Plant-insect interactions patterns in three European paleoforests of the late-Neogene – early-Quaternary

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Plants and insects are constantly interacting in complex ways through forest communities since hundreds of millions of years. Those interactions are often related to variations in the climate. Human activity may have disturbed these relationships in modern ecosystems. Therefore, observations of plant-insect interactions made in current ecosystems could be impacted by human influence. Consequently, studies based on past ecosystems, through fossil leaf assemblages, are essential to complement modern day human influenced studies in order to better understand changes of plant-insect interactions in environments throughout time. The goal of this study is to discuss the possible causes of the differences of plant-insect interactions’ patterns in European paleoforests from the Neogene – Quaternary transition. This was accomplished through three fossil leaf assemblages: Willershausen, Berga (both from the late Neogene of Germany) and Bernasso (from the early Quaternary of France). In Willershausen it has been measured that half of the leaves presented insect interactions, 35% of the fossil leaves were impacted by insects in Bernasso and only 25% in Berga. The largest proportion of these interactions in Bernasso were categorized as specialist (mainly due to galling) while in Willershausen and Berga those ones were significantly more generalist. Contrary to previous studies, this study did not support the hypothesis that the mean annual precipitation (MAP) and temperature (MAT) were the main factors that impacted the different plant-insect interactions’ patterns. However, for the first time in the fossil record, our results tend to support that the hydric seasonality and the mean temperature of the coolest months could be potential factors influencing plant insect interactions.

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ABSTRACT

Plants and insects are constantly interacting in complex ways through forest communities since hundreds of millions of years. Those interactions are often related to variations in the climate. Human activity may have disturbed these relationships in modern ecosystems. Therefore, observations of plant-insect interactions made in current ecosystems could be impacted by human influence. Consequently, studies based on past ecosystems, through fossil leaf assemblages, are essential to complement modern day human influenced studies in order to better understand changes of plant-insect interactions in environments throughout time. The goal of this study is to discuss the possible causes of the differences of plant-insect interactions' patterns in European paleoforests from the Neogene – Quaternary transition. This was accomplished through three fossil leaf assemblages: Willershausen, Berga (both from the late Neogene of Germany) and Bernasso (from the early Quaternary of France). In Willershausen it has been measured that half of the leaves presented insect interactions, 35% of the fossil leaves were impacted by insects in Bernasso and only 25% in Berga. The largest proportion of these interactions in Bernasso were categorized as specialist (mainly due to galling) while in Willershausen and Berga those ones were significantly more generalist. Contrary to previous studies, this study did not support the hypothesis that the mean annual precipitation (MAP) and temperature (MAT) were the main factors that impacted the different plant-insect interactions' patterns. However, for the first time ~~in the fossil record~~, our results tend to support that the hydric seasonality and the mean temperature of the coolest months could be potential factors influencing ~~plant insect~~ interactions.

INTRODUCTION

Climate is a major factor affecting the extension, structure, and composition of terrestrial ecosystems (Taylor et al., 2012; Frank et al., 2015). Hence, past climatic oscillations are of special importance for understanding and interpreting biotic changes in the past (e.g., DeChaine & Martin, 2006) and are of interest in terms of forecasting the biotic response to future global warming (Meehl et al., 2007). Nowadays, it is clear that Human activities have now reached a global impact affecting components of the Earth system as a whole (Turner et al., 1990; Heller & Zavaleta, 2009). In terrestrial ecosystems, arthropods are one of the most important components in biodiversity (Yang & Gratton, 2014) and their interactions with plants are essential for terrestrial food webs (Forister et al., 2015). Many modern ecological studies are focusing on these interactions between plants and insects but interpretations may be limited, therefore a combination with studies focused on the fossil record is necessary (Wilf, 2008). Studies on fossil insect herbivory have provided a variety of ecological and evolutionary information over long periods of time, such as climate (e.g., Wappler, 2010; Wappler et al., 2012), the evolutionary impact of plant radiations (e.g.,Labandeira, 2012; Labandeira & Currano, 2013), food web dynamics (e.g., Wappler & Grímsson, 2016), extinction patterns (e.g., Labandeira, 2002; Labandeira, Johnson & Wilf, 2002; Donovan et al., 2016), and ecosystem recovery after extinction events (e.g., Wappler et al., 2009; Labandeira, Kustatscher & Wappler, 2016). They have also shown that biodiversity loss may greatly impede trophic interactions and change the overall food web structure of ecological systems (Haddad et al., 2009). Moreover, there is increasing concern about the loss of biological diversity from ecosystems (Hooper et al., 2012). The large amount of Plio–Pleistocene fossil records offers an exceptional possibility for conducting meta-analysis for the estimation of the evolution and dynamics of associations between plant species and their dependent insect-herbivore species, as descriptions of Plio–Pleistocene floral changes (Tzedakis, Hooghiemstra & Pälike, 2006; Médail & Diadema, 2009; Postigo Mijarra et al., 2009; Magri, 2010; Migliore et al., 2012). Although a few isolated records of specialized phytophagy categories have been reported from the Pliocene (Straus, 1977; Givulescu, 1984; Titchener, 1999), only a single systematic survey of plant–arthropod interactions has been carried out on an early Pleistocene flora (Adroit et al., 2016). Thus, an ideal setting for the evaluations of relationships among global climate and biodiversity under conditions warmer than today, but with a similar paleogeographic configuration (Raymo *et* *al*. 2011; Rohling *et al*. 2009) is possible throughout the famous upper Pliocene fossil Lagerstätten Willershausen (3.2 – 2.6 Ma; MN 16/17) (Hilgen, 1991; Mai, 1995) and the comparisons with Berga (Germany, late Pliocene) and the French Pleistocene locality of Bernasso (Adroit et al., 2016). Willershausen and Berga outcrops are of similar age (Piacenzian) and are located in the surroundings of the Harz Mountains, Germany (Figure 1). The Willershausen paleoforest was dominated by typical taxa of hilly mesophytic woodland (Ferguson & Knobloch, 1998; Knobloch, 1998) such as *Acer, Aesculus, Carpinus, Fagus, Quercus, Sassafras, Tilia* (Mai, 1995; Knobloch, 1998) and other taxa such as *Parrotia, Zelkova* and *Liquidambar* were also characteristic elements of Willershausen (Mai, 1995). All of these taxa were also found in Berga (Mai & Walther, 1988). The presence of these taxa indicates relatively warmer conditions in Europe than today during the late Pliocene (Uhl et al., 2007; Thiel, Klotz & Uhl, 2012). Most plant fossil evidence from Central Europe outcrops (Haywood, Sellwood & Valdes, 2000; Uhl et al., 2007; Williams et al., 2009; Thiel, Klotz & Uhl, 2012) and data from marine isotopes, geological evidences (Driscoll & Haug, 1998; Haug, Tiedemann & Keigwin, 2004) are directing in the same way. Bernasso is younger than the German outcrops, estimated around 2.16 Ma – 1.96 Ma (Suc, 1978; Leroy & Roiron, 1996). It is located in southern France, 5km far away from Lunas in the department of Hérault (Suc, 1978; Leroy & Roiron, 1996; Adroit et al., 2016). The Bernasso fossil leaf assemblages is mainly dominated by the genera *Carpinus*, *Parrotia*, *Acer* and *Sorbus* (Leroy & Roiron, 1996; Adroit et al., 2016) wherein many plant species are in common with the German fossil leaf assemblages. Detailed descriptions are available in Roiron & Leroy (1996) and Adroit et al. (2016). The decreasing temperatures, from ca. 18°C to 14°C throughout the Pliocene (Thunell, 1979; Ravelo et al., 2004; Hansen et al., 2013) lead to the dominant European vegetation changing gradually from highly diverse subtropical and warm-temperate forests to temperate deciduous forests with East Asian and partly North American affinities (Mai, 1995).

Through the comparison of three European forest plant communities of the Plio-Pleistocene, the aim of this study was to understand how climatic parameters could have impacted plant-insect interactions of fossil leaves. It has been expected that the difference of estimated mean annual temperatures between those paleoforests could have a major impact on the quantity of the plant-insect interactions (Coley & Aide, 1991; Zvereva & Kozlov, 2006; Currano, Labandeira & Wilf, 2010). Moreover, the estimated mean annual precipitations of the fossil outcrops should be negatively related to the galls proportions observed on the fossil leaves (Fernandes & Martins, 1985; Fernandes & Price, 1988; Price et al., 1998; Lara, Fernandes & Gonçalves-Alvim, 2002). Our results provide the first approach on plant-insect interactions from the Plio-/Pleistocene in European paleoecosystems.

STUDY AREA

Willershausen, Lower-Saxony, Germany

Geological studies of Willershausen dated back to the end of the 19th century (e.g., Wegele, 1914); see details in (Wegele, 1914; Ferguson & Knobloch, 1998; Meischner, 2000). The absence of bioturbation gave rise to one of the exceptionally well preserved floras and faunas (Briggs et al., 1998). The Willershausen site was a lake which developed in a pond due to the dissolution of underlying Permian evaporites and tumbled of the Triassic and Early Jurassic sediments (Briggs et al., 1998; Meischner, 2000; Kolibáč et al., 2016). Today, Willershausen is an abandoned clay mining operation and it is included in the Geopark Harz, Braunschweiger Land, Ostfalen since 2012. This paleolake was ca. 200m in diameter and approximately 10m deep with a narrow sand beyond which the sides inclined abruptly towards the bottom of the lake (Meischner, 2000). Willershausen geology has been described by (Von Koenen, 1895) and detailed compilations can be found in (Vinken, 1967; Ferguson & Knobloch, 1998; Meischner, 2000).

The leaves used in this study are stored in different museum collections in Germany. The majority (6546 leaves) is located at the Geoscience center of the University of Göttingen (GZG.W collection). Additional fossil leaves are stored in the Staatliches Museum für Naturkunde Stuttgart (SMNS.W collection, 957 leaves), in the collections of TU Clausthal of Clausthal-Zellerfeld (320 leaves), in the Naturkundemuseum im Ottoneum of Kassel (NMOK.W, 236 leaves) and in the Senckenberg Natural History Collections Dresden (14 leaves). Some of the best well-preserved fossil specimens are presented in Figure 2.A. The flora from Willershausen comprised a rich vegetation community including the presence of *Acer, Alnus, Betula, Carpinus, Carya, Fagus, Pterocarya*, *Populus, Quercus, Tilia, Ulmus, Zelkova* (Straus, 1977; Ferguson & Knobloch, 1998; Knobloch, 1998). The vertebrates *Anancus (Mastodon) arvernensis* and *Tapirus* were found in Willershausen and seems to indicated a Piacenzian age (late Pliocene, ca. 3.2 – 2.4 Ma; MN 16/17; (Mai, 1995), which is corroborated by the presence of *Parrotia persica* and *Liquidambar europaeum* (Mai, 1995).

Most plant fossil evidence from Willershausen indicates warmer conditions than today (Ferguson & Knobloch, 1998). The mean annual temperature (MAT) in Willershausen was estimated between 10.6°C and 15.6°C on the base of the leaf morphology and of diversity of plant species niches (Table S1; Uhl et al., 2007); these different approaches explain the wide range of temperature estimated. The mean temperature of the coldest month (CMMT) is estimated between 0.6°C and 3.2°C and the mean annual precipitation (MAP) between 897 and 1151 mm per year (Table S1; Uhl et al., 2007; Thiel, Klotz & Uhl, 2012).

Berga, Thüringen, Germany

Berga was a lake in which compressions and impressions (some with the cuticles preserved) of leaves were found in silty sediments (Mai & Walther, 1988). It is 70km far from the Willershausen outcrop. The stratigraphic age of the Berga sediments is estimated on the basis of sedimentological correlations referring to the Piacenzian (ca. 3 Ma – 2.6 Ma) (Bachmann et al., 2008).

This leaf collection (534 specimens) is housed in the collection of the Senckenberg Natural History Collections Dresden, Germany. It contained many fossils of different origins (Mai & Walther, 1988), including 30 angiosperms leaf taxa (Figure 2.B). They represent different environments: a freshwater plant community, a swamp and riparian associations and a zonal mixed broadleaved conifer forest (which dominates the taphocoenosis). The temperatures were estimated with the same approach as Willershausen; MAT is estimated between 7.4°C and 16.6°C, the CMMT is between -4.3°C to +0.6°C and the MAP is between 897 and 1297 mm per year (Table S1; Uhl et al., 2007; Thiel, Klotz & Uhl, 2012).

Bernasso, France

Bernasso was a lake developed when a basaltic flows shut off a canyon valley (Leroy & Roiron, 1996). Diatomites were formed and fossil leaves, often with rest of cuticle, were preserved. It is located close to Lunas (Hérault, southern France) (Leroy & Roiron, 1996; Adroit et al., 2016). The fossil deposit is dated from the early Pleistocene on the basis of K/Ar analysis (Ildefonse et al., 1972) on a basaltic dyke that crosses the diatomite layers. A complementary analysis on cyclostratigraphy (Suc & Popescu, 2005) and paleomagnetism (Ambert et al., 1990) corroborated these results and estimated an age around 2.16 to 1.96 Ma.

The collection included 800 fossil leaves and 535 specimens well-preserved were described in (Adroit et al., 2016). These same specimens were also used for comparisons in the present study. The fossil leaves were conserved the Institut des Sciences de l'Evolution de Montpellier in France. Different preparation of fossil leaves were done by (Leroy & Roiron, 1996) and their impact on interpretation were discussed in (Adroit et al., 2016). The flora represents a mesothermic forest, mixing Mediterranean and Caspian elements (Suc, 1978; Leroy & Roiron, 1996). The MAT in Bernasso was estimated about 14-15°C and the MAP is around 1500 mm/y (Table S1; Leroy & Roiron, 1996). It is important to note that CLAMP results in Bernasso suggest a possibly lower temperature (Table S1; Girard et al., in review).

DATA ANALYSES

Plant-insect interaction identifications

The plant-insect interactions were identified following the Guide to Insect (and Other) Damage Types on Compressed Plant Fossils (Labandeira et al., 2007). The damages type (DTs) are easily recognizable thanks to the black reaction mark surrounding them (Labandeira, 2002; Labandeira et al., 2007). They are divided in 7 Functional Feeding Groups (FFG): hole feeding, margin feeding, skeletonization, surface feeding, piercing & sucking, mining and galling. Leaves without damage were also categorized in an eighth FFG called the undamaged leaves. The leaves undamaged has been take into account as a proxy of the non-palatability of the leaves, thus can be considered as another FFG. The leaves were examined under a binocular Leica MZ95 and all photographed with a Canon EOS 350D camera fitted with a Canon EF-S 60-mm f/2.8 macro lens. A Nikon Coolpix E4500 was used sometimes for precise pictures through the binocular. All pictures were developed using Abode Lightroom CC v.2015 especially in order to improve contrast of the leaf. The insect interactions on leaves were scored according to the richness, frequency and distribution on the different plant species for each outcrop. For each DT, a host specificity value has been attributed by Labandeira et al. (2007) that allowed to classify our DTs into generalist interactions (made by polyphagous organisms) and specialized interactions (made by monophagous organisms) (Labandeira, 2002). Detailed plates of fossil leaves from Willershausen are available in Table S2 including the original descriptions of the plant-insect interactions made by (Straus, 1977) and our actual updates with the guide of insect (and other) damage types on compressed plant fossils (Labandeira et al., 2007).

The results obtained for Willershausen and Berga were compared to those recently published for the outcrop of Bernasso (Adroit et al., 2016). For some comparisons with Bernasso, new values were calculated based on raw data.

Statistical analyses

For each outcrop, the statistical analyses were performed on two different databases as described in Knor et al. (2012). The first one is the whole assemblage of plant-insect interactions. The second one considers only the interactions of the species that are significantly represented (more than 20 leaves). The quantitative analyses were done in R version 3.1.2 (R Development Core Team, 2014). The differences among the proportions of occurrences from all FFG were tested with Chi-squared-test. The remaining information needed for this test was obtained by using the generalized linear model of binominal distribution. Sample-based rarefaction curves were done to compare the different damage richness and the different plant richness between the outcrops (Gotelli & Colwell, 2001). At last, in order to observe the distributions of plant species according to the FFG among the different fossil leaf assemblages, principal component analysis (PCA) were performed with the software Past3 (v3.14) (Hammer, Harper & Ryan, 2001) in a biplot. PCA in a biplot has been useful to measure the directions where each plant species had the most variance according to the different FFG. The data matrices used for it considered the frequency of each eight FFGs for each plant species of each outcrop (i.e. for each outcrops a matrix such as FFG frequency x leaf morphotype).

RESULTS

Comparisons of insect interactions and plant species richness.

In Willershausen 50.4% of the leaves are damaged, and only 25.1% in Berga. This percentage was 34.6% in Bernasso (Adroit et al., 2016). These differences are statistically significant (p<0.001) (Figure 3, Table 1).

The frequencies of generalist interactions are 42.8% for Willershausen, 17.8% for Berga and 19.8% for Bernasso (Adroit et al., 2016). Only Willershausen frequency is significantly different from the others (p<0.001). Willershausen leaves have especially much more hole feedings

(26.9%) and margin feedings (9.9%) than Berga (respectively 12.7% and 1.9%) and Bernasso leaves (respectively 9.8% and 7%) (Figure 3).

The frequencies of specialized interactions are 11.2% for Willershausen, 8.4% for Berga and 17.9% for Bernasso (Adroit et al., 2016). Only the Bernasso frequency is significantly different from the others (*p*<0.001). This difference is mainly due to the important quantity of galling in Bernasso (12%) which is significantly higher than in Willershausen and Berga, respectively 7% and 6% (*p*<0.01) (Figure 3).

Rarefaction tests on plant species richness highlight that Willershausen has more plant species (>100) than Berga (33) and Bernasso (20) (Figure 4). However, the DT richness in Willershausen (36 DTs) and Berga (25 DTs) are lower than in Bernasso (40 DTs) (Figure 4).

Structure of the paleoforests with the damage distribution on plant species

Figure 5 presents the different PCA realized for the 3 outcrops with the data of plant and DT diversities. For each outcrop, only the first two axes are presented as for Willershausen they represent 77% (Figure 5A), for Berga 93% (Figure 5B) and for Bernasso 91% (Figure 5C) of the whole distribution.

For Willershausen (Figure 5A) the FFGs hole feeding and skeletonization are positively correlated with PCA-axis 1 (respectively 0.76 and 0.61) and undamaged is negatively correlated with this axis (-0.97) (Data S1). Skeletonization and galling are positively correlated with PCA-axis 2 (respectively 0.62 and 0.63) while hole feeding is negatively correlated with this axis (-0.73) (Data S1). Concerning the species, three pools of plant species can be distinguished. The *Tilia* (*T. saportae, T.* cf. *saviana*), the *Ulmus* (*U. caprinifolia, U. campestris*), the *Fagus* (*F. grandifolia, F. pliocenica*), *Acer integerrimum* and *Quercus roburoides* are all along the positive part of the PCA-axis 1. The leaves of these species have the highest DT frequency of hole feeding and skeletonization. A second set of taxa is composed, for the most evident species, by *Acer cappadocicum, A. laetum, Carya minor*, cf. *Magnolia* sp1 and 2, *Populus willershausensis*, *Quercus praeerucifolia* and *Zelkova ungeri*. They are along the negative part of the PCA-axis 1 and along the positive part of the PCA-axis 2. They are mainly affected by the FFG galling (specialized interaction) or have no damage. At last, the third set of species is composed of Fagales (*Fagus sylvatica*, all the *Quercus*, *Alnus* and *Betula* species) and is in the negative part of the PCA-axis 2. These leaves are mainly undamaged or only impacted by hole feeding (generalist interaction).

For Berga (Figure 5B) the FFGs hole feeding, skeletonization and undamaged are positively associated with the PCA-axis 1, respectively with a correlation of 0.56, 0.73 and 0.99 (Data S1). Hole feeding and skeletonization are also correlated with the PCA-axis 2, negatively for hole feeding (-0.79) and positively for skeletonization (0.66) (Data S1). Concerning the species, we can note that *Taxodium dubium, Zelkova ungeri, Cercidiphylum crenatum* and *Acer integerrimum* are correlated with this undamaged category. *Fagus attenuata, Acer tricuspidatum* and *Quercus* sp. are mainly correlated with hole feeding.

For Bernasso (Figure 5C; (Adroit et al., 2016), the skeletonization and galling are positively correlated with the PCA-axis 1 (0.82 and 0.92) while undamaged is negatively correlated with this axis (-0.94) (Data S1). Hole feeding and skeletonization are positively correlated with PCA-axis 2 (0.92 and 0.25) while undamaged and galling are negatively correlated with this axis (-0.25 and -0.37) (Data S1). *Acer monspessulanum* and *Sorbus domestica* are in the positive part of the PCA-Axis 1 while the other are in the negative one (to note that *Parrotia persica* is close to zero). Concerning the PCA-axis 2, *Acer monspessulanum, Carpinus orinetlais* and *Carya minor* are in the positive part of the PCA-axis 2 while the others are in the negative part (to note that *Zelkova ungeri* is close to zero).

Furthermore, *Zelkova ungeri* is a species found in the 3 outcrops (Figure 5) and comparing to its position in the different PCAs, we can note that *Z. ungeri* is mostly associated with the FFG undamaged. However, for other common plant species, their relative position on the PCAs could be different. *Acer integerrimum* in Berga (Figure 5B) is mostly associated with the FFG undamaged while in Willershausen is opposite to this FFG as it is mainly associated to the FFG hole feeding and more weekly with skeletonization, margin and galling (Figure 5A).

Comparing Willershausen and Bernasso (Figure 5A & 6C), *A. monspessulanum* is mainly associated to skeletonization and galling in Willershausen (Figure 5.A) and in Bernasso it is with skeletonization, galling too but also with hole feeding (Figure 5C).

Sorbus domestica and *Carpinus orientalis* are both associated to the FFG undamaged in Willershausen (Figure 5A). In Bernasso, *S. domestica* is associated with galling and skeletonization and *C. orientalis* is associated with hole feeding and undamaged (Figure 5C). *Carya minor* is associated to skeletonization and galling in Willershausen (Figure 5A) while in

Bernasso it is associated to undamaged and hole feeding (Figure 5C). *Parrotia persica* is associated to galling and undamaged in Bernasso (Figure 5C) while in Willershausen, it is associated with the FFGs skeletonization and galling (Figure 5A).

DISCUSSION

Floristic richness and herbivory representativeness

All genera and at least 22 plant species from Berga leaf assemblage are also present in the Willershausen assemblage (Table S3). It can be explained by the geographical and stratigraphical proximity of the two outcrops. Bernasso had nearly the same composition of plant genera found in Willershausen (except *Ilex* only found in Bernasso) and also the majority of plant species (Table S3) despite its geographical situation and its younger age. There is quite a difference of plant richness between Bernasso and Berga, but the genera are the same (Table S3). This may suggest a difference in specific richness between those paleoforests. Rarefaction data indicated for the Willershausen leaf assemblage a highest plant species richness than the ones of Berga and Bernasso (Figure 4). However, the original sample size is ~~strongly~~ larger in Willershausen and could have led to artificial differences of plant species richness between the outcrops (Table 1). However, a bias due to the sample size is unlikely as Bernasso has the highest DT richness while the plant species and the quantity of leaves are lower than the one of the Willershausen assemblage.

Sampling effort tests indicate that enough specimens were taken into account to have a representative overview of the interactions on plant species found into the different outcrops (Figure 4). The large standard deviation observable on the Willershausen rarefaction curves on Figure 4 is due to this size of the fossil collection that includes around 8.000 specimens while the others are only 534 for Berga and 535 for Bernasso.

Relations between herbivory and the different mean annual temperatures estimated

Climatic conditions seem to be in relation with variations in richness and frequency of plant-insect interactions (Currano, Labandeira & Wilf, 2010). If an increase of temperature seems to stimulate insect herbivory (Coley & Aide, 1991; Coley & Barone, 1996; Zvereva & Kozlov, 2006; Currano, Labandeira & Wilf, 2010), it is still difficult to understand the complete role of temperature in the modulation of herbivory (DeLucia et al., 2012).

Thiel, Klotz & Uhl (2012) indicated, through leaf morphological analyses, that temperatures estimated for Willershausen were approximately 3°C higher than those for Berga. These paleoforests were geographically very close to each other (less than 70 km) and at a similar latitude (51°N) (Figure 1). Today the nearest meteorological stations of these locations (Willershausen: Göttingen, Lower-Saxony; Berga: Nordhausen, Thüringen) indicates the same mean annual temperature also for the coldest and warmest months over the last years (www.worldweatheronline.com). Such current similarities make the argument for similar paleoclimates of the two fossil localities if they were strictly of the same age. However, between 3 Ma and 2.5 Ma, CO_2 concentration progressively decreased (Kürschner et al., 1996; van de Wal et al., 2011) implicating a continuous decrease of mean annual temperatures (Willeit et al., 2015). Consequently, as Willershausen was warmer than Berga, the paleoforest of Willershausen grew under higher atmospheric CO_2 concentration than the Berga paleoforest. It seems to corroborate by the higher damage frequency observed in Willershausen that can have been favored by an increase of C/N ratios and an increase of photosynthesis rates (due to the high CO_2 concentration) (Bezemer & Jones, 1998; Stiling & Cornelissen, 2007; DeLucia et al., 2012). However, Willershausen and Berga had different sedimentological contexts and the preservation of the fossil leaves did not follow the same taphonomical constrains in the two outcrops. This could have influenced interpretation of the climate through morphological analyses.

For this reason, Thiel, Klotz & Uhl (2012) were in favor of the Coexistence Approach for climate interpretation which estimated similar temperature for Berga and Willershausen. It has been highlighted that the diversity of insects is often correlated to richness of plant species (Siemann, Tilman & Haarstad, 1996; Wright & Samways, 1998; Knops et al., 1999; Mulder et al., 1999) and should be expected to have higher damage richness in the more diverse paleoforest (Price, 1991, 2002). Thus, the higher richness and frequency of damage in Willershausen than in Berga could also be due to a higher insect diversity. Nevertheless, despite its higher plant richness Willershausen had less DT richness than Bernasso (Figures 3, 4). Bernasso had also more damage richness and frequency than Berga (Figures 3, 4). Thus, these observations make this assumption unsustainable for our study. It is also conceivable that the relative abundance of a plant species in those paleoforests could partly explain the herbivory measured; Indeed, more plant species are represented in the forest community, then more individuals have had a chance to be damaged by insect feeding (Feeny, 1976). However, for the fossil record, it is not possible to support this assumption because the leaf quantity of a plant species from an outcrop cannot be correlated to the relative abundance of this plant species in the paleoforest.

For Bernasso, the latitudinal position is different from Berga and Willershausen, as it located 1,000 km to the South. It has been highlighted that the insect diversity increases getting closer to the tropics (Hutchinson, 1959; Klopfer, 1959; Klopfer & MacArthur, 1960; MacArthur, 1972; Coley & Barone, 1996; Fraser, 2017). The southern position of Bernasso could partly explain the measured damage type richness. Nevertheless, the quantity of damage is not exclusively ~~link~~ to the insect diversity (Currano, Labandeira & Wilf, 2010). Latitudinal differences could led to a difference of thermal seasonality (Saikkonen et al., 2012) which is the key to the latitudinal gradient of insect diversity (Archibald et al., 2010). Leroy & Roiron (1996) indicated that Bernasso paleoforest grew under temperatures of 14–15°C and precipitations around 1500 mm/a. Recently, Girard et al. (in review) re-estimated Bernasso climate with different approaches and some results, based on leaf morphological traits, estimated temperatures in Bernasso to be cooler than estimations of Leroy & Roiron (1996), while the pollen analysis from the same study tend to corroborate previous estimations done by Leroy & Roiron (1996).

Relations between herbivory rates and temperatures of the coldest months and

Berga has a low temperature of the coldest months (from -6.4°C to 2°C) compared to Willershausen which had the highest temperatures (from -0.5°C to 5.1°C) (Uhl et al., 2007; Thiel, Klotz & Uhl, 2012). These lower temperatures during the cold period could explain the lowest damage frequency observed in Berga. Indeed, insects are poikilotherms, meaning that their body temperature is extremely dependent to the environment temperature (Meglitsch, 1972). Cooler temperatures decrease the insect metabolism (leading to diapause of insects) and the quantity of generations per year (Archibald et al., 2010), consequently it could also reduce the herbivory rates during the year (Bale & Hayward, 2010). Concerning Bernasso, the different estimations of temperatures, included the CMMT, are lower than those of Willershausen (Uhl et al., 2007; Thiel, Klotz & Uhl, 2012), thus the lowest frequency of damage could also be due to a lower insect metabolism in Bernasso than in Willershausen. The lowest frequency of damage in Berga than in Bernasso could also be due to insect diapause in the case of coolest temperatures being lower in Berga. However, the estimated temperatures of Bernasso overlap with the ones of

Berga (especially for the coolest temperatures) and therefore complicate any interpretations about the damage frequency between these two fossil leaf assemblages.

Moreover, it is important to note that no data about insect richness of these different paleoforests are available. Although it could be assumed that insect richness between Willershausen and Berga could be similar because outcrops are geographically and temporally similar, the insect richness of Bernasso could be quite different. Consequently, in cases of differences in the insect faunas, the previous relation could be disturbed as some insects, such as larvae of *Thaumetopoea pityocampa,* feed on plants during the winter season (Battisti et al., 2005; Buffo et al., 2007), when others insects have no or lower activity (Hahn & Denlinger, 2007).

More precision provided by proportion of generalized/specialized damages

The comparison of plant-insect interaction between different locations or through different time periods could still be upset by local disturbances (fires, floodings, etc.) or other constraints (such as different soils) that are not perceptible in fossil record and could impacted damage pattern in general (Currano et al., 2011; García, Castellanos & Pausas, 2016). Moreover, taphonomic biases, especially fossil preservation and different excavation histories, could also interfere with our analyses. For example, the damage frequency observed in fossil record could be partly distorted because the damaged leaves had less chance to be preserved in the fossil record than the complete and undamaged leaves (Ferguson, 2005). For all these reasons, we suggested complementing analyses by comparison of the proportions of generalized and specialized damage patterns.

Leckey et al. (2014) indicate that the proportion of generalist and specialist herbivores may change between different forests because the difference of abiotic parameters (such as climate). There are the lowest proportions of specialist interactions (mainly based on galling) in Willershausen and Berga, and conversely the highest proportion is in Bernasso (Figure 3), this may due to climatic factors (e.g., Leckey et al., 2014). Indeed, precipitation in Bernasso was higher than in Willershausen and Berga (Leroy & Roiron, 1996; Uhl et al., 2007) and hydric seasonality was probably more important in Bernasso (Girard et al., in review). This is in agreement with the proposed Mediterranean climate for Bernasso that provided heavy constrain to plants here due to less water availability during the dry season (Bagnouls & Gaussen, 1957; Daget, 1977, 1984). The higher seasonality conditions in Bernasso compared to conditions proposed for Berga and Willershausen could also be supported the idea that regional conditions of Northern Atlantic realm were more marked by higher ~~seasonlity~~ during Pleistocene than the Pliocene (Williams et al., 2009; Hennissen et al., 2015; Utescher et al., 2017). Water stress should have a positive impact on galling quantity, as many studies already mentioned that galling is an adaptation of stressful environment (Fernandes & Martins, 1985; Fernandes & Price, 1988, 1992; Price et al., 1998; Lara, Fernandes & Gonçalves-Alvim, 2002). In addition, Cuevas-Reyes et al. (2003), who studied the development of galling, showed that it exists a negative correlation between gall-forming insect species richness and plant species richness. It could also partly explain the highest proportion of specialized interactions in Bernasso. Additionally, a forest in its late successional stage, as it has been proposed for Bernasso (Leroy & Roiron, 1996; Adroit et al., 2016), tend to favor the richness of gall-inducing insects (that increase the proportion of specialized interaction) (Fernandes, Almada & Carneiro, 2010; Adroit et al., 2016).

Inputs of the comparisons between the common plant species from the different outcrops

This global comparison of specialized and generalized damages between the fossil leaf assemblage of Bernasso, Willershausen and Berga are also observable precisely on the common plant species statistically represented ~~on~~ each outcrop. However, the FFGs and especially the undamaged feature on some plant taxa are similar or could be slightly different between the fossil leaf assemblage (Figure 5). It tends to confirm that the abiotic parameters are important determinant factors involving significant variation of herbivory between different paleoenvironments (Cuevas-Reyes et al., 2004, 2003; Leckey et al., 2014). Biotic parameters can also be involved in the difference of interaction structures. For example, a decrease in food quality caused by higher concentration of carbon in plants could also have a negative impact on herbivory (Stiling & Cornelissen, 2007), but in general, it is compensated by an increase of insect feeding (Bezemer & Jones, 1998). The impact of biotic factors seems to be further confirmed as in Willershausen we can note that most Fagales (Betulaceae: *Alnus, Betulus, Carpinus*; Fagaceae: *Fagus, Quercus*; Juglandaceae: *Carya, Juglans*) are all associated to hole feeding and to undamaged feature (Figure 5A). This measurement cannot be due to hazard but it probably reflects an effect of some biotic parameters (such as genetic background, plant competition, host specificity, etc.).

CONCLUSION

Despite their similar plant species and their relative geographical and stratigraphical proximity (at least for Berga and Willershausen), trophic structures of those paleoforests were different. The relationships between the mean annual temperature and precipitation on one hand and the plant-insect interactions on the other hand as defined by previous studies have not been observed in this study. The comparison of the fossil records of Willershausen, Berga and Bernasso allowed discussion about the potential impacts of seasonality of the precipitation on the high proportion of galling. In addition, results suggested also that the herbivory rates could be impacted by the mean temperature of the coldest months of these paleoenvironments. Such observations can be related to the insects' response to climatic variation, ~~whichis~~ very sensitive (Bale & Hayward, 2010). Further analyses are needed to improve the possible links between plant-insect interactions and accurate climatic parameters. Concerning European paleoforests, the studies of other late Pliocene outcrops such as the one of Frankfurt-am-Main in Germany (Thiel et al., 2012) or Fossano in Italy (Macaluso et al., 2017) should provide interesting data to answer this question.

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REFERENCES

Adroit, B., Wappler, T., Terral, J.-F., Ali, A.A., Girard, V., 2016. Bernasso, a paleoforest from the early Pleistocene: New input from plant–insect interactions (Hérault, France). Palaeogeogr. Palaeoclimatol. Palaeoecol. 446, 78–84. https://doi.org/10.1016/j.palaeo.2016.01.015

Ambert, P., Boven, A., Leroy, S.A.G., Løvlie, R., Seret, G., 1990. Révision chronostratigraphique de la séquence paléobotanique de Bernasso (Escandorgue, Midi de la France). CRAS Paris 413–419.

Archibald, S.B., Bossert, W.H., Greenwood, D.R., Farrell, B.D., 2010. Seasonality, the latitudinal gradient of diversity, and Eocene insects. Paleobiology 36, 374–398.

Bachmann, G.H., Ehling, B.C., Eichner, R., Schwab, M., 2008. Geologie von Sachsen-Anhalt, Schweizerbart. ed.

Bagnouls, F., Gaussen, H., 1957. Les climats biologiques et leur classification. Ann. Géographie 66, 193–220. https://doi.org/10.3406/geo.1957.18273

Bale, J.S., Hayward, S. a. L., 2010. Insect overwintering in a changing climate. J. Exp. Biol. 213, 980–994. https://doi.org/10.1242/jeb.037911

Battisti, A., Stastny, M., Netherer, S., Robinet, C., Schopf, A., Roques, A., Larsson, S., 2005. Expansion of geographic range in the pine processionary moth caused by increased winter temperatures. Ecol. Appl. 15, 2084–2096.

Bezemer, T.M., Jones, T.H., 1998. Plant-Insect Herbivore Interactions in Elevated Atmospheric CO 2 : Quantitative Analyses and Guild Effects. Oikos 82, 212. https://doi.org/10.2307/3546961

Briggs, D.E., Stankiewicz, B.A., Meischner, D., Bierstedt, A., Evershed, R.P., 1998. Taphonomy of arthropod cuticles from Pliocene lake sediments, Willershausen, Germany. Palaios 13, 386–394.

Buffo, E., Battisti, A., Stastny, M., Larsson, S., 2007. Temperature as a predictor of survival of the pine processionary moth in the Italian Alps. Agric. For. Entomol. 9, 65–72.

Coley, P.D., Aide, T.M., 1991. Comparisons of herbivory and plant defenses in temperate and tropical broad-leaved forests. Plant-Anim. Interact. Evol. Ecol. Trop. Temp. Reg. 25–49.

Coley, P.D., Barone, J.A., 1996. Herbivory and Plant Defenses in Tropical Forests. Annu. Rev. Ecol. Syst. 27, 305–335. https://doi.org/10.1146/annurev.ecolsys.27.1.305

Cuevas-Reyes, P., Quesada, M., Hanson, P., Dirzo, R., Oyama, K., 2004. Diversity of gall-inducing insects in a Mexican tropical dry forest: the importance of plant species richness, life-forms, host plant age and plant density. J. Ecol. 92, 707–716.

Cuevas-Reyes, P., Siebe, C., Martínez-Ramos, M., Oyama, K., 2003. Species richness of gall-forming insects in a tropical rain forest: correlations with plant diversity and soil fertility. Biodivers. Conserv. 12, 411–422.

Currano, E.D., Jacobs, B.F., Pan, A.D., Tabor, N.J., 2011. Inferring ecological disturbance in the fossil record: A case study from the late Oligocene of Ethiopia. Palaeogeogr. Palaeoclimatol. Palaeoecol. 309, 242–252. https://doi.org/10.1016/j.palaeo.2011.06.007

Currano, E.D., Labandeira, C.C., Wilf, P., 2010. Fossil insect folivory tracks paleotemperature for six million years. Ecol. Monogr. 80, 547–567.

Daget, P., 1984. Introduction à une théorie générale de la méditerranéité. Bull. Société Bot. Fr. Actual. Bot. 131, 31–36. https://doi.org/10.1080/01811789.1984.10826644

Daget, P., 1977. Le bioclimat Mediterraneen: analyse des formes climatiques par le systeme d'Emberger. Vegetatio 34, 87–103.

DeChaine, E.G., Martin, A.P., 2006. Using coalescent simulations to test the impact of quaternary climate cycles on divergence in an alpine plant-insect association. Evolution 60, 1004. https://doi.org/10.1554/05-672.1

DeLucia, E.H., Nabity, P.D., Zavala, J.A., Berenbaum, M.R., 2012. Climate Change: Resetting Plant-Insect Interactions. PLANT Physiol. 160, 1677–1685. https://doi.org/10.1104/pp.112.204750

Donovan, M.P., Iglesias, A., Wilf, P., Labandeira, C.C., Cúneo, N.R., 2016. Rapid recovery of Patagonian plant–insect associations after the end-Cretaceous extinction. Nat. Ecol. Evol. 1, 0012. https://doi.org/10.1038/s41559-016-0012

Driscoll, N.W., Haug, G.H., 1998. A Short Circuit in Thermohaline Circulation: A Cause for Northern Hemisphere Glaciation? Science 282, 436–438.

Feeny, P., 1976. Plant apparency and chemical defense. Plenum Press 1–40.

Ferguson, D.K., 2005. Plant Taphonomy: Ruminations on the Past, the Present, and the Future. PALAIOS 20, 418–428. https://doi.org/10.2110/palo.2005.P05-25p

Ferguson, D.K., Knobloch, E., 1998. A fresh look at the rich assemblage from the Pliocene sink-hole of Willershausen, Germany. Rev. Palaeobot. Palynol. 101, 271–286.

Fernandes, G.W., Almada, E.D., Carneiro, M.A.A., 2010. Gall-Inducing Insect Species Richness as Indicators of Forest Age and Health. Environ. Entomol. 39, 1134–1140. https://doi.org/10.1603/EN09199

Fernandes, G.W., Martins, R.P., 1985. As galhas: tumores de plantas. Ciênc. Hoje 4, 58–64.

Fernandes, G.W., Price, P.W., 1992. The adaptive significance of insect gall distribution: survivorship of species in xeric and mesic habitats. Oecologia 90, 14–20.

Fernandes, G.W., Price, P.W., 1988. Biogeographical gradients in galling species richness. Oecologia 76, 161–167.

Forister, M.L., Novotny, V., Panorska, A.K., Baje, L., Basset, Y., Butterill, P.T., Cizek, L., Coley, P.D., Dem, F., Diniz, I.R., 2015. The global distribution of diet breadth in insect herbivores. Proc. Natl. Acad. Sci. 112, 442–447.

Frank, Dorothea, Reichstein, M., Bahn, M., Thonicke, K., Frank, David, Mahecha, M.D., Smith, P., van der Velde, M., Vicca, S., Babst, F., Beer, C., Buchmann, N., Canadell, J.G., Ciais, P., Cramer, W., Ibrom, A., Miglietta, F., Poulter, B., Rammig, A., Seneviratne, S.I., Walz, A., Wattenbach, M., Zavala, M.A., Zscheischler, J., 2015. Effects of climate extremes on the terrestrial carbon cycle: concepts, processes and potential future impacts. Glob. Change Biol. 21, 2861–2880. https://doi.org/10.1111/gcb.12916

Fraser, D., 2017. Can latitudinal richness gradients be measured in the terrestrial fossil record? Paleobiology 43, 479–494. https://doi.org/10.1017/pab.2017.2

García, Y., Castellanos, M.C., Pausas, J.G., 2016. Fires can benefit plants by disrupting antagonistic interactions. Oecologia 182, 1165–1173. https://doi.org/10.1007/s00442-016-3733-z

Girard, V., Fauquette, S., Adroit, B., Ahmed, A., Paya, A., Ali, A.A., Paradis, L., Roiron, P., Leroy, S.A.G., Suc, J-P., in review, Fossil macro- and micro-flora from Bernasso (Early

Pleistocene, Southern France): new palaeoclimatic data and comparison of methods. Comptes Rendus Palevol, 1-14.

Givulescu, R., 1984. Pathological elements on fossil leaves from Chiuzbaia (galls, mines and other insect traces). Dari Seama Ale Sedintelor Intitutul Geol. Si Geofızica 68, 123–133.

Gotelli, N.J., Colwell, R.K., 2001. Quantifying biodiversity: procedures and pitfalls in the measurement and comparison of species richness. Ecol. Lett. 4, 379–391.

Haddad, N.M., Crutsinger, G.M., Gross, K., Haarstad, J., Knops, J.M.H., Tilman, D., 2009. Plant species loss decreases arthropod diversity and shifts trophic structure. Ecol. Lett. 12, 1029–1039. https://doi.org/10.1111/j.1461-0248.2009.01356.x

Hahn, D.A., Denlinger, D.L., 2007. Meeting the energetic demands of insect diapause: Nutrient storage and utilization. J. Insect Physiol., VIII European Congress of Entomology - Physiology and Endocrinology 53, 760–773. https://doi.org/10.1016/j.jinsphys.2007.03.018

Hammer, Ø., Harper, D.A.T., Ryan, P.D., 2001. PAST: Paleontological statistics software package for education and data analysis. Palaeontologia Electronica. Palaeontol. Electron. 4, 1–9.

Hansen, J., Sato, M., Russell, G., Kharecha, P., 2013. Climate sensitivity, sea level and atmospheric carbon dioxide. Philos. Trans. R. Soc. Math. Phys. Eng. Sci. 371, 20120294–20120294. https://doi.org/10.1098/rsta.2012.0294

Haug, G.H., Tiedemann, R., Keigwin, L.D., 2004. How the Isthmus of Panama put ice in the Arctic. Oceanus 42, 94–97.

Haywood, A.M., Sellwood, B.W., Valdes, P.J., 2000. Regional warming: Pliocene (3 Ma) paleoclimate of Europe and the Mediterranean. Geology 28, 1063–1066.

Heller, N.E., Zavaleta, E.S., 2009. Biodiversity management in the face of climate change: A review of 22 years of recommendations. Biol. Conserv. 142, 14–32. https://doi.org/10.1016/j.biocon.2008.10.006

Hennissen, J.A.I., Head, M.J., De Schepper, S., Groeneveld, J., 2015. Increased seasonality during the intensification of Northern Hemisphere glaciation at the Pliocene–Pleistocene boundary ~2.6 Ma. Quat. Sci. Rev. 129, 321–332. https://doi.org/10.1016/j.quascirev.2015.10.010

Hilgen, F.J., 1991. Astronomical calibration of Gauss to Matuyama sapropels in the Mediterranean and implication for the geomagnetic polarity timescale. Earth Planet Sci Lett 104, 226–244.

Hooper, D.U., Adair, E.C., Cardinale, B.J., Byrnes, J.E.K., Hungate, B.A., Matulich, K.L., Gonzalez, A., Duffy, J.E., Gamfeldt, L., O'Connor, M.I., 2012. A global synthesis reveals biodiversity loss as a major driver of ecosystem change. Nature. https://doi.org/10.1038/nature11118

Hutchinson, G.E., 1959. Homage to Santa Rosalia or why are there so many kinds of animals? Am. Nat. 93, 145–159.

Ildefonse, J.-P., Bellon, H., Pantaloni, A., Philippet, J.C., 1972. Mise en évidence de la transition paléomagnétique Gauss-Matuyama dans les formations volcaniques de l'Escandorgue, Hérault, France. Earth Planet Sci Lett 14, 249–254.

Klopfer, P.H., 1959. Environmenta determinants of faunal diversity. Am. Nat. XCIII, 337–342.

Klopfer, P.H., MacArthur, R.H., 1960. Niche size and faunal diversity. Am. Nat. XCIV, 293–300.

Knobloch, E., 1998. Der pliozäne laubwald Laubwald von willershausen Willershausen am harz Harz (Mitteleuropa). Doc. Naturae 120, 1–302.

Knops, J.M., Tilman, D., Haddad, N.M., Naeem, S., Mitchell, C.E., Haarstad, J., Ritchie, M.E., Howe, K.M., Reich, P.B., Siemann, E., others, 1999. Effects of plant species richness on invasion dynamics, disease outbreaks, insect abundances and diversity. Ecol. Lett. 2, 286–293.

Knor, S., Prokop, J., Kvaček, Z., Janovský, Z., Wappler, T., 2012. Plant–arthropod associations from the Early Miocene of the Most Basin in North Bohemia—Palaeoecological and palaeoclimatological implications. Palaeogeogr. Palaeoclimatol. Palaeoecol. 321–322, 102–112. https://doi.org/10.1016/j.palaeo.2012.01.023

Kolibáč, J., Adroit, B., Gröning, E., Brauckmann, C., Wappler, T., 2016. First record of the family Trogossitidae (Insecta, Coleoptera) in the Late Pliocene deposits of Willershausen (Germany). PalZ. https://doi.org/10.1007/s12542-016-0316-6

Kürschner, W.M., van der Burgh, J., Visscher, H., Dilcher, D.L., 1996. Oak leaves as biosensors of late Neogene and early Pleistocene paleoatmospheric CO2 concentrations. Mar. Micropaleontol. 27, 299–312.

Labandeira, C.C., 2012. Evidence for Outbreaks from the Fossil Record of Insect Herbivory. Oxf. Wiley-Blackwell, Oxford: Wiley-Blackwell 267–290.

Labandeira, C.C., 2002. The history of associations between plants and animals. Plant–Animal Interact. Evol. Approach 248, 261.

Labandeira, C.C., Currano, E.D., 2013. The Fossil Record of Plant-Insect Dynamics. Annu. Rev. Earth Planet. Sci. 41, 287–311. https://doi.org/10.1146/annurev-earth-050212-124139

Labandeira, C.C., Johnson, K.R., Wilf, P., 2002. Impact of the terminal Cretaceous event on plant–insect associations. Proc. Natl. Acad. Sci. 99, 2061–2066.

Labandeira, C.C., Kustatscher, E., Wappler, T., 2016. Floral Assemblages and Patterns of Insect Herbivory during the Permian to Triassic of Northeastern Italy. PLoS ONE 11, e0165205. https://doi.org/10.1371/journal.pone.0165205

Labandeira, C.C., Wilf, P., Johnson, K.R., Marsh, F., 2007. Guide to insect (and other) damage types on compressed plant fossils. Smithson. Inst. Natl. Mus. Nat. Hist. Dep. Paleobiology Wash. DC.

Lara, A.C.F., Fernandes, G.W., Gonçalves-Alvim, S.J., 2002. Tests of hypotheses on patterns of gall distribution along an altitudinal gradient. Trop. Zool. 15, 219–232. https://doi.org/10.1080/03946975.2002.10531176

Leckey, E.H., Smith, D.M., Nufio, C.R., Fornash, K.F., 2014. Oak-insect herbivore interactions along a temperature and precipitation gradient. Acta Oecologica 61, 1–8. https://doi.org/10.1016/j.actao.2014.08.001

Leroy, S.A., Roiron, P., 1996. Latest Pliocene pollen and leaf floras from Bernasso palaeolake (Escandorgue Massif, Hérault, France). Rev. Palaeobot. Palynol. 94, 295–328.

Macaluso, L., Martinetto, E., Vigna, B., Bertini, A., Cilia, A., Teodoridis, V., Kvaček, Z., 2017. Palaeofloral and stratigraphic context of a new fossil forest from the Pliocene of NW Italy. Rev. Palaeobot. Palynol. https://doi.org/10.1016/j.revpalbo.2017.08.005

MacArthur, R.H., 1972. Geographical Ecology: Patterns in the Distribution of Species, Biology / Princeton University Press. Princeton University Press.

Magri, D., 2010. Persistence of tree taxa in Europe and Quaternary climate changes. Quat. Int. 219, 145–151. https://doi.org/10.1016/j.quaint.2009.10.032

Mai, D.H., 1995. Tertiäre Vegetationsgeschichte Europas: Methoden und Ergebnisse. Fischer.

Mai, D.H., Walther, H., 1988. Die pliozänen Floren von Thüringen, Deutsche Demokratische Republik. Quartärpaläontologie 7, 55–297.

Médail, F., Diadema, K., 2009. Glacial refugia influence plant diversity patterns in the Mediterranean Basin. J. Biogeogr. 36, 1333–1345. https://doi.org/10.1111/j.1365-2699.2008.02051.x

Meehl, G.A., Stocker, T.F., Collins, W.D., Friedlingstein, P., Gaye, A.T., Gregory, J.M., Kitoh, A., Knutti, R., Murphy, J.M., Noda, A., Raper, S.C.B., Watterson, I.G., Weaver, A.J., Zhao, Z.-C., 2007. Global Climate Projections, in: Climate Change 2007: The Physical Science Basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. S. Solomon, D. Qin, M. Manning, Z. Chen, M. Marquis, K.B. Averyt, M. Tignor, H.L. Miller, Cambridge, United Kingdom and New York, NY, USA.

Meglitsch, P.A., 1972. Invertebrate Zoology. Oxford University Press, New York.

Meischner, D., 2000. Europäische Fossillagerstätten, in: Der Pliozäne Teich von Willershausen Am Harz. Dieter Meischner, New-York, pp. 223–228 and 261.

Migliore, J., Baumel, A., Juin, M., Médail, F., 2012. From Mediterranean shores to central Saharan mountains: key phylogeographical insights from the genus Myrtus: Phylogeography of the genus Myrtus. J. Biogeogr. 39, 942–956. https://doi.org/10.1111/j.1365-2699.2011.02646.x

Mulder, C.P.H., Koricheva, J., Huss-Danell, K., Högberg, P., Joshi, J., 1999. Insects affect relationships between plant species richness and ecosystem processes. Ecol. Lett. 2, 237–246.

Postigo Mijarra, J.M., Barrón, E., Gómez Manzaneque, F., Morla, C., 2009. Floristic changes in the Iberian Peninsula and Balearic Islands (south-west Europe) during the Cenozoic. J. Biogeogr. 36, 2025–2043. https://doi.org/10.1111/j.1365-2699.2009.02142.x

Price, P.W., 2002. Resource-driven terrestrial interaction webs. Ecol. Res. 17, 241–247.

Price, P.W., 1991. The plant vigor hypothesis and herbivore Attack. Oikos 62, 244–251. https://doi.org/10.2307/3545270

Price, P.W., Fernandes, G.W., Lara, A.C.F., Braw, J., Barrios, H., Wright, M.G., Ribeiro, S.P., Rothcliff, N., 1998. Global patterns in local number of insect galling species. J. Biogeogr. 25, 581–591.

R Development Core Team, 2014. R: a language and environment for statistical computing. Vienna: R Foundation for Statistical Computing.

Ravelo, A.C., Andreasen, D.H., Mitchell, L., Lyle, A.O., Wara, M.W., 2004. Regional climate shifts caused by gradual global cooling in the Pliocene epoch. Nature 429, 263.

Saikkonen, K., Taulavuori, K., Hyvönen, T., Gundel, P.E., Hamilton, C.E., Vänninen, I., Nissinen, A., Helander, M., 2012. Climate change-driven species’ range shifts filtered by photoperiodism. Nat. Clim. Change 2, 239–242. https://doi.org/10.1038/nclimate1430

Siemann, E., Tilman, D., Haarstad, J., 1996. Insect species diversity, abundance and body size relationships. Nature 704–706.

Stiling, P., Cornelissen, T., 2007. How does elevated carbon dioxide (CO_2) affect plant–herbivore interactions? A field experiment and meta-analysis of CO_2-mediated changes on plant chemistry and herbivore performance. Glob. Change Biol. 13, 1823–1842. https://doi.org/10.1111/j.1365-2486.2007.01392.x

Straus, A., 1977. Gallen, Minen und andere Fraßspuren im Pliozän von Willershausen am Harz. Verhandlungen Bot. Ver. Prov. Brandenbg. 41–80.

Suc, J., Popescu, S., 2005. Pollen records and climatic cycles in the North Mediterranean region since 2.7 Ma. Spec. Publ.-Geol. Soc. Lond. 247, 147.

Suc, J.-P., 1978. Analyse pollinique de dépots Plio-Pléistocènes du sud du massif basaltique de l’Escandorgue (Site de Bernasso, Lunas, Hérault, France). Pollen Spores 20, 497–512.

Taylor, L.L., Banwart, S.A., Valdes, P.J., Leake, J.R., Beerling, D.J., 2012. Evaluating the effects of terrestrial ecosystems, climate and carbon dioxide on weathering over geological time: a global-scale process-based approach. Philos. Trans. R. Soc. B Biol. Sci. 367, 565–582. https://doi.org/10.1098/rstb.2011.0251

Thiel, C., Klotz, S., Uhl, D., 2012. Palaeoclimate estimates for selected leaf floras from the late Pliocene (Reuverian) of Central Europe based on different palaeobotanical techniques. Turk. J. Earth Sci. 21, 263–287.

Thunell, R.C., 1979. Pliocene-Pleistocene paleotemperature and paleosalinity history of the Mediterranean Sea: Results from DSDP Sites 125 and 132. Mar. Micropaleontol. 4, 173–187.

Titchener, F.R., 1999. Leaf feeding traces from the upper Pliocene fossil Lagerstätte of Willershausen, lower saxony, Germany. Acta Palaeontol. 5, 403–409.

Turner, B.L.I., Clark, W., C., Kates, R., W., Richards, J., F., Mathews, J., T., Meyer, W., B., 1990. The Earth was formed by Human Action: Global and Regional Changes in the Past 300 Years. Camb. Univ Press Camb.

Tzedakis, P.C., Hooghiemstra, H., Pälike, H., 2006. The last 1.35 million years at Tenaghi Philippon: revised chronostratigraphy and long-term vegetation trends. Quat. Sci. Rev. 25, 3416–3430. https://doi.org/10.1016/j.quascirev.2006.09.002

Uhl, D., Klotz, S., Traiser, C., Thiel, C., Utescher, T., Kowalski, E., Dilcher, D.L., 2007. Cenozoic paleotemperatures and leaf physiognomy — A European perspective. Palaeogeogr. Palaeoclimatol. Palaeoecol. 248, 24–31. https://doi.org/10.1016/j.palaeo.2006.11.005

Utescher, T., Dreist, A., Henrot, A.-J., Hickler, T., Liu, Y.-S. (Christopher), Mosbrugger, V., Portmann, F.T., Salzmann, U., 2017. Continental climate gradients in North America and Western Eurasia before and after the closure of the Central American Seaway. Earth Planet. Sci. Lett. 472, 120–130. https://doi.org/10.1016/j.epsl.2017.05.019

van de Wal, R.S.W., de Boer, B., Lourens, L.J., Köhler, P., Bintanja, R., 2011. Reconstruction of a continuous high-resolution CO_2 record over the past 20 million years. Clim. Past 7, 1459–1469. https://doi.org/10.5194/cp-7-1459-2011

Vinken, R., 1967. Kurzer Überblick über die Geologie der Umgebung von Willershausen. Ber. Naturhistorischen Ges. Zu Hann. 115, 5–14.

Von Koenen, A., 1895. Blatt Westerhof. Erl. Geol. Specialkt. Preußen und Thüringischen Staaten, 71. Lief. Grababtheilung 55, 18.

Wappler, T., 2010. Insect herbivory close to the Oligocene–Miocene transition — A quantitative analysis. Palaeogeogr. Palaeoclimatol. Palaeoecol. 292, 540–550. https://doi.org/10.1016/j.palaeo.2010.04.029

Wappler, T., Currano, E.D., Wilf, P., Rust, J., Labandeira, C.C., 2009. No post-Cretaceous ecosystem depression in European forests? Rich insect-feeding damage on diverse middle Palaeocene plants, Menat, France. Proc. R. Soc. B Biol. Sci. 276, 4271–4277. https://doi.org/10.1098/rspb.2009.1255

Wappler, T., Grímsson, F., 2016. Before the 'Big Chill': Patterns of plant-insect associations from the Neogene of Iceland. Glob. Planet. Change 142, 73–86. https://doi.org/10.1016/j.gloplacha.2016.05.003

Wappler, T., Labandeira, C.C., Rust, J., Frankenhäuser, H., Wilde, V., 2012. Testing for the Effects and Consequences of Mid Paleogene Climate Change on Insect Herbivory. PLoS ONE 7, e40744. https://doi.org/10.1371/journal.pone.0040744

Wegele, H., 1914. Stratigraphie und tektonik der tertiären ablagerungen. Druck von A. Rüttgerodt, Göttingen.

Wilf, P., 2008. Fossil angiosperm leaves: Paleobotany's difficult children prove themselves. Paleontol Soc Pap. 14, 319–333.

Willeit, M., Ganopolski, A., Calov, R., Robinson, A., Maslin, M., 2015. The role of CO2 decline for the onset of Northern Hemisphere glaciation. Quat. Sci. Rev. 119, 22–34. https://doi.org/10.1016/j.quascirev.2015.04.015

Williams, M., Haywood, A.M., Harper, E.M., Johnson, A.L.., Knowles, T., Leng, M.J., Lunt, D.J., Okamura, B., Taylor, P.D., Zalasiewicz, J., 2009. Pliocene climate and seasonality in North Atlantic shelf seas. Philos. Trans. R. Soc. Math. Phys. Eng. Sci. 367, 85–108. https://doi.org/10.1098/rsta.2008.0224

Wright, M.G., Samways, M.J., 1998. Insect species richness tracking plant species richness in a diverse flora: gall-insects in the Cape Floristic Region, South Africa. Oecologia 115, 427–433.

Yang, L.H., Gratton, C., 2014. Insects as drivers of ecosystem processes. Curr. Opin. Insect Sci. 2, 26–32. https://doi.org/10.1016/j.cois.2014.06.004

Zvereva, E.L., Kozlov, M.V., 2006. Consequences of simultaneous elevation of carbon dioxide and temperature for plant-herbivore interactions: a metaanalysis. Glob. Change Biol. 12, 27–41. https://doi.org/10.1111/j.1365-2486.2005.01086.x

Figure 1(on next page)

The location of Willershausen and Berga outcrops from the late Pliocene.

A- The location of Germany in Europe. B- The locations of both outcrops in Germany. C- Zoom on the area near Göttingen. On the scale, each dash (black or white) represents 5km. The data from the maps A and B come from Natural Earth database (http://www.naturalearthdata.com)

Origin data : Natural Earth
Coordinate system : WGS84 et ED1950utm

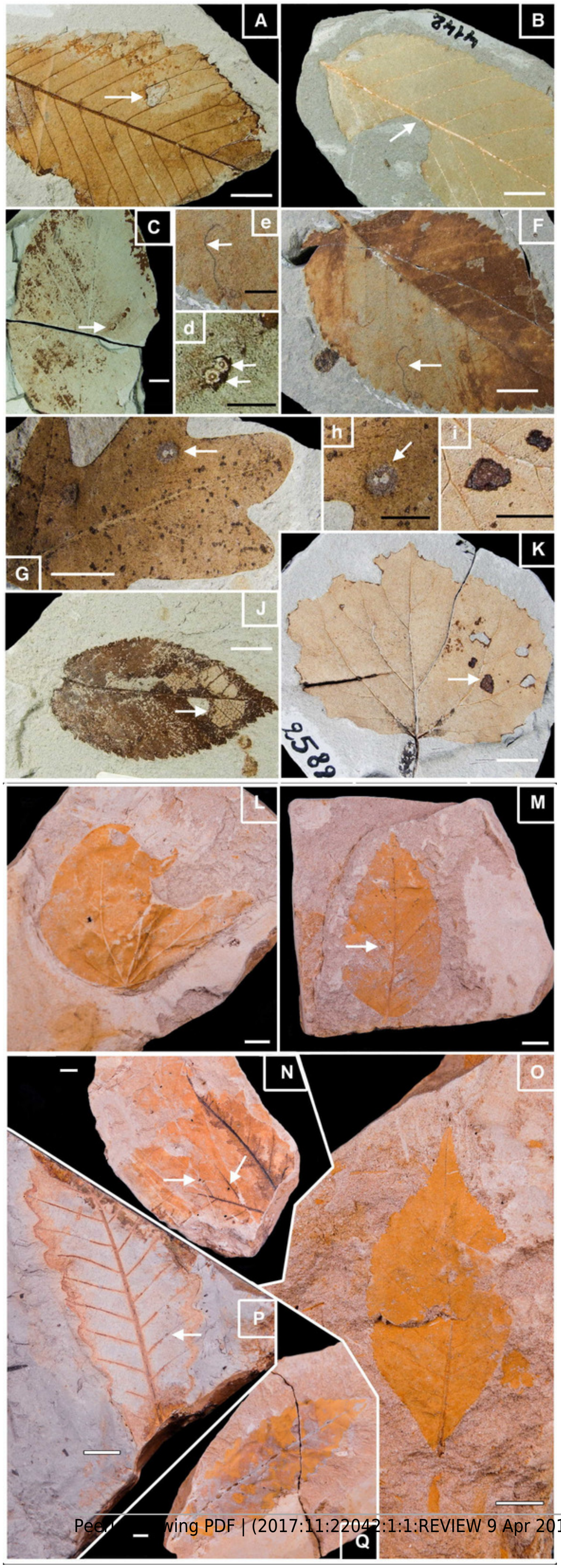
Figure 2

Fossil leaves from the German outcrops

Plate 1. Fossil leaves from Willershausen (Göttingen coll.). A- *Ulmus caprinifolia* with Hole feeding (DT05). B- *Alnus spaethii* with Margin feeding (DT14). C- *Fagus* sp with Piercing & Sucking (DT168). enlarged in ‘d’. F- *Ulmus campestris* with Mining (DT109) enlarged in ‘e’. G- *Quercus praeerucifolia* with Galling (DT145) enlarged in ‘h’. J- *Ulmus caprinifolia* with Skeletonization (DT17). K- *Populus tremula* with Surface feeding (DT30) enlarged in ‘i’.

Plate 2. Fossil leaves from Berga (Dresden coll.). L- *Cercidiphyllum crenatum*. M- *Fagus attenuata* with Hole feeding (DT01). N- *Juglans* sp. with Galls (DT34) . O- *Pterocarya paradisiaca*. P- *Quercus pseudocastanea* with Galling (DT116). Q- *Quercus castaneifolia.* White scale bar represents 1cm, black scale bar represents 0.5cm.

Photographs by Benjamin Adroit.


A
B
C
e
d
F
G
h
i
J
K
L
M
N
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P
Q

Figure 3

Quantitative distribution of plant-insect interactions from Willershausen, Berga outcrops (late Pliocene) and the fossil deposit of Bernasso (early Pleistocene).

The data of Bernasso come from Adroit et al., (2016). For each damage frequency, significant difference (alpha <0.05) from an outcrop to another one is marked by an asterisk. The percentage of generalized and specialized damages are computed only with the damaged leaves, consequently their sum on each outcrop is 100% in this figure. According to the whole amount of leaves the percentage of generalist interactions are 42.8% for Willershausen, 17.8% for Berga and 19.8% for Bernasso and the percentage of specialist interactions are 11.2% for Willershausen, 8.4% for Berga and 17.9% for Bernasso.

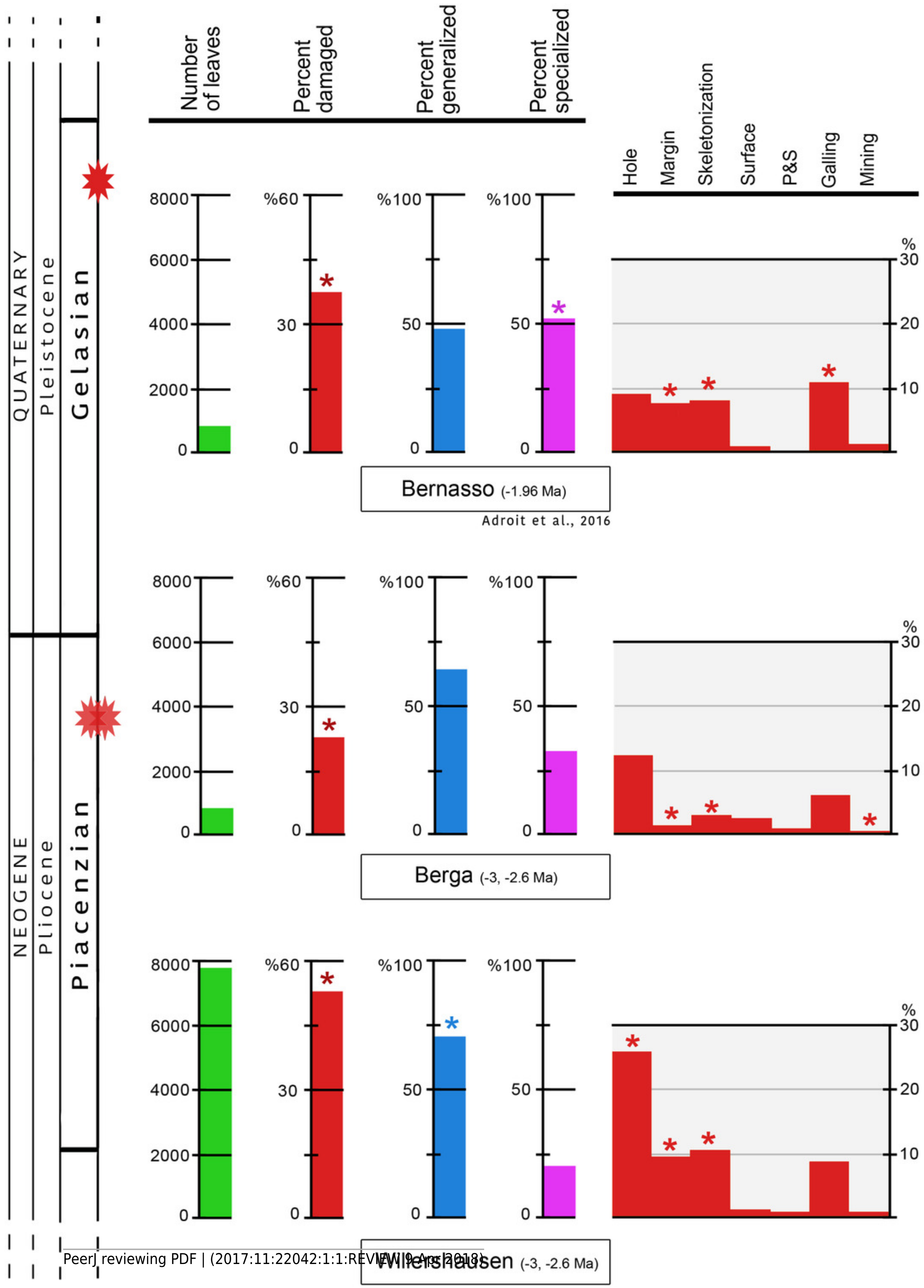
QUATERNARY
Pleistocene
Gelasian
NEOGENE
Pliocene
Piacenzian
Number of leaves
Percent damaged
Percent generalized
Percent specialized
Hole
Margin
Skeletonization
Surface
P&S
Galling
Mining
8000
6000
4000
2000
0
%60
30
0
%100
50
0
%
30
20
10
*
Bernasso (-1.96 Ma)
Adroit et al., 2016
Berga (-3, -2.6 Ma)
Willershausen (-3, -2.6 Ma)

Figure 4

Rarefaction curves on the leaves from Willershausen, Berga and Bernasso (Adroit et al. 2016).

The grey curves represent Berga (Germany), the blue curves represent Willershausen (Germany) outcrop and the orange curves represent Bernasso (France). The shaded area represents the standard deviation below and above the average of the resamples, with the method from Heck et al. (1975). Rarefaction curves represent the number of specimens by: A- Richness of plant species; B- Richness of damage type (DT); C- Richness of generalized damage; D- Richness of specialized damage

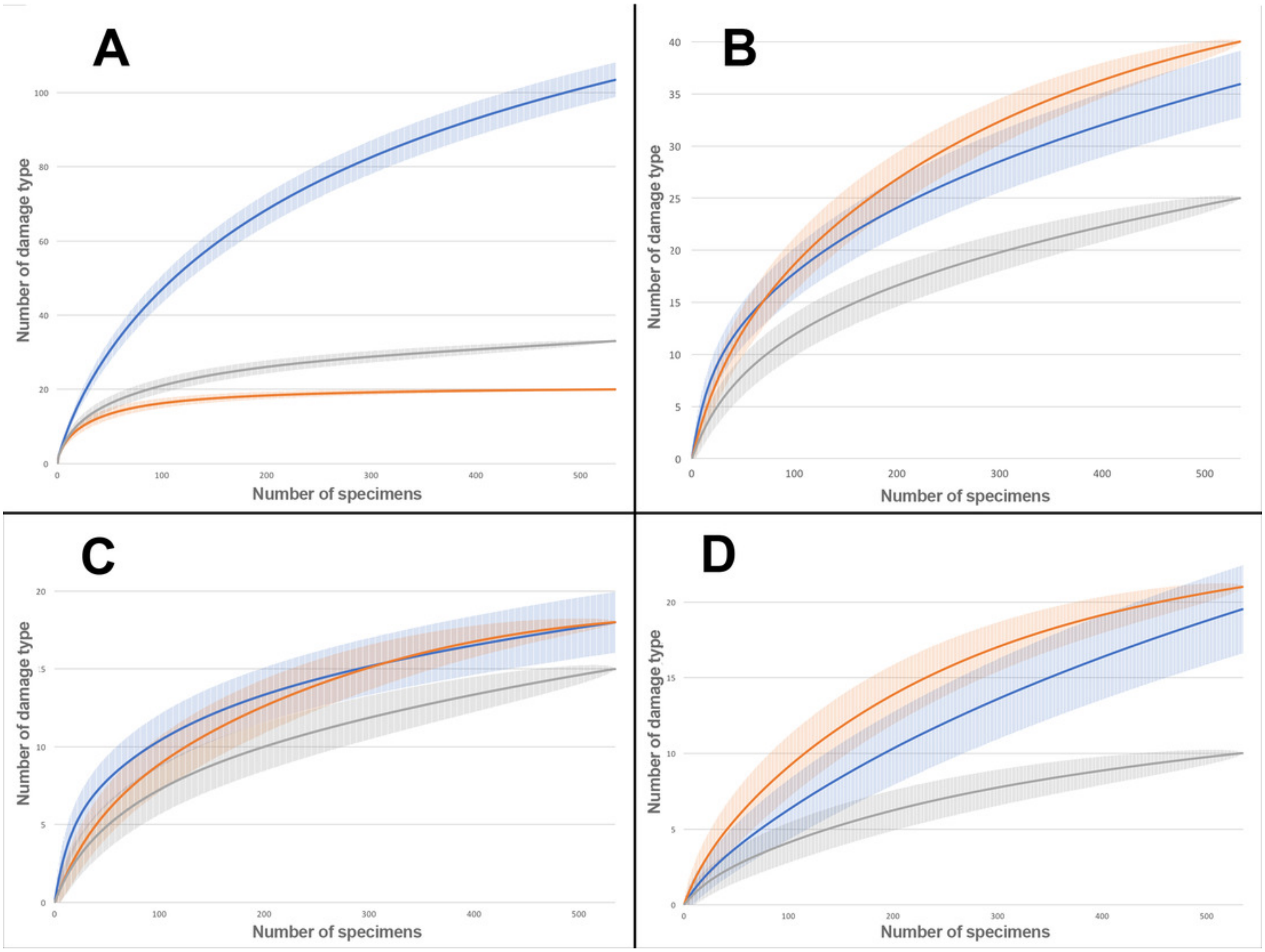

Figure 5(on next page)

Principal components analysis (PCA) based on the proportion (in percentage) of the FFG on each plant species.

A- Willershausen (Germany, late Pliocene), B- Berga (Germany, late Pliocene), C- Bernasso (France, early Pleistocene). Due to large amount of plant species names on A- Willershausen, the species names near the axes intersection were replace by alphabetic letter for visibility concerns: a- *Malus pulcherrima*, b- *Prunus mahaleb*, c- *Fagus sylvatica*, d- *Sorbus gabbrensis*, e- *Populus tremula*, f- *Populus willershausensis*, g- *Zelkova caprinifolia*, h- *Betula pubescens*, i- *Betula_sp1*, j- cf_*Toona*.


A
cf_Magnolia
Parrotia_persica
Skeletonization
Acer_paleosaccharinum
Galling
Acer_laetum
cf_Magnolia_sp2
cf_Magnolia_sp1
Acer_monspessulanum
Mining
Aesculus_hippocastanum
Crataegus_meischneri
Quercus_praeerucifolia
Carya_minor
Acer_cappadocicum
Surface
Zelkova_ungeri
F
Margin
Quercus_libani
E
G
H
Fagus_cf_pliocenica
Quercus_roburoides
Acer_integerrimum
Sorbus_domestica
D
P&S
Fagus_grandifolia
Populus_populina
Carpinus_betulus
A
I
J
Ulmus_campestris
Ulmus_carpinifolia
B
C
Carpinus_orientalis
Carpinus_sp1
Quercus_praecastaneaefolia
Tilia_cf_savania
Tilia_saportae
Undamaged
Carya_cf_Pterocarya
Alnus_spaethii
Quercus_castaneaefolia
Alnus_serrulata
Carpinus_grandis
Betula_alnoides
Betula_maximowicziana
Betula_lenta
Quercus_iberica
Quercus_macranthera
Hole
0.225
0.150
0.075
-0.075
-0.150
-0.225
-0.300
-0.300
-0.225
-0.150
-0.075
0.075
0.150
0.225
0.300
0.375
Component 2 (20%)
Component 1 (57%)
B
0.20
0.15
0.10
0.05
-0.05
-0.10
Alnus_gaudinii
Cercidiphylum_crenatum
Skeletonization
Margin
Taxodium_dubium
Undamaged
Mining
Galling
Surface
Zelkova_ungeri
Acer_integerrinum
-0.20
-0.15
-0.10
-0.05
0.05
0.10
0.15
0.20
0.25
P&S
Fagus_attenuata
Quercus_sp
Acer_tricuspidatum
Hole
Component 2 (22%)
Component 1 (71%)
C
0.20
0.15
0.10
0.05
-0.05
-0.10
-0.15
-0.20
Carya_minor
Hole
Skeletonization
Acer_monspessulanum
Carpinus_orientalis
Margin
Zelkova_ungeri
Mining
Surface
-0.150
-0.075
0.075
0.150
0.225
0.300
0.375
P&S
Sorbus_domestica
Undamaged
Parrotia_persica
Galling
Fraxinus_ornus
Component 2 (16%)
Component 1 (75%)

Table 1(on next page)

Frequency of the leaves damaged per FFG based on the whole flora.

It happens that there is more than one FFG on a leaf damaged, consequently the sum of the percent of galling, mining, margin feeding, hole feeding, skeletonization, surface feeding and piercing & sucking exceed the value of the damaged leaves. The Data for Bernasso originate from Adroit et al. (2016). Detailed of damaged leaves per species are presented in Table S3.

1

Outcrops	*#leaves*	**Damaged**	**Generalist**	**Specialist**	**External_ Specialized**	Galling	Mining	MarginF	HoleF	Skeletonization	SurfaceF	P&S
WILLERSHAUSEN	*7932*	50.43	42.80	10.16	1.11	7.01	1.59	9.86	26.94	11.01	1.64	1.10
BERGA	*534*	25.09	17.79	7.12	1.31	6.18	0.19	1.87	12.73	2.62	2.06	0.94
BERNASSO	*535*	34.58	19.81	15.70	2.24	11.78	1.68	7.10	9.72	7.66	0.93	0.00