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Bernasso, a paleoforest from the early Pleistocene: new input from plant-insect interactions (Hérault, France).

Benjamin Adroit^{1,2,*}, Torsten Wappler¹, Jean-Frederic Terra², Adam A. Ali², Vincent Girard²

¹ Steinmann Institute for Geology, Mineralogy and Palaeontology, Division Palaeontology, University of Bonn, Nussallee 8, D-53115 Bonn, Germany

² Institut des Sciences de l'Evolution, UMR 5554 Université de Montpellier, CNRS, IRD, EPHE. Place Eugène Bataillon, 34095 Montpellier cedex 05, France

* Corresponding author. Tel: +49228733336. E-mail address: badroit@uni-bonn.de (B. Adroit).

Abstract

Plants and insects have interacted for millions of years in terrestrial ecosystems and are essential to the function of the food web. These interactions can be classified based on different functional feeding groups and damage types, and provide information about the relationship between plants and insects. We focused this study on the Pleistocene deposit of Bernasso (Hérault, southern France). The Bernasso fossil leaf assemblage has been dated from 2.16-1.96 Ma and corresponds to a deciduous forest comprising both continental and Mediterranean species such as *Caprinus orientalis* Mill., *Parrotia persica* C. A. Mey. and *Acer monspessulanum* L., *Sorbus domestica* L., respectively. Twenty different plant species are represented by 590 fossil leaves among which more than 36% present insect damage. Thirty-nine different damage types were found with galling being the most prominent. These types of interactions enable discussion about the ecological settings that prevailed at this epoch (forest age, precipitation, temperature). For instance, the mean annual precipitation was certainly higher than today in the region. Moreover, the results of this study offer the possibility to get new insights into the ecological paleoforest community.

Keywords: paleoecology, paleoforest structure, Pleistocene, fossil leaves, insect damage

1. Introduction

There is no doubt that human activities in recent decades have impacted the climate (Ellis, 2011). Nowadays, some of these anthropogenic impacts have reached a tipping point, generating a “global change” (Turner et al., 1990; Heller and Zavaleta, 2009). The study of past climate thus can be very helpful to evaluate the impacts of the global change on multitrophic interactions and biodiversity (Dowsett et al., 2009; Salzmann et al., 2011). Regionally, for the northern Mediterranean realm and western Europe the highly diverse forest vegetation implies MATs 3-6°C higher than today with a respectively increased in mean annual precipitation (MAP) by 400 mm and 230 mm respectively (Utescher et al., 2000; Contoux et al., 2012). The current climate change will probably upset ecological processes, especially between vegetation and insects.

Interactions between plants and insects have played an important role in the structure of the food web and the study of their evolution could help to reconstruct climate dynamics (May, 1988; Wilf, 2008). Plant-insect interactions have also been shown to virulently impact entire modern ecosystems (Cornelissen, 2011). Distinctive signs found on fossil leaves are thus important to understand the evolution of interactions (Labandeira and Phillips, 1996; Wilf et al., 2001, 2006; Currano et al., 2008; Wappler et al., 2009, 2012; Labandeira, 2013). Therefore, the fossil record offers a unique possibility for conducting meta-analysis and for analyzing the dependence between climate parameters and plant-insect interactions through geologic time (Wilf et al., 2001; Currano et al., 2010; Wappler, 2010; Knor et al., 2012; Wappler et al., 2012; Labandeira and Currano, 2013).

Bernasso, a locality near Lunas (Hérault, southern France), provides a new snapshot of early Pleistocene multitrophic interactions between insects and plants. Previous studies on pollen records (Suc 1978) and fossil leaf assemblages (Leroy and Roiron 1996) suggest that Bernasso's climate was warmer and wetter than today. However, a recent study based on CLAMP analysis, still not published yet, could suggest a climate certainly wetter but cooler than today (unpublished data). Moreover, Bernasso provides a rare glimpse into multitrophic interactions between insects and plants in a time period slightly wetter and under different temperatures, as well as a similar geographic configuration to today (Rohling et al., 2009; Raymo et al., 2011). Leroy and Roiron (1996) showed that Bernasso fossil leaves fossilized in a lacustrine deposit, represented a deciduous forest mainly dominated by *Parrotia*

persica C. A. Mey., *Carpinus orientalis* Mill and other important species as e.g. *Zelkova ungerii* Spach., *Acer Monspessulanum* L., *Fraxinus ornus* L., *Sorbus domestica* L. and *Carya minor* Nutt. Today, there is no exact modern analog for the Bernasso paleoforest anywhere in the world. Bernasso was a combination between typical Mediterranean plants species (*A. monspessulanum*, *F. ornus*...) and some Caucasian tree species such as *P. persica* (its last natural population is located around the Caspian Sea in Iran; Akhani et al., 2010).

The Bernasso deposits were formed 2.16-1.96 Ma ago, just after the beginning of glacial/interglacial cycles (-2.6Ma). Pollen assemblages show that during the time lapse of deposition, two glaciations occurred separated by an interglacial event (Suc, 1978; Leroy and Roiron, 1996). Presently, studies show the possible impact of the glacial/interglacial cycles on precise species (DeChaine and Martin, 2006; DeLucia et al., 2012). For example, the genetic diversification of *Myrtus communis* in the Mediterranean region was possible because of a glaciation event around 2 Ma ago (Médail et al., 2012). In parallel, other studies suggest that the variability in abundance and diversity of plant-insect interactions could be due to temperature fluctuations (Currano et al., 2010), plant defense strategies (Coley and Barone, 1996), and age of the forest (Fernandes et al., 2010). Furthermore, Nicole (2002) has shown that there is also a strong correlation between some plant species and interactions amongst them.

In the present study, interaction structure of the paleoforest of Bernasso was investigated. This study corresponds to one of the first that focus on the herbivory structure of a forest ecosystem at the beginning of glaciation cycles. We tried to highlight, with the example of Bernasso, if interaction structures were already in agreement with climatic conditions or if forest ecosystems were still slowly acclimatized to the recurrent climatic fluctuation of that period.

2. Material & Methods

2.1 Study area

The lacustrine Bernasso deposits (43°43'34"N, 3°15' 25"E) (Figure 1) are located 5km to the east of Lunas (Hérault, southern France) at an altitude of 512m asl (Suc, 1978; Leroy et Roiron, 1996). Bernasso is characteristic of the supra-mediterranean bioclimatic zone. The MAT is around 12.05°C and the MAP is 1070mm (Kessler and Chambraud, 1990). This region is now mostly dominated by

deciduous taxa with maples (*Acer monspessulanum*, *A. campestre* L.), oaks (*Quercus pubescens* Willd) and ashes (*Fraxinus oxyphylla* L., *F. excelsior* L.).

Bernasso lacustrine deposits, composed of diatomites, were formed 2 Ma ago. They are rich in plant remains (pollen and fossil leaves) (Suc, 1978; Leroy and Roiron, 1996). Basaltic flows (due to the activity of the Escandorgue volcano) shut a canyon valley and allowed the development of a lake in which diatom floras developed. Lacustrine deposits correspond to the early Pleistocene (Hilgen, 1991). Dating was done by the K/Ar analysis (Ildefonse et al., 1972) on the diatomite and completed with cyclostratigraphy (Suc and Popescu, 2005) and paleomagnetism work (Ambert et al., 1990). For more details, see Leroy and Roiron (1996).

The Bernasso palaeoflora was mainly dominated by *Carpinus orientalis*, *Parrotia persica*, *Zelkova ungeri* and *Carya minor* and indicate that modern Caucasian flora could serve as models for such vegetation (Leroy and Roiron, 1996). Mediterranean species such as *Acer monspessulanum*, *Sorbus domestica*, *Tilia* sp. and *Vitis* sp. are examples of the macrofossils (Leroy and Roiron, 1996), *Buxus* L., *Olea* L., Ericaceae and Poaceae (Suc, 1978) are also preserved in the microfossils. Pollen analyses have shown a succession of three different climatic periods (Suc, 1978). The base and top of the sequence correspond to cold periods (glaciations) and the regional vegetation was steppic. The intermediate period was warmer (interglacial) and allows the development of woody vegetation. The leaf fossils came from this last climatic period.

Beside plant micro- and macrofossils, Bernasso diatomites provided few others fossils. For example, only two fossil insects have been found at Bernasso (a coleoptera and an orthoptera) (pers. obs. B.Adroit, 2013).

2.2 Fossil leaves collection

The collection includes more than 800 fossil leaves, collected between 1995 and 2012. Only 590 were used for this study exclusively because the complete or almost completely preserved specimens were chosen. All the specimens are conserved in the leaf paleontological collection of Institut des Sciences de l'Evolution de Montpellier, France (ISEM). The collection is split into two sets according to the kind of preservation of the fossil. The first group contains 321 specimens (54.4% of all) preserved on the extracted rocks (hereafter Ro). The second group contains 269 specimens (45.6% of all) totally removed from the diatomite with the peeling method

(hereafter BDC). With this method, the dried fossil leaf is fixated on a plastic film and placed between two glass slides with Canada balsam (fir resin). Only terrestrial angiosperms were taken into account in this study. The aquatic species *Ceratophyllum* L., ferns and conifers were excluded. For the complete flora list and illustrations, see Leroy and Roiron (1996).

2.3 Fossil analysis and classification of damages

The fossils were observed under a Leica MZ7.5 equipped with a Leica DFC300Fx camera. The BDC fossils were investigated under an Olympus MX51 microscope. All of the plant-insect interactions were identified using the Guide to Insect Damage Types (DT) by Labandeira et al. (2007). Functional feeding group (FFG) and host specificity (HS) defined by Labandeira et al. (2007) were determined for each damage type indicated, host specificity is an index assigned for every DT between 1 and 3 (the higher the HS is, the more specialized the damage is). Two kinds of interactions are recognized: specialized damages that include galling, mining, piercing & sucking, and oviposition (damages with mainly HS=2/3) and external damages that comprise holes feeding, margin feeding, surface feeding, and skeletonization (damages with mainly HS=1). All data were compiled in a spreadsheet including leaf species, leaf quantity, DT, FFG (Table S1).

2.4 Statistic analysis

The R software (R Core Team, 2012) and its vegan package (Oksanen et al., 2013) were used to perform rarefaction test in order to analyze diversity of FFG according to leaf quantity. A rarefaction test was also performed on the proportion of specialized damage types in regard to all damages. As the data does not follow a normal distribution, a Wilcoxon test was used when necessary to check if there was a significant difference between two species. At last, a Chi-squared test ($\alpha < 0.1\%$) was used to compare the proportion of DTs to the kind of fossils (Ro or BDC). The goal was to determine if the type of fossil preservation influenced conservation and/or detection of DTs. The statistical analyses were performed on leaf species represented by at least 20 specimens. Seven species were analyzed by statistics.

3. Results

3.1 Damage diversity and distribution on the leaf species

37% of the Bernasso leaves (220/590) displayed interactions with insects. 39 different DTs were identified. These DTs are organized into external foliage feeding (with seven types of holes feeding, eight types of margin feeding, eight types of skeletonization, three types of surface feeding and one undetermined DT) and specialized foliage feeding (with four types of mining, seven types of galling and a single type of oviposition) (Figure 2). Oviposition is represented by a unique occurrence detected on a leaf of *P. persica*. Figure 3 illustrates the distribution of the seven FFGs within the different leaf species. *P. persica* and *C. orientalis* (the most abundant plant species) have the most important quantities of damages. *P. persica* has a noticeable dominance of galling while *C. orientalis* has a dominance of holes feeding and margin feeding. Galling represents half of the specialized damages ($p < 2.2e-16$) (Figure 4) and its diversity is seven DTs. Moreover, specialized damages (21 DTs), in which galling is included, are significantly more diversified than external damage (18DTs) ($p = 2.534e-12$).

At Bernasso four FFGs represent 90% of the total DTs: galling being 30% of the total interaction observed, hole feeding 24%, margin feeding 18% and skeletonization 18%. Galling is statistically more present than hole feeding (Wilcox-test: $p < 2.2e-16$), margin feeding (Wilcox-test: $p = 1.56e-12$) and skeletonization (Wilcox-test: $p < 2.2e-16$). Hole feeding and margin feeding are statistically equally distributed (Wilcox-test: $p = 0.1881$) and skeletonization is less representative than hole feeding among the Bernasso fossil leaf collection (Wilcox-test: $p = 3.116e-05$). No statistically significant difference between margin feeding and skeletonization is observable (Wilcox-test: $p.value = 0.078$). Concerning the distribution of these four FFGs over the palaeoflora (Figure 3), galling is represented in 19 different species but is abundant only in four leaf species: *P. persica* (34% of the galling), *A. monsepessulanum* (10%), *S. domestica* (10%) and *F. ornus* (9%). Galling is more important on *P. persica* than *A. monsepessulanum* ($p = 3.472e^{-05}$), *S. domestica* ($p = 3.472e^{-05}$) and *F. ornus* ($p = 0.01231$). The most important galling DT is DT32 (60% of all galling). It is also the main galling found on *P. persica* and *A. monspessulanum* (these 2 species contain 64% of DT32). Another important galling type is DT80 that represents 23% of all galling. Among this damage type DT80, 53% of its occurrences are present on *S. domestica* and *F. ornus* plant species. Skeletonization is present on 15 different species and 45% of this FFG is represented by DT16. This FFG is widely distributed but leaves of *Acer* have the largest proportion (36%) of total

skeletonization. Lastly, hole feeding and margin feeding are respectively found on 11 and 14 species. These two FFGs are homogeneously present on all leaf species. Hole feeding is in correspondence to two DTs DT01 (25%) and DT02 (29%), whereas 63% of margin feeding is represented by DT12 only.

3.2 Conservation bias

First of all, there are statistically more fossil leaves within the Ro (54%) than the BDC (46%) dataset (Chi-squared test: $p = 0.03$; $\alpha = 1\%$; $ddl = 1$). BDC fossil leaves contain 70% of the total DTs detected while Ro fossils contain only 30% of the DTs (Figure 5). This difference is significant (Chi-squared test: $p < 0.01$; $\alpha = 0.1\%$; $ddl = 1$) and is mostly due to two FFGs: margin feeding and galling (Figure 5). These two FFGs are at least twice more abundant within BDC fossils than Ro fossils (Chi-squared test: $p < 0.01$; $\alpha = 0.1\%$; $ddl = 1$). Concerning the two other important FFGs (hole feeding and skeletonization), the difference between BDC and Ro fossil is not significant (Chi-squared test: $p = 0.27$ and $p = 0.26$; $\alpha = 0.1\%$; $ddl = 1$).

4. Discussion

4.1 Representative and taphonomic bias

Rarefaction data shows that all the species exhibit damage. The sampling effort during the collecting field trips was sufficient for representing plant diversity from 300 fossil leaves (19.21 ± 0.74)

Also the significant difference between the kinds of fossil preparation (BDC and Ro) could result from biases generated by the BDC preparation. This particular method could lead to an overestimation of margin feeding. During the transfer, parts of leaves could be torn. Moreover, the Canada balsam tends to blacken the margin of the leaves, mimicking the reaction after an insect feeding attack. On the contrary, an under-estimation of galling could occur for the Ro fossils. Most galls were more readily observed on BDC fossils, possibly due to the transparency of these fossils.

4.2 Damage abundance on the paleoforest

The two most abundant species *P. persica* and *C. orientalis* have had 40% of their leaves attacked. However, FFGs on these two dominant species are completely different. *C. orientalis* mostly contains hole feeding and margin feeding that are

general types of damage caused by polyphagous insects (Currano et al., 2008; Wilf, 2008; Wappler, 2010). *P. persica* mainly have galling, a specialized interaction caused by oligophagous insects (Currano et al., 2010). *P. persica* also has a lot of generalist interactions (e.g., DT01, DT02, DT12), probably produced by polyphagous insects (Currano et al., 2008). These results are not exactly congruent with the hypothesis of Currano et al. (2010) that in modern ecosystems insects feed more on the dominant plant species (Novotny et al., 2006). Proportionally *P. persica* and *C. orientalis* are not the two most damaged species. The size of these two species can provide some explanation. Modern *P. persica* and *C. orientalis* in Iran can grow to the height of 15-20m (Abedi M., pers. comm. 2015). If it is estimated that sizes of these two species were the same at Bernasso 2 Ma ago, *P. persica* and *C. orientalis* should have been the dominate trees of the canopy in the Bernasso paleoforest and their leaves were subject to more intense sunlight than other species. This probably resulted in a more efficient photosynthesis for *P. persica* and *C. orientalis* and therefore an increase of C/N ratio in their leaves (Bazzaz, 1990; Barradas and Jones, 1996). These two phenomena are both unfavorable for herbivorous organisms (Yamasaki and Kikuzawa, 2003).

Furthermore, *P. persica* and *C. orientalis* have both specialist and generalist interactions. According to Currano et al. (2008), the physiology and/or anatomy of these two plants should be directly involved in their diversity of interactions. Proportionally *A. monspessulanum* and *S. domestica* are more attacked than *P. persica* and *C. orientalis*. 59% of *A. monspessulanum* leaves and 54% of *S. domestica* leaves possess damages. These proportions have to be compared to the number of leaves of each species. *C. orientalis* and *P. persica* are represented by more than 100 specimens, *A. monspessulanum* and *S. domestica* are only represented by ca. 20 specimens. It is necessary to have more *A. monspessulanum* and *S. domestica* specimens to statistically test these differences. However, it seems that the majority of insects adapted to feed on the two dominant species (*C. orientalis* and *P. persica*) and more specialized insects adapted for feeding on the other less represented plant species (*A. monspessulanum* and *S. domestica*).

4.3 Community structure of the paleoforest: ecological parameters

Specialized damages, especially galling, are an interesting tool to discuss a paleoforest community structure (Fernandes and Price, 1992; Fernandes et al.,

2010). Figure 6 compares the quantity and the diversity of galling at Bernasso according to the theories of Fernandes et al. (1992, 2010). Three ecological parameters were analyzed: the age of Bernasso forest, the temperature and the precipitation at Bernasso ca. 2 Ma ago. The aim of figure 6 is to synthesize the following interpretation.

At Bernasso, specialized damages, mainly due to galling, are important but undiversified (Figure 6). DTs 32 and 80 represent nearly 90% of the total galling diversity and they were found on almost all species (Table S1). Moreover their HS is low (Labandeira et al., 2007) even if galling, in general, represents a specialized FFG (e.g., Knor et al., 2013). This low diversity connected to the large quantity of galling is in contrast with Fernandes et al. (2010) (Figure 6). Indeed, when compared to the modern forests in the Hyrcanian region (Iran) (Leroy and Roiron, 1996), Bernasso could be considered as a late-successional forest. *P. persica*, *Z. carpinifolia* and *C. orientalis* are known in Iran to be the main species of late-successional forests (Djamali et al., 2008). According to Fernandes et al. (2010), in old forest such as Bernasso, there should be an expected important quantity and diversity of galling. In the case of Bernasso, the large quantity of galling may be explained by the age of the forest (Figure 6). Also, Fernandes and Price (1992) suggest that a cold temperature implies a low diversity and quantity of galling. Thus, the low diversity of galling at Bernasso could suggest low mean annual temperature (Figure 6). Lastly, precipitation may also be correlated with diversity and quantity of galling. A high ratio of precipitation seems to imply a low quantity and diversity of galling (Fernandes and Price, 1992). Thus, the high mean annual precipitation (Suc, 1978; Leroy and Roiron, 1996) could also explain the low diversity of galling at Bernasso. All these information and data may support the results of the unpublished CLAMP data.

More generally, Bernasso fossils (590 specimens/20 species) have a particularly high diversity of different DTs (39 DTs), which is comparable to other fossil localities. The Oligocene locality of Quegstein (404 specimens/36 species) has 4.5 ± 1.6 DT for 50 leaves when Bernasso have 12.23 ± 2.38 DT for 50 leaves (Wappler, 2010). The locality of Rott, another Oligocene site (2476 specimens/112 species) only has 7.5 ± 2.1 DT for 50 leaves (Wappler, 2010). Even if it is difficult to quantify insect diversity (because it is not always correlated to the diversity of damage; Currano et al., 2008), the large quantity of DTs at Bernasso could suggest an increased diversity of insects. However, 85% (34 DTs) of damage types are rare

and only 15% of them (6DTs: DT01, 02, 12, 16, 32, 80) are dominant. Carvalho et al. (2014) argued that the abundance of rare DTs could mainly be the result of the activity of multi-damaging insects. This may indicate that the Bernasso DT diversity could be biased by the presence and diversity of multi-damaging insects (Novotny et al., 2002; Currano et al., 2010).

5. Conclusion

This study set up a snapshot of plant-insect interactions during the onset of the glacial/interglacial cycles. The Bernasso fossil leaf assemblage contained a large diversity of DTs, even if only 6 DTs represent more than 56% of the total of interactions. The most important damage evidenced is galling, but it is represented mostly by a unique DT (DT32). Based on previous studies (Fernandes and Price, 1992; Fernandes et al., 2010), it appears that galling is undiversified despite its abundance.

The overwhelming presence of galling at Bernasso can be explained by the community structure of a late-successional forest. Furthermore, the low diversity of galling could suggest that the mean annual precipitation was relatively high as indicated in previous studies (Suc, 1978; Leroy and Roiron, 1996) and the temperatures was cooler than modern day and could confirm the recent unpublished results (unpublished data)

Finally, a Pliocene locality from southeast France (such as Armissan [Aude, France]; Saporta, 1893) would make for an interesting comparison to Bernasso and to bring out evolutionary trends or perturbation of plant-insect interactions due to installation of glaciation cycles. Also, it could be interesting to study galling patterns in the current Mediterranean and Caucasian forests to compare with Bernasso paleoforest. This level of comparison should highlight links between galling, temperature and precipitation and therefore, by extension, relationships between paleoclimatic fluctuations and changes in plant-insect interactions.

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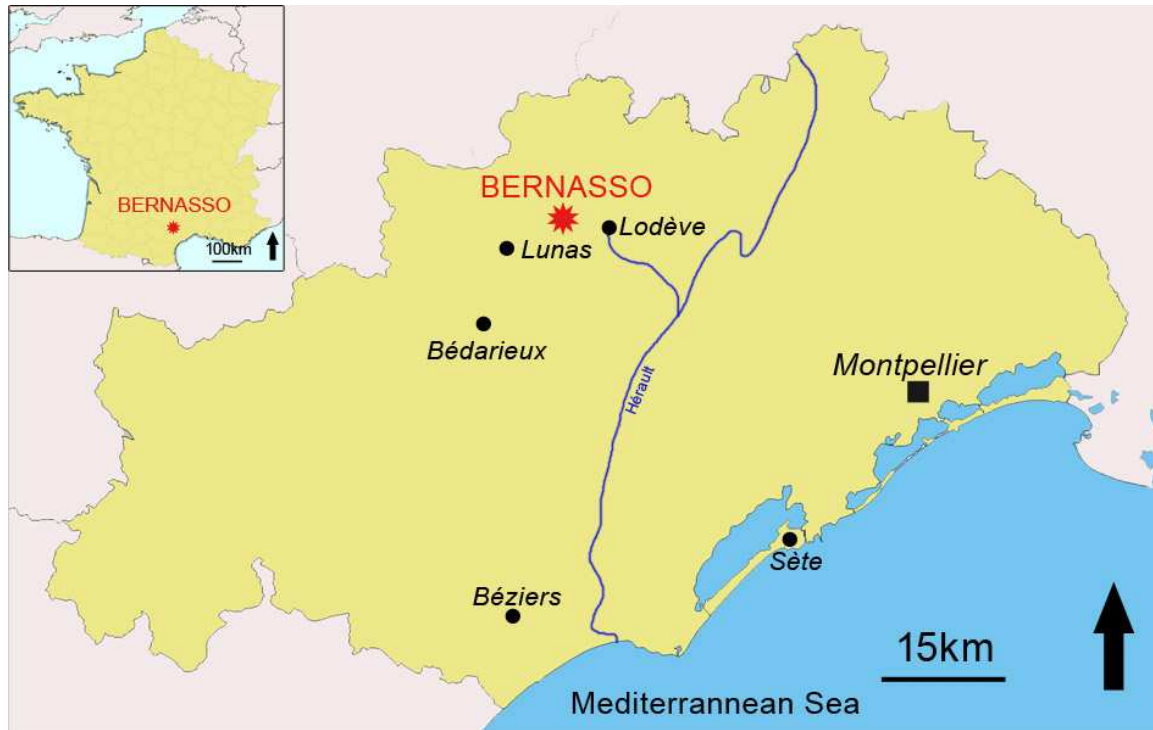


Figure 1

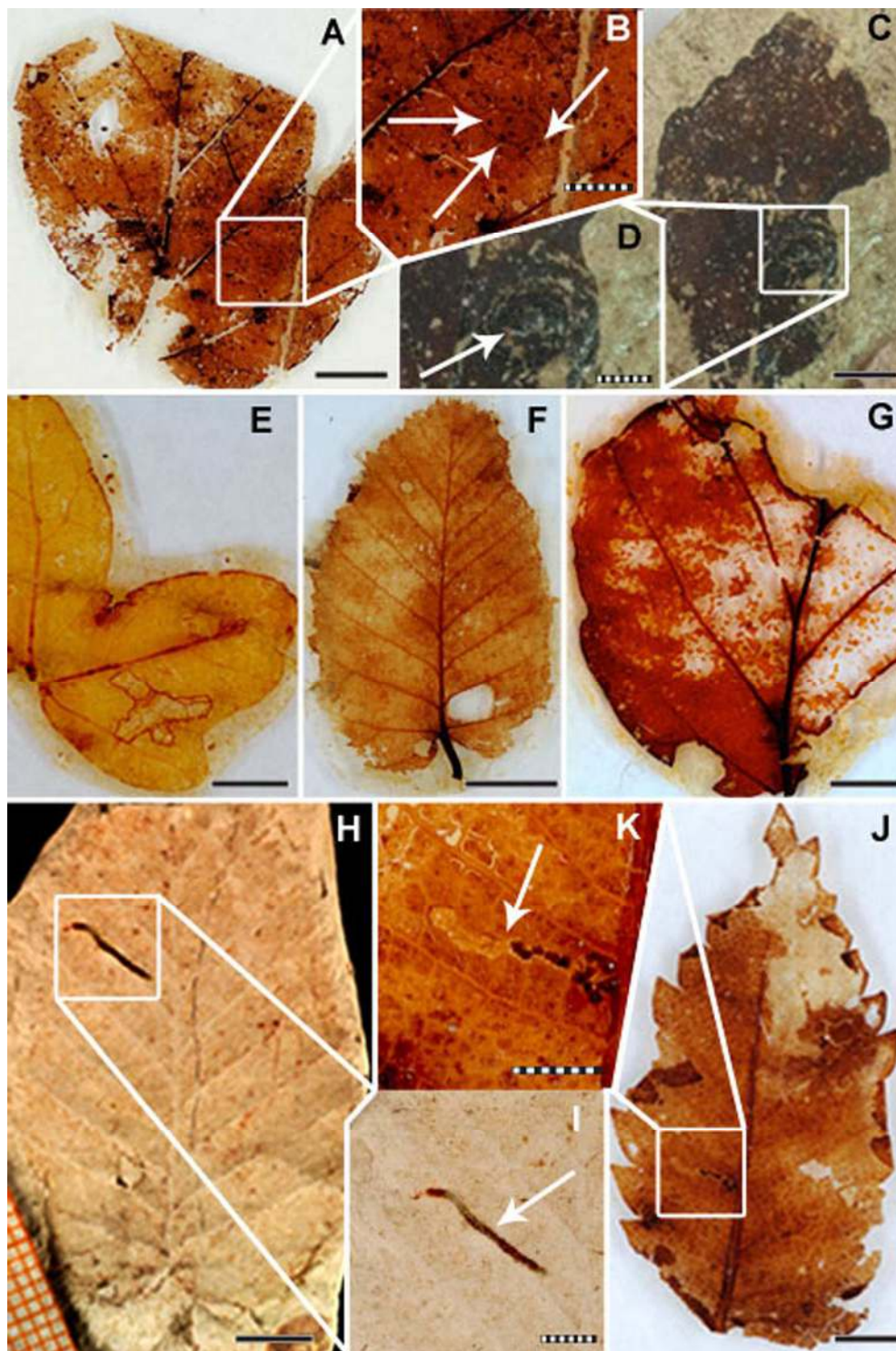


Figure 2

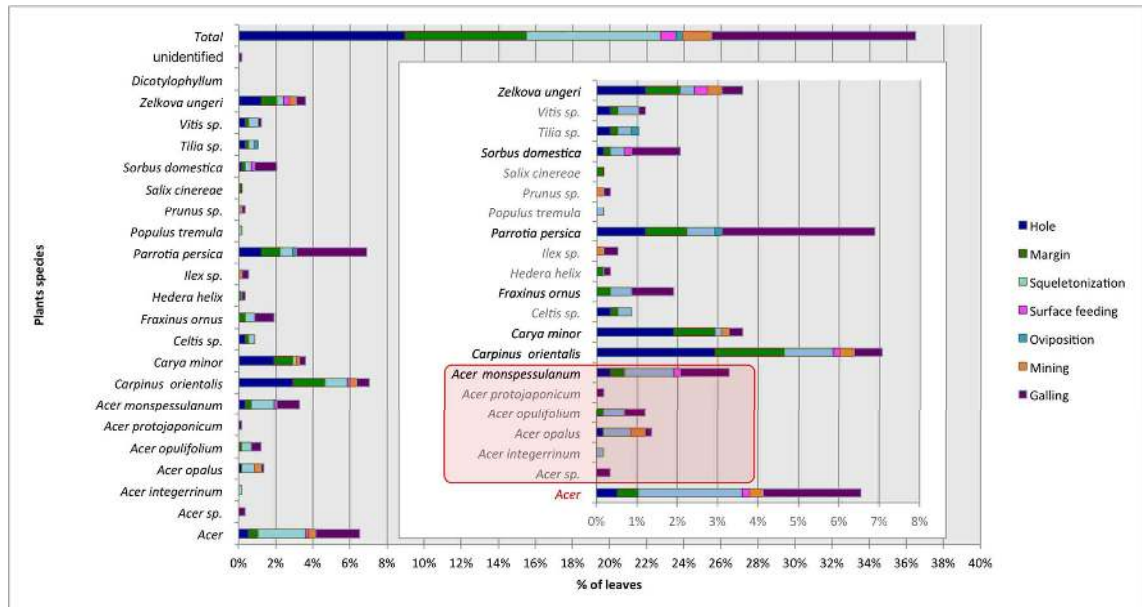


Figure 3

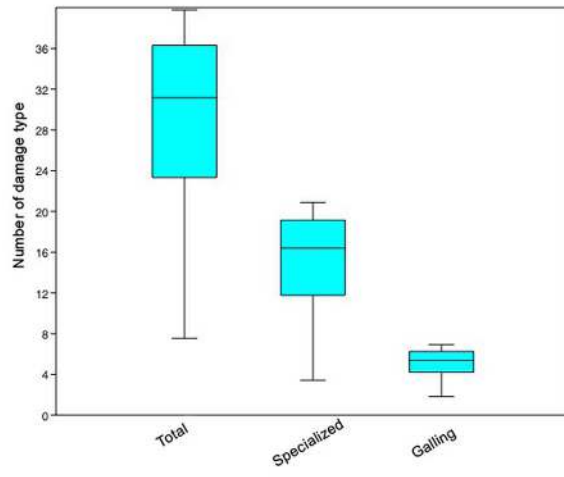


Figure 4

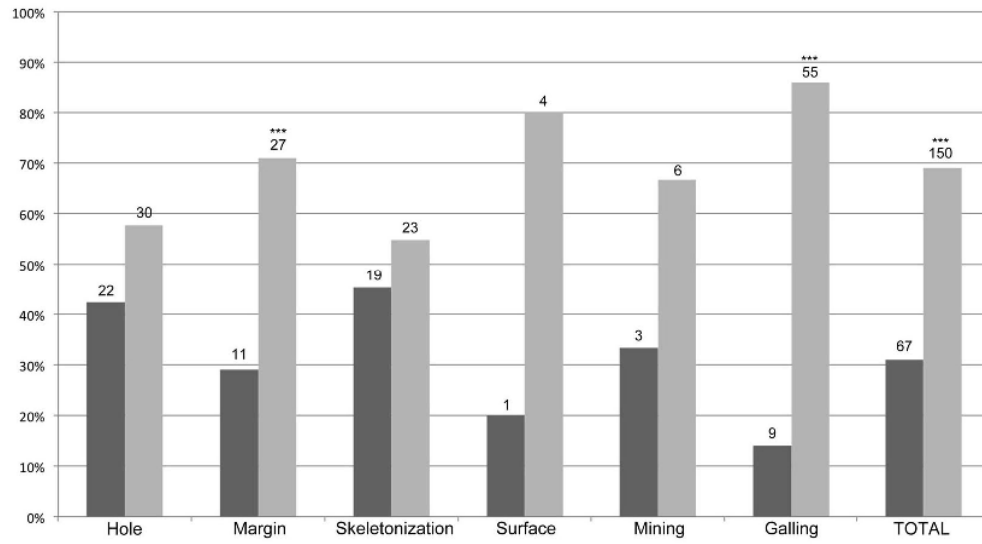


Figure 5

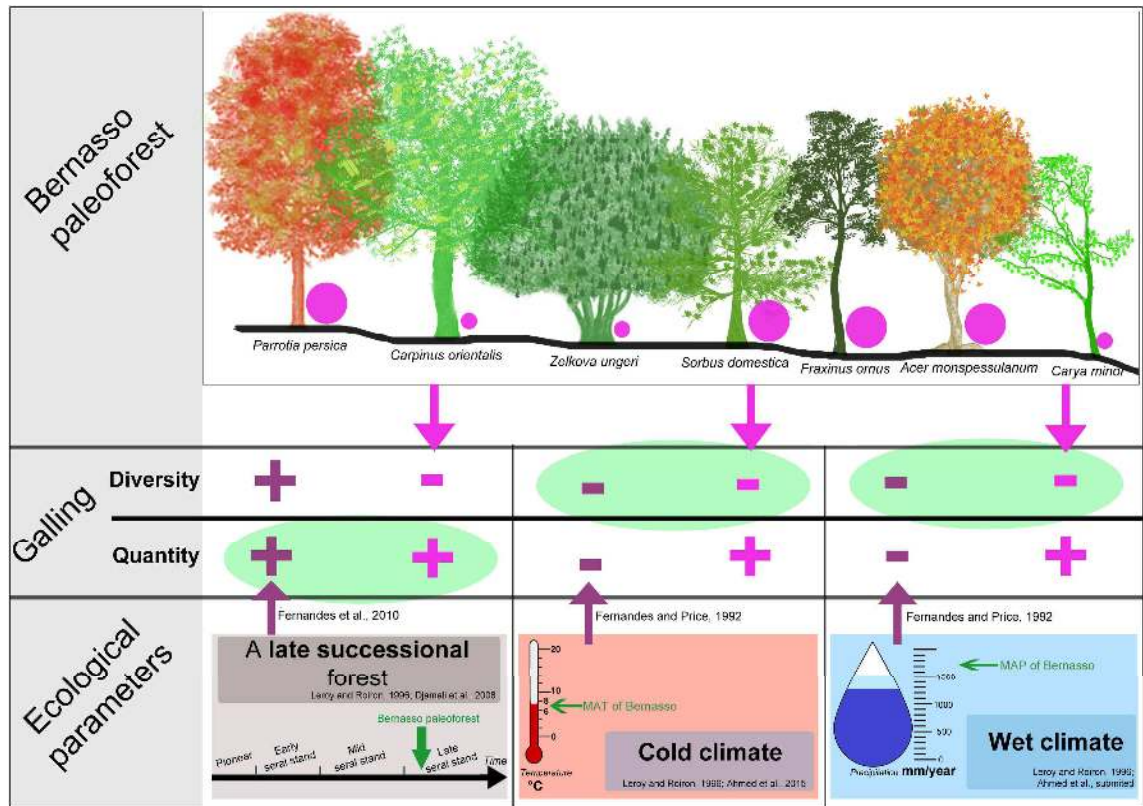


Figure 6

Figure 1: Map of the Hérault region showing the fossil locality of Bernasso (simplified from Leroy and Roiron, 1996).

Figure 2: Representative insect damages on Bernasso leaves. A- Gallings (DT32) on *Parrotia persica* preserved by Canada balsam method (BDC fossil); enlarged in B, DT32 are the small black dot, plenty on this leaf. C- Gallings on *Parrotia persica* preserved by (Ro fossil); enlarged in D. E- Skeletonization (DT20) on *Acer opalus*; F- Hole (DT78) on *Carpinus orientalis*; G- Margin feeding (DT12) on *Parrotia persica*; H- Surface feeding (DT130) on *Carpinus orientalis*; enlarged in I; J- Mining (DT90) on *Zelkova ungeri*; enlarged in K.

Scale bars: black= 0.5cm / stripes= 0.25cm.

Figure 3: Interactions distribution (FFG) according Bernasso plant species. Box on the right is a highlight of the big one behind. Species in bold are the main 7 species with more than 20 specimens. Due to the diversity of maples, the genus *Acer* was individualized.

Figure 4: Mean diversity of all damage types, specialized damage types and gallings damages on Bernasso floras. The box-plots are based on the rarefaction data for each group of interactions considered for the database.

Figure 5: Distribution of FFGs according to the fossil preparation. The grey bars represent the fossils preserved with Canada balsam method; the black bars represent the fossils preserved on the rock.

The numbers are the exact quantity of damages. Chi-squared test enables to see the significance of the difference between Ro and BDC per FFG.

: alpha =0.1%. Lack of “*” means there is no significant difference.

Figure 6: Overview of Bernasso forest. The different trees represent the 7 main species with more than 20 specimens. The purple circles near each tree represent the gallings. The 2 different sizes of the circles indicate that there are more than 20% of leaves with gallings for the bigger size, and less than 20% for the smaller.

Information about gallings was obtained comparing Bernasso Gallings and data from Fernandes and Price (1996) and Fernandes et al. (2010). The symbols + and -

respectively means high and low.

3 ecological parameters were used: {Left to the right} Age of Bernasso's forest, Mean annual temperature at Bernasso (MAT), Mean annual precipitation at Bernasso (MAP).

Highlights

- First studies of plant-insect interaction for the Pleistocene period in Europe.
- Provides insights for herbivory pattern at the onset glacial/interglacial cycles.
- *Parrotia persica* and *Carpinus orientalis* were the dominant species at Bernasso.
- The Hyrcanian forest in Iran is a modern analogous of Bernasso paleoforest.