-15 mm in *C. sativa* or *C. sativa* × *C. crenata* (Breisch and Streito, 2004).

The volume of leaf, stem and leaf stipule galls of chestnut varieties are shown in Fig. 7. The gall volume varied significantly among varieties ($p < 0.05$). The leaf and leaf stipule gall volumes of varieties were 0.84-1.80 cm^3 and 0.25-0.56 cm^3, respectively. Stem galls (2.12-2.19 cm^3) had the largest volume.

When all gall types were evaluated together, the 'Marigoule' and wild trees had larger volume galls than the 'Hacıömer' and 'Bursakestanesi' cultivars. However, some researchers observed similar values (0.2-2.5 cm^3) (Nugnes *et al.*, 2018) or higher values (0.06-10.46 cm^3) (Panzavolta *et al.*, 2012) than our findings. These differences might be due to various factors such as host plant, population density or oviposition strategy (Miyashita *et al.*, 1965; Kato and Hijii, 1993; Nugnes *et al.*, 2018). When the relationships between gall volume and the chamber numbers were analyzed, there were significant and positive correlation in stem galls ($R^2 = 0.698$, $p < 0.001$), and leaf galls ($R^2 = 0.635$, $p < 0.001$). Similarly, Panzavolta *et al.* (2012), found a positive correlation between the gall volume and the number of chambers.

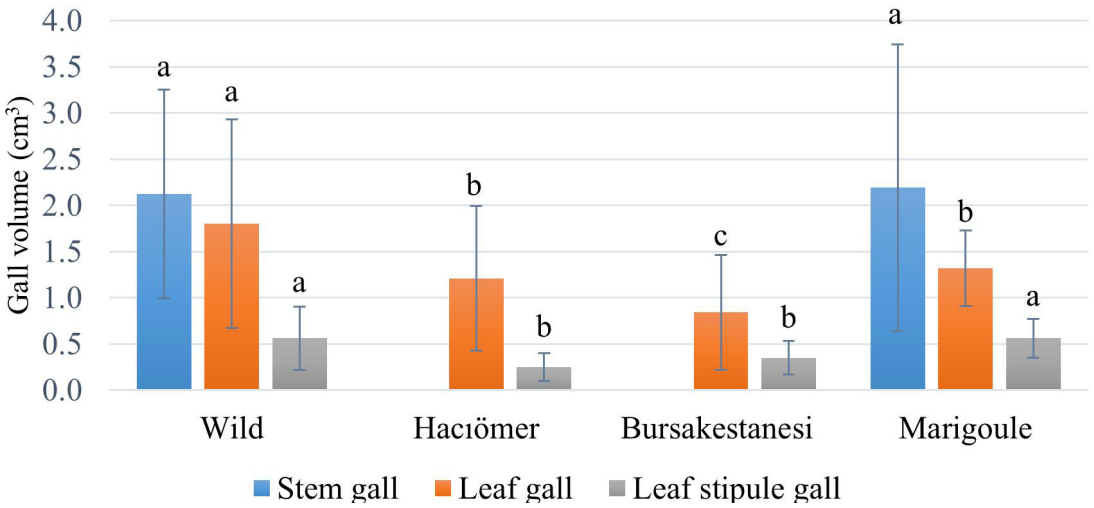

Fig. 7. The stem, leaf and leaf stipule gall volumes of chestnut varieties

Conclusions

As a result, there were significant differences in terms of gall type ratios, sizes, volumes and chamber numbers depending on the varieties. Leaf gall ratios were considerably higher in the 'Hacıömer' (71.28%) and 'Bursakestanesi' (65.53%) variety whereas stem gall ratios were higher in the 'Marigoule' (45.52%) and wild chestnut trees (31.1%). Chamber numbers per gall ranged from 2-28, 1-12 and 1-3 in the stem, leaf and leaf stipule gall, respectively. A positive relationship was found between the gall dimensions and the chamber numbers. These results indicated that gall wasp preferred wild chestnut trees and 'Marigoule' variety.

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