Structures des paléoforêts européennes de la fin du Cénozoïque : apport des interactions plante-insecte

Benjamin Adroit

THÈSE POUR OBTENIR LE GRADE DE DOCTEUR DE L'UNIVERSITÉ DE MONTPELLIER

En EERGP - Écologie, Evolution, Ressources Génétique, Paléobiologie

École doctorale GAIA - Biodiversité, Agriculture, Alimentation, Environnement, Terre, Eau

Unité de recherche Institut des Sciences de l'Evolution de Montpellier UMR 5554

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Présentée par Benjamin ADROIT

Mars 2018

Sous la direction de Jean-Frédéric TERRAL de l'Université de Montpellier et Torsten WAPPLER de l'Université de Bonn

Devant le jury composé de

Mme. Brigitte MEYER-BERTHAUD, Directrice de recherche, Université de Montpellier	Présidente du jury
M. Dieter UHL, apl. Professeur, Senckenberg Research Institute Frankfurt am Main	Rapporteur
M. Romain GARROUSTE, Ingénieur de recherche, MNHN à Sorbonne Universités	Rapporteur
Mme. Sylvie HURTREZ-BOUSSÈS, Professeure, Université de Montpellier	Examinatrice
M. Jes RUST, Professeur, Université de Bonn	Examinateur
M. Maximilian WEIGEND, Professeur, Université de Bonn	Membre du jury
M. Torsten WAPPLER, Maître de conférence, Université de Bonn	Membre du jury
M. Jean-Frédéric TERRAL, Professeur, Université de Montpellier	Membre du jury
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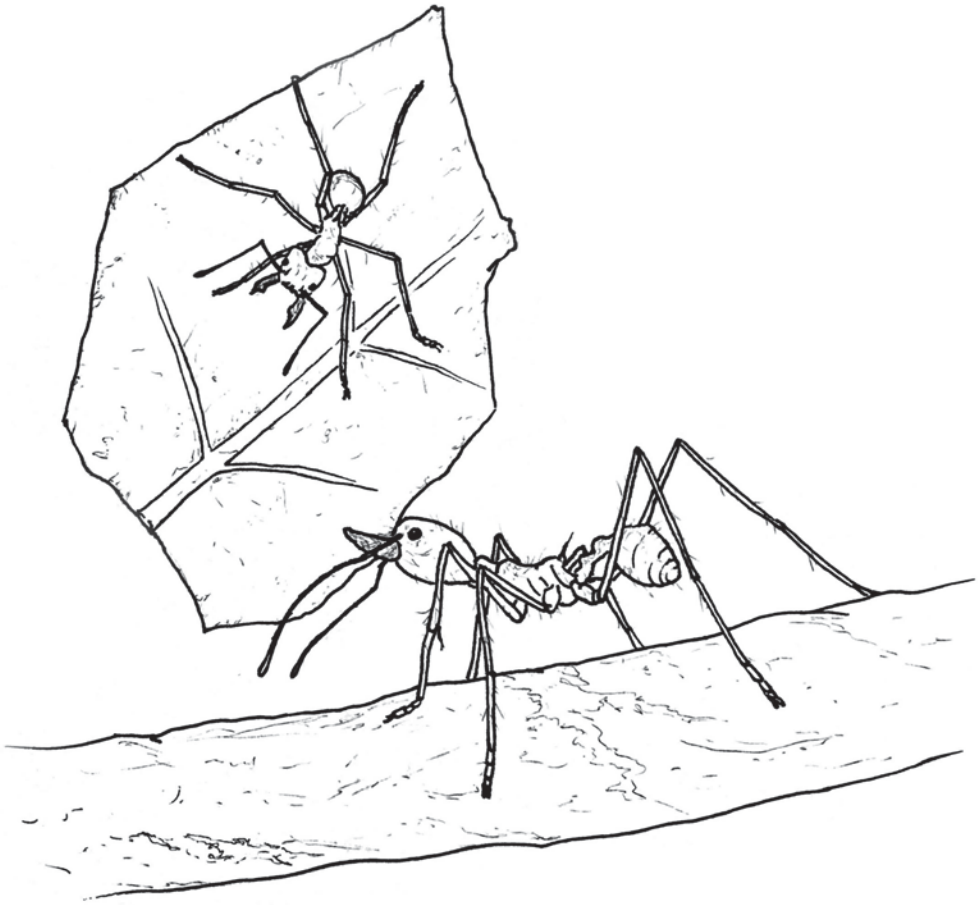
To my grand-father

Dr. Jacques Adroit



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PREFACE

This thesis is composed in three main chapters dealing with plant-insect interactions, each having been published or in publishing process. An additional chapter on specific insect damage, still in work for future publication, was also included. A general introduction of the thesis' topic and a final conclusion were also edited.

The first article has the purpose of discuss locally about plant-insect interactions and the potential relations with abiotic and biotic parameters. The outcrop of Bernasso in southern France was selected for this article. In addition, for the first time, the specific fossil preparation of certain fossil leaves from this outcrop were debate concerning their impact on insect damages identification.

In a second article, the famous Lagerstätte of Willershausen in Germany was studied through the plant-insect interactions. It has been compared to a similar other outcrop, near geographically, the outcrop of Berga in Germany. Results from the previous article were also added in this second article for further measurements. Those comparisons highlighted the importance of coolest month temperatures and climate seasonality as factors impacting the plant-insect interactions.

Finally, a last comparison of plant-insect interactions on a common plant species between fossil and modern record were produced in a third article. The modern Hyrcanian forest in Iran was selected as it is the closest analogue forest from the paleoforest studied in the previous articles. Accurate measurements in this article tend to supported the observations made in the previous articles. The presence of an unknown insect feeding on the modern leaves also observed in the fossil record was focus in a distinct article, still in preparation.

The plan of this thesis is done in such a way that each article includes the results of the previous ones. Some redundancies, especially in material and method, can be observed between chapters due to the structuration of the thesis in article format.

Chapter 2: Adroit, B., Wappler, T., Terral, JF., 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. 10.1016/j.palaeo.2016.01.015

Chapter 3: Adroit, B., Girard, V., Kunzmann, Lutz., Terral, JF., Wappler, T., *submitted* 2017, Plant-insect interactions patterns in three European paleoforests of the late-Neogene – early-Quaternary. PeerJ, 1-25.

Chapter 4: Adroit, B., Malekhosseini, M., Girard, V., Abedi, M., Rajaei, H., Terral, JF., Wappler, T., *submitted* 2017, Changes on herbivory pattern on the Persian ironwood (Parrotia persica) over the last 3 million years. Rev. Palaeobot. Palynol., 1-24.

Chapter 5: Adroit, B., Girard, V., Labandeira, C., Marsh, F., Terral, JF., Wappler, T., *in preparation*, A new damage type on fossil and modern leaves of *Parrotia persica*.

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ABSTRACT

"*If all mankind were to disappear, the world would regenerate back to the rich state of equilibrium that existed ten thousand years ago. If insects were to vanish, the environment would collapse into chaos.*"

Edward O. Wilson

Insects are the most diverse animals on Earth, and neatly associated with plants they represent two of the major groups of organisms both in species diversity and biomass quantity. The majority of their interactions involves insect feeding and insect parasitism (such as galls forming) mostly on leaves. Plant and insect compose one of the main trophic levels in ecosystems over the 325 million years. Today, the continuous and fast rising of temperature mostly due to human activities since the last century is disturbing the balance of ecosystems on Earth. This is named "global change". Consequently, to understand the role of plant and insect interactions, through time but also trophic networks, becomes crucial. It is in this general context that the aim of the thesis pursued.

The fossil record is an exceptional opportunity to survey responses of plant-insect interaction to climate variations over long time interval through traces of plants reactions caused by interaction with insects, as Earth has already experienced many climate changes. For the last 3 million years (Ma), oscillations between long cold periods, marked by the rifting of ice cap in Northern Hemisphere, and short warm periods have occurred. Europe ecosystems has been particularly impacted by these climatic variations. Therefore, thesis emphasized an interest on some fossil outcrops in Europe during this period.

The famous Lagerstätte of Willershausen (middle Germany) was specifically study. It is an exceptional fossil outcrop that contains around 200 angiosperm species for a total of ca. 8000 collected fossil leaves. These leaves testify a paleoforest developed there around 3 — 2.6 Ma ago in a climate warmer than today (mean temperature ca. +5°CUnder these conditions, many plant species typical of the Mediterranean ecosystems were settled there, such as Montpellier maple (*Acer monspessulanum* L.) or Olive tree (*Olea* sp.). For comparison, other paleoforests were studied: Berga (similar in age and geographically close to Willershausen) and Bernasso (younger than Willershausen (2.16 — 1.96 Ma) and located in southern France close to Mediterranean. These forests were compared as many common plant taxa were similar between each other. Furthermore, some species today endemic to the Caucasian region, such as Persian ironwood (*Parrotia persica* (DC.) C.A.Mey) or Caucasian elm (*Zelkova carpinifolia* (Pall.) Dippel), were also present in these outcrops. The aim of this study is to determine how far the

climate differences could be involved in the changes of plant-insect interactions in European paleoforests of the late Pliocene – early Pleistocene.

Results highlighted the impacts of both hydric and temperature seasonality, hitherto underestimated in the fossil record, on the patterns of plant-insect interaction in the European paleoforests. It appeared that ecosystems subject to intense hydric seasonality (i.e. important variations of precipitation during the year) could led to higher specialization of plant-insect interaction inferred by higher rate of observed damages du to 'specialists insects'. In parallel, the coolest temperature during the year seems to be a major factor in the low diversity of damage in paleoforest, presumably due to lower insect metabolism. Absence of convergent correlations between plant richness and damage richness could suggested that influence of climatic factors override impact of these local biotic factors.

In order to understand the whole parameters that could have an impact on plant-insect interactions (such as interspecific competition, soil components, taphonomic biases, etc.), our current knowledges are still insufficient to conclude about the relationships between climate and patterns of plant-insect interactions observed in these European paleoforests. It would be wise to make more investigations on modern forests with the methods as applied in fossil record community structure studies. These investigations could help to understand the factors potentially involved in the establishment of a pattern of plant-insect interactions. It is in this perspective that a part of this study was precisely focused on one plant species (*P. persica*) currently endemic to the Hyrcanian forest (Iran). This forest is supposed to be an analogue forest of the European paleoforests as those studied in this thesis. For now, observations made in Iran tend to corroborate our interpretation. However, the discovery of a new plant-insect interaction only found on leaves of *Parrotia persica* from both fossil and current forests suggests that historical factors could also have influenced plant-insect interactions observed in the different forests.

Finally, the studies on plant-insect interactions in past and extant ecosystems, combined with the study of climatic changes, should permit us to better characterize the relations between plants and insects in forests through time.

Keywords: Herbivory, Pliocene-Pleistocene, Hyrcanian forest, Fossil leaves, Insect Damage, Climatic Gradient

KURZFASSUNG

"*If all mankind were to disappear, the world would regenerate back to the rich state of equilibrium that existed ten thousand years ago. If insects were to vanish, the environment would collapse into chaos.*"

Edward O. Wilson

Insekten spielen im komplexen Netz von Wechselwirkungen in terrestrischen Ökosystemen eine Schlüsselrolle. Dort repräsentieren sie die Hauptgruppe von Organismen in Bezug auf spezifische Diversitäten und Biomassen. Ferner sind sie bedeutende Konsumenten von Pflanzenbestandteilen und dienen als Nahrung für größere Tiere oder parasitieren diese. In den letzten Jahren hat sich gezeigt, das vor allem die Interpretationen von Pflanzen-Insekten Interaktionen vergangener Ökosysteme von großer Bedeutung sind, da sie uns ein tieferes Verständnis für den Ursprung und die Dynamik der terrestrischer Biodiversitätsmuster erlaubt. Dies ist möglich vor allem möglich, da zwischen Pflanzen und Insekten seit über 325 Millionen Jahren wichtige koevolutive Beziehungen bestehen. Heute hat der schnelle und ständige Temperaturanstieg, der hauptsächlich durch menschliche Aktivitäten in den vergangenen Jahrhunderten verursacht wurde, das Gleichgewicht der Ökosysteme auf der Erde gestört. Daher ist es wichtig, die Rolle von Interaktionen zwischen Pflanzen und Insekten im Laufe der Zeit sowie bei Nahrungsketten zu verstehen, was ein Schwerpunktthema der vorliegenden Arbeit ist.

Hierbei bietet der Fossilienbericht die außergewöhnliche Gelegenheit, um Reaktionen der Pflanzen-Insekten-Interaktionen unter schwankenden klimatischen Bedingungen zu untersuchen, wissend, dass die Erde bereits vielen Klimaänderungen ausgesetzt war. Vor allem während der letzten drei Millionen Jahre (Ma) waren diese Schwankungen der Umweltbedingungen durch rasche und dramatische Wechsel des Klimas gekennzeichnet. Europäische Ökosysteme sind von diesen klimatischen Schwankungen besonders betroffen. Daher konzentrierte sich diese Arbeit auf bestimmte fossile Vorkommen in Europa während dieser Zeit.

Hier bietet die oberpliozäne Fossillagerstätte Willershausen wegen ihres Artenreichtums (ca. 200 Arten) und ihrer hervorragenden Erhaltungsqualität von Pflanzenfossilien (ca. 8000 Blattfossilien) und Fraßspuren günstigste Voraussetzungen für Untersuchungen zur Dynamik vergangener Ökosysteme und die Entwicklung ihrer Diversität, unter Bedingungen deutlich wärmer als heute (ca. + 5°C), aber mit einer ähnlichen Paläogeographie, weshalb es nicht

verwundert, das viele als Fossilien vorkommende Arten, heute im mediteranen Raum und dem Nahen Osten zu finden sind, wie z.B. der Französische Ahorn (*Acer monspessulanum* L.) oder der Olivenbaum (*Olea* sp.). Um Die Ergebnisse von Willershausen besser einordnen zu können, wurden zwei weitere Fundstellen vergleichbaren und jüngeren Alters untersucht: Berga (gleichaltrig und geographisch nahe Willershausen) und Bernasso (jünger als Willershausen (2.16 – 1.96 Ma) im Süden Frankreichs. Diese Vergleichs-Taphozönosen sind insbesondere wegen der vielen gemeinsamen Taxa sehr interessant, von denen einige heute z.B. in der Kaukasusregion endemisch sind, wie der Persischer Eisenholzbaum (*Parrotia persica* (DC.) C.A. Mey) oder die Kaukasische Ulme (*Zelkova carpinifolia* (Pall.) Dippel), vorkommen und somit allgemeine Aussagen über die Auswirkungen auf multitrophische Interaktionen für den Zeitraum vom Ende des Pliozäns bis in das frühe Pleistozän ermöglichen.

Die erzielten Ergebnisse ermöglichten es, die in den ausgeführten Fossilstudien bislang unterschätzten Auswirkungen der Saisonalität der Temperaturen und Niederschläge als Einflussfaktoren auf Pflanzen-Insekten-Interaktionen der neogenen europäischen Wälder hervorzuheben. Ökosysteme, die jedoch einer starken saisonbedingten Wasserabhängigkeit unterliegen, zeigen eine stärkere Spezialisierung in der Zusammensetzung ihrer Fraßspurmuster. Parallel dazu scheinen die kältesten Temperaturen während des Jahres ein wichtiger Faktor für die geringe Schädigungsvielfalt zu sein, wahrscheinlich aufgrund eines geringen Metabolismus der meisten Insekten. Hinweise auf eine enge Korrelation zwischen der Pflanzendiversität und der Artenzahl der tierischen Organismen besteht, konnten nicht bestätigt werden, was darauf hindeutet, das der Einfluss klimatischer Faktoren die potenziellen Auswirkungen lokaler biotischer Interaktionen überprägt. Für all diese Parameter, die Auswirkungen auf Pflanzen-Insekten-Interaktionen haben könnten (wie interspezifischen Wettbewerb, Bodennährstoffe, taphonomische Filtermechanismen, etc.), ist unser aktuelles Wissen immer noch unzureichend, um auf die Beziehungen zwischen Klima und Pflanzen-Insekten-Interaktionen, wie wir sie in europäischen Paläo-Wäldern beobachtet konnten, in allen Einzelheiten zu klären.

Zu diesem Zweck wurde eine Vergleichsstudie basierend auf Pflanzen-Insekten Interaktionen am Persischer Eisenholzbaum (*Parrotia persica*), einer heute endemisch im Hyrcanischen Wald, einem Gebirgswald in der Nähe der südlichen Küste des Kaspischen Meeres in Aserbaidschan und im Iran, vorkommenden Bauart durchgeführt, die auch fossil aus den Lagerstätten Willershausen und Bernasso bekannt ist. In beiden Fällen konnten vergleichsbare Fraßspuren nachgewiesen werden. Darunter auch eine Fraßspur, die bislang nur auf fossilen

Blättern aus Willershausen bekannt war. Dies mag verdeutlichen, das derartige Studien Aussagen über die Stabilität von Wirtspflanzen-Assoziationen über mehrere Millionen Jahre hinweg ermöglichen, aber auch in welchem Maße fossilen Pflanzen/Insekten Assoziation zum Verständnis klimatischer Veränderungen beitragen können, die wesentlich kompliziertere Ursachen haben können, als die einfache Grundannahme vermuten lässt.

Schlüsselwörter: Herbivorie, Pliozän-Pleistozän, Hyrcanischer Wald, Fossile Blätter, Insektenschaden, Klimatischer Gradient

RÉSUMÉ

"*If all mankind were to disappear, the world would regenerate back to the rich state of equilibrium that existed ten thousand years ago. If insects were to vanish, the environment would collapse into chaos."*

Edward O. Wilson

Les insectes sont les animaux les plus diversifiés de la Terre, associés aux plantes, ils représentent deux des principaux groupes d'organismes en termes de diversités spécifiques et de biomasses. La majorité de leurs interactions est alimentaire ou parasitaire (formation de galles) et touchent principalement des feuilles. Les plantes et les insectes forment l'un des principaux niveaux trophiques des écosystèmes au cours des 325 derniers millions d'années. Aujourd'hui, l'augmentation rapide et continuelle de la température principalement causée par l'activité humaine depuis les derniers siècles, perturbe la balance des écosystèmes sur Terre. Ceci est dénommé « changement global ». En conséquence, comprendre le rôle des interactions entre les plantes et les insectes, à travers le temps mais aussi les réseaux trophiques, est essentiel. C'est dans ce contexte général que s'inscrit cette thèse.

Le registre fossile est une opportunité exceptionnelle d'examiner les réponses aux interactions plante-insecte lors de longues variations climatiques et à travers des traces de réaction de la plante sachant que la Terre a déjà été soumise à de nombreux changements climatiques. Durant les derniers trois millions d'années (Ma), des oscillations entre de longues périodes froides, marquées par l'avancée sur les continents de la calotte glaciaire dans l'hémisphère nord et de courtes périodes chaudes ont eu lieu. Les écosystèmes Européens ont particulièrement été impactés par ces oscillations climatiques. Ainsi, cette thèse s'est intéressée à certains gisements fossiles d'Europe durant cette période.

Le célèbre Langerstätte (i.e. dépôt sédimentaire contenant une grande diversité de fossiles complets) de Willershausen (centre de l'Allemagne) a été particulièrement étudié. C'est un gisement fossile exceptionnel contenant environ 200 espèces d'angiospermes pour un total avoisinant les 8000 feuilles fossiles. Ces feuilles relatent d'une paléoforêt ayant existé il y a 3-2,6 Ma dans un climat plus chaud qu'aujourd'hui (environ +5°C). Dans ces conditions climatiques, de nombreuses espèces d'écosystèmes Méditerranéens étaient présentes, telles que l'Érable de Montpellier (*Acer monspessulanum* L.) ou l'Olivier (*Olea* sp.). En comparaison, d'autres paléoforêts ont été prise en compte : Berga (du même âge et proche de Willershausen) et Bernasso (plus jeune que Willershausen (2,16 — 1,96 Ma) localisée dans le sud de la France

près de la Méditerranée. Ces forêts sont comparables notamment du fait des nombreux taxons communs qu'elles partagent. En outre, certaines de ces espèces sont aujourd'hui endémiques de la région du Caucase, telles que le Parrotie de Perse (*Parrotia persica* (DC.) C.A.Mey) ou encore l'orme du Caucase (*Zelkova carpinifolia* (Pall.) Dippel). Le but de cette étude a été de déterminer en quoi les différences climatiques peuvent être impliquées dans les changements des interactions plante-insecte au sein des paléoforêts Européennes de la fin du Pliocène - début du Pléistocène.

Les résultats obtenus ont permis de mettre en évidence les impacts de la saisonnalité des températures et précipitations, jusqu'ici sous-estimées dans les études fossiles, comme facteurs impactants les interactions plante-insecte des paléoforêts Européennes. Il est apparu que les écosystèmes sujets à d'intenses saisonnalités hydriques ont pu engendrer une plus grande spécialisation des interactions plante-insecte déduite d'un fort taux d'interactions spécialistes observées. En parallèle, les températures les plus froides durant l'année semble être un facteur important dans la faible diversité de dégâts, probablement dû à un faible métabolisme de la majorité des insectes. L'absence de corrélation convergente entre la richesse des plantes et la richesse des interactions pourrait suggérer que l'influence des facteurs climatiques surpasse l'impact potentiel des interactions biotiques locales. Pour l'ensemble de ces paramètres qui ont pu avoir un impact sur les interactions plante-insecte (comme par exemple la compétition interspécifique, les nutriments du sol, les biais taphonomiques, etc.), nos connaissances actuelles sont encore insuffisantes pour clairement conclure. Il serait intéressant de focaliser davantage d'études sur les forêts modernes avec des méthodes appliquées dans le fossile. C'est dans cette intention qu'une partie de cette étude a précisément étudié une espèce de plante (*P. persica*) actuellement endémique de la forêt Hyrcanienne (Iran). Cette forêt est supposée être une forêt analogue des paléoforêts Européennes étudiées dans cette thèse. Pour le moment, les observations qui ont été faites en Iran semblent corroborer notre interprétation. Néanmoins, la découverte d'une nouvelle interaction plante-insecte uniquement retrouvée sur les feuilles fossiles et actuelles de *Parrotia persica* suggère que des facteurs historiques peuvent également avoir une influence sur les interactions plante-insecte observées dans deux forêts différentes.

Au final, les études sur les interactions plante-insecte des forêts anciennes et actuelles, combinés avec les études de changements climatiques, pourraient nous permettre de mieux caractériser les relations entre les insectes et les plantes au sein d'une forêt.

Mots-clés : Herbivore insect, Pliocène-Pleistocène, Forêt Hyrcanienne, Feuilles fossiles, Dégât d'insectes, Gradient Climatique

LIST OF ABBREVIATIONS

Ali: Aliabad-e Katul, Iran

Ast: Astara, Iran

BDC: Canada balsam (comes from French abbreviation “Baume du Canada”)

C/N ratio: Carbone/Nitrogen ratio

CLAMP: Climate Leaf Analysis Multivariate Program (Wolfe, 1993)

CMMT: Mean temperature of the coldest month

DT: Damage type (Labandeira et al., 2007)

$DT297_{cu}$: Damage type 297 on current leaves

$DT297_{foA}$: Damage type 297 on fossil leaves

$DT297_{foB}$: Enlarge version of damage type 297 on fossil leaves

FFG: Functional feeding group (Labandeira et al., 2007)

HS: Host specificity (Labandeira et al., 2007)

IRIMO: Iran meteorological organization

LMA: Leaf mass per area (Royer et al., 2007)

Ma: Million year

MAP: Mean annual precipitation

MAT: Mean annual temperature

Mol: Molla Kala, Iran

Pas: Pasand, Iran

PCA: Principal component analysis

Rud: Rudsar, Iran

WMMT: Mean temperature of the warmest month

LIST OF DEFINITIONS

Galling: The gall is a reaction induced by a larval arthropod and it products tissues rich in nutrient usually consummate by the insect (more details in Labandeira, 2002). Examples in Appendix D.

Herbivory: This is an interaction, a degradation most part of the time, from an animal (arthropod in the case of the thesis) with the alive leaf tissue.

Hole feeding: An external feeding generated by a larval or adult insect within the leaf blade and for which a part of the leaf is removed. Examples in Appendix D.

Margin feeding: An external feeding generated by a larval or adult insect whereby the margin of the leaf is removed. Examples in Appendix D.

Mining: Mine is a consumption of specific foliar tissue from a larval insect characterized by a serpentine trace often ended in enlarged cavity used for pupation (more details in Labandeira, 2002). Examples in Appendix D.

Piercing & sucking: This is performed by some insects which first pierce the leaf blade and second inject saliva into the plant tissue which decompose a part of the leaf tissue. Examples in Appendix D.

Plant-insect interaction: This is the initiative to decompose, to feed the leaf tissue; the interactions linked with pollination are excluded of this definition for this thesis.

Seasonality: It deals with temperature or precipitation and means the amplitude between minimal and maximal values of those climatic parameters during a year. For example, it is mentioned a high seasonality when the minimum and maximum temperatures are highly distant.

Skeletonization: An external feeding generated by a larval or adult insect which feed the leaf blade tissue without removed the leaf venation. Examples in Appendix D.

Surface feeding: The surface fluid-feeders remove a part of the plant tissue, on one side of the leaf blade, due to the presence of nutrients contents came from diverse origins such as extra-floral nectaries (more details in Labandeira, 2002). Examples in Appendix D.

CHAPTER I

General Introduction

Climate modifications are essential in the evolution of ecosystems. (Blois et al., 2013; Scholze et al., 2006). The current climatic changes have undeniable major effect on numerous living beings on the surface of the Earth (Tylianakis et al., 2008), especially since these variations are particularly faster than usual (Sala et al., 2000). Consequently, the main challenge of the 21st century is to understand climatic variations which have reached a tipping point, generating a "global change" (Heller and Zavaleta, 2009; Turner et al., 1990) and for which human activities undoubtedly had the most important impact (IPCC, 2007). In this context, plants and insects, which represent the largest taxa on the Earth and whence their food webs contribute at least to 75% of the terrestrial biodiversity (Price, 2002), are the clearly impacted (Bale et al., 2002). Nevertheless, the Earth's ecosystems have already been subject to climatic changes and warmer conditions than today, such as Paleogene which could reached 8°C warmer than today during some interval (Zachos et al., 2001). Lately, since the mid-Miocene the climate is globally cooling (e.g., for the last 14 Ma) marked, though, by climatic oscillations (Zachos et al., 2001). Most recently, at the late Pliocene the mean annual temperature (from the sea-surface) was around 3°C warmer than today at mid-latitudes (Dowsett et al., 2009; Fedorov et al., 2013) and up to 6°C warmer in higher latitude (Dowsett et al., 2009). There has been a transition in the world from a warm global climate towards a colder climate proceeding during the Piacenzian epoch (3.6—2.58 Ma) (Mudelsee and Raymo, 2005; Ravelo et al., 2004). Glacial amplitudes increased at the end of this time interval which resulted in substantial waning of Northern Hemisphere ice sheets, then characterizing the Pleistocene (Gibbard et al., 2010; Raymo et al., 2006; Zachos et al., 2001). The glacial/interglacial cycles consists of oscillations between long cold periods and short warm periods (also including periodical variations) in a time interval from 40,000 up to 100,000 years correlated to the Milankovitch cycles (Penck and Brückner, 1909). The glacial/interglacial cycles and their consequences are well-known in the Northern Hemisphere, especially in Europe through the analysis of macrofossils and pollen records (e.g., Mai, 1995; Suc and Popescu, 2005; Tallis, 1991). The climatic oscillations due to long glacial periods and short interglacial periods govern Earth's ecosystems for the last 2-3 Ma and many studies have shown possible impacts on certain ecosystems or plant species especially in Europe (e.g., Comes and Kadereit, 1998; DeChaine and Martin, 2006; Médail and Diadema, 2009). In the Northern Hemisphere the tree diversity considerably differs along the temperate forests, and today the diversity of European forests are the poorest compared to North American or Eastern Asian forests (Campbell, 1982; Grubb, 1987; Huntley, 1993; Latham and Ricklefs, 1993a). These differences are mainly due to various rates of speciation and extinction of plant species and constitute the main causal factor for the current tree diversity in Europe (Latham and

Ricklefs, 1993a, 1993b; Mai, 1995; Qian and Ricklefs, 2000; Ricklefs et al., 1999; Tallis, 1991; Wen et al., 2014), as they cannot be the result of the current climate (Latham and Ricklefs, 1993a; Ricklefs et al., 1999). In Europe, a marked decrease of atmospheric CO_2 concentration during the mid-Pliocene (Ravelo et al., 2004) leading to a reduction of mean annual temperature (MAT) of approximately 2 to 4°C (Thunell, 1979) started to cause notable changes in plant diversity (Médail and Diadema, 2009; Svenning, 2003). In the northwest of Europe, many subtropical terrestrial plant species, such as palms, completely disappeared at the end of the Branussumian (Van der Hammen et al., 1971). The Mediterranean coastal forests formerly dominated by conifers such as mainly cypresses (*Cupressus* L. sp.) vanished in favor of oaks and other plant species that were better adapted to a higher seasonal climate, marked especially by a relative dry summer season (Suc, 1984). Subsequently, after warmer climate conditions in Europe between 3 to 2.6 Ma (Thunell, 1979), a new cooling episode occurred at 2.58 Ma and marked the transition to the modern ice ages (Gibbard et al., 2010; Herbert et al., 2015). Thereafter, during the Praetiglian stage (2.4 to 2.3 Ma interval) the larger cycles were set up and providing the first appearance of really substantial ice sheets (Shackleton et al., 1984; Zimmerman, 1984). Consequently, in the Mediterranean region, forest decreased and more steppe vegetation, including weedy plants, have been expanded (Suc, 1984) simultaneously the subtropical Malayan elements disappeared in the northwestern Europe (Van der Hammen et al., 1971). Successively, many climatic cycles occurred but never exceed intensity from those of the beginning of the Gelasian (Raymo et al., 1989; Ruddiman et al., 1989). Finally, during the last 0.9 Ma, many larger cycles reached amplitudes considerably higher than the ones from previous cycles (Shackleton and Opdyke, 1976) and triggered rapid deglaciations (Broecker and Van Donk, 1970). These periods were marked by decreasing plant diversity including some extinctions of taxa (Lang, 1994; Tallis, 1991) and even removed certain endemic species (Leopold, 1967). The main explanation of all these major extinctions in Europe is due to the geographical conditions. The flora and fauna migrations during cold periods were very complicated because there are confined by the mountains along southern Europe and also by Mediterranean Sea and deserts in northern Africa (Huntley, 1993). In addition, the dry climate of central Asia during these same cold periods also prohibited this European flora migration (Reid and Reid, 1915). All these conditions were fatal for some warm-adapted plant species (Reid and Reid, 1915). However, some plant taxa survived in small refuges in Europe, mainly in southerly areas (Tallis, 1991; Tzedakis, 1993; Willis, 1996). Furthermore, the climatic oscillations also allowed establishment of some species in European regions, such as the introduction of the Myrtle (*Myrtus communis* L.) in northern Mediterranean 2 Ma ago (Médail

et al., 2012). A large proportion of plant taxa present in Europe during the Pliocene disappeared, others survived elsewhere in the Northern Hemisphere (Martinetto, 2001; Tallis, 1991; Van der Hammen et al., 1971). Many of them, more widely distributed, are surviving today in North America such as many species of *Carya* or in Asia such as numerous plant species of *Zelkova* (Lang, 1994). The impact of glacial cycles in the Hyrcanian forest in northern Iran has been lower for the flora, because the complex topography of the region, and today Hyrcanian forest is considered as a relictual forest of the former European Pliocene paleoforests (Leroy and Roiron, 1996; Noroozi et al., 2008; Probst, 1981; Tralau, 1963; Zohary, 1973). Many relict plant species from this period are nowadays endemic of the Hyrcanian forest such as *Zelkova carpinifolia* (Pall.) C. Koch, *Quercus castaneifolia* C. A. Mey or *Parrotia persica* C. A. Mey (Akhani et al., 2010; Maharramova et al., 2015) representing the potential natural vegetation in Central Europe during Pliocene times.

Nonetheless, despite all these crucial observations we still do not really have specific information about the structures of these ecosystems. An ecosystem is not only defined by the diversity of organisms but also by the interactions between organisms and those between organisms and their environment (Barbault, 1992). More than the species *stricto sensu*, ecosystems are defined by their structure organization driven by the climate, primary production and species heterogeneity (Dajoz, 2006) which is mostly impacted by temperature and relative humidity (Park, 1954). For example, the Mediterranean ecosystems are mostly characterized by the climate. The Mediterranean climate can be defined by a dry and hot summer (drought period or period of water deficit) (Bagnouls and Gaussen, 1957). The Mediterranean biome, presents in six main areas in the world, is one of the major biome classified by Köppen-Geiger (updated in Kottek et al., 2006) and constitute one of the major hotspots of biodiversity in the world. The structure of the biome is similar everywhere; specifically composed by four ecosystems (garrigue, pinewood, maquis, oakwood) (Quézel and Médail, 2003). Each one are composed by diverse plant species in the different regions but with similar ecosystem function (Cowling et al., 1996) such as evergreen oaks – *Quercus ilex* L. in the Mediterranean Basin and *Quercus wislizeni* A.DC. in California (Specht, 1969) – that represent ecological vicarious species (i.e. taxa occupying a similar ecological niche in geographically separate areas). This statement show how the structure of ecosystems and climate may be linked through similar biogeographical and speciation patterns. Therefore, it may be argued that climatic oscillations during the Plio-Pleistocene boundary could also affect the structures of the European ecosystems which in short-term also influenced the relation

between organisms. In this respect, plants and insect herbivores are essentials in terrestrial ecosystems and their interactions have a major part in structuring ecosystem (Schoonhoven et al., 2005). Hence there is a serious interest to understand variations of plant-insect interaction in response to climatic changes (Bale et al., 2002; Brooker, 2006; Labandeira and Currano, 2013). The good preservation of plant macrofossils (i.e. leaves) could provide great local observations of the past and could estimate what have been shaped ecosystems (Louys et al., 2012; Uhl et al., 2006; Utescher et al., 2000). Nowadays, some studies have referenced insect traces found on fossil leaves and indicate how climate variations during long geological time periods could modified plant-insect interaction (Currano et al., 2008; Knor et al., 2012; Wappler, 2010; Wilf et al., 2001; Wilf, 2008; Wilf and Labandeira, 1999). Thus, the aim of this manuscript is to understand how locally, relatively, and precisely the patterns of plant-insect interaction could differ according to external factors, in order to answer to the following question:

"What could be the causes of the differences of plant-insect interaction
in European forests for 3 Ma?"

It is suggested that climatic oscillations which have been impacted European ecosystems, mainly triggered abiotic factors (e.g., temperature, precipitation) but also some biotic factors, like plant species richness, and could have been significant impacts on the plant-insect interactions in those forests. For this purpose, we focused on three areas different on both geographical and temporal scales and on a current analogue of the European paleoforest from the Plio-Pleistocene interval. The study areas were selected to this end.

1.1 STUDIED AREAS

The three studied fossil outcrops are located in Germany (Willershausen and Berga located around Harz mountain (Willershausen: Knobloch (1998); Berga: Mai and Walther (1988)) and France (Bernasso located in Escandorgue massif; (Suc, 1978)) (Figure 1). Germany localities are today under continental climate and the French locality has a Mediterranean climate. German localities are dated from late Pliocene (Ferguson and Knobloch, 1998) and the French one from the early Pleistocene (Ildefonse et al., 1972). This configuration allowed us to observe ecosystems from before and after the Praetiglian stage (2.4 Ma), meaning the first major extent of ice since the beginning of Quaternary (Shackleton et al., 1984; Zimmerman, 1984). To compare the fossil outcrops, a current forest in Iran was studied because its status of relictual forest from the Neogene European ones. This forest includes many plant taxa existed also in

Europe during the late Neogene and the early Quaternary. It allowed comparison between fossil forests and a close analogous forest (e.g., Probst, 1981; Zohary, 1973).

Figure 1 Representation of the main spots studied for this thesis.

Red symbol means fossil location, green symbol means modern location. The different scales are present on each map. Photography of Bernasso outcrop was shoot by Aicha Ahmed in 2013. Photography of Willershausen outcrop was shoot by Benjamin Adroit in 2015. Photography of Pasand in Iran was shoot by Mehdi Abedi. Please note that no access to the Berga outcrop was permit during the thesis, consequently there is no personal photo presented. *Nota bene*: the complete and administrative name of Aliabad in Iran is Aliabad-e Katol.

1.1.1 Willershausen (Pliocene, -3.2 to -2.6 Ma)

The famous Lagerstätten Willershausen (3.2 – 2.6 Ma; MN 16/17; Hilgen, 1991; Mai, 1995) is an ancient clay mine, now inactive and classified as a natural monument in 1977. Today it is included in the Geopark Harz, Braunschweiger Land, Ostfalen (Germany). The outcrop is located in the village of the same name, in the western Harz Mountains (51.7839°N, 10.1133°E) (Figure 1). Fossils were discovered around 1920's and researches were conducted during many years by paleontologists from University of Göttingen. More than 50 000 fossils were found including vertebrates (fishes, turtles, amphibians (frogs and giant salamanders)), mammals (including the proboscis *Anancus (Mastodon) arvernensis*) arthropods (grasshoppers, crickets, bees, etc.) and a huge amount of plants material including the fossil leaves studied here

(www.willershausen-harz.de). The main works on the fossil leaves were done by Straus (1977) and Knobloch (1998). Willershausen was a paleoforest included at least 200 angiosperm species including typical taxa from European paleoforests such as *Zelkova, Carpinus, Acer, Parrotia, Carya, Quercus, Pterocarya* etc. These paleoforest grew under warmer climate than today (ca. +5°C than today). The paleoenvironment was globally warmer than today (c.a. +5°C than today) and recently, a study based on plant species and morphologies of the leaves estimated more precisely mean annual temperature (MAT), mean temperature of the coldest month (CMMT), mean temperature of the warmest month (WMMT) and mean annual precipitation (MAP) (Thiel et al., 2012; Uhl et al., 2007). An artistic illustration of Willershausen can be found in the Appendix A.

1.1.2 Berga (Pliocene, -3.2 to 2.6 Ma)

Berga (3.2 – 2.6 Ma; Mai and Walther, 1988) is an ancient clay pit close the village of the same name in Saxony-Anhalt (Germany). It is located at the edge of the village (Figure 1), 60km in the east of Willershausen (51.4604°N, 10.9817°E). This is a smaller outcrop with approximately 600 fossil leaves including 160 taxa, described by Mai and Walther (1988). Almost all the taxa in Berga paleoforest are similar to the ones from Willershausen and the major ones are *Fagus attenuata* Goeppert., *Alnus gaudinii (Heer)* Knobloch & Kvaček*, Acer integerrimum* Viviani. The forests grew under cooler temperature than the Willershausen, but the climate was still warmer than today (Thiel et al., 2012; Uhl et al., 2007).

1.1.3 Bernasso (Pleistocene, -2.16 to -1.96 Ma)

Bernasso (2.16 – 1.96 Ma; Hilgen, 1991) is located in Hérault (southern France) close to the city of Lunas (43.7261°N, 3.2569°E) (Figure 1). The outcrops include both fossil leaves and pollen material (Leroy and Roiron, 1996; Suc, 1978). The fossil leaf collection contains approximatively 800 specimens with about 20 taxa (Leroy and Roiron, 1996). Majority of angiosperms from Bernasso were also found in Willershausen. The main angiosperm taxa are *Carpinus orientalis* Mill., *Parrotia persica*, *Zelkova ungeri* Spach. The first climatic analysis estimated warmer and wetter condition than today (Leroy and Roiron, 1996). Recently, re-estimation of Bernasso climatic conditions confirmed the high amount of precipitations, but suggested lower temperatures (Girard et al., in review).

1.1.4 Iran (Holocene, today)

The Hyrcanian forest is located in the northern Iran and covered about 50 000 km^2 (Akhani et al., 2010). It expands from the Golestan National Park to the border with the Azerbaijan and is contained between the Alborz mountain to the south and the Caspian Sea to the north (Miller, 1994) (Figure 1). The extinct taxa from Europe such as *Parrotia* or *Pterocarya* have some plant species retained only in this forest (Akhani et al., 2010; Maharramova et al., 2015). Besides, figure 2 presents the actual distribution of those extinct plant taxa in Europe paleoecosystems. The mean annual temperatures in the Hyrcanian region are around 16°C (Akhani et al., 2010). The climate in this region seems to be globally warmer than the paleoclimate from Willershausen, Berga and Bernasso. The mean annual precipitations are really heterogeneous from west to east, from ca. 1600 mm to 500 mm.

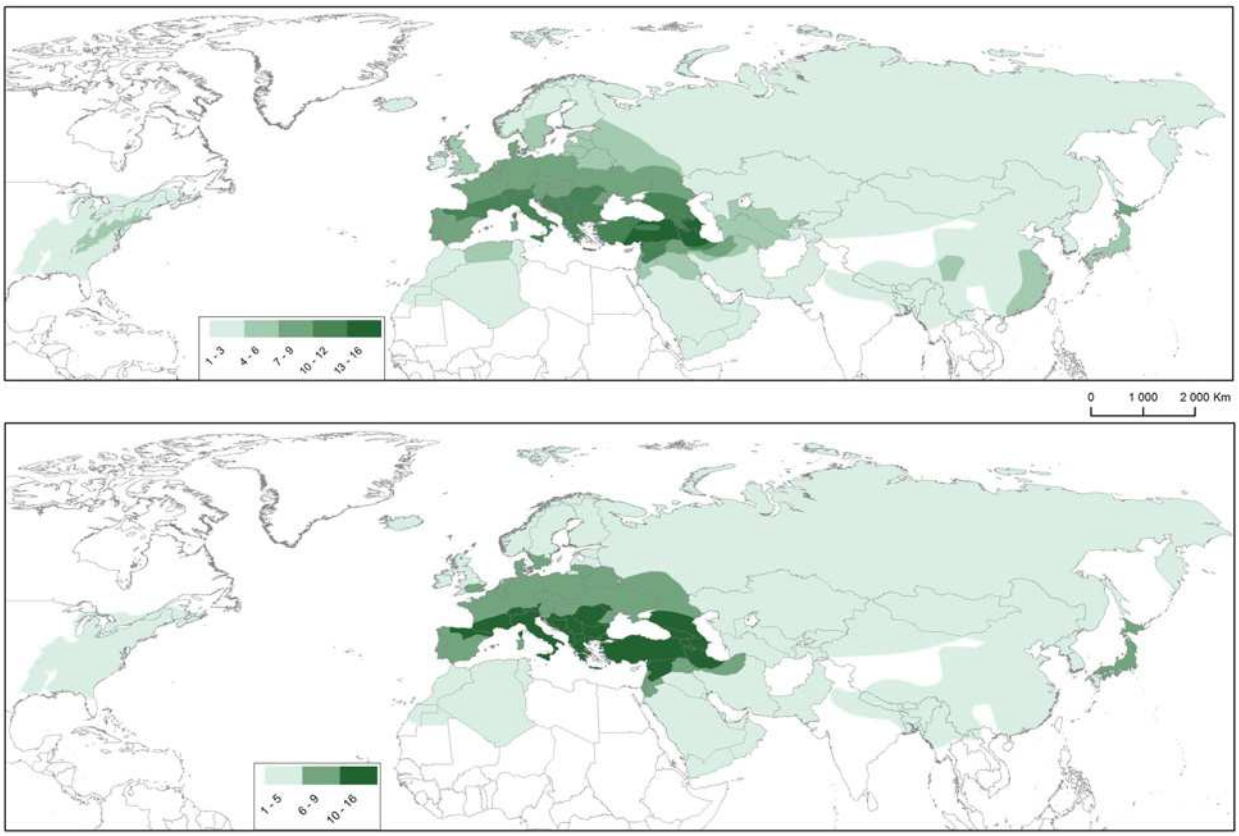

Figure 2: Actual distribution of plant taxa found in Europe there are 3 Ma, based on the statistical plant fossil recorded in Willershausen.

Mapping has been realized by Laure Paradis (ISEM, Montpellier) according to the datasets included in Coombes and Debreczy (2015). As suggested in the thesis and in many different studies, the Caucasian region, especially the Hyrcanian forest in Iran/Azerbaijan, includes the larger quantity of relictual plant taxa presented in Europe during the Pliocene (Arpe and Leroy, 2007; Noroozi et al., 2008; Probst, 1981; Tralau, 1963; Zohary, 1973). The map to the top has subdivided the superposed area distribution of plant taxa in five distinct groups, the map to the bottom has subdivided in 3 groups.

1.2 PLANT-INSECT INTERACTIONS

The species diversity and composition of plant communities may vary across different biogeographic zones, which correspond to different abiotic and biotic parameters (Gurevitch and Fox, 2006). Consequently, the structure of the forests based on plant-insect interactions, *inter alia*, also change (Agrawal et al., 2006). There are two kinds of these interactions.

The entomophile pollination, which is the relationship between insect and plants that that we could qualify as mutualisms (Bronstein et al., 2006). The concept is generally the fecundation of plants by means of insects, in exchange insect mostly feed flowers nectars (or other components in some cases) during the process (Faegri and Pijl, 2013; Gurevitch and Fox, 2006). These mutualistic interactions between plant and insect species has been molded by millions of years of coevolution and already have an accurate attention to the scientific community (Bronstein, 1994; Wappler et al., 2015).

Herbivory (or phytophagy) is the other plant-insect interaction. This is mostly binding for the plant, because the insects collect energy from the plants which lose this energy (Gurr et al., 2003; Singer et al., 2004). Unlike pollination, herbivory can provide a unique record of the plant-insect interactions in the past as their traces on leaf blade are observable on the plants (Labandeira, 2006, 2013). Up to now, arthropods/insects and plants have been often considered separately, this work is a rare opportunity to develop researches on biotic interactions through insect feeding traces.

1.2.1 Relationships and correlations between plant-insect interaction and environmental factors

Fossil leaves are known to be potential indicators of past climates (Wilf, 1997). Insect feeding observable on fossil leaves could be an important source of information (Labandeira, 2006). Thus, relation between abiotic factors (such as climate) and ecosystems structure could be partly estimated by investigations on plant-insect interaction (e.g., Labandeira, 2002; Lewinsohn and Roslin, 2008). Researches highlighted some possible association between climatic parameters and plant-insect interactions.

Fernandes and Price (1992) showed a negative correlation between amount of precipitations and galling as the fungi (more efficient in wetter conditions in most cases) are the main organisms responsible of gall-makers insects death (Carroll, 1988). On the contrary, leaf mining seems to be better adapted in wetter environments (e.g., Yarnes and Boecklen, 2005). The impacts of the temperature are diverse on plant-insect interaction. However, variation of temperature depends on many factors (such as CO_2 atmospheric concentration),

then the impacts of temperature on the plant-insect interactions are still unclear. Generally, warmer temperatures allow an increase in insect development and reproduction rate (Ayres and Lombardero, 2000). Moreover, Virtanen and Neuvonen (1999) measured on Macrolepidoptera in Finland that warmer temperatures lead to an increasing of insect species diversity. Consequently, it expected to observe higher insect feeding consumption on leaves (e.g., Lemoine et al., 2014; O'Connor et al., 2011; Zvereva and Kozlov, 2006). Furthermore, insect feeding is mostly related to different plant defense strategies based on chemical components that many abiotic factors such as temperatures and atmospheric CO_2 concentration could disturb (e.g., Zavala et al., 2008). Indeed, studies have shown that elevated CO_2 concentrations tend to decrease jasmonic concentration (plant defense hormone) on plants, then increasing exposure to insect feeding (in: DeLucia et al., 2012). On the contrary, the high CO_2 concentration could also increase salicylic acid concentration potentially allowing greater defense against herbivores (in: DeLucia et al., 2012). Moreover, a combination of CO_2 elevation with temperature elevation lead to decrease the nitrogen (N) concentration in the leaves (Bezemer and Jones, 1998; Stiling and Cornelissen, 2007). That is resulting a less palatability of the leaf (Stiling and Cornelissen, 2007) and causing insects to feed on more foliage to compensate this diminution of nutritional resources (Bezemer and Jones, 1998). Thus, consequences of high temperaturse combined with elevated atmospheric $C0_2$ concentration on the plant-insect interaction is hardly predictable. Furthermore, the high specialization of some herbivorous insects on plants reveals long time persistence of some of plant-insect interactions (Labandeira, 2006, 2002; Wilf, 2008). It could potentially disturb some interpretations in the fossil record between plant-insect interaction and abiotic factors such as climate. Investigations on paleontological studies could provide better indications on relations between insect feeding traces and climate (e.g., Currano et al., 2008; Wappler et al., 2009; Wilf et al., 2001; Wilf and Labandeira, 1999). For example, a study showed during the Paleocene-Eocene Thermal Maximum a positive correlation between herbivory and the combination of both high temperature and atmospheric CO_2 concentration (Currano et al., 2008, 2016). However, most studies on the changes of plant-insect interaction according to the climate have not paid attention to how organisms could acclimatize on temperature and CO_2 changes (Shaw and Etterson, 2012).

Furthermore, it is important to note that plant-insect interactions could also be related to some biotic factors. A high diversity of plants should involve a higher diversity of plant-insect interaction (e.g., Price, 2002). Fernandes and Price (1988) suggested that the richness of gall-making insect is often positively correlated with the plant species richness, a correlation

later detected in South Africa by Wright and Samways (1998). It has also been shown that a richness of gall-inducing insect species correlates to the forest age and health (Fernandes et al., 2010).

1.2.2 Reconstruction of the plant-insect interactions in the fossil record

In order to identify, compare and discuss difference of plant-insect interactions, it is indispensable to adopt a standardized approach, regardless of the locality studied. The preservation of an insect trace represents one behavior of an insect (Genise, 2017). It reflects how insect interacted with its environment that can correspond, for example, to traces of movement, of reproduction and feeding (Genise, 2017). In the fossil record, these traces, and especially the insect feeding traces, can be preserved and observable on the fossil leaves (Genise, 2017). These plant-insect interactions can provide the sole evidence for the paleoecological interpretation or they can be used to supplement other fossil resources (such as pollen, wood charcoal, etc.). Nevertheless, until the late twentieth century there was no formal icnotaxonomic system to designate traces of insects on fossil leaves. Previously, some authors named taxonomically certain traces but these were not grouped into a system of hierarchical structures (Amerom, van H.W.J., 1966; Friĉ, 1901; Kinzelbach, 1970; Straus, 1977).
Nowadays, three classification systems exist to label fossil traces with different approaches, and even classified under different systems.

The first was established by Vialov (1975). He decided to include all the traces that showed the consumption of plant matter made by insects in the group Phagophytichnidea, which in turn was further divided into two subgroups Phagophytichnida (with three families) and Phagophylignichnida, to differentiate those damages produced in leaves of those produced in fossil woods.

Another method was proposed by Krassilov and Rasnitsyn (2008), which includes 72 icnoespecies that include oviposition, galls, mines, buds and exceptional examples of non-marginal excisions.

The last, and most common method, corresponds to an informal classification system proposed by Labandeira (2002), that has been enhanced later (Labandeira et al., 2007). In this case, the classification is based on the concept of a guild (in the sense of a functional grouping) and has proved to be a valuable tool in ecology (e.g.,Simberloff and Dayan, 1991). In this classification, there are categories that group the traces according to the feeding behavior of the insect (Labandeira et al., 2007). These damage types (DT) can be classified into seven functional feeding groups: hole feeding (HF), margin feeding (MF), skeletonization (S), surface

feeding (SF), galling (G), mining (M), and piercing-and-sucking (PS). All of these interactions are considered to be herbivory because they use plant tissue as a ressource (Labandeira et al., 2007). The DTs can also be classified as most likely to be specialized (made by monophagous and/or olyphagous insects that consume only one or a few closely related plant species) or generalized (typically made by polyphagous insects, e.g., most of external damages such as HF or MF) (Labandeira et al., 2007). Specialized feeding is delineated by evidence from extant analogue feeders, morphologically stereotyped damage patterns, and restricted occurrences confined to particular host-plant species or tissue types in either fossil or extant host taxa (Labandeira, 2002). Moreover, today 70% of insect damage results from monophagous or oliphagous insect (Bernays and Chapman, 1994) that making their consideration particularly important. This system also allows to make the distinction in the dataset between the damage richness (i.e. the number of different DT represented in a dataset) and the damage frequency (i.e. the relative quantity of DT per dataset). This system, because of its completeness and of its simplicity, was used in the complete study presented in this manuscript.

1.3 AIM OF THE THESIS

The general purpose of this thesis is to better understand differences in plant-insect interactions in three past environments. This study allows for a better understanding of the insect damage patterns observable in paleoenvironments. For this goal, the study focuses on European paleoenvironments from the Late Pliocene and the Early Pleistocene. It allows accurate comparisons with the plant-insect interactions knowledges on both current and fossil record. This thesis is built according to three geographical areas which were linked to three different time period observations.

"Chapter 2" (Adroit et al., 2016) is focusing in Bernasso (southern France). Patterns of interactions in this outcrop was correlated to climatic conditions inferred for plants (Leroy and Roiron, 1996; Girard et al., in review). The results provide first analyses of plant-insect interaction pattern for the early Pleistocene. In parallel, a part of this chapter debates on different fossil leaves preparations and the consequences of them.

"Chapter 3" (Adroit et al., submitted a) focuses on relative interpretations of plant-insect interactions in the outcrops of Willershausen and Berga, both from the Late Pliocene of Germany). Results on Bernasso (Chapter 2) were also used for comparison. These outcomes better highlight interpretations of abiotic parameters and taphonomic bias are also discussed.

"Chapter 4" (Adroit et al., submitted) focuses on the analyze of plant-insect interactions on modern and fossil leaves of *Parrotia persica*. Modern leaves of *P. persica* were collected in the Hyrcanian forest (northern Iran). Results from this study allow the comparison of interaction pattern on *Parrotia* leaves under different current and past climatic conditions. Additionally, a complementary study ("Chapter 5") about the discovery of a new DT exclusively found on *P. persica* in the Willershausen paleoforest and the Hyrcanian forest is in progress (Adroit et al., in prep).

This thesis ends by a Chapter 6 that try to mixed the information obtained in the 3 first chapters and to answer to the thesis problematic.

CHAPTER II

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

Published

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2.1 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.

2.2 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.

2.3 MATERIAL & METHODS

2.3.1 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 paleoflora 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).

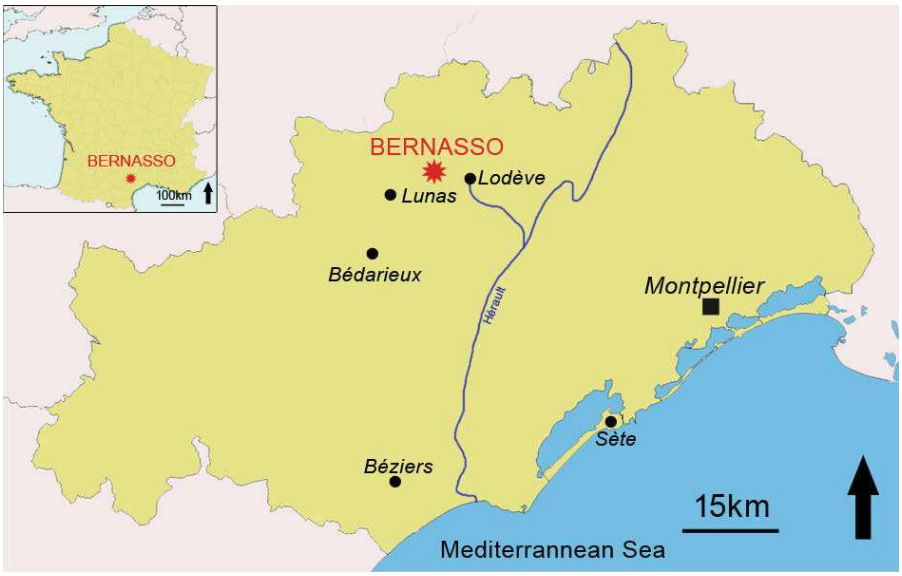

Figure 1: Map of the Hérault region showing the fossil locality of Bernasso (simplified from Leroy and Roiron, 1996).

2.3.2 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).

2.3.3 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 (Appendix B).

2.3.4 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.

2.4 RESULTS

2.4.1 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 ($p < 2.2e\text{-}16$) (Figure 4) and

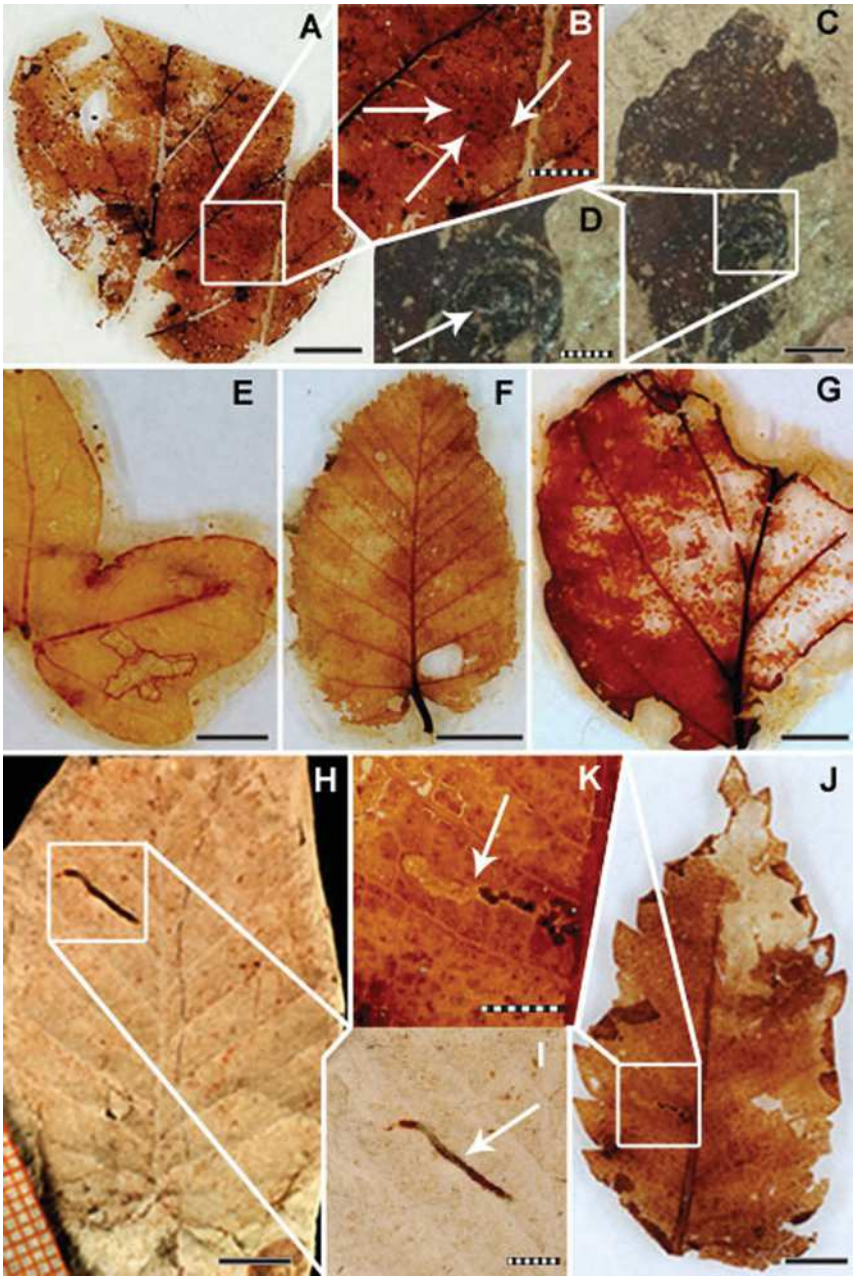

Figure 2: Representative insect damages on Bernasso leaves. A- Galling (DT32) on *Parrotia persica* preserved by Canada balsam method (BDC fossil); enlarged in B, DT32 are the small black dot, plenty on this leaf. C- Galling on *Parrotia persica* preserved by (Ro fossil); enlarged in D. E- Skeletonization (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.

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).

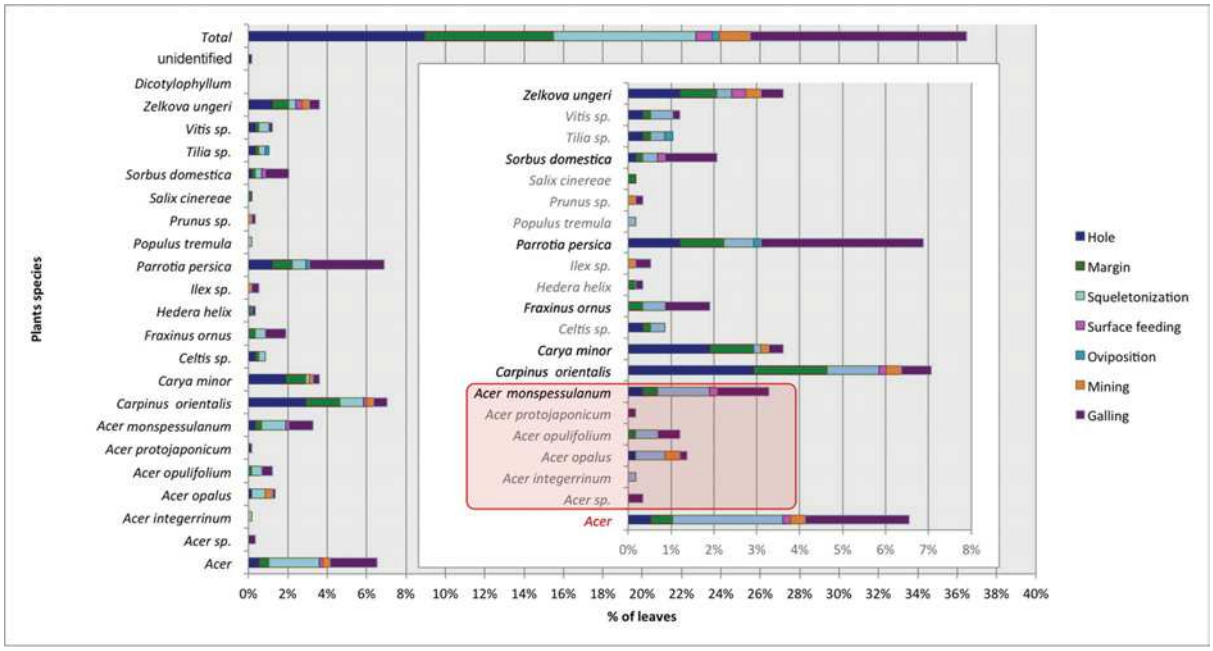

Figure 4: Interactions distribution (FFG) according Bernasso plant species. Box on the right is a highlight of the big one behind. Species in bold are the main 7 species with more than 20 specimens. Due to the diversity of maples, the genus Acer was individualized.

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 paleoflora (Figure

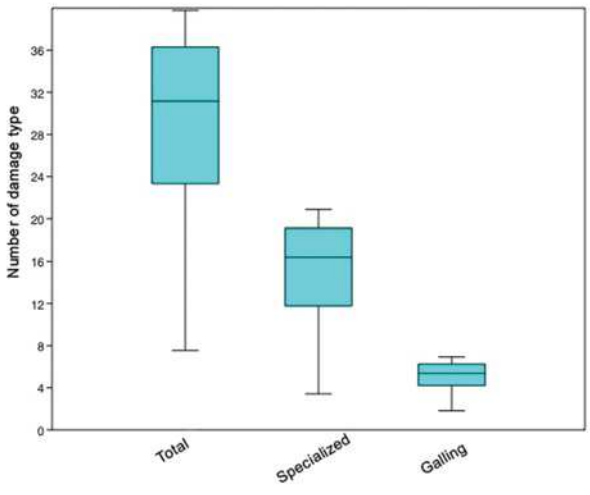

Figure 3: Mean diversity of all damage types, specialized damage types and galling damages on Bernasso floras. The box-plots are based on the rarefaction data for each group of interactions considered for the database.

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.

2.4.2 Conservation bias

First of all, there are statistically more fossil leaves within the Ro (54%) than the BDC (46%) dataset (Chi-squared test: $p = 0.03$; $\alpha = 1\%$; $ddl = 1$).

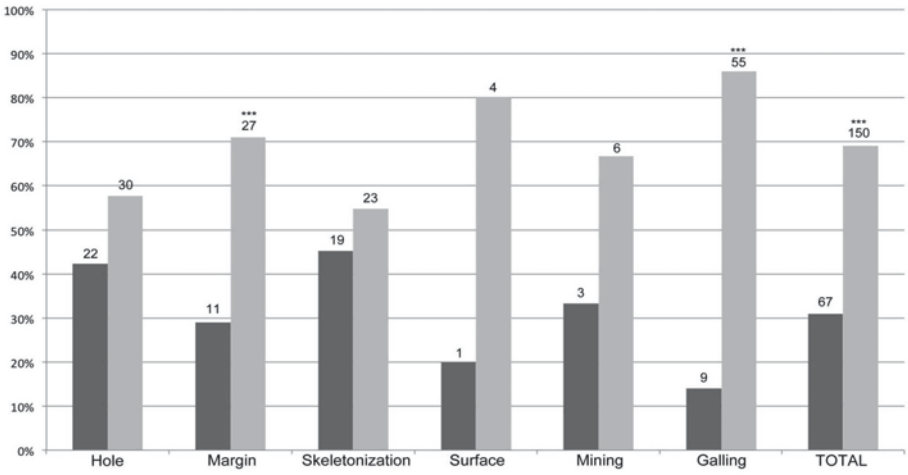

Figure 5: Distribution of FFGs according to the fossil preparation. The grey bars represent the fossils preserved with Canada balsam method; the black bars represent the fossils preserved on the rock.
The numbers are the exact quantity of damages. Chi-squared test enables to see the significance of the difference between Ro and BDC per FFG.
: alpha =0.1%. Lack of "" means there is no significant difference.

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 $p = 0.26$; $\alpha = 0.1\%$; $ddl = 1$).

2.5 DISCUSSION

2.5.1 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.

2.5.2 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*).

2.5.3 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 synthetize 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 (Appendix B). 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

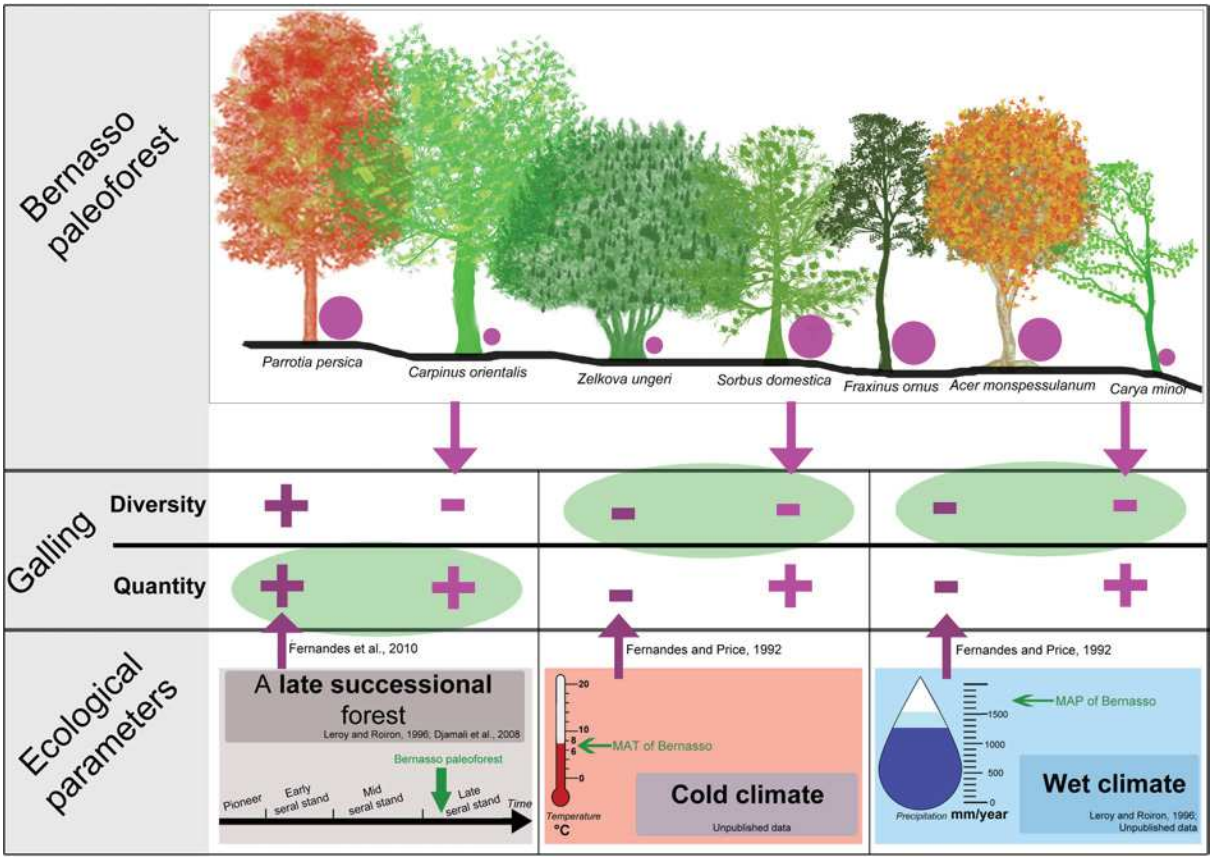

Figure 6: Overview of Bernasso forest. The different trees represent the 7 main species with more than 20 specimens. The purple circles near each tree represent the galling. The 2 different sizes of the circles indicate that there are more than 20% of leaves with galling for the bigger size, and less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

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).

2.6 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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CHAPTER III

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

Submitted

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3.1 ABSTRACT

Since hundred millions of years the interactions between plants and insect are essential in terrestrial ecosystems, as they composed one of the main trophic level in forest communities. Plant-insect interactions are correlated with many different environmental factors (such as the diversity of plants or climatic parameters) but these relations are still poorly understood, especially since human activities may have disturbed these relationships. This study put forward the first comparison between three European fossil leaves assemblages from late Cenozoic (Willershausen, Berga) and early Quaternary (Bernasso). The plant-insect interactions were estimated through insect activity traces identified on the leaf blade. Half of the leaves showed some kind of interaction in Willershausen (Germany) while 25% and 35% of the leaves were affected by insects in Berga (Germany) and Bernasso (35%) respectively. Proportions of specialized damages are significantly higher in Bernasso, which may suggest a more important thermal and hydric seasonality than in Willershausen and Berga. Convergent results reveal that temperature and precipitation seasonality seem to have a larger influence on plant-insect interaction difference than the annual average of these climatic parameters as it traditionally discussed. This is the first time in fossil record that seasonality is highlighted to be a potential factor impacting the plant-insect interaction.

3.2 INTRODUCTION

Climate is a major factor affecting the extension, structure, and composition of terrestrial ecosystems (Frank et al., 2015; Taylor et al., 2012). Hence, past climatic oscillations are of special importance for understanding and interpreting biotic changes in the past (e.g., DeChaine and 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 (Heller and Zavaleta, 2009; Turner et al., 1990) while these impacts on terrestrial biotas are still poorly understood (Knight and Harrison, 2012). Nevertheless, previous studies on fossil insect herbivory have provided a variety of ecological and evolutionary information, such as climate (e.g., Wappler, 2010; Wappler et al., 2012), the evolutionary impact of plant radiations (e.g., Labandeira, 2012; Labandeira and Currano, 2013), food web dynamics (e.g., Wappler and Grímsson, 2016), extinction patterns (e.g., Donovan et al., 2016; Labandeira, 2002; Labandeira et al., 2002), and ecosystem recovery after extinction events (e.g., Labandeira et al., 2016; Wappler et al., 2009). They have also shown that biodiversity loss may greatly impede trophic interactions and change the overall food web structure of ecological systems (e.g., Haddad et al., 2009). Moreover, there is increasing concern about the loss of biological diversity from ecosystems (Hooper et al., 2012). The Plio–Pleistocene fossil record offers an exceptional possibility for conducting a 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 (e.g., Magri, 2010; Médail and Diadema, 2009; Migliore et al., 2012; Postigo Mijarra et al., 2009; Tzedakis et al., 2006). Although a few isolated records of specialized phytophagy categories have been reported from the Pliocene (e.g., Givulescu, 1984; Straus, 1977; 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 and Knobloch, 1998; Knobloch, 1998) such as *Acer, Aesculus, Carpinus, Fagus,*

Quercus, Sassafras, Tilia (Knobloch, 1998; Mai, 1995) and other taxa such as *Parrotia, Zelkova* and *Liquidambar* were also characteristic elements of Willershausen (Mai, 1995). All of these taxa were also find in Berga (Mai and Walther, 1988). The presence of these taxa indicates relatively warmer conditions in Europe than today during the late Pliocene (Thiel et al., 2012; Uhl et al., 2007). Most plant fossil evidence from Central Europe outcrops (e.g., Haywood et al., 2000; Thiel et al., 2012; Uhl et al., 2007; Williams et al., 2009) and data from marine isotopes, geological evidences (Driscoll and Haug, 1998; Haug et al., 2004) are directing in the same way. The decreasing temperatures throughout the Pliocene (Ravelo et al., 2004; Thunell, 1979) 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).

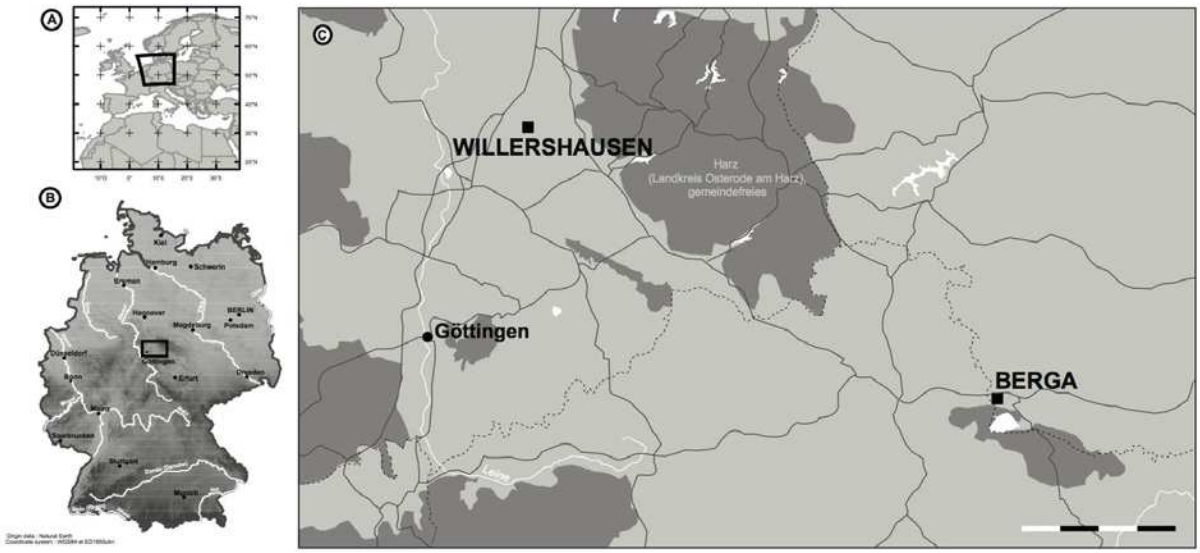

Figure 1: The location of Willershausen and Berga outcrops.
A- The location of Germany in Europe. B- The locations of both outcrops in Germany. C- Zoom on the area near Göttingen. On the scale, each dash (black or white) represents 5km. The data from the maps A and B come from Natural Earth database (http://www.naturalearthdata.com)

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 insect interactions of fossil leaves. It has been expected to observe different patterns of plant-insect interactions according to the climatic conditions in each fossil leaf assemblage. Variations of insect herbivory between the three fossil plant communities were statistically correlated plant diversity and climatic parameters to highlight similarities and differences between the 3 contexts. Our study will present a baseline data on the categories of arthropod interactions among plant species at the Pliocene-Pleistocene transition. This will provide a basis

for quantitative analyses of individual biotas and improved reconstructions of Plio-/Pleistocene trophic webs in the future.

3.3 STUDIED AREA

3.3.1 Willershausen, Lower-Saxony, Germany

Geological studies of Willershausen dated back to the end of the XIX century (e.g., Wegele, 1914; see details in Ferguson and 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 pond due to the dissolution of underlying Permian evaporites and tumbled of the Triassic and Early Jurassic sediments (Briggs et al., 1998; Kolibáč et al., 2016; Meischner, 2000). Today, Willershausen is an abandoned clay mining 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 and Knobloch (1998) and 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.Y. 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* (Ferguson and Knobloch, 1998; Knobloch, 1998; Straus, 1977). 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 corroborate by the presence of *Parrotia persica* and *Liquidambar europaeum* (Mai, 1995).

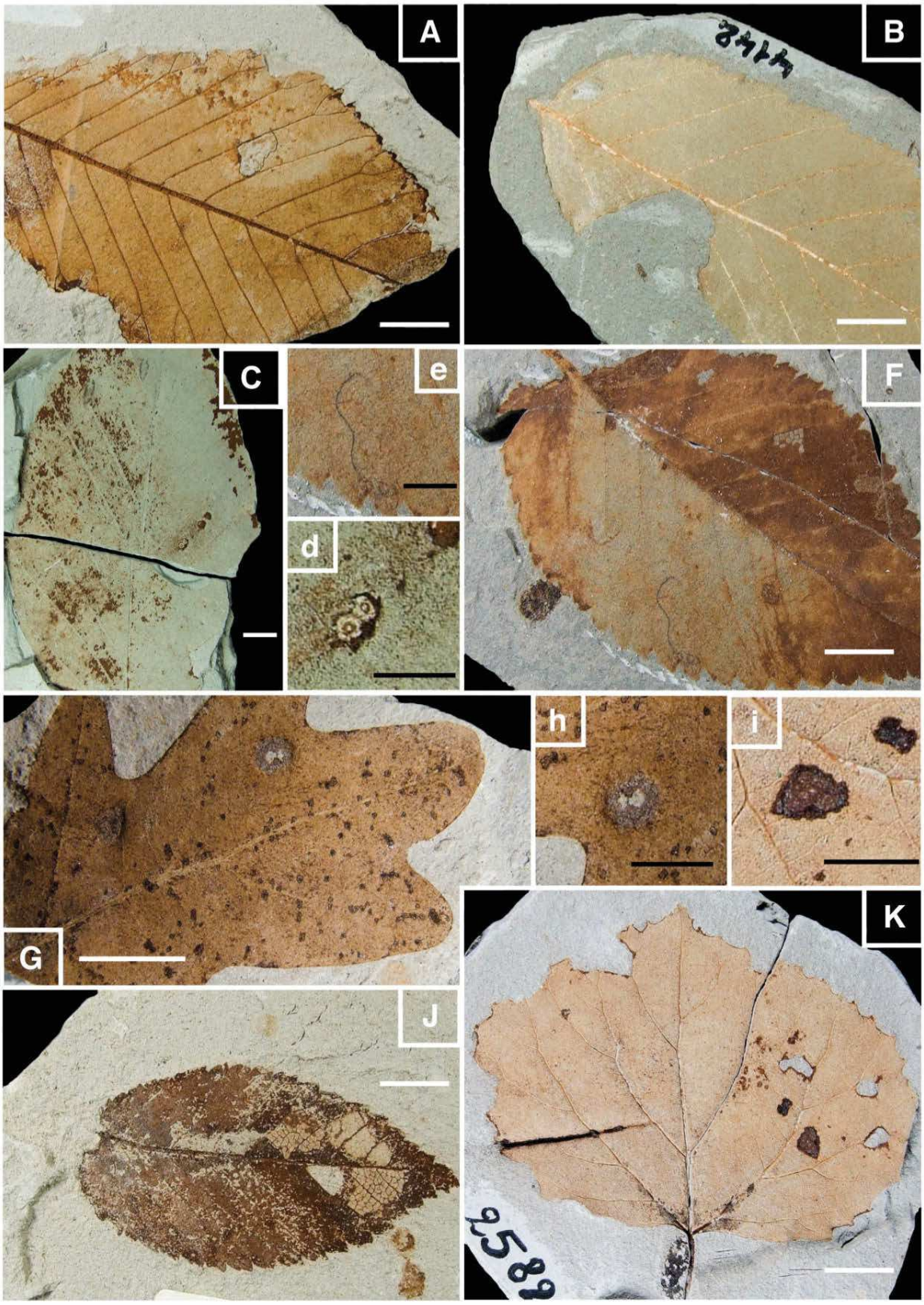

Figure 2: Plate Y. Fossil leaves from Willershausen (Göttingen coll.).

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'.

Most plant fossil evidence from Willershausen (*vide infra*) indicates warmer conditions than today (Ferguson and 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 (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 (for more details, see Thiel et al., 2012; Uhl et al., 2007).

3.3.2 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 and 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 and Walther, 1988), including 30 angiosperms leaf taxa (Figure 2.Z). They represent different environments: a freshwater plant community, a swamp and riparian associations and a to zonal mixed broadleaved conifer forest (which dominates the taphocoenosis). The temperatures were estimated with the same approaches than Willershausen; MAT is comprised 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 (Thiel et al., 2012; Uhl et al., 2007).

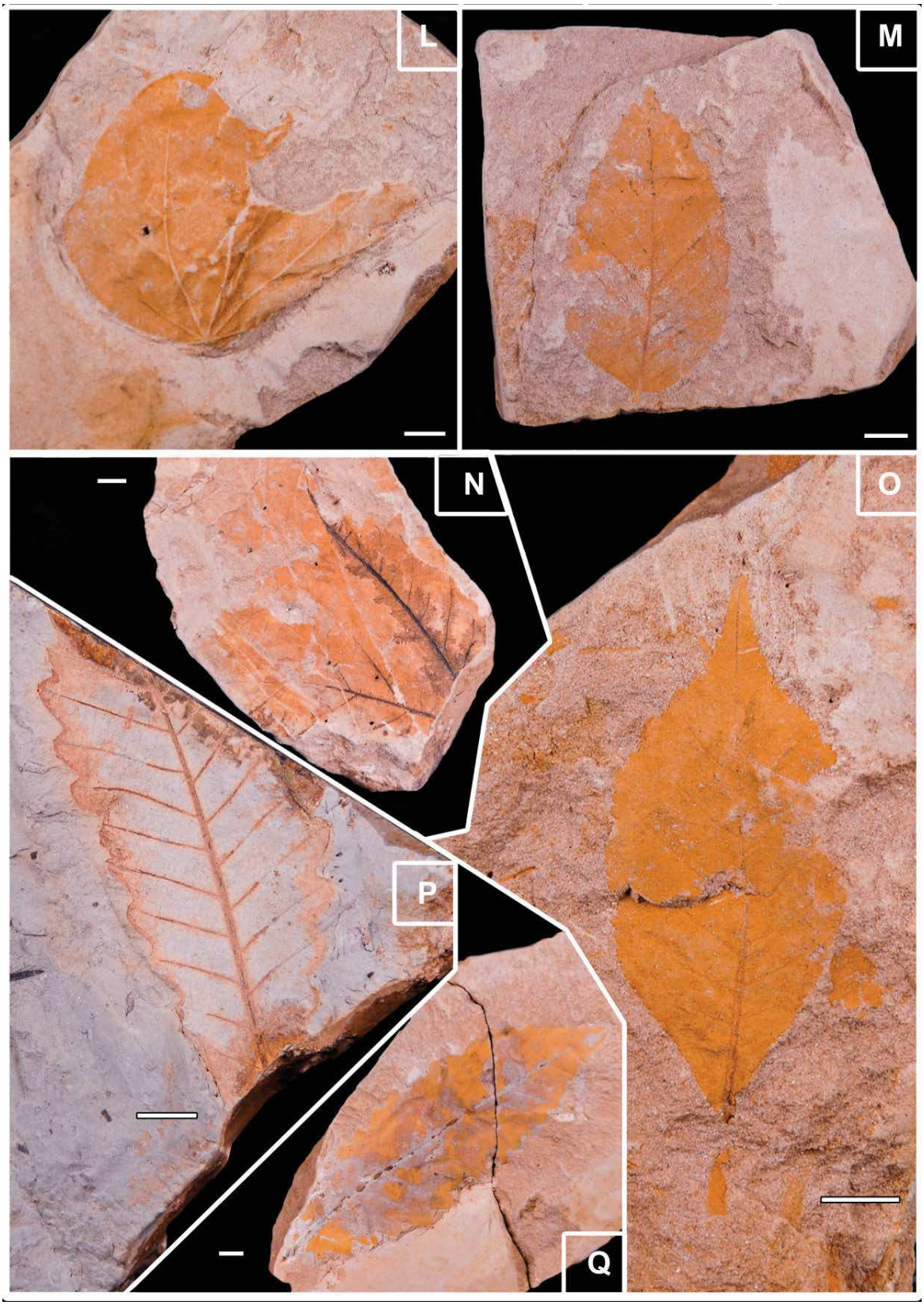

Figure 2: Plate Z. Fossil leaves from Berga (Dresden coll.).

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*.

3.3.3 Bernasso, France

Bernasso was a lake developed when a basaltic flows shut on a canyon valley (Leroy and Roiron, 1996). Diatomites were formed and fossil leaves, often with rest of cuticle, were preserved. It is located close to Lunas (Hérault, southern France) (Adroit et al., 2016; Leroy and Roiron, 1996). 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. An complementary analysis on cyclostratigraphy (Suc and 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 and 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 (Leroy and Roiron, 1996; Suc, 1978). The MAT in Bernasso was estimated about 14-15°C and the MAP is around 1500 mm/y (Leroy and Roiron, 1996). It is important to note that CLAMP results in Bernasso suggest a probably lower temperature (Girard et al., in review, see Appendix C).

3.4 DATA ANALYSES

3.4.1 Plant-insect interaction identifications

The plant-insect interactions were identified following the Damage Type Guide (Labandeira et al., 2007). The damages type (DTs) 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 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 Appendix D 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.

3.4.2 Leaf Mass per Area

Leaf mass per area (LMA) was estimated as there is a robust relationship between petiole width squared and leaf mass standardized by leaf area (Royer et al., 2007). Generally, plants with a low LMA have thinner leaves with a short lifespan and should also have high nutrient concentrations, thus being more palatable for insects (Coley and Barone, 1996; Royer et al., 2007). Because of the difficulties in having specific measurements needed to obtain the LMA, we considered plant species statistically represented, i.e. when at least 10 specimens were measured (as it was done in Currano et al., 2016).

3.4.3 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^2-test. The remaining information needed for this test was obtained by using the generalized linear model of the binominal distribution. Sample-based rarefaction curves were done to compare the different damage richness between the different outcrops (Gotelli and Colwell, 2001; Tipper, 1979). At last, in order to observe the different distributions of plant-insect interactions among the paleoforests, principal component analysis (PCA) were performed with the software Past3 (v3.14) (Hammer et al., 2001) in a biplot way. The data matrix used for it comprises the diversity of each eight FFGs on every plant species for each outcrop.

3.5 RESULTS

3.5.1 Comparisons of plant-insect interactions

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, Appendix E).

The frequencies of generalist interactions are of 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).

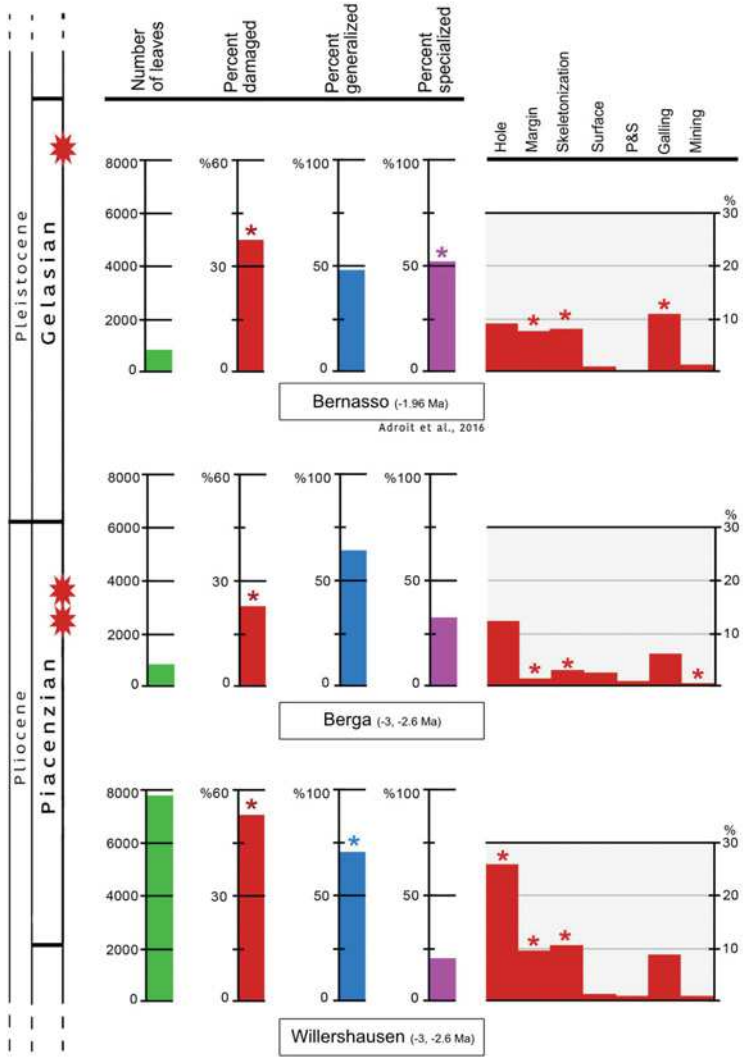

Figure 3: Quantitative distribution of plant-insect interactions from Willershausen, Berga outcrops and the fossil deposit of Bernasso (from Adroit et al., 2016).

Significant difference (alpha <0.05) from an outcrop to another one is marked by an asterisk. The percent of generalized and specialized damages are computed with the damaged leaves, thus their sum is 100%.

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 diversity 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).

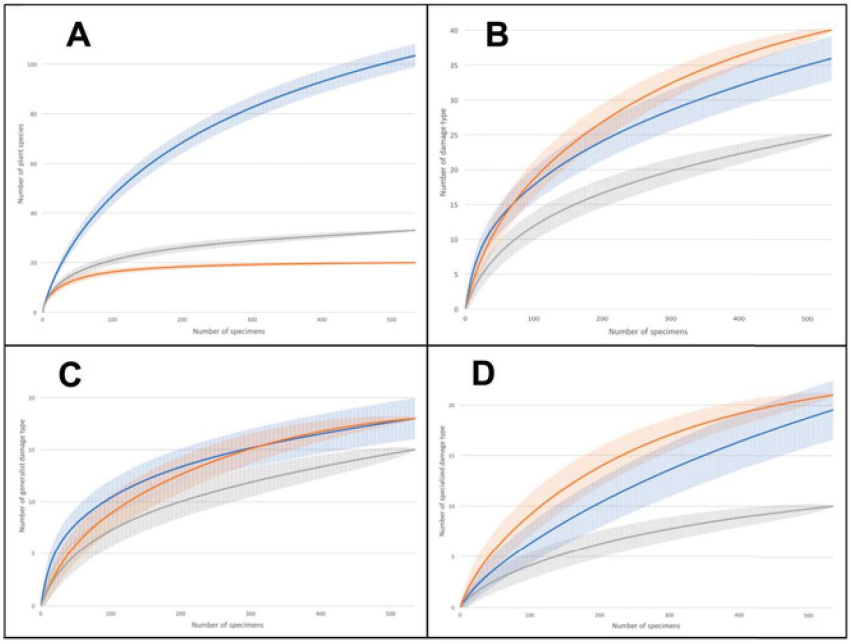

Figure 4: Rarefaction curves on the leaves from Willershausen, Berga and Bernasso (from Adroit et al. 2016)**.**
The grey curves represent Berga, the blue curves represent Willershausen outcrop and the orange curves represent Bernasso. The shaded area represents the standard deviation below and above 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

3.5.2 LMA per species at Willershausen

The LMA calculations in Bernasso and Berga were not possible due to the low quantity of leaves per species and the bad preservation of many fossil leaves. The LMA was calculated only on leaves in Willershausen. For the 10 species on which LMA was determinable, values in Willershausen vary from 46.18 g/m^2 to 79.26 g/m^2 (Figure 5, Appendix F). For the same 10 species, the percentage of damaged leaves varies between 38.8 and 71.2 % (Figure 5, Appendix F). Lastly, on the figure 5, we clearly observed (with also standard deviation in Appendix F) that there is no significant difference of herbivory according to the different values of LMA.

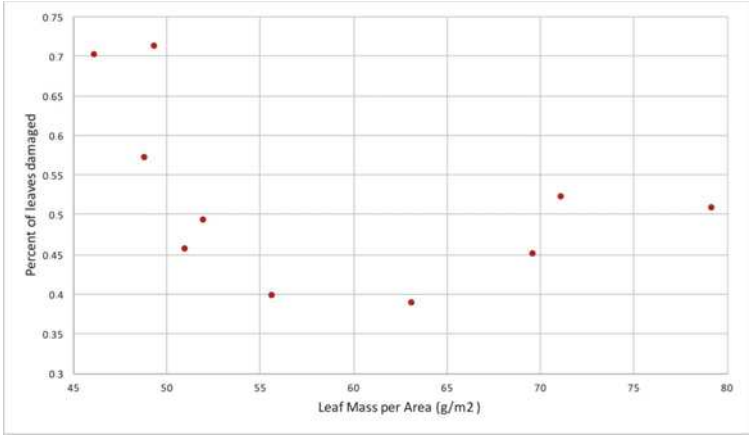

Figure 5: Percent of leaves damaged according to the leaf mass per area (LMA).
The LMA was estimated using the method of Royer et al. (2007). Each dot represents a plant species. Each species was considered only if at least 10 leaf from the species were measurable.

3.5.3 Structure of the paleoforests with the damage distribution on plant species

The figure 6 presents the different PCA realized for the 3 outcrops with the data of plant and DT diversities. For each outcrop, only the 2 first axes are presented as for Willershausen they represent 77% (Figure 6A), for Berga 93% (Figure 6B) and for Bernasso 91% (Figure 6C) of the whole distribution.

For Willershausen (Figure 6A) 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) (Appendix G). 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) (Appendix G). 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 diversity 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 6B) 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 (Appendix G). Hole feeding and skeletonization are also correlated with the PCA-axis 2, negatively for hole feeding (-0.79) and positively for skeletonization (0.66) (Appendix G). 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 6C; 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) (Appendix G). 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) (Appendix G). *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

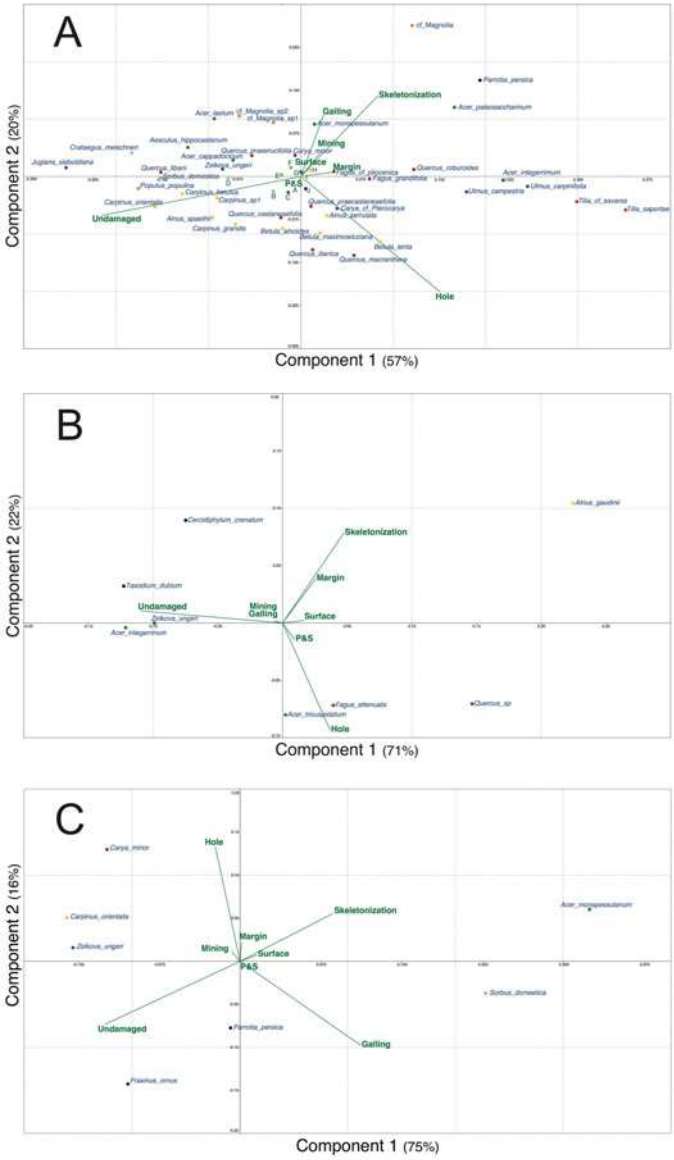

Figure 6: Principal components analysis (PCA) of plant species according to the diversity of damage type (DT).

A- Willershausen
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*

B- Berga
C- Bernasso

Detailed significance of PCA's axes are presented in Appendix G.

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 6) 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 6B) 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 6A).

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

Sorbus domestica and *Carpinus orientalis* are both associated to the FFG undamaged in Willershausen (Figure 6A). In Bernasso, *S. domestica* is associated with galling and skeletonization and *C. orientalis* is associated with hole feeding and undamaged (Figure 6C). *Carya minor* is associated to skeletonization and galling in Willershausen (Figure 6A) while in Bernasso it is associated to undamaged and hole feeding (Figure 6C). *Parrotia persica* is associated to galling and undamaged in Bernasso (Figure 6C) while in Willershausen, it is associated with the FFGs skeletonization and galling (Figure 6A).

3.6 DISCUSSION

3.6.1 Floristic richness and herbivory representativeness

All genera and many plant species from Berga are also present in Willershausen (Appendix H) that can be expected because they have similar age and geographic proximity. 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 (Appendix H) 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 (Appendix H). This may suggest a difference in specific richness between those paleoforests. However, it could also be due to the fossil collections themselves. The great difference of collection size between the three outcrops could have led to artificial difference of plant species richness (Appendix E). However, rarefaction data tests indicate that there is in fact a highest richness of plant species in the Willershausen outcrop compared to Berga and Bernasso outcrops when the collections are similarly re-sized (Figure 4).

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.

3.6.2 Absence of correlation between LMA and plant-insect interaction

In Willershausen, no significant correlation between the LMA of plant species used and the herbivory was observed (Figure 5) while previous studies (Currano et al., 2009, 2008; Wappler et al., 2009) showed a negative correlation between the LMA and the damage on the leaves.

These correlations were measured on leaf assemblages including both deciduous and evergreen plant species. In the case of Willershausen, only delicious plant species have been measured. Finally, the relationship between LMA and damaged leaf by insect feeding is not such evident (Poorter et al., 2009). For example, a small increase of secondary defense compounds is not really affecting LMA but could significantly reduce the herbivory rate (Coley, 1988). Moreover, under different resource availabilities for plants, Züst and Agrawal (2017) shown that LMA values could be similar between leaves while their nutrient concentrations were totally different.

3.6.3 Parameters involved in insect damages differences

Climatic conditions seem to be in relation with variations in richness and frequency of plant-insect interactions (Currano et al., 2016). If an increase of temperature seems to stimulate insect herbivory (Zvereva and Kozlov, 2006), it is still complex to understand the complete role of temperature in the modulation of herbivory (DeLucia et al., 2012).

Thiel et al. (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 the 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 could argue 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 be 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 and Jones, 1998; DeLucia et al., 2012; Stiling and Cornelissen, 2007). 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 trough morphological analyses.

For this reason, Thiel et al., (2012) were in favor of the Co-existence 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 diversity of plant species (Knops et al., 1999; Mulder et al., 1999; Siemann et al., 1996; Wright and Samways, 1998) and should be expected higher damage richness in the more diverse paleoforest (Price, 2002, 1991). 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 diversity 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 case study.

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 (Coley and Barone, 1996; Fraser, 2017; Hutchinson, 1959; Klopfer, 1959; Klopfer and MacArthur, 1960; MacArthur, 1972). 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 et al., 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 (e.g., Archibald et al., 2010). Leroy and Roiron (1996) indicated that Bernasso paleoforest grew under temperatures of 14–17°C and precipitations of 1300-1500 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 and Roiron (1996), while the pollen analysis from the same study tend to corroborated previous estimations done by Leroy and Roiron (1996).

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) (Thiel et al., 2012; Uhl et al., 2007). 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 and Hayward, 2010). Concerning Bernasso, the different estimations of temperatures, included the CMMT, are lower than those of Willershausen (Girard et al., in review; Thiel et al., 2012; Uhl et al., 2007), 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 are lower in Berga. However, the estimated temperatures of Bernasso overlap the ones of Berga (especially for the coolest temperatures) and therefore complicate any interpretations about the damage frequency between these two paleoforests.
Moreover, it is important to note that no data about insect richness of these different paleoforests are available. Although as 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 case of differences in the insect faunas, the previous relation could be disturbed as some insects, such as larvae of *Thaumetopoea pityocampa,* feed plant during winter season (Battisti et al., 2005; Buffo et al., 2007), when others insects have no or lower activity (e.g., Hahn and Denlinger, 2007).

3.6.4 More precisions 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 (e.g., Currano et al., 2011; García et al., 2016). Moreover, taphonomic biases, especially fossil preservation and different excavation histories, could also interfere 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 (e.g., 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 and 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 and

Gaussen, 1957; Daget, 1984, 1977). 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 (Hennissen et al., 2015; Utescher et al., 2017; Williams et al., 2009). Water stress should have a positive impact on galling diversity (especially the frequency), as many studies already mentioned that galling is an adaptation of stressful environment (Fernandes and Martins, 1985; Fernandes and Price, 1992, 1988; Lara et al., 2002; Price et al., 1998). 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 and Roiron, 1996), tend to favor the richness of gall-inducting insects (that increase the proportion of specialized interaction) (Adroit et al., 2016; Fernandes et al., 2010).

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 6). 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 and Cornelissen, 2007), but in general, it is compensated by an increase of insect feeding (Bezemer and 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 6A). 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.).

3.7 CONCLUSION

In the actual context where plant-insect interactions are essential to understand the food web of the paleo/-forests (Cornelissen, 2011; May, 1988; Wilf, 2008), this study allows the first comparison of the plant-insect interaction structure of three Plio-Pleistocene leaf assemblages. Such a comparison highlighted that relationships between climate parameters (such as temperature and precipitation) and plant-insect interactions are not evident in European paleoforests around the Neogene – Quaternary transition. Temperatures and precipitations undoubtedly have major impacts on herbivory rate and our results suggested that the annual variation of those climatic parameters (i.e. seasonality) could have a greater influence on plant-insect interactions than the average annual of these parameters. It has sense as the insects' responses to climate variation are very sensitives (e.g., Bale and Hayward, 2010) and could be predicted with their feeding behavior on leaves (Bale et al., 2002).

These statements about the impacts of seasonality on plant-insect interactions should to be considered for the further studies in the fossil record.

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CHAPTER IV

Changes on herbivory pattern on the Persian ironwood (*Parrotia persica*) over the last 3 million years

Submitted

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4.1 ABSTRACT

The Hyrcanian forest is composed of many Arcto-Tertiary plant species that since the beginning of the Glacial cycles (early Quaternary) have decreased their distribution until they became endemic to the coasts of the Caspian Sea in northern part of Iran. Today, the Hyrcanian forest is considered as the closest similar remnant of the European paleoforests during the Plio-Pleistocene times. One of the most emblematic plant species is the Persian Ironwood (*Parrotia perisca* C. A. Mey) known to be also an important representatives of the genus *Parrotia* in the world. This study presents the first comparison of herbivory pattern among this plant species in its current endemic environment and its fossil opponents known from Willershausen (Germany) and Bernasso (France) also indicating a larger paleontological distribution during the Plio-Pleistocene boundary. We wanted to know if the ecological relationships of *P. persica* with its environment were similar or changed during these last millions of years, through the analyzed of their plant-insect interaction. Fifteen sites were selected in the Hyrcanian forest along temperature and precipitation gradients. A total of 2160 leaves were collected, measured, and examined through some leaf characteristics and their plant-insect interaction. 84% of the leaves were damaged and a total of 3507 damage occurrences were found. A significant lower proportion of specialized damage were found on leaves from *P. persica* in the Hyrcanian forest compared to we observed among the samples from the two fossil outcrops, which could suggest a “non-suitable” environment for *P. persica* today in its current endemic distribution.

4.2 INTRODUCTION

The Hyrcanian forest represents the most important woodland in Iran, covering a total area of ca. 50.000 km^2. This deciduous mixed forest is delimited by the coast of Caspian Sea compressional depression to the North (Berberian, 1983) and by the Alborz mountains to the South (Stoecklin, 1968) (Figure 1). Along the Caspian Sea, the forest spreads from the Talish region in Azerbaijan to the Golestan National Park in Iran (Miller, 1994). The Hyrcanian forest extends over an altitudinal gradient from sea level to ca. 2.000 m above sea level (a.s.l.) and suffers a drought gradient increasing from west to east (Djamali et al., 2011; Talebi et al., 2014). Precipitations in the Hyrcanian region are not distributed equally over the forest. In the western part, most of the precipitations occur in fall and no regular dry season happens while in the eastern part, temperature are higher, precipitations are lower and an obvious dry season is observed (Akhani et al., 2010; Djamali et al., 2008; Talebi et al., 2014). It has been established that precipitation seasonality seems to be the main bioclimatic proxy for distribution and ecology of the different plant communities in Iranian vegetation especially in the Hyrcanian forest (Akhani and Ziegler, 2002; Djamali et al., 2010).

Forests in this region are mainly composed by Arcto-Tertiary relict flora elements (Mai, 1995) which covered a large part of the Northern Hemisphere in the Paleogene, but since the late Neogene and the onset of glacial cycles it has been reduced to the south which de facto have led to isolation of vegetation groups within refuge zones (Kozlowski et al., 2012; Leroy and Arpe, 2007; Milne, 2006; Milne and Abbott, 2002; Petit et al., 2005). Particularly, due to the complex topography of the Hyrcanian region (Djamali et al., 2012; Favre et al., 2015; Wen et al., 2014), its forest is considered today as relictual of the former European paleoforests (Arpe and Leroy, 2007; Leroy and Roiron, 1996; Noroozi et al., 2008; Probst, 1981; Tralau, 1963; Zohary, 1973). For example, the Hyrcanian forest has been considered as a possible analogue of the early Pleistocene Bernasso paleoforest in France (Suc, 1978; Leroy and Roiron, 1996) and late Pliocene Willershausen paleoforest in Germany (Ferguson and Knobloch, 1998; Knobloch, 1998). Indeed, numerous relict plant species found in both paleoforests, that are today endemics of the Caucasian region and the Hyrcanian forest representing dominating tree species of the potential natural vegetation in Central Europe such as *Zelkova carpinifolia* (Pall.) C. Koch., *Alnus subcordata* C. A. Mey., *Quercus castaneifolia* C. A. Mey., *Acer velutinum* Boiss., *A. cappadocicum* Gled. and *Parrotia persica* C. A. Mey (Akhani et al., 2010; Djamali et al., 2008; Maharramova et al., 2015). This indicates that possible modern analogues for fossil insect traces (plant-insect interaction) of the Plio-Pleistocene European forests could also be found in forests in the Caspian region.

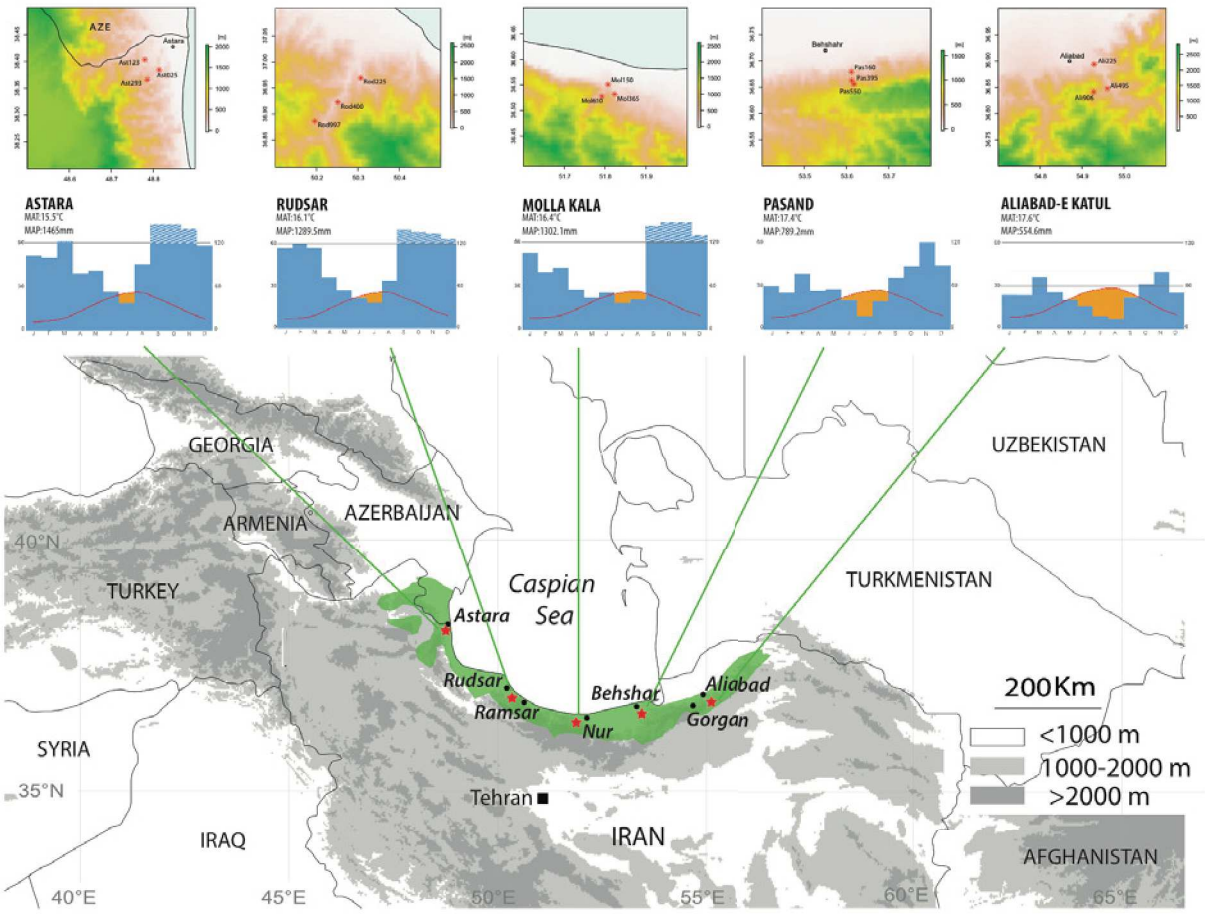

Figure 1: Map of the Hyrcanian forest in the Caucasian region.

The green zone represents the Hyrcanian forest located in the Northern part of Iran, southern Caspian Sea. The red stars represent the five localities choose for our study from West to East: Astara, Rudsar, Molla Kala, Pasand and Aliabad-e Katul.

Above the map, the climatographs of each locality, realized by the climatic data of the Iran Meteorological Organization (IRIMO) from 1995 to 2005. The orange area indicates the dry season. The hatched blue area indicates the extremes values of precipitation rate at Astara, Rudsar and Molla Kala, then it does not respect the scale of the Y axis.

Overhead, zoom on locality that showing the three sites at different altitudes. The maps were obtained with the Wordclim dataset (available at http://www.worldclim.org/download; Hijmans et al., 2005) at a resolution of 30 arc-second. The nomenclature of the different sites indicates their exact altitude, for example, Ast293 means the site at Astara from 293 above the sea-level.

In order to compare the plant-insect interactions on leaves between these European fossil paleoforests and their possible Hyrcanian analogue forest, we focused our study on one of the most emblematic and important plant species from Iran, *Parrotia persica* (Hajizadeh, 2016). *P. persica* was also present in European Plio-Pleistocene paleoenvironments from where it gradually disappeared since the Holstein (0.4 Ma) (Bińka et al., 2003; Jiménez-Moreno et al., 2010; Biltekin et al., 2015) before to become endemic of the Hyrcanian forest (Bińka et al., 2003; Sefidi et al., 2011). It is a deciduous tree belonging to the Hamamelidaceae and represents one of the two species of the genus *Parrotia*. The other *Parrotia* species (*P. subaequalis* H.T. Chang, R.M. Hao et H.T. Wei) was recently described from eastern China (Geng et al., 2015;

Li and Zhang, 2015). The Persian ironwood (*P. persica*) is found from sea level up to 1.200 m a.s.l., while it is uncommon to find it at higher altitudes (Sefidi et al., 2011). *P. persica* has several characteristics that could represent a defense strategy against its herbivores, such as the presence of phenolic compounds (Nabavi et al., 2008; Ahanjan et al., 2009). It still supports an extensive insect fauna although the extent of feeding damage may vary across longitudinal and altitudinal ranges.

Presence of *P. perscia* into analogous past and modern forests allows to analyze the similarities and/or differences of insect herbivory traces on its leaves and to try to interpreted the observed variations. Many studies have shown obvious correlations between the insect herbivory and abiotic parameters (Adroit et al., 2016; Bale et al., 2002; Blanche and Ludwig, 2001; Currano et al., 2016; Fernandes et al., 2010; Fernandes and Price, 1992; Janzen, 1970; Leckey et al., 2014; Price et al., 1998; Su et al., 2015; Wappler, 2010; Wappler and Denk, 2011; Wilf et al., 2001). Firstly, climatic variations observed in the today Hyrcanian forest will help to better understand the parameters that influence insect herbivory on *P. persica* leaves. Secondly plant insect interactions on fossil *P. persica* will be analyze in regard to the result on the modern leaves in order to highlight changes in the pattern of insect herbivory since 3 million years.

4.3 MATERIAL AND METHODS

4.3.1 An overview to the study area

The studied area in the Hyrcanian forest is expanded from Astara (west) to the Golestan national park (east). It covers ca. 800 km from west to east and 110 km from north to south for a total of 1.85 million ha (i.e. approximately 15% of the Hyrcanian zone and 1.1% of the country's area; Talebi et al., 2014). The climate of the Hyrcanian region is modulated by the complex topography of the region, the proximity of the Caspian Sea and the Westerlies from Black Sea and Mediterranean Sea (Domroes et al., 1998; Sharifi et al., 2015; Talebi et al., 2014). This region is characterized by (1) an increasing of the mean annual temperature from east (MAT: 15.5 °C) to west (MAT: 17.7 °C) (Sabeti, 1969; Talebi et al., 2014), (2) a decrease of the mean annueal precipitation from west (MAP: 1465.6 mm) to east (MAP: 554.6 mm), (3) an absence of dry season in the western part (mesic habitat), due to both the Westerlies from Black Sea and Mediterranean Sea which increase significantly the rainfall in the western part of the Hyrcanian forest (Sharifi et al., 2015) (Figure 1).

4.3.2 Characteristics of the Persian ironwood (*P. persica*)

Parrotia persica is a deciduous single or sometimes multi-stemmed tree (Coombes and Debreczy, 2015). According to its different shapes, the Persian ironwood can vary in general between 8 to 25 meters in height. The leaves are oblong to obovate, 15 cm long and 6 cm wide, with 5 to 8 pairs of secondary veins. The apex is slightly rounded, tapering from above to the middle and the base is obtuse or sometimes slightly asymmetric with an entire margin (Coombes and Debreczy, 2011; Knobloch, 1998; Leroy and Roiron, 1996). The petiole is really short, around 1 cm long (Coombes and Debreczy, 2015). Furthermore, the Persian Ironwood

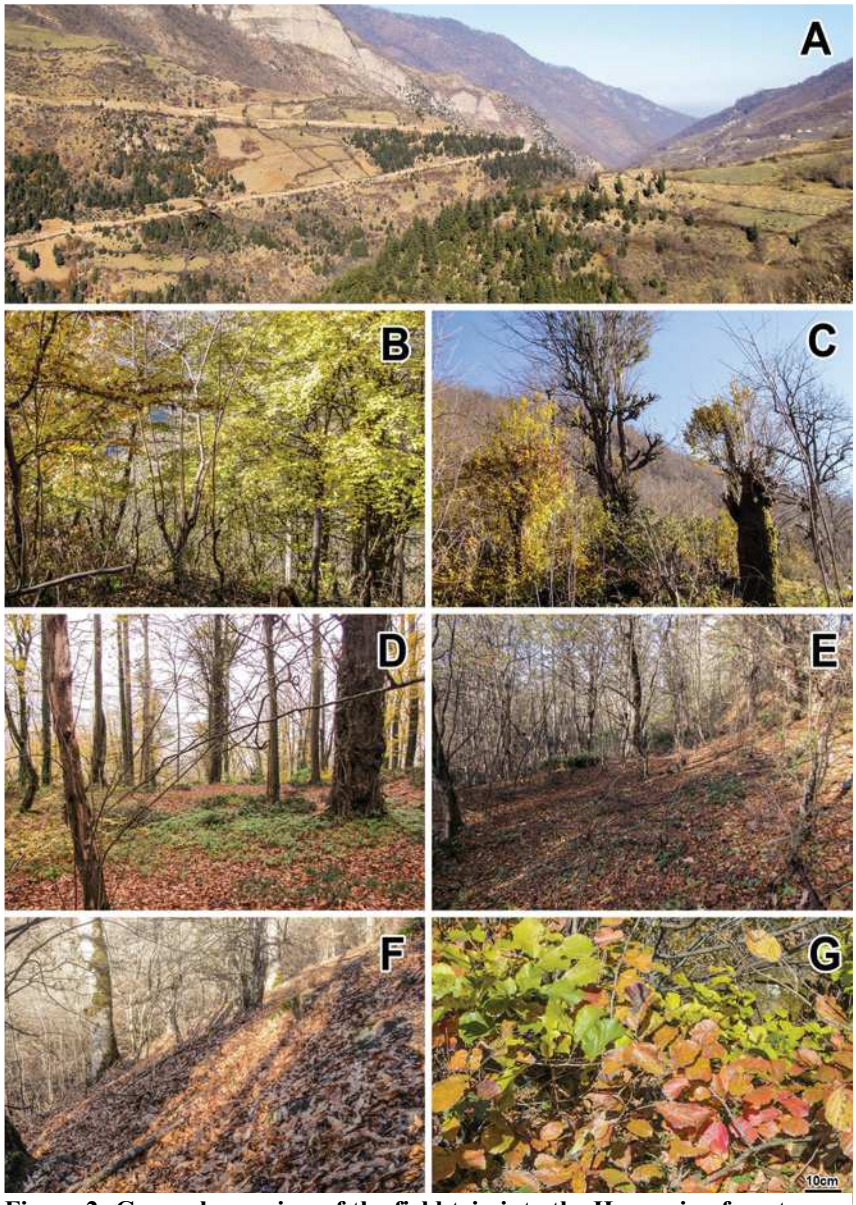

Figure 2: General overview of the field trip into the Hyrcanian forest.

(A) Global overview showing a part of the forest enclosed in the valley of the Alborz mountains.

From West to East: (B) Astara, (C) Rudsar, (D) Molla Kala, (E) Pasand and (F) Aliabad-e Katul.

(G) *Parrotia persica* leaves, colors range from green to bright red, characteristic of the Persian Iroonwood leaves, the scale for this picture is 10cm.

produces colorful foliage during the fall season; the deciduous leaves turn purple to brilliant red (Nicholson, 1989) (Figure 2).

In the Hyrcanian forest, *P. persica* is always associated with *Carpinus betulus* as both compose one of the most important forest group of the Hyrcanian forest: *Parrotio-Carpinetum* (Assadollahi, 1980; Talebi et al., 2014). This group is dominated by these aforementioned species and it also comprises *Zelkova crenata, Colutea persica* and *Quercus castaneifolia* but only at lower altitudes. At higher altitudes this dominance, especially for of *P. persica*, decreases and *Quercus pubescens* seems to be dominant (Assadollahi, 1980). The plant association *Parrotio-Carpinetum* is mainly found on mull brown soil and which has a clay-silty texture or even only clayey with a pH variation from 5.7 to 7.7 (Assadollahi, 1980; Talebi et al., 2014).

4.3.3 Sites and sampling method

In order to cover the gradients of temperature and precipitation we studied five localities from west to east (Figures 1 and 2). Localities studied by Talebi et al., (2014) were re-used. For each locality, three different altitudes up to 1.000 m a.s.l. (called thereafter "site") were sampled in order to compare as faithfully as possible the localities along the longitudinal gradient. We classified sites of similar altitude (less than 100 m different) into different groups: low altitude (115 to 150 m), low mid altitude (225 to 293 m), mid altitude (365 to 395 m), high mid altitude (452 to 503 m) and high altitude (above 900 m). A GPS (GARMIN gps map 62s) was used to register the coordination and altitude of each locality (Table 1).

A frame (50 cm x 50 cm) was set up on the ground 3 m away a Persian ironwood trunk and leaves were randomly collected from the upper leaf-litter layer inside the frame (*sensu* Smith and Nufio, 2004; Leckey et al., 2014). Leaves from each plot were put in a separate zip bag and labeled. A total of 150 leaves per site were sampled (five replicates of 30 leaves). According to Reynolds and Crossley (1997), Cornelissen and Stiling (2005), Leckey et al. (2014) and Su et al. (2015) fallen leaves are more representative than the ones in the canopy, as herbivory is not evenly distributed throughout a tree's canopy, leaf litter samples allow for greater standardization of samples between trees. As we additionally compare those samples with fossil leaf assemblages, the upper leaf-litter collection method most reliably represents taphonomically influenced condition of the fossil material.

To assess the differences of local climate conditions among the five transects (Astara, Rudsar, Molla Kala, Pasand and Aliabad-e Katul) (Figure 1), climatic parameters were used from the Iran meteorological organization (IRIMO). Bioclimatic variables of our different sites (such as monthly temperatures, monthly precipitations, warmer and coolest temperatures) published in Djamali et al. (2011) and downloaded from WorldClim dataset at a resolution of 30 arc-second (available at http://www.worldclim.org/download); (Hijmans et al., 2005) were used in our study and notably compiled in Figure 1. The altitude-dependent mean annual temperature, and temperatures of the coldest and warmest months, for each site, are presented in Appendix I (according to Meyer, 1991). The neighboring species found in the plot were also recorded.

Sample localities	MAT (°C)	T°min (°C)	T°max (°C)	MAP (cm/y)	Altitude (m)	Latitude (N)	Longitude (E)	Date of samling
Astara	15.5	2.8	29.6	1465.6	25	38.384	48.814	22/12/2015
					123	38.403	48.780	
					293	38.365	48.786	
Rudsar	16.1	2.4	30.3	1289.6	225	36.969	50.306	23/12/2015
					452	36.923	50.251	
					997	36.886	50.195	
Molla Kala	16.4	3.9	28.7	1302.1	150	36.550	51.807	19/12/2015
					365	36.532	51.823	
					610	36.527	51.792	
Pasand	17.4	4.3	31	789.2	115	36.679	53.611	20/11/2015
					395	36.662	53.612	
					550	36.655	53.617	
AliAbal-e Katul	17.7	3.4	32.6	554.6	225	36.894	54.928	26/12/2015
					495	36.847	54.960	
					906	36.841	54.927	

Table 1: Details for sample localities in the Hyrcanian forest.
For each locality, mean annual temperature (MAT), minimal temperature (T°min), maximum temperature (T°max) and the mean annual precipitation (MAP) were obtained thanks to the climatic data from IRIMO (Iran meteorological organization).
For each site, the altitude, latitude and longitude were measured with a GPS (GARMIN gps map 62s). The altitude-dependent temperatures are available in Table S1.

4.3.4 Damage types

Each collected leaf was photographed with a digital camera (Sony TX1). Both leaf sides were observed to identify all damage types (DTs) among the different functional feeding groups (FFGs) on the leaves. For identification and classification of damage types, the guide from Labandeira et al. (2007) (Version 3.0) was used. This guide was originally made for fossil material and was reused in this study on fresh materials in order to compare modern and fossil leaves. Only the damages made by insect feeding were considered, these ones being easily identifiable by black reactions scars (Figures 3 and 4).


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Figure 3: Examples of external foliage feeding on *Parrotia persica*.
A, Small-sized circular perforations (DT1), indicated by arrow (loc. Astara). B, Medium-sized circular perforations (DT2) (loc. Astara). C, Large-sized, polylobate perforations (DT5) (loc. Astara). D. Center feeding (DT63) and shallow to deeper excision of the leaf margin (DT12) (loc. Molla Kala). E, Apex feeding (DT13) (loc. Molla Kala). F, Trenched excision of the leaf blade that expands inwardly from the leaf margin (DT15) (loc. Aliabad-e Katul). G, Broad swaths of interveinal tissue are absent and highest orders of venation removed (DT21) (loc. Astara). H, Apex feeding (DT13) (loc. Molla Kala). I, Extensively and completely removed interveinal tissue surrounded by remaining veinal stringers (DT26) (loc. Molla Kala). J, Perforation at the divergence point of secondary veins from primary veins (DT57) (loc. Molla Kala). K, Detail of hole feeding in J, showing reaction rim and flaps of necrotic tissue (arrow) along the outer margin of the reaction rim. L, M, Cuspate margin excisions (arrows) separated by a short segment of leaf margin (DT143) (loc. Aliabad-e Katul). N, Removal of interveinal tissue resulting in retention of some vein network but with a well-developed surrounding reaction rim (DT17) (loc. Aliabad-e Katul). O, Removal of interveinal tissue resulting in retention of some vein network but with a poorly developed surrounding reaction rim (DT16) (loc. Aliabad-e Katul). P, Same skeletonization pattern as seen in O (DT16) (loc. Molla Kala). Q, Skeletonization were the highest orders of venation removed; broad swaths of interveinal tissue are absent (DT21) (loc. Molla Kala). R, Small to medium sized circular perforations arranged in a curved chain with prominent dark edges to the margin (DT297) (loc. Rudsar). S, Magnification of the long-bent concatenation of small holes in R, surrounded by a prominent dark reaction rim. T, long bent concatenation of small holes (DT297) (loc. Aliabad-e Katul). U, Elongate, curvilinear to rectilinear strings of skeletonized tissue in the apical part of the leaf (DT20) and interveinal skeletonization with a poorly developed surrounding reaction rim (DT16) (loc. Pasand). Solid scale bars indicate 4 mm. DT in captions refer to damage types listed in Labandeira et al. (2007).

The guide allows for distinguishing generalist damages (caused by polyphagous insects, as a majority of external damage) and specialized damages (mostly caused by monophagous or oliphagous insects, as galls and mines). Specialized plant-insect interactions are defined by comparison with existing known insect feeding traces and restricted area of the occurrences limited to specific host-plant species (Labandeira, 2002). A compilation of the different observed damages on *P. persica* leaves can be found in Figures 3 and 4. In order to compare the herbivory distributions according to the abiotic parameters we split the proportion of interaction between the generalist and specialist damages (Labandeira et al., 2007). The frequencies and richness of plant-insect interaction according to the localities and sites were taken into account separately and summarized in one file using the statistical software R 2.15.1 (R Core Team 2014) and code developed by Gunkel and Wappler (2015) in order to obtain statistical resume and rarefaction dataset of each localities and sites. We also calculated the percentage of destruction per leaf with Adobe Photoshop CS and ImageJ (Mangels et al., 2015) to identify the leaves the more destroyed in regard of the insect interactions.

Figure 4: Examples of endophytic interaction and external foliage feeding on *Parrotia persica*.

A, Foliar gall (DT32) (loc. Molla Kala). B, Detail of the circular gall in A, occurring on the interveinal region of the leaf lamina. C, Foliar and midrib gall (DT23,33) (loc. Molla Kala). D, Magnification on the galls in C, occurring on the interveinal region of the leaf lamina and on the primary vein of the leaf. E, Blotchy, skeletonized areas on the upper leaf surface, originated by instar leaf tissue feeding (DT38) (loc. Pasand). F, Regular dark-brown blotch mine on the edge of the leaf that crosses major leaf veins (DT35) and a blotch mine (weak edges marked by arrows) lacking a central chamber, containing dispersed, small spheroidal coprolites (DT36) (loc. Aliabad-e Katul). G, Detail of the blotch mine in F, showing the slightly folded epidermal layer of a bulging blotch-type mine. H, Large blotch of necrotic tissue with peripheral holes (DT154) (loc. Pasand). I, Detail of the blotch in H, showing that the outer margin of the blotch is surrounded by a weakly developed reaction front. Solid scale bars indicate 4 mm. DT in captions refer to damage types listed in Labandeira et al. (2007).

4.3.5 Leaf characteristics

The leaf mass per area (LMA) for modern and fossil leaves was estimated on a sampling of 30 leaves (randomly selected) for each site in following the protocol of (Royer et al., 2007). LMA is a proxy for the plant physiology because it differs according to the nutriments concentration in the leaf (de la Riva et al., 2016) that can lead to variations of herbivory.

Furthermore, for the Iranian sites, as the shape of the leaf could potentially vary along the gradients due to different climate conditions and because it could have an impact on herbivory, we determined and calculated a shape ratio. We did not estimate in the fossil record as the shape of the leaf could vary and be modified because the different process of fossilization. This ratio is the quotient of L1/L2, where L1 is the length from the leaf base and the part where the leaf is the largest, and L2 is total length of the leaf blade.

4.3.6 Statistical tests

To compare all the leaf measurements and interactions, we used different common statistical tests to show the significant difference between our values. The Shapiro test was used before all other tests to check the normalization of the samples, followed by a Fisher test to check the variance equality (R Development Core Team, 2014). We also used Student tests (with and without Welsh correction) and ANOVA tests, along with the Tuckey test for simultaneous comparisons of damage richness at the different sites. A Chi-2 test (χ^2) was done to compare the frequency of certain specialist and generalist damages between sites at same altitude in Iran. It was also used to compare the proportion of specialist damages on the same locality at the different altitudinal sites. In this last case, linear regression was also done to show dependence between altitude and specialist damage frequency (Past 3, Hammer et al., 2001). Principal component analysis (PCA) was established in order to compare the frequency distribution of the plant-insect interactions (i.e. FFG frequency per locality) from the five Iranian localities with the ones from European fossil sites. The damage richness was related between all the locations by rarefaction curves made with the vegan package (Oksanen et al., 2013) in R (R Development Core Team, 2014).

4.4 RESULTS

4.4.1 Measurements of Persian Ironwood leaves

In all Iranian localities, *Parrotia persica* is associated with *Quercus infectoria* and *Zelkova carpinofolia*. Some *Acer* species (e.g. *Acer tataricum, A. heldreichii, A. velutinum, A. cappadocicum)* are also found at Astara, Molla Kala and Pasand.

The shape ratio L1/L2 (Appendix J) varies between 0.5 to 0.6 and the different standard error bars overlap each other. The variation range of the removed area in the different sites is large (Appendix K), varying to 15% in average and 30% considering the extreme values. At each site, the percentage of removed area of the leaf blades have huge variations, but no statistical tests showed significant differences between the sites. Only the leaves collected at

the highest altitude (Rud_3, Ali_3) are statistically less damaged than the leaves in others sites (Appendix K).

The LMA is significantly similar in the different sites and localities, along the longitudinal gradient, the values are varying between 80 and 100 g/m^2 (Appendix L). LMA from Willershausen seems to be significantly lower than the one of the Iranian localities (Appendix L). However, the LMA from Bernasso could not be estimated as there were not enough specimens to represent a fair average.

4.4.2 Overall patterns of herbivory

A total of 450 leaves were collected for each locality of Aliabad-e Katul, Molla Kala and Rudsar, 420 at Pasand and 390 at Astara. In the forest 84.5% of the leaves show insect feeding traces. In Willershausen 517 fossil leaves of *P. persica* were found (71% were damaged) and 106 were found in Bernasso (37% were damaged). Five FFGs, as defined by (Labandeira et al., 2007), are present in all the localities, included a total of 32 DTs in the Hyrcanian mixed forests, 40 DTs in Willershausen and only 18 DTs in Bernasso. Surface feeding was not found in Bernasso and piercing and sucking was only observed in Willershausen (Appendix M). Generalist damages (80% of the leaves in Iran, 36% in Willershausen and 11% in Bernasso) correspond to external damages such as hole feeding, margin feeding, skeletonization and surface feeding. Nevertheless, few DTs of these FFG belongs to specialized damages. However, the main specialized damages (16% in Iran, 29% in Willershausen and 23% in Bernasso) mostly correspond to galling and mining.

4.4.3 Distribution of the plant-insect interaction

The rarefaction curve (Figure 5) indicates that the leaf sample size used in every locality is enough large to cover all the DT richness present in the Hyrcanian forest and can be compare with the fossil leaves. The highest richness of damage is at Willershausen (Figure 5). It seems that Bernasso damage richness is higher than in Iran, but it cannot be confirmed statistically because the quantity of leaves at Bernasso is not sufficient to be clearly compare with other locations. The details of the damage proportion per sites presented below, are compiled in Appendix M and illustrated in the Figure 6.

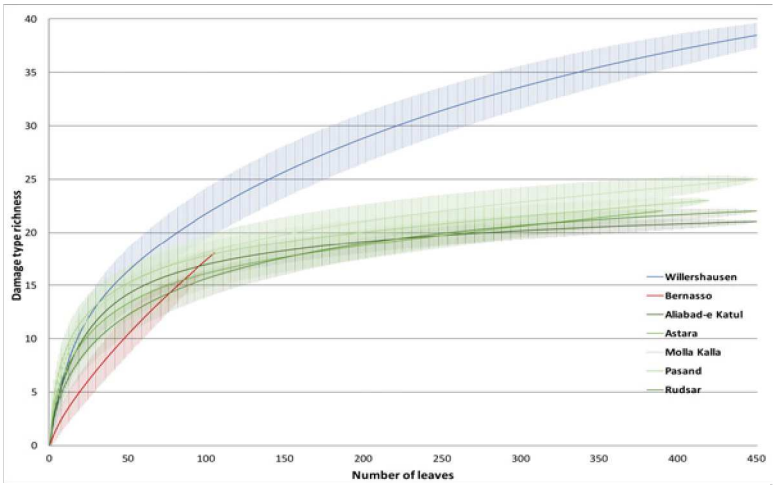

Figure 5: Rarefaction curves for insect damage richness on damaged leaves.
The shaded area represents the standard deviation below and above the average of the resamples, with the method from Heck et al. (1975).

4.4.3.1 Margin feeding

This is one of the most common FFG in Iran. It is represented on 58% of the total of the leaves. The highest proportion is observed in Pasand (74%) and the lowest frequency is at Rudsar (36%). At Astara, Molla Kala and Aliabad-e Katul margin feeding is found on respectively 61%, 56% and 64% on the leaves. Inversely correlated with altitude, the frequency of margin feeding decreases significantly in most sites, from 72% to 33% at Aliabad-e Katul or 83% to 62% at Pasand for example (χ^2, p<0.01). Such a trend is not observed at Aliabad-e Katul.
In Willershausen 10% of leaves were impacted by margin feeding and only 6% at Bernasso.

4.4.3.2 Hole feeding

This is the most common external FFG with a frequency of 56% in total Iran. Pasand had the highest proportion with 74% on its leaves. Rudsar and Aliabad-e Katul are the least impacted by hole feeding with 40% and 33% respectively. At Astara and Molla Kala the hole feeding is found on 70% and 65% respectively. Regarding the relative altitude, hole feeding frequency decreased significantly with an increase in altitude, from 43% to 22% at Aliabad-e Katul (χ^2, p<0.01) and from 76% to 48% at Molla Kala (χ^2, p<0.01). In the other localities, no specific trend was observed with altitude (χ^2, p>0.5).
Hole feeding is present on 26% of the leaves from Willershausen and 7% in Bernasso.

4.4.3.3 Skeletonization

This FFG is present on only 14% of the total on Hyrcanian leaves. Pasand and Molla Kala are the most impacted with 19% and 18% of their leaves affected by skeletonization. Aliabad-e Katul and Rudsar have 15% and 13.5% of this FFG. Only 4% of the leaves from Astara are affected by skeletonization.

In Willershausen it is found on 25.7% of the leaves and in Bernasso only on 4% of the fossil leaves.

4.4.3.4 Surface feeding

This rare FFG affects only 4.5% of the leaves from Iran. On the leaves of Astara, only 3% are impacted and it is significantly the same in Willershausen (χ^2, $p>0.5$). In the other locations, surface feeding is found between 4.5 to 5% of the respective floras of each locality.

4.4.3.5 Galling

Galling has a frequency of 6.3% on the leaves from the Hyrcanian forest, that makes galling the most important specialist FFG. The frequency is clearly lower than the main functional feeding group cited before. However, it is important to note that galling is a highly-specialized interaction which, consequently, is always represented lower in comparison to external damage. Despite this, the frequency of galling is the most important of the specialists' interactions in our dataset. It affects about 3% of the leaves at the different locations, except at Molla Kala, where its frequency is more than 16%. Regarding the relative altitudinal gradient, galling frequency does not vary, excepted at Molla Kala, which it increases with altitude from 3% to 35% (χ^2, $p<0.01$). 13% of the Willershausen leaves and 22% of the Bernasso ones were impacted by galling

4.4.3.6 Mining

Only two mines in Iran were found, one (DT224) at Molla Kala at 150 m a.s.l. and one (DT60) at Rudsar at 225 m a.s.l. 11% of Willershausen leaves have mining and just 1% in Bernasso.

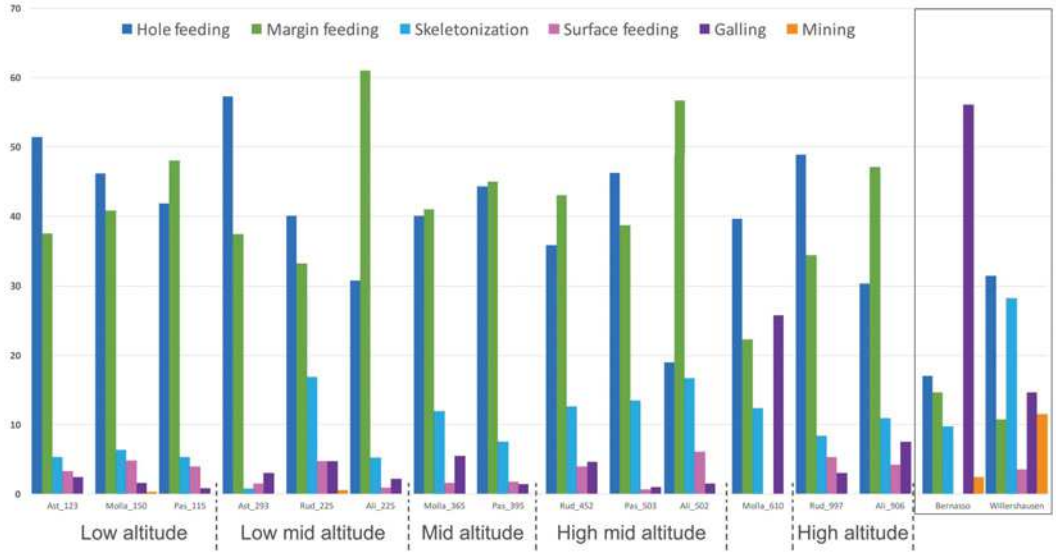

Figure 6: Frequency of *Parrotia persica* leaves with each Functional Feeding Group (FFG) at each site.

For comparison, on the framed to the right side, is presented the frequency of each FFG from the fossil outcrops of Bernasso and Willershausen. For each location, the sum of the percentage is equal to 100%. The nomenclature of the different Iranian sites indicates their exact altitude, for example, Ast293 means the site at Astara from 293 above the sea-level. *Nota bene*: We did not consider the site Ast_025 because it is not comparable to other sites based on the frequency of damage.

4.4.4 Patterns of plant-insect interaction among the locations

The PCA axis 1 represents by itself 86% of the variance while PCA axis 2 represents only 8% (Figure 7). Four of the five Iranian localities are located to the positive side of the PCA axis 1. The fifth Iranian locality (Rudsar) are close to the axes intersection, slightly in the negative side of the PCA axis 1. Both fossil outcrops are mainly correlated to the negative part of the PCA axis 1. The PCA axis 2 seems to only impacted Aliabad-e Katul location and a bit the fossil outcrop of Willershausen. PCA axe 1 seems to be mainly positively correlated with hole, margin and surface feeding while it is mainly negatively correlated with galling, mining

Figure 7: Principal components analysis (PCA) of *Parrotia persica* from each locality according to the diversity of damage type (DT) observed.

The significances of each axes are presented in Appendix N.

and piercing & sucking (Appendix N). The positive correlation with skeletonization is mainly observable along the PCA axis 2.

4.4.5 Detailed variation of plant-insect interaction along both altitudinal and longitudinal gradients in Iran

It appears that the main frequency and richness of interaction are located at the mid-altitude zones between 225 to 500 m a.s.l. At the highest altitude (Rud_997, Ali_906), the frequency is the lowest. At the relative highest site of Astara (Ast_293), the frequency of interaction is also significantly low (χ^2, p<0.01). In the lowest sites of all localities, the frequency of damages is similar to the mid-altitude zones but the damage richness is lower (Student test, p>0.05).

4.4.5.1 Frequency according to the gradients (altitude, longitude)

At Astara, the frequency of specialist interactions decreases significantly with the altitude, from 52% around 25 m a.s.l. to 15% around 293 m a.s.l. (χ^2, p<0.05). The linear regression trends show a dependence (r^2= 0.96, p= 0.1) between the altitude and the frequency decrease of specialist damages. At Rudsar the larger frequency of specialist interactions are also located at the lowest altitude, from 52% at 225 m a.s.l. to 15.4% at 997 m a.s.l. (χ^2, p<0.05). A correlation seems to exist between the altitude and the decrease of frequency of specialist interactions (r^2= 0.93, p= 0.1). Conversely, at Molla Kala the specialist interactions, that are mostly galling, increase significantly with the altitude (p<0.01), from 19.7% at 150 m a.s.l. to 49.2% at 610 m a.s.l. A correlation also seems to exist between the altitude and the frequency of specialist damage (r^2= 0.99, p= 0.06). At Pasand and Aliabad-e Katul (the eastern localities), no correlation between the altitude and the frequency of specialist damages was determined. At Pasand, the frequency does not change with altitude while at Aliabad-e Katul increases from 24% to 53% from the low site to the mid altitude site but it decreases at the high altitude to 22%.

Furthermore, for the mid-altitude zones (225 to 500 m a.s.l.) it appears that all the sites have significant differences in the frequency of specialist damages (p<0.05). On the contrary, at low altitudes and high altitudes, the frequencies of specialist interactions are similar for all sites (p>0.05).

4.4.5.2 Richness according to the gradients (altitude, longitude)

Astara and Aliabad-e Katul, the western and the eastern localities, have their damage richness that increases from the lowest altitude sites to the mid-altitude ones (Pairwise t test, p<0.001)

(Figure 8). This increase of richness is mainly due to the specialist interactions (p<0.001). At Ali-Aliabad-e Katul, this richness decreases at the highest altitude, i.e. 905 m a.s.l. (p<0.001).

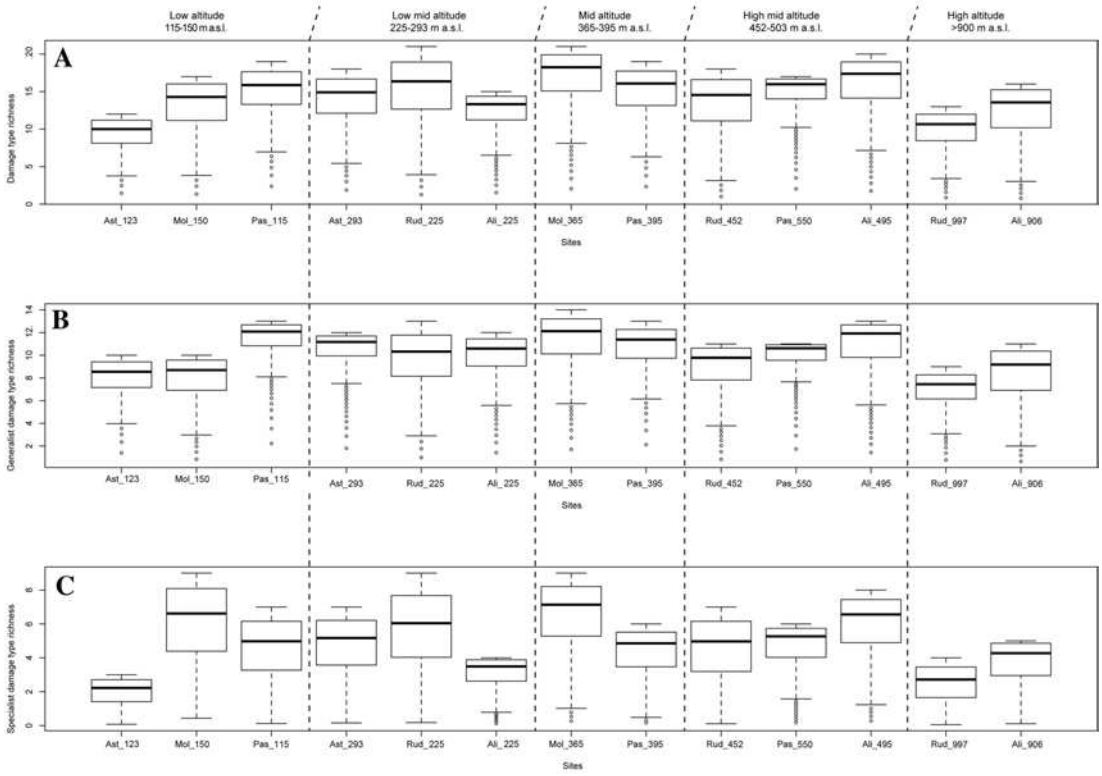

Figure 8: Box-plot of the mean richness of damage type (DT) at each site.
The sites are grouped by similar altitude and from West to East. (A) Mean damage richness; (B) Mean external damage richness; (C) Mean specialized damage richness.
Nota bene: We did not consider the sites Ast_025 and Mol_610 because they are not comparable to other sites in terms of DT richness according to altitude.

For this altitude, no data at Astara are available (Figure 8). At Rudsar we observe a decrease of damage type richness, due to the specialist interactions, from the mid altitude to the highest one (p<0.001) (Figure 8). At Molla Kala, an increase of DT richness is statistically confirmed from the lowest altitude to the mid altitude, mainly due to generalist interactions (p<0.001). From west to east, at low-mid altitude (under 400 m a.s.l.), the richness of specialist interactions is significantly more important in the center of the forest (Rudsar, Molla Kala, Pasand) than to the borders (Aliabad-e Katul, Astara) (p<0.001) (Figure 8). To the contrary, from 450 m a.s.l., this trend is reversed because we observed the richness of specialist damages is more important at Aliabad-e Katul (i.e. in the east) than in the sites of the center of the studied area (Rudsar, Pasand) (p<0.001) (Figure 8).

4.5 DISCUSSION

4.5.1 *P. persica* similarities between the locations

The richness of others plant species that we found during our sampling are similar in all sites. This is confirming that we are in the same forest formation, the *Parrotio-Carpinetum,* among our sites (Assadollahi, 1980; Talebi et al., 2014). Many studies suggest that the diversity of neighboring plants is a main determinant of insect attack and, consequently, affects plant-insect interaction (Agrawal et al., 2006; Bachelot and Kobe, 2013; Janovský et al., 2016). In our case, damage variation is probably not affected by such a parameter.

The leaf shape of *Parrotia persica* does not suggest a gradient of shape modification (Appendix J) contrary to other species which could vary according to its development or to some climate conditions (e.g. some of *Quercus* sp.) (Leckey et al., 2014). Eventually, only one study showed a slight plasticity from the leaves of *P. persica* in the angle of the apex (among many characters measured) according to the altitudinal change (Yosefzadeh et al., 2010) but it can be considered as trivial for the herbivory variation. The high similarity of the leaves of *P. persica* is not astonishing because a previous study already observed that in natural parcels the Persian Ironwood had same height and a very slight different of trunk diameter (Sefidi et al., 2015). According to this last study from Sefidi et al. (2015), it is probably because as an endemic species without plan conservation, *P. persica* probably could not reach an old-growth stage where multiple age cohorts are possible and then allows a high diameter differentiation (Jimu et al., 2013; Oliver and Larson, 1996). In the same way, the LMA, known to have an influence of the palatability of the leaves and thus to insect herbivory (Coley and Barone, 1996; Currano et al., 2008; Royer et al., 2007; Soh et al., 2017; War et al., 2012) are not significantly different in our Iranian studied sites (Appendix L). All these results confirm that general shape variations of *P. persica* could not be the cause of herbivory variations in Iran. However, we must to point out that LMA value of Willershausen could be significantly lower than the LMA values from Iranian sites (Appendix L) then could affect our interpretations. But this difference of result could also be caused by fossil leaf fossilization and by measurement on fossil leaf which is more imprecise than on fresh leaf.

4.5.2 Leaf-removed area

The low percentage of removed leaf area at high altitudes can correspond to a decrease of insect herbivory in general. (Roslin et al., 2017) shown that decrease of insect herbivory is correlated to elevation. The decrease of temperature with altitude is a factor that could affect directly the populations of insect (Bale et al., 2002; Deutsch et al., 2008) and thus herbivory while (Leckey

et al., 2014) observed on oak lineages no such a correlation between lower temperature and lower herbivory. However, a general tendency is the increasing of leaf thickness with the altitude (Hultine and Marshall, 2000; Zhao et al., 2014) which could also negatively affect the leaf removed area caused by insect feeding.

4.5.3 Variation of plant-insect interaction along abiotic gradients

Along the Hyrcanian forest, the frequency and the richness of plant-insect interactions seems to be influenced by both altitudinal and longitudinal gradients. In all localities, the damage frequency is more important at the mid-altitude zones. This is consistent with previous studies which showed a peak of insect diversity at middle altitudes, in temperate (Straw et al., 2009) and tropical forests (Janzen, 1970), and are responsible for high frequencies of damage. In the Hyrcanian forest sites the variation of plant-insect interaction is mainly due to the specialized damages. The proportion of specialist interactions is crucial for the structure of a forest and could significantly vary according to the climate variations (Ding et al., 2015; Kašparová et al., 2015), as a difference in response of both frequency and richness of the specialized interactions observed according to the elevation and the longitude (Figures 6 and 8, Appendix M). On *P. persica*, the specialized interactions correspond mainly to galling and some external damages (Figure 6).

In the Hyrcanian forest, under 400 m a.s.l., the frequency and the richness of specialized interactions on *P. persica* is higher in the western sites (Rudsar, Molla Kala and Astara but significant only for the frequency) where the MAP is the most important. This could also be observed in the highest sites at Molla Kala, also the wettest locality, where the frequency of galling shows the highest values (Figure 6). The results for galling are opposite than expected as galling should decrease in mesic habitats due to the high amount of precipitation that generates an increasing in fungi, main predator of gall-making insect (Carroll, 1988; Fernandes and Price, 1992, 1988; Lara et al., 2002). Moreover, it was suggested and observed by different studies, galling is an adaptation to harsh xeric environment (Fernandes and Martins, 1985; Fernandes and Price, 1988; Lara et al., 2002; Price et al., 1998) which is seemed not confirm with our observations. Above 400 m a.s.l. the richness of specialized interaction increases from west to east, following the general drought gradient, but these specialist damages are mainly external DTs. This is also not in agreement with previous studies which observed that external specialist DT was positively correlated with higher precipitation (Fernandes and Price, 1988).

However, these results which seems to be totally opposite and rather unexpected could be moderate by Veldtman and McGeoch (2003) who suggest that there still is no accepted

hypothesis to explain the richness of gall-making insect species at a particular location. Moreover, Fernandes and Price (1988) conclude that if gall-forming insects could be successful in drier habitats, this does not explain why the gall-forming insects could not also be abundant in the mesic habitat, as in our main observations. Furthermore, it is accepted that relictual distribution areas of plant species, as the one of *P. persica* today in the Hyrcanian forest, not necessarily reflect complete climatic and ecological capacity of the taxa (Bocsi et al., 2016). The reduction of *P. persica* distribution since at least the Pliocene (Probst, 1981; Tralau, 1963) seems to have forced *P. persica* to grow into very restricted environmental conditions. Such circumstances could had led to affect both richness and frequency features of plant-insect interactions.

4.5.4 Comparison of herbivory pattern though time

The high percentage of damage on Iranian leaves compared to the fossil leaves can be due to taphonomic and collect bias. Indeed, a damaged leaf could have less chance to be preserved in fossil record than a complete leaf due to different process of fossilization (Ferguson, 2005). Moreover, as the main goal of fossil leaf sampling was to obtain well-preserved specimens, it could happen than the most destroy leaves were voluntarily not collected. Lastly, the LMA values were not well known for Bernasso and were significantly different between Willershausen and Hyrcanian sites, it is preferable to not compare the percent of undamaged leaves between fossil and current locations; because LMA is correlated with the thickness and the density of the leaves and it is a parameter which can impact the frequency of leaves damaged by insect feeding (Currano et al., 2008; de la Riva et al., 2016).

Thus, to avoid some bias due to fossil preservation, we mainly focus our comparison on the damaged leaves and the proportion of the different FFGs. No common herbivory pattern between fossil and current *P. persica* is observable. European fossil leaves have higher proportions of specialized damage than the Iranian locality (Figure 7). There too, we did not expect these results.

Firstly, because specialization of damage should increase through time simultaneously with an increase of overall insect feeding (Ding et al., 2015) which is not the case for *P. persica*. The reduction of area distribution of *P. persica* since de the last millions of years (Probst, 1981; Tralau, 1963) could have also constrained many specialized interactions to decrease. Nevertheless, the geographic positions of the compared localities are different and other (local) factors could have impacted the ratio of specialist interactions. Secondly, Bachelot and Kobe (2013) indicated that closer is the latitude to the tropics, higher should be the diversity of

specialized plant-insect interaction (thus proportion of specialist damages), due inter alia to higher plant species richness. Lastly and above all, the climatic conditions in Iran seems to be more favorable for higher herbivory and especially for galling development (specialized damage). Indeed, the mean annual temperatures and also the temperatures of the coldest and warmest months from the Hyrcanian forest (Table 1, Appendix I) are all warmer than the ones estimated in Bernasso (Girard et al., in review; Suc, 1978; Leroy and Roiron, 1996) and Willershausen (Thiel et al., 2012). This could have reduced the diapause of insect and thus increased diversity of plant-insect interaction (Archibald et al., 2010). Furthermore, in northern Iran (especially on the eastern localities) tougher environmental conditions due to drought, should favor specialist damages (at least galling) (Fernandes and Martins, 1985; Fernandes and Price, 1988; Price et al., 1998).

As suggested by previous studies (Bocsi et al., 2016), specificities of *P. persica* are possibly due to its current restricted distribution area. Especially its pattern of insect interactions was different in Europe there are 3 million years ago from those present in the Hyrcanian forest. Nevertheless, similarities still exist between fossil and modern interactions on leaves of *Parrotia persica*. Indeed, the recent discovery of a new damage type only found on *P. persica* leaves at Willershausen and in the Hyrcanian forest (unpublished data) suggests that despite the historical events that happened on *P. persica*, some intimate interactions between *P. persica* and insects subsisted during the last 3 million years. This suggests that ancient plant-insect interactions, not really sensitive to climatic change, could be the source of some unexpected observations (Lara et al., 2002).

4.6 CONCLUSION

This work shows that both frequency and richness of plant-insect interaction must be considered separately as their response to climatic variation could be different.

Finally, it is clear that the gall-forming insects and free-feeding insects (generating the external damages) respond in different ways according to climate variation (e.g., Fernandes and Price, 1988; Leckey et al., 2014) and also to altitude (Leckey et al., 2014) on *P. persica* in the Hyrcanian forest. In contrast with previous studies, the plant-insect interaction on *P. persica* in Iran tend to not support that specialized interaction especially gall including is an adaptation to harsh xeric environment (Fernandes and Martins, 1985; Fernandes and Price, 1988; Lara et al., 2002; Price et al., 1998).

No similar pattern of plant-insect interaction between the fossil European outcrops and the different localities in the Hyrcanian forest. The history of *P. persica* taxa suggests that the

modern Persian Ironwood is not in its ideal environment that could have disturbed plant-insect interactions as our observation seems to support. Human activities could also be contributed to this reduction of distribution area and according to the vulnerability of 25 major biodiversity hotspots (Caucasus included), could lead to extinction all in all of c.a. 40% of endemic species (Malcolm et al., 2006).

However, more studies are needed in order to show if the variations observed are specific to *P. persica* or if it is a general trend in the Hyrcanian forest, at least on some others relict plant species. More investigations on chemical compounds in leaves (we know that nitrogen concentration could limit the herbivory on the plant (Langley and Megonigal, 2010; Slansky and Feeny, 1977; Soh et al., 2017; Strong et al., 1984), including galling (Abrahamson and Weis, 1987)) are necessary to comprehensively understand plant-insect interactions. Moreover, the absence of LMA variation on *P. persica* in our sampling did not necessary significate an absence of variation of nutrient concentration, as the relation between LMA and nutrient concentration is not so evident (de la Riva et al., 2016).

Finally, recent discovered on very specialized damages on *P. persica* in both fossil and current forest suggests deeper investigations

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CHAPTER V

A new damage type on fossil and modern leaves of *Parrotia persica*

In preparation

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NB: list of authors is preliminary

5.1 PREAMBLE

This part is the pre-draft of a future article. The article is not completely finished and need complements (especially for the discussion) and further figures before submission. A complementary work could possibly be made if measurements prove the presence of another new damage type on Parrotia subaequalis (H.T. Chang) R.M. Hao et H.T. Wei in China.

5.2 INTRODUCTION

Plants and insects greatly contribute to the biodiversity of Earth (Mitter et al., 1988). Together, they represent more than 70% of organisms present on Earth (Schoonhoven et al., 2005; Wilson, 1999). Today, the studies of plant-insect interactions is very important in ecology (Wappler and Ben-Dov, 2008). These interactions are recognized as primordial factors affecting the evolution of both taxa and more generally the development of the complexity of the terrestrial ecosystems (Krassilov and Rasnitsyn, 2008). Since the beginning of the plant-insect association, at least from the Devonian, many strategies constantly evolved until nowadays (e.g., Labandeira, 2006; Pinheiro et al., 2016; Wheat et al., 2007). It can correspond mainly to pollination (e.g., Crepet, 1983) and to herbivory interactions (e.g., Coley, 1988; Strauss and Agrawal, 1999) (non-exhaustive list). The latter are the topic of the present study. If these herbivory interactions can be studied in modern ecosystems (e.g., Sauvion et al., 2013), they are also investigable in the fossil record (Labandeira, 2002). In the fossil record, plant-insect interactions are mostly determined by traces on fossil leaves (e.g., Labandeira, 2002; Labandeira et al., 1994; Schoonhoven et al., 2005; Scott et al., 2004) and each identifiable interaction is called damage type (DT) (Labandeira et al., 2007). The guide of insect damage types on compressed fossil leaves from Labandeira et al. (2007) classified all the damages found in the fossil records into several functional feeding groups (FFG) such as hole feeding, skeletonization, piercing and sucking, etc. Most of the studies made on fossil plant-insect interactions throughout the last decade (e.g., Adroit et al., 2016; Currano et al., 2010; Donovan et al., 2014; Wappler et al., 2009) used this guide.

The guide of Labandeira et al. (2007) compiles most of the known fossil plant insect interactions, but new ones can always be found or referenced. This study presents an updated version of the first observations made by Straus (1977) in the Pliocene locality of Willershausen, Germany. He described an insect damage looking like a cord of multiples holes, more or less straight on the fossil leave of *Parrotia persica* C.A. Mey (Persian Ironwood). Originally this damage on the fossil record was named *Phagophytichnus*

catellarius ichnosp.nov and its holotype could be found in the Willershausen fossil collection of Göttingen in Germany (Nr. 2993/a; Holotypus, Z 10, F.339, D).

Today, *Parrotia persica* is an endemic species of the Hyrcanian forest in Iran (Akhani et al., 2010; Talebi et al., 2014), a forest considered by many studies as an analogue forest to the European paleoforests during the late Neogene – Quaternary (Arpe and Leroy, 2007; Noroozi et al., 2008; Probst, 1981; Tralau, 1963; Zohary, 1973) such as Willershausen. *Parrotia persica* is a deciduous tree belonging to the Hamamelidaceae. The genus *Parrotia* has only two species nowadays, *P. persica* and *P. subaquelis* (recently updated in eastern China; Geng et al., 2015; Li and Zhang, 2015). In the Hyrcanian forest, *P. persica* constitutes the dominant complex Parrotio-Carpinetum (in association with *Carpinus betulus* L) (Izadi et al., 2017; Talebi et al., 2014) and other plants composed also this vegetal complex such as *Zelkova carpinifolia* (Pall.) K.Koch or *Quercus castaneifolia* C.A.Mey (Assadollahi, 1980; Djazirei, 1965). In the Hyrcanian area, the climate is warm Mediterranean in the east and semi-temperate Mediterranean in the western and central part (Djamali et al., 2011; Sabeti, 1969).

During the Pliocene and the Pleistocene, *Parrotia persica* was present in Europe, in a climate relatively warmer than today (e.g., Haywood et al., 2000; Thiel et al., 2012; Uhl et al., 2007; Williams et al., 2009). Fossil leaves of *P. persica* were found in Andance (Late Miocene, France; Brice, 1965), in Willershausen (Late Pliocene, Germany; Knobloch, 1998; Straus, 1977), in Crespià (Early Pleistocene, Catalogna; Roiron, 1983), in Bernasso (Early Pleistocene, France; Leroy and Roiron, 1996), in Western Hungary (Pliocene, Hungary; Hably and Kvacek, 1998). Moreover, pollen was also recorded in the Podlasie region (Mid Pleistocene, Poland; Bińka et al., 2003) and in some others Pliocene-Pleistocene localities in Europe (Biltekin et al., 2015).

The recent reinvestigation of herbivory interactions on the fossil leaves of Willershausen (Adroit et al., submitted a) and the comparison of fossil and modern leaves of *Parrotia persica* (Adroit et al., submitted b) led to discover this similar insect damage originally observed in the fossil record (Straus, 1977) on the last and endemic population of *P. persica* existing nowadays. This damage was unknown in the guide of Labandeira et al. (2007) and was never mentioned in the current studies (Figure 1). In this article, this new damage will be described and discussed in regards to *Parrotia persica* ecology. A particular attention will be paid to the specific association of this new damage with *Parrotia persica* at least since the last 3 million years despite of the changes in environmental conditions of *P. persica*.

Figure 1: Drawing of *Parrotia persica* leaf with insect damage, including an interpretation of the DT297.

Illustration realized by Dorothea Kranz, Steinmann Institut, Bonn.

5.3 MATERIAL & METHODS

5.3.1 Leaf materials

Today, Persian Ironwood develops from the sea level until 1.000m a.s.l. (Nicholson, 1989), it is rarer to find it at an upper altitude in the Hyrcanian forest (Talebi et al., 2014). The trees can grow up to 20-30 meters tall and the wood is extremely hard (Coombes and Debreczy, 2015). The leaves are easily distinguishable as they are mainly oblong and simple, with an alternate phyllotaxy (e.g., Leroy and Roiron, 1996). Leaf margin is wavy with rounded teeth above the middle and the apex is rounded (Coombes and Debreczy, 2015). Their size are on average 15cm long and 6cm large (Coombes and Debreczy, 2015). *Parrotia persica* leaves were collected during the fall season (from October to December 2015) in five different localities along a longitudinal gradient from West to East, marked by hydric and thermic variations, respectively Astara, Rudsar, Molla Kala, Pasand and Aliabad-e Katul (Adroit et al.,

submitted b). Those locations are well-know as they were the focus of previous studies about climatic conditions, soil and plant taxa (resumed in Talebi et al., 2014). Detailed climatic information can be found in Djamali et al. (2011). For each location, three different sites through altitudinal gradient were sampled following the protocol from Adroit et al. (submitted b). In total, 2160 leaves were collected on the ground. A compact camera Sony TX1 was used to photograph the leaves.

Fossil specimens are originated from the famous Lagerstätte of Willershausen (Germany) (Ferguson and Knobloch, 1998; Knobloch, 1998; Straus, 1977). This is a lacustrine clay pit containing more than 130 plant species clearly determined through 77 diverse genera (Ferguson and Knobloch, 1998). The paleoforest represented by this fossil leaf assemblage is dated from 3.2 – 2.6 Ma; MN 16/17 (Hilgen, 1991; Mai, 1995). Many information about the paleoforest are known as its development, hydrography and taphonomy (Meischner, 2000). The presence of certain taxa, such as *Carpinus, Quercus, Fagus, Zelkova* and *Parrotia*, indicated a relative warmer climate (mean annual temperature around 15°C) than today (Thiel et al., 2012; Uhl et al., 2007). Detailed climate estimations can be found in Thiel et al. (2012). The fossil leaves of *P. persica* are mostly housed in the Göttingen museum collection (GZG.W collection) and few of them are in Stuttgart (SMNS.W collection), Kassel (NMOK.W collection) and Clausthal (TU Clausthal-Zellerfeld collection) collections, in Germany. Those fossil leaves were photographed with a Canon EOS 350D camera equipped with a Canon EF-S 60-mm f/2.8 macro lens. Moreover, a Nikon Coolpix E4500 was used through the binocular for accurate photography of the new damage type. All the photos were developed in Adobe Lightroom CC v.2015.

5.3.2 Measurements

On each leaf from the fossil and modern record, different characteristics were measured. In order to ensure a precise comparison, the methods usually applied on fossil leaf record were also used on the fresh leaf, the reverse not being possible. Leaf mass per area (LMA) of the leaf were determined through an established correlation between the width of the petiole and the length of the leaf blade (Royer et al., 2007). LMA is an index which corresponds to the relation between the thickness and the density of the leaf (de la Riva et al., 2016). Thickness of the leaf positively varies with the LMA; it is also the same with the leaf density and the LMA (de la Riva et al., 2016). The size of the leaves was categorized according to the leaf size classes from Webb (1959), detailed in Ellis et al. (2009). Shape of the leaf was estimated with the quotient between the length of the leaf and the length from the base of the leaf to the

part where the leaf blade is the largest (more details in Adroit et al, submitted b). The latter was only computed on the modern leaf as the leaf shape could strongly modify during the fossilization process (Adroit et al., submitted b).

The guide of (Labandeira et al., 2007) was used to determine all the damage found on the modern and fossil leaves. During this investigation, the new damage was found. A first qualitative description was established compared to preliminary observations made by Straus, (1977). Afterward, a quantitative description was computed with the measurements of length and width of the specific damages found on leaves, with photos of specimens handled on the software ImageJ.

5.3.3 Statistics analyzes

Statistical tests were used to support the significant differences in the measurements done on the fossil and modern leaves. A Shapiro test was used at first to check if the values for each measurement were normal, followed by a Fisher test to check the equality of the variances. According to the preliminary tests, a Student test or Student test with Welch correction were performed in order to support or not any significant differences in measurements of the damages between the leaves. To distinguish different sizes of the new damage between the fossil and the modern leaves, the truncated mean was used. This method was used mainly to avoid some vagueness during the measurements with ImageJ and also some errors due to the potential deformation during the processes of fossilization.

5.4 RESULTS

5.4.1 Overview of the sampling

This new damage type is now referenced as DT297 for a future updated version of the actual guide of insect damage types on fossil leaves (Labandeira et al., 2007). On the 517 leaves of *P. persica* in Willershausen, 71% are damaged. On the leaves damaged by insect feeding, 37 fossil leaves included the new damage type specialized interaction DT297 (7.1%) (see selected examples in Appendix O). In the Hyrcanian forest, out of all the *P. persica* leaves, 85% displayed plant-insect interaction. The damage type DT297 was only observed on 7 leaves (0.3%). One of those was found in Astara, on the site at 25m a.s.l., another one was found at 610m a.s.l. in Molla Kala, two of them in Pasand, both at 160m a.s.l. and three of them was observed in Aliabad-e Katul at 495m a.s.l.

5.4.2 Morphological description of the new Damage Type

There is no specific position of the DT297 on the leaf blade, neither a specific quantity of this damage per leaf. It has been defined as long bent concatenation of circular perforations less than 5mm in diameter. The curved chain of holes does not exceed 7cm long. Overall it is formed by less than 10 small holes. No increase of the width. The line that determines the holes is very thin, sometimes inconspicuous. The path of the damage is not affected by the leaf venation. The margin is strongly marked by dark edges. Furthermore, two kinds of the damage type DT297 have been noted in the fossil record. Their morphological description is the same, only the size is different. Henceforth, there is a distinction between $DT297_{foA}$ (DT297 fossil A) and $DT297_{foB}$ (DT297 fossil B). Consequently, to make the distinction, the damage in the current record is called $DT297_{cu}$.

5.4.3 Measurements of DT297 in fossil and modern records

The mean area of the $DT297_{foA}$ is 4.375 cm^2 and it is 3.47 cm^2 for $DT297_{cu}$. The normal distribution of the area values for $DT297_{foA}$ was confirmed ($p = 0.922$, $\alpha = 5\%$) as well as the normal distribution for $DT297_{cu}$ ($p = 0.96$, $\alpha = 5\%$). However, the variances equality between both $DT297_{foA}$ and $DT297_{cu}$ was not established ($p = 0.09$, $\alpha = 5\%$). Consequently, a Student test with a Welch correction was performed to compare their respective area. Those areas were not significantly different ($p = 0.31$, $\alpha = 5\%$).

This procedure was repeated for the comparison of the length values between $DT297_{foA}$ and $DT297_{cu}$ which respectively measured an average of 5.75 cm and 4.89 cm. Normal distribution was also confirmed by a Shapiro test for both $DT297_{foA}$ and $DT297_{cu}$ (respectively $p = 0.90$ and $p = 0.98$, $\alpha = 5\%$). As previously calculated, the variances were also significantly different ($p = 0.002$). Student test with Welch correction was also computed and revealed no significant difference between the length of the DT297 in the fossil ($DT297_{foA}$) and in the modern record ($p = 0.45$, $\alpha = 5\%$).

Finally, an average of the three measurements of the width along each damage was calculated. The mean width of $DT297_{foA}$ was about 0.67 cm and about 0.56 cm for $DT297_{cu}$. Normality for both width values distribution was checked ($p_{foA} = 0.80$ and $p_{cu} = 0.97$, $\alpha = 5\%$) and the Fisher test confirmed the equality of their variances ($p = 0.48$). Thus, Student test was performed and found no significant difference of width between $DT297_{foA}$ and $DT297_{cu}$ ($p = 0.03$, $\alpha = 10\%$).

5.4.4 Enlarger version of $DT297_{foA}$ in the fossil record: $DT297_{foB}$

The comparisons made with $DT297_{foB}$ were only computed with $DT297_{foA}$ as this larger version of DT297 was only observed in the fossil record. The mean surface area of $DT297_{foB}$ is 17.65 cm^2, the mean length is 10.81 cm and the average for the widths is about 1.53 cm. Similar procedures were computed to compare these measurements and obviously, Student

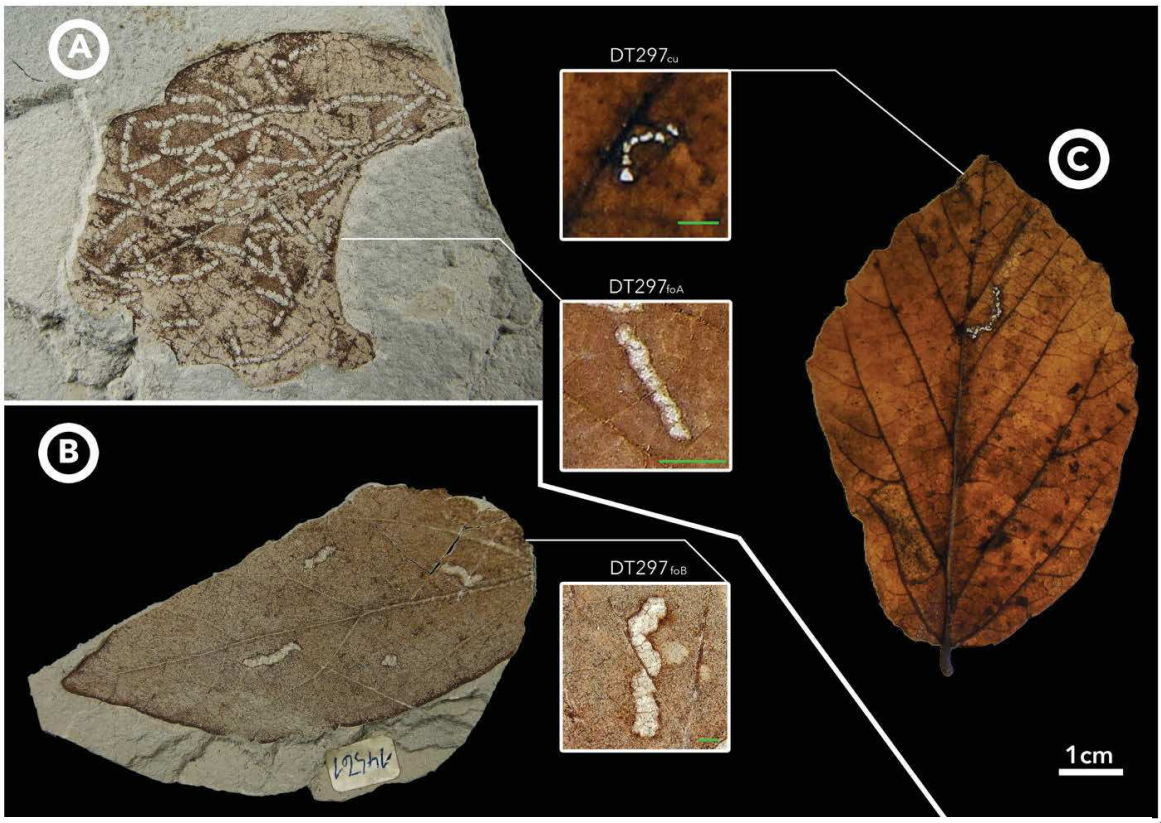

Figure 2: *Parrotia persica* leaves from the fossil record of Willershausen, Germany (A, B) and modern record of the Hyrcanian forest in Iran (C).

Photos of the leaves are in the same scale. White scale bar represents 1cm for the three leaf photos.
Enlargement of the diverse versions of the damage type DT297 are not represent with the same scale, due especially, to the different quality of camera used for the photography. Green scale bars represent 0.25cm.
Photos of the fossil leaves were shot by Benjamin Adroit and further ones can be observed in Appendix O.
Photos of the modern leaf were shot by Mahdieh Malekhosseini.

tests reveals a significant difference of these between $DT297_{foB}$ and $DT297_{foA}$ (p <00.1, α = 1%). The mean surface area of the $DT297_{foB}$ is approximately 4 times bigger than the mean area of the $DT297_{foA}$. The mean length of $DT297_{foB}$ is around 2 times longer than the $DT297_{foA}$'s and the average of width of $DT297_{foB}$ is also around 2 times wider than the mean width of $DT297_{foA}$.

Thereafter, in order to check if those multipliers are working with each value measured on each leaf, division of every measurements (length, area and width) of each $DT297_{foB}$ by each one of $DT297_{foA}$ were computed. Afterward, results of all these calculations were averaged

using the truncated means to exclude the extreme measurements. Thus, averages are 4.13 for the area, 1.86 for the length and 2.35 for the width. Consequently, the differences with the expected values are respectively about 0.09, 0.019 and 0.070. Differences between expected and observed values are less than 0.1, thence negligible.

5.5 PRELIMINARY DISCUSSION AND CONCLUSIONS

Contrary to preliminary observations from Straus (1977), our study testifies that DT297 also exists on *P. persica* modern leaves (Figure 2). In addition to the similar morphological description made on the modern DT297, statistical analysis confirmed no significant difference between $DT297_{foA}$ and $DT297_{cu}$. Consequently, this study assumes that it is the same damage type between fossil and modern record (Figure 2). As Straus (1977), we believed that this interaction could be made by a beetle (Coleoptera) from the *Chrysomeloidea* family even the subfamilies of *Halticinae* or *Hispinae*. Statistical measurement also confirmed the significant difference between $DT297_{foA}$ and $DT297_{foB}$. The distinction between two size categories of this damage type was also notified by Straus (1977) and he also believed that it was the same plant-insect interaction. There are two main possibilities that could be the source of this observation.

It could be from an ecological origin. This could be due to different larval stage. An increase of the mandible could explain a significant increase of the global size, especially the width, of this special damage type.

Another explanation could be taphonomic. It could be suggested that a difference in rock compression during the fossilization could lead to a flattening of the damage on the leaf, then involving an increase of the damage area. Numerous observations could support this theory. The black reaction rim surrounding the damage is darker and thinner on $DT297_{foA}$ than on $DT297_{foB}$ which suggests that this latter could has been flattened more intensely and thus generates a larger and less dark mark than $DT297_{foA}$. Moreover, both $DT297_{foA}$ and $DT297_{foB}$ were never observed on the same fossil leaves which could suggest different fossilization process along time. However, it is impossible to know if the Willershausen fossil leaves come from different layers, in which case it could support this hypothesis, or from the same fossil layer and thus disproves definitely this hypothesis. Furthermore, $DT297_{foB}$ was not observed on the modern record. This could support that the difference of leaf preservation could be the origin of the different observations between modern and fossil leaves and spearhead of $DT297_{foB}$.

The evidence of the presence of DT297, now exclusively on *P. persica* in Europe during the late Pliocene and on the same plant species endemic of the Hyrcanian forest nowadays, tends to confirm an anterior connection between the Caucasian and European paleoforests for at least 3 Ma. Additional comparisons should be made with other fossil traces of *P. persica* in Europe throughout the Neogene. For example, *P. persica* leaves were found in Dufort (Saporta, 1879). Expanding the comparison to the genus *Parrotia*, for example on *Parrotia pristina* (Kvaček et al., 2004) or *Parrotia subaequalis* (Geng et al., 2015; Li and Zhang, 2015), could also be relevant. Similar traces of DT297 seemed to be observed on *P. subaequalis* in China (Appendix P), deeper investigations are upcoming.

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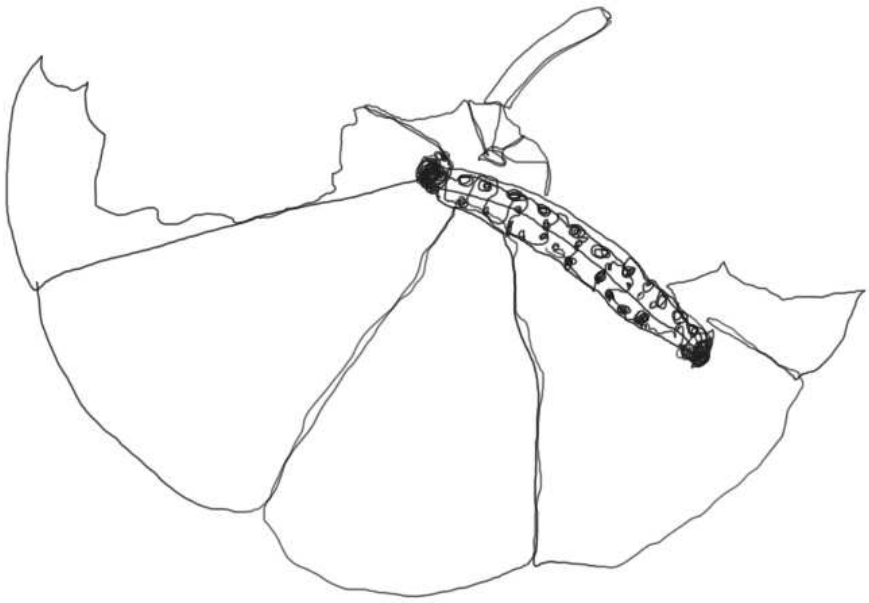
CHAPTER VI

Discussions and Conclusions



This thesis allowed a first assessment on plant-insect interaction from the late Neogene to the early Pleistocene in Western Europe. The mid-Neogene marked the last main floras change until nowadays, especially observed in western and southern Europe (Jiménez-Moreno et al., 2007; Jiménez-Moreno and Suc, 2007). Consequently, this study was focused on paleoecosystems quite comparable to the modern ecosystems and proposes to characterize the main factors involved on the differences of plant-insect interaction. Abiotic factor (such as climate) and some biotic factor (such as plant diversity) were discussed.

6.1 EVIDENT CAUSES ON THE DIFFERENCES OF PLANT-INSECT INTERACTION IN THE EUROPEAN PALEOFORESTS

As previous studies already noticed on both fossil and current leaves, it is undeniable that the climate was a crucial parameter which had significantly impacted plant-insect interactions in the paleoforests presented in this thesis (Chapters 3, 4). The following paragraphs intend to present convergent conclusions observed through the different chapters of the thesis.

6.1.1 Potential consequences of climate variations on fossil leaves

Previous studies already indicated that temperature is one of the most important parameters which could have an impact on plant-insect interactions (Bale et al., 2002; Currano et al., 2016, 2010; Fernandes and Price, 1992; Jamieson et al., 2012). Through fossil record studies, it is generally considered that an increase of temperatures lead to increase herbivory rates (e.g., Currano et al., 2008; Wappler et al., 2012). Ecological studies indicated that high temperature causes higher quantity of net primary production for the plants (Norby and Luo, 2004; Rustad et al., 2001) and generates higher quality of foliar tissue (Hunter and Lechowicz, 1992; Nealis and Nault, 2005). This could have an impact on plant-insect interaction as insect feeding is closely related to the foliar quality (Scriber and Slansky, 1981). Warmer climate also increases intensity and occurrences of dry season, which could affect plant-insect interactions in different ways (e.g., Huberty and Denno, 2004). For example, drier conditions tend to increase sugar quantity within the leaves that is benefit for insect feeding (Koricheva et al., 1998; Mattson and Haack, 1987).

Nonetheless, knowledge about temperature and secondary metabolites is limited (Bidart-Bouzat and Imeh-Nathaniel, 2008). Some experimental studies suggested that warmer climate conditions lead to reduced phenolic compounds in plants, like tannins, but could also enhance others such as terpenoids, both being repulsive for herbivorous insects (Bidart-Bouzat and Imeh-Nathaniel, 2008; Zvereva and Kozlov, 2006). Climate variation could also have an impact to the tolerance of plants to insect feeding (Jamieson et al., 2012). For example, it has been analyzed on some birch tree species that warm temperatures increased the tolerance capacity of the plants

against insect feeding (Huttunen et al., 2007). This is certainly because the increase of temperature is rising the net primary production and the time of growth season, both are beneficial for plants to compensate herbivory (Jamieson et al., 2012). Thus, it is not surprising if some studies, such as the one from Leckey et al. (2014) on oak lineages, suggested that mean annual temperature has no evident effects on the leaf removed area by insect feeding. In accordance to this latter and contrary to many studies (e.g., Currano et al., 2008; Wappler et al., 2012), no relation between the mean annual temperature and the plant-insect interaction was measured through the three fossil outcrops (Chapter 3).

6.1.2 Consequences of coldest temperatures on herbivory rates

The estimated temperatures of the coolest months could be an important factor in the difference of plant-insect interactions (Chapter 3). Between Willershausen and Berga, and between Willershausen and Bernasso it appeared that the cooler are the coldest periods then the lower are herbivory rates. These observations are in accordance with diapause time of insects during cold climatic conditions (Gilbert et al., 2005; Tauber et al., 1986). Consequently, a higher quantity of insect in diapause could reduce insect damage frequencies (Chapter 3). Nevertheless, as insect are ectothermic organisms (May, 1979; Meglitsch, 1972), meaning they are extremely sensitive to temperature variations, their metabolism is synchronous with the phenology of their host plants (Bale and Hayward, 2010), and climate change will be a major factor affecting this synchronism (Bale et al., 2002; Parmesan and Yohe, 2003). It is in agreement with Roy et al. (2004) that noted various responses of insect herbivores on their plant hosts to the "global change" then could cause different patterns of plant-insect interaction among plant species.

However, this relation could not be confirmed between Bernasso and Berga as the estimated cold temperature for Bernasso overlap the ones estimated for Berga, consequently it is not possible to know which of those paleoforests had the coldest temperatures in wintertime (Chapter 3). Moreover, it is important to note that while the plant richness is similar between the fossil leaf assemblages, the insect richness is not clearly known and insect species may have different requirements during diapause (Hahn and Denlinger, 2007). For example, the black blowfly (*Phormia regina* Meigen) could feed during diapause, while others insect had no activity during diapause. Although the consumption of black blowfly is lower than 80% to compare when they are not in diapause (Stoffolano, 1975).

6.1.3 Consequences of thermic and hydric seasonality on specialized plant-insect interactions

Leckey et al. (2014) also observed that precipitations mainly affected the damage richness, and especially the proportion of generalized and specialized damages in an ecosystem. These

observations are supported by different previous studies also which suggested that drought stress conditions on plants should affect responses amongst generalist and specialist insects (Gutbrodt et al., 2011; Huberty and Denno, 2004; Koricheva et al., 1998). Diversity of galling (specialized damage) seems inversely correlated to the amount of precipitations (Fernandes and Price, 1992; Lara et al., 2002) because in humid conditions, fungi, the major factor of gall-maker insects' death, develop better (Carroll, 1988). Contrary to the expectations, neither among the fossil outcrops (Chapters 2,3) nor on modern *P. persica* (Chapter 4) the diversity of galling was inversely correlated to the mean annual precipitations. For example, Bernasso had the highest mean annual precipitations of the three studied fossil localities (Girard et al., in review; Thiel et al., 2012; Uhl et al., 2007) and it has also the highest proportion of galls (Chapters 2,3). However, the distribution of precipitation in Bernasso during a year should have been very heterogeneous with an enhanced seasonal contrast and a marked dryness during summertime as southern Europe was in Pleistocene (Biltekin et al., 2015; Popescu et al., 2010; Suc, 1984; Suc et al., 1995) and as the Mediterranean climatic regime is nowadays (Bagnouls and Gaussen, 1957; Daget, 1977). Girard et al. (in review) supposed for Bernasso a hydric seasonality with a probable marked humid season. In the Hyrcanian forest, comparable measurements were made (Chapter 4). For example, the site Molla Kala had the highest galling proportion and the highest mean annual precipitations of all the studied sites. Also in Molla Kala, the distribution of the precipitations along the year is the most heterogeneous of the five studied Iranian localities, suggesting a high stress environment for the plants but also for their consuming insect communities (Chapter 4). As for Bernasso, the distribution of precipitations, which could lead to higher stress environment, seems essential in the differences of galling diversity (Chapters 3,4).

Finally, in this thesis the climate seasonality was mainly distinguishable by the estimated distribution of the precipitations during the year and also the amplitude of temperature (Chapters 2,3,4). Indeed, the higher seasonality of temperature supposed in Bernasso than in Willershausen and Berga (Chapter 3) could be reinforced by studies which reveal an increase of temperature seasonality during the intensification of glaciation at the Plio-Pliocene boundary (Hennissen et al., 2015), notably observed in southern Europe (Popescu et al., 2010; Suc, 1984). Moreover, the reduced temperature seasonality during the Pliocene was also confirmed by Williams et al. (2009). This difference of climate seasonality could be the main factors of the highest diversity of galling in Bernasso (Chapter 3). Some studies shown that galling was well-acclimated to harsh environments, especially marked by dry season (Fernandes and Martins, 1985; Fernandes and Price, 1992, 1988; Lara et al., 2002; Price et al., 1998) such as could have been the case in Bernasso (Chapter 3, Girard et al., in review). Contrary to Veldtman and McGeoch (2003) which suggested that there was no admitted assumption to justify the richness of insect gall-markers species in

particular location, observations made in some European paleoforests and reinforced by observations made in their current analogue in Iran, tend to suggest that galling differences could be mainly drive by high hydric seasonal during the year (Chapters 3,4).

6.2 LIMITS FOR INTERPRETATIONS

6.2.1 Biotic parameters causing changes on insect damage rates

Biotic parameters could also have an impact on the change of plant-insect interactions (such as the plant species diversity) (Mulder et al., 1999; Price, 2002, 1991, 1991; Price et al., 1995).

Firstly, on some common plant species between the paleoforests, similar patterns of plant-insect interaction were identified in regard to their FFG proportions, while the climate between these locations are supposed to be different (Chapter 3). There are many relation very specific between some insect and plants (e.g., Logan et al., 2003; Powell and Bentz, 2009) which could probably explain similar pattern of plant-insect interaction in two different environments.

Secondly, it has been shown that plant diversity difference could be a cause of the difference of damage richness (Price, 2002, 1991). In Willershausen the significant higher damage richness than in Berga could consequently be due to the important diversity of plant in Willershausen (Chapter 3). However, this relationship is not working with Bernasso as this paleoforest had the highest damage richness but the lowest plant diversity (Chapter 3). The absence of convergent relationship about this biotic parameter could be due to two major explanations. (1) The impact of biotic parameter is less important than the climate impact, but it is clearly impossible to prove that, especially in the fossil record. Moreover, it sounds possible that the late successional age of a forest (i.e. biotic parameter) (Leroy and Roiron, 1996) could be one of the main cause explaining the large diversity of galling (Fernandes et al., 2010) as observed for Bernasso (Chapter 2). (2) Taphonomic bias and fossil sampling could have an impact in the observations.

6.2.2 Taphonomic and methodological biases

Everything that separates the moment when the leaf fell down and when it has been analyzed could have disturbed the observations that have been made. Unfortunately, it is complicated (perhaps impossible) to determine the importance of these biases.

Fossilization may be different. It appeared that the fossil leaves from Berga outcrop were preserved on very poor condition comparing to Bernasso and Willershausen outcrops (Chapter 3). This is probably due to a difference of fossilization process and that could affected our observations (Alroy, 2010), especially when the aim is to quantify the leaves damaged by insect feeding. Indeed, Ferguson (2005) suggested that higher the leaf is damaged and lower chance it has to be well-

preserved during fossilization. This could potentially distort the proportion of leaves damaged and undamaged measured in “Chapter 2” and “Chapter 3”.

Fossil collection may be different. As it seen in the thesis, the number of leaf analyzed strongly differs especially between Willershausen and, Berga and Bernasso (Chapter 2,3), also the quantity of *P. persica* leaves was different for the comparison between modern and fossil leaves (Chapter 4). Thus, diversity of plant-insect interactions could be misinterpreted. Consequently, instead of extrapolate proportions of taxa, rarefaction curves with replacement (Gunkel and Wappler, 2015) were used. This allows a normalization of the data and a fairer comparison (Gotelli and Colwell, 2001). However, methodological process could vary from a researcher to another one (Alroy, 2010), meaning that while diversity curves were calculated, it did not necessary mean a fair sampled of the paleoforest (Newell, 1952; Simpson, 1949).

Fossil preparation may be different. A part of the fossil leaves from Bernasso outcrop were opt out of the rock and put between two glass slides with Canada balsam (Chapter 2). It has been measured significantly more galls and margin feeding on the leaves prepared with Canada balsam (Chapter 2). It suggested that potentially galls could be underestimated on the leaves still preserved on the rock because they were more easily observable (Chapter 2). Conversely, the fossil preparation lead to overestimated the margin feeding because Canada balsam tends to darken the leaf borders, and it could be confused with margin feeding damage (Chapter 2). Finally, the leaf fossil preparation could significantly affect insect damages observation, so it must to be used carefully. These statements were taken into account for fossil comparison between different outcrops. Furthermore, these black reactions rims, mentioned above for margin feeding for example, are essential to distinguished the feeding traces done when the leaves were alive (mostly by insect) from the removed area caused by detritivores once the dead leaves were on the ground (Labandeira, 2002). However, it must to be admitted that other organisms than insects could also do a tiny amount of “feeding traces”. Nevertheless, for the different observations in this thesis, an updated (and unpublished yet) version of the guide from Labandeira et al. (2007) was used and it distinguished many “feeding traces” done by fungi; additionally a FFG called *incertae sedis* is used when the trace origin is not certain. These precautions tend to minimalize the chances of a misinterpretation about damages observed on the leaves.

6.2.3 Plants physiology

The leaf shape could be a good indicator for the plant growth and strategies of plants (Wang et al., 2015). Recently, Zhang et al. (2017) measured that leaf shape of *Quercus acutissima* Carruth. could vary according to nitrogen (N) and phosphorus (P) compounds in soil. In this way, "Chapter 4" partly estimated the leaf shape (through a ratio of lamina measures) of the *P. persica* along the

different sites in the Hyrcanian forest, but no significant difference of the shape of the leaves were measured.

Leaf mass per area (LMA) is also a good indicator for leaf compounds in the leaves (e.g., de la Riva et al., 2016; Zhang et al., 2005). Leaf mass per area is generally positively correlated to the thickness of the leaf and low nutrients concentration, i.e. to a lower palatability of the leaf (Wright et al., 2004). It has been measured that high LMA could lead to low insect feeding (Coley and Barone, 1996; Royer et al., 2007). Willershausen leaves did not indicate a significant correlation between LMA and herbivory rates (Chapter 3), but negative correlations between herbivory rates and LMA were already computed in the fossil record (e.g., Currano et al., 2008; Royer et al., 2007). In fact, the leaf mass per area depends on both leaf thickness and leaf density (de la Riva et al., 2016) which could respond differently to change of many chemical compound concentration (Garnier and Laurent, 1994; Villar et al., 2013). Leaf thickness is mainly positively correlated to an increase of carbon (C) into the leaf (Coley, 1983; de la Riva et al., 2016; Royer et al., 2007; Westoby et al., 2002). Leaf density is negatively correlated to the nitrogen concentration (de la Riva et al., 2016; Garnier and Laurent, 1994; Villar et al., 2013; Wright et al., 2004). Thus, an increase of leaf mass per area leads to an increase in the C/N ratio in the leaf. Moreover, the C/N ratio is positively correlated to the herbivory as the insect should feed more to compensate the low nitrogen concentration (Watt et al., 1995). These astonishing correlations could nevertheless be explained by different plant strategies. Firstly, some studies show that on certain plant species (such as *Linaria dalmatica* (L.) Mill) and their associated herbivores (such as *Calophasia lunula* Hufnagel) the variation of nitrogen is not impacting plant defense and herbivory (Jamieson and Deane Bowers, 2012). Secondly, the correlation mentioned between leaf mass per area and herbivory rate is in most cases due the differences between the evergreen and deciduous plants strategies (de la Riva et al., 2016). That could partly explain the non-significant results observed in Willershausen because most of the species are deciduous (Ferguson and Knobloch, 1998; Knobloch, 1998).

All these observations indicate that the difference in strategy for resource acquisition from the plants species could impact the difference of plant-insect interaction. For example, in Bernasso, *P. persica* and *C. orientalis* which are the co-dominant species of the forest group *Parrotio-Carpinetum* (Assadollahi, 1980; Bazdid Vahdati, 2014; Djazirei, 1965; Dorostkar and Noirfalise, 1976; Hejazi and Sabeti, 1961; Izadi et al., 2017; Mossadegh, 1981; Talebi et al., 2014) are present on the same soil category (rendzina soils) (Izadi et al., 2017; Talebi et al., 2014) and they presented a total opposition of the proportion of generalized and specialized damages. Indeed, *P. persica* on 37% of damaged leaves contained 23% with specialized damage and 11% with generalized damaged, while *C. orientalis* on 30% of damaged leaves presented the inverse ratio (respectively

11% and 22%) (Chapter 2). Another example, in Willershausen the plant species *Leguminosites strausii* E. Knobloch (Fabaceae) had less than half of the leaves damaged by insect feeding. Fabaceae are well-known to be excellent nitrogen-fixing (Wink, 2013) and as N-fixing plants, the Fabaceae should be subjected to relatively more insect consumption (Vance et al., 1979), that was certainly not the case in Willershausen (Chapter 3). These two examples observed in different fossil leaf assemblages (Chapters 2,3) could not be more discussed for now as the correlations between insect feeding and plant species are still not enough quite known for these precise relationships. However, it was already confirmed that resources, such as nitrogen in soils, could be a cause of interspecies competition in an environment (e.g., Kaye and Hart, 1997) which tended to maintain the community balance (Leroy, 2009) and the plant-insect interactions could be impacted by this competition (Strauss and Agrawal, 1999).

6.3 NEW PERSPECTIVES

For now, only few studies offered general overviews in the fossil record about notable difference of insect damage and its associated factors (e.g., Currano et al., 2016; Labandeira et al., 2002; Wappler et al., 2012, 2009; Wilf, 2008; Wilf et al., 2001). Studies on "recent" geological time periods are less common but offer greater possibilities to be compared with the actual ecosystems (e.g., Su et al., 2015). This thesis contributes to the first studies made on late Pliocene — early Pleistocene time period, with a focus on European paleoforests.

6.3.1 Increasing knowledge on the history of the European paleoforests

In order to enhance and to improve the understanding of European paleoforests structure from the Neogene until the present, it is essential to analyze more fossil outcrops with fossil leaves. Numerous fossil outcrop including leaves in Europe are available such as Crespià, Spain (Roiron, 1983), Murat, France (Roiron, 1991), Pincina, Slovakia (Sitár et al., 1989) from the Neogene and Leffe, Italia (Ravazzi, 1995) or Meyrargues, France (Magnin et al., 1990) from the Quaternary; a detailed and non-exhaustive list of European fossil leaf assemblage could be found in different publications (e.g., Kovar-Eder, 2003; Kovar-Eder et al., 2008; Martinetto, 1999; Suc and Zagwijn, 1983). Moreover, new sites continue to be discovered as one from the Pliocene in northern Italy (Macaluso et al., 2017), besides their first results suggest similar plant species richness than from Willershausen, Berga and Bernasso (Chapters 2,3).

These multiple comparisons could be also an opportunity to discuss about other correlations between plant-insect interaction and environmental parameters which have not been measured in the studied outcrops. For example, neither in Bernasso (Chapter 2) nor in Willershausen and Berga (Chapter 3) mining was significantly represented to be compare statistically between the outcrops;

yet during the Neogene leaf-mining insect clades were relatively diverse and abundant in Europe (Givulescu, 1984; Straus, 1977). Mining, as galling, is a very specialized plant-insect interaction (Connor and Taverner, 1997; Labandeira, 2002). An advantage of feeding within a leaf mine is to be protected from the desiccation and the effects of UV radiation (Connor and Taverner, 1997). Mine feeding traces could also be related to diverse parameters such as plant phenology (Faeth et al., 1981) or leaf area (Basset, 1991) for example and some studies were already focused on mining interaction through different time periods and geographic locations (e.g., Ding et al., 2014; Donovan et al., 2014; Opler, 1974; Robledo et al., 2016). It could be interesting to discuss about the relative low proportion of mining in Willershausen, Berga and Bernasso, comparing their results to other European paleoecosystems in order to improve the understanding of the structure of those paleoenvironments.

Additionally, as the Hyrcanian forest is considered to be a possible modern analogue of European forests from the early Quaternary (Leroy and Roiron, 1996; Noroozi et al., 2008; Probst, 1981; Tralau, 1963; Zohary, 1973), studying mechanism of plant-insect interactions in the overall forest should bring out relevant information. The comparison of damage patterns from both fossil and current *P. persica* leaves contributed to a first step in this way (Chapter 4). Finally, plant-insect interaction patterns should be studied in the current European plant communities with the same approach used in "Chapter 4" in order to estimate evolution of European forest structures for the last millions of years. Theoretically, based on Price (2002, 1991), it could be expected to observe a general lower richness of plant-insect interaction today as the plant species diversity in the forests decreased in Europe since the late Pliocene (Campbell, 1982; Grubb, 1987; Huntley, 1993; Latham and Ricklefs, 1993). However, expectations are complicated to be estimated because the “global change” disturbed numerous ecological process, for example it allowed the invasion of new insect species in some European temperate regions (Chapman et al., 2012; Sparks et al., 2005). Moreover, pollution caused by human activities generates an increasing of herbivore insect populations on plants (Hain, 1987). At last, “global change” also favor the increase of both precipitation and temperature seasonality (IPCC, 2007) which could potentially impact plant-insect interactions (Chapters 2,3,4).

Furthermore, plant-insect interactions related also to a coevolution history between the species (Farrell et al., 1992). During this thesis, a new damage type (DT297) was discovered exclusively on modern and fossil *P. persica* leaves (Chapter 5). This discovered on such specific taxa suggests that information about historical evolution of forests could be analyzed with the comparison between fossil paleoforest and current forest. For example, this specific damage on *P. persica* (i.e. DT297) suggests an anterior forests connection between the Caucasian and western-European regions there are, at least, 3 Ma ago. In addition, a new species of *Parrotia* (*Parrotia*

subaequalis (H.T. Chang) R.M. Hao et H.T. Wei) was recently updated in southeastern China (Geng et al., 2015; Li and Zhang, 2015). A preliminary cooperation suggests the probable presence of DT297 on *P. subaequalis*, (Adroit et al., unpublished data). If the results are to be proven, that could improve the knowledge of forest evolution of whole Eurasia.

6.3.2 A better understanding of climatic impact

As seen throughout the thesis, relationships between plant-insect interactions and climate are still misunderstood. For example, some studies focused on gall-making insects, presented some opposite responses to climate variation of those insects according to dry or humid forest habitats (Cuevas-Reyes et al., 2004, 2003). It seems that a climatic change in different ecosystems could have different impacts on plant-insect interactions. Consequently, it should be relevant to focusing analyzes on plant-insect interaction on similar ecosystems in order to measure if damage patterns are also similar. There are many other paleoecosystems from the Neogene which have been characterized in Europe (Kovar-Eder, 2003) and multiple comparisons to Willershausen and Berga (Chapter 3) could provide more information about the factors involved on plant-insect interactions patterns. In order to obtain accurate interpretations, such comparisons should also be done on current ecosystems (e.g., Su et al., 2015).

6.3.3 Improving comparison of plant-insect interactions among paleoforests

As stated in the thesis, there are also some limits in the interpretation of fossilized plant-insect interactions by the presence and absence of particular feeding types due to various taphonomic filters (Chapters 2,3). In general, those mechanisms are still weakly understood (Grimes et al., 2001). Next perspectives in taphonomy should be to estimate as much as possible the distorts in interpretation of plant-insect interaction on the fossil leaves. Investigations on taphonomic problems were already conducted especially on plants. Fossil assemblages are impacted by many different factors such as the distance of the tree from the deposit area or also the size, shape, or thickness of the leaves (Chaney, 1924; Chaney and Sanborn, 1933). Experimental studies, based on pyritization of the plant, simulated fossilization process and they showed how fast could be the fossilization in anaerobic surroundings and how it could affected the fidelity of the taxonomic preserved fossil (Grimes et al., 2001). Reproduce these kinds of experiments on fossil leaves could be relevant to show how damaged leaves are generally preserved and which impact it could have on the damage preservation. Moreover, for a comparison of the plant-insect interactions from different outcrops, it would be wise to firstly compare the percentage of leaf destroy area. The percentage of leaf destroy area would correspond to the difference between the complete filled fossil leaf (reconstituted) and the observed fossil leaf shredded by insect feeding and scavenger

activities. As previously mentioned in the thesis, more a leaf is shredded and less the leaf has chance to be preserved (Ferguson, 2005). Various fossilization processes impact differently those preservations. Thus, a higher leaf destroy area in an outcrop rather another one, increase the chance to observe more plant-insect interaction which could distort interpretations. The comparison of the leaf destroy area on common plant species between different outcrops, should allow estimating how the difference of fossilizations could affect interpretation about plant-insect interactions.
Furthermore, it is essential that a common method for identification of plant-insect interaction must to be used in the way to allow general comparisons between diverse fossil leaf assemblages. Indeed, the guide of insect (and other) damage types on compressed plant fossils (Labandeira et al., 2007) prove to be the best suited. However, it has to be continually improve obliviously by an increase of sampling, as the recent discovery made on *P. persica* leaves (Chapter 5).

Moreover, in order for this guide to be used in the most studies, the vocabulary has to be more accurate. This thesis suggests that the functional feeding group (FFG) has to be rename as functional interaction group for example. Indeed, according to the galling definition in Labandeira (2002), the plant tissues resulting to the sting of the insect, for its larvae development, is developing without there being any herbivory. It is only after, when the insect larva develops, that the larvae may potentially feeds the nutrient contained in the gall (Labandeira, 2002), thus insect feeding is only secondary in this case. One has to admit that the term functional "feeding" group could influence the readers or future researchers interested in plant-insect interaction of fossil (and modern) leaf records, then it could affect ecological interpretations, especially if the identifications of new damage types were to increase.

6.3.4 Outlooks

To conclude, the relationships between plants, insects and abiotic parameters (such as climatic factors) have major implications on global biosphere cycles such as carbon, nitrogen or water (McDowell et al., 2011). In the global context, the fast increase of global temperature will probably force plants to adjust their growth more than improving their defenses (Herms and Mattson, 1992). Therefore, increase of insect feeding in forests could possibly accelerate the global change, because the forests are the major carbon reserve and will therefore release it into the atmosphere, then increasing greenhouse effect (DeLucia et al., 2012; Hicke et al., 2012). As it has been suggested in this thesis, variation of specialized/generalized damages should be the main focus in future studies about plant-insect interactions and their correlations to climate parameters such as temperature and precipitation.

Moreover, as recently suggested by Carré and Cheddadi (2017), this thesis corroborates the importance of the climate seasonality in the difference of ecological patterns, here the plant-insect

interactions. Indeed, the amplitudes of both precipitations and temperatures condition the plant growth, especially the leaf development over the year (van Asch et al., 2007). The low variation of these climatic parameters between daytime and nighttime under tropics favor the leaf development (Wright et al., 2017) in order to, *inter alia*, overcompensate the high herbivory rate (McNaughton, 1983; Wright et al., 2017). However, for now the estimated relationships between generalized and specialized plant-insect interactions triggered by seasonality of climatic parameters (and their indirect consequences) seemed to be more complex than previously thought.

Consequently, it is essential to study plant-insect interactions trough different time periods in order to understand their evolutionary mechanisms. Pinheiro et al., (2016) already studied variation of herbivory pattern through long time period from the Devonian to the Miocene periods. Subsequent to this previous study, this thesis proposes to focus on the variation of plant-insect interaction on “recent” paleoecosystems as they are central for an understanding of food web dynamics and biodiversity patterns for the future.

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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 galling. The 2 different sizes of the circles indicate that there are more than 20% of leaves with galling for the bigger size, and less than 20% for the smaller. Information about galling was obtained comparing Bernasso Galling and data from Fernandes and Price (1996) and Fernandes et al. (2010). The symbols + and - respectively means high and low. Three 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).

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

Figure 1: The location of Willershausen and Berga outcrops.
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)

Figure 2:
Plate Y. Fossil leaves from Willershausen (Göttingen coll.).
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Plate Z. Fossil leaves from Berga (Dresden coll.).
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Figure 3: Quantitative distribution of plant-insect interactions from Willershausen, Berga outcrops and the fossil deposit of Bernasso (from Adroit et al., 2016).
Significant difference (alpha <0.05) from an outcrop to another one is marked by an asterisk. The percent of generalized and specialized damages are computed with the damaged leaves, thus their sum is 100%.

Figure 4: Rarefaction curves on the leaves from Willershausen, Berga and Bernasso (from Adroit et al. 2016).
The grey curves represent Berga, the blue curves represent Willershausen outcrop and the orange curves represent Bernasso. 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: Percent of leaves damaged according to the leaf mass per area (LMA).
The LMA was estimated using the method of Royer et al. (2007). Each dot
represents a plant species. Each species was considered only if at least 10 leaf from the species were measurable.

Figure 6: Principal components analysis (PCA) of plant species according to the diversity of damage type (DT).
A- Willershausen
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;*
B- Berga; C- Bernasso
Detailed significance of PCA's axes are presented in Appendix G.

Chapter IV: Changes on herbivory pattern on the Persian ironwood (Parrotia persica) over the last 3 million years

Figure 1: Map of the Hyrcanian forest in the Caucasian region.
The green zone represents the Hyrcanian forest located in the Northern part of Iran, southern Caspian Sea. The red stars represent the five localities choose for our study from West to East: Astara, Rudsar, Molla Kala, Pasand and Aliabad-e Katul. Above the map, the climatographs of each locality, realized by the climatic data of the Iran Meteorological Organization (IRIMO)

from 1995 to 2005. The orange area indicates the dry season. The hatched blue area indicates the extremes values of precipitation rate at Astara, Rudsar and Molla Kala, then it does not respect the scale of the Y axis.

Overhead, zoom on locality that showing the three sites at different altitudes. The maps were obtained with the Wordclim dataset (available at http://www.worldclim.org/download; Hijmans et al., 2005) at a resolution of 30 arc-second. The nomenclature of the different sites indicates their exact altitude, for example, Ast293 means the site at Astara from 293 above the sea-level.

Figure 2: General overview of the field trip into the Hyrcanian forest.

(A) Global overview showing a part of the forest enclosed in the valley of the Alborz mountains. From West to East: (B) Astara, (C) Rudsar, (D) Molla Kala, (E) Pasand and (F) Aliabad-e Katul. (G) *Parrotia persica* leaves, colors range from green to bright red, characteristic of the Persian Iroonwood leaves, the scale for this picture is 10cm

Figure 3: Examples of external foliage feeding on *Parrotia persica*.

A, Small-sized circular perforations (DT1), indicated by arrow (loc. Astara). B, Medium-sized circular perforations (DT2) (loc. Astara). C, Large-sized, polylobate perforations (DT5) (loc. Astara). D. Center feeding (DT63) and shallow to deeper excision of the leaf margin (DT12) (loc. Molla Kala). E, Apex feeding (DT13) (loc. Molla Kala). F, Trenched excision of the leaf blade that expands inwardly from the leaf margin (DT15) (loc. Aliabad-e Katul). G, Broad swaths of interveinal tissue are absent and highest orders of venation removed (DT21) (loc. Astara). H, Apex feeding (DT13) (loc. Molla Kala). I, Extensively and completely removed interveinal tissue surrounded by remaining veinal stringers (DT26) (loc. Molla Kala). J, Perforation at the divergence point of secondary veins from primary veins (DT57) (loc. Molla Kala). K, Detail of hole feeding in J, showing reaction rim and flaps of necrotic tissue (arrow) along the outer margin of the reaction rim. L, M, Cuspate margin excisions (arrows) separated by a short segment of leaf margin (DT143) (loc. Aliabad-e Katul). N, Removal of interveinal tissue resulting in retention of some vein network but with a well-developed surrounding reaction rim (DT17) (loc. Aliabad-e Katul). O, Removal of interveinal tissue resulting in retention of some vein network but with a poorly developed surrounding reaction rim (DT16) (loc. Aliabad-e Katul). P, Same skeletonization pattern as seen in O (DT16) (loc. Molla Kala). Q, Skeletonization were the highest orders of venation removed; broad swaths of interveinal tissue are absent (DT21) (loc. Molla Kala). R, Small to medium sized circular perforations arranged in a curved chain with prominent dark edges to the margin (DT297) (loc. Rudsar). S, Magnification of the long-bent concatenation of small holes in R, surrounded by a prominent dark reaction rim. T, long bent concatenation of small holes (DT297) (loc. Aliabad-e Katul). U, Elongate, curvilinear to rectilinear strings of skeletonized tissue in the apical part of the leaf (DT20) and interveinal skeletonization with a poorly developed surrounding reaction rim (DT16) (loc. Pasand). Solid scale bars indicate 4 mm. DT in captions refer to damage types listed in Labandeira et al. (2007).

Figure 4: Examples of endophytic interaction and external foliage feeding on *Parrotia persica.*

A, Foliar gall (DT32) (loc. Molla Kala). B, Detail of the circular gall in A, occurring on the interveinal region of the leaf lamina. C, Foliar and midrib gall (DT23,33) (loc. Molla Kala). D, Magnification on the galls in C, occurring on the interveinal region of the leaf lamina and on the primary vein of the leaf. E, Blotchy, skeletonized areas on the upper leaf surface, originated by instar leaf tissue feeding (DT38) (loc. Pasand). F, Regular dark-brown blotch mine on the edge of the leaf that crosses major leaf veins (DT35) and a blotch mine (weak edges marked by arrows) lacking a central chamber, containing dispersed, small spheroidal coprolites (DT36) (loc. Aliabad-e Katul). G, Detail of the blotch mine in F, showing the slightly folded epidermal layer of a bulging blotch-type mine. H, Large blotch of necrotic tissue with peripheral holes (DT154) (loc. Pasand). I, Detail of the blotch in H, showing that the outer margin of the blotch is surrounded by a weakly developed reaction front. Solid scale bars indicate 4 mm. DT in captions refer to damage types listed in Labandeira et al. (2007).

Figure 5: Rarefaction curves for insect damage richness on damaged leaves.

The shaded area represents the standard deviation below and above the average of the resamples, with the method from Heck et al. (1975).

Figure 6: Frequency of *Parrotia persica* leaves with each Functional Feeding Group (FFG) at each site. For comparison, on the framed to the right side, is presented the frequency of each FFG from the fossil outcrops of Bernasso and Willershausen. For each location, the sum of the percentage is equal to 100%. The nomenclature of the different Iranian sites indicates their exact altitude, for example, Ast293 means the site at Astara from 293 above the sea-level. Nota bene: We did not consider the site Ast_025 because it is not comparable to other sites based on the frequency of damage.

Figure 7: Principal components analysis (PCA) of *Parrotia persica* from each locality according to the diversity of damage type (DT) observed.

The significances of each axes are presented in Appendix N.

Figure 8: Box-plot of the mean richness of damage type (DT) at each site.

The sites are grouped by similar altitude and from West to East. (A) Mean damage richness; (B) Mean external damage richness; (C) Mean specialized damage richness.

Nota bene: We did not consider the sites Ast_025 and Mol_610 because they are not comparable to other sites in terms of DT richness according to altitude.

Table 1: Details for sample localities in the Hyrcanian forest.

For each locality, mean annual temperature (MAT), minimal temperature (T°min), maximum temperature (T°max) and the mean annual precipitation (MAP) were obtained thanks to the climatic data from IRIMO (Iran meteorological organization).

For each site, the altitude, latitude and longitude were measured with a GPS (GARMIN gps map 62s). The altitude-dependent temperatures are available in Table S1.

Chapter V: A new damage type on fossil and modern leaves of *Parrotia persica*

Figure 1: Drawing of Parrotia persica **leaf with insect damage, including an interpretation of the DT297.** Illustration realized by Dorothea Kranz, Steinmann Institut, Bonn.

Figure 2: *Parrotia persica* leaves from the fossil record of Willershausen, Germany (A, B) and modern record of the Hyrcanian forest in Iran (C).

Photos of the leaves are in the same scale. White scale bar represents 1cm for the three leaf photos.

Enlargement of the diverse versions of the damage type DT297 are not represent with the same scale, due especially, to the different quality of camera used for the photography. Green scale bars represent 0.25cm.

Photos of the fossil leaves were shot by Benjamin Adroit and further ones can be observed in Appendix O. Photos of the modern leaf were shot by Mahdieh Malekhosseini.

Appendix A: Artist painting of the supposed landscape of Willershausen, realized by August Ahlborn (1877-1951).

The original paintwork is exhibited in the natural museum of Göttingen in Germany.

Species	#leaves	%DMG	%Spec	%Gall	%Mine	%External	%MarginF	%HoleF	%Skeleton
Acer integerrinum	4	0.25	0	0	0	0	0	0	0.25
Acer opalus	12	0.5	0.42	0.17	0.17	0.08	0	0.08	0.33
Acer opulifolium	11	0.45	0.27	0.18	0	0.09	0.09	0	0.27
Acer potojaponicum	2	0.5	0.5	0.5	0	0	0	0	0
Acer monspessulanu	22	0.59	0.45	0.32	0	0.18	0.09	0.09	0.32
Acer sp	7	0.29	0.29	0.29	0	0	0	0	0
Carpinus orientalis	123	0.3	0.11	0.03	0.02	0.22	0.08	0.14	0.06
Carya minor	51	0.39	0.1	0.04	0.02	0.31	0.12	0.22	0.02
Celtis sp	18	0.17	0	0	0	0.11	0	0.11	0.11
Fraxinus ornus	35	0.26	0.23	0.17	0	0.09	0.09	0	0.03
Hedera helix	3	0.67	0.33	0	0	0.33	0.33	0	0.33
Ilex sp	4	0.5	0.5	0.5	0	0	0	0	0
Parrotia persica	106	0.37	0.23	0.22	0.01	0.11	0.06	0.07	0.04
Populus tremula	9	0.11	0	0	0	0	0	0	0.11
Prunus sp	9	0.22	0.22	0.11	0.11	0	0	0	0
Salix cinereae	1	0,00	0	0	0	0	0	0	0
Sorbus domestica	22	0.59	0.41	0.32	0	0.18	0.09	0.05	0.09
Tilia sp	18	0.28	0	0	0	0.17	0.06	0.11	0.11
Vitis sp	11	0.36	0.18	0	0	0.18	0.09	0.09	0.27
Zelkova ungeri	67	0.3	0.12	0.06	0.03	0.22	0.07	0.12	0.03
Total	535	0.35	0.18	0.12	0.02	0.17	0.07	0.1	0.08

Appendix B: Matrix of the Bernasso data

%SurfaceF	%PS	%Ovi	DTs	SpecDTs	GDTs	MDTs	ExtDTs	MFDTs	HFDTs	SKDTs
0	0	0	1	0	0	0	0	0	0	1
0	0	0	8	6	2	2	1	0	1	3
0	0	0	6	3	2	0	1	1	0	3
0	0	0	1	1	1	0	0	0	0	0
0.05	0	0	10	5	2	0	5	2	2	3
0	0	0	2	2	2	0	0	0	0	0
0.01	0	0	17	8	2	1	8	3	4	6
0	0	0	11	4	1	1	8	3	5	1
0	0	0	3	0	0	0	2	0	2	1
0	0	0	6	5	3	0	2	2	0	1
0	0	0	3	1	0	0	1	1	0	2
0	0	0	1	1	1	0	0	0	0	0
0	0	0.01	18	7	4	1	7	3	4	3
0	0	0	1	0	0	0	0	0	0	1
0	0	0	2	2	1	1	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0
0.05	0	0	7	4	2	0	3	1	1	2
0	0	0.06	5	0	0	0	3	1	2	1
0	0	0	4	1	0	0	2	1	1	2
0.03	0	0	17	8	3	2	11	5	4	1
0.01	0	0	40	21	7	4	18	8	7	8

SFDTs	PSFDTs	OviDTs	#FFGs	DTOAll	DTOSpec	DTOGall	DTOMine	DTOExternal	DTOMF	DTOHF
0	0	0	1	1	0	0	0	0	0	0
0	0	0	4	9	7	2	2	1	0	1
0	0	0	3	6	3	2	0	1	1	0
0	0	0	1	1	1	1	0	0	0	0
1	0	0	5	19	10	7	0	5	2	2
0	0	0	1	2	2	2	0	0	0	0
1	0	0	6	41	13	4	2	28	10	17
0	0	0	5	21	5	2	1	17	6	11
0	0	0	2	4	0	0	0	2	0	2
0	0	0	3	10	8	6	0	3	3	0
0	0	0	2	3	1	0	0	1	1	0
0	0	0	1	2	2	2	0	0	0	0
0	0	1	5	44	26	23	1	13	6	7
0	0	0	1	1	0	0	0	0	0	0
0	0	0	2	2	2	1	1	0	0	0
0	0	0	0	0	0	0	0	0	0	0
1	0	0	5	13	9	7	0	4	2	1
0	0	1	3	6	0	0	0	3	1	2
0	0	0	3	5	2	0	0	2	1	1
2	0	0	6	23	9	4	2	15	5	8
3	0	1	6	213	100	63	9	95	38	52

DTOSK	DTOSF	DTOPS	DTOOvi	DTnumbers
1	0	0	0	16,00
4	0	0	0	2;16;20;21;32;80;164;170
3	0	0	0	12;16;17;20;32;80
0	0	0	0	32,00
7	1	0	0	1;12;15;16;17;20;25;32;148;213
0	0	0	0	32;80
7	1	0	0	1;2;3;12;16;17;20;24;26;32;61;62;78;79;130;164;214
1	0	0	0	1;2;3;5;10;12;13;14;20;62;210
2	0	0	0	1;2;16
1	0	0	0	12;24;32;62;80;142
2	0	0	0	12;16;61
0	0	0	0	80,00
4	0	0	1	2;3;10;12;13;16;17;21;32;67;73;106;142;144;145;148;164;218
1	0	0	0	16,00
0	0	0	0	32;210
0	0	0	0	
2	1	0	0	1;12;16;19;25;32;80
2	0	0	1	2;5;12;16;67
3	0	0	0	2;12;16;20
2	2	0	0	1;2;3;12;15;16;25;26;30;32;78;80;81;90;210;213;214
				1;2;3;5;10;12;13;14;15;16;17;19;20;21;24;25;26;30;32;61;62;67;73;78;79;80;81;9
42	5	0	2	0;106;130;142;144;145;148;164;170;210;213;214;218

Fossil macro- and micro-flora from Bernasso (Early Pleistocene, Southern France): new palaeoclimatic data and comparison of methods

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Abstract

The Early Pleistocene palaeoflora of Bernasso was described during the 1990's. The fossil assemblage was interpreted as association of Mediterranean taxa and Caucasian taxa. Based on the most abundant taxa (*Carpinus orientalis*, *Parrotia persica*, *Acer* sp. and *Carya* sp.), Bernasso palaeoflora was interpreted as a mesothermic flora that developed during an interglacial stage of the beginning of the Pleistocene. However no precise quantification of climatic parameters was done. In the present study, climatic reconstruction using CLAMP and LMA methods based on the macroflora on one hand and using the Climatic Amplitude Method (CAM) based on the microflora on the other hand have been realised to verify the first climatic estimation based on the comparison between palaeovegetation data and similar modern ecosystems. CLAMP and LMA analyses show that Bernasso climate was colder than the present-day climate in Southern France and in the Hyrcanian and Euxinian regions. CAM shows mean annual temperatures higher than the modern Bernasso temperature and equivalent to the temperature of the Hyrcanian and Euxinian regions. For what concerns mean temperature of the coldest and warmest months, CLAMP and LMA indicate also largely lower values than the Climatic Amplitude Method. Mean annual precipitation and relative humidity reconstructed by CLAMP and CAM are in agreement: the climate was wetter than today at Bernasso. The differences between the 3 methods highlights important methodological biases that could be solved by new calibration sites in Europe and in Mediterranean region.

Key-words: Leaf morphology; CLAMP analyses; LMA; Pollen; Climatic Amplitude Method

Introduction

The beginning of the Pleistocene (2.58 Ma, Cohen and Gibbard, 2016) has been marked by the installation of glacial/interglacial cycles. It is clear that these cycles had important impacts on ecosystems, for example controlling the latitudinal distribution of the biomes (Suc 1984; Bertini 2010; Fauquette and Bertini 2003; Fauquette et al., in press). However we have little data about their initial impact.

The Early Pleistocene fossil locality of Bernasso is remarkable as it corresponds to one of the rare well dated site that registered rapid vegetational changes due to the rise of glacial-interglacial cycles of the Plio-Pleistocene. Bernasso palaeoflora thus represents a rare and precious case of fossil flora (leaves and pollen) that allows the characterisation of this key period. The site of Bernasso corresponds to lacustrine deposits (diatomites and clays) that formed in a volcanic context. The Escandorgue Massif, in which Bernasso is located, was active at the end of the Pliocene and beginning of Pleistocene. Between the effects of this volcanic activity, a basalt flow completely blocked a palaeovalley that become the lake of Bernasso in which pollen and fossil leaves were preserved (Ildefonse, 1970; Ildefonse et al., 1972, 1976). The age of entire sequence was estimated to 2.05-1.96 Ma (Ambert et al., 1990). On the basis of pollen record (Suc, 1978), three different vegetation zones have been defined. The oldest (pollen zone I) and the youngest (pollen zone III) were dominated by a steppe environment and corresponded to dry and/or cold periods. The intermediate zone (pollen zone II) was dominated by a leafy vegetation that developed under a wetter and warmer climate. Pollen zones I and III only preserved pollen, the pollen zone II is characterised by the presence of an abundant macrofloral assemblage including leaves and fruits (Ildefonse et al., 1976). Leroy and Roiron (1996) described in detail this fossil leaf assemblage. Among the 800 macrofossils collected, Leroy and Roiron (1996) identified 23 taxa. The most abundant macroremains correspond to the species *Carpinus orientalis* Mill., *Parrotia persica* (DC) C.A. Mey., and the genera *Acer* L. and *Carya* Nutt. The other leaves are mostly angiosperms (15 taxa) and only few corresponds to ferns (1 taxon) and gymnosperms (only 2 needles). For more details about this fossil assemblage, see Leroy and Roiron (1996) and the appendix 1. Comparison to modern floras highlighted similarities between Bernasso palaeoflora and the modern flora of the Hyrcania (south of the Caspian sea) and the Euxinia (east of the Black sea) (Leroy and Roiron, 1996). The climate under which Bernasso palaeoflora grew was thus interpreted as similar to those of the two regions, i.e. characterised by a mean annual temperature of 14-17°C and high precipitation (more than 800 mm per years). However, presence of different taxa suggests that Bernasso palaeoflora was exposed to climatic conditions that made possible the development of

typical Mediterranean elements (those later requesting different climatic conditions than those of the Hyrcania and Euxinia). In the macroflora (Leroy and Roiron, 1996), such taxa are for example *Carpinus orientalis*, *Acer monspessulanum* L., *Celtis australis* L., *Fraxinus ornus* L. and in the microflora (Suc, 1978), *Buxus* L., *Olea* L., Ericaceae and Gramineae.

If Bernasso palaeoflora remains well known, its climatic significance must be improved. In particular we do not know the reason that allows the development of a mixed vegetation at Bernasso at the beginning of the Pleistocene. Coexistence of Caucasian and Mediterranean taxa is a mixed association that needs specific climatic conditions (combination of Caucasian climatic conditions and western Mediterranean climatic conditions). So, in order to understand the development of the curious palaeoflora of Bernasso, Leaf Margin Analysis (LMA; Wilf, 1997), Climate Leaf Analysis Multivariate Program (CLAMP; Wolfe, 1993, 1995) analysis and Climatic Amplitude Method (CAM, Fauquette et al., 1998a and b) based on pollen data were performed on macro and micro-fossil assemblages to better characterise the climatic conditions that controlled the vegetation development at Bernasso at the beginning of the Pleistocene. This study was the occasion to compare the climatic estimates obtained through three methods based on leaf material and pollen data.

Material and Methods

Site of Bernasso

The fossil locality of Bernasso (43°43'34"N, 3°15'25"E) is located in the north-western part of the department Hérault (France), at an altitude of 512 m asl (Fig. 1). This fossil locality formed when the palaeocanyon of Bernasso filled by lacustrine deposits (diatomites and clays) after the enclosure of the palaeocanyon by lava flows (due to the activity of Escandorgue Massif). For more details about the geological and sedimentological contexts, see following references: Ildefonse, 1970; Ildefonse et al., 1972, 1976; Suc, 1978; Ambert et al., 1990; Leroy, 1990; Leroy and Seret, 1992, Roiron, 1992; Leroy and Roiron, 1996.

The fossil macroflora

More than 800 fossil leaves were collected at Bernasso. They were attributed to 23 different taxa (for details, read Leroy and Roiron, 1996). These leaves are conserved in the collection of fossil leaves of ISEM (contact: V. Girard – vincent.girard@umontpellier.fr) that formed part of the palaeontogical collection of the Montpellier University (https://collections.umontpellier.fr/; contact: S. Jiquel – suzanne.jiquel@umontpellier.fr). Only 590 leaves from the total collections were used in this study.

A Leaf Margin Analysis (LMA) was performed on the ISEM collection in order to specify the

climatic conditions that controlled the development of Bernasso palaeoflora. Different equations derived for leaf margin analysis reconstruction of mean annual temperature (Wolfe, 1979; Wilf, 1997; Gregory-Wodzicki, 2000; Greenwood et al., 2004; Traiser et al., 2005; Miller et al., 2006). These different equations were used because the only one available from Europe (Traiser et al., 2005) is the equation with the weakest correlation between mean annual temperature and the proportion of entire margined leaves (Traiser et al., 2005).

A CLAMP analysis was also performed on the ISEM collection. The application CLAMP online was used for this (Wolfe, 1993, 1995; http://clamp.ibcas.ac.cn/). Since its initial development, CLAMP online was amended several times (Kovach and Spicer, 1995; Wolfe and Spicer, 1999; Spicer et al., 2004, 2009, Spicer et al., 2007, 2008) and requires now 31 morphological characters of leaves concerning the shape and size of the leaf, its margin (presence or absence of teeth), the shape of the leaf apex, base and surface. For each morphological character, a value between 0 and 1 was attributed according the description made on the web page of CLAMP online. The obtained data was included the dataset of CLAMP online (that corresponds to the leaf morphological characters of 144 modern floras). This new dataset was analysed thanks to canonical correspondence analysis (CCA). The 2 main axes of the CCA represent 70% of the variation of the dataset and no other axe were used for the present study. Axis 1 is mostly related to mean annual temperature and Axis 2 to precipitation (Wolfe, 1993). CLAMP scores are provided in Appendix 1. We tested the different CLAMP calibrations that concern the Northern and Southern hemispheres to provide ranges of values for different environmental parameters from Bernasso. The CLAMP analysis is based only on the deciduous angiosperms and thus 2 plant species from Bernasso were not taken into account (*Aspidium meyeri*, Filicophyta; *Ceratophyllum demersum*, Ceratophyllaceae, aquatic angiosperm; Leroy and Roiron, 1996).

The fossil microflora

In the present study we focus on the intermediate pollen zone (pollen zone II, Suc, 1978; Leroy and Roiron, 1996), which represents the same time slice than the macroflora layer. In this zone, pollen data point out an environment dominated by a leafy vegetation. This zone is rich in arboreal taxa such as *Carya*, *Pterocarya*, *Celtis*, *Ulmus-Zelkova*, *Carpinus orientalis* type, *Acer*, *Parrotia persica*, *Alnus*, …Gymnosperms like *Tsuga*, *Abies*, *Picea* are also present in the pollen data (Suc, 1978), but they were certainly not developing around the lake. Indeed no specimen has been found in the macroflora (Leroy and Roiron, 1996), and they should come from high altitudinal vegetation belts. Pollen zone II has been related to a warm and wet climatic phase (Suc, 1978; Leroy and Roiron, 1996).

Based on these pollen data, the palaeoclimate has been reconstructed using the Climatic Amplitude

Method developed by Fauquette et al. (1998a, b) specifically to quantify the climate of periods with no modern analogue for the pollen spectra. In this method, the past climate is estimated by transposing the climatic requirements of the maximum number of modern taxa to the fossil data. This method takes into account pollen percentages and allows, despite some biases, for a greater refinement and precision of the climatic estimates because taxa with very low pollen percentages are not always significant. The most probable climate for a set of taxa is estimated as the climatic interval suitable for the highest number of taxa and a 'most likely value' corresponding to a weighted mean. *Pinus* pollen grains are excluded from the pollen sum as they are generally over-represented due to a prolific production and over-abundance due to long distance air and water transport (Beaudouin et al., 2007). High latitude/altitude taxa are also excluded from the reconstruction process to avoid a cold bias linked to pollen transport from higher elevations (Fauquette et al., 1998a). The excluded taxa are defined on the basis of their occurrence in modern vegetation zones (Fauquette et al., in press): vegetation types described for the Neogene and Lower Pleistocene are found today in south-eastern China (Wang, 1961). There, the vertical distribution of vegetation is characterized from the base to the top of the massifs by evergreen broad-leaved forest, mixed evergreen and deciduous broad-leaved forest, *Picea*/*Tsuga* forest, *Abies* forest and high mountain meadows (Wang, 1961; Hou, 1983). This stratification allows us to exclude the modern high altitude taxa from the climatic reconstruction in order to infer the low to middle-low altitude climate (Fauquette et al., 1998a).

Results

LMA results

Table 1 provides the different Mean Annual Temperatures obtained with LMA considering that Bernasso palaeoflora contains 25.00% untoothed leaf taxa (Appendix 1).

CLAMP analysis

Table 2 provides the climate quantification under which Bernasso palaeoflora grew, quantification given by the correlations between morphological characteristics and the environmental parameters of the CCA made from the CLAMP analysis. Mean annual temperature is comprised between 5.32 and 8.37 °C, mean temperature during the coldest and warmest months between -8.11 and -2.91 °C and 20.24 and 21.98 °C, respectively. The precipitation during the 3 driest months were estimated to 93.6 and 500.4 mm and during the 3 wettest months to 471.8 and 695.8 mm. Lastly, the annual mean relative humidity has been estimated to 40.64 and 70.31 %.

Climatic Amplitude Method results (Table 3)

The climatic conditions reconstructed from pollen data of the leaf level (Suc, 1978) are summarized

in table 3. Mean annual temperatures are comprised between 11 and 20°C (with a most likely value around 15°C), temperatures of the coldest month between -2 and 5°C (with a most likely value around 1.6°C), temperatures of the warmest month between 24 and 27°C (with a most likely value most likely value around 25°C), mean annual precipitation between 700 and 1350 mm (with a most likely value around 1100 mm) and finally a relative humidity comprised between 66 and 80% (with a most likely value around 75%).

The climate reconstructed from pollen data of the borehole BN I (Leroy and Roiron, 1996) shows similar values *i.e.* mean annual temperature between 11 and 20°C (most likely value around 15.5°C), temperatures of the coldest month between -2.2 and 8°C (most likely value around 3.2°C), temperatures of the warmest month between 24 and 27.7°c (most likely value around 25.7°C), mean annual precipitation between 700 and 1320 mm (most likely value around 1055 mm) and relative humidity between 66 and 81% (most likely value around 75%).

One must note that CLAMP gives the mean temperatures of the three coldest months (CMMT) and the three warmest months (WMMT) whereas the CAM gives the mean temperatures of the coldest month (MTC) and warmest month (MTW).

Discussion

Palaeoclimate at Bernasso

Today climatic data at Lunas or Joncels (altitudes of 371 and 373 m respectively), the nearest towns at ~5 km from Bernasso, show a mean annual temperature of 12.8°C, a mean temperature of the coldest month of 4.8°C, a mean temperature of the warmest month of 21.3°C, mean annual precipitation around 720 mm (source: https://fr.climate-data.org).

Ambert et al. (1995) and Leroy and Roiron (1996) compared Bernasso leaf flora to the modern floras that develop in the Caucasian region. They also noticed the Bernasso palaeoflora was characterised by the presence of typical Mediterranean taxa, both in the macrofossils (Leroy and Roiron, 1996) and microfossils (Suc, 1978). Leroy and Roiron (1996) estimated palaeoclimate by comparison with modern climatic data from diverse stations of the Caucasian region (see Table 4 in Leroy and Roiron, 1996). The Hyrcanian region shows mean annual temperatures varying between 14.7°C and 17.5°C at low altitude and the Euxinian region has more homogeneous temperatures, around 14.3 °C, precipitation are between 880 and 2350 mm at low altitude in the two regions. Leroy and Roiron (1996) thus suggested that Bernasso palaeoflora grew under warmer (+ 2 to 5 °C) and more humid (at least + 300 to 500 mm per year) climate than today. However, these authors indicate that their model (the Hyrcanian and Euxinian regions) is not a perfect analogue to Bernasso palaeoflora. Indeed, the presence of typical Mediterranean taxa in the palaeoflora was not taken into

account and thus Leroy and Roiron (1996) could have misestimate Bernasso palaeoclimate.

The use of CLAMP analysis, LMA and Climatic Amplitude Method in the present study allows to make quantitative instead of qualitative reconstructions to verify previous interpretations.

Concerning Bernasso temperature, CLAMP results indicate a mean annual temperature (5.52-8.37°C) largely lower than the modern temperature observed in the Caucasian region and, than the one reconstructed by the Climatic Amplitude Method (11-20°C with a most likely value around 15°C). These latest values are furthermore in agreement with the temperature deduced by Leroy and Roiron (1996). However, the CLAMP mean annual temperature is confirmed by LMA that provides a mean annual temperature of 4.63 to 9.76°C. LMA mean annual temperature obtained from European regression (8.36°C) established by Traiser et al. (2005) is equivalent to the higher value of the MAT interval determined by CLAMP.

For what concerns mean temperature of the coldest and warmest months, CLAMP and LMA indicate once again largely lower values than the Climatic Amplitude Method, but mean annual precipitation and relative humidity are in agreement. CLAMP provides data about the precipitation during the 3 wettest and driest months (Table 2). These data allow to estimate the mean annual precipitation. It can be estimated in an interval comprised between a low value of precipitation (LMP) and a high value (HMP). The low value can be calculated as follow: LMP = 3*3DRY + 3WET where 3DRY corresponds to the precipitation during the 3 driest months and 3WET to the precipitation during the 3 wettest months. The high value can be calculated as follow: HMP = 3DRY + 3*3WET. LMP and HMP provide an interval of precipitation between a very low value (9 “dry” months and 3 “wet” months) and a very high value of precipitation (3 “dry” months and 9 “wet” months), the real mean annual precipitation being between these two extreme values. According to the CLAMP results, Bernasso annual precipitation should have been comprised between 963.2 and 2493.4 mm. This interval is slightly higher than the CAM values based on pollen data (700 to 1350 mm). Our results are included in the precipitation range of the two modern analogues as the Hyrcanian and Euxinian regions have precipitation varying from 880 mm to 2357 mm at low altitude (Leroy and Roiron, 1996). Results from our study confirm the hypothesis of Leroy and Roiron (1996) and show that Bernasso palaeoflora grew under a humid (perhaps very humid) climate. Suc (1978), on the basis of pollen data, interpreted the climate of the pollen phase II (during which macrofossils formed) as warm and wet with only a weak hydric seasonality. CLAMP results do not completely confirm this last conclusion and show that the hydric seasonality could have been well marked, with a ratio between wettest and driest months according some CLAMP calibrations of varying from ca. 1.3:1 to 6:1. Suc (1978) and Leroy and Roiron (1996) indicate a low seasonality with just a weak fall of precipitation during summer. The question of possible seasonal drought is still unsolved. It could had been the case as it is today in the

Mediterranean region, but no reconstruction, whatever the method used, allow to confirm it and no leaf specimens present functional adaptation to drought.

In the Climatic Amplitude Method, high latitude/altitude taxa are excluded from the reconstruction process to avoid a cold bias linked to pollen transport from higher vegetation belts. As no intense tectonic events occurred in Bernasso region during the last 2 million years, the topography did certainly not evolve a lot. The Bernasso valley, as today, was a long, narrow and steep sided valley and had a maximal altitude of ca. 700 m. The palaeoforest was thus growing from ca. 500 m (lake level) to ca. 700 m, on the canyon peaks. Suc (1978) and Leroy and Roiron (1996) suggested that it existed an altitudinal zonation of the Bernasso palaeoflora. The mid-altitudinal vegetation belt on the slopes of the Bernasso palaeocanyon was composed by mixed forests of *Carpinus orientalis*, *Parrotia persica* and several species of *Acer*. *Carya* and *Zelkova* should have grown only in sheltered areas of the palaeocanyon. The conifer forest, only highlighted by pollen data (presence of pollen grains of *Abies*, *Picea*, *Tsuga*), was developing at higher altitude.

However, taking into account the high percentages of *Tsuga* in the pollen data (Suc, 1978; Leroy and Roiron, 1996), although this taxa is considered as a middle to high altitude/latitude taxa and is not present in the macroflora (Leroy and Roiron, 1996), we can eventually assume that *Tsuga* was developing very close to the lake. We thus made a climate reconstruction with the Climatic Amplitude Method involving the climatic requirements of this taxa. The obtained temperatures are lower than the previous ones, with mean annual temperatures between 11 and 15°C (most likely value around 13°C), mean temperatures of the coldest month between -2 and 5°C (most likely value around 1.3°C), mean temperatures of the warmest month between 23 and 27°C (most likely value around 24.8°C). Mean annual precipitation and relative humidity are equivalent to values without *Tsuga*. In any case the temperatures are never as low as CLAMP and LMA temperatures.

We are aware that all methodologies proposing climatic reconstructions based on biological palaeoproxies have their own biases influencing the results. However, it is now well known that reconstructions based on leaf physiognomy (LMA, CLAMP) methods give generally lower temperature values (e.g., Utescher et al. 2000; Uhl et al. 2003, 2006, 2007; Peppe et al., 2011; Thiel et al., 2012). In fact the modern datasets used to calibrate the relationships are principally made up of sites in the USA, Canada, China and Japan. For CLAMP, the modern data sets available do not include any data of Europe and Middle East (http://clamp.ibcas.ac.cn). It is really problematic as many authors has shown that temperate floras of different continents may have different leaf-climate relationships (e.g. Gregory-Wodzicki, 2000; Greenwood et al., 2004) that led to differences in temperature estimates up to 5°C or more (Jordan, 2011). The case of the Bernasso macroflora fall into this gap as it is composed by a mixture of temperate taxa living today in the Mediterranean region and in Eurasia that are not covered by the modern data compiled in the database.

Moreover, the modern dataset mainly incorporate dry land sites although fossil macrofloras are predominantly deposited in wet land sites that leads also to discrepancies. Due to this bias, Kowalski and Dilcher (2003) indicate that physiognomic methods lead to an underestimation of temperatures between 2.5 and 10°C. With the results obtained by CLAMP and LMA method in this study, one can note that the low temperatures estimated for Bernasso are found today in Norway that can be very surprising. Applying the range of underestimation of the mean annual temperature given by Kowalski and Dilcher (2003), we obtained a corrected mean annual temperature from Bernasso comprised between 8°C and 17°C, a range of mean annual temperature more coherent with the results given by the Climatic Amplitude Method, even if it is still slightly lower than the CAM results. Real mean annual temperatures should have been probably between this CLAMP corrected values and the one given by CAM.

Peppe et al. (2011) made reconstructions using "digital leaf physiognomy" calibrated on modern data corresponding to 92 sites sampled in biomes where fossil leaves are generally preserved. The correlations between the chosen leaf's characters and climate parameters show that these physiognomic variables are linked to climate. When applying this calibration on fossil floras the authors obtained better results than CLAMP results. In particular, the estimated climate is warmer and wetter, similar to that estimated by other independent palaeo-proxies. However, it is noteworthy that improvements can be made in particular calibrating the model on supplementary sites that are absent from this database (for instance from Europe, Africa, etc; Peppe et al., 2011).

All these evidences highlight that CLAMP method is not optimal to reconstruct the climate of the Early Pleistocene in the Mediterranean region because of the lack of modern data to calibrate the method on Europe and the Mediterranean region ecosystems. Bernasso temperature estimated by CLAMP and LMA are lower than those given by the Climatic Amplitude Method. Also as no comparison with other outcrops with fossil leaves of the same age exists, it is difficult to see if Bernasso results are similar or different to other regional results. Applying the range of underestimation the mean annual temperature given by CLAMP, we obtained a corrected mean annual temperature from Bernasso comprised between 8°C and 17°C, a range of mean annual temperature more coherent with the results given by the Climatic Amplitude Method, even if it is still slightly lower. The values obtained with CAM are in agreement with the climate reconstructed at Garraf in Northern Spain for the same period (Fauquette et al., 1998a). It thus appears that, at a regional scale, CAM method provides coherent results and should be privileged, almost in the South European region. However calibrations are still needed for this last method in order to have a better precision on the presence of some taxa in the local flora deduced from pollen data and to get more accurate climatic reconstruction.

Conclusion

The fossil site of Bernasso is a remarkable site recording both leaves and pollen grains that allows to apply different climatic reconstruction methods and to compare the results. Climatic reconstruction using CLAMP and LMA methods based on the macroflora on one hand and using the Climatic Amplitude Method (CAM) based on the microflora on the other hand have been realised to verify the values given in an earlier study (Leroy and Roiron, 1991) based on the comparison between palaeovegetation data and similar modern ecosystems. In their study they compared the climate at Bernasso to the modern climate of the Hyrcanian and Euxinian regions.

The comparisons of the three methods used in this study highlight their complementarity as LMA and CLAMP are based on leaf physiognomy and ignore the taxonomy of the plants present in the sediment level whereas CAM is based on the modern climatic requirements of the pollen taxa fossilized in the same sediment level. LMA and CAM are interesting tools to get information about temperatures (mean annual temperatures and temperatures of the coldest and warmest months) and CLAMP may help to discuss these parameters and also provides supplementary data about precipitation pattern (*e.g.* precipitation during the three wettest and driest months) that can be partly compared to the CAM results (*i.e.* mean annual precipitation).

However, despite this possible complementary, the results of this study highlight important differences between the three methods. CLAMP and LMA analyses show that Bernasso climate was colder than the present-day climate in Southern France and in the Hyrcanian and Euxinian regions. Against all expectations, the obtained values are found today in Norway. CAM show mean annual temperatures higher than the modern Bernasso temperature and equivalent to the temperature of the Hyrcanian and Euxinian regions. For what concerns mean temperature of the coldest and warmest months, CLAMP and LMA indicate again lower values than the Climatic Amplitude Method. The only parameters in agreement between CLAMP and CAM are mean annual precipitation and relative humidity: the climate was wetter than today at Bernasso with the existence of a weak precipitation seasonality highlighted in CLAMP results.

It is now commonly admitted that physiognomic methods lead to an underestimation of temperatures from 2.5 to 10°C certainly due to problems of calibration. Absence of calibration sites in Europe and in the Mediterranean region and also the lack of calibration sites corresponding to biomes where fossil leaves are generally preserved are a key problem in the underestimation by CLAMP (even if LMA results for mean annual temperature is coherent with CLAMP results). New calibrations should solved the methodological biases inherent to each climatic reconstruction method.

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Figure 1 – Location map of Bernasso (with altitude indication) (A) and Bernasso outcrop (B).

Table 1 – Mean Annual Temperature (MAT) obtained from the different LMA equations.

Table 2 – CLAMP results obtained from Bernasso palaeoflora.
The calibrations Calib1 and Calib2 correspond to Physg3brcAZ calibration (based on 144 modern vegetation sites from the Northern Hemisphere temperate regions; Calib2 was obtained thanks to a meteorological correction applied in Calib1). The calibrations Calib3 and Calib4 correspond to Physg3arcAZ calibration (based on 173 modern vegetation sites from the Northern Hemisphere including samples from areas with pronounced winter cold; Calib4 was obtained thanks to a meteorological corrections applied in Calib3). For more details, see CLAMP online: http://clamp.ibcas.ac.cn/
MAT – Mean annual temperature; CMMT – Mean temperature of the 3 coldest months; WMMT – Mean temperature of the 3 warmest months; GSP – Precipitation during the growing season; 3DRY – Precipitation during the 3 driest months; 3WET – Precipitation during the 3 wettest months; LPM – Lower estimation of the mean annual precipitation; HPM – Higher estimation of the mean annual precipitation; RH – Relative humidity.
LPM and HPM were respectively estimated as (3*3DRY + 3WET) and (3DRY + 3WET).

Table 3 – Climatic estimates obtained with the Climatic Amplitude Method using pollen data of the leaf level and of the borehole BNI studied by Leroy and Roiron (1996). MAT – mean annual temperature; MTC – mean temperature of the coldest month; MTW – mean temperature of the warmest month; MAP – mean annual precipitation; RH – relative humidity. All parameters are given as an interval and a Most Likely Value which corresponds to a weighted mean.

Appendix 1 - Data matrix input to on-line CLAMP analysis tool (http://clamp.ibcas.ac.cn/Clampset2.html). Scores are the consensus of four independent scorers. CLAMP scores 32 character states; only those characters represented in the Bernasso flora are presented here. Descriptions of all character states and the details of the scoring method can be found on the CLAMP web site (http://clamp.ibcas.ac.cn).

Table 1.

Regression equation	Standard error of estimate (°C)	Sources	Region	MAT (°C)
MAT = 30.6P + 1.14	0.8	Wolfe, 1979	East Asia	8.79
MAT = 28.6P + 2.24	2.0	Wilf, 1997	North America, Caribbean	9.39
MAT = 27.5P + 1.36	3.3	Gregory-Wodzicki, 2000	North America, Japan, Carribbean, South Pacific	8.24
MAT = 24.9P + 3.53	2.2	Gregory-Wodzicki, 2000	North America, Japan, Carribbean, South Pacific	9.76
MAT = 22.0P + 1.32	3.0	Greenwood et al., 2004	Eastern Australia	6.82
MAT = 27.0P – 2.12	2.2	Greenwood et al., 2004	Eastern Australia	4.63
MAT = 31.4P + 0.512	1.7	Traiser et al. 2005	Europe	8.36
MAT = 28.99P + 1.32	0.12	Miller et al., 2006	Central and North America	8.57

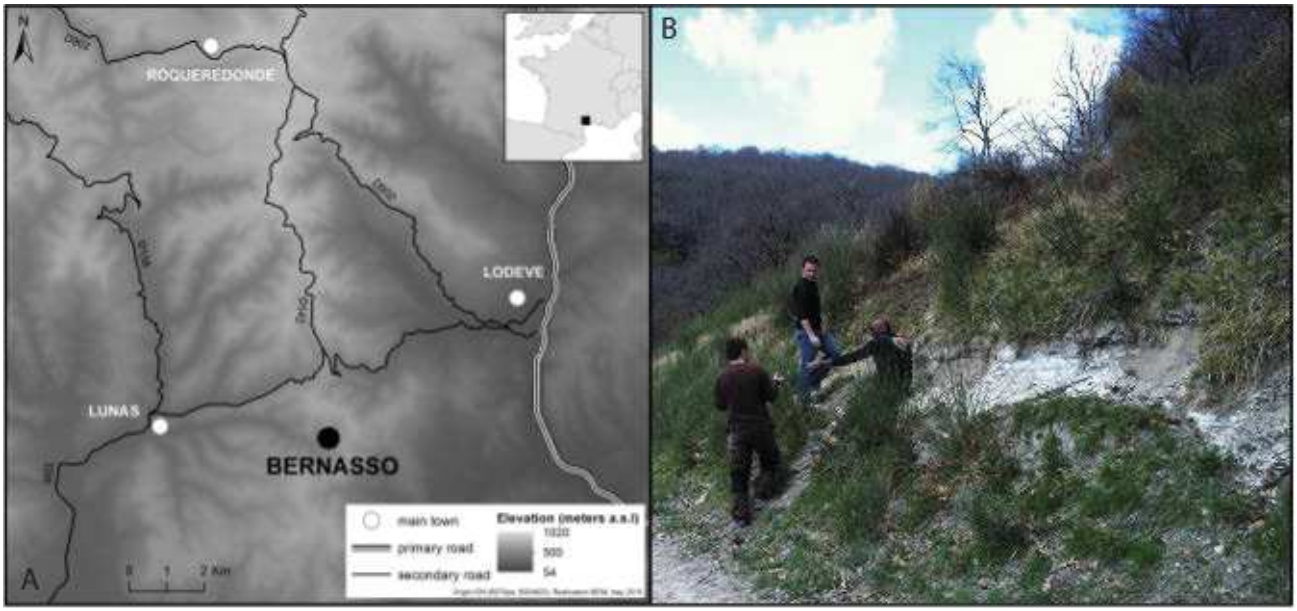
Table 2.

Parameter	MAT (°C)	CMMT (°C)	WMMT (°C)	3DRY (mm)	3WET (mm)	LMP (mm)	HMP (mm)	RH (%)
Calib1	5.52	-8.11	20.24	144.8	528.8	963.2	1731.2	68,38
Calib2	6.10	-7.26	20.26	500.4	662.7	2163.9	2488.5	61,91
Calib3	6.31	-6.90	20.69	129.3	608.4	996.3	1954.5	65,25
Calib4	7.37	-5.57	21.21	490.0	667.8	2137.8	2493.4	62,82
Calib5	6.82	-7.39	21.19	110.6	471.8	830.6	1526.0	67.24
Calib6	8.37	-2.91	20.86	93.6	691.1	971.9	2166.9	70.31
Calib7	8.31	-5.12	21.98	120.7	695.8	1057.9	2208.1	40.64
Standard Error	1.16	1.90	1.40	3.18	14.49	24.03	46.65	0.44

Table 3.

Parameter	MAT (°C)	MTC (°C)	MTW (°C)	MAP (mm)	RH (%)
Leaf level	11 – 15 – 20	-2 – 1.6 – 5	24 – 25 – 27	700 – 1100 – 1350	66 – 75 – 80
Borehole	11 – 15.5 – 20	-2.2 – 3.2 – 8	24 – 25.7 – 27.7	700 – 1055 – 1320	66 – 75 – 81

Figure 1


N
ROQUEREDONDE
LODEVE
LUNAS
BERNASSO
main town
primary road
secondary road
Elevation (meters a.s.l)
1020
500
54
0 1 2 Km
A
B

Appendix D: Fossil record of fungal/plant-insect interaction described by A. Straus from the Willershausen quarry

NB: This appendix is an extended version of the one submitted in the scientific journal. This version includes a historical and methodical summary, presented below.

Insect trace fossils represent the preservation of an insect's behaviour ranging from its reproduction technique (summarized in Genise, 2017*);* how it survived by feeding, shelter or movement; and how the insect interacted with other organisms in its environment. Some trace fossils can be preserved within the sediment itself; these include locomotion and resting traces (summarized in Genise, 2017), or are secondary traces found on other preserved materials such as plant remains, bones or vertebrate coprolites (summarized in Genise, 2017*)*. They can provide the sole evidence for palaeoecological interpretation or they can be used to supplement other fossil material. Trace fossils can provide a unique and direct record of the plant-insect interactions in the past (e.g., Labandeira, 2006, 2013). Nevertheless, until the late twentieth century there was no formal icnotaxonomic system to designate traces of insect insects on fossil leaves. Previously, some authors named taxonomically certain traces but these were not grouped into a system of hierarchical structures (e.g., Friĉ, 1882, 1901; von Heyden, 1862; Lesquereux, 1892; Potonié, 1893, 1921; Hering, 1930; van Amerom, 1966; Kinzelbach, 1970; van Amerom & Boersma, 1971).

There are currently three classification systems in place to designate fossil traces with different approches, and even classified under different systems:

1. the first was established by Vjalov in 1975, who decided to include all the traces that showed the consumption of vegetal matter made by insects in the group Phagophytichnidea, which in turn was further divided in two subgroups Phagophytichnida (with three families) and Phagophylignichnida, to differentiate those damages produced in leaves of those produced in fossil woods;
2. the second type corresponds to an informal classification system proposed by Labandeira (2002), which was complemented later (Labandeira et al., 2007). In this case the classification is based on the concept of a guild (in the sense of a functional grouping) has proved to be a valuable tool in ecology (e.g., Simberloff & Dayan, 1991). In this classification there are categories that group the traces according to the feeding behavior of the insect. These damage types (DTs) can be classified into seven functional feeding groups: hole feeding (HF), margin feeding (MF), skeletonization (S), surface feeding (SF), galling (G), mining (M), and piercing-and-sucking (PS). The DTs can also be classified (Labandeira et al., 2007) as most likely to be specialized (made by insects that typically consume only one or a few closely related plant species) or generalized (typically made by polyphagous insects, e.g., most holes). Specialized feeding is delineated by evidence from extant analogue feeders, morphologically stereotyped damage patterns, and restricted occurrences confined to particular host-plant species or tissue types in either fossil or extant host taxa (Labandeira 2002);
3. the last system was proposed by Krassilov & Rasnitsyn (2008), which includes 72 icnoespecies that include ovipositions, galls, mines, buds and exceptional examples of non-marginal excisions.

Currently the principles of icnology are included within the ICZN (International Code of Zoological Nomenclature), although some authors have proposed to create an Icnological Code independent of the Codes of

Botanical and Zoological Nomenclature (Sarjeant & Kennedy, 1973; Sarjeant, 1979), arguing that fossil traces are not only the result of animal activity (e.g., Bertling et al., 2006). A different proposal is that of Krassilov & Rasnitsyn (2008), who suggest to include this type of traces ["phyllostigmas" according to Krassilov & Rasnitsyn (2008)] within the botanical code, since the substrate where they are the vegetal tissue.

All this may illustrate that there is no formal classification theme and that those traces are either named or unnamed in literature and described for different purposes using different approches. For example, van Ameron (1966) introduced the ichnotaxon *Phagophytichnus* for sub-circular or oval-margin excisions, which later was used for plant/insect interactions from different time periods (e.g., Cariglino & Gutiérrez, 2011; Dieguez et al., 1996; Ianuzzi & Labandeira, 2008; Kustatscher et al., 2014; Peñalver, 2002; Robledo, Sarzetti & Anzótegui, 2016; Sarzetti, Labandeira & Genise, 2008, Sarzetti et al., 2009; Wedmann, Wappler & Engel, 2009). However, non-marginal excisions were included by some authors within the ichnotaxon *Phagophytichnus* (Straus, 1977; Givulescu, 1984), although this inclusion does not agree with van Amerom's (1966) intension.

In general, Straus (1977) classified most of the leaf leasions with names of their putative producers and following hereby the classical work of Buhr (1964-1965) [for galls], whereas those modern-day attributable trace are ending with the addendum *fossilis*. For those non-attributable galls Straus (1977) choose the name *Phyllocecidium* for leaf gall, and for petiolus galls the ichnotaxon name *Petiolocecidium*.
Following the classification theme of von Kéler (1963) Straus choose the term *nomus* for mines, so that the term *Cuniculonomus* is a reference to linear mines and *Loconomus* refered to blotch mines. Kernbach (1967) defined *Stigmellites* and *Stigmella* for mines from Willershausen made by a representatives of an extant Lepidopteran genus. Later, Straus (1977) extended the knowledge of mining traces by adding the ichnogenera *Fenusa*, *Profenusa*, *Fenusites* for saw fly mines and *Lithocolletis* for lepidopteran (Sohn et al., 2012; Doorenweerd et al., 2015) and *Phytomizites* for dipteran mines (Winkler et al., 2010), mainly by following the classification of mining damage based on the work of Hering (1957).
As this is one of the most important and comprehensive contributions for a Pliocene locality so far we summarize the descriptions made by A. Straus in the following table with all the available informations on holotypes, ichnospecies taxonomy, possible culprits, the depository, and try to figure all the available type material from the Straus collection deposited in the Geoscience Centre, University of Göttingen, Germany (Plates A–E). Furthermore, we re-classified the visible damage patter according to the actual Damage Type Catalog by Labandeira et al. (2007).

Plant-Insect interaction and herbivory pattern observerd and originally described by Adolf Straus (1977) in his first comprehensive work on leaf interactions from Willershausen.

[¶] not found in the collections; [*] new ichnospecies type; [§] named after recent species with the addendum *fossilis*; H holotype; P paratype;

Coll. No.	Ichnotaxon	Type	Straus orginal Ref.	Plate Ref.	DT	FFG	Host plant	Culprit	Comments
17381[§]	cf. *Taphrina* sp.		Straus (1977; Taf. 1, Fig. 19)		16	Galling	*Parrotia* sp.	Ascomycetes: Exoascaceae	
18419[§]	cf. *Taphrina* sp.				2	Fungal	*Populus* sp.	Ascomycetes: Exoascaceae	
Fu 174	*Gymnosporangium* sp.				80	Fungal	*Sorbus graudifolia*	Basiodiomycetes: Pucciniaceae	
275[§]	cf. *Eriophyes lateannulatus fossilis*		Straus (1977; Taf. 4, Fig. 78)		32	Galling	*Tillia* sp.	Acarina: Eriophyidae	
7257/a[§]	*Eriophyes exilis fossilis* vel *Typhlodromus* sp. *fossilis*				34,16	Galling	*Tillia* sp.	Acarina: Laelaptidae	
18059[§]	*Eriophyes exilis fossilis* vel *Typhlodromus* sp. *fossilis*				32,33	Galling	*Tillia* sp.	Acarina: Laelaptidae	
7087/a[¶,§]	*Aceria aroniae fossilis* (*=Eriophyes pyri*)					Galling	*Amelanchier* sp.	Acarina: Eriophyidae	
2614/a[§]	*Aceria aroniae fossilis* (*=Eriophyes pyri*)				32	Galling	*Crataegus meischnerii*	Acarina: Eriophyidae	
11661[§]	*Aceria erinea fossilis*		Straus (1977; Taf. 1, Fig. 27)		12,3	Galling	*Juglans* sp.	Acarina: Eriophyidae	
18419[§]	*Aceria erinea fossilis*		Straus (1977; Fig. 5)		2	Galling	*Populus* sp.	Acarina: Eriophyidae	
9160[¶,§]	*Aceria erinea fossilis*		Straus (1977; Taf. 1, Fig. 26)			Galling	*Carya* sp./ *Pterocarya* sp.	Acarina: Eriophyidae	
3201/a[§]	*Aceria inangulis fossilis* (*=Eriophyes inangulis*)		Straus (1977; Fig. 6)		33	Galling	*Alnus nepalensis*	Acarina: Eriophyidae	
9159, [§]	Aceria leionota fossilis (*=Eriophyes leionotus*)		Straus (1977; Taf. 1, Fig. 17)	**Plate B, Fig. A**	32,3	Galling	*Betula* sp.	Acarina: Eriophyidae	
9444/a[§]	*Aceria leionota fossilis* (*=Eriophyes leionotus*)			**Plate B, Fig. B**		Galling	?*Carpinus* sp.	Acarina: Eriophyidae	
17758[¶,§]	cf. *Aceria macrochela fossilis*		Straus (1977; Taf. 2, Fig. 41)			Galling	*Acer* sp.	Acarina: Eriophyidae	
3116[§]	cf. *Aceria macrochela fossilis*			**Plate B, Fig. C**	?	Galling	*Acer laetum*	Acarina: Eriophyidae	
6560[§]	cf. *Aceria macrochela fossilis*		Straus (1977; Taf. 4, Fig. 71)	**Plate B, Fig. D**	80	Galling	*Acer laetum*	Acarina: Eriophyidae	
19779[§]	cf. *Aceria macrochela fossilis*			**Plate B, Fig. E**	32	Galling	*Acer laetum*	Acarina: Eriophyidae	
30808[§]	cf. *Aceria macrochela fossilis*			**Plate B, Fig. F**	32	Galling	*Acer* sp.	Acarina: Eriophyidae	
30914[§]	*Aceria nervisequa faginea fossilis*		Straus (1977; Taf. 3, Fig. 50)	**Plate B, Fig. G**	80	Galling	*Fagus* sp.	Acarina: Eriophyidae	
21979[§]	*Aceria nervisequa faginea fossilis*		Straus (1977; Fig. 2)	**Plate B, Fig. H**	unidentified	Galling	*Fagus* sp.	Acarina: Eriophyidae	

Coll. No.	Ichnotaxon	Type	Straus orginal Ref.	Plate Ref.	DT	FFG	Host plant	Culprit	Comments
30815[§]	*Aceria nervisequa faginea fossilis*			**Plate B, Fig. K**	46	Galling	*Fagus sylvatica*	Acarina: Eriophyidae	
30815[¶,*]	*Phyllocecidium internervellum*	H				Galling	*Fagus* sp.	Acarina: Eriophyidae	
13748/a[§]	*Aceria nervisequa nervisequa fossilis*		Straus (1977; Taf. 1, Fig. 20)	**Plate B, Fig. L**	unidentified	Galling	*Fagus* cf. *pliocenica*	Acarina: Eriophyidae	
22848/a[§]	*Aceria nervisequa nervisequa fossilis*			**Plate B, Fig. M**	145	Galling	*Fagus sylvatica*	Acarina: Eriophyidae	
38/150[¶,§]	*Aceria stenaspis stenaspis fossilis*		Straus (1977; Fig. 8)			Galling	*Fagus* sp.	Acarina: Eriophyidae	
8281[¶,§]	*Aceria tenella fossilis*		Straus (1977; Taf. 3, Fig. 46)			Galling	*Carpinus* sp.	Acarina: Eriophyidae	
5778[¶,§]	*Schizoneura ulmi fossilis*					Galling	*Ulmus* sp.	Hemiptera: Eriostomatidae	
2179[§]	*Schizoneura ulmi fossilis*		Straus (1977; Fig. 4)	**Plate B, Fig. I**	not observed	Galling	*Ulmus* sp.	Hemiptera: Eriostomatidae	
13419[¶,§]	*Schizoneura ulmi fossilis*		Straus (1977; Taf. 1, Fig. 31)			Galling	*Ulmus* sp.	Hemiptera: Eriostomatidae	
23403[¶,§]	cf. *Dasyneura ruebsaameni fossilis* vel *Cecidophyes reniformis fossilis*		Straus (1977; Fig. 1)			Galling	*Carpinus* sp.	Diptera: Cecidomyiidae vel Acarina: Eriophyidae	
3765[¶,§]	cf. *Dasyneura ruebsaameni fossilis* vel *Cecidophyes reniformis fossilis*					Galling	*Carpinus* sp.	Diptera: Cecidomyiidae vel Acarina: Eriophyidae	
23992/a[§]	cf. *Dasyneura ruebsaameni fossilis* vel *Cecidophyes reniformis fossilis*			**Plate A, Fig. W**	32	Galling	*Carpinus orientalis*	Diptera: Cecidomyiidae vel Acarina: Eriophyidae	
14652[§]	cf. *Dasyneura fraxini fossilis*		Straus (1977; Taf. 1, Fig. 16)	**Plate B, Fig. J**	87,1	Galling	*Fraxinus* sp.	Diptera: Cecidomyiidae	
18935[¶,§]	cf. *Myzus* sp.		Straus (1977; Taf. 4, Fig. 83)			Galling	*Prunus* sp.	Hemiptera: Aphididae	
2928[¶,§]	cf. *Dreyfusia* sp. *fossilis*					Galling	Pinaceae?	Hemiptera: Adelgidae	
2425[§]	*Neuroterus albipes fossilis* (= *N. laeviusculus*)		Straus (1977; Taf. 2, Fig. 39)	**Plate B, Fig. O**	32	Galling	*Quercus praeerucifolia*	Hymenoptera: Cynipidae	
2737/a[¶,§]	cf. *Andricus curvator fossilis*		Straus (1977; Taf. 1, Fig. 30)			Galling	*Quercus praeerucifolia*	Hymenoptera: Cynipidae	
9161[¶,§]	cf. *Andricus curvator fossilis*					Galling	*Quercus praeerucifolia*	Hymenoptera: Cynipidae	
14200[§]	*Andricus quercus-radicis fossilis*		Straus (1977; Taf. 1, Fig. 18)	**Plate B, Fig. N**	117	Galling	*Quercus roburoides*	Hymenoptera: Cynipidae	
7244[§]	cf. *Andricus* sp.		Straus (1977; Taf.1, Fig. 25)	**Plate B, Fig. S**	12,32	Galling	*Quercus roburoides*	Hymenoptera: Cynipidae	*Neuroterus* as possible culprid could not be excluded. Berger (1949) reported comparable galling structures on Pliocene angiosperm leaves
13732[§]	cf. *Andricus* sp.			**Plate B, Fig. X**	32	Galling	*Quercus praeerucifolia*	Hymenoptera: Cynipidae	*Neuroterus* as possible culprid could not be excluded. Berger (1949) reported comparable galling structures on Pliocene

Coll. No.	Ichnotaxon	Type	Straus orginal Ref.	Plate Ref.	DT	FFG	Host plant	Culprit	Comments
									angiosperm leaves
15091[§]	cf. *Andricus* sp.		Straus (1977; Taf. 1, Fig. 21)	**Plate B, Fig. Y**	32	Galling	*Quercus praeerucifolia*	Hymenoptera: Cynipidae	*Neuroterus* as possible culprid could not be excluded. Berger (1949) reported comparable galling structures on Pliocene angiosperm leaves
17076[§]	cf. *Andricus* sp.			**Plate B, Fig. W**	32	Galling	*Quercus praeerucifolia*	Hymenoptera: Cynipidae	*Neuroterus* as possible culprid could not be excluded. Berger (1949) reported comparable galling structures on Pliocene angiosperm leaves
22705[§]	cf. *Andricus* sp.		Straus (1977; Taf. 1, Fig. 24)	**Plate B, Fig. Z**	142,12	Galling	*Quercus praeerucifolia*	Hymenoptera: Cynipidae	*Neuroterus* as possible culprid could not be excluded. Berger (1949) reported comparable galling structures on Pliocene angiosperm leaves
5021[§]	cf. *Mikiola fagi fossilis* vel *Hartigiola annulipes fossilis*		Straus (1977; Taf. 1, Fig. 22)	**Plate B, Fig. AA**	unidentified	Galling		Diptera: Cecidomyiidae	
5786[¶,§]	cf. *Mikiola fagi fossilis* vel *Hartigiola annulipes fossilis*					Galling		Diptera: Cecidomyiidae	
8717[§]	cf. *Didymomyia reaumuriana fossilis*		Straus (1977; Fig. 3)	**Plate B, Fig. T**	78,19	Galling	*Tillia* sp.	Diptera: Cecidomyiidae	
8717[*]	*Fenusites tiliae*	H	Straus (1977; Fig. 3)	**Plate B, Fig. T**	78,19	Mining	*Tiliia* sp.	Hymenoptera: Tenthredinidae	
15650*	*Fenusites tiliae*			**Plate D, Fig. A**	2,16	Mining	*Tilia saportae*	Hymenoptera: Tenthredinidae	
21695/a[¶,*]	*Fenusites tiliae*					Mining	Tiliia sp.	Hymenoptera: Tenthredinidae	
22549/a[*]	*Fenusites tiliae*			**Plate D, Fig. B**	2,16	Mining	*Tilia* cf. *savania*	Hymenoptera: Tenthredinidae	
3042[§]	cf. *Contarinia carpini fossilis*		Straus (1977; Taf. 1, Fig. 28)	**Plate B, Fig. U**	32	Galling	*Populus willershausensis*	Diptera: Cecidomyiidae	Steinbach (1967) and Straus (1962) determined these galls earlier as hymenopteran galls belonging to *Andricus curvator*
8907[¶,§]	cf. *Phegomyia fagicola fossilis*					Galling		Diptera: Cecidomyiidae	Steinbach (1967) and Straus (1962) determined these galls earlier as hymenopteran galls belonging to *Andricus curvator*
12110/a[¶,§]	cf. *Phegomyia fagicola fossilis*		Straus (1977; Taf. 2, Fig. 34)			Galling		Diptera: Cecidomyiidae	Steinbach (1967) and Straus (1962) determined these galls earlier as hymenopteran galls belonging to *Andricus curvator*
19960/a[¶,§]	cf. *Phegomyia fagicola fossilis*					Galling		Diptera: Cecidomyiidae	Steinbach (1967) and Straus (1962) determined these galls earlier as hymenopteran galls belonging to *Andricus curvator*

Coll. No.	Ichnotaxon	Type	Straus orginal Ref.	Plate Ref.	DT	FFG	Host plant	Culprit	Comments
30810*	*Petiolocecidium aceris*	H	Straus (1977; Taf. 2, Fig. 38)	**Plate B, Fig. P**	unidentified	Galling	*Acer integerrinum*	Hemiptera: Pemphigidae	Resemble those structures made by *Pemphigus spirotheca* (comp. Mädler, 1936)
20197/a*	*Petioloceoidium hamamelidacearum*	H	Straus (1977; Taf. 2, Fig. 42)	**Plate C, Fig. A**	unidentified	Galling	*Parrotia* sp.		
10060*	*Petioloceoidium hamamelidacearum*		Straus (1977; Taf. 1, Fig. 32)	**Plate B, Fig. Q**	33	Galling	*Parrotia* sp.		
10959¶,*	*Petioloceoidium hamamelidacearum*		Straus (1977; Taf. 1, Fig. 29)			Galling	cf. *Parrotiopsis* sp.		
13164§	*Petioloceoidium hamamelidacearum*			**Plate B, Fig. AB**	16,3	Galling	*Parrotia persica*		
3388/a*	*Phyllocecidium alni-tuberculosum*	H	Straus (1977; Taf. 2, Fig. 33)	**Plate B, Fig. R**	32	Fungal / Galling	*Alnus incana*	Ascomycetes: Exoascaceae vel. Diptera: Cecidomyiidae	
12143*	*Phyllocecidium comma*	H	Straus (1977; Taf. 2, Fig. 36)	**Plate B, Fig. V**		Galling	*Quercus* sp.		
20161/a*	*Phyllocecidium comma*			**Plate C, Fig. B**	32	Galling	*Quercus praeerucifolia*		
23862*	*Phyllocecidium comma*			**Plate C, Fig. C**	32	Galling	*Quercus praeerucifolia*		
7691¶,*	*Phyllocecidium cuniculatum*	H	Straus (1977; Taf. 3, Fig. 47)			Galling	cf. *Quercus*		
30815*	*Phyllocecidium internervellum*		Straus (1977; Taf. 2, Fig. 43)	**Plate B, Fig. K**	46	Galling	*Fagus sylvatica*		comp. also with *Hartigiola* and *Aceria nervisequa faginea fossilis*
18421/a*	*Phyllocecidium medionervisequm*	H	Straus (1977; Fig. 9)	**Plate C, Fig. D**	53	Galling	*Quercus praeerucifolia*	Hymenoptera: Cynipidae (*Neuroterus* sp.)	
30845/a*	*Phyllocecidium medionervisequm*			**Plate C, Fig. E**	194	Galling	*Quercus roburoides*		
13156*	*Phyllocecidium parrotiae*	H	Straus (1977; Taf. 4, Fig. 82)	**Plate C, Fig. F**	145	Fungal / Galling	Parrotia sp.		comparapble to fungal infection caused by *Rhytisma*. Probably also on *Parratiopsis*
2603*	*Phyllocecidium parrotiae*			**Plate C, Fig. G**	1	Fungal / Galling	*Parrotia* sp.		comparapble to fungal infection by *Rhytisma*. Probaly also on *Parratiopsis*
7217*	*Phyllocecidium parrotiae*			**Plate C, Fig. H**	32	Fungal / Galling	*Parrotia* sp.		comparapble to fungal infection caused by *Rhytisma*. Probably also on *Parratiopsis*
7253*	*Phyllocecidium parrotiae*			**Plate C, Fig. I**	210	Fungal / Galling	*Parrotia* sp.		comparapble to fungal infection caused by *Rhytisma*. Probably also on *Parratiopsis*
7411*	*Phyllocecidium parrotiae*			**Plate C, Fig. J**	33	Fungal / Galling	*Parrotia* sp.		comparapble to fungal infection caused by *Rhytisma*. Probably also on *Parratiopsis*
9214*	*Phyllocecidium parrotiae*		Straus (1977; Taf. 3, Fig. 48)	**Plate C, Fig. K**	120	Fungal / Galling	*Parrotia* sp.		comparapble to fungal infection caused by *Rhytisma*. Probably

Coll. No.	Ichnotaxon	Type	Straus orginal Ref.	Plate Ref.	DT	FFG	Host plant	Culprit	Comments
									also on *Parratiopsis*
9673/a*	*Phyllocecidium parrotiae*			**Plate C, Fig. L**	32,17	Fungal / Galling	*Parrotia* sp.		comparaple to fungal infection caused by *Rhytisma*. Probably also on *Parratiopsis*
10196*	*Phyllocecidium parrotiae*		Straus (1977; Taf. 2, Fig. 40)	**Plate C, Fig. M**	2,78	Fungal / Galling	*Parrotia* sp.		comparaple to fungal infection caused by *Rhytisma*. Probably also on *Parratiopsis*
12733¶,*	*Phyllocecidium parrotiae*					Fungal / Galling	*Parrotia* sp.		comparaple to fungal infection caused by *Rhytisma*. Probably also on *Parratiopsis*
12818¶,*	*Phyllocecidium parrotiae*					Fungal / Galling	*Parrotia* sp.		comparaple to fungal infection caused by *Rhytisma*. Probably also on *Parratiopsis*
13217§	*Phyllocecidium parrotiae*			**Plate C, Fig. N**	146	Fungal / Galling	*Parrotia* sp.		comparaple to fungal infection caused by *Rhytisma*. Probably also on *Parratiopsis*
16732*	*Phyllocecidium parrotiae*			**Plate C, Fig. O**	33	Fungal / Galling	*Parrotia* sp.		comparaple to fungal infection caused by *Rhytisma*. Probably also on *Parratiopsis*
17689*	*Phyllocecidium parrotiae*			**Plate C, Fig. P**	128	Fungal / Galling	*Parrotia* sp.		comparaple to fungal infection caused by *Rhytisma*. Probably also on *Parratiopsis*
17715*	*Phyllocecidium parrotiae*		Straus (1977; Taf. 2, Fig. 37)	**Plate C, Fig. Q**	33	Fungal / Galling	*Parrotia* sp.		comparaple to fungal infection caused by *Rhytisma*. Probably also on *Parratiopsis*
20318/a*	*Phyllocecidium parrotiae*			**Plate C, Fig. R**	12,16	Fungal / Galling	*Parrotia* sp.		comparaple to fungal infection caused by *Rhytisma*. Probably also on *Parratiopsis*
20892*	*Phyllocecidium parrotiae*			**Plate C, Fig. S**	not observed	Fungal / Galling	*Parrotia* sp.		comparaple to fungal infection caused by *Rhytisma*. Probably also on *Parratiopsis*
21920/a§	*Fenusa ulmi fossilis*		Straus (1977; Fig. 15)	**Plate C, Fig. U**	78,2,17	Mining	*Sorbus gabbrensis*	Hymenoptera: Tenthredinidae	A comparable mining type is also figured on *Ulmus longifolia* by Jakubowskaja (1955)
12§	*Fenusa ulmi fossilis*			**Plate C, Fig. V**	78	Mining	*Fagus* cf. *pliocenica*	Hymenoptera: Tenthredinidae	
3183§	*Fenusa ulmi fossilis*			**Plate C, Fig. W**	78	Mining	*Ulmus* sp.	Hymenoptera: Tenthredinidae	A comparable mining type is also figured on *Ulmus longifolia* by Jakubowskaja (1955)
30837¶,§	*Fenusa ulmi fossilis*					Mining	*Ulmus* sp.	Hymenoptera: Tenthredinidae	A comparable mining type is also figured on *Ulmus longifolia* by Jakubowskaja (1955)
14535/a*	*Fenusites denckmanni*	H	Straus (1977; Taf. 3, Fig. 58)	**Plate C, Fig. X**	42	Mining	*Magnolia* sp. vel. *Syringa* sp.	Hymenoptera: Tenthredinidae	
17885*	*Fenusites denckmanni*				78	Mining	*Magnolia* sp. vel. *Syringa* sp.	Hymenoptera: Tenthredinidae	

Coll. No.	Ichnotaxon	Type	Straus orginal Ref.	Plate Ref.	DT	FFG	Host plant	Culprit	Comments
20987/a*	*Fenusites betulacearum*	H	Straus (1977; Taf. 3, Fig. 54)	**Plate C, Fig. Y**	78	Mining	Betulaceae indet.	Hymenoptera: Tenthredinidae	*Fenusa* type
18874*	*Fenusites betulacearum*			**Plate C, Fig. T**	78,21	Mining	Betulaceae indet.	Hymenoptera: Tenthredinidae	*Fenusa* type
18906*	*Fenusites betulacearum*			**Plate D, Fig. C**	32,63	Mining	Betulaceae indet.	Hymenoptera: Tenthredinidae; Lepidoptera: Coleophoridae	*Fenusa* type
20468/a¶,*	*Fenusites betulacearum* + cf. *Coleophora* sp.		Straus (1977; Taf. 2, Fig. 45)			Mining	Betulaceae indet.	Hymenoptera: Tenthredinidae	*Fenusa* type
17924*	*Fenusites celtis*	H	Straus (1977; Taf. 3, Fig. 53)		21	Mining	*Celtis* sp.	Hymenoptera: Tenthredinidae	*Fenusa* type
21926*	*Fenusites fagi*	H	Straus (1977; Fig. 13)		15,5	Mining	*Fagus* sp.	Hymenoptera: Tenthredinidae	*Fenusa* type
11339*	*Fenusites fagi*				78,16	Mining	*Fagus* cf. *pliocenica*	Hymenoptera: Tenthredinidae	*Fenusa* type
19782¶,*	*Fenusites fagi*					Mining	*Fagus* sp.	Hymenoptera: Tenthredinidae	*Fenusa* type
22508*	*Fenusites parrotiae*	H	Straus (1977; Fig. 11)		16,78	Mining	*Parrotia* sp.		additional frass on the leaf caused by Coleoptera: Hispinae vel. Halticidae
30807¶,*	*Fenusites parrotiae*	P				Mining	*Parrotia* sp.		
30813¶,*	*Fenusites zelkovae*	H	Straus (1977; Taf. 4, Fig. 73)			Mining	*Zelkova* sp.	Hymenoptera: Tenthredinidae	*Fenusa* type
8068/a¶,*	*Fenusites zelkovae*					Mining	*Zelkova* sp.	Hymenoptera: Tenthredinidae	*Fenusa* type
30805¶,*	*Fenusites zelkovae*					Mining	*Zelkova* sp.	Hymenoptera: Tenthredinidae	*Fenusa* type
30806*	*Fenusites zelkovae*					Mining	*Quercus praeerucifolia*	Hymenoptera: Tenthredinidae	*Fenusa* type
30814/a¶,§	cf. *Profenusa pygmaea fossilis*					Mining	*Quercus petrae* vel. *Quercus iberica*	Hymenoptera: Tenthredinidae	*Fenusa* type
19783¶,§	cf. *Profenusa pygmaea fossilis*					Mining	*Quercus petrae* vel. *Quercus iberica*	Hymenoptera: Tenthredinidae	*Fenusa* type
18429 vel 18422§	cf. *Bucculatrix thoracella fossilis*		Straus (1977; Taf. 3, Fig. 61)		12,32	Mining	*Ampelopsis cordataeformis*	Lepidoptera: Lyonetiidae	
21040§	cf. *Coleophora* sp.				5	Mining	*Betula maximowicziana*	Lepidoptera: Coleophoridae	
21695/a§	cf. *Coleophora* sp.				4,5, 80	Mining	*Tilia* sp.	Lepidoptera: Coleophoridae	
22549/a§	cf. *Coleophora* sp.				2,16	Mining		Lepidoptera: Coleophoridae	
22858§	cf. *Coleophora* sp.		Straus (1977; Taf. 3, Fig. 56)			Mining		Lepidoptera: Coleophoridae	
22907¶,§	cf. *Coleophora* sp.					Mining		Lepidoptera: Coleophoridae	
22996/a§	cf. *Coleophora* sp.				3	Mining		Lepidoptera: Coleophoridae	

Coll. No.	Ichnotaxon	Type	Straus orginal Ref.	Plate Ref.	DT	FFG	Host plant	Culprit	Comments
30809[¶,§]	cf. *Coleophora* sp.					Mining		Lepidoptera: Coleophoridae	
22788[§]	cf. *Caloptilia alchimiella fossilis*				1,3	Mining	*Fagus* cf. *orientalis*	Lepidoptera: Gracilariidae	
22440[§]	cf. *Caloptilia roscipenella fossilis*		Straus (1977; Taf. 4, Fig. 76)		152, 21	Mining	*Fagus* sp.	Lepidoptera: Gracilariidae	
30838[§]	cf. *Coriscium* sp.		Straus (1977; Taf. 3, Fig. 60)		142	Mining	*Magnolia* sp. vel. *Syringa* sp.	Lepidoptera: Gracilariidae	
15876/a[§]	cf. *Parornix* sp.		Straus (1977; Taf. 3, Fig. 49)		15	Mining	*Carpinus orientalis*	Lepidoptera: Gracilariidae	
30057[§]	*Lithocolletis maestingella fossilis*		Straus (1977; Taf. 3, Fig. 59)			Mining	*Fagus* sp.	Lepidoptera: Gracilariidae	
15026[§]	*Lithocolletis maestingella fossilis*					Mining	*Fagus* sp.	Lepidoptera: Gracilariidae	
15427[§]	cf. *Incurvaria oehlmanniella fossilis*		Straus (1977; Taf. 2, Fig. 44)		36	Mining	cf. *Vaccinium* sp.	Lepidoptera: Incurvariidae	
21313[§]	cf. *Incurvaria* sp.		Straus (1977; Taf. 3, Fig. 55)		32,7	Mining	*Berberis* sp.	Lepidoptera: Incurvariidae	comp. Hering (1957: 166; Nr. 770)
12724/a[§]	cf. *Recurvaria nanella*		Straus (1977; Taf. 3, Fig. 51)		32	Mining	*Sorbus torminalia fossilis*	Lepidoptera: Gelechiidae	
3050[¶,*]	*Stigmella pliotityrella*	H				Mining	*Fagus sylvatica*	Lepidoptera: Nepticulidae	Holotype described by Kernbach (1967: 105-106, Fig. 4)
9111[§]	*Stigmella ulmivora fossilis*					Mining	*Ulmus* sp.	Lepidoptera: Nepticulidae	comp. Kernbach (1967: 105-106, Fig. 5)
17738[§]	*Stigmella ulmivora fossilis*		Straus (1977; Fig. 12)	**Plate E, Fig. A**	36	Mining	?*Sorbus domestica*	Lepidoptera: Nepticulidae	
22763[§]	*Stigmellites carpini-orientalis*	H	Straus (1977; Taf. 4, Fig. 80)	**Plate E, Fig. B**	94	Mining	Carpinus sp.	Lepidoptera: Nepticulidae	
22134[§]	*Stigmellites carpini-orientalis*	P	Straus (1977; Taf. 3, Fig. 62)	**Plate E, Fig. C**	94	Mining	Carpinus sp.	Lepidoptera: Nepticulidae	
11137[¶,*]	*Stigmellites heringi*	H				Mining	Berberis sp.	Lepidoptera: Nepticulidae	Holotype described by Kernbach (1967: 104-106, Fig. 3)
23973[¶,*]	*Stigmellites zekovae*	H	Straus (1977; Fig. 14)			Mining	Zelkova sp.	Lepidoptera: Nepticulidae	
19753/a	Diptera mine undetermined			**Plate E, Fig. F**	90	Mining	Fraxinus sp. Fraxinus ornus	Diptera vel Coleoptera	most probably these mines belong to mines produced by Diptera larvae (comp. Hering (1957: 456; Nr. 2242)). Alternatively, comparable mines could also be produced by weevils (Coleoptera: Curculionidae) from the genus *Steronychus* feeding on *Fraxinus* sp.
8764/a[¶,§]	*Phytomya ranunculi fossilis*					Mining	?*Ranunculus repens*	Diptera: Agromyzidae	see also discussion in Winkler et al. (2010)
10965[§]	*Phytomyzites corni*	H	Straus (1977; Taf. 3, Fig. 52)	**Plate E, Fig. D**	5	Mining	Fabaceae	Diptera: Agromyzidae	

Coll. No.	Ichnotaxon	Type	Straus orginal Ref.	Plate Ref.	DT	FFG	Host plant	Culprit	Comments
30816¶,§	cf. *Phytagromyza populicola fossilis*	H				Mining	Populus latior	Diptera: Agromyzidae	
30818¶,§	cf. *Phytagromyza populicola fossilis*		Straus (1977; Taf. 4, Fig. 75)			Mining	Populus latior	Diptera: Agromyzidae vel Coleoptera: Curcuionidae	this mine could also be produced by *Rhynchaenus* cf. populi (Coleoptera: Curculionidae)
19471§	*Cuniculonomus carpini*	H	Straus (1977; Taf. 3, Fig. 57)	**Plate E, Fig. E**	185	Mining	*Carpinus betulus*		comp. Engelhardt (1876) and Kinzelbach (1970)
30836¶,§	*Cuniculonomus carpini*	P	Straus (1977; Taf. 4, Fig. 79)			Mining		Coleoptera: Curculionidae (*Rhychaenus* sp.)	
30607¶,§	*Loconomus vitis*	H	Straus (1977; Taf. 4, Fig. 66, 77)			Mining	*Vitis* sp.		blotch mine habitus
30821¶	*Loconomus* sp.		Straus (1977; Taf. 4, Fig. 81)			Mining	cf. *Toona*	?Diptera: Agrpmyzidae (*Agromyza*, *Phytagromyza*, *Dibolia*)	comp. To mines produced by Agromyza, Phytagromyza, Dibolia
2993/a*	*Phagophytichnus catellarius*	H	Straus (1977; Fig. 10)	**Plate A, Fig. T**	297	Skeletonization	Parrotia persica	Coleoptera	produced by Coleoptera: Chrysomelidae: Halticinae and/or Hispinae
10626*	*Phagophytichnus catellarius*	P	Straus (1977; Fig. 7)	**Plate A, Fig. U**	297	Skeletonization	*Parrotia persica*	Coleoptera	produced by Coleoptera: Chrysomelidae: Halticinae and/or Hispinae
13076*	*Phagophytichnus catellarius*			**Plate A, Fig. V**	298	Skeletonization	*Parrotia persica*	Coleoptera	produced by Coleoptera: Chrysomelidae: Halticinae and/or Hispinae
12917*	*Phagophytichnus circumsecans*			**Plate A, Fig. A**	1,297	Hole	*Parrotia persica*	Coleoptera?	interpreted by Straus (1977) as the intitial phase (oviposition site) of *Phagophytichnus catellarius*
21482*	*Phagophytichnus nervillos-reliquens*	H	Straus (1977; Taf. 4, Fig. 69)	**Plate A, Fig. Q**	17	Skeletonization	*Parrotia persica*	?Coleoptera vel Hymenoptera	
7612¶,*	*Phagophytichnus nervillos-reliquens*					Skeletonization			
11490/a*	*Phagophytichnus nervillos-reliquens*			**Plate A, Fig. C**	17,12, 2	Skeletonization		?Coleoptera vel Hymenoptera	
21140*	*Phagophytichnus nervillos-reliquens*			**Plate A, Fig. S**	17	Skeletonization		?Coleoptera vel Hymenoptera	
30892/a*	*Phagophytichnus nervillos-reliquens*			**Plate A, Fig. R**	17	Skeletonization	*Parrotia persica*		
11490*	*Phagophytichnus marginis-folii*			**Plate A, Fig. C**	17,12, 2	Margin	*Quercus* sp.		
11490*	*Phagophytichnus circumsecans*			**Plate A, Fig. C**	2	Hole	*Quercus* sp.		
12231a*	*Phagophytichnus circumsecans* + *(Phagophytichnus catellarius)*	H	Straus (1977; Taf. 4, Fig. 70)	**Plate A, Fig. B**	17,2	Hole	*Parrotia persica*	Coleoptera?	Coleoptera: Curculionidae or Coleoptera: Chrysomelidae are able to produce such pattern
13386*	*Phagophytichnus marginis-folii*	H		**Plate A, Fig. E**	297	Margin	*Parrotia persica*	?Coleoptera: Curculionidae vel Chrysomelidae	Coleoptera: Curculionidae (*Rhynchaenus* or *Otiorhynchus*)

Coll. No.	Ichnotaxon	Type	Straus orginal Ref.	Plate Ref.	DT	FFG	Host plant	Culprit	Comments
									or Coleoptera: Chrysomelidae (larvae of Halticinae or Hispinae) are able to produce such pattern
2132/a*	*Phagophytichnus marginis-folii*			**Plate A, Fig. F**	297	Margin	*Parrotia persica*	?Coleoptera: Curculionidae vel Chrysomelidae	Coleoptera: Curculionidae (*Rhynchaenus* or *Otiorhynchus*) or Coleoptera: Chrysomelidae (larvae of Halticinae or Hispinae) are able to produce such pattern
3532*	*Phagophytichnus marginis-folii*		Straus (1977; Taf. 4, Fig. 67)	**Plate A, Fig. D**	unidentified	Margin		?Coleoptera: Curculionidae vel Chrysomelidae	Coleoptera: Curculionidae (*Rhynchaenus* or *Otiorhynchus*) or Coleoptera: Chrysomelidae (larvae of Halticinae or Hispinae) are able to produce such pattern
3612*	*Phagophytichnus marginis-folii*			**Plate A, Fig. H**	1	Margin	*Fraxinus* sp	?Coleoptera: Curculionidae vel Chrysomelidae	Coleoptera: Curculionidae (*Rhynchaenus* or *Otiorhynchus*) or Coleoptera: Chrysomelidae (larvae of Halticinae or Hispinae) are able to produce such pattern
7227*	*Phagophytichnus marginis-folii*			**Plate A, Fig. G**	81,16	Margin	*Cedrela heliconia*	?Coleoptera: Curculionidae vel Chrysomelidae	Coleoptera: Curculionidae (*Rhynchaenus* or *Otiorhynchus*) or Coleoptera: Chrysomelidae (larvae of Halticinae or Hispinae) are able to produce such pattern
13298*	*Phagophytichnus marginis-folii*			**Plate A, Fig. J**	15,1	Margin		?Coleoptera: Curculionidae vel Chrysomelidae	Coleoptera: Curculionidae (*Rhynchaenus* or *Otiorhynchus*) or Coleoptera: Chrysomelidae (larvae of Halticinae or Hispinae) are able to produce such pattern
20544*	*Phagophytichnus marginis-folii*		Straus (1977; Taf. 4, Fig. 64)	**Plate A, Fig. K**	15,1	Margin		?Coleoptera: Curculionidae vel Chrysomelidae	Coleoptera: Curculionidae (*Rhynchaenus* or *Otiorhynchus*) or Coleoptera: Chrysomelidae (larvae of Halticinae or Hispinae) are able to produce such pattern
20615[¶,*]	*Phagophytichnus marginis-folii*					Margin		?Coleoptera: Curcu,lionidae vel Chrysomelidae	Coleoptera: Curculionidae (*Rhynchaenus* or *Otiorhynchus*) or Coleoptera: Chrysomelidae (larvae of Halticinae or Hispinae) are able to produce such pattern

Coll. No.	Ichnotaxon	Type	Straus orginal Ref.	Plate Ref.	DT	FFG	Host plant	Culprit	Comments
21266*	*Phagophytichnus marginis-folii*			**Plate A, Fig. I**	12,1	Margin	*Fraxinus ornus*	?Coleoptera: Curculionidae vel Chrysomelidae	Coleoptera: Curculionidae (*Rhynchaenus* or *Otiorhynchus*) or Coleoptera: Chrysomelidae (larvae of Halticinae or Hispinae) are able to produce such pattern
22686*	*Phagophytichnus marginis-folii*		Straus (1977; Taf. 4, Fig. 65)	**Plate A, Fig. L**	15	Margin	*Parrotia persica*	?Coleoptera: Curculionidae vel Chrysomelidae	Coleoptera: Curculionidae (*Rhynchaenus* or *Otiorhynchus*) or Coleoptera: Chrysomelidae (larvae of Halticinae or Hispinae) are able to produce such pattern
30811¶,*	*Phagophytichnus marginis-folii*					Margin		?Coleoptera: Curculionidae vel Chrysomelidae	Coleoptera: Curculionidae (*Rhynchaenus* or *Otiorhynchus*) or Coleoptera: Chrysomelidae (larvae of Halticinae or Hispinae) are able to produce such pattern
30819*	*Phagophytichnus marginis-folii*		Straus (1977; Taf. 4, Fig. 72)	**Plate A, Fig. N**	14,8	Margin	*Fraxinus ornus*	?Coleoptera: Curculionidae vel Chrysomelidae	Coleoptera: Curculionidae (*Rhynchaenus* or *Otiorhynchus*) or Coleoptera: Chrysomelidae (larvae of Halticinae or Hispinae) are able to produce such pattern
30955*	*Phagophytichnus marginis-folii*			**Plate A, Fig. M**	12	Margin	*Tilia saportaea*	?Coleoptera: Curculionidae vel Chrysomelidae	Coleoptera: Curculionidae (*Rhynchaenus* or *Otiorhynchus*) or Coleoptera: Chrysomelidae (larvae of Halticinae or Hispinae) are able to produce such pattern
11710*	*Phagophytichnus nervos-mutans*	H	Straus (1977; Taf. 4, Fig. 63)	**Plate A, Fig. O**	12,32	Margin	*Carya minor*		
9781*	*Phagophytichnus nigromarginatus*	H	Straus (1977; Taf. 4, Fig. 68)	**Plate A, Fig. P**	8	Hole	*Laburnum* sp.	?Coleoptera: Chrysomelidae	comparable to *Phagophytichnus catellarius*

Plate A

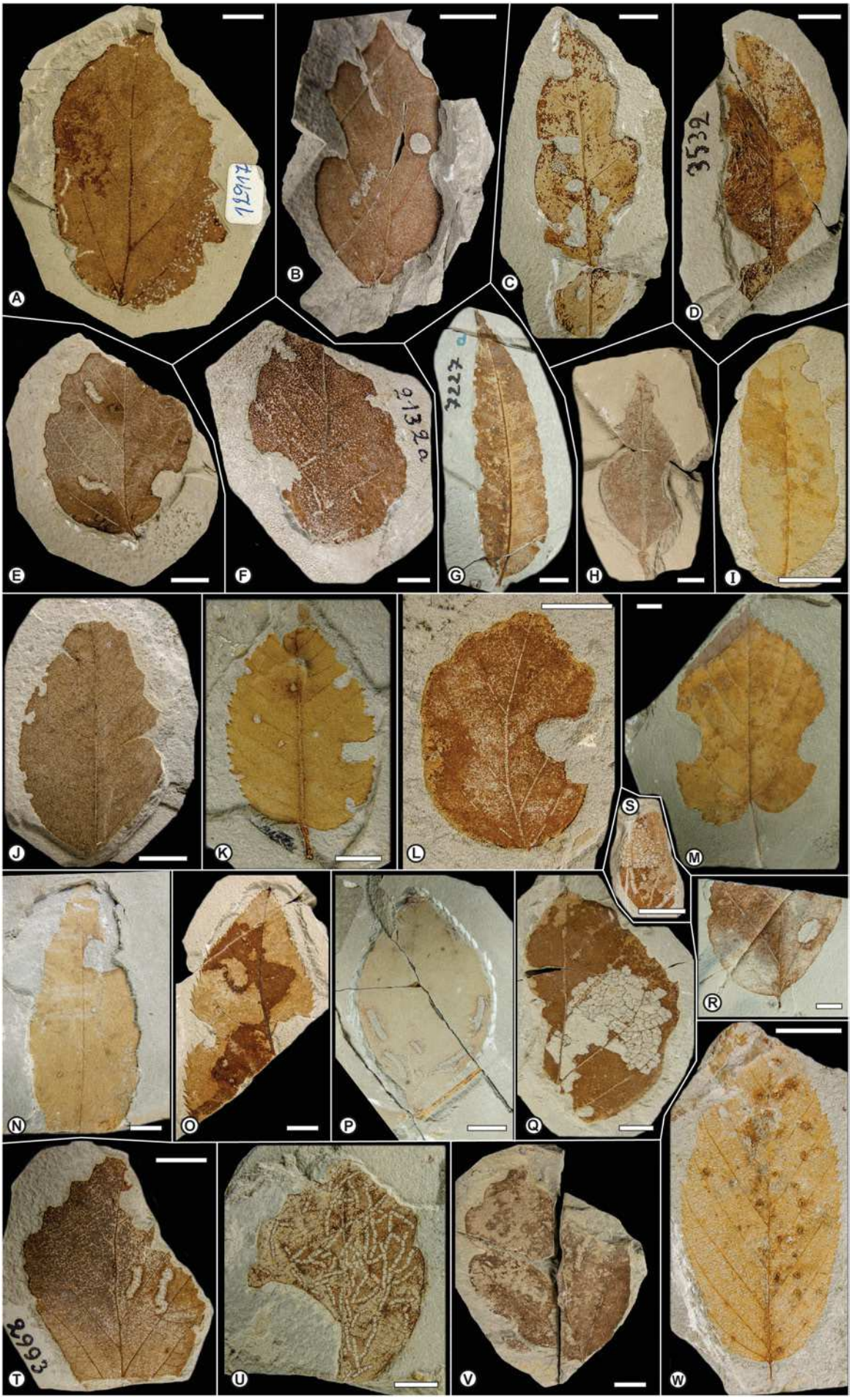


Plate A. Types of hervivorized leaves as described by A. Strauss (1977) from the Pliocene of Willershausen (type material is stored in the Willershausen locality, showing herbivory pattern. The material is deposited in the Geoscience Centre, University of Göttingen (GZG). **A.** *Phagophytichnus circumsecans* / *Phagophytichnus catellarius* (GZG.W 12917); **B.** *Phagophytichnus circumsecans* (Holotype, GZG.W 12231); **C.** *Phagophytichnus marginis-folii* / *Phagophytichnus circumsecans* (GZG.W 11490); **D.** *Phagophytichnus marginis-folii* (GZG.W 3532); **E.** *Phagophytichnus marginis-folii* (Holotype, GZG.W 13386); **F.** *Phagophytichnus marginis-folii* (GZG.W 2132/a); **G.** *Phagophytichnus marginis-folii* (GZG.W 7227); **H.** *Phagophytichnus marginis-folii* (GZG.W 3612); **I.** *Phagophytichnus marginis-folii* (GZG.W 21266); **J.** *Phagophytichnus marginis-folii* (GZG.W 13298); **K.** *Phagophytichnus marginis-folii* (GZG.W 20544); **L.** *Phagophytichnus marginis-folii* (GZG.W 22686); **M.** *Phagophytichnus marginis-folii* (GZG.W 30955); **N.** *Phagophytichnus marginis-folii* (GZG.W 30819); **O.** *Phagophytichnus nervos-mutans* (Holotype, GZG.W 11710); **P.** *Phagophytichnus nigromarginatus* (Holotype, GZG.W 9781); **Q.** *Phagophytichnus nervillos-reliquens* (Holotype, GZG.W 21482); **R.** *Phagophytichnus nervillos-reliquens* (GZG.W 30892/a); **S.** *Phagophytichnus nervillos-reliquens* (GZG.W 21140); **T.** *Phagophytichnus catellarius* (Holotype, GZG.W 2993/a); **U.** *Phagophytichnus catellarius* (Paratype, GZG.W 10626); **V.** *Phagophytichnus catellarius* (GZG.W 13076); **W.** cf. *Dasyneura ruebsaameni fossilis* vel *Cecidophyes reniformis fossilis* (GZG.W 23992/a). Scale bars represent 10mm.

Plate B

Plate B. Types of hervivorized leaves as described by A. Strauss (1977) from the Pliocene of Willershausen (type material is stored in the Willershausen locality, showing herbivory pattern. The material is deposited in the Geoscience Centre, University of Göttingen (GZG). **A.** *Aceria leionota fossilis* (GZG.W 9159); **B.** *Aceria leionota fossilis* (GZG.W 9444/a); **C.** cf. *Aceria macrochela fossilis* (GZG.W 3116); **D.** cf. *Aceria macrochela fossilis* (GZG.W 6560); **E.** cf. *Aceria macrochela fossilis* (GZG.W 19779); **F.** cf. *Aceria macrochela fossilis* (GZG.W 30808); **G.** *Aceria nervisequa faginea fossilis* (GZG.W 30914); **H.** *Aceria nervisequa faginea fossilis* (GZG.W 21979); **I.** *Schizoneura ulmi fossilis* (GZG.W 2179); **J.** cf. *Dasyneura fraxini fossilis* (GZG.W 14652); **K.** *Aceria nervisequa faginea fossilis* (GZG.W 30815); **L.** *Aceria nervisequa nervisequa fossilis* (GZG.W 13748/a); **M.** *Aceria nervisequa nervisequa fossilis* (GZG.W 22848/a); **N.** *Andricus quercus-radicis fossilis* (GZG.W 14200); **O.** *Neuroterus albipes fossilis* (GZG.W 2425); **P.** *Petiolocecidium aceris* (Holotype, GZG.W 30810); **Q.** *Petioloceoidium hamamelidacearum* (GZG.W 10060); **R.** *Phyllocecidium alni-tuberculosum* (Holotype, GZG.W 3388/a); **S.** cf. *Andricus* sp. (GZG.W 7244); **T.** cf. *Didymomyia reaumuriana fossilis* (GZG.W 8717) / *Fenusites tiliae* (Holotype, GZG.W 8717); **U.** cf. *Contarinia carpini fossilis* (GZG.W 3042); **V.** *Phyllocecidium comma* (Holotype, GZG.W 12143); **W.** cf. *Andricus* sp. (GZG.W 17076); **X.** cf. *Andricus* sp. (GZG.W 13732); **Y.** cf. *Andricus* sp. (GZG.W 15091); **Z.** cf. *Andricus* sp. (GZG.W 22705); **AA.** cf. *Mikiola fagi fossilis* vel *Hartigiola annulipes fossilis* (GZG.W 5021); **AB.** *Petioloceoidium hamamelidacearum* (GZG.W 13164). Scale bars represent 10mm.

Plate C

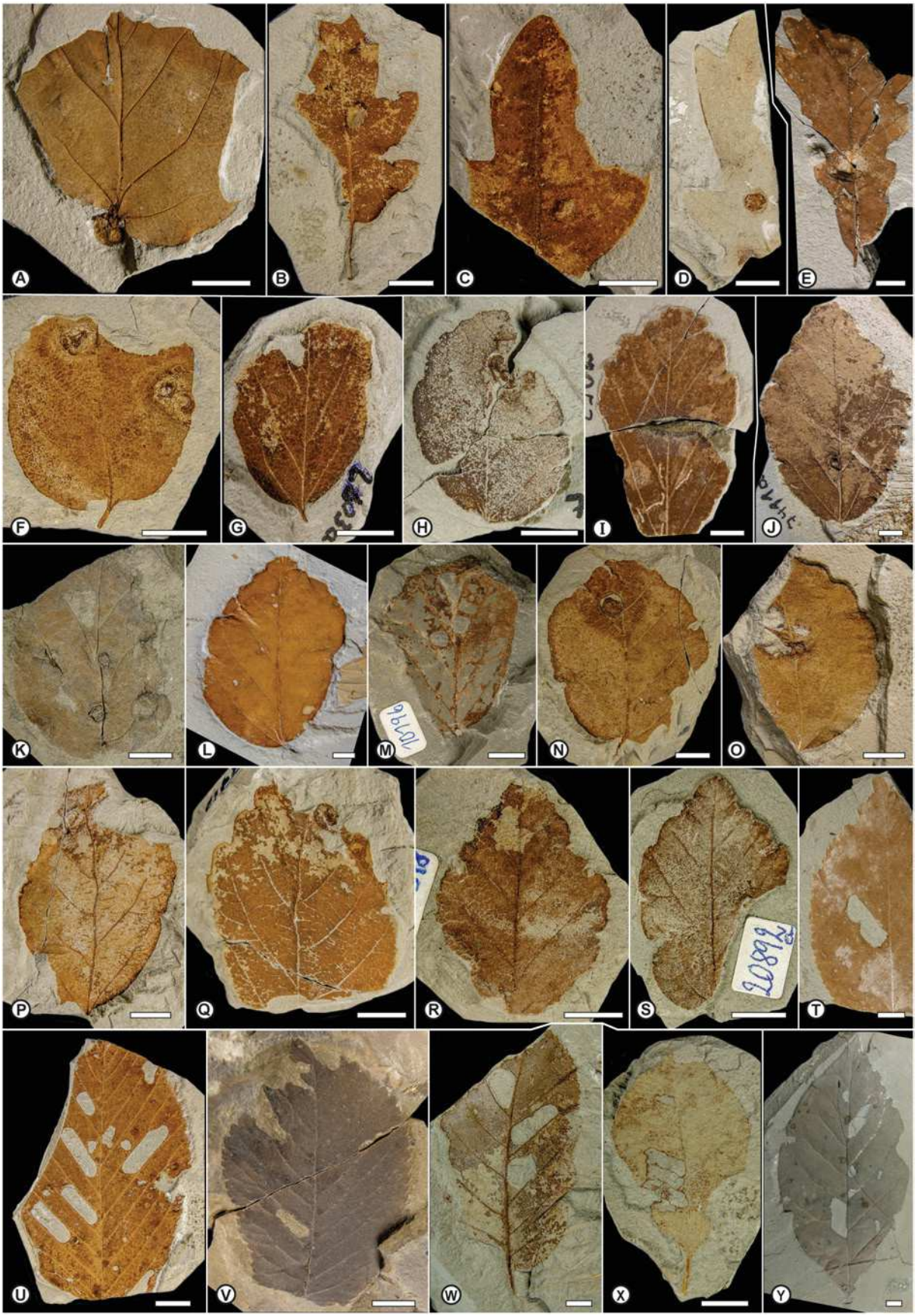


Plate C. Types of hervivorized leaves as described by A. Strauss (1977) from the Pliocene of Willershausen (type material is stored in the Willershausen locality, showing herbivory pattern. The material is deposited in the Geoscience Centre, University of Göttingen (GZG). **A.** *Petioloceoidium hamamelidacearum* (Holotype, GZG.W 20197/a); **B.** *Phyllocecidium comma* (Paratype, GZG.W 20161/a); **C.** *Phyllocecidium comma* (Paratype, GZG.W 23862); **D.** *Phyllocecidium medionervisequm* (Holotype, GZG.W 18421/a); **E.** *Phyllocecidium medionervisequm* (GZG.W 30845/a); **F.** *Phyllocecidium parrotiae* (Holotype, GZG.W 13156); **G.** *Phyllocecidium parrotiae* (GZG.W 2603); **H.** *Phyllocecidium parrotiae* (GZG.W 7217); **I.** *Phyllocecidium parrotiae* (GZG.W 7253); **J.** *Phyllocecidium parrotiae* (GZG.W 7411); **K.** *Phyllocecidium parrotiae* (GZG.W 9214); **L.** *Phyllocecidium parrotiae* (GZG.W 9673/a); **M.** *Phyllocecidium parrotiae* (GZG.W 10196); **N.** *Phyllocecidium parrotiae* (GZG.W 13217); **O.** *Phyllocecidium parrotiae* (GZG.W 16732); **P.** *Phyllocecidium parrotiae* (GZG.W 17689); **Q.** *Phyllocecidium parrotiae* (GZG.W 17715); **R.** *Phyllocecidium parrotiae* (GZG.W 20316/a); **S.** *Phyllocecidium parrotiae* (GZG.W 20892); **T.** *Fenusites betulacearum* (GZG.W 18874); **U.** *Fenusa ulmi fossilis* (GZG.W 21920/a); **V.** *Fenusa ulmi fossilis* (GZG.W 12); **W.** *Fenusa ulmi fossilis* (GZG.W 3183); **X.** *Fenusites denckmanni* (Holotype, GZG.W 14535/a); **Y.** *Fenusites betulacearum* (Holotype, GZG.W 20987/a). Scale bars represent 10mm.

Plate D

Plate D. Types of hervivorized leaves as described by A. Strauss (1977) from the Pliocene of Willershausen (type material is stored in the Willershausen locality, showing herbivory pattern. The material is deposited in the Geoscience Centre, University of Göttingen (GZG). **A.** *Fenusites tiliae* (GZG.W 15650); **B.** *Fenusites tiliae* and cf. *Coleophora* sp. (GZG.W 22549/a); **C.** *Fenusites betulacearum* (GZG.W 18906); **D.** *Fenusites celtis* (Holotype, GZG.W 17924); **E.** *Fenusites fagi* (Holotype, GZG.W 21926); **F.** *Fenusites fagi* (GZG.W 11339); **G.** *Fenusites parrotiae* (Holotype, GZG.W 22508); **H.** *Fenusites zelovae* (Holotype, GZG.W 30806); **I.** cf. *Bucculatrix thoracella fossilis* (GZG.W 18422); **J.** cf. *Coleophora* sp. (GZG.W 21040); **K.** cf. *Coleophora* sp. (GZG.W 21695/a); **L.** cf. *Coleophora* sp. (GZG.W 22549/a); **M.** cf. *Coleophora* sp. (GZG.W 22858); **N.** cf. *Coleophora* sp. (GZG.W 22996/a); **O.** cf. *Caloptilia alchimiella fossilis* (GZG.W 22788); **P.** cf. *Caloptilia alchimiella fossilis* (GZG.W 22440); **Q.** cf. *Coriscium* sp. (GZG.W 30838); **R.** cf. *Parornix* sp. (GZG.W 15876/a); **S.** *Lithocolletis maestingella fossilis* (GZG.W 30057); **T.** cf. *Incurvaria oehlmanniella fossilis* (GZG.W 15427); **U.** cf. *Incurvaria* sp. (GZG.W 21313); **V.** cf. *Recurvaria nanella* (GZG.W 12724/a); W. *Stigmella ulmivora fossilis* (GZG.W 9111). Scale bars represent 10mm.

Plate E

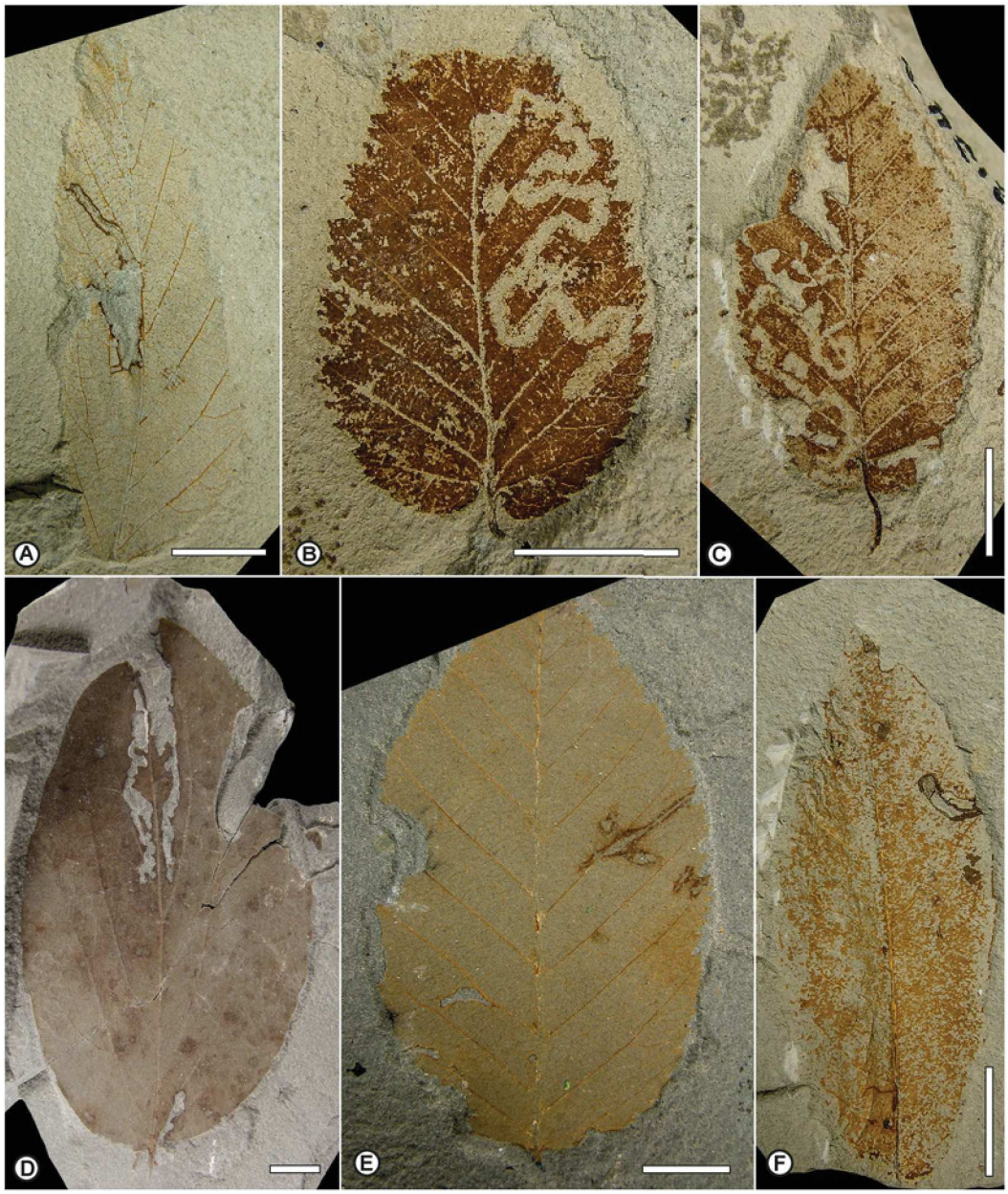


Plate E. Types of hervivorized leaves as described by A. Strauss (1977) from the Pliocene of Willershausen (type material is stored in the Willershausen locality, showing herbivory pattern. The material is deposited in the Geoscience Centre, University of Göttingen (GZG). **A.** *Stigmella ulmivora fossilis* (GZG.W 17738); **B.** *Stigmellites carpini-orientalis* (Holotype, GZG.W 22763); **C.** *Stigmellites carpini-orientalis* (Paratype, GZG.W 22134); **D.** *Phytomyzites corni* (Holotype, GZG.W 10965); **E.** *Cuniculonomus carpini* (Holotype, GZG.W 19471); **F.** Diptera mine undetermined (GZG.W 19753/a). Scale bars represent 10mm.

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Appendix E: Frequency of the damaged leaves per FFG.

Data for Bernasso originate from Adroit et al. (2016)

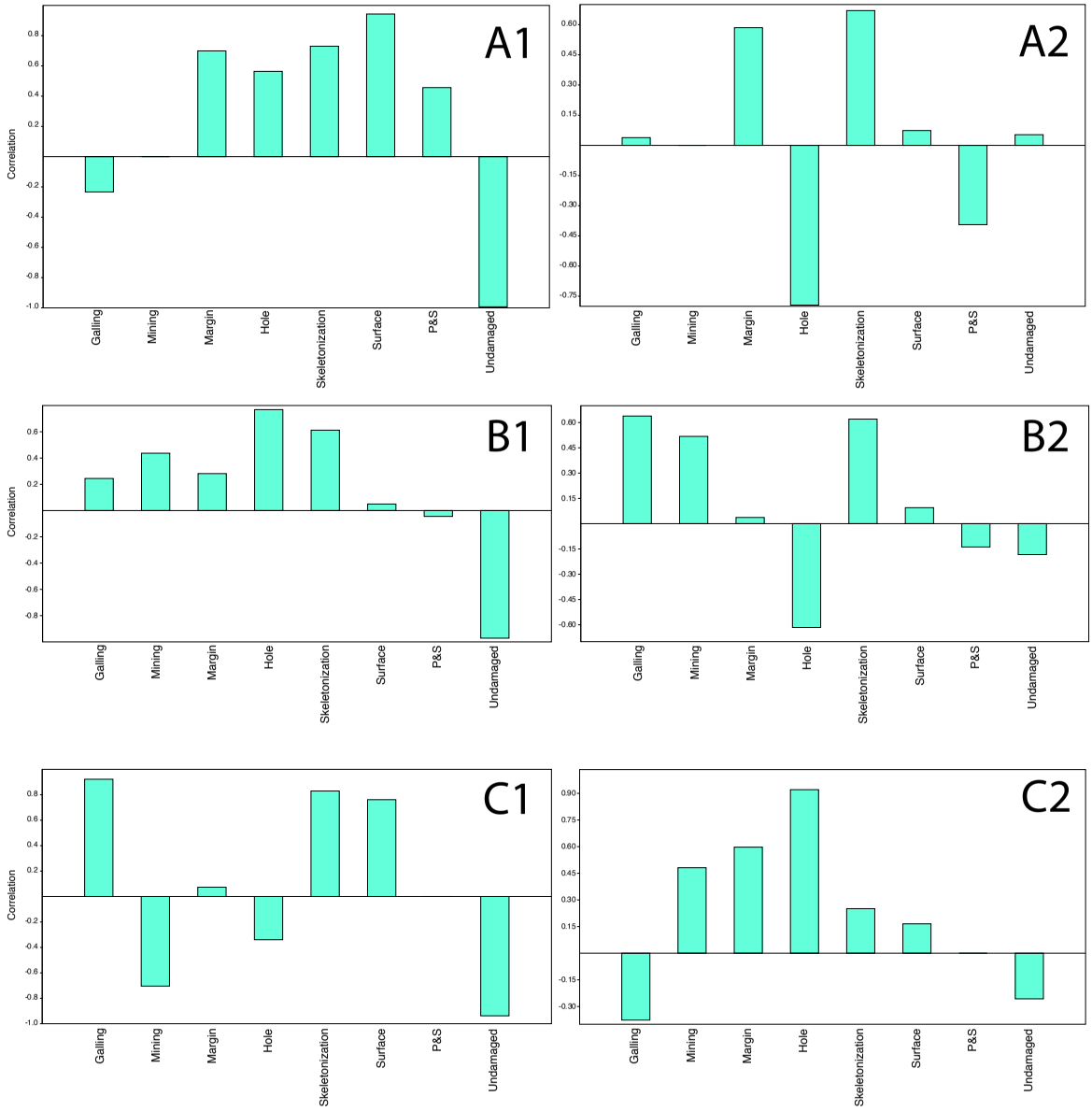
Outcrops	*#leaves*	**Damaged**	**Generalist**	**Specialist**	**External_ Specialized**	Galling	Mining	MarginF	HoleF	Skeletonization	SurfaceF	P&S
WILLERSHAUSEN	*7932*	50.43	42.80	10.16	1.11	7.01	1.59	9.86	26.94	11.01	1.64	1.10
BERGA	*534*	25.09	17.79	7.12	1.31	6.18	0.19	1.87	12.73	2.62	2.06	0.94
BERNASSO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

Appendix F: Percent of damaged leaves according to LMA per species measured.

Detailed values of Figure 5.

Plant ID	Number of specimens	Percent of damaged leaves	LMA (g/m^2)	95% PI top (mean)	95% PI bottom (mean)
Acer integerrinum	15	0.701	46.183	104.19	20.47
Acer laetum	20	0.456	51.066	115.17	22.64
Carpinus orientalis	15	0.388	63.173	142.43	28.02
cf "Magnolia" sp1	22	0.521	71.173	160.45	31.57
cf "Magnolia" sp2	13	0.506	79.261	178.65	35.17
Fagus graudifolia	15	0.57	48.872	110.24	21.67
Fagus sylvatica	97	0.492	52.041	117.36	23.08
Parrotia persica	75	0.712	49.365	110.83	21.78
Sorbus domestica	12	0.397	55.715	125.63	24.71
Zelkova ungeri	19	0.449	69.671	157.05	30.91

Appendix G: Significance of the axes 1 and 2 from the PCAs presented in Figure 6.



Appendix H: General spreadsheet of plant species considered statistically (more than 20 specimens) in our study.

Results are expressed in percent. The plant species with less than 20 specimens were taken into account for the plant species richness and the general result of FFGs in each fossil leaf assemblages.

Outcrops	Species	Leaves	Damaged	Specialized	Gall	Mine	External Spz	Generalist	MarginF	HoleF	Skeleton	SurfaceF	P&S
	Zelkova_ungeri	948	0.449	0.06	0.052	0.004	0.0042	0.403	0.086	0.228	0.12	0.014	0.005
	Fagus_sylvatica	549	0.492	0.084	0.067	0.009	0.0146	0.439	0.077	0.302	0.113	0.009	0.011
	Parrotia_persica	517	0.712	0.282	0.133	0.108	0.0155	0.509	0.099	0.263	0.257	0.033	0.01
	Quercus_praeerucifolia	322	0.478	0.096	0.081	0.003	0.0031	0.41	0.075	0.233	0.14	0.006	0.016
	Carpinus_orientalis	206	0.388	0.092	0.063	0.015	0.0146	0.306	0.092	0.233	0.015	0.015	0.01
	Carpinus_grandis	176	0.449	0.057	0.045	0,000	0.0057	0.409	0.085	0.301	0.04	0.011	0.006
	Carpinus_betulus	173	0.405	0.069	0.058	0.006	0,0000	0.353	0.052	0.243	0.075	0.006	0.006
	Betula_maximowicziana	164	0.518	0.116	0.085	0.006	0.0061	0.439	0.03	0.372	0.049	0.024	0.03
	cf_Magnolia_sp1	121	0.521	0.157	0.107	0.025	0.0083	0.372	0.074	0.198	0.157	0.008	0,000
	Acer_integerrimum	107	0.701	0.168	0.084	0.019	0.0374	0.598	0.196	0.383	0.15	0.009	0.047
	Fagus_grandifolia	100	0.57	0.09	0.06	0.01	0.01	0.51	0.14	0.32	0.15	0,000	0.02
	Ulmus_carpinifolia	94	0.713	0.096	0.053	0.021	0.0106	0.649	0.117	0.415	0.191	0.032	0.011
	Carya_minor	90	0.533	0.089	0.078	0,000	0,0000	0.467	0.178	0.233	0.111	0.022	0.011
	cf_Magnolia_sp2	81	0.506	0.21	0.148	0.062	0.0123	0.346	0.148	0.16	0.086	0.012	0,000
	Quercus_macranthera	79	0.519	0.025	0,000	0,000	0.0127	0.494	0.063	0.418	0.101	0.013	0.013
	Populus_tremula	72	0.5	0.069	0.069	0,000	0.0278	0.458	0.139	0.264	0.111	0.028	0,000
	Acer_laetum	68	0.456	0.103	0.074	0.015	0.0147	0.368	0.103	0.162	0.176	0.015	0,000
	Sorbus_domestica	68	0.397	0.059	0.029	0.015	0.0147	0.338	0.147	0.191	0.074	0.029	0.015
	Alnus_spaethii	66	0.439	0.136	0.106	0.015	0,0000	0.348	0.045	0.288	0,000	0.015	0.015
	cf_Toona	65	0.538	0.108	0.062	0.015	0.0154	0.446	0.169	0.277	0.062	0.062	0.015
	Betula_lenta	64	0.563	0.094	0.094	0,000	0,0000	0.484	0.031	0.422	0.063	0,000	0,000
	Populus_willershausensis	63	0.492	0.143	0.095	0,000	0,0000	0.397	0.048	0.286	0.159	0,000	0.016
	Malus_pulcherrima	59	0.525	0.153	0.085	0.017	0.0169	0.441	0.034	0.288	0.085	0.102	0.051
	Carya_cf_Pterocarya	58	0.552	0.052	0.034	0.017	0,0000	0.534	0.259	0.31	0.052	0.017	0,000
	Quercus_iberica	56	0.5	0.036	0.018	0,000	0,0000	0.482	0.107	0.375	0.054	0,000	0.018
	Quercus_roburoides	54	0.611	0.148	0.167	0,000	0.037	0.519	0.111	0.352	0.13	0,000	0,000
	Sorbus_gabbrensis	52	0.462	0.096	0.038	0,000	0,0000	0.404	0.115	0.231	0.096	0.038	0.038

Willershausen	*Tilia_cf_savania*	47	0.745	0.043	0.043	0,000	0.0213	0.702	0.085	0.468	0.213	0.064	0,000
	Ulmus_campestris	45	0.644	0.111	0.089	0.022	0.0222	0.578	0.133	0.4	0.156	0.022	0,000
	Fagus_cf_pliocenica	44	0.545	0.136	0.068	0.045	0.0455	0.409	0.091	0.295	0.136	0.045	0.023
	Alnus_serrulata	38	0.553	0.079	0.026	0,000	0.0263	0.447	0.158	0.316	0.053	0,000	0.026
	cf_Magnolia	38	0.684	0.263	0.184	0.053	0.0263	0.474	0.053	0.158	0.289	0.026	0,000
	Prunus_mahaleb	38	0.5	0.026	0,000	0,000	0,0000	0.5	0.132	0.263	0.105	0.079	0.026
	Betula_pubescens	36	0.556	0.056	0.056	0.028	0,0000	0.528	0.167	0.25	0.083	0,000	0,000
	Quercus_libani	36	0.389	0.056	0.028	0,000	0,0000	0.361	0.111	0.194	0.111	0,000	0.028
	Acer_monspessulanum	33	0.545	0.182	0.182	0,000	0,0000	0.424	0.061	0.242	0.152	0.03	0,000
	Tilia_saportae	33	0.758	0.152	0.061	0.061	0.0303	0.697	0.182	0.515	0.212	0.03	0.03
	Carpinus_sp1	30	0.433	0.033	0.033	0,000	0,0000	0.4	0.033	0.267	0.1	0.033	0,000
	Betula_sp1	29	0.552	0.034	0,000	0,000	0,0000	0.517	0.172	0.241	0.103	0,000	0.034
	Betula_alnoides	28	0.5	0.036	0.036	0,000	0,0000	0.464	0.107	0.321	0.036	0,000	0,000
	Zelkova_caprinifolia	28	0.536	0.036	0.036	0,000	0,0000	0.5	0.179	0.25	0.107	0.036	0,000
	Crataegus_meischneri	27	0.407	0.148	0.074	0,000	0,0000	0.259	0,000	0.148	0.074	0.037	0.074
	Quercus_castaneaefolia	27	0.481	0.074	0.074	0,000	0,0000	0.444	0.037	0.333	0.074	0,000	0,000
	Quercus_praecastaneaefo	26	0.538	0.077	0.038	0,000	0,0000	0.462	0.077	0.308	0.077	0,000	0.038
	Acer_paleosaccharinum	25	0.68	0.2	0.16	0.04	0,0000	0.56	0.16	0.28	0.2	0,000	0,000
	Acer_cappadocicum	24	0.5	0.125	0.125	0,000	0,0000	0.375	0.083	0.208	0.042	0.042	0,000
	Juglans_sieboldiana	24	0.333	0.042	0.042	0,000	0,0000	0.292	0.083	0.125	0.042	0.042	0,000
	Aesculus_hippocastanum	23	0.478	0.087	0.043	0,000	0.0435	0.391	0.217	0.13	0.043	0.043	0.043
	Populus_populina	23	0.348	0,000	0,000	0,000	0,0000	0.348	0.043	0.217	0.13	0,000	0,000
	Betula_pendula	22	0.455	0.091	0.045	0,000	0,0000	0.409	0.045	0.318	0.045	0.045	0,000
	Leguminosites_strausii	21	0.476	0.333	0.286	0.048	0,000	0.238	0.048	0.143	0.095	0,000	0,000
	Populus_gregorii	21	0.667	0.19	0.143	0.048	0.048	0.524	0.095	0.429	0.095	0,000	0,000
	Carpinus_caroliniana	20	0.45	0.15	0.15	0,000	0,000	0.45	0.1	0.35	0.1	0,000	0,000
	Corylus_avellana	20	0.45	0,000	0,000	0,000	0,000	0.45	0.05	0.45	0.1	0,000	0,000
	Populus_alba	20	0.35	0.1	0.1	0,000	0,000	0.25	0.1	0.15	0.05	0,000	0,000

Berga	*Fagus_attenuata*	148	0.25	0.101	0.081	0,000	0.02	0.169	0,000	0.162	0,000	0.014	0,000
	Quercus_sp	52	0.346	0.115	0.058	0,000	0,000	0.212	0,000	0.173	0.038	0.038	0.058
	Alnus_gaudinii	48	0.375	0.063	0.021	0,000	0.042	0.25	0.104	0.104	0.188	0.042	0,000
	Acer_tricuspidatum	45	0.2	0.022	0.022	0,000	0,000	0.178	0,000	0.178	0,000	0,000	0,000
	Acer_integerrinum	35	0.086	0.057	0.029	0,000	0.029	0.086	0,000	0.086	0,000	0,000	0,000
	Cercidiphylum_crenatum	34	0.147	0.088	0.088	0,000	0,000	0,000	0,000	0,000	0.059	0,000	0,000
	Zelkova_ungeri	26	0.115	0.038	0.038	0,000	0,000	0.077	0,000	0.077	0,000	0,000	0,000
Bernasso	*Carpinus_orientalis*	123	0.301	0.106	0.033	0.016	0.016	0.22	0.081	0.138	0.057	0.008	0,000
	Parrotia_persica	106	0.368	0.226	0.217	0.009	0.019	0.113	0.057	0.066	0.038	0,000	0,000
	Zelkova_ungeri	67	0.299	0.119	0.06	0.03	0.045	0.224	0.075	0.119	0.03	0.03	0,000
	Carya_minor	51	0.392	0.098	0.039	0.02	0.02	0.314	0.118	0.216	0.02	0,000	0,000
	Fraxinus_ornus	35	0.257	0.229	0.171	0,000	0.029	0.086	0.086	0,000	0.029	0,000	0,000
	Acer_monspessulanum	22	0.591	0.455	0.318	0,000	0.091	0.182	0.091	0.091	0.318	0.045	0,000
	Sorbus_domestica	22	0.591	0.409	0.318	0,000	0.045	0.182	0.091	0.045	0.091	0.045	0,000

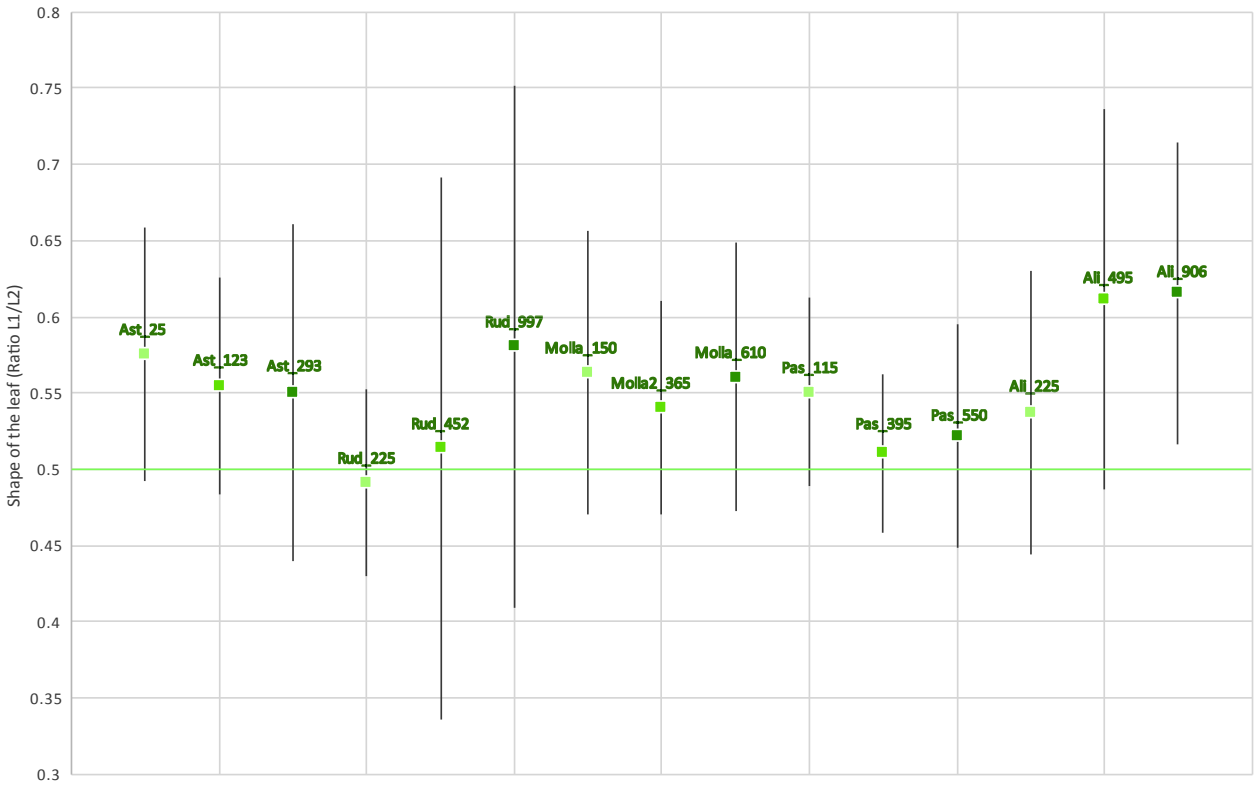
Appendix I: Altitude-dependent mean annual temperature (MAT), warmest month temperature (WMT) and coolest month temperature (CMT). The results were obtained using the regression for MAT of 5.42°C/km, for WMT of 5.36°C/km and for CMT of 5.33°C/km. All the lapse rate came from actual Georgian stations studied by Meyer (1992).

The mean annual temperatures came from weather station close to the sea level and were record by the Iran Meteorological Organization (IRIMO) from 1995 to 2005. The nomenclature of the different sites indicates their exact altitude, for example, Ast293 means the site at Astara from 293 above the sea-level.

Sites	MAT (°C) (sea level)	MAT (°C) (corr:-0.99. p.value=0.999)	WMT (°C) (sea level)	WMT (°C) (corr:-0.978. p.value=0.999)	CMT (°C) (sea level)	CMT (°C) (corr:-0.955. p.value=0.999)
Ast_025		15.5		29.6		2.8
Ast_123	15.5	14.958	29.6	29.064	2.8	2.267
Ast_293		13.874		27.992		1.201
Rud_225		15.016		29.228		1.934
Rud_400	16.1	13.932	30.3	28.156	3	0.868
Rud_997		10.68		24.94		-2.33
Mol_150		15.587		27.896		3.1005
Mol_365	16.4	14.503	28.7	26.824	3.9	2.0345
Mol_610		13.148		25.484		0.702
Pas_160		16.587		30.196		3.5005
Pas_395	17.4	15.232	31	28.856	4.3	2.168
Pas_550		14.419		28.052		1.3685
Ali_225		16.616		31.528		2.334
Ali_495	17.7	14.99	32.6	29.92	3.4	0.735
Ali_906		12.822		27.776		-1.397

Appendix J: *Parrotia persica* leaf shape at each site.

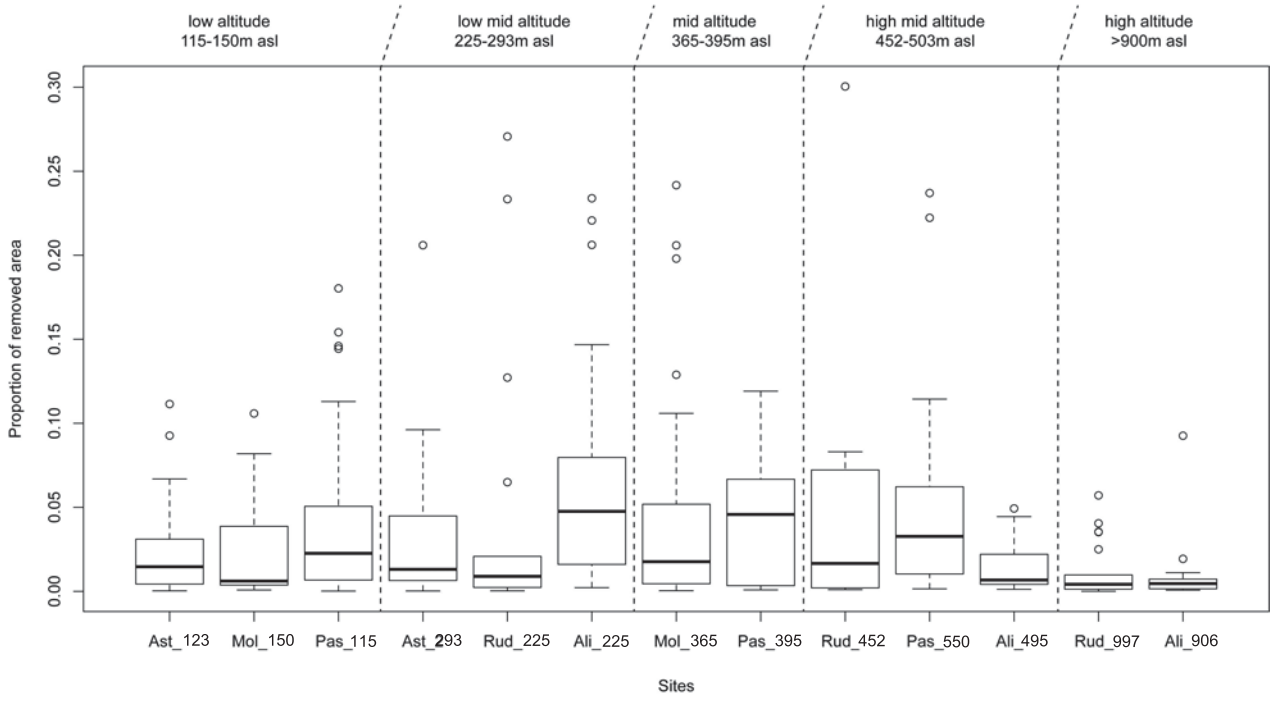
The shape of the leaf is calculated by the length from the basis of the leaf blade to the widest part of the leaf (L1) divided by the total length of the leaf (L2). The green horizontal line (Y axis = 0.5) shows the limit from which the leaf is considered as obovate. All the leaves from each site can be considered obovate. The nomenclature of the different sites indicates their exact altitude, for example, Ast293 means the site at Astara from 293 above the sea-level.



Appendix K: Box-plot of removed area caused by herbivory on the leaves of *Parrotia persica* at each site.

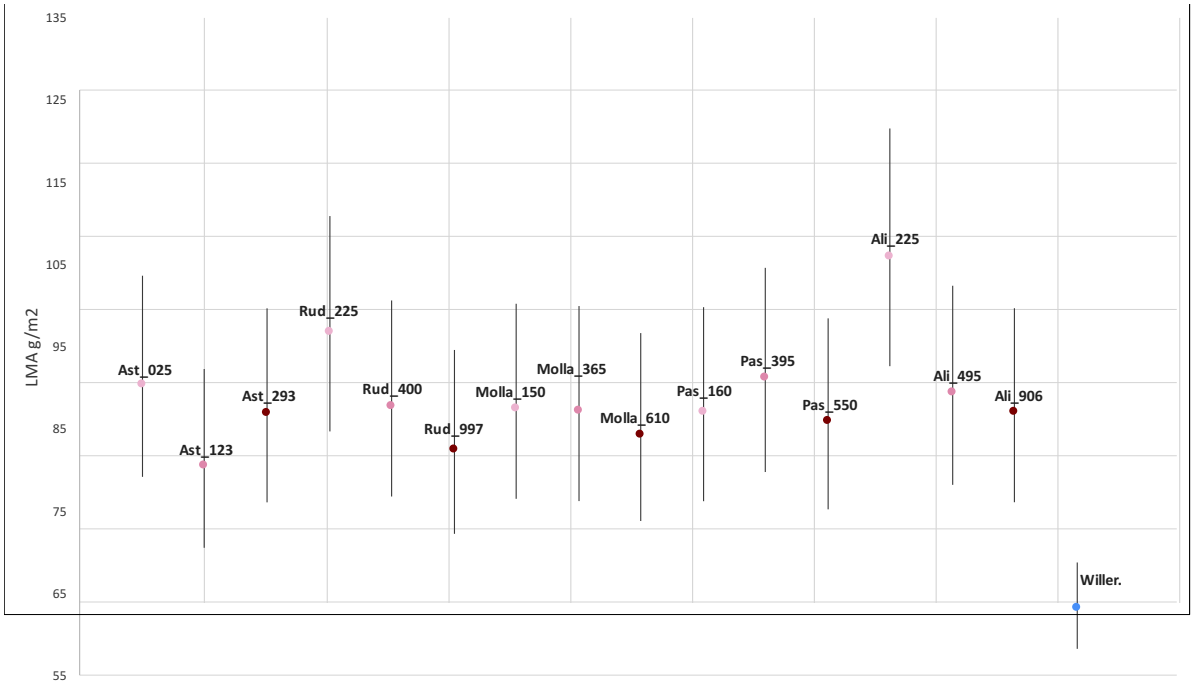
The sites are grouped by similar altitude and from West to East for each group. The removed area is obtained by the difference between the reconstructed complete leaf and the observed destroyed leaf. Only the part of the leaf destroyed by insect feeding was considered. All measurements were done with Image J and Adobe Photoshop CS.

NB: We did not consider the sites Ast_025 and Mol_610 because not comparable to other site for the removed area according to altitude



Appendix L: LMA of *Parrotia persica* leaf at each Iranian sites and addition Willershausen outcrop.

The LMA is estimated using the method of Royer et al. (2007). The errors bars represent the 95% prediction interval. The nomenclature of the different Iranian sites indicates their exact altitude, for example, Ast293 means the site at Astara from 293 above the sea-level.



Appendix M: Occurrences of the damage type (DT) into the different actual and fossil locations.

The nomenclature of the different Iranian sites indicates their exact altitude, for example, Ast293 means the site at Astara from 293 above the sea-level.

The meaning of each DT could be found in the guide from Labandeira et al. (2007).

		DT1	DT2	DT3	DT4	DT5	DT6	DT7	DT8	DT9	DT10	DT11	DT12	DT13	DT14	DT15	DT16	DT17	DT18	DT19	DT20	DT21	DT22	DT23	DT24	DT25	DT26	DT27	DT28	DT29	DT30
1	AST_025	21	24	63	4	11	0	0	0	0	0	0	75	2	6	5	13	0	0	0	0	0	0	0	0	0	2	0	0	1	7
2	AST_123	7	46	14	0	8	0	0	0	0	0	0	36	9	1	2	1	0	0	0	0	0	0	0	0	0	1	0	0	0	2
3	AST_293	10	83	19	0	23	0	0	0	0	0	0	94	7	12	11	1	1	0	0	1	0	2	0	0	0	4	0	0	0	2
4	RUD_225	7	30	33	1	4	0	0	0	1	0	0	52	5	1	1	17	1	0	0	10	2	2	0	0	0	1	0	0	0	9
5	RUD_400	5	28	16	2	3	0	0	0	0	0	0	53	1	5	2	14	1	0	0	1	1	2	0	0	0	4	0	0	0	6
6	RUD_997	7	43	9	0	4	0	0	0	0	0	0	43	1	1	0	8	0	0	0	1	0	2	0	0	0	0	0	0	0	7
7	MOL_150	8	57	5	0	9	0	0	0	0	0	0	35	4	2	2	17	0	0	0	3	4	1	0	0	0	2	0	0	0	0
8	MOL_365	7	75	28	1	11	1	0	0	1	0	0	95	14	8	4	20	3	0	0	1	8	5	0	0	0	4	0	0	0	5
9	MOL_610	5	83	30	0	22	0	0	0	0	0	0	94	16	7	8	15	1	0	0	0	3	1	0	0	0	3	0	0	0	15
10	PAS_160	12	69	40	0	26	0	0	0	0	0	0	111	32	13	8	16	1	0	0	1	1	0	0	0	0	4	0	0	1	13
11	PAS_395	28	58	30	1	7	0	0	0	0	0	0	84	24	8	5	12	2	0	0	5	1	0	0	0	0	4	0	0	0	4
12	PAS_550	23	70	20	5	23	0	0	0	0	0	0	77	23	10	4	19	6	0	0	7	8	1	0	0	0	4	0	0	0	2
13	ALI_225	4	38	19	0	10	0	0	0	0	0	0	101	17	3	10	10	0	0	0	0	0	2	0	0	0	2	0	0	1	1
14	ALI_495	5	30	4	0	8	0	0	0	0	0	0	120	4	1	5	15	1	0	0	19	1	7	0	0	0	2	0	0	12	4
15	ALI_906	2	22	9	0	3	0	0	0	0	0	0	46	4	1	3	8	0	0	0	3	2	0	0	0	0	0	0	0	1	4
16	BERNASSO	0	3	2	0	0	0	0	0	0	1	0	4	1	0	0	1	2	0	0	0	1	0	0	0	0	0	0	0	0	0
17	WILLERSHAUSEN	49	31	43	0	10	0	11	0	0	0	0	42	4	2	3	59	53	0	2	18	0	0	1	2	0	0	0	0	14	1

		DT31	DT32	DT33	DT34	DT35	DT36	DT37	DT38	DT39	DT40	DT41	DT42	DT43	DT44	DT45	DT46	DT47	DT48	DT49	DT50	DT51	DT52	DT53	DT54	DT55	DT56	DT57	DT58	DT59	DT60
1	AST_025	0	4	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	AST_123	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	AST_293	0	1	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	RUD_225	0	8	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1
5	RUD_400	0	6	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6	RUD_997	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
7	MOL_150	0	49	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0
8	MOL_365	0	15	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
9	MOL_610	0	4	0	1	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	4	0	0	0
10	PAS_160	0	2	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
11	PAS_395	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
12	PAS_550	0	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
13	ALI_225	0	5	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
14	ALI_495	0	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
15	ALI_906	0	7	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
16	BERNASSO	0	20	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
17	WILLERSHAUSEN	0	52	5	2	1	5	0	0	0	0	0	0	0	0	0	0	1	0	1	0	0	0	2	0	0	0	0	0	0	0

		DT61	DT62	DT63	DT64	DT65	DT66	DT67	DT68	DT69	DT70	DT71	DT72	DT73	DT74	DT75	DT76	DT77	DT78	DT79	DT80	DT81	DT82	DT83	DT84	DT85	DT86	DT87	DT88	DT89	DT90
1	**AST_025**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	**AST_123**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	**AST_293**	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	**RUD_225**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5	**RUD_400**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6	**RUD_997**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7	**MOL_150**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
8	**MOL_365**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
9	**MOL_610**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10	**PAS_160**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
11	**PAS_395**	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
12	**PAS_550**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
13	**ALI_225**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
14	**ALI_495**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0
15	**ALI_906**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
16	**BERNASSO**	0	0	0	0	0	0	1	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
17	**WILLERSHAUSEN**	3	3	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6	1	3	0	0	0	0	0	0	0	0	0	1

		DT91	DT92	DT93	DT94	DT95	DT96	DT97	DT98	DT99	DT100	DT101	DT102	DT103	DT104	DT105	DT106	DT107	DT108	DT109	DT110	DT111	DT112	DT113	DT114	DT115	DT116	DT117	DT118	DT119	DT120
1	**AST_025**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	**AST_123**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	**AST_293**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	**RUD_225**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5	**RUD_400**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6	**RUD_997**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7	**MOL_150**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
8	**MOL_365**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
9	**MOL_610**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10	**PAS_160**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
11	**PAS_395**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
12	**PAS_550**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
13	**ALI_225**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
14	**ALI_495**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
15	**ALI_906**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
16	**BERNASSO**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
17	**WILLERSHAUSEN**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1

		DT121	DT122	DT123	DT124	DT125	DT126	DT127	DT128	DT129	DT130	DT131	DT132	DT133	DT134	DT135	DT136	DT137	DT138	DT139	DT140	DT141	DT142	DT143	DT144	DT145	DT146	DT147	DT148	DT149	DT150
1	**AST_025**	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
2	**AST_123**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	**AST_293**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	5	0	0	0	0	0	0	0
4	**RUD_225**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0
5	**RUD_400**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6	**RUD_997**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7	**MOL_150**	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
8	**MOL_365**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0
9	**MOL_610**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10	**PAS_160**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	3	0	0	0	0	0	0	0
11	**PAS_395**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0
12	**PAS_550**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
13	**ALI_225**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	8	0	0	0	0	0	0	0
14	**ALI_495**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	17	0	0	0	0	0	0	0
15	**ALI_906**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0
16	**BERNASSO**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	1	1	0	0	1	0	0
17	**WILLERSHAUSEN**	0	0	0	0	0	0	0	2	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	2	1	0	0	0	0

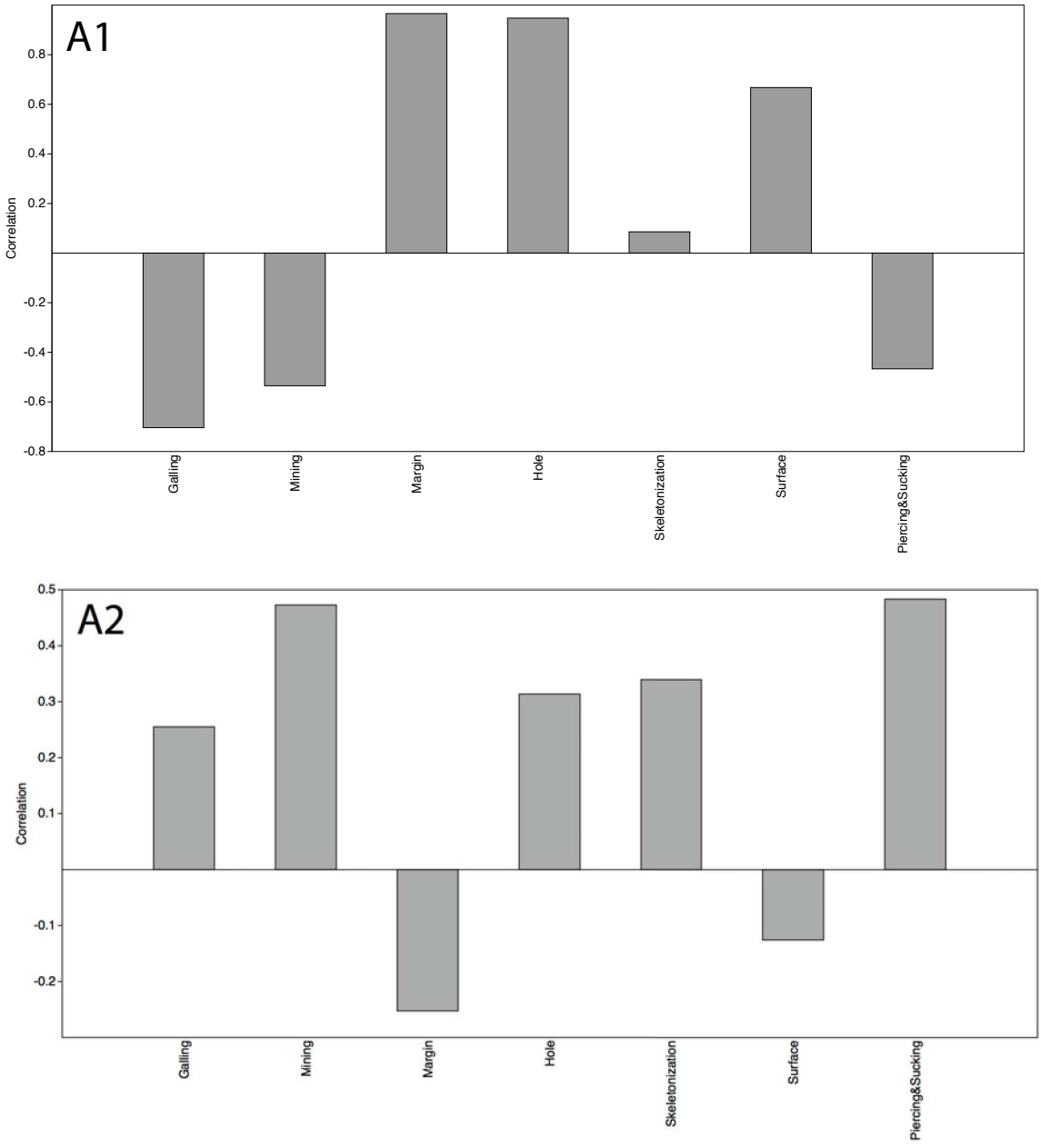
		DT151	DT152	DT153	DT154	DT155	DT156	DT157	DT158	DT159	DT160	DT161	DT162	DT163	DT164	DT165	DT166	DT167	DT168	DT169	DT170	DT171	DT172	DT173	DT174	DT175	DT176	DT177	DT178	DT179	DT180
1	**AST_025**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	**AST_123**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	**AST_293**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	**RUD_225**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5	**RUD_400**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6	**RUD_997**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7	**MOL_150**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
8	**MOL_365**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
9	**MOL_610**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10	**PAS_160**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
11	**PAS_395**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
12	**PAS_550**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
13	**ALI_225**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
14	**ALI_495**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
15	**ALI_906**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
16	**BERNASSO**	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
17	**WILLERSHAUSEN**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0

		DT181	DT182	DT183	DT184	DT185	DT186	DT187	DT188	DT189	DT190	DT191	DT192	DT193	DT194	DT195	DT196	DT197	DT198	DT199	DT200	DT201	DT202	DT203	DT204	DT205	DT206	DT207	DT208	DT209	DT210
1	**AST_025**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
2	**AST_123**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	**AST_293**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	**RUD_225**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5	**RUD_400**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6	**RUD_997**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7	**MOL_150**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
8	**MOL_365**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
9	**MOL_610**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
10	**PAS_160**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
11	**PAS_395**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
12	**PAS_550**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
13	**ALI_225**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
14	**ALI_495**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
15	**ALI_906**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
16	**BERNASSO**	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
17	**WILLERSHAUSEN**	0	0	0	0	4	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	45

		DT211	DT212	DT213	DT214	DT215	DT216	DT217	DT218	DT219	DT220	DT221	DT222	DT223	DT297
1	**AST_025**	0	0	0	0	0	0	0	0	0	0	0	0	0	1
2	**AST_123**	0	0	0	0	0	0	0	0	0	0	0	0	0	0
3	**AST_293**	0	0	0	0	0	0	0	0	0	0	0	0	0	0
4	**RUD_225**	0	0	0	0	0	0	0	0	0	0	0	0	0	0
5	**RUD_400**	0	0	0	0	0	0	0	0	0	0	0	0	0	0
6	**RUD_997**	0	0	0	0	0	0	0	0	0	0	0	0	0	0
7	**MOL_150**	0	0	0	0	0	0	0	0	0	0	0	0	0	0
8	**MOL_365**	0	0	0	0	0	0	0	0	0	0	0	0	0	0
9	**MOL_610**	0	0	0	0	0	0	0	0	0	0	0	0	0	1
10	**PAS_160**	0	0	0	0	0	0	0	0	0	0	0	0	0	2
11	**PAS_395**	0	0	0	0	0	0	0	0	0	0	0	0	0	0
12	**PAS_550**	0	0	0	0	0	0	0	0	0	0	0	0	0	0
13	**ALI_225**	0	0	0	0	0	0	0	0	0	0	0	0	0	0
14	**ALI_495**	0	0	0	0	0	0	0	0	0	0	0	0	0	3
15	**ALI_906**	0	0	0	0	0	0	0	0	0	0	0	0	0	0
16	**BERNASSO**	0	0	0	0	0	0	0	1	0	0	0	0	0	0
17	**WILLERSHAUSEN**	0	0	0	0	0	0	0	0	0	0	0	0	0	37

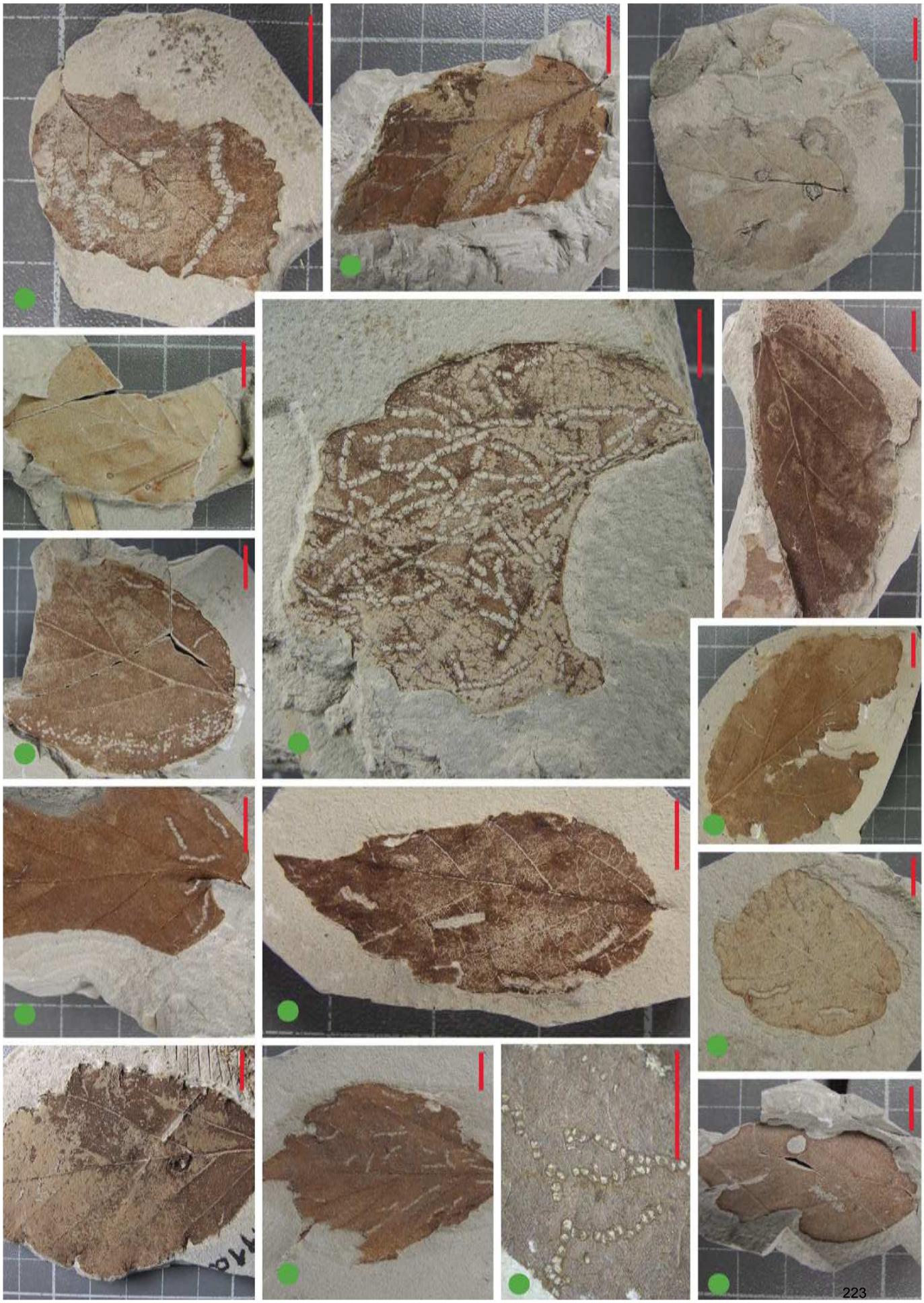
Appendix N: Significance of the Principal Components Analysis correlation for axes 1 and 2 correlation.

The PCA is presented in figure 7, chapter 4



Appendix O: Compilation of fossil leaves of *Parrotia persica* from Willershausen including specialized damage type.

The photos marked with a green circle include DT297. Red bars represent 1cm.



Appendix P: Photo of *Parrotia subaequalis* presenting an insect damage looking like the DT297.

Further investigations are upcoming

RÉSUMÉ SUBSTANTIEL

Les écosystèmes sont en constante évolution. Leurs transformations au cours du temps sont principalement dues à des forçages climatiques, des principales causes des changements écosystémiques (Blois et al., 2013; Scholze et al., 2006). Aujourd'hui, les variations du climat ont un effet d'autant plus significatif sur les écosystèmes et ses êtres vivants, qu'ils sont particulièrement rapides car liées aux activités humaines (IPCC, 2007; Sala et al., 2000; Tylianakis et al., 2008). Par conséquent, les principaux défis du XXI^{ème} siècle sont de comprendre l'impact des changements globaux sur les écosystèmes marins et terrestres et de tenter de réduire leurs impacts (Heller et Zavaleta, 2009; Turner et al., 1990). Dans ce contexte, les plantes et les insectes qui représentent à eux seuls les macroorganismes terrestres les plus importants sur Terre en terme de diversité et d'interactions trophiques et constituant environ 75% de la biodiversité terrestre (Price, 2002) sont indéniablement impactés par ces changements climatiques (Bale et al., 2002).

Néanmoins, il est crucial de souligner que la Terre a déjà connu de nombreux changements climatiques importants au cours du temps. Depuis le milieu du Néogène (*i.e.* 14 Ma), de nombreuses oscillations climatiques se sont succédées et ont engendré des modifications significatives des températures annuelles moyennes (Zachos et al., 2001). À la fin du Pliocène (environ 3 Ma), la température annuelle moyenne sur Terre était d'environ 3°C plus élevée qu'actuellement aux latitudes moyennes, et jusqu'à 6°C plus élevée au niveau des latitudes les plus hautes (e.g., Dowsett et al., 2009). Cet âge, le Piacenzien (-3,6 – -2.58 Ma), est caractérisée à l'échelle mondiale par une transition d'un climat chaud vers un climat plus froid (Mudelsee et Raymo, 2005; Ravelo et al., 2004). Cela a marqué progressivement les débuts du Pléistocène, époque au début de laquelle les amplitudes des périodes glaciaires ont commencé à augmenter, entraînant un recul important des calottes glaciaires de l'hémisphère nord (Gibbard et al., 2010; Raymo et al., 2006; Zachos et al., 2001).

En Europe, la diminution significative des températures annuelles moyennes d'environ 2 à 4°C (Thunell, 1979) a eu des répercussions importantes sur la diversité végétale (Médail et Diadema, 2009; Svenning, 2003). Dans la région méditerranéenne par exemple, la forêt a reculé au profit de végétations steppiques (Suc, 1984). Une partie des taxons végétaux, présents dans cette région et plus globalement en Europe ont disparu suite à ces variations climatiques, d'autres ont pu cependant survivre dans certaines zones européennes, les zones refuges (Martinetto, 2001; Tallis, 1991; Van der Hammen et al., 1971). Enfin, beaucoup de taxons plus largement distribués existent encore aujourd'hui, par exemple en Asie et particulièrement dans la région du Caucase. L'impact qu'ont pu avoir les cycles glaciaires sur la végétation en Europe durant la fin du Pliocène et depuis le Pléistocène a été bien moindre

voire quasi nul dans la région du Caucase ; Cela dû notamment à la position latitudinale relativement basse de cette région et une topographie particulière entre mer (*i.e.* la Caspienne) et montagne (*i.e.* l'Alborz) et ouverte sur l'Anatolie permettant la migration de nombreuses espèces végétales depuis l'Europe (e.g., Zohary, 1973). D'ailleurs, l'actuelle forêt Hyrcanienne présente au nord de l'Iran et bordant la mer Caspienne, est considérée comme relictuelle de ces forêts Européennes de la fin du Pliocène et début du Pleistocène (e.g., Leroy et Roiron, 1996; Noroozi et al., 2008; Probst, 1981; Tralau, 1963; Zohary, 1973). De nombreuses espèces végétales autrefois présentes en Europe durant ces périodes et désormais disparues d'Europe, sont aujourd'hui endémiques de la forêt Hyrcanienne, comme par exemple *Zelkova carpinifolia* (Pall.) C. Koch, *Quercus castaneifolia* CA Mey ou encore *Parrotia persica* CA Mey (e.g., Akhani et al., 2010; Maharramova et al., 2015). Malgré ces espèces communes entre les paléoécosystèmes Européens de la fin de Pliocène et l'actuelle forêt Hyrcanienne, nous n'avons à ce jour très peu (voire pas) d'informations concernant la structure générale de ces écosystèmes. En effet, un écosystème dans son ensemble se définit par deux facteurs clés ; d'une part par sa diversité des organismes vivants, observable directement via les espèces identifiées, et d'autre part, par ses différents niveaux de structures (verticales, trophiques, etc.), observables de façon plus complexe et indirecte. Ces derniers sont notamment régies par le climat, la production primaire du milieu et l'hétérogénéité des espèces (Dajoz, 2006). Dans ce contexte, les interactions entre les plantes et les insectes herbivores sont primordiaux dans le réseau trophique de ces écosystèmes et jouent par conséquent un rôle essentiel dans ces écosystèmes (Schoonhoven et al., 2005). Par conséquent, la compréhension des interactions plante-insecte et leurs variations en réponse aux changements climatiques (Bale et al., 2002; Brooker, 2006; Labandeira et Currano, 2013) s'avère importante afin de mieux caractériser les écosystèmes et leurs transformations. Dans cette optique, l'étude des macrofossiles végétaux (en l'occurrence des feuilles fossiles) doit permettre de modéliser, du moins en partie, la structure des écosystèmes passés en fonction des divers paramètres pouvant l'influencer (Louys et al., 2012; Uhl et al., 2006; Utescher et al., 2000). D'ailleurs, certaines recherches ont déjà commencé à référencer des traces d'herbivorie commises par les insectes sur les feuilles fossiles ; celles-ci ont permis d'amorcer des premières discussions sur les interactions plante-insecte et de leurs variations au cours des temps géologiques en lien avec les changements climatiques (e.g., Currano et al., 2008; Wappler, 2010; Wilf, 2008; Wilf et al., 2001; Wilf et Labandeira, 1999). C'est dans ce contexte que cette thèse s'inscrit. L'objectif est de comprendre : En quoi, localement, les patrons observables des interactions plante-insecte sur les feuilles fossiles ont pu différer, compte tenu des facteurs externes mesurables ? Notre hypothèse de départ s'est basé sur le fait que si la diversité des espèces

peut varier dans les différentes zones biogéographiques à cause de paramètres abiotiques comme le climat et biotiques comme la compétition (e.g., Gurevitch et Fox, 2006), alors la structure trophique des forêts, appréhendée par les interactions plante-insecte, peut-elle aussi significativement varier (Agrawal et al., 2006).

Les interactions plante-insecte, dans le cadre de cette thèse, réfèrent principalement à tout ce qui directement ou indirectement est lié à l'herbivorie de la feuille par des insectes. Par herbivorie, il a été repris la définition suivante : « L'insecte exploite la plante à son seul bénéfice et la plante subit une déprédation, un dégât préjudiciable à sa physiologie ou son développement. Le dégât direct est une lésion, la destruction d'un ou plusieurs types d'organes (racine, tige, feuille, fleur, fruit...) ou la spoliation de sève lors de la prise alimentaire ou de la ponte » (thèse de M. Durand-Gillmann, 2014, INRA/Aix-Marseille). Afin d'identifier ces traces observables sur les feuilles et de pouvoir les comparer, il est indispensable d'adopter une approche standardisée quel que soit l'écosystème étudié. Pour ce faire, la classification des interactions plante-insecte de Labandeira et al. (2007) a été utilisée. Son usage catégorisé des interactions plante-insecte, nommés FFG (pour Functional Feeding Group, c'est-à-dire groupe fonctionnel de nutrition), et également le fait que ce guide soit utilisé dans la majorité des recherches s'intéressant aux interactions plante-insecte dans le registre fossile, ont confirmé le choix de son utilisation. Plusieurs FFG sont référencés selon le catalogue : les trous, les marges, la squelettisation, la surface, la succion & piercing, les galles et les mines. Chacune de ces FFG est subdivisée en plusieurs types de dégât nommés DT (pour Damage Type ou type de dommage). Chacun de ces DT est défini à travers une description morphologique précise et, pour chacun, un indice de spécificité renseignant sur la spécialisation de l'interaction y est associé, en d'autres termes un lien spécifique plus ou moins marqué entre la plante et l'insecte qui a causé le dégât. Cet indice nommé HS (pour Host Specificity, soit indice de spécificité) est important à prendre en compte dans la caractérisation de la structure de la forêt.

Pour cette thèse, trois gisements fossiles ont été étudiés. Deux se trouvent au centre de l'Allemagne autour de la montagne Harz, Willershausen (Knobloch, 1998) et Berga (Mai et Walther, 1988), le dernier se situe dans le sud de la France près de la Méditerranée : Bernasso (Leroy et Roiron, 1996; Suc, 1978). Cette étude a notamment mis en avant la première comparaison entre trois assemblages européens de feuilles fossiles datant du Cénozoïque supérieur. De plus, dû à sa particularité d'être considérée comme le meilleur analogue actuel des paléoforêts citées précédemment (e.g., Knobloch, 1998; Leroy et Roiron, 1996; Zohary, 1973), la forêt Hyrcanienne située au nord de l'Iran a elle-aussi fait l'objet d'une étude centrée

sur une espèce très représentée en Europe il y a 3 Ma, le Parrotie de Perse (*Parrotia persica*), aujourd'hui endémique de la forêt Hyrcanienne (e.g., Nicholson, 1989).

À Bernasso, l'assemblage de feuilles fossiles, daté de 2,16 – 1,96 Ma, correspond à une forêt de feuillus incluant à la fois des espèces plutôt continentales telle que le cormier (*Sorbus domestica* L.) et d'autres typiquement méditerranéennes comme l'érable de Montpellier (*Acer monspessulanum* L.) par exemple. Au total, vingt espèces végétales ont été identifiées sur un total de 590 feuilles fossiles parmi lesquelles 36% présentent des dégâts causés par les insectes, des DT. L'assemblage de feuilles fossiles contient une grande diversité de DT, bien que seulement 6 DT représentent à eux seuls plus de la moitié de cette diversité. Le FFG le plus diversifié sont les galles avec 39 DT identifiés, mais un type en particulier (le DT32) représente l'écrasante majorité des galles. Au total 18% des feuilles à Bernasso présentent des interactions spécialistes.

À Willershausen, l'assemblage de feuilles fossiles est daté 3 – 2,6 Ma. L'intégralité de la richesse spécifique présente à Bernasso est comprise à Willershausen, dans une flore globale comprenant plus de 150 espèces identifiées. Sur une collection de 8000 feuilles fossiles, l'assemblage de feuilles fossiles de Willershausen présente des interactions sur la moitié de celles-ci. Ici, la proportion de galles est significativement plus faible qu'à Bernasso, et seulement 11% des feuilles sont affectées par des interactions spécialistes.

À Berga, daté lui aussi de 3 – 2,6 Ma, sur une collection de feuilles fossiles d'environ 600 unités, seulement 25% présentent des traces d'herbivorie. C'est l'assemblage fossile qui, parmi les trois, est significativement le moins impacté par des interactions plante-insecte. Les genres des plantes identifiés par les feuilles sont similaires à Bernasso et Willershausen, on y retrouve les taxons *Carpinus* (charme), *Acer* (érable)*, Parrotia* ou encore *Zelkova* pour n'en citer que certains. Tout comme à Willershausen, les feuilles fossiles de Berga sont significativement moins affectées par des interactions spécialistes (comme les galles), puisque seulement 8% des feuilles y sont confrontées.

Les différentes estimations climatiques, obtenues via diverses méthodes basées sur la morphologie foliaire (*i.e.* CLAMP, LMA) ou encore par une méthode de Coexsistence des espèces (CoA) basée sur l'identification par les feuilles, montrent que Berga était sûrement la localité subissant les plus basses températures annuelles moyennes des mois les plus froids (autrement dit, l'hiver le plus froid) avec des températures estimées descendant jusqu'à -6,5°C. Bernasso et Willershausen seraient caractérisées par des températures hivernales moyennes estimées à environ –3°C et 5°C respectivement (Adroit et al., accepté; Thiel et al., 2012; Uhl et al., 2007). Cette configuration pourrait notamment expliquer la plus faible quantité d'interactions mesurée à Berga. En effet, les insectes sont des animaux poïkilothermes, ce qui

signifie que leur température corporelle est strictement dépendante de la température de l'environnement (e.g., Meglitsch, 1972), ainsi, plus les températures sont basses et plus le métabolisme des insectes diminue. En dessous d'un certain seuil de température, les insectes entrent en diapause, c'est-à-dire que leur métabolisme et développement s'arrêtent temporairement, engendrant par conséquent une diminution de la quantité de générations annuelles (Archibald et al., 2010). De facto, plus les températures sont basses à l'année et moins le taux d'herbivorie observable devrait être important (Bale et Hayward, 2010). C'est une observation que l'on peut mesurer également pour Bernasso, puisque les températures hivernales y sont significativement plus froides qu'à Willershausen (Girard et al., soumis; Thiel et al., 2012). Néanmoins, il est important de noter qu'entre Bernasso et Berga, cette relation, bien que plausible, est plus difficile à mesurer du fait que les estimations des températures entre ces deux localités se chevauchent selon les études et les méthodes utilisées. De plus, aucune donnée sur la richesse spécifique des insectes n'est disponible dans ces registres fossiles. Bien que l'on puisse supposer que la richesse spécifique en insecte était similaire à Berga et à Willershausen du fait de leurs fortes similitudes géographiques, végétales et temporelles, celle de Bernasso pourrait être sensiblement différente. Ces potentielles différences d'entomofaune, impossible à mesurer directement, ne doivent pas être ignorées comme facteur plausible des différents patterns d'interactions plante-insecte mesurés.

Les précipitations annuelles pour chaque localité peuvent, elles aussi, constituer un facteur clé justifiant des différences des interactions plante-insecte mesurées. En effet, Leckey et al. (2014) ont suggéré que les proportions d'insectes herbivores spécialistes (insectes engendrant des galles) par rapport à ceux généralistes pouvaient varier en fonction de certains paramètres abiotiques, notamment en fonction des précipitations. Ainsi, une relation négative entre le taux de précipitation et la diversité des insectes responsables des galles a été mise en évidence. Cette relation repose sur le fait que plus il y a de précipitations, plus les champignons, qui sont la première cause de mortalité des insectes faisant les galles, se développent (Lara et al., 2002). Pourtant, ce résultat n'a pu être vérifié dans via nos mesures. Au contraire, c'est à Bernasso, avec les précipitations annuelles relatives les plus importantes (Girard et al., soumis; Leroy et Roiron, 1996; Thiel et al., 2012; Uhl et al., 2007), qu'a été mesuré la plus forte proportion de galles. L'hypothèse majeure qui se dégage de ces mesures est que la saisonnalité des précipitations durant l'année serait finalement le facteur primordial concernant la proportion d'insectes cécidogène (et donc de galles observées sur les feuilles). Des précipitations annuelles fortes ne signifient pas nécessairement que les précipitations soient réparties de façon homogène durant toute l'année.

Les estimations climatiques faites directement *via* les feuilles fossiles ainsi que les données déjà publiées (Hennissen et al., 2015; Leroy et Roiron, 1996; Thiel et al., 2012; Uhl et al., 2007; Utescher et al., 2017; Williams et al., 2009), convergent pour supporter le fait que la saisonnalité des précipitations était significativement plus marquée à Bernasso qu'à Berga et à Willershausen. Le stress hydrique plus important à Bernasso, lié à la situation du site en contexte méditerranéen (Bagnouls et Gaussen, 1957; Daget, 1984, 1977; Leroy et Roiron, 1996), a probablement eu un impact important sur la forte proportion de galles observées, la présence de galles étant souvent lié à des environnements stressants (Fernandes et Price, 1992, 1988; Lara et al., 2002; Price et al., 1998). Néanmoins, il est important de souligner que le développement des galles pouvait être négativement corrélé à la richesse des espèces végétales de l'environnement (Cuevas-Reyes et al., 2003). Ce facteur peut également justifier de la forte présence de galles à Bernasso qui présente la collection de feuilles fossiles la moins riche. Cependant, il n'est pas possible d'affirmer clairement que la faible richesse de feuilles fossiles à Bernasso témoigne d'une faible richesse des espèces de plantes dans la paléoforêt, l'assemblage de feuilles fossiles ne représentant pas nécessairement l'intégralité de la flore qui a existé dans cet environnement. La saisonnalité du climat (ici appréhendée par les estimations des précipitations) a d'autant plus de sens que les insectes sont extrêmement sensibles à la moindre variation du climat (Bale et Hayward, 2010) et justement, l'une des réponses des insectes à ces variations climatiques annuelles pourraient être leur comportement alimentaire, visible *via* les traces d'herbivorie observées sur les feuilles (Bale et al., 2002).

Suite à ces limites d'interprétations imposées par le registre fossile, qu'une partie de cette thèse s'est également portée sur l'observation des traces d'herbivorie sur les feuilles actuelles de l'espèce *Parrotia persica,* espèce endémique de la forêt Hyrcanienne au nord de l'Iran (Adroit et al., soumis). Le long d'un gradient de précipitations est-ouest (secondairement de températures également) quinze sites au travers cinq localités ont été sélectionnés afin de collecter les feuilles de *P. persica*. Au total, 2160 feuilles ont été échantillonnées. Chacune d'entre elles a été mesurée (*i.e.* largeur, longueur, largeur du pétiole, périmètre, épaisseur du limbe) et leurs interactions plante-insecte identifiées. 84% des feuilles ont été endommagées par des insectes pour un total de 3507 occurrences d'interactions identifiées. En forêt Hyrcanienne, la proportion de galles apparaît encore reliée positivement avec le taux de précipitations annuel moyen, allant à l'encontre des hypothèses faites entre autres par Lara et al. (2002). Néanmoins, le taux de galles sur les feuilles est d'autant plus nombreux que la saisonnalité des précipitations est marquée. En comparant les feuilles de *P. persica* actuelles et fossiles, il n'est pas possible de mesurer significativement un pattern commun d'interaction

plante-insecte. La conservation des feuilles, drastiquement différentes entre fossile et actuel, peut aisément expliquer ces différences de patterns, notamment quant aux différentes quantités des interactions mesurées. En revanche, des similitudes très marquées de certaines interactions plante-insecte, propre à *P. persica*, ont pu être mesurées. En effet, un type de dégât, qui a été exclusivement retrouvé sur *P. persica* dans le registre fossile de Willershausen et jamais observé auparavant dans tout autre gisement fossile, a également été retrouvé sur les feuilles de *P. persica* en forêt Hyrcanienne. Il s'agit d'une chaîne de petits trous, large de 0,5 mm de diamètre. Cette découverte permet de soulever deux perspectives intéressantes concernant l'étude des interactions plante-insecte et plus spécifiquement l'étude de la structure des forêts européennes des 3 derniers millions d'années. Tout d'abord, cette interaction très spécifique à *P. persica* suggère une histoire biogéographique complexe et commune entre *P. persica* disparu d'Europe de nos jours et *P. persica* encore existant et endémique du nord de l'Iran. Cette observation laisse également à penser que des interactions entre des plantes et des insectes spécifiques peuvent exister depuis des millions d'années, et être très probablement indépendantes de paramètres environnants comme le climat par exemple. Ainsi, on peut penser qu'une partie, vraisemblablement minime, des patterns d'interactions plante-insecte serait indépendante des paramètres environnementaux aussi bien abiotiques et biotiques mesurables dans les registres fossiles.

Dans le contexte de changements globaux actuel, l'augmentation des températures à grande échelle, devrait surtout engendrer un ajustement de la croissance des plantes (afin de s'adapter aux rapides changements des conditions climatiques), plutôt qu'une amélioration ou une adaptation de leurs systèmes de défense contre l'herbivorie (Herms et Mattson, 1992). De ce fait, le taux d'herbivorie (notamment des insectes) favorisera la libération du carbone séquestré majoritairement dans les forêts et donc favorisera l'effet de serre, amplifiant ainsi les effets du changement climatique global (DeLucia et al., 2012). En conséquence, mieux comprendre la complexité des interactions entre les plantes et les insectes sur des périodes de temps relativement longues devrait permettre de mieux appréhender les changements des structures trophiques des écosystèmes forestiers, potentiellement brutaux en réponse au changement global. Dans cette thèse, l'hypothèse selon laquelle la saisonnalité climatique est un facteur de première importance est pour la première fois discutée dans le registre des interactions plante-insecte observable sur les feuilles fossiles. D'après Carré & Cheddadi (2017), la saisonnalité du climat passé est un facteur primordial qui doit constituer le prochain saut qualitatif dans la compréhension des écosystèmes actuels. Cette thèse s'inscrit clairement dans cette perspective. Aussi, il est essentiel de référencer et d'étudier les variations des interactions plante-insecte au cours des temps géologiques, comme l'ont réalisé

Pinhero et al. (2016) du Dévonien jusqu'au début du Miocène, pour en comprendre les mécanismes adaptatifs sous-jacents. Cette thèse constitue une première étape dans la suite de l'étude précédente (citée ci-dessus). Il est primordial de s'intéresser à ces paléoécosystèmes « récents » allant de la fin du Miocène jusqu'à nos jours pour comprendre et caractériser la dynamique des réseaux trophiques qui a conditionné et conditionnera les écosystèmes forestiers à venir.

RÉSUMÉ

"If all mankind were to disappear, the world would regenerate back to the rich state of equilibrium that existed ten thousand years ago. If insects were to vanish, the environment would collapse into chaos."
Edward O. Wilson

Les insectes sont les animaux les plus diversifiés de la Terre, associés aux plantes, ils représentent deux des principaux groupes d'organismes en termes de diversités spécifiques et de biomasses. La majorité de leurs interactions est alimentaire ou parasitaire (formation de galles) et touchent principalement des feuilles. Les plantes et les insectes forment l'un des principaux niveaux trophiques des écosystèmes au cours des 325 derniers millions d'années. Aujourd'hui, l'augmentation rapide et continuelle de la température principalement causée par l'activité humaine depuis les derniers siècles, perturbe la balance des écosystèmes sur Terre. Ceci est dénommé « changement global ». En conséquence, comprendre le rôle des interactions entre les plantes et les insectes, à travers le temps mais aussi les réseaux trophiques, est essentiel. C'est dans ce contexte général que s'inscrit cette thèse.

Le registre fossile est une opportunité exceptionnelle d'examiner les réponses aux interactions plante-insecte lors de longues variations climatiques et à travers des traces de réaction de la plante sachant que la Terre a déjà été soumise à de nombreux changements climatiques. Durant les derniers trois millions d'années (Ma), des oscillations entre de longues périodes froides, marquées par l'avancée sur les continents de la calotte glaciaire dans l'hémisphère nord et de courtes périodes chaudes ont eu lieu. Les écosystèmes Européens ont particulièrement été impactés par ces oscillations climatiques. Ainsi, cette thèse s'est intéressée à certains gisements fossiles d'Europe durant cette période.

Le célèbre Langerstätte (i.e. dépôt sédimentaire contenant une grande diversité de fossiles complets) de Willershausen (centre de l'Allemagne) a été particulièrement étudié. C'est un gisement fossile exceptionnel contenant environ 200 espèces d'angiospermes pour un total avoisinant les 8000 feuilles fossiles. Ces feuilles relatent d'une paléoforêt ayant existé il y a 3 — 2,6 Ma dans un climat plus chaud qu'aujourd'hui (environ +5°C). Dans ces conditions climatiques, de nombreuses espèces d'écosystèmes Méditerranéens étaient présentes, telles que l'Érable de Montpellier (*Acer monspessulanum* L.) ou l'Olivier (*Olea* sp.). En comparaison, d'autres paléoforêts ont été prise en compte : Berga (du même âge et proche de Willershausen) et Bernasso (plus jeune que Willershausen (2,16 — 1,96 Ma) localisée dans le sud de la France près de la Méditerranée. Ces forêts sont comparables notamment du fait des nombreux taxons communs qu'elles partagent. En outre, certaines de ces espèces sont aujourd'hui endémiques de la région du Caucase, telles que le Parrotie de Perse (*Parrotia persica* (DC.) C.A.Mey) ou encore l'orme du Caucase (*Zelkova carpinifolia* (Pall.) Dippel). Le but de cette étude a été de déterminer en quoi les différences climatiques peuvent être impliquées dans les changements des interactions plante-insecte au sein des paléoforêts Européennes de la fin du Pliocène — début du Pleistocene.

Les résultats obtenus ont permis de mettre en évidence les impacts de la saisonnalité des températures et précipitations, jusqu'ici sous-estimées dans les études fossiles, comme facteurs impactants les interactions plante-insecte des paléoforêts Européennes. Il est apparu que les écosystèmes sujets à d'intenses saisonnalités hydriques (i.e. large variation des précipitations durant une année) ont pu engendrer une plus grande spécialisation des interactions plante-insecte déduite d'un fort taux d'interactions spécialistes observées. En parallèle, les températures les plus froides durant l'année semble être un facteur important dans la faible diversité de dégâts, probablement dû à un faible métabolisme de la majorité des insectes. L'absence de corrélation convergente entre la richesse des plantes et la richesse des interactions pourrait suggérer que l'influence des facteurs climatiques surpasse l'impact potentiel des interactions biotiques locales. Pour l'ensemble de ces paramètres qui ont pu avoir un impact sur les interactions plante-insecte (comme par exemple la compétition interspécifique, les nutriments du sol, les biais taphonomiques, etc.), nos connaissances actuelles sont encore insuffisantes pour clairement conclure sur les relations entre le climat et les interactions plante-insecte observées dans les paléoforêts Européennes. Il serait intéressant de focaliser davantage d'études sur les forêts modernes avec des méthodes appliquées dans le fossile. C'est dans cette intention qu'une partie de cette étude a précisément étudié une espèce de plante (*P. persica*) actuellement endémique de la forêt Hyrcanienne (Iran). Cette forêt est supposée être une forêt analogue des paléoforêts Européennes étudiées dans cette thèse. Pour le moment, les observations qui ont été faites en Iran semblent corroborer notre interprétation. Néanmoins, la découverte d'une nouvelle interaction plante-insecte uniquement retrouvée sur les feuilles fossiles et actuelles de *Parrotia persica* suggère que des facteurs historiques peuvent également avoir une influence sur les interactions plante-insecte observées dans deux forêts différentes.

Au final, les études sur les interactions plante-insecte des forêts anciennes et actuelles, combinés avec les études de changements climatiques, pourraient nous permettre de mieux caractériser les relations entre les insectes et les plantes au sein d'une forêt.

Mots-clés : Herbivore insect, Pliocène-Pleistocène, Forêt Hyrcanienne, Feuilles fossiles, Dégât d'insectes, Gradient Climatique

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ABSTRACT

Insects are the most diverse animals on Earth, and neatly associated with plants they represent two of the major groups of organisms both in species diversity and biomass quantity. The majority of their interactions involves insect feeding and insect parasitism (such as galls forming) mostly on leaves. Plant and insect compose one of the main trophic levels in ecosystems over the 325 million years. Today, the continuous and fast rising of temperature mostly due to human activities since the last century is disturbing the balance of ecosystems on Earth. This is named "global change". Consequently, to understand the role of plant and insect interactions, through time but also trophic networks, becomes crucial. It is in this general context that the aim of the thesis pursued.

The fossil record is an exceptional opportunity to survey responses of plant-insect interaction to climate variations over long time interval through traces of plants reactions caused by interaction with insects, as Earth has already experienced many climate changes. For the last 3 million years (Ma), oscillations between long cold periods, marked by the rifting of ice cap in Northern Hemisphere, and short warm periods have occurred. Europe ecosystems has been particularly impacted by these climatic variations. Therefore, thesis emphasized an interest on some fossil outcrops in Europe during this period.

The famous Lagerstätte of Willershausen (middle Germany) was specifically study. It is an exceptional fossil outcrop that contains around 200 angiosperm species for a total of ca. 8000 collected fossil leaves. These leaves testify a paleoforest developed there around 3 — 2.6 Ma ago in a climate warmer than today (mean temperature ca. +5°C). Under these conditions, many plant species typical of the Mediterranean ecosystems were settled there, such as Montpellier maple (*Acer monspessulanum* L.) or Olive tree (*Olea* sp.). For comparison, other paleoforests were studied: Berga (similar in age and geographically close to Willershausen) and Bernasso (younger than Willershausen (2.16 — 1.96 Ma) and located in southern France close to Mediterranean. These forests were compared as many common plant taxa were similar between each other. Furthermore, some species today endemic to the Caucasian region, such as Persian ironwood (*Parrotia persica* (DC.) C.A.Mey) or Caucasian elm (*Zelkova carpinifolia* (Pall.) Dippel), were also present in these outcrops. The aim of this study is to determine how far the climate differences could be involved in the changes of plant-insect interactions in European paleoforests of the late Pliocene – early Pleistocene.

Results highlighted the impacts of both hydric and temperature seasonality, hitherto underestimated in the fossil record, on the patterns of plant-insect interaction in the European paleoforests. It appeared that ecosystems subject to intense hydric seasonality (i.e. important variations of precipitation during the year) could led to higher specialization of plant-insect interaction inferred by higher rate of observed damages du to 'specialists insects'. In parallel, the coolest temperature during the year seems to be a major factor in the low diversity of damage in paleoforest, presumably due to lower insect metabolism. Absence of convergent correlations between plant richness and damage richness could suggested that influence of climatic factors override impact of these local biotic factors.

In order to understand the whole parameters that could have an impact on plant-insect interactions (such as interspecific competition, soil components, taphonomic biases, etc.), our current knowledges are still insufficient to conclude about the relationships between climate and patterns of plant-insect interactions observed in these European paleoforests. It would be wise to make more investigations on modern forests with the methods as applied in fossil record community structure studies. These investigations could help to understand the factors potentially involved in the establishment of a pattern of plant-insect interactions. It is in this perspective that a part of this study was precisely focused on one plant species (*P. persica*) currently endemic to the Hyrcanian forest (Iran). This forest is supposed to be an analogue forest of the European paleoforests as those studied in this thesis. For now, observations made in Iran tend to corroborate our interpretation. However, the discovery of a new plant-insect interaction only found on leaves of *Parrotia persica* from both fossil and current forests suggests that historical factors could also have influenced plant-insect interactions observed in the different forests.

Finally, the studies on plant-insect interactions in past and extant ecosystems, combined with the study of climatic changes, should permit us to better characterize the relations between plants and insects in forests through time.

Keywords: Herbivory, Pliocene-Pleistocene, Hyrcanian forest, Fossil leaves, Insect Damage, Climatic Gradient