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

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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/cm^2). 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	*C sativa*	France
Bouche de Betizac	*C. sativa* x *C. crenata*	France
Bourrue de Juillac	*C. sativa*	France
Bracalla	*C. sativa*	North-West Italy
Brunette	*C. crenata* x *C. sativa*	France
Canepina	*C. sativa*	Central Italy
Cervaschina	*C. sativa*	North-West Italy
Colossal	*C. sativa* x *C. crenata*	U.S.A.

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

Rapuca	*C. sativa*	Spain
Riggiola	*C. sativa*	South Italy
Russaia	*C. sativa*	North-West Italy
Sardonne	*C. sativa*	France
Savoye	*C. sativa*	South West France
Siria	*C. sativa*	North-West Italy
Tempuriva	*C. sativa*	North-West Italy
Torcione Nero	*C. sativa*	Switzerland
Verdale	*C. sativa*	France
Verdeisa	*C. sativa*	North-West Italy
Vignols	*C. crenata* x *C. sativa*	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/cm^2) 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.

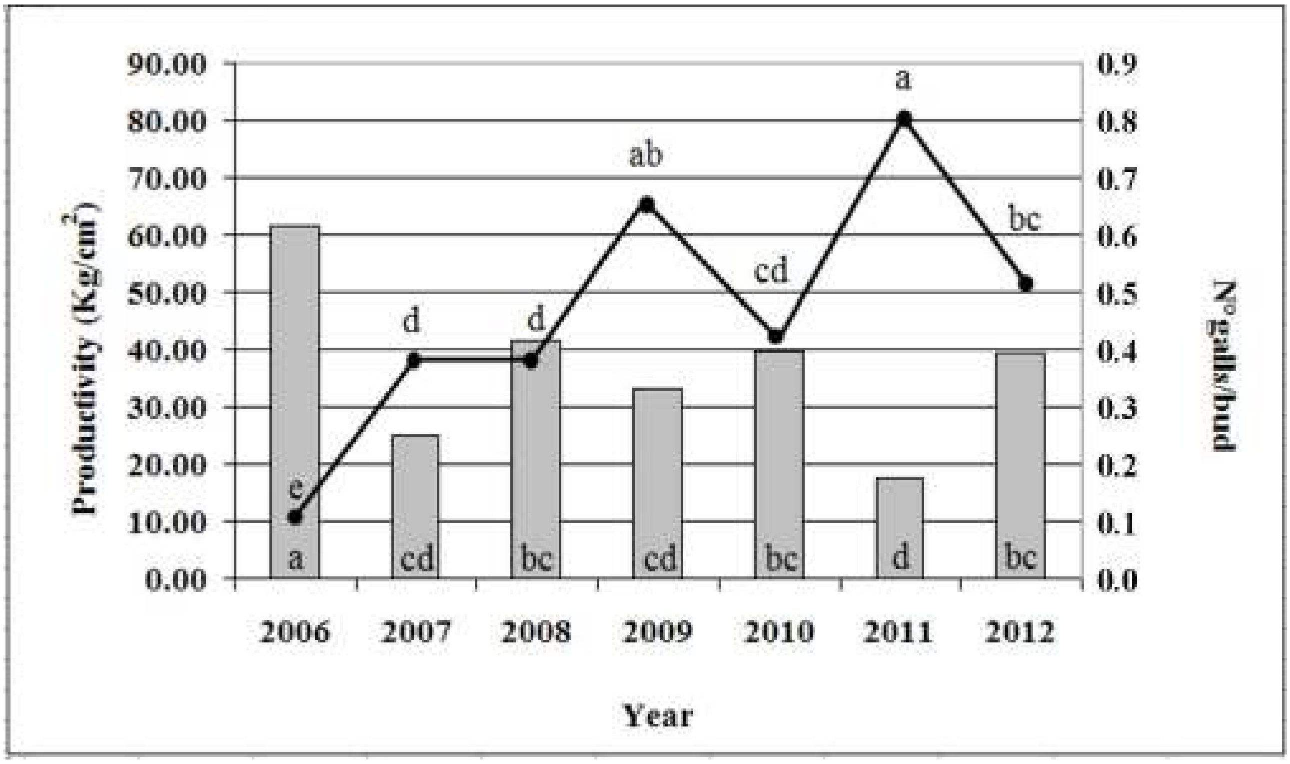

Fig. 2: Chart of yield (productivity index, bars) and infestation levels (black line) observed in the years 2006-2012.

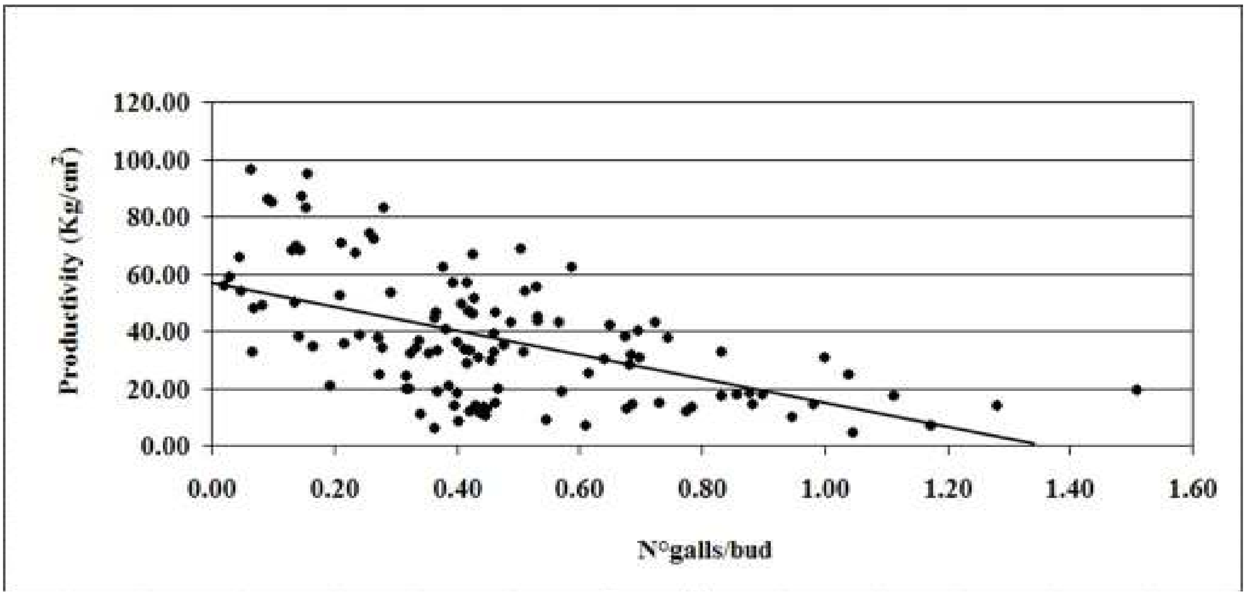

Fig. 3: Correlation between productivity (kg/cm^2) and infestation (No. galls/bud) of data collected in the years 2006 - 2012 (R = 0.56*)

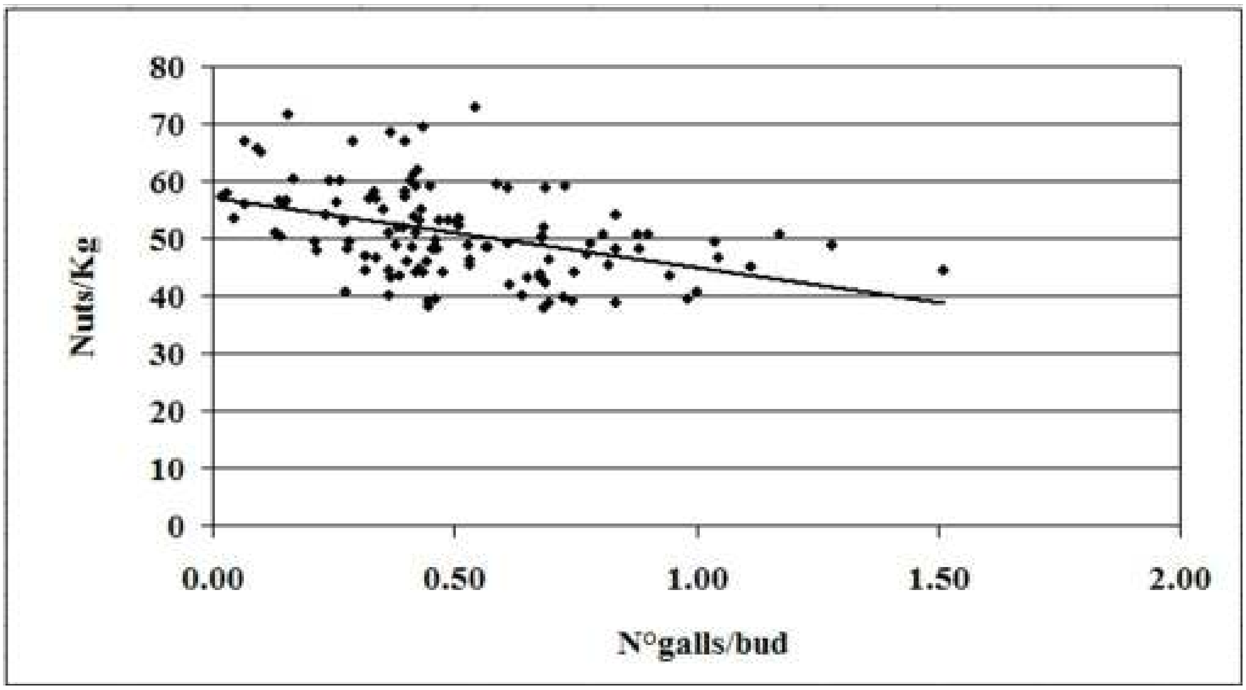

Fig. 4: Correlation between nut size (No. nuts/kg) and infestation index (No. galls/bud)

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/cm^2; medium: 32.74 kg/cm^2; low: 58.97 kg/cm^2).

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 'Savoye'. 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 mm^3) compared with smaller buds (approximately 3 mm^3).

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).

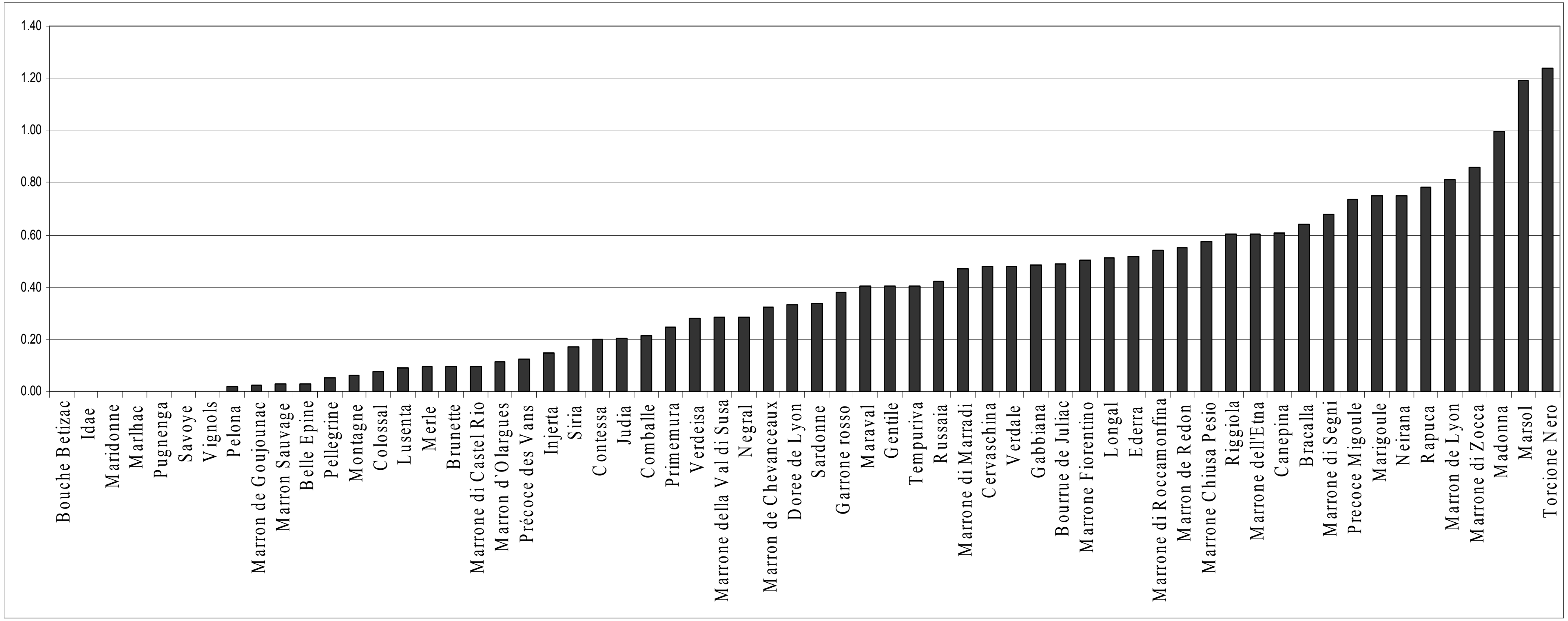

Fig. 5: Susceptibility (ratio No. of galls/No. of buds, G/B) to gall wasp in 62 cultivars of chestnut.

3.3. Gall impact on leaf characteristics

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

The weight values obtained for healthy leaves (1.27±0.5 g) and leaves with gall (0.98±0.46 g) were not significantly different. As reported by Kato and Hijii (1997) the effect of the gall presence on the leaf of *Castanea crenata*, leads to a surface reduction of about 50%, and consequently to a reduction of the photosynthetic apparatus. Moreover the gall, while being an organ capable of operating the photosynthesis, at least in a first phase of its development, appears to have a lower chlorophyll content compared to healthy leaves (Andersen and Mizell, 1987). In our study galled leaves had a mean area of 19,5 cm^2 against 95,5 cm^2 of healthy leaves showing a drastic surface reduction (80%) due to gall development.

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

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

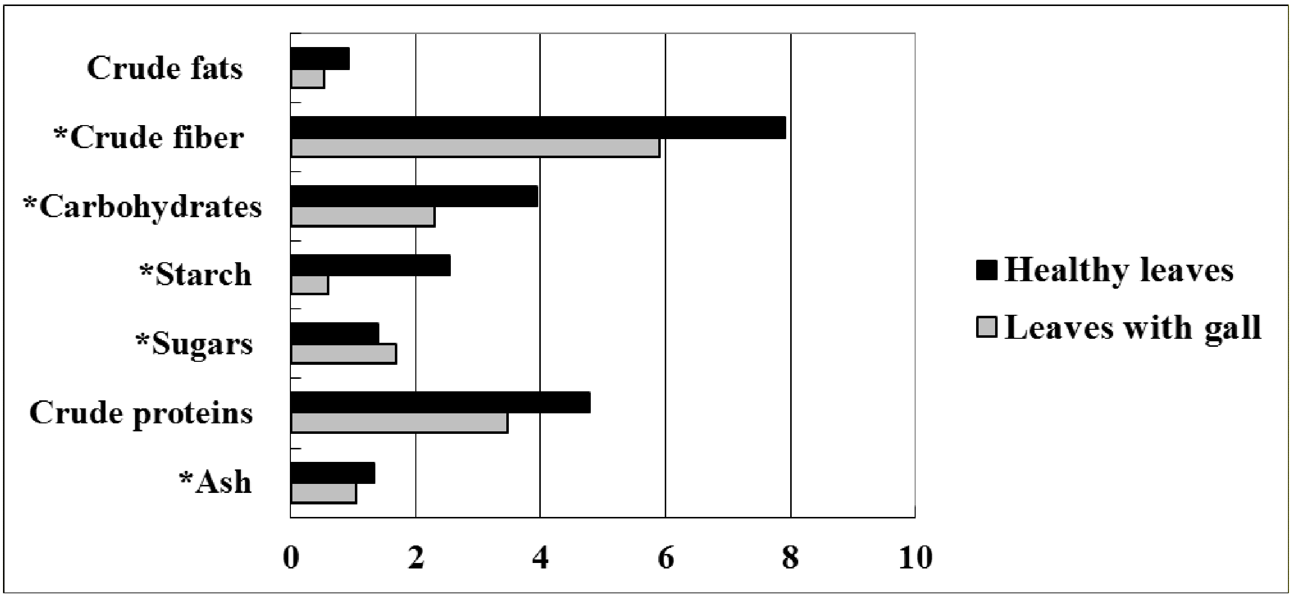

Fig. 6: Ash content, protein, sugars, starch, total carbohydrates (sugars+starch) and crude oils and fats of the healthy leaf and the leaf with gall (g/100g fresh weight) *p = 0.05.

4. CONCLUSIONS

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

EFSA (2010) elaborated a risk assessment of the oriental chestnut gall wasp *Dryocosmus kuriphilus* for the EU territory, stating that: “The potential for yield reduction in *Castanea* and negative effects on production is estimated as moderate. Although reported as high in the literature, there is a high level of uncertainty relating to this estimate in the absence of quantitative data confirming the yield reduction attributed directly to *D. kuriphilus*". In this paper we show that the yield loss can be potentially very high in highly susceptible cultivars in the absence of biological control. These information will be useful for evaluating yield loss in orchards and elaborate policy of support and strategies of pest containment, in particular in those Countries where the insect was only recently found.

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

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LEGENDA OF TABLES AND FIGURES

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

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

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

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

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

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