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Do Castanea sativa wild provenances influence *Dryocosmus kuriphilus* Yasumatsu (Hymenoptera: Cynipidae) infestations?

Mario CONTARINI[1], **Luca ROSSINI**[1,*], **Romina CACCIA**[1], **Silvia MORELLI**[1], **Isacco BERITOGNOLO**[2], **Muriel GAUDET**[2], **Fiorella VILLANI**[2], **Bruno PAPARATTI**[1], **Stefano SPERANZA**[1]

[1]Department of Agricultural and Forest Sciences (DAFNE), University of Tuscia, Viterbo, Italy
[2]Institute of Research on Terrestrial Ecosystems (IRET), National Research Council of Italy (CNR), Porano, Italy

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Abstract: The Asian chestnut gall wasp (ACGW), *Dryocosmus kuriphilus*, native to China was accidentally introduced into many countries worldwide, including Italy. Different susceptibilities have been reported in literature among cultivated plants belonging to different gene pools worldwide, but this aspect has not been sufficiently explored among wild chestnut populations. The aims of this multiyear study were (i) to assess differences in susceptibility to ACGW in wild *C. sativa* plants coming from different parts of Europe grown in an experimental plot in Central Italy, (ii) to preliminarily analyse the relationships between temperature, relative humidity and rain variables of winter and summer and *D. kuriphilus* infestation. Provenances coming from Greece reported a significantly smaller number of galls than the Italian and Spanish ones in the three-years of survey, showing a lower susceptibility to ACGW attacks in respect of plants coming from Italy and Spain. Interannual variation of ACGW infestation in the chestnut provenances may be influenced by the local environmental conditions. A hot and dry summer, occurred in 2015, could be responsible for a marked decrease in ACGW population density during 2016. The results lead to suppose that the different genotypes considered have different reactions depending on environmental conditions and ACGW attacks.

Key words: Chestnut gall wasp, invasive species, chestnut genotypes, meteorological factors

1. Introduction

Invasive insect species have significant ecological impacts on natural ecosystems and agriculture because of their effects on conservation of biodiversity and their ability to cause remarkable economic loss each year (Biondi et al., 2018). Among these harmful pests the Asian chestnut gall wasp (ACGW), *Dryocosmus kuriphilus* Yasumatsu, 1951 (Hymenoptera: Cynipidae), infests chestnut trees, induces galls on growing shoots and leaves, causes massive yield reductions in different species of the genus *Castanea* (Bernardo et al., 2013; Sartor et al., 2015). Native to China, ACGW was accidentally introduced in Japan (1941), Korea (1963), USA (1974) and many other countries worldwide.[1] In Europe, ACGW was first recorded in 2002 in Piedmont (Italy) (Brussino et al., 2002), from where it rapidly spread and established in the Italian peninsula and in several continental countries through dissemination of infected grafting materials (Quacchia et al., 2008; Avtzis et al., 2019). The formation of galls induced by ACGW affects normal plant development, inducing a condition of stress in chestnut trees. Battisti et al. (2014) reported yield losses in some cases as high as 80% of the total production. Losses can also be amplified by fungal pathogens such as *Gnomoniopsis castanea*, associated with *D. kuriphilus*, that can cause necrosis of galls and surrounding plant tissues (Vannini et al., 2017; Morales-Rodriguez et al., 2019).

ACGW is a univoltine species. Usually, between June and July (Bernardo et al., 2013), thelytokous females lay eggs into chestnut buds where, in optimal conditions, eggs hatch after few days. Larvae, protected by chestnut buds, overwinter and in the next spring induce a prompt formation of greenish-red galls, with a size of 5–15 mm. *D. kuriphilus* pupate and become adult inside the galls at the beginning of summer (Otake, 1980; Sartor et al., 2009).

Temperature, relative humidity and rain can influence ACGW development and behaviour (Bonsignore and Bernardo, 2018; Gil-Tapetado et al., 2018), particularly during the flight period (June–July), when adults fly for oviposition becoming more susceptible to biotic and abiotic factors (Bosio et al., 2010). Additionally, as ACGW overwinters in larval form, low winter temperatures may induce larval mortality as it is the case for many other

[1] CAB International (2021). Dryocosmus kuriphilus (Oriental chesnut gall wasp) [online]. Website https://www.cabi.org/isc/datasheet/20005 [07 January 2021].

* Correspondence: luca.rossini@unitus.it

insect species having the similar overwintering pattern (Turnock and Fields, 2005).

Chemical control of preimaginal stages and adults of *D. kuriphilus* has proven to be ineffective; most field trials failed to show significant results (Moriya et al., 1989; Stacchiotti, 2009; Bernardo et al., 2013). On the other hand, substantial results were obtained using an ACGW parasitoid, *Torymus sinensis* Kamijo,1982 (Hymenoptera: Torymidae), released in Japan (1975) and in Italy (2005) (Moriya et al., 2002; Quacchia et al., 2008). After its release, *T. sinensis* successfully reduced *D. kuriphilus* infestations and also showed edits capability of effective establishment in new environments (Moriya et al., 2002; Cascone et al., 2018; Ferracini et al., 2019). In addition to the biological control by parasitoids, the identification of chestnut germplasm resistant, tolerant or less susceptible to *D. kuriphilus* is a valuable approach in reducing the impact of ACGW on chestnut trees (Quacchia et al., 2008).

A high genetic diversity in traditional and local varieties on chestnut is reported in many studies (Martín et al., 2017), leaving ample scope in the identification of cultivars, ecotypes, varieties, etc., that may combine different agronomic aspects with the lowest susceptibility to insect pests. In this frame, several genetic studies, firstly in Asia and then in Europe, were promoted to find and breed varieties of chestnut tolerant to *D. kuriphilus* (Kajiura and Machida, 1961; Moriya et al., 2002; Shimura, 1973; Sartor et al., 2015; Nugnes et al., 2018). Most studies on the susceptibility of *C. sativa* to ACGW were conducted on grafted varieties or cultivated germplasm, whereas few data are available on the wild genetic resources of this species.

The aims of this multiyear study were: i) to evaluate the variation in susceptibility to ACGW in geographic provenances of wild *C. sativa* trees grown in an experimental plot located in Central Italy; and ii) to preliminarily analyse the possible relationships among temperature, relative humidity and rain variables in winter and summer over consecutive years along with infestation level and population dynamics of *D. kuriphilus*.

2. Materials and methods

2.1. Experimental site and plant material

The experimental field is composed, to date, of 1003 chestnut plants from six European provenances, and it is located in Castelgiorgio municipality (Terni, Central Italy), latitude 42.675°N, longitude 12.000°E (Figure 1), elevation 620 m a.s.l. The field has approximately a surface of 10 ha, is moderately sloped, with north-east orientation and characterized by a volcanic soil (pH 6.23). The surrounding environment is composed by open fields alternated to vegetation dominated by *Quercus cerris* L., 1753. The experimental field locates in a temperate Mediterranean climate area, with typical summer droughts. This experimental field was established by the European Project CASCADE (2003) with provenances randomly sampled from natural populations of *C. sativa* from six European sites (Figure 1). The provenances are from Spain, (Coruña, henceforth Sp1 and Malaga, henceforth

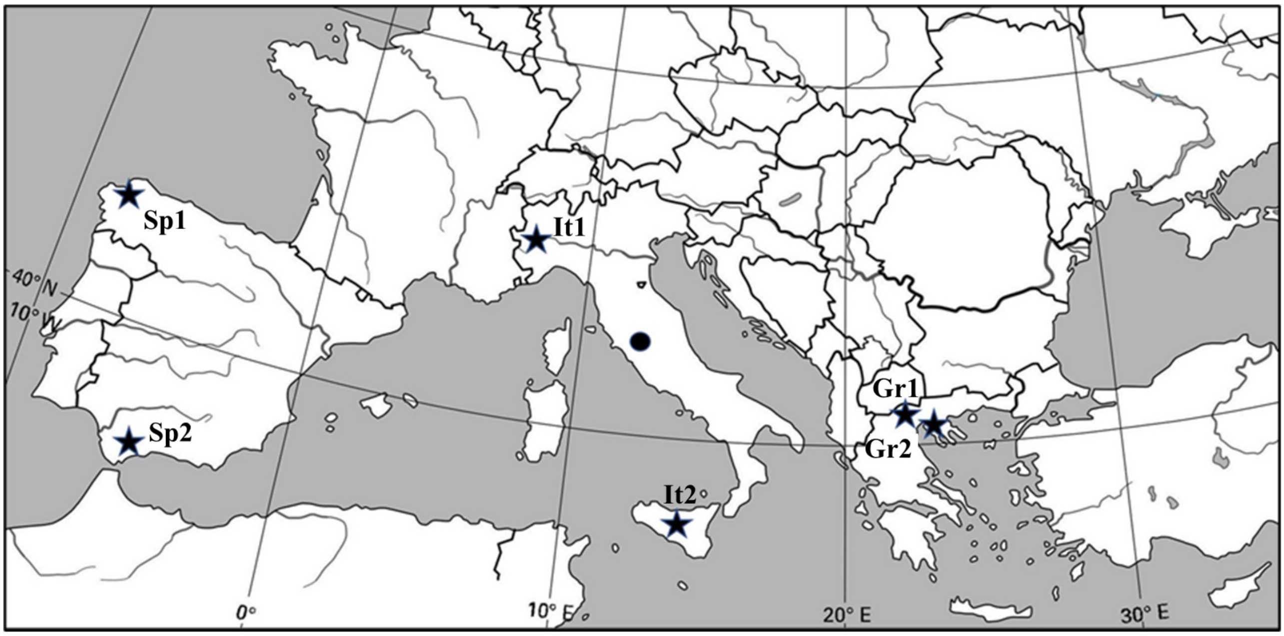


Figure 1. South Europe map localising the experimental site in Castel Giorgio (Italy) (black dot) where the investigation was conducted. Black stars and codes identify the provenance areas of the chestnuts that were considered in this study.

Sp2), Italy (Pellice, henceforth It1 and Petralia Sottana, henceforth It2), and Greece (Paiko, henceforth Gr1 and Hortiatis, henceforth Gr2) (Pliura and Eriksson, 2002) (Table 1). These provenances are characterized by different climatic conditions: those identified by the number 1 were characterized by mesic conditions while the provenances 2 had more xeric traits. Chestnut fruits were collected from individual mother trees in the sites of origin in 2001, then germinated and grown in nursery. Seedlings were planted in the plot in the spring of 2002. The plot trial was colonized by *D. kuriphilus* in 2008–2009. The *D. kuriphilus* population was assessed with preliminary field observations in 2014 and with more in-depth field surveys in 2015 and 2016.

2.2. Preliminary analysis of *D. kuriphilus* infestation

A preliminary and explorative investigation was carried out in October 2014 on all the 1003 chestnut plants in the experimental plot to evaluate the level of *D. kuriphilus* infestation (Table 2). The infestation level was evaluated by a sampling of four 1-m long branches per plant for quantification of buds and galls. Apparently healthy branches were selected randomly at a breast height and, in any case, at a height that never exceeded 2 m from the ground. Galls were counted once a spherical shape and a minimum dimension of 5 mm of diameter was reached, and they were not distinguished basing on type (sensu Kato and Hijii, 1997) and size. Thereafter, the plants were ranked into a four-class system based on the following percentage *R*:

$$R = \frac{N_G}{N_B} \cdot 100 \qquad (1)$$

where N_G is the number of galls and N_B is the number of buds per branch. With the expression (1) plants were assigned into four different classes defined adapting the classification reported by Sartor et al. (2009). Notably, class 1, no infestation (0%), class 2, low infestation (<19.99%), class 3, medium infestation (20%–49.99%), class 4, high infestation (>50%).

The preliminary observations of the *D. kuriphilus* infestation recorded in 2014 were used to have an estimation of how many plants of classes 1 and 2 displayed a relatively low susceptibility to *D. kuriphilus* (Table 2). Since there were wide differences in the number of trees belonging to classes 1 and 2, the choice for 2015 and 2016 was to continue to focus the study only on the less infested plants that each year (after checking all the 1003 plants) fell into the classes 1 and 2.

2.3. 2015 and 2016 samplings

In July 2015 and 2016, a detailed survey focused on plants selected in the infestation classes 1 and 2, with infestation rates lower than 20%. To determine the plants belonging to each class, all the 1003 plants were surveyed each year, following the methodology presented in subsection 2.2. According to the surveys, a total of 100 and 176 plants satisfied this condition in the 2015 and 2016 seasons, respectively (Table 2), thus were considered for the experimentation. Differences among the provenances in terms of the number of buds available for *D. kuriphilus* oviposition were assessed by randomly selecting four 1-m long branches per plant and counting the total number of buds (100 × 4 and 176 × 4 branches). The number of buds has been considered for two reasons: i) it was necessary to assign plants among the infestation classes and, ii) if the number of buds does not differ among the provenances, the infestation can be expressed with the number of galls N_G instead of using the expression (1).

The number of cells per gall, i.e. the larval chambers where ACGW develops, was assessed by randomly collecting a total of 580 galls in 2015 and 450 galls in 2016. To do that, 58 and 45 plants in 2015 and 2016, respectively, were randomly selected among the plants belonging to all the provenances within the infestation classes 1 and 2. From each plant, 10 galls were collected, brought to laboratory, dissected with a scalpel and cells were counted under a stereomicroscope.

Table 1. Sites of origin and new codes of the *C. sativa* provenances analysed in the study.

Site	Country	Geographic coordinates	New code
Paiko	Greece	40.961°N 22.371°E	Gr1
Hortiatis	Greece	40.378°N 23.164°E	Gr2
Coruña	Spain	43.287°N 8.373°W	Sp1
Malaga	Spain	36.536°N 5.305°W	Sp2
Pellice	Italy	44.816°N 7.150°E	It1
Petralia Sottana	Italy	37.822°N 14.088°E	It2

2.4. Meteorological data

Meteorological data from the years 2013, 2014, and 2015 were provided by the agrometeorological station of Grotte di Castro, managed by ARSIAL agency.[2] The raw dataset was composed of the daily average temperature and

[2] ARSIAL Regional Agency for the Development of Innovation and Agriculture in Lazio (2021). SIARL Servizio Agrometeorologico della Regione Lazio [online]. Website http://www.arsial.it/portalearsial/agrometeo/index.asp [accessed 07 January 2021].

Table 2. Total number of chestnuts presents in the experimental field belonging to classes 1 and 2 of infestation, divided per provenance, and tested in the survey conducted in 2014, 2015, and 2016. Provenances are identified by an alphanumeric code in which Gr stands for Greece, It for Italy and Sp for Spain while the numbers 1 or 2 identify mesic or more xeric climatic conditions, respectively.

	Total number of plants tested	Number of plants belonging to classes of infestation 1 and 2	
Provenance	2014	2015	2016
Gr1	71	12	8
Gr2	106	35	18
It1	166	9	39
It2	281	12	55
Sp1	241	5	35
Sp2	138	27	21
Total	1003	100	176

humidity values, and the total amount of rain per day. These data were used to highlight the differences between the years of surveys, in the meteorological variables of the summer months, June and July, and the winter months, December, January and February.

2.5. Data analysis

2.5.1. Preliminary analysis: year 2014

Differences in i) the number of buds produced by plants, ii) the percentage of infestation and iii) the number of cells per gall were analysed with the Kruskal–Wallis nonparametric test, followed by Dunn's pairwise comparison, with R software (R Core Team, 2018). The analysis was performed considering $\alpha = 0.05$.

2.5.2. 2015 and 2016 data

Differences in number of buds produced by plants belonging to different provenances were assessed with the Kruskal–Wallis nonparametric test, followed by Dunn's pairwise comparison. Differences (without distinction among provenances) in number of buds between the two years of survey were assessed using Mann–Whitney test.

The number of galls were analysed with a GLM with a Poisson distribution and Bonferroni posthoc test. In particular, provenances and years were considered as factors, while the numeric values concerned the number of galls and the number of cells within the galls. The analysis was performed using R software (R Core Team, 2018), and considering $\alpha = 0.05$.

2.5.3. Meteorological data

Temperature and relative humidity data were analysed using the ANOVA test followed by a posthoc Tukey HSD test, with R software (R Core Team, 2018) and considering $\alpha = 0.05$. The total rainfall was analysed with a x^2-test calculated with EntoSim software (Rossini et al., 2019a, 2020a). Although ANOVA and x^2 test are not usually considered when analyzing meteorological data, they were used in the present study for two main reasons: i) it may be possible to assume that the trend in the measured daily values is negligible over the short periods of the year considered, ii) the meteorological and population dataset did not allow for a more refined analysis.

3. Results

3.1. Preliminary survey of *D. kuriphilus* infestation

The preliminary investigations carried out in 2014 revealed a significant variation (Kruskal–Wallis test, $p < 0.0001$) in the percentage of infestation among the provenances and highlighted that Gr2 plants were the least infested by *D. kuriphilus*, in comparison to the other provenances (Figure 2a).

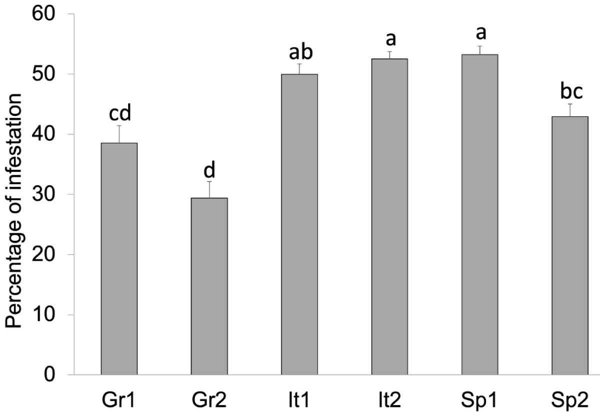
Percentage of plants sorted into the different classes of infestations can be seen in Figure 2b. The proportion of plants belonging to the infestation class 1 was much higher in Gr2 provenance than in the other provenances, as 32% of Gr2 plants did not have any galls. On the opposite end of the scale, the lowest percentage of the infestation class 1 was found in Sp1 (1.2%) and It1 (2.4%) provenances.

3.2. Multiyear analysis of chestnut buds and *D. kuriphilus* infestation

3.2.1. Buds available for oviposition

In the 2015 season, the number of buds formed by chestnut trees was not significantly different among the six provenances (Kruskal–Wallis test, $p = 0.43$).

In a similar way, no significant differences among provenances were observed in 2016 (Kruskal–Wallis test, p



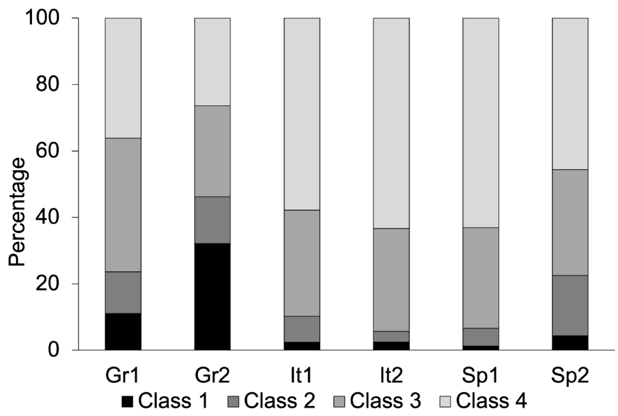


Figure 2. Panel a). Percentage (mean ± SE) of *D. kuriphilus* infestation observed in 2014 on *C. sativa* provenances. Different letters indicate significant differences at the Dunn's multiple comparison test, (p < 0.0001) among the provenances. Panel b). Percentage contribution of each class of infestation (1–4). Gr, Greece; It, Italy, Sp, Spain; the numbers 1 or 2 identify mesic or more xeric climatic conditions, respectively.

= 0.26). Accordingly, the six provenances formed a similar number of buds available for *D. kuriphilus* oviposition into each year of survey.

On the whole, the number of buds formed by all chestnut plants was significantly higher in 2016 than in 2015 (Mann–Whitney test, p < 0.0001).

3.2.2. ACGW infestation

The number of galls recorded in two consecutive years are presented in Figures 3a and 3c. In the 2015 survey (Figure 3a), the provenances showed highly significant differences in the infestation caused by *D. kuriphilus* (GLM, p < 0.0001). The plants belonging to Gr1 and Gr2, showed the lowest number of galls, 3 ± 1 and 3.4 ± 0.8 (mean ± se), respectively, which was a significantly different result from all the other provenances (GLM, p < 0.0001). The number of galls detected on the Italian provenances, It1 and It2, was 16 ± 3 and 9 ± 2 (mean ± se), respectively, while Spanish ones, Sp1 and Sp2, had values of 17 ± 3 and 7 ± 1 (mean ± se), respectively. However, there was a significant difference between the couples It1, Sp1 and It2, Sp2 (GLM, p < 0.0001).

The survey conducted in 2016 (Figure 3c) again showed significant differences in the number of galls among the provenances (GLM, p < 0.0001) confirming that Gr1 and Gr2 were the least infested. In particular, Gr2 showed an extremely low number of galls, 1.0 ± 0.6 (mean ± se). Similar to the 2015 survey, the highest number of galls was observed in the Italian It1 and Spanish Sp1 provenances, with values of 5 ± 1 and 5 ± 1 (mean ± se), respectively.

On the whole, the total infestation in terms of number of galls in plants belonging to class 1 and 2 decreased significantly between the two years of investigation (GLM, p < 0.0001).

3.2.3. Analysis of cells

The results of microscope analysis on dissected galls are presented in Figures 3b and 3d. Significant differences among the provenances in terms of the number of cells per gall were observed in both the years of survey (GLM, p < 0.0001).

Notably, in the year 2015 Gr1 showed the lowest number of cells per gall (1.6 ± 0.1, mean ± se), while the highest number was recorded in Sp1 provenance (3.7 ± 0.3, mean ± se).

Statistical differences among provenances were found in 2016 survey, also (GLM, p < 0.0001). As already observed in 2015, even in 2016 Gr1 showed the lowest number of cells (2.0 ± 0.2, mean ± se) but in this year the same value has been reported by Sp2 (2.0 ± 0.1, mean ± se) also. The highest number was recorded in It1 provenance (2.4 ± 0.1, mean ± se).

Overall, the number of cells per gall recorded on plants belonging to classes 1 and 2 decreased significantly between the two years of the survey (GLM, p < 0.0001).

3.3. Multiyear analysis of meteorological data

Summer meteorological data were analysed focusing on June and July in 2013, 2014 and 2015 (see supplementary material), since, in our assumption, the infestation of the year is a consequence of what happened the year before. These two months are relevant for the phase of flight and oviposition of *D. kuriphilus*.

The warmest summer by a significant margin was recorded in 2015, while there were no significant differences between 2013 and 2014.

An analogous situation was assessed in relative humidity, with the summer 2015 being significantly more humid than in 2013 and 2014.

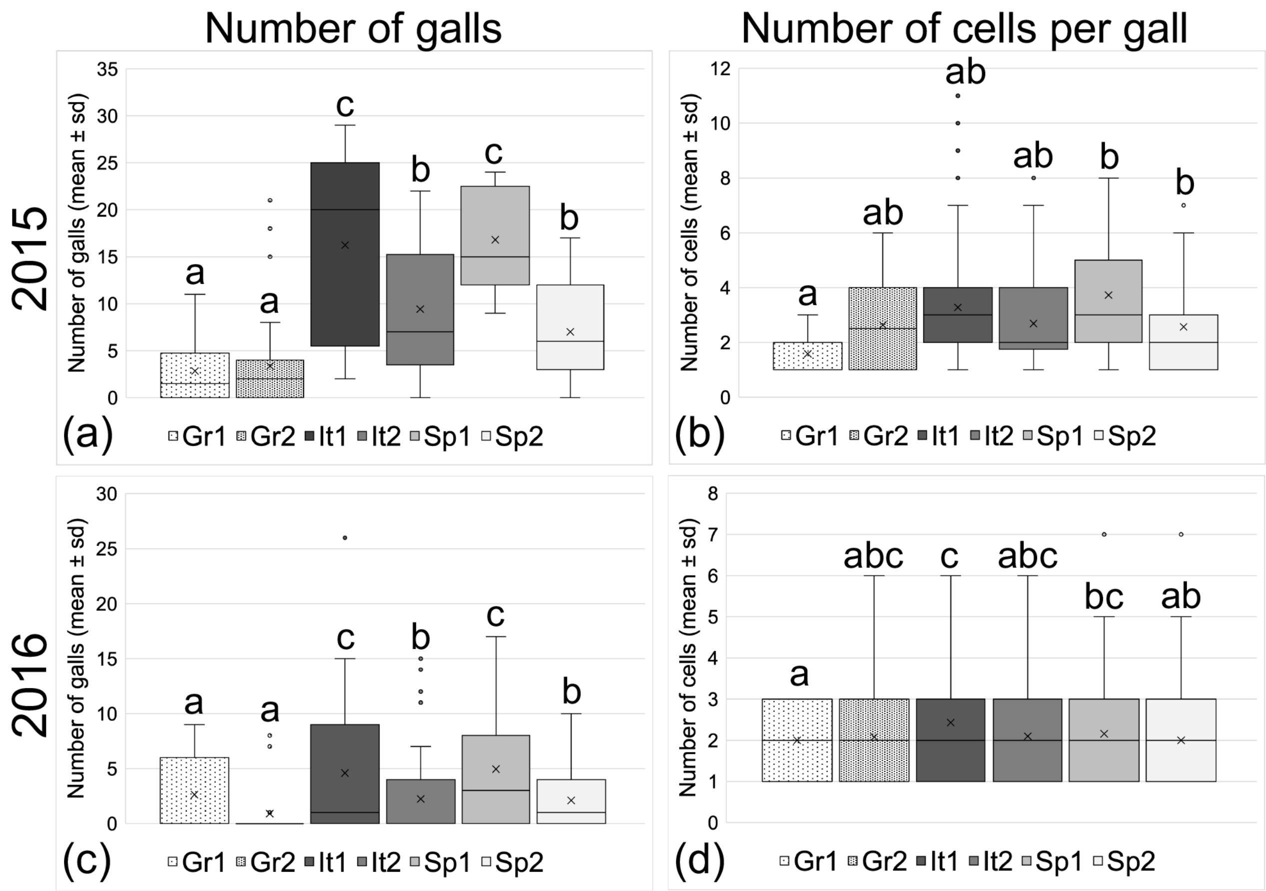


Figure 3. Infestation by *D. kuriphilus* on six *C. sativa* provenances grown in common garden field trial, during the years 2015 (top plots) and 2016 (bottom plots). Panels a) and c) number of gallsper shoot/branch; b) and d) number of cells per gall. Bars represent mean and standard error. Within each plot, different letters indicate significant difference between provenances after Bonferroni posthoc test at p < 0.05.

The total rainfall in June and July was significantly different ($p < 0.05$) among the three years of investigation, with the summer 2014 being the wettest summer, and the year 2013 being the driest one.

On the whole, the summer 2014 was the coldest and dampest, while the summer 2015 was the warmest and driest one, in terms of relative humidity.

Winter climatic data were analysed considering the interval between December and February of each year (see supplementary material). These three months may be relevant for the phase of overwintering of *D. kuriphilus* larvae. In particular, temperature, relative humidity and total amount of rain were considered.

The 2015–2016 winter was the warmest one, the 2014–2015 winter was the coldest one and the 2013–2014 winter had an intermediate temperature, not significantly different from the others.

On the other hand, the data of relative humidity show a different pattern. The 2013–2014 winter was the dampest one, while the following 2014–2015 winter was the driest one. An intermediate relative humidity was recorded in 2015–2016 winter, not significantly different from the others.

The total rain recorded during the three winters showed that the 2013–2014 winter was significantly wetter than the winter of the two consecutive years.

Overall, the meteorological data indicated two different scenarios for the two growing seasons 2015 and 2016 that were considered in this study.

4. Discussion and conclusion

The results obtained in this study provide evidence of a difference in galls formation due to *D. kuriphilus* activity among European wild provenances of *C. sativa* (coming from Greece, Italy and Spain) cultivated in an experimental field and tested in three consecutive years. In particular, the provenances coming from Greece displayed a significantly lower formation of galls in comparison to the provenances coming from Italy and Spain. This trend has been consistently recorded during three years of observation. In the 2014 survey, the Gr1 and Gr2 provenances contained the highest number of plants belonging to the class 1, with no infestation. On the contrary, the It1, It2, Sp1 and Sp2 provenances showed a high proportion of plants belonging to the fourth class of infestation, with more than 50% of infested buds. Although the year 2014 was considered as a year of preliminary investigation, it was coherently aligned with the results of 2015 and 2016 if the number of galls is considered, and with the results obtained by Bombi et al. (2019) for the year 2016. The number of galls, in fact, confirms the susceptibility level assessed during 2014 even though this year of survey cannot be compared at all with the other two, since the survey was conducted in a different season. Collecting data in the month of October, as experienced in 2014, instead of the month of July can bring to an underestimation of the effective infestation level.

The results lead to suppose that the variation in susceptibility to ACGW is associated with the geographic origin of the chestnut plants, even though further genetic studies are needed.

Previous studies have investigated the susceptibility to *D. kuriphilus* in the genetic resources of various *Castanea* species. Research and breeding programmes were conducted in Japan on *C. crenata* (Shimura, 1973) and in Italy on *C. sativa* in order to analyse differences in susceptibility to *D. kuriphilus* (Bracalini et al., 2019). In particular, Sartor et al. (2015) assessed the susceptibility to *D. kuriphilus* in 62 cultivated and wild *Castanea* spp. accessions that ranged from totally resistant to highly susceptible. In addition, it has been reported that plants belonging to the Euro-Japanese hybrid "Bouche de Betizac" (Sartor et al., 2009, 2015) showed a hypersensitivity reaction involved in resistance to the ACGW activity (Dini et al., 2012). More recently, Nugnes et al. (2018) reported the low susceptibility of a *C. sativa* ecotype present in Southern Italy and highlighted that the resistance to ACGW is caused by a high mortality rate among the insect larvae.

The experience of this study suggests that the number of galls can be considered, with good approximation, as the main indicator of infestation and plant susceptibility. The number of galls, in fact, provides a direct estimation of the damages that ACGW provokes on chestnut plants, and an indirect indication of the population abundance. However, the number of cells becomes fundamental when a more precise estimation of the population abundance is necessary, since it can be obtained multiplying the mean number of galls and the mean number of cells per gall.

Graziosi et al. (2014) reported an average number of 250–300 eggs laid by each female in a short period ranging between 1 and 3 days after adult emergence. This leads to the assumption that in cases of high population density, females lay eggs in already colonized buds because of the low availability of noncolonized buds. According to this reasoning, it is possible to hypothesize that in summer 2014 more females lay eggs in the same bud, confirming the results obtained by Kato et al. (2001). This fact could explain the higher statistical differences among the provenances reported in 2015 in terms of number of cells per gall. The year 2015, in fact, had a higher population abundance, estimated in terms of number of galls, in respect of 2016, where less statistical differences among the provenances emerged in terms of number of cells per gall. The reduction of the population abundance, both in terms of number of galls and number of cells per gall, assessed in 2016 may be explained by meteorological factors.

According to Bonsignore et al. (2019), in fact, environmental alterations of the seasonal temperature may induce modifications in the ACGW behaviour, which could affect the flight activity and accordingly oviposition, also. ACGW adults, in central and southern Italy, emerge and lay their eggs mainly in the months of June and July (Bosio et al., 2010; Bernardo et al., 2013). The relatively hot and dry summer that occurred in 2015, in comparison to 2014, may be responsible for a marked decrease in ACGW population density in the year 2016, resulting in a lower infestation observed in year and among the provenances.

It is also likely that winter climatic conditions affect ACGW mortality since this insect overwinters as first instar larva in chestnut buds, and galls are formed after the start of plant growth in spring. Our results from two years of observations do not confirm such a hypothesis and do not lead to suppose any relationships between winter climatic conditions and a change in ACGW population. However, further multiyear studies on this aspect are desirable.

The low susceptibility to ACGW of Gr1 and Gr2 provenances is likely to be determined by a genetic basis, because the susceptibility traits were phenotypically stable and confirmed by independent experiments (Mattioni et al., 2013). Previous studies on the neutral genetic diversity and structure of *C. sativa* populations highlighted that the Greek chestnut gene pool is genetically distinct from the gene pool of Western Europe, including Italy and Spain (Mattioni et al., 2013). The distinct genetic background of the Greek chestnut could bring functional genes selected for evolutionary adaptation to abiotic and biotic stresses, which could account for the lower susceptibility to ACGW observed in this study.

A secondary but noteworthy pattern of variation can be observed within the Spanish and Italian provenances that belong to the Western Europe gene pool. The provenances Sp2 and It2, which originated from Southern sites (and that show more xeric conditions), displayed a lower ACGW susceptibility than the provenances Sp1 and It1, which originated from northern mesic sites of the same country. In a previous common garden study, the chestnut plants that originated from sites with more xeric conditions (Sp2 and It2) showed different water use efficiency than the provenances Sp1 and It1, respectively (Lauteri et al., 2004). The statistical difference in ACGW susceptibility (in terms of number of galls) between mesic and more xeric provenances It1, Sp1 and It2, Sp2 was consistently observed in both years, thereby supporting the hypothesis that genetic susceptibility to ACGW could be associated with the evolutionary adaptation to drought-prone climates. Different values and higher phenotypic plasticity of water use efficiency, measured by carbon isotope discrimination (Δ), indicate a wider variation of adaptive response to drought in more xeric sites. This comparative field trial represents a good starting point for future studies to identify genes for susceptibility to ACGW and to investigate mechanisms of host-parasite interaction.

The better response to *D. kuriphilus* attacks displayed by the Gr1 and Gr2 provenances indicate that wild genetic resources of *C. sativa* could be valuable for new chestnut plantations and for breeding programmes to improve the existing germplasm. Since *D. kuriphilus* presence has been recently assessed in Greece (Michaelakis et al., 2016) it will be interesting to verify whether the assessed low susceptibility of plants coming from Greece will be confirmed by other studies carried out on the native chestnut population.

As future perspective, understanding better how the meteorological factors influences *D. kuriphilus* phenology on different chestnut varieties is fundamental for understanding the effects of the natural enemies complex (Bonsignore and Bernardo, 2018). Physical factors, such as temperature and relative humidity, in fact, drive insects population dynamics (Castex et al., 2018; Rossini et al., 2020c, 2020b, 2020a) by influencing development rate, fertility, survival, fitness and the abundance (Büntgen et al., 2020) and plant responses to external stresses (Gil-Tapetado et al., 2021).

In conclusion, as demonstrated in this study, the continued research of appropriate genotypes can be helpful in reducing the impact of *D. kuriphilus* in chestnut cultivations. A correct interpretation of climate conditions may provide an additional support mechanism to predict and contrast ACGW and other insect pests in general. This integrated approach can be extended to other invasive species, such as the ambrosia beetle *Xylosandrus germanus* (Blandford, 1894) (Coleoptera: Curculionidae) recently detected in the same extended area (Rassati et al., 2020), and it will be fundamental in the protection of the chestnut trees for the economic and environmental value of this species.

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Supplementary Material

Table S1. Summer climatic data analysis. Temperature and relative humidity were compared with ANOVA test with a posthoc Tuckey HSD test. Letters indicate significant differences among the summers ($p < 0.05$). Rain values are the sum of total rain in the months of June–July of each respective year. In this case, letters were assigned with a $x^{!}$-test and indicate significant differences with $p < 0.05$.

Summer	Temperature (°C ± *SE*)	Relative humidity (*RM* ± *SE*)	Rain (*mm*)
June–July 2013	20.81 ± 3.26^{b}	66.82 ± 10.60^{a}	78.6^{a}
June–July 2014	20.33 ± 2.55^{b}	70.93 ± 12.48^{a}	132.0^{b}
June–July 2015	23.47 ± 3.09^{a}	61.49 ± 11.23^{b}	110.4^{c}

Table S2. Winter climatic data analysis. Temperature and relative humidity were compared with ANOVA test with a posthoc Tuckey HSD test. Letters indicate significant differences among the winters ($p < 0.05$). Rain values are the sum of total rain in the months of December–February of each respective winter. In this case letters were assigned with a $x^{!}$-test and indicates significant differences with $p < 0.05$.

Winter	Temperature (°C ± *SE*)	Relative humidity (*RM* ± *SE*)	Rain (*mm*)
Dec 2013–Feb 2014	6.59 ± 2.21^{ab}	87.16 ± 13.15^{a}	368.4^{a}
Dec 2014–Feb 2015	5.99 ± 2.79^{b}	80.66 ± 15.69^{b}	266.2^{b}
Dec 2015–Feb 2016	7.14 ± 2.51^{a}	84.69 ± 13.30^{ab}	248.2^{b}

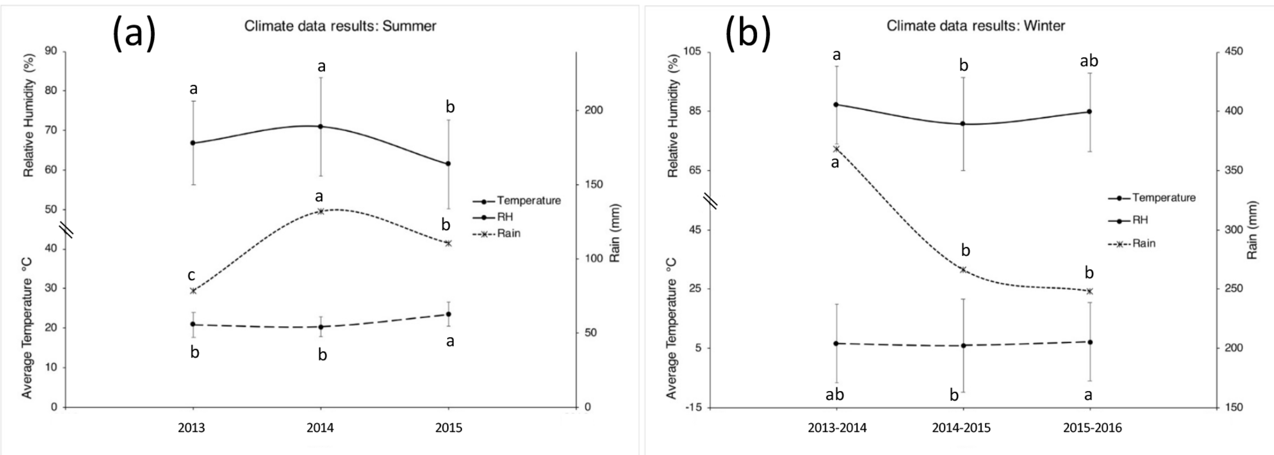

Figure S1. Results of climatic data analysis of the interval June–July (left panel a) and December–February (right panel b) in 2013, 2014, 2015, and 2016. Temperature and relative humidity indicate mean value (dot) and standard error (bars). Rain is the sum of total rainfall in the interval, without confidence interval. The left vertical axis is divided in two sections, the average temperature at bottom, and the relative humidity at the top, respectively. Different letters indicate significant differences between years at $p < 0.05$. Differences between years in temperature and relative humidity were tested with ANOVA and posthoc Tukey HSD test ($p < 0.05$). Difference in rainfall between years were tested with $x^{!}$-test ($p < 0.05$).