

AperTO - Archivio Istituzionale Open Access dell'Università di Torino

Impact of the Asian wasp *Dryocosmus kuriphilus* (Yasumatsu) on cultivated chestnut: Yield loss and cultivar susceptibility

This is the author's manuscript

Original Citation:

Availability:

This version is available <http://hdl.handle.net/2318/1528910> since 2015-11-20T17:23:11Z

Published version:

DOI:10.1016/j.scienta.2015.10.004

Terms of use:

Open Access

Anyone can freely access the full text of works made available as "Open Access". Works made available under a Creative Commons license can be used according to the terms and conditions of said license. Use of all other works requires consent of the right holder (author or publisher) if not exempted from copyright protection by the applicable law.

(Article begins on next page)



UNIVERSITÀ DEGLI STUDI DI TORINO

This Accepted Author Manuscript (AAM) is copyrighted and published by Elsevier. It is posted here by agreement between Elsevier and the University of Turin. Changes resulting from the publishing process - such as editing, corrections, structural formatting, and other quality control mechanisms - may not be reflected in this version of the text. The definitive version of the text was subsequently published in [<http://dx.doi.org/10.1016/j.scienta.2015.10.004>].

You may download, copy and otherwise use the AAM for non-commercial purposes provided that your license is limited by the following restrictions:

- (1) You may use this AAM for non-commercial purposes only under the terms of the CC-BY-NC-ND license.
- (2) The integrity of the work and identification of the author, copyright owner, and publisher must be preserved in any copy.
- (3) You must attribute this AAM in the following format: Creative Commons BY-NC-ND license (<http://creativecommons.org/licenses/by-nc-nd/4.0/deed.en>), [[+http://dx.doi.org/10.1016/j.scienta.2015.10.004](http://dx.doi.org/10.1016/j.scienta.2015.10.004)]

IMPACT OF THE ASIAN WASP DRYOCOSMUS KURIPHILUS (YASUMATSU) ON CULTIVATED CHESTNUT: YIELD LOSS AND CULTIVAR SUSCEPTIBILITY

Sartor C.¹⁾, Dini F.²⁾, Torello Marinoni D.¹⁾, Mellano M.G.¹⁾, Beccaro G.L.¹⁾, Alma A.¹⁾, Quacchia A.³⁾, Botta R.¹⁾

- 1) Dipartimento di Scienze Agrarie, Forestali, Alimentari – DISAFA, Università degli Studi di Torino, L.go P. Braccini 2, 10095 Grugliasco (Torino), Italy.
- 2) Former grant holder at Dipartimento di Colture Arboree, Università degli Studi di Torino, L.go P. Braccini 2, Grugliasco (TO)
- 3) Former grant holder at DIVAPRA, Università degli Studi di Torino, L.go P. Braccini 2, Grugliasco (TO)

chiara.sartor@unito.it,

francesca.dini@unito.it,

daniela.marinoni@unito.it,

gabriella.mellano@unito.it,

gabriele.beccaro@unito.it,

alberto.alma@unito.it,

roberto.botta@unito.it)

Corresponding author: Roberto Botta

postal address: L.go P. Braccini 2, 10095 Grugliasco (TO), Italy.

e-mail address: roberto.botta@unito.it

telephone: +39 011 6708800

fax: +39 011 6708658

ABSTRACT

Dryocosmus kuriphilus is the most impactful alien pest of chestnut currently reported in almost the whole Europe after its accidental introduction in Piemonte (North-west Italy) where it was found for the first time in 2002. The Piemonte Region Administration funded a project aimed to find control solutions based on both the biological control of gall-wasp and the study of plant response. This work was carried out from 2004 to 2013 and reports studies on assessment of production loss (2006-2012), cultivar susceptibility (2004-2013) and amount of nutrients subtraction caused by the insect (2012).

The assessment of yield loss showed that infestation values (G/B=No. galls/bud) lower than 0.3 G/B caused no significant losses; values between 0.3-0.6 G/B originated a moderate decrease in productivity. A drastic decrease of productivity was observed for values above 0.6 G/B.

A second objective was to assess varietal susceptibility in 62 cultivars. The susceptibility trait showed a wide range of variation from total resistance (7 cultivars: two *C. sativa*, one *C. crenata* and 4 Euro-Japanese hybrids) to high susceptibility (>0.6 G/B; 14 cultivars).

Finally, size and proximate differences in galled and healthy leaves were studied to assess the changes due to infestation. Significant differences for leaf area, moisture, dry matter, ash, sugars, starch, and total carbohydrates were observed between the two types of leaves indicating a deep influence of the infestation on leaf functionality and on its photosynthetic capacity.

KEYWORDS: *Castanea*, gall wasp, cynipid, resistance, leaf, chemical composition

1. INTRODUCTION

Chestnut tree (*Castanea sativa* Miller) is a multipurpose species with the role of fruit tree, wood resource and mountain landscape element in many areas of the Northern Hemisphere, where it is also interesting from a social point of view. However, in the last centuries, the sweet chestnut has been affected by major diseases, such as ink disease (*Phytophthora* spp.) and canker blight

(*Chryphonectria parasitica* (Murr.) Barr.), that have heavily changed its cultivation, production and economy. Among pests, *Dryocosmus kuriphilus* Yasumatsu (Hymenoptera: Cynipidae) is considered as the most invasive insect for chestnut; native of China, it was accidentally introduced in Japan (1941), Korea (1959) and North America (1974); more recently (spring 2002) its presence was firstly reported in Europe in the chestnut orchards and woods of Cuneo Province (Piemonte Region, Italy) (Brussino *et al.*, 2002).

Japan, being the first Country to face the invasion of this alien species, was first in starting breeding programs (Pereira-Lorenzo *et al.*, 2010). Oho *et al.* (1970) cite a report of 1948 by Shirakami recounting that some cultivars belonging to *C. crenata* ('Akanaka', 'Shikatsume', 'Kishine' and 'Ginyose') were found without damage following the gall wasp infestation. In 1952 the Horticultural Research Station of Tsukuba began a chestnut-breeding program with the goal of developing cultivars resistant to the chestnut gall wasp. This program and some private breeding projects, released the cultivars 'Tanzawa', 'Tsukuba', and 'Ishizuchi' and other resistant varieties; in few years the chestnut production recovered. In spite of this initial success, the resistance was eventually overcome by ecotypes of the insect and cultivars became still susceptible to the chestnut gall wasp (Moriya *et al.*, 2003). This problem induced researchers to evaluate a different strategy, based on biological control using the parasitoid *Torymus sinensis* Kamijo, with successful results. Yet, the development of resistant cultivars continued until the 1980s when varieties with increased resistance, such as 'Kunimi' and 'Shiho', were released (Saito, 2009).

The studies on genotypes susceptibility were accompanied, over the years, by observations on the lifecycle of the insect, and by biochemical studies carried out to identify compounds responsible for the different cultivars reaction to the phytophagous (Oho and Shimura, 1970). In particular, Oho and Shimura (1970) considered the levels of tannins and the content of flavonols in bark. No significant differences in relation to the degree of susceptibility of the cultivars were found, among samples for tannins, flavonol content, instead, varied significantly.

The damage of the infestation directly affect the leaves and shoots and, indirectly, the whole biomass. The leaf surface is reduced, yellowing is earlier and the amount of vegetative buds is, year by year, decreasing (Kato and Hijii, 1997). According to Dixon et al. (1986) the interruption of growth and fruiting, results in production losses up to 50-70% in the species *C. mollissima*, *C. crenata* and *C. dentata*. The infestation rarely causes plant death, but can favour it when other pathogens are present (Payne et al., 1975). Despite these studies, still extremely important for the determination of damages caused by the chestnut gall wasp, the difference in composition between healthy tissues and infected tissues remains unknown. In fact, there are no studies at the biochemical level on chestnut galls, but only for oak and rose ones. In these plants, affected by several species of gall wasps (but not *D. kuriphilus*), the gall, in addition to being a source of nutrients for the insect, also defends it from the attack of herbivores, increasing the concentration of phenols in the outer layers (Allison and Schultz, 2005). Regarding the characteristics of the tissues inside the gall, some authors claim "the nutritive hypothesis" according to which all the plant defence mechanisms are suppressed in the tissues of which the insect feeds (Price et al., 1987; Bronner, 1992). The gall wasp is also adept at controlling the levels of nitrogen that keep the host within the usual limits for survival, even in cases in which the plant is fertilized (Hartley and Lawton, 1992). Cynipid gall formation is due to an extremely complex interaction between the insect and the host plant, in which the wasp communicates with the host to redirect normal plant development, providing shelter, nutrients and protection for the developing wasp larva in the form of a gall (Stone et al., 2002).

Following the introduction of the cynipid in Italy in 2002, the Piedmont Region Administration promoted and founded a project aimed to find lasting solutions to control the pest. It was based on two strategies: the biological control of gall-wasp by the introduction from Japan of *Torymus sinensis* Kamijo (Hymenoptera: Torymidae) (Gibbs et al., 2011) and the study of plant response, both assessing production loss and determining cultivars susceptibility to the insect.

In this paper the results of the work carried out from 2004 to 2013 to study plant response to gall wasp are presented. Data on production losses were recorded (2006-2012) in a chestnut orchard located in a highly infested area and were used to classify chestnut response in terms of yield. Furthermore, gall wasp infestation susceptibility was studied on both local and international cultivars under controlled conditions (2004-2013). In 2012 we used chemical analyses to determine the amount of the basic constituents present in healthy leaves compared with leaves with galls of the same plant, to evaluate the decrease of resources available to the plant for the production of the fruit and for the vegetative growth.

2. MATERIALS AND METHODS

2.1. Yield loss evaluation in orchard

Yield loss resulting from infestation has been assessed in the period 2006-2012 in a specialized orchard (4ha) located in the municipality of Busca (CN), at the base of the Maira Valley and the edge of the Cuneo plain (Piemonte, Italy; alt. 500 m a.s.l.; lat. 44°29'12.8"N long. 7°27'46.0"E). Chestnut trees of the cultivars 'Bouche de Bétizac', 'Precoce Migoule', 'Marsol' and 'Marrone di Castel del Rio' were grown in the orchard; 'Marsol' was chosen for the trial for its high susceptibility to the insect. In this area, gall wasp was reported for the first time in 2004. In 2006 the infestation was light and primarily localized at the borders of the orchard.

Twenty 'Marsol' plants (eight years old in 2006) located in different parts of the orchard were selected. Each year, the plant circumference was measured at the end of the vegetative period, at 20 cm above the grafting line.

In autumn, for six years, the production of each tree was separately collected, weighed and evaluated for nut size (no. of nuts/kg). The weight of the production of each plant was related to the trunk area of the tree section, in order to obtain a productivity index that takes into account the size of the plant (production/trunk area in kg/cm²). Finally, production data were correlated with the

infestation index, calculated as the number of galls/bud (G/B) observed along 10 branches randomly selected around the canopy.

From the data collected, a model was constructed to relate infestation level and production changes.

2.2. Evaluation of susceptibility of chestnut cultivars

2.2.1. Plant material

Every year, during the months of January and February, scions were collected from Euro-Japanese hybrid cultivars and *C. sativa* trees in different Italian regions and European countries (Gobbin et al., 2007; Torello Marinoni et al., 2013; Mellano et al., 2012; Pereira-Lorenzo et al., 2006; Pereira-Lorenzo et al., 2011), and grafted on seedlings of *C. sativa* in pot (Ø 20 cm). Grafts were performed by whip and tongue technique in February and March.

2.2.2. Experimental design

In Spring (April), four screenhouses were set up in the forest nursery of Chiusa Pesio (CN, Piedmont, Italy) to isolate the chestnut accessions from the external environment, each of them consisting of a metal structure on which it was stretched an anti aphid net (mesh: 0.75x0.27 mm) which allowed air circulation and rain flow. From May, the modules were covered with black shading net in order to reduce the irradiation and the increase of the internal temperature.

Irrigation was manual using buckets or watering cans.

A total of 62 cultivars (table 1) was evaluated over a period of eight years (2005-2012).

At least three replicates (plants in pot) of each cultivars were put randomly in the screenhouses and data were considered reliable when at least 100 buds/cultivar were subjected to infestation.

In summer (June – July) the gall wasps, obtained by a mass rearing of galls collected in the forest, were released into the modules with a proportion of 1 insect per 5 buds.

In autumn, the screenhouses were removed before the first snowfall and the accessions were moved to a protected area of the nursery.

2.2.3. *Dryocosmus kuriphilus* rearings

In order to obtain the adult cynipids to be released inside the cages, new formed galls were collected in infested woods. Time of collection was chosen after monitoring the insect stage inside the galls. Galls were indeed collected at the stage of black pupa (the closer to the emergence) in order not to compromise the natural development of the insects. The galls were isolated inside net cages and kept under natural condition at the DISAFA facilities. The cages were checked every morning and any *Dryocosmus kuriphilus* that emerged was collected, counted and isolated in glass test tubes. The tubes were moved the same day of collection, from the laboratories to the nursery inside thermic bags in order to avoid heat stress. Insects were released inside the cages by opening the tubes and letting them exit spontaneously.

2.2.4. Infestation check

In the following spring the physiological state and the effect of the infestation by *D. kuriphilus* was checked, evaluating the number, type (Kato and Hijii, 1997) and size of the galls developed on the new shoots. The galls were classified in three classes based on their diameter: small= diameter < 0.5 cm, medium= diameter between 0.5 and 1 cm, large= diameter > 1 cm, and in five classes in relation to their position: type A: at the base of leaf, type B: at the base of the shoot, type C: on the leaf, type D: at the inflorescence base, type E: on the whole bud (Fig. 1).

The total gall number (G) counted on each tree was related with the total number of buds (B) registered the previous year, and the infestation index (G/B) was used to measure the susceptibility of cultivars to the insect. Data were analyzed by ANOVA, followed by Tukey's test.

Table 1 List of 62 *Castanea* spp. cultivars evaluated in this study and their geographic origin

Cultivar	Species	Origin
Belle Epine	<i>C. sativa</i>	France
Bouche de Betizac	<i>C. sativa</i> x <i>C. crenata</i>	France
Bourrue de Juillac	<i>C. sativa</i>	France
Bracalla	<i>C. sativa</i>	North-West Italy
Brunette	<i>C. crenata</i> x <i>C. sativa</i>	France
Canepina	<i>C. sativa</i>	Central Italy
Cervaschina	<i>C. sativa</i>	North-West Italy
Colossal	<i>C. sativa</i> x <i>C. crenata</i>	U.S.A.

Contessa	<i>C. sativa</i>	North-West Italy
Doree de Lyon	<i>C. sativa</i>	France
Ederra	<i>C. crenata</i>	France
Gabbiana	<i>C. sativa</i>	North-West Italy
Garrone rosso	<i>C. sativa</i>	North-West Italy
Gentile	<i>C. sativa</i>	North-West Italy
Idae	<i>C. crenata</i>	South Korea
Injerta	<i>C. sativa</i>	Spain
Judia	<i>C. sativa</i>	Portugal
Longal	<i>C. sativa</i>	Portugal
Lusenta	<i>C. crenata</i> x <i>C. sativa</i>	North-West Italy
Madonna	<i>C. sativa</i>	North-West Italy
Maraval	<i>C. crenata</i> x <i>C. sativa</i>	France
Maridonne	<i>C. sativa</i> x <i>C. crenata</i>	France
Marigoule	<i>C. crenata</i> x <i>C. sativa</i>	France
Marlhac	<i>C. sativa</i> x <i>C. crenata</i>	France
Marron Comballe	<i>C. sativa</i>	France
Marron d'Olargues	<i>C. sativa</i>	France
Marron de Chevanceaux	<i>C. sativa</i>	France
Marron de Goujounac	<i>C. sativa</i>	France
Marron de Lyon	<i>C. sativa</i>	France
Marron de Redon	<i>C. sativa</i>	France
Marron Sauvage	<i>C. sativa</i>	France
Marrone Chiusa Pesio	<i>C. sativa</i>	North-West Italy
Marrone della Val di Susa	<i>C. sativa</i>	North-West Italy
Marrone dell'Etna	<i>C. sativa</i>	South Italy
Marrone di Castel Rio PGI	<i>C. sativa</i>	Central Italy
Marrone di Marradi	<i>C. sativa</i>	Central Italy
Marrone di Roccamonfina	<i>C. sativa</i>	South Italy
Marrone di Segni	<i>C. sativa</i>	Central Italy
Marrone di Zocca	<i>C. sativa</i>	Central Italy
Marrone Fiorentino	<i>C. sativa</i>	Central Italy
Marsol	<i>C. crenata</i> x <i>C. sativa</i>	South West France
Merle	<i>C. sativa</i>	France
Montagne	<i>C. sativa</i>	France
Negral	<i>C. sativa</i>	Spain
Neirana	<i>C. sativa</i>	North-West Italy
Pellegrine	<i>C. sativa</i>	France
Pelona	<i>C. sativa</i>	Spain
Précoce des Vans	<i>C. sativa</i>	France
Precoce Migoule	<i>C. crenata</i> x <i>C. sativa</i>	France
Primemura	<i>C. sativa</i>	North-West Italy
Pugnenga	<i>C. sativa</i>	North-West Italy

Rapuca	<i>C. sativa</i>	Spain
Riggiola	<i>C. sativa</i>	South Italy
Russaia	<i>C. sativa</i>	North-West Italy
Sardonne	<i>C. sativa</i>	France
Savoie	<i>C. sativa</i>	South West France
Siria	<i>C. sativa</i>	North-West Italy
Tempuriva	<i>C. sativa</i>	North-West Italy
Torcione Nero	<i>C. sativa</i>	Switzerland
Verdale	<i>C. sativa</i>	France
Verdeisa	<i>C. sativa</i>	North-West Italy
Vignols	<i>C. crenata</i> x <i>C. sativa</i>	France



a. Gall at the base of leaf



b. Gall at the base of the shoot



c. Gall on the leaf



d. Gall at the inflorescence base



e. Gall on the whole bud

Fig. 1. Gall types observed in chestnut following infestation by *Dryocosmus kuriphilus*.

2.3. Gall impact on leaf characteristics

There are not information on changes of the proximate composition of chestnut leaves that develop galls. In May 2012, samples from healthy and infested (gall bearing) leaves were harvested from a susceptible cultivar of *C. sativa*. The collected leaves were stored at 4 °C during transfer. For each sample type, 3 replicates weighing 200g for were frozen in liquid Nitrogen and stored at -28 °C for chemical analyses. In addition, 20 galled leaves and 20 without gall were weighed and measured (length, width, surface area).

Moisture, dry matter, ash, crude proteins, sugars, starch, total carbohydrates, crude fiber and crude fats were evaluated using the following methods:

Moisture and dry matter: EC Reg 152/2009 27/01/2009 OJ EC L 54 Annex III A

Ash: EC Reg 152/2009 27/01/2009 OJ EC L 54 Annex M III;

Proteins: ISO 1871:2009;

Sugars: Reports ISTISAN 1996/34 p. 63;

Crude fiber: EC Reg 152/2009 27/01/2009 OJ EC L 54 Annex III I;

Crude fat: MI0236 rev.9/2009 Soxhlet extraction after hydrolysis;

Starch: EC Reg 900/2008 16/09/2008 OJ EC L248 / 8 17/09/2008 Annex 1;

Statistical analysis was performed by ANOVA.

3. RESULTS AND DISCUSSION

3.1. Yield loss evaluation in orchard

The evaluation of the yield loss in orchard started in 2006 and was conducted on 20 plants of the cultivar 'Marsol'. Across years, 4 plants become infected by canker blight and were excluded by the trial. Infestation status was detected in late summer considering 10 branches for each plant; the amount of galls present was correlated with the number of buds of the previous year in order to assess the infestation level as No. of galls/bud (G/B). The vertical axis of the chart in fig. 2 represents the average productivity index (kg/cm²) and the average infestation observed each year

during the test period (2006-2012, horizontal axis). The infestation across years, varied from a minimum level of 0.1 G/B, found in 2006, to maximum level of 0.8 G/B in 2011, but showed remarkable fluctuations across years. This could be due to environmental conditions during the development of the larva that may influence infestation rates in the following season (Bosio et al., 2009).

The fluctuations of productivity index and infestation show that, in general, in the years when the infestation was lower (2006, 2008, 2010, 2012), the production was higher, as expected.

The fig. 3 illustrates the correlation between productivity and infestation of each plant. Trees showed a wide variability in yield. Three different levels of response can be identified in relation to the infestation range: values less than 0.3 G/B showed no significant losses. Infestation in the range of 0.3-0.6 G/B caused a moderate decrease in productivity, while a drastic decrease of productivity is observed for infestation values superior to 0.6 G/B. This effect is also revealed by the regression line ($R=0.56^*$). The productivity data analyzed at the three levels of infestation are significantly different.

There was an apparent increase in nut size following the increase of infestation (less nuts: bigger size; fig. 4) but the correlation between the two factors was not statistically significant. In fact, nut size is primarily genetically determined (it is a distinctive trait of cultivars), although influenced to some extent by environmental factors and crop load. In this case the presence of the cynipid reduces both yield and canopy efficiency, without a statistically significant effect on nut

size.

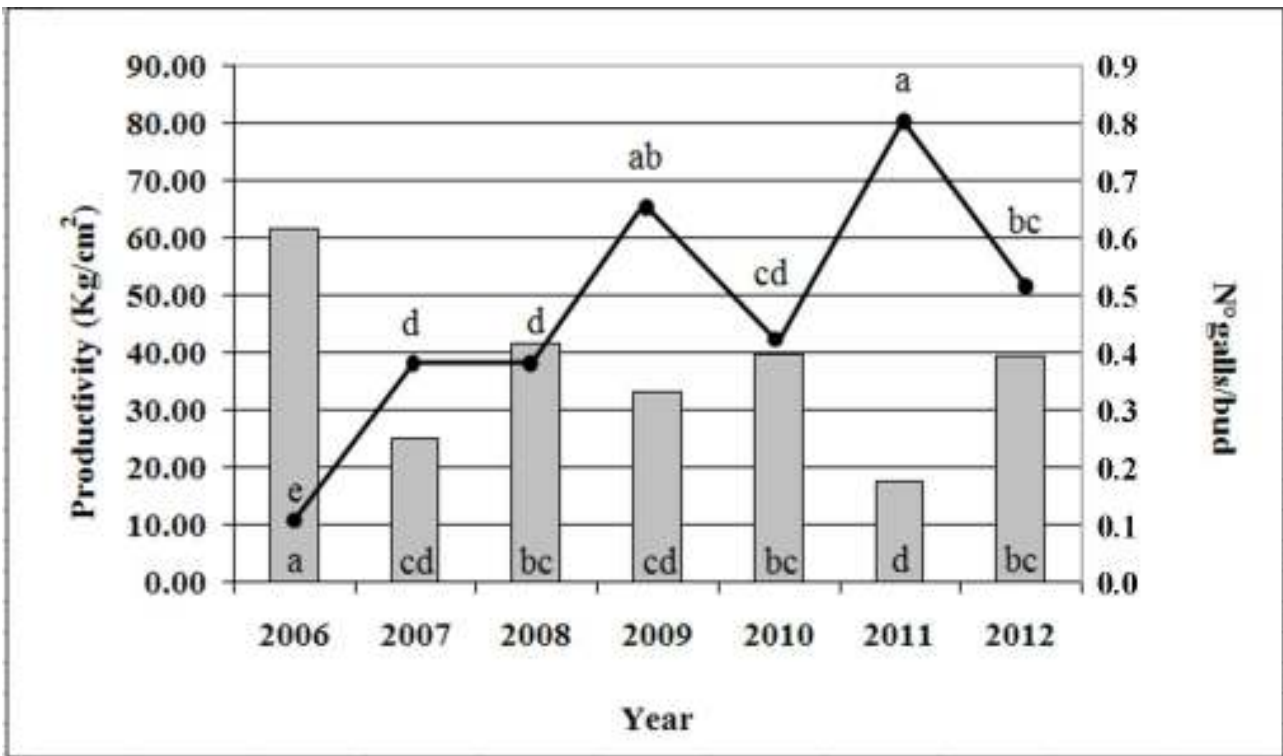


Fig. 2: Chart of yield (productivity index, bars) and infestation levels (black line) observed in the years 2006-2012.

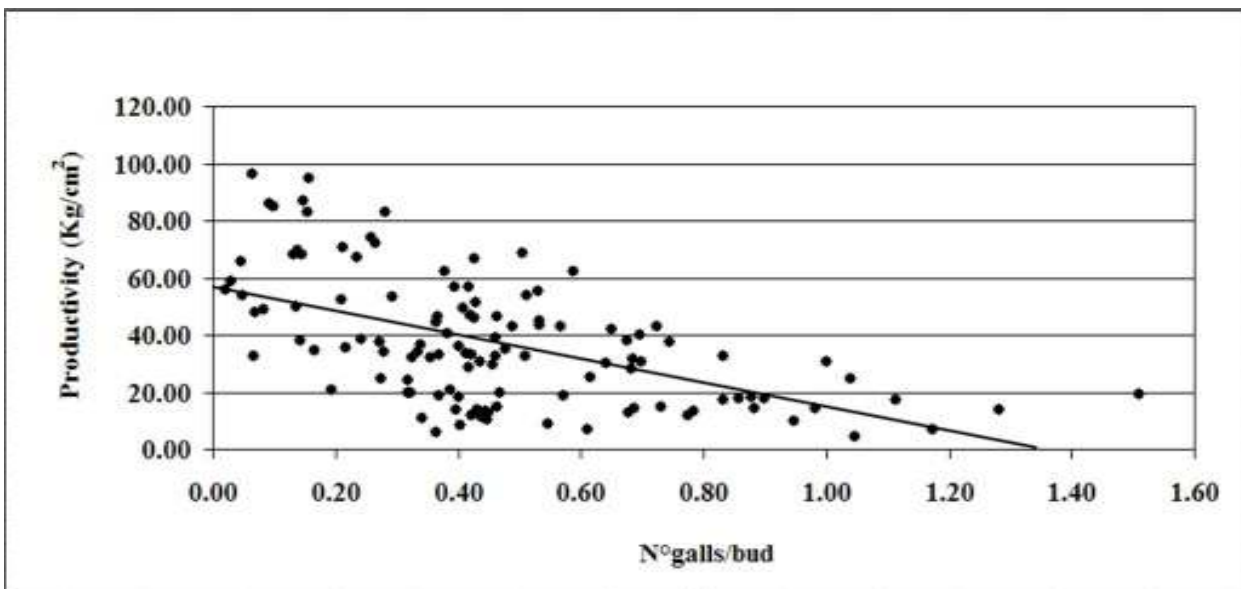


Fig. 3: Correlation between productivity (kg/cm²) and infestation (No. galls/bud) of data collected in the years 2006 - 2012 ($R = 0.56^*$)

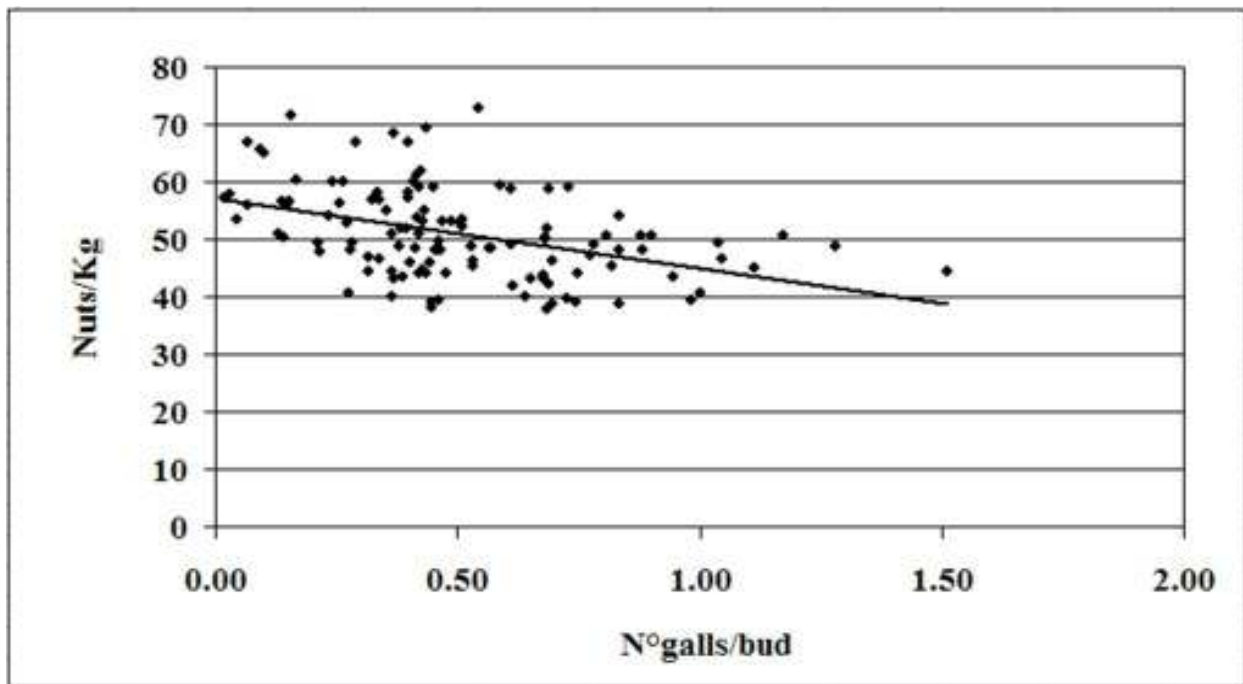


Fig. 4: Correlation between nut size (No. nuts/kg) and infestation index (No. galls/bud)

3.2. Evaluation of susceptibility of chestnut cultivars

Susceptibility to *Dryocosmus kuriphilus* was evaluated in 62 cultivars over a period of eight years (2005-2012); susceptibility was measured as infestation index (No galls/bud). The results obtained after controlled infestation of *D. kuriphilus* revealed that most of the chestnut cultivars were sensitive to gall wasp attack, underlining the dangerousness of this pest.

Due to the variability observed within the cultivars, we have chosen to split the data into classes of susceptibility (high=infestation > 0.6 G/B; medium=infestation between 0.3 and 0.6 G/B; low=infestation < 0.3 G/B) according to the information obtained from the work of yield loss evaluation in orchard (3.1). Statistical analysis showed that there are significant differences of yield ($P=0.05$) between the classes (high: 21.92 kg/cm²; medium: 32.74 kg/cm²; low: 58.97 kg/cm²).

As shown in fig. 5, 14 cultivars resulted very susceptible, with an average G/B > 0.6; among them, ‘Marsol’, an Euro-Japanese hybrid, and ‘Torcione Nero’, a major cultivar in Switzerland, developed an average of over one gall per bud. They were followed by a group of 19 cultivars showing

medium susceptibility; 18 of them were *C. sativa* cultivars, with G/B between 0.3 and 0.6. A third large low susceptibility group (22 cultivars) was observed for G/B values lower than 0.3 G/B.

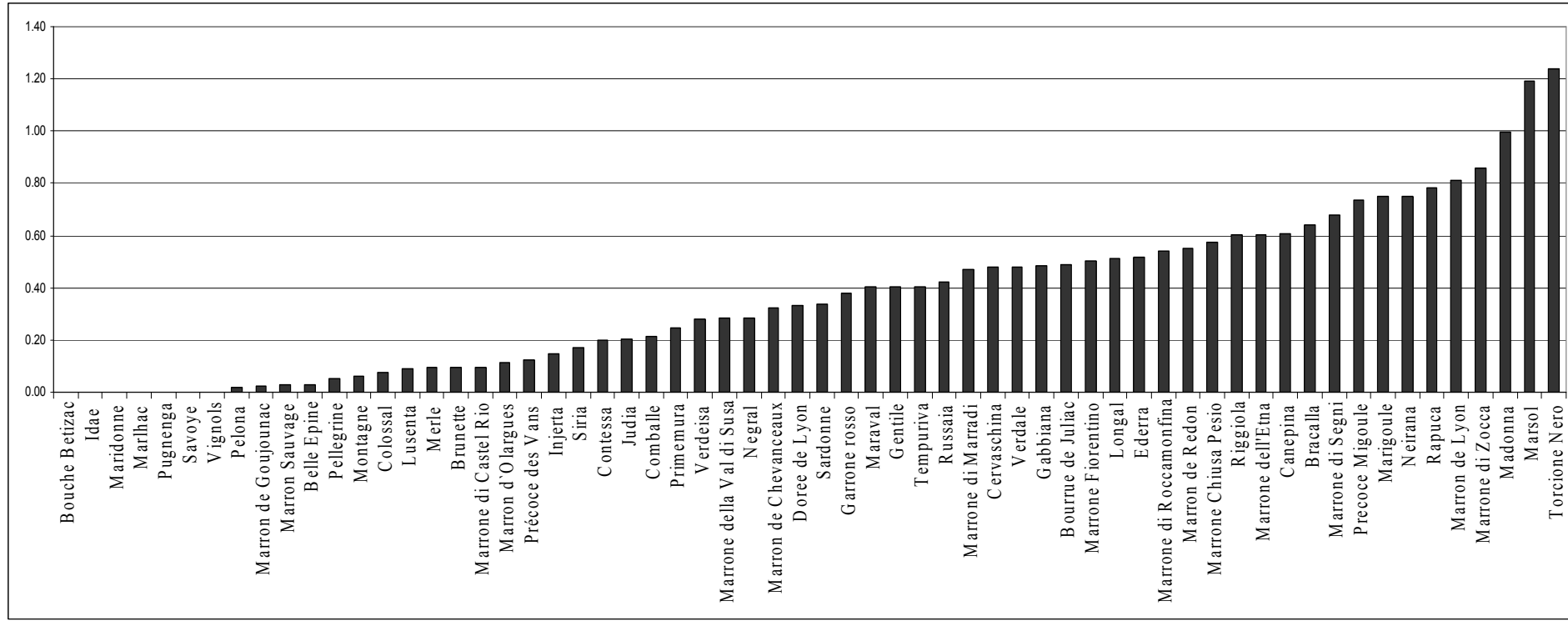
Seven cultivars resulted resistant (no gall development in spring) and were further tested in screenhouse under gall-wasp pressure (2 insects/bud released inside the screenhouse) where their response was confirmed. Only 2 were cultivars of *C. sativa*: 'Pugnenga' and 'Savoie'. The first is an Italian cultivar native of Cuneo Province (Piedmont Region), the second is a French cultivar native of Midi-Pyrenees Region. The presence of individuals belonging to *C. sativa* among cultivars without galls, appears to be a particularly important finding in view of the direct transmission of resistance from this species. In addition, the identification of these individuals is a very positive result considering that the resistant cultivars, so far isolated in Japan and Korea, are all from *C. crenata* or its hybrids, except for the Chinese cultivar 'LM' which belongs to *C. mollissima* (Anagnostakis, 2000). The group of 7 resistant genotypes also included a *C. crenata* cultivar ('Idae') and 4 Euro-Japanese hybrids ('Bouche de Bétizac', 'Marlhac', 'Maridonne' and 'Vignols'); for 'Bouche de Bétizac', 'Vignols' and 'Maridonne', we can assume that it is the selection 'CA04' (*C. crenata*, selected by INRA Bordeaux), the common male parent of the crossing, that passed the resistance trait. These results confirm the actual involvement of *C. crenata* in the mechanism of inhibition of larva and gall development: the resistant trait was further studied in 'Bouche de Bétizac' and was found to have a simple Mendelian inheritance (Botta et al., 2012). Histological examinations revealed the presence of eggs and larvae at the first instar in 'Bouche de Bétizac' but larvae failed to reach the second instar in spring due to a hypersensitive reaction by the plant (Dini et al., 2012).

Data show that wasp lays eggs in all cultivars although with different degrees of preference, probably due to their different levels of attractiveness. The factors involved are still unknown, but it can be assumed that they include bud size and bud texture and the presence of volatile substances on the bark (Huang et al., 1990).

Furthermore, the effect of the infestation by *D. kuriphilus* was studied evaluating the type (Kato and Hijii, 1997) and size of the galls. The most frequent gall types were C type, gall on the leaf (52.5 %) and type A, gall at the base of the leaf (mean 34.5 %). Yet, a considerable percentage (mean 10.9 %) of galls causing unsuccessful bud development was counted (type E: on the whole bud). The other two classes (type B: at the base of the shoot and D: at the inflorescence base) were present in only 5 cultivars with a lower incidence. The different distribution of vegetative and mixed bud along the shoot justifies the lower presence of gall types A, C and E rather than those of type B and D (linked to mixed buds). In the conditions of the trial the highest presence of galled shoots was observed in the medium part of the branch of the previous year. According to Panzavolta et al. (2012) the mean number of eggs per bud tends to decrease from the apical bud toward the basal bud and is related with bud size. *D. kuriphilus* prefers to lay eggs in larger buds (approximately 6 mm³) compared with smaller buds (approximately 3 mm³).

As concerns the gall size, medium-sized galls resulted prevalent (mean 44 %), while small- and large-sized galls were on average 31.5% and 24.5%, respectively. Females may tend to lay fewer eggs per bud to give rise to smaller galls because the fertility of emerging adults increases with decreasing gall size (Kato and Hijii, 1997). On the other hand, increasing gall size could represent a defensive strategy against parasitism, because the two factors are negatively correlated with each other (Cooper and Rieske, 2010).

1



2

3 Fig. 5: Susceptibility (ratio No. of galls/No. of buds, G/B) to gall wasp in 62 cultivars of chestnut.

4 3.3. Gall impact on leaf characteristics

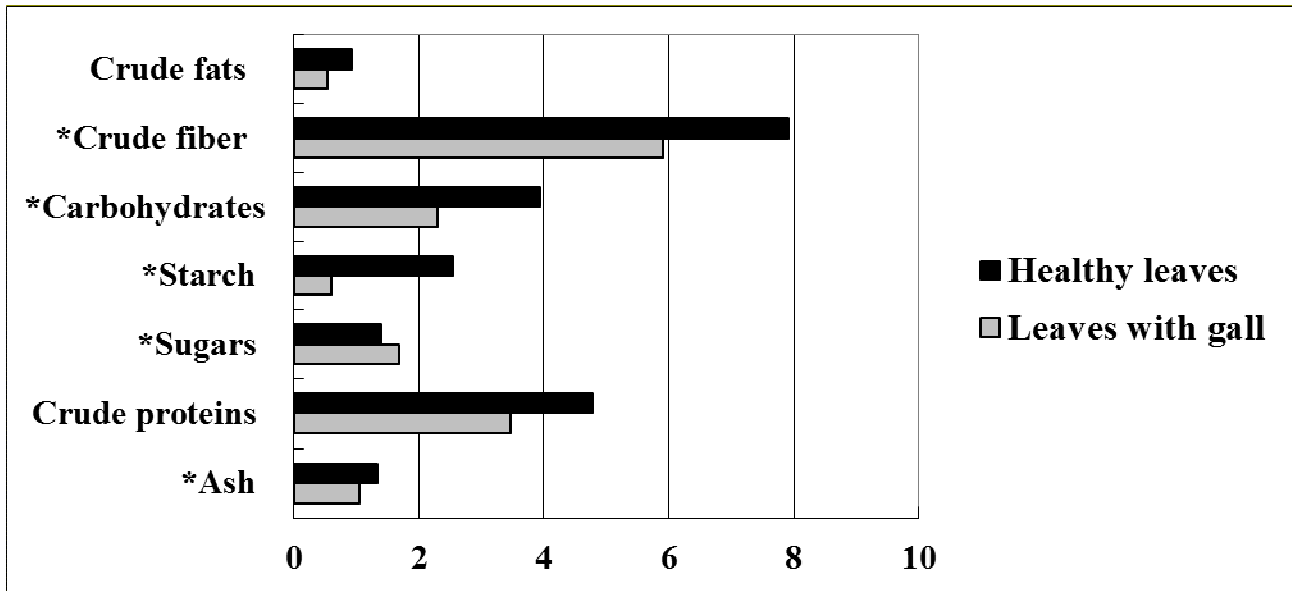
5 The incidence of chestnut gall wasp infestation on productivity is evident from the results
6 previously described. Here we want to assess the changes in leaf characteristics, in particular size
7 and proximate composition. In fact, as showed before, leaves are the most affected by the
8 infestation with consequences on their photosynthetic capacity.

9 The weight values obtained for healthy leaves (1.27 ± 0.5 g) and leaves with gall (0.98 ± 0.46 g) were
10 not significantly different. As reported by Kato and Hijii (1997) the effect of the gall presence on
11 the leaf of *Castanea crenata*, leads to a surface reduction of about 50%, and consequently to a
12 reduction of the photosynthetic apparatus. Moreover the gall, while being an organ capable of
13 operating the photosynthesis, at least in a first phase of its development, appears to have a lower
14 chlorophyll content compared to healthy leaves (Andersen and Mizell, 1987). In our study galled
15 leaves had a mean area of 19,5 cm² against 95,5 cm² of healthy leaves showing a drastic surface
16 reduction (80%) due to gall development.

17 Observing the chart in fig. 6, it is evident that there are differences in the amounts of the various
18 compounds examined. The ANOVA analysis revealed highly significant differences in moisture
19 and, consequently, dry matter content and significant differences for ash, sugars, starch, and total
20 carbohydrates (starch+sugars) contents. These differences between the two leaf types are index of
21 the *D. kuriphilus* negative impact, due to the subtraction of nutrients both for the pest growth and
22 for gall structure formation. In particular, highly different values in total carbohydrates can be
23 justified by the reduction of photosynthetic activity (less starch accumulation) and by the fact that
24 the larva in the growth phase uses photosynthates as a source of nourishment, interfering with both
25 translocation of sugars and accumulation of starch. This supports the hypothesis on the nutritional
26 function of the gall inner surface proposed by Harper (2004) on the basis of studies carried out in
27 *Quercus robur*. The oak gall is constituted by outer epidermis, from a bark of sclerenchyma and
28 from one or more rooms coated by two different types of tissue: one is a parenchyma nutrient rich
29 in starch and the other is composed of cells with high nutritive concentrations of lipids and proteins

30 (Harper et al., 2004). It also appears that the amount of moisture is much higher in the galled leaf
 31 (77.2% against 66.9%), due to the spongy parenchyma that characterize the gall structure,
 32 containing a large amount of water (Harper et al., 2004).

33



34

35 Fig. 6: Ash content, protein, sugars, starch, total carbohydrates (sugars+starch) and crude oils and
 36 fats of the healthy leaf and the leaf with gall (g/100g fresh weight) *p = 0.05.

37

38

39 4. CONCLUSIONS

40 *Dryocosmus kuriphilus* has recently become a problem of the whole Europe, since the presence of
 41 the insect has been observed in most of the chestnut producing Countries, from Portugal to Turkey,
 42 and recently also in the UK. Piemonte (Northwest Italy) was the first area infested and the first to
 43 start a research program for the control of the pest; part of the study carried out is presented in this
 44 paper and can contribute to clarify the impact of gall wasp infestation in chestnut orchards.

45 EFSA (2010) elaborated a risk assessment of the oriental chestnut gall wasp *Dryocosmus kuriphilus*
 46 for the EU territory, stating that: “The potential for yield reduction in *Castanea* and negative effects
 47 on production is estimated as moderate. Although reported as high in the literature, there is a high

48 level of uncertainty relating to this estimate in the absence of quantitative data confirming the yield
49 reduction attributed directly to *D. kuriphilus*". In this paper we show that the yield loss can be
50 potentially very high in highly susceptible cultivars in the absence of biological control. These
51 information will be useful for evaluating yield loss in orchards and elaborate policy of support and
52 strategies of pest containment, in particular in those Countries where the insect was only recently
53 found.

54 The biological control with *Torymus sinensis* will probably reduce the infestation to levels
55 compatible with the cultivation of most varieties but the finding of resistant genotypes is of relevant
56 interest for studies on host-pathogen interaction and for future breeding programs.

57

58

59 **ACKNOWLEDGMENTS**

60 The research was funded by the Regione Piemonte Administration and by the cooperation program
61 Italy-France Alcotra 2007-2013. Authors thanks colleagues that sent plant material for the cultivar
62 susceptibility evaluation and, in particular, Dr. Bernard Hennion (Ctifl, Lanxade).

63

64

65 **REFERENCES**

66

67 Allison, S. D., Schultz, J. C., 2005. Biochemical responses of chestnut oak to a galling cynipid. Journal of
68 Chemical Ecology. 31:151-166.

69

70 Anagnostakis, S. L., 2000. Chestnut cultivar names department of plant pathology and ecology the
71 connecticut agricultural experiment station.

72

73 Andersen, P.C., Mizell, R.F., 1987. Physiological effects of galls induced by *Phylloxera notabilis*
74 (Homoptera: Phylloxeridae) on pecan foliage. Environ. Entomol. 16:264-268.

75 Belsky, A.J., 1986. Does herbivory benefit plants? A review of the evidence. *Am. Nat.* 127:870–892.

76

77 Bosio, G., Gerbaudo, C., Piazza E., 2009. *Dryocosmus kuriphilus* Yasumatsu: an outline of seven years after

78 the first report in Piedmont (Italy). In: A global serious pest of chestnut trees, *Dryocosmus kuriphilus*:

79 yesterday, today and tomorrow. Proceedings of the Japan-Italy joint international symposium, Tsukuba,

80 Japan, November 24-25, 2009. :3-13.

81

82 Botta R., Sartor C., Dini F., Costa R., Santos C., Quacchia A., Alma A., Torello Marinoni D., 2012.

83 Susceptibility to *Dryocosmus kuriphilus* Yasumatsu and molecular analysis of chestnut genotypes. Book of

84 abstracts IUFRO 2012 Conference “Genetics of *Fagaceae* and *Nothofagaceae*” Talence, France, 9-12

85 October:20-21.

86

87 Bronner, R., 1992. The role of nutritive cells in the nutrition of cynipids and cecidomyiidae. *Biology of*

88 *Insect Induced Galls* (eds J.D. Shorthouse and O. Rohfritsch). Oxford University Press, New York :118-140.

89 Brussino G., Bosio G., Baudino M., Giordano R., Ramello F., Melika G. 2002. Pericoloso insetto esotico per

90 il castagno europeo. *L'Informatore Agrario* 37:59-61.

91

92 Cooper, W.R., Rieske, L.K., 2010. Gall structure affects ecological associations of *Dryocosmus kuriphilus*

93 Yasumatsu (Hymenoptera: Cynipidae). *Environmental Entomology*. 39 :787-797.

94

95 Dini, F., Sartor, C., Botta, R., 2012. Detection of a hypersensitive reaction in the chestnut hybrid ‘Bouche de

96 Bétizac’ infested by *Dryocosmus kuriphilus* Yasumatsu. *Plant Physiology and Biochemistry*. 60:67-73.

97 ISSN: 0981-9428, doi: 10.1016/j.plaphy.2012.07.023

98

99 Dixon, W.N., Burns, R.E., Stange, L.A., 1986. Oriental chestnut gall wasp. *Dryocosmus Kuriphilus*. *Plant*

100 *Industry*. Florida Department of Agriculture and Consumer Services, Gainesville (US). Division of

101 Entomology Circular no. 287:1–2.

102

103 EFSA, 2010. Risk assessment of the oriental chestnut gall wasp, *Dryocosmus kuriphilus* for the EU territory
104 and identification and evaluation of risk management options. EFSA Journal 8(6):1619 (114 pp.).

105 Gibbs M., Schönrogge K., Alma A., Melika G., Quacchia A., Stone G.N., Aebi A., 2011. *Torymus sinensis*:
106 a viable management option for the biological control of *Dryocosmus kuriphilus* in Europe? *Biocontrol*,
107 56:527-538.

108

109 Gobbin D., Hohl L., Conza L., Jermini M., Gessler C., Conedera M., 2007. Microsatellite-based
110 characterization of the *Castanea sativa* cultivar heritage of southern Switzerland. *Genome* 50: 1089-1103.

111

112 Harper, L.J., Schönrogge, K., Lim, K.Y., Francis, P., Lichtenstein, C.P., 2004. Cynipid galls: insect induced
113 modifications of plant development create novel plant organs. *Plant, Cell and Environment*. 24:327-335.

114

115 Hartley, S.E., Lawton, J.H., 1992. Host plant manipulation by Gall Insect: a test of the nutrition hypothesis.
116 *The Journal of Animal Ecology*. 61:113-119.

117

118 Huang, H.W., Norton, J.D., Smith, D.A., Slayden, P.W., 1990. Characteristics of chestnut gall wasp
119 Resistant Chinese chestnut - Annual report - Northern Nut Growers' Association. 81;29-32.

120

121 Kato, K., Hijii, N., 1997. Effects of gall formation by *Dryocosmus kuriphilus* Yasumatsu (Hym., Cynipidae)
122 on the growth of chestnut trees. *J. Appl. Entomol.* 121:9-15.

123

124 Mellano M.G., Beccaro G.L., Donno D., Torello Marinoni D., Boccacci P., Canterino S., Cerutti A.K.,
125 Bounous G., 2012. *Castanea* spp. biodiversity conservation: collection and characterization of the genetic
126 diversity of an endangered species. *Genetic Resources and Crop Evolution*, published online. doi:
127 10.1007/s10722-012-9794-x.

128

129 Moriya, S., Shiga, M., Adachi, I., 2003. Classical biological control of the chestnut gall wasp in Japan.
130 Proceedings, 1st International Symposium on Biological Control of Arthropods ed. by R. G. Van Driesche.
131 United States Department of Agriculture, Forest Service, Washington, District of Columbia :407–415.
132

133 Oho, N., Shimura, I., 1970. Process of study on *Dryocosmus kuriphilus* Yasumatsu (Hymenoptera:
134 Cynipidae) and several problems about recent infestation. Shokubutsu Boeki (Plant Protection). 24:421–427.
135

136 Panzavolta T. Bracalini M. Croci F. Campani C., Bartoletti T. Miniati G., Benedettelli S., Tiberi R (2012)
137 Asian chestnut gall wasp in Tuscany: gall characteristics, egg distribution and chestnut cultivar
138 susceptibility. Agricultural and Forest Entomology 14(2):139–145.
139

140 Payne, J.A., Menke, A.S., Schroeder, P.M., 1975. *Dryocosmus kuriphilus* Yasumatsu, (Hymenoptera:
141 Cynipidae), an Oriental chestnut gall wasp in North America. USDA Cooperative Economic Insect Report
142 25:903–905.
143

144 Pereira-Lorenzo S., Ballester A., Corredoira E., Vieitez A.M., Agnanostakis S., Costa R., Bounous G., Botta
145 R., Beccaro G.L., Kubisiak T.L., Conedera M., Krebs P., Yamamoto T., Sawamura Y., Takada N., Gomes-
146 Laranjo J., Ramos-Cabrer A.M. 2010. Chestnut Breeding. In: Fruit breeding / Springer Series "Handbook of
147 Plant Breeding" M.L. Badenes and D. Byrne eds. Springer, Hidelberg 8:729-770.
148

149 Pereira-Lorenzo, S., Lourenço Costa R.M., Ramos-Cabrer A.M., Ciorda-Ara M., Marques Ribeiro C.A.,
150 Borges O., Barreneche T. 2011. Chestnut cultivar diversification process in the Iberian Peninsula, Canary
151 Islands and Azores. Genome, 54: 301-315. Doi:10.1139/g10-122
152

153 Pereira-Lorenzo, S.; Diaz-Hernandez, M.B.; Ramos-Cabrer, A.M., 2006. Use of highly discriminating
154 morphological characters and isoenzymes in the study of Spanish chestnut cultivars. Journal of the American
155 Society for the Horticultural Science, 131(6):770-779
156

157 Price, P.W., Fernandes, G.W., Waring, G.L., 1987. Adaptive nature of insect galls. Environmental
158 Entomology. 16:15-24.
159

160 Saito, T., 2009. Japan. Book chapter in Following Chestnut Footprints (*Castanea spp.*) - Cultivation and
161 Culture, Folklore and History, Tradition and Uses. Scripta Horticulturae. 9:96-99.
162

163 Schönrogge, K., Harper, L.J., Lichtenstein, C.P., 2000. The protein content of tissues in cynipid galls
164 (hymenoptera: Cynipidae): Similarities between cynipid galls and seeds. Plant Cell and Environment.
165 23:215-222.
166

167 Stone, G.N., Schönrogge, K., Atkinson, R.J., Bellido, D., Pujade-Villar, J., 2002. The population biology of
168 oak gallwasps (Hymenoptera: Cynipidae). Ann. Rev. Entomol. 47:633–668.
169

170 Torello Marinoni D., Akkak A., Guaraldo P., Boccacci P., Ebone A., Viotto E., Bounous G. Ferrara A.M.,
171 Botta R., 2013. Genetic and morphological characterization of chestnut (*Castanea sativa* Mill.) germplasm in
172 Piedmont (north-western Italy). Tree Genetics & Genomes 9:1017-1030. DOI: 10.1007/s11295-013-0613-0
173

174 **LEGENDA OF TABLES AND FIGURES**

175 Table 1: List of 62 *Castanea* spp. cultivars evaluated in this study and their geographic origin

176

177

178 Fig. 1: Chart of yield (productivity index, bars) and infestation levels (black line) observed in the
179 years 2006-2012.

180 Fig. 2: Correlation between productivity (kg/cm²) and infestation (No. galls/bud) of data collected
181 in the years 2006 - 2012 (R = 0.56*)

182 Fig. 3: Correlation between nut size (n^o nuts/kg) and infestation index (No. galls/bud)

183 Fig. 4: Susceptibility (ratio No. of galls/No. of buds, G/B) to gall wasp in 62 cultivars of chestnut.

184 Fig. 5: Ash content, protein, sugars, starch, total carbohydrates and crude oils and fats of the healthy
185 leaf and the leaf with gall (100g fresh weight) *p = 0.05 **p = 0.01.

186

187

188

189