

Insect Herbivores and Neighbourhood Effects in Plant Communities of Al Jabal Al Akhdar, Libya

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FOR MY PARENTS AND MY FAMILY



Abstract

Neighbouring plants in the locale of an individual plant may help or hinder it in the task of defence against herbivores, depending on their levels of defence (chemical or physical), and their interactions with potential herbivores. Such ‘neighborhood effects’ are part of the complex network of species interactions that structure ecological communities. This thesis sets out to test whether there are neighbourhood effects on insect herbivory among the plants of the Al Jabal Al Akhdar region of Libya. Having chosen to concentrate on the two main species of three study sites, *Juniperus phoenicea* and *Pistacia atlantica*, nine plots were mapped in detail and the insect herbivores sampled from focal plants, and then from all plants. Leaves were sampled for chemical analysis of their phenol (tannin) content.

The set of insect herbivores collected from plants in the plots were identified to species using the expertise of the staff of the Natural History Museum in London. Some insects recorded are new to Libya, and there are several species not previously recorded as feeding on either of the two plant species studied.

Tannin levels were much higher in *Pistacia* than in *Juniperus*, and there were effects of elevation as well: plants from middle elevation plots had the highest levels, while those from the lowest elevation at the coast had the lowest levels of tannins.

There were clear effects of neighbouring plants on the insects of individual plants, in both *Pistacia* and *Juniperus*. These were much more complex effects in *Pistacia*, but both sets of predictors of insect herbivore density or species richness contained clear signs of neighbourhood effects, where the existence of close neighbours reduced the herbivore load on individual plants. There were no signs of any protective neighbourhood effects of tannin levels.

Thus in the plant communities of Al Jabal Al Akhdar, associational avoidance appears to be the major mechanism of neighbourhood effects, rather than associational resistance.

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Chapter 1: Insect-plant relationships

1.1 History

For more than 150 years, biologists have sought to understand the diversity of life, how it changes with time, and why differences occur among taxa and environments. Over the last fifty years, plants and their herbivores, particularly insects, have been under intense scrutiny because they constitute more than half of all known species, and play highly significant roles in ecology (Futuyma & Agrawal 2009). The enormous diversity of so-called ‘secondary compounds’ or ‘secondary metabolites’ has been central to this study. Secondary compounds do not perform core metabolic functions of plants, but it has become clear that they are often a means of defence by plants against their natural enemies (Futuyma & Agrawal 2009). We now think about these chemicals under the umbrella of ‘coevolution’.

Research into coevolution has developed from the description of reciprocal adaptations in interacting species into an investigative science concerned with the models and mechanisms of parallel evolution. Studies of many systems (predators and prey, parasites and hosts, herbivores and plants) have shown that these interactions are subject to an identifiable set of mutual forces that affect the strength of selection and the direction of change. The term ‘coevolution’ has developed into a necessary part of the lexicon of evolutionary biology in describing the processes and mechanisms of mutual evolutionary change of two or more related species, each adapting to changes in the other (Thompson 1989). Of course, Darwin discussed much the same concept, that he called ‘coadaptation’, for example leading to the

evolution of flowers with long corollas and moths with a long proboscis (Darwin 1859, 1862).

Modern ideas started with models of defence and virulence between plants and pathogens (Flor 1942, Mode 1958), and suggestions that mutual genetic shifts might govern interacting populations (Pimentel 1961). Ehrlich & Raven (1964) then used the idea on a large scale, altering the concept to one of mutual adaptation and speciation among the interacting species. 'Coevolution' is now used consistently to mean mutual evolutionary alterations among interacting species (Thompson 1989).

Though the common usage of the coevolution has become more consistent, it has become clear that the word coevolution is a cover for a range of mechanisms and the outcomes of mutual evolutionary shift (Thompson 1989). Some conditions must be satisfied for coevolution to progress in this pair of interacting species: There must be genetic variation for characters in both plant and insect that influence the interaction between the species, each species must be a selective force on the other species (such as affect the other's fitness), and there must a response to selection in each species.

As Thompson (1989) clarified, there are five definitions that organize the variety of processes and outcomes that we collectively call coevolution. (a) **Gene-for-gene coevolution** was developed from the idea that parasites and hosts have supplementary loci for the relative capacity of a pathogen to overcome host defenses and resistance. This may be rare in nature, although the genes involved in host choice by adult insects are probably linked with those affecting larval performance. (b) **Specific coevolution** is the mutual adaptation between two species without specifying any particular genes. The variety of possible outcomes includes 'evolutionary arms races', character displacement in competing species, and convergence of traits in mutualisms. Demonstrating specific coevolution between

two species is not a simple task, because it is hard to show that both species have evolved in response to the interaction. Perhaps one of the best examples is the interaction between wild parsnips (*Pastinaca sativa*) and the parsnip webworm (*Depressaria pastinacella*), mediated by the toxic furanocoumarins of the parsnip plant (Berenbaum 1981, Berenbaum & Zangerl 1998). (c) **Group, guild or diffuse coevolution** involves reciprocal evolutionary adaptations between groups instead of pairs of species, and includes the evolution of mimicry complexes, and interactions between frugivorous birds and fleshy-fruited plants, and pollinators and grazing mammals with herbs. (d) **Diversifying coevolution** is where speciation occurs as a result of the interaction. (e) **Escape-and-radiation coevolution** was devised by Ehrlich & Raven (1964) to describe how coevolution works in butterflies and their host plants, and involves evolution into enemy-free space. Plants evolve new defensive chemicals that allow them to escape and hence to radiate into a new adaptive zone, soon followed by the evolution of new ways of overcoming these novel defences by their herbivores, with subsequent radiation.

Ehrlich & Raven's (1964) paper stimulated a huge outpouring of research into the relationship between plants and their insect herbivores. The idea was fruitful because it explained a great deal of the patterns of host use by insects. Plant species evolve chemical defences as efficient counters against herbivores, enabling them to escape most or all of the herbivores linked with them. By an indeterminate mechanism, this feature allows the plant to radiate into different species, which all share the new defence, generating the pattern of shared secondary compounds among related plant species. After a period of time, one or more species of insect manage to establish feeding relationships by adapting to these plants, perhaps evolving from

feeding on chemically similar, though unrelated plant hosts (Futuyma & Agrawal 2009). These insects are able to utilize the ‘empty niches’ provided by this different clade of chemically characterized plants, and because of this are able to radiate themselves. Such a process results in a set of related insects able utilize a clade of related plant hosts, a pattern long recognized by entomologists and in butterflies in particular. Ehrlich & Raven (1964) suggested that recurrence of such episodes of adaptive radiation through time in both plants and herbivores accounts for much of the distribution of biodiversity (Futuyma & Agrawal 2009).

At the time when Ehrlich & Raven’s (1964) paper was published in the 1960s, there was still an argument about whether or not secondary compounds played any role at all, and if they did, whether it was to protect. Afterwards, the focus moved from describing the variety and distribution of such compounds to the costs and benefits of changing types and levels of investment in defence. Feeny (1976) suggested that investment ought to be greater in ‘obvious’ or ‘apparent’ (large, long-lived common) plants than less obvious (rarer, smaller, or ephemeral) species; Coley et al. (1985) suggested that allocation to defence would be especially high in plants that grow slowly due to limited resources. These ecological or microevolutionary approaches, based on the assumption of optimal adaptation, were supplemented by studies of selection within populations, particularly using the methods of quantitative genetics. A variety of studies showed that secondary compounds are heritable, herbivores do indeed exert selection for defence, and negative genetic correlations often exist that imply tradeoffs in investment (Van der Meijden et al. 1988). An important outcome from such studies was the recognition that plants can adapt to herbivory not only by ‘resistance’, but also by the ability to tolerate damage, using stored resources to grow and reproduce (Nunez-Farfan et al. 2007).

Research on insect herbivores includes sensory, physiological and behavioural features required to discover and select host plants for oviposition, as well as their subsequent performance. Such data are essential in efforts to answer the question of whether or not such choices are adaptations to different host plants, the dominant hypothesis to explain the occurrence of specialized host relations (Scheirs et al. 2005).

The macroevolutionary heart of Ehrlich & Raven's (1964) hypothesis, the history of speciation events, has been slow in developing, perhaps because the related fossil record is quite sparse, and methods of estimating phylogenies and speciation rates were only just starting in the 1960s. The whole approach could only be crude while phylogenies depended on non-molecular data. Today the analysis of phylogenies is more sophisticated than ever: using the ever-increasing mass of sequence data we can now both estimate and evaluate our confidence in not only the order of branching, but also the approximate timing and evolution of the characters of interest (Futuyma & Agrawal 2009). This step-change in the importance of phylogenies leads us increasingly to appreciate the impact of deep evolutionary history on the features of organisms, including features that influence host-plant associations (Wiens & Donoghue 2004).

1.2 The evolution of plant defence

Herbivores are now assumed to exert selection on plants. For example, the Milkweed (*Asclepias syriaca*) has five native insect herbivores (a seed bug, a leaf-mining fly, a leaf-feeding caterpillar [the monarch butterfly], and two beetles: Agrawal 2007), which may even have caused the plant's habitat specialization (Fine & Mesones 2004). The defensive function of plant secondary chemicals is now

routinely assumed (Futuyma & Agrawal 2009). However, this does not mean that all secondary compounds have a defensive purpose, and principles for identifying these are still not fully developed. Comparisons among species via phylogenies can detect patterns of convergent evolution, which may give evidence of adaptation, and some have sought sets of plant features in “defence syndromes” (Agrawal & Fishbein 2006) that might indicate adaptation to particular suites of herbivores and environments (Futuyma & Agrawal 2009). Many classes of compounds appear to have evolved repeatedly from widely shared biosynthetic pathways, suggesting that relatively minor changes in gene regulation may be entailed, or else they are obtained either directly or by lateral gene transfer from symbiotic fungi (Wink 2008).

The adaptations of plants to herbivores can be classified in various ways according to different principles, for instance, the pathway of biosynthesis, the level of outlay, or the impact on target organisms. Tolerance rather than resistance may be more useful if resources are comparatively abundant (Fine & Mesones 2004). Chemically-based resistance can occur via toxins, inhibitors of digestion, or deterrents. The extent to which these classes of defence vary with phylogeny or ecological context is not known (Futuyma & Agrawal 2009). One can easily envisage changes in many characteristics of a plant which modify or delete stimuli necessary for oviposition or feeding by particular specialized insects; compounds that act like this form barriers to insects, and could be more variable among plant taxa than toxins (Futuyma 2000). There is plenty of evidence that specialist insects are deterred by a wide variety of compounds (Koul 2008), but the phylogenetic analysis of features of plant defence has only just started because we are only now really getting to grips with plant phylogenies (Bernays & Chapman 1994, Futuyma & Agrawal 2009). Many articles address the effect of ecological associations on the

macroevolution of plant defence, ranging from biotic defense by ants to investment in various chemical classes (Kursar et al. 2009; Heil et al. 2009), which may indicate adaptation to particular herbivores or constraints of the abiotic environment (Futuyma & Agrawal 2009). We are only just beginning to learn about such constraints: for example, Johnson et al. (2009) showed that the repeated evolution of asexual reproduction had detectable impacts on defensive traits against herbivores in the Onagraceae.

Via phylogenetic reconstruction, we are now beginning to be able to test some of the main predictions from the theory of coevolutionary arms races. For example, does continuing pressure from progressively adapting herbivores result in escalation in the effectiveness or diversity of plant chemical defense, or mainly the evolution of novel defences? Novelty clearly does occur, as in the diagnostic sulphur-based glucosinolate-myrosinases of the Brassicaceae, with some genera also evolving new classes of compound such as the tropane alkaloids (Brock et al. 2006) and cardenolides that have arisen in other plant families (Futuyma & Agrawal 2009). The evolution of furanocoumarins in Apiaceae is thought to have entailed progression toward more toxic forms, but we need a robust phylogenetic framework to be sure. Phenolic compounds and tolerance to herbivory via regrowth rates seem to have increased during the evolutionary history of milkweeds (*Asclepias* spp.), but their toxic cardenolides and latex production have decreased (Agrawal & Fishbein 2008; Agrawal et al. 2009). In the evolution of *Bursera* (Burseraceae), the diversity of terpenoid defences has increased through evolutionary time (Becerra et al. 2009). The patterns we know about do not support the idea of a coevolutionary arms race resulting in a pattern of phylogenetic escalation or the progressive addition of novel defences. Instead, we see old defences being substituted by new ones, suggesting that

costs and tradeoffs can constrain the macroevolution of defence (Adler 2008; Futuyma & Agrawal 2009), although not invariably (Koricheva et al. 2004; Agrawal & Fishbein 2006). Compounds that are taxonomically generally distributed, and hence presumed to be plesiomorphic, show lower toxicity than taxonomically limited compounds, presumed to be more recently evolved (Cornell & Hawkins 2003; Futuyma & Agrawal 2009), pointing again to the process whereby older defences become less effective as herbivores adapt to them. Over macroevolutionary timescales, tradeoffs seem to occur in strategy (for example, between chemical toxins and tolerance to herbivory) rather than between single chemicals (Kursar et al. 2009; Heil et al. 2009).

Are chemical defences effective against a wide or narrow variety of herbivores (Futuyma & Agrawal 2009)? The high specificity of insect feeding might imply pairwise coevolution of specific plant characteristics and specific enemies. Alternatively, if defense against a range of enemies were positively genetically correlated, coevolution would be “diffuse” (Strauss et al. 2005), and new defences would evolve from the collective impact of all herbivores. The few attempts to measure the effects of particular defensive compounds across a wide array of insects suggest these are not highly correlated across herbivores, phenotypically or genetically (Futuyma & Agrawal 2009). Do novel defences result in plants escaping from some or all of their herbivores, as Ehrlich & Raven (1964) assumed? Although likely, there are few data: ancestral defences of Brassicaceae (glucosinolates) have been overcome by many specialist insects, but not the latest new defences (Brock et al. 2006). The second part of Ehrlich & Raven’s (1964) hypothesis involves radiation after escape from natural enemies, although why this should stimulate radiation is not clear. Again, very few data exist, but Farrell et al. (1991) showed that the evolution

of latex- or resin-bearing canals significantly elevated the speciation rate across many plant lineages. Agrawal et al. (2009) proved the first evidence that trait diversity within a lineage evolves early in the diversification process: there was a correlation between changes in defence chemistry and the rate of diversification in species of *Asclepias*.

1.3 The evolution of herbivory

Most mammalian herbivores are generalized, but most insects are relatively or very specialized, limited to plants in one family or to a small number of closely related species. Many insect clades show phylogenetic conservatism, with unchanging association with the same plant taxa for several millions of years: only 8% of speciation events include a host shift to a different plant family (Winkler & Mitter 2008). There are almost cases of cospeciation or congruence between the phylogenies of plant and insect clades (for example, the radiation of pierine butterflies started about 10 million years after the diversification of glucosinolate-containing brassicas: Wheat et al. 2007), although there is intriguing evidence for this happening after a time lag (Futuyma & Agrawal 2009).

The few detailed studies of the phylogeny of phytophagous taxa indicate that specialists give rise to generalists as often as vice versa. Specialists maintain the physiological capacity to use ancestral hosts, so that re-including them can serve as an evolutionary bridge to new specialized associations (Janz & Nylin 2008). Although herbivory seems to enhance diversification rates (herbivorous clades are significantly more species-rich than non-herbivorous clades: Mitter et al. 1988), only about half the speciation events in known phylogenies involve changes of plant host

(Winkler & Mitter 2008). For example, in the diversification of 14 species of *Asphondylia* gall midges, all involve changes in the plant tissue used within a single host (creosote bush, *Larrea tridentata*: Joy & Crespi 2007). The great diversity of insect herbivores may be attributed to the sheer diversity of ecological niches provided by the great diversity of plants, although speciation may be promoted by divergent host use in some cases (Winkler & Mitter 2008).

The insect lineages that adapt to diverse chemically distinct plant clades should undergo adaptive radiation at an enhanced rate of diversification, a key premise of Ehrlich & Raven's (1964) escape-&-radiation coevolution. There are still too few studies of this prediction but there are one or two: for example, the Pierinae is more diverse than its sister group, perhaps because of the evolution of a particular enzyme that detoxifies the glucosinolate defences of their brassica hosts (Wheat et al. 2007). Evolution into enemy-free space may facilitate this process: thus the evolution of adaptive differences and speciation in herbivores may be greatly enhanced by escape from their own natural enemies and host plant traits. There is very little known about this (Futuyma & Agrawal 2009).

As discussed above, the term "coevolution" has several meanings, depending upon whether the relationship is relatively specific or pairwise, or more diffuse among many interacting species (Strauss et al. 2005), and whether the interaction is continuous, with a relatively short time lag, or with successive bursts of adaptation and diversification (Ehrlich & Raven 1964). Although adaptations of specialized insects to their hosts have been carefully described, it is more difficult to determine the specificity of plant defence traits. There are some examples, however. European *Barbarea vulgaris* (Brassicaceae) are polymorphic for resistance to a major flea beetle herbivore (*Phyllotreta nemorum*), and the beetle similarly shows a simply

inherited polymorphism for usage of *B. vulgaris* (Nielsen & Jong 2005). Furanocoumarins profiles among populations of *Pastinaca* are related to the detoxification profile of the local population of *Depressaria pastinacella* (Zangerl & Berenbaum 2003).

However, it is hard to prove that these differences are the result of the interaction rather than some other environmental factor without reciprocal transplants and the measurement of selection on relevant traits. Related plants are chemically similar but vary a lot in the identity and levels of the compounds, sometimes known to have been driven by selection (e.g. glucosinolate synthesis in crucifers: Benderoth et al. 2006). Adaptive divergence in the detoxifying abilities of insects has also been registered, for example in the cytochrome P450's of *Papilio* butterflies (Li et al. 2003). Many issues remain to be tested. At the macroevolutionary level, Futuyma & Agrawal (2009) note that phylogenies of insects rarely match closely those of their host plants. The most frequent pattern seems to be that of sequential evolution (Jermy & Szentesi 2003).

The role of history in explaining the composition and organization of ecological communities is increasingly appreciated, with the invention and use of phylogenetic methods in community analyses (Ives & Godfray 2006; Lavender et al. 2009). The subset of herbivores that feed on particular host plants in one area is influenced by all sorts of historical events, both local (timing of arrival) and global (the history of herbivore diets). Some insect clades appear to be prevented completely from feeding on some plant clades, for largely unknown reasons.

The revolution in the generation and utilization of phylogenies is providing many new insights into old or unexamined evolutionary premises (Agrawal et al. 2009; Agrawal 2007). An example is escalation, the directional trend in the diversity,

density or quantity of defensive traits (Agrawal & Fishbein 2008; Agrawal et al. 2009), a special case of Ehrlich & Raven's (1964) "escape and radiate" premise (Agrawal 2007). Agrawal et al. (2009) tested this hypothesis using the phenol metabolic pathways in milkweeds, where several defence compounds arise from two major pathways, including cardenolides. While tradeoffs among these defences are expected among individuals within species, over evolutionary time the persistence of such tradeoffs would indicate constraints on the independent evolution of the components of the pathways. They found that overall phenol levels increased through evolutionary time, but cardenolide levels decreased (perhaps because specialist herbivores have overcome this defence and sequester them). Evolutionary tradeoffs were evident among some defences that were components of a single metabolic sequence, whereas positive evolutionary relationships occurred when two defences were both derived from branches of a common precursor.

The consumption of the host plant normally is carried out by the larval stage of holometabolous insect herbivores, whilst it is the adult ovipositing female that normally selects the host plant species. One way of organising thoughts about the link between selection by the female and feeding by the larva is the preference-performance hypothesis (Jaenike 1978; Berenbaum & Feeny 2008), although there are others (eg Courtney et al. 1981). According to this hypothesis, ovipositing females should maximize their fitness by selecting plants on which offspring survival will be high; thus over a range of potential host plants, adult female preference should be correlated with larvae performance. Evidence in support of this hypothesis has been mixed, and especially poor for Lepidoptera (Thompson 1988; Scheirs & deBruyn 2002; Berenbaum & Feeny 2008), often because of the fact that larval

survival from feeding is only one element of fitness: the hypothesis should be formulated in terms of fitness rather than survival (Sadeghi & Gilbert 2000). There are many other evolutionary forces affecting performance: predation and parasitism (Heisswolf et al. 2005), the frequency of the most-suitable hosts (Rausher 1980), recent invasions into the plant community (Wiklund 1975), phenological mismatches (Scriber 2002), phenotypic plasticity in host-plant selection (Mercader & Scriber 2005), and even parent-offspring conflict where adults are also herbivores.

There has been more than 60 years of arguments about the evolution of herbivore specialization because, as Dethier pointed out in 1948, the first barrier to be overcome in the insect-plant relationship is a behavioural one: the insect must sense and discriminate before nutritional and toxic factors become operative (Berenbaum & Feeny 2008). However, host shifts can only happen when both preference and performance components are both present, so arguments about which came first are not productive (Berenbaum 1990), and some elements may be epigenetic or non-genetic, rendering optimality hypotheses less useful (Berenbaum & Feeny 2008). The role of oviposition ‘mistakes’ by ovipositing females has always been controversial: the idea that larval feeding conditions adult sensors in their search for host plants (Hopkins’ host selection principle) has repeatedly failed to be supported when tested, although some aspects of this ‘chemical legacy’ idea are plausible (van Emden et al 1996).

Females search visually and by odour first, and then by contact chemoreception. They encounter a wide variety of chemical signals from which they must distinguish the occasional host plant. The larvae, in contrast, rarely need to distinguish among potential host plants, but are usually in constant contact with food. Memory and

neural processing limitations may limit adult performance in their discrimination task and hence influence the evolution of host range (Bernays 2001).

Kairomone (attractants) and allomone (deterrent) complexes play an important role in preference and performance in insect herbivores. (These terms are less and less useful as we discover more details about the chemical environments of adult and larval herbivores.) Larvae can eat their body weight in plant material every day, and are thus exposed to host plant chemicals more or less constantly throughout larval life, including massive amounts of often harmful complexes (Berenbaum & Feeny 2008). Most herbivores avoid eating some plant parts by concentrating on particular tissues (e.g. skeletonisers and leaf miners), but still must ingest large quantities of defence compounds. The occurrence and processing activities of detoxification enzymes are assumed to limit the ability of larvae to deal with the chemical content of the ingested food, particularly the cytochrome P450 monooxygenases. Although broadly substrate specific in vertebrates, they nearly always have very narrow specificity in the mostly oligophagous insect herbivores (Berenbaum 1999, 2002; Mao et al. 2006). Since the plant chemical profile changes markedly with time, an ovipositing female can have very little information on which to base her decision about whether to lay or not. For example, the parsnip webworm *Depressaria pastinacella* only eats the developing buds, flowers, and fruits of its only host plant *Pastinaca sativa*, and its performance depends on the levels of furanocoumarins (Berenbaum & Zangerl 1993). However, the number of eggs laid by females is independent of furanocoumarin content in these reproductive structures (Zangerl & Berenbaum 1992) because females lay in late spring before the flowering stalk has appeared, a time when the only tissue available for evaluation is the leaf. The furanocoumarin content of foliage is uncorrelated with that of the later reproductive

structures (Berenbaum & Feeny 2008). Larvae probably discriminate their food from the leaf material using the octyl esters present in reproductive structures, but absent from the leaves, even though these compounds are toxic (Carroll et al. 2000).

Given the difficulties of measurement, it is perhaps not surprising that in Lepidoptera preference and performance are uncorrelated in the majority of studies (Berenbaum & Feeny 2008). What data we have does not support a genetic link between the two. Interspecific differences in female preference are X-linked (Thompson & Pellmyr 1991); since the female in Lepidoptera is the heterogametic sex, preference can evolve quickly because recessive mutations are exposed to selection in females. Thus female preference may indeed be the key to host shifts during evolution. Intraspecific variation in female preference does not seem to be sex-linked (Janz 1998).

1.3.1 Host use in Papilionidae

Berenbaum & Feeny (2008) summarise a great deal of literature about preference and performance in the Swallowtail butterflies, and it is useful to outline their findings here as a backdrop to the study in Libya. Host use is remarkably conservative: only five plant families are used (Annonaceae, Apiaceae, Aristolochiaceae, Lauraceae, and Rutaceae), just 21% of species with known host associations use more than a single plant family. Two papilionid families (Troidini and Zerynthiini) are limited to Aristolochiaceae, from which their larvae sequester toxic aristolochic acids to carry over into the adult to use as chemical defense against predators. Over 75% of species in the Papilionini use Rutaceae and Apiaceae, with a few polyphagous species such as the *P. glaucus* complex.

Visual signals dominate in long-distance of orientation to host plants, while olfaction of host-plant volatiles is important during close approach. Even so, one third of non-hosts are only rejected after landing and contact chemoreception. Learning modifies responses to visual cues, and the olfactory responses to host-plant volatiles can be reduced or abolished by the presence of some non-host volatiles. For example, female Black Swallowtail *Papilio polyxenes* were stimulated by host volatiles added to artificial leaves to land more frequently and lay more eggs, but activity levels, rates of landing and number of eggs were greatly reduced in the presence of volatiles from a non-host, cabbage (Feeny et al. 1989).

Once a female has landed on a leaf, contact stimulants perceived by the tarsal chemoreceptors (some of which are tuned to characteristic chemicals of host plants) are vital in determining whether a potential oviposition site is accepted or rejected (Berenbaum & Feeny 2008). Mixtures of both volatile and contact chemicals are more stimulatory than single compounds, and these responses are mostly innate, permanent experience, although some experiments demonstrate a role for learning here. Oviposition ‘mistakes’ often seem to involve volatiles where the mistaken plant is a food plant of related insect species, and contains chemical elements of the true host plant. Since we know that volatiles from non-hosts can disrupt accuracy in finding the true host plants, neighbouring plants clearly play a role in the ability of herbivores to discover good oviposition sites.

Contact stimulants for oviposition are known in many species (described in Berenbaum & Feeny 2008). Mixtures are usually but not always involved, and are quite different in species of different tribes, interpretable as an ancient set of responses to flavonoids followed by secondary losses in derived clades. In swallowtails these oviposition stimulants are usually innocuous compounds derived

from a single metabolic pathway (the phenylpropanoid pathway), whereas in pierids (glucosinolates) and troidine swallowtails (aristolochic acids) they are plant defence chemicals. Some cases are known where the same chemical acts as an oviposition stimulant for adult females and a feeding deterrent for the larvae (e.g. Ono et al. 2004).

The host plants of more than three-quarters of the many species of *Papilio* contain furanocoumarin allomones, toxic to many organisms because they bind to DNA and protein and interfere with metabolism when activated by UV (Berenbaum 1983, 1985). There are two structures of furanocoumarins, linear (abundant in the Rutaceae and Apiaceae) and angular (restricted to two tribes of Apiaceae, plus a few other genera). Species of the Papilionini, which normally encounter furanocoumarins, are the only swallowtails that can tolerate normal concentrations of these compounds in their diet. Among species of *Papilio*, toleration of furanocoumarins is correlated with encounter frequency in host plants; some can deal with concentrations of 1% dry weight, and in one species survival is improved with furanocoumarins in the diet (Berenbaum & Feeny 2008). This pattern of adaptation to encountered defensive compounds extends to mixtures of chemicals as well (e.g. furanocoumarins and myristicin: Neal & Wu 1994). A recurrent theme now emerging from these kinds of studies is that species retain the ability to tolerate the chemical defences of ancestral host plants even though they themselves never normally use them. Gene duplication is an obvious mechanism whereby this could arise.

Despite theoretical predictions, there does not seem to be any genetic linkage between preference and performance. In the few species investigated, oviposition preference has at least one locus on the X chromosome, whereas larval performance

has no contribution at all from the X chromosome (but may be Y-linked, or have a maternal effect) (Thompson et al. 1990). Cytochrome P450 genes appear to be associated with both preference and performance, but these effects do not seem to be correlated. P450s constitute the main detoxification system for dealing with plant defence chemicals, and also play a key role in chemosensory systems probably by destroying molecules of plant volatiles that are bound to the receptors, thereby renewing the sensors. Studies of the activities of particular P450s in studied species of swallowtail show constitutive and inducible responses consistent with their role in detoxifying the particular furanocoumarins normally encountered in the diet. It is interesting that very few genetic changes result in a substantial broadening of the substrate specificity of P450s in *Papilio* species. In addition, conserved promotor regions may cause furanocoumarin inducibility even in species that do not normally encounter these toxins in their diet (McDonnell et al. 2004).

Enough is therefore known about preference and performance in swallowtail butterflies to suggest that their joint evolution may involve P450 genes that play roles in adult olfaction, larval gustation and the detoxification of plant defensive toxins. If such is the case, then rapid evolution is possible. In the context of this thesis, we can see that the olfactory environment surrounding plants is an important component of oviposition accuracy.

1.4 The approach of this thesis

A plant's chances of detection and colonization by herbivorous insects depend not only on the plant's traits but also on the identity of neighbours with which it grows (Feeny, 1975). Some assumptions are often made when studying relationships between plant neighbourhoods and herbivore dynamics: (a) plants sometimes escape

detection beneath or in the odour umbrella provided by other more conspicuous plants; and (b) floral and extra floral nectaries on neighbouring plants are assumed to be beneficial because they provide resources sought by predators and parasites of herbivores. Thus plants with flowering neighbours can gain protection from herbivory, called associational resistance. However, associational susceptibility is also a possibility, where neighbouring plants attract herbivores to attack focal plants.

This thesis explores the role of neighbourhood effects on the plants and their herbivores in the dry Mediterranean environment of the Al Jabal Al Akhdar region of Libya, by studying the distribution of plants and their association with herbivores within plots, and measuring phenol concentrations in the leaves of selected plants. I have written the thesis as a set of manuscript papers, and therefore the background to the study region and to the ideas that lie behind neighbourhood effects are given in the relevant chapters rather than here in the introduction.

The aims of this study are to study the effects of the local plant neighbourhood on insect herbivores and their hosts. The hypotheses I shall address are the following:

- 1 Sites, elevation and plant neighbourhood all affect the density and the amount of damage by insect herbivores on individual plants.
- 2 Local insect herbivore community structure can predict damage rates.
- 3 Herbivory rates are affected both by plant traits (such as levels of chemical defences) and by the local effects of neighbouring plants.

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Chapter 2: The plant communities of Al Jabal Al Akhdar.

2.1 Introduction

In this chapter, I review the plant communities of my study site, and then describe how I measured and mapped the plants of a number of study plots chosen to represent the three main elevations of the area. The mapping of plants is the fundamental bedrock data for the study of neighbourhood effects.

The Mediterranean Basin is documented as one of the 18 world “hotspots” where outstanding concentrations of biodiversity arise (Ozden et al. 2008). The Mediterranean flora is one of the richest in the Old World, comprising more than 25,000 species of flowering plants (Ozden et al. 2008). Its invertebrate fauna is the richest in Europe in terms of species richness; 75% of the total European insect fauna are established within the Basin (Ozden et al. 2008).

Libya lies along the southern coast of the Mediterranean; about of between latitudes 10° and 33° north and longitude 9° and 25° east. Libya covers an area of about 1,760,000 km², of which more than 90% is desert: only the coastal narrow region and the Al Jabal Al Akhdar area are different (Boulos 1975). Libya is significant biogeographically because of the presence of the Mediterranean to the north, and the Saharan desert to the south; it functions as a bridge between the western and eastern parts of these regions. The current climatic conditions are characteristic of the Mediterranean area, characterized by changeability and

unpredictability. The rainfall is erratic in quantity, rate and distribution (Al-Idrissi et al. 1996; El-Darier & Mogaspi 2009).

Except for descriptions of some new species and a few other works, our information of the flora of Libya stems principally from the Italian colonial era, ca. 1911-1934 (Bologna 1991). The recorded number of vascular plant species varies from 1900 and 2059 (World Conservation Monitoring Centre 1992; Sheriff & Ben-Othman 1992); this cannot be considered a very rich flora in view of the huge area of the country (El Darier & El Mogaspi 2009). Interest in Libyan plants started with the work of Ascherson (1881) on the grasses of Kufra: he listed 57 grasses from the Libyan region. Since that time, a good deal of information on Libyan plants has accumulated significantly (Zunni 1977). The annotated lists of these publications depend mainly on the authors' own specimens and previous study of the Libyan flora.

A very significant step was the publication by Keith (1965), a complete taxonomic treatment of the North African flora, unfortunately largely unavailable in Libya (Zunni 1977). He incorporated plants that had actually been introduced throughout the late 1920s and early 1930s by the Italians, but lack of proof of the status of these plants means that they are now only of historical value (Faruqi 1980). More recently, H Scholz published a sequence of papers and on the Libyan flora (Faruqi 1980).

The Al Jabal Al Akhdar region contains 90% of the Libyan flora because it is the only area with reasonable amounts of rain. It is a regional hotspot of Mediterranean biodiversity. The major plant communities of the Al Jabal Al Akhdar region are dominated by dense Mediterranean maquis scrub, with cover reaching 70-80%. The plant communities resemble those of Crete and the western Mediterranean, rather

than those of the eastern Mediterranean (Boulos 1972). The most dominant plant is juniper *Juniperus phoenicea*, along with lentisc *Pistacia lentiscus* and laurustine *Viburnum tinus*. There are basically three major plant habitats: maquis, coastal areas and wadis.

At elevations under 300 m the maquis contains *Juniperus phoenicea* scrub/woodland with a patchwork of garigue containing rich herbaceous communities; associated plants are cypress *Cupressus sempervirens*, two species of lentisc *P. lentiscus* and *P. atlantica*, myrtle *Myrtus communis*, wild olive *Olea europea*, buckthorn *Rhamnus* spp., carob *Ceratonia siliqua*, sumac *Rhus tripartita* and boxthorn *Lycium europaeum*. Above 300 m other species become significant components: strawberry tree *Arbutus pavarii*, heath *Erica multiflora*, globe-daisy *Globularia alypum*, and rockrose *Cistus salvifolius*. In areas where the scrub is thinner or absent there are communities dominated by Batha (Thorny burnet *Sarcopoterium spinosum*), with woolly sage *Phlomis floccosa* and various kinds of thistles (*Carthamnus lanatus*, *Notobasis syriaca*, and *Carlina corymbosa*) and grasses (*Briza* spp. and *Ligurus ovatus*) (Boulos 1975; ACSAD 1979).

The Batha community is common in the coastal plain where the soil is less than 15 cm deep (Zunni 1977). Wide areas are covered with the plant itself *Sarcopoterium spinosum* and its associates *Phlomis floccosa*, *Ballota pseudo-dictamnus*, *Urginea maritima*, *Eryngium campestre* and *Thapsia garganica*, with other species at higher elevations, such as *Cistus villosus*, *Onosis* spp, *Tolpis virgata*, *Echinops spinosus*, *Scilla autumnalis*, *Calamintha* spp, *Micromeria* spp, *Reaumuria mucronata*, *Thymus capitatus* and *Cyclamen rohlfsianum*. South of the Jabal Al Akhdar region, however, *Juniperus* dominates the coastal plain with an open community containing *Ammophila arenaria*, colonies with *Sporobolus* spp, *Crucianella rupestris*,

Limonium spp. and *Suaeda vermiculata*, as well as shrubs such as *Zygophyllum album*. Beach communities tend to form dense scrub, which can be high in some Southern areas; other species show for instance *Ononis vaginalis*, and isolated patches of *Cichorium spinosum* (Zunni 1977).

The deep gorges of the wadis contain real forests dominated by the genera *Quercus*, *Laurus*, *Arbutus*, *Olea*, *Cupressus*, *Juniperus*, *Pistacia* and *Periploca*. *Cupressus sempervirens* and oak *Quercus coccifera* trees grow up to 10 m high in the more protected areas. The shrubs *Smilax aspera*, *Viburnum tinus* and *Pistacia lentiscus* are common amongst the rocks. The increased humidity and shade allow the growth of *Samolus valerandi*, *Parietaria judaica* and *Adiantum capillus-veneris* in the caves and crevices, and *Putoria calabrica* and *Capparis spinosa* among exposed rocks (Boulos 1972; ACSAD 1979). Where rainfall is less, the vegetation becomes more open, where *Asphdelus microcarpus*, *Sarcopoterium spinosum* and *Artemisa herba-alba* dominate (Al Hamedi 1999).

Vegetation tends to become more xenomorphic with increasing elevation, particularly noticeable in Mediterranean-type climates despite the hills receiving equal amounts or more rainfall than nearby lowlands. There are a number of factors which contribute: summers are dry, and up to 1000 m daytime summer temperatures are often no lower and sometimes even higher than at sea level; solar radiation flux is greater in the hills than at lower elevations; and reduced pressure increases of evapotranspiration (Gale, 1972).

The whole area of forest used to extend about 500,000 hectares, of which 35% was destroyed to convert to agricultural crops. Therefore the true area of these natural forests is about of 320,000 ha (Al-Idrissi et al. 1996). However, most types of

natural forests in this region have been damaged by human actions. As a result, many local native species such as cypress (*Cupressus sempervirens*), pine (*Pinus halepensis*), oak (*Quercus coccifera* and *Q. ilex*), lentisc (*Pistacia atlantica*) and juniper (*Juniperus phoenicea*) have declined (Qaisar & El Gadi 1994).

The most important trees/shrubs of the forest regions, Juniper or Al Arar (*Juniperus phoenicea*) and Lentisc or Al Batom (*Pistacia atlantica*) were chosen to test for neighbourhood effects of insect herbivores and their variation in different environmental conditions. These species occur in a wide variety of plant communities throughout the Al Jabal Al Akhdar region, from sea level to the highest mountain areas, because they can grow on both acid and alkaline soils.

Juniperus phoenicea L. (Pinales: Cupressaceae), called Arâr in Arabic, is a species of juniper distributed across the Mediterranean area, from Morocco and Portugal east to Italy, Turkey and Egypt, south to the mountains of Lebanon, Israel, Jordan, and in western Saudi Arabia near the Red Sea. It also occurs on Madeira and the Canary Islands. It mainly grows at low elevations near to the coast, but reaches 2,400 m elevation in the south of its range in the Atlas Mountains. *Juniperus phoenicea* is a large shrub or small tree reaching a height of 2–12 m, with a trunk up to 1 m wide and a rounded or irregular crown. The leaves are of two forms, juvenile needle-like leaves 8–10 mm long on seedlings, and adult scale leaves 0.5–2 mm long on older plants; they are arranged in opposite decussate pairs or whorls of three. It is largely monoecious, but some individual plants are dioecious. The cones are 6–14 mm in diameter, berry-like, orange-brown, and include 3–8 seeds; they mature in about 18 months. The male cones are 2–4 mm long, and shed their pollen in early spring.

Pistacia atlantica Desf. (Sapindales: Anacardiaceae) is a species of pistachio tree native to Eurasia from the Iranian plateau to North Africa, where it was once

common. It is a deciduous tree up to 7 m tall, and old trees have thick trunks covered in fissured bark. The leaves are pinnate, each with 7 to 9 lance-shaped leaflets. The leaves and branches often have galls produced by characteristic species of aphids. The tree is dioecious with male and female trees producing different types of flowers. The oblong, fleshy, oily fruit borne by the female tree is 6 to 8 mm long, and pink ripening to blue (İsfendiyaroğlu & Özekera 2009).

2.2 Materials & methods

The Al Jabal Al Akhdar (“Green Mountain”) region in Cyrenaica is the study area of the present fieldwork (Figure 2-1). It is situated directly behind the coastal strip in the north-eastern region of Libya, in Cyrenaica. It extends for about 300 km along the coast, and climbs to an elevation of 881 m above sea level. The massif is rocky intersected by several Wadis (dry rivers). Rainfall ranges between 250-600 mm per year, a respectable amount for this hyper-arid country, the happy result of the winds from the west coming across the Mediterranean, picking up moisture and depositing it on the mountainous massif. Heavy red-clay (terra rossa) soils are common (El-Darier & Mogaspi 2009). Recently the area has been suffering from a drought which may be a signal of climate change, or may be a normal component of long-term fluctuations. There is a debate among academics in the region whether the drought is causing vegetation change or not. Temperatures are moderate with an annual average of 16-18 °C; the hottest temperatures are in May and June, with averages between 25 and 33 °C.

There are three layers of elevations going from the coast to the high mountains, effectively three ‘steps’. The coastal strip is about 3 km wide and 0 - 200 m above sea level in elevation. Then the land rises to the second ‘step’, a middle strip lying at

200 - 400 m above sea level. Finally there is a steep gradient up to the high mountains 400 - 880 m above sea level.

We chose three plots at each of the elevations, making nine plots in total (Figure 2-2), all close to the site of Cyrene, one of the most famous of all cities in the Ancient world of Greece and Rome. The economy of this city was based on collecting and exporting a medicinal plant called *Silphium*, a kind of umbellifer. It was collected to extinction.

Each plot was 50 by 50 m (Figure 2-3); in each plot, the first corner was chosen by random coordinates, and the others chosen so that one side was parallel to the sea. These corners were mapped using GPS. The plot size was chosen to be large enough so that there were at least 30 individuals of each focal plant species in the least-dense plot. Permanent corner posts were painted red to help find them again, and there were temporary posts at 10-m intervals within the plot; a sighting compass ensured the lines were straight. We recorded general plot conditions such as aspect, gradient, altitude, and soil type and soil depth.

We then mapped the positions of the centres of every *Juniperus* and *Pistacia* plant, plus other trees and shrubs (defined as any woody plant higher than 0.5 m) of all species within the plot, using distances and angles from the seaward posts. For trees, we measured the circumference (perimeter) of the stem at 1.3 m height. For shrubs, we recorded the maximum width (a), the longest perpendicular width (b) and the height (c). The position of each shrub was calculated as shown in (Figure 2-4). The 'size' (= volume) of each shrub was calculated as half the volume of the ellipsoid created from the measurements, calculated as $\frac{1}{2}((4\pi abc)/3)$.

The data were analysed using Generalized Linear Models in SPSS16 with either normal or Poisson errors, as appropriate, and using two predictors of elevation (bottom, middle, top) and plot (1 to 9) in the model of *elevation + plot-within-elevation*. We used subplots as the replicates for these analyses.

2.3 Results

The species recorded in the plots are listed in (Table 2-1). The main components were *Pistacia atlantica* and *Juniperus phoenicea*. The density of *Pistacia atlantica* at all plots ranged from 42 to 156 trees, and of *Juniperus phoenicea* from 67 to 132 trees (Table 2-2). The density of *Pistacia* was higher than *Juniperus*, but in some areas there were almost pure *Juniperus* stands with few other plant species, especially *Pistacia*, and vice versa. There were 1902 trees of all kinds in all the sites, and the densities varied among sites and altitudes.

In addition to the two selected trees/shrubs, plots at the lowest elevation contained *Periploca laevigata*, *Ceratonia siliqua* and *Phillyrea angustifolia*; plots in the middle elevation contained many species of plants, especially *Sarcopoterium spinosum*, *Calicotome villosa*, *Phlomis floccosa*, *Ceratonia siliqua* and *Olea europea*; and plots at the highest elevation contained *Arbutus pavarii*. There were noticeable differences in plant height, with the tallest plants at the lowest elevation in *Juniperus* (Wald $\chi^2_2 = 189.6$, $p < 0.001$) and in *Pistacia* (Wald $\chi^2_2 = 27.0$, $p < 0.001$). Plant species densities at middle elevations, especially of *Pistacia atlantica* (Figure 2.17), were greater than at the highest or lowest, although *Juniperus* density was reduced at the highest elevation (Figure 2-16).

Maps of each plot are reproduced in (Figure 2-5 to Figure 2-13) with summary statistics in (Table 2-2). At all elevations there were big gaps between plants because of aggregated distributions.

Top elevation - plot 1 (Figure 2-5): *Pistacia* and then *Juniperus* dominated this plot, while *Arbutus* was very rare, with only a single individual in the plot. By plotting sizes as well as positions (not shown) it was obvious that *Pistacia* size increased when it grew in isolated positions, while *Juniperus* size decreased.

Top elevation - plot 2 (Figure 2-6): Only *Pistacia* and *Juniperus* occurred in this plot, but *Pistacia* was less dominant than in Plot 1. A proportion of the plot lacked trees and shrubs altogether (top right-hand corner of Figure 6).

Top elevation - plot 3 (Figure 2-7): The number of *Juniperus* and *Pistacia* were nearly equal in this plot, and there were a few individuals of *Arbutus*.

Middle elevation - plot 4 (Figure 2-8): This elevation quite clearly contains a different set of plant species. In this plot there were six species, with *Pistacia* and *Juniperus* dominant, but also with a reasonable number of *Calicotome villosa*; a few *Phlomis floccosa*, *Ceratonia siliqua* and *Olea europaea* were also present. *Juniperus* were distinctly smaller here than in the high-elevation plots.

Middle elevation - plot 5 (Figure 2-9): There are four plant species in this plot, but the majority of plants are *Pistacia* and *Juniperus*. Again, mean sizes are low for both species.

Middle elevation - plot 6 (Figure 2-10): Tree/shrub density was much lower in this plot mainly because the number of *Pistacia* plants was almost half that of the other mid-elevation plots. Interestingly, the size of the *Juniperus* was much larger, a

possible indication of competition between these two dominant species.

Bottom elevation - plot 7 (Figure 2-11): In this plot, as with the other low-elevation plots, overall density was lower because there were many fewer *Pistacia* plants, coupled with fairly low numbers of *Juniperus*. These junipers were on average much larger, again possibly indicating a role for resource competition between *Pistacia* and *Juniperus*. *Phillyrea* was also recorded in this and the other low-elevation plots, but was absent from all of the higher-elevation plots.

Bottom elevation - plot 8 (Figure 2-12): This plot had the lowest numbers of *Pistacia* of any of the nine plots, but the largest plants of this species coupled with the second-largest plants of *Juniperus*.

Bottom elevation - plot 9 (Figure 2-13): Like plot 8, the final plot 9 had very low numbers of *Pistacia* plants. There was the largest number of *Ceratonia* trees in this plot, and also the largest number of *Phillyrea* shrubs.

The density of plants per ha varied significantly among levels (Figure 2-14, Table 2-3, with the highest densities in the middle plots, and the lowest in the bottom coastal plots. The number of species per ha also varied among levels (Figure 2-15, Table 2-4), again with middle-level plots containing the greatest species richness per ha.

The numbers of *Juniperus* per ha varied a lot among plots within levels, and the highest mean number per ha occurred in the middle-elevation plots (Figure 2-16), but the differences among levels were not significant (Table 2-5). The numbers of *Pistacia* were less variable among plots within levels, and bottom-elevation plots were clearly less dense than mid- or top-elevation plots (Figure 2-17): this difference was significant (Table 2-6).

2.4 Discussion

Pistacia and *Juniperus* dominated all plots, but were less frequent at the bottom elevation, especially *Pistacia*. There were a number of other shrubs growing in the plots, but only *Calicotome villosa* was at all frequent, and only in mid-elevation plots: all others were rare. Plant size was generally highest at the lowest elevation in both species, and there was an indication of a negative relationship between the two species in height, perhaps due to competition for resources (Zunni 1977). Middle-elevation plots were clearly the most diverse and the most densest. Our data confirm those of Zunni (1977), who also found that *Pistacia* sp and *Juniperus* sp dominated all of his plots. He also found that *Juniperus phoenicea* is found in the region from sea level up to 600 m; it is highly resistant to arid conditions, and is associated with *Pistacia*.

The density of plants varied significantly among levels, with the highest densities in the middle plots, and the lowest in the bottom coastal plots. Reasons why this is the case include many factors not measured here, and it is difficult to speculate in a vacuum. The reasons for the pattern with elevation were not the focus of my study, and hence no environmental data were collected specifically to explain the pattern. The simplest explanation might involve patterns of rainfall, but there are very few data available to investigate this possibility. In Zunni's (1977) study, *Sarcopoterium spinosum*, *Pistacia*, *Juniperus* and other species were common in the plant communities of all his plots, but suggested the community attains better development on the high plateau where it may more properly be considered a 'disclimax'.

The number of species per ha also varied among levels, again with middle-level plots containing the greatest species richness. Variation in species richness with elevation is thought to result from the effects of many interacting factors: plant productivity, competition, geographical area, historical or evolutionary development, regional species dynamics, and regional species pool, environmental changeable and human activity (Zobel, 1997; Criddle et al., 2003). Rahbek (1995, 1997) discussed three main models: a monotonic decline in species richness from low to high elevation, a hump-shaped pattern with a maximum at mid-elevation; or essentially a constant from the lowlands to mid -elevations followed by a strong decline further up. Zunni (1977) also found that the middle-level plant community was dominated by shrubs, with trees generally lacking. It was dominated by *Pistacia*, with some areas containing many widely scattered *Ceratonia* and a few scattered trees or tall shrubs of *Juniperus*, *Arbutus* and *Olea*. He confirmed that the bottom level has a rich ground layer of *Sarcopoterium spinosum*, *Phlomis floccosa*, *Ballota* spp, *Urginea*, *Eryngium*, and *Thapsia garganica* in the openings between shrubs

The important carob tree *Ceratonia siliqua* did not grow on the top level in our plots; this tree is known to be a thermophilous plant that does not generally grow above an elevation of 300 to 500 m on mountain slopes (Zohary 1973; Zunni 1977). Although not in our plots, *Ceratonia* does grow high up on the northern plateau of Cyrenaica in the Al Jabal Al Akhdar. It seems probable that in this region temperature may not be a limiting factor for its occurrence since the temperature rarely drops below zero. It is likely that in Al Jabal Al Akhdar, precipitation is the most important factor limiting its distribution. Other factors can compensate for a lack of rainfall: for example, in some areas with low annual rainfall, carob trees can

be found growing luxuriantly in the beds of valleys where the soil moisture is high and the water table is accessible to the root system.

The numbers of *Juniperus* varied a lot among plots within levels; although the highest mean number occurred in the middle-elevation plots, the differences among levels were not significant. The numbers of *Pistacia* were less variable among plots within levels, and bottom-elevation plots were clearly significantly less dense than mid- or top-elevation plots. Zunni's (1977) data were similar.

The forests of Cyrenaica were identified by Médail & Quézel (1997) as one of the ten hotspots of plant diversity in the Mediterranean basin, urgently in need of conservation. A study on the endemic vascular flora of Cyrenaica suggested that about 10% of the flora was endemic (Bartolo et al. 1977), and these represent about 50% of the total number of plants endemic to Libya (59 species). The unique physiographic and climatic conditions of the Al Jabal Al Akhdar region isolate it from the rest of the country, providing an ecological refuge from the gradual aridification of the Sahara over the last 10,000 years (El Darier & El Mogaspi, 2009). Forest vegetation actually follows the coast for about 300 km thus it extends beyond the mountains across the western slopes of Al Jabal Al Akhdar and covers most of the northern part of Benghazi plain. The width of this forest vegetation varies but does not extend inland much more than 60 km (Zunni 1977). The distribution of forest tree species is generally limited to the Al Jabal Al Akhdar and its coastal belt, but a few exceptions occur where isolated clumps patches or strips of trees that grow in the valley beds within the northern part of the desert. This geographical isolation gives the plant communities some of the characteristics of island vegetation (Zunni 1977). Similar lush vegetation occurs to the west in the Atlas Mountains of Morocco

and the highlands of Algeria and Tunisia, and in the Nile Delta to the east. The Atlas Mountains share some species with Al Jabal Al Akhdar, and may have been linked via a continuous belt of similar vegetation in wetter times in the past (Zunni 1977).

This chapter shows the results of mapping all the shrubs and trees of nine plots at three elevations in the study site of Al Jabal Al Akhtar. *Pistacia* and *Juniperus* dominated all plots. There were a number of other shrubs growing in the plots, but only *Calicotome villosa* was at all common, and only in mid-elevation plots: all others were uncommon. Plant size was greatest at the lowest elevation in both the main species. Plant density and species richness differed considerably among levels, with the highest values of both in the mid-elevation plots. In next chapter I study the distribution and density of insect herbivores on host plants.

2.5 References

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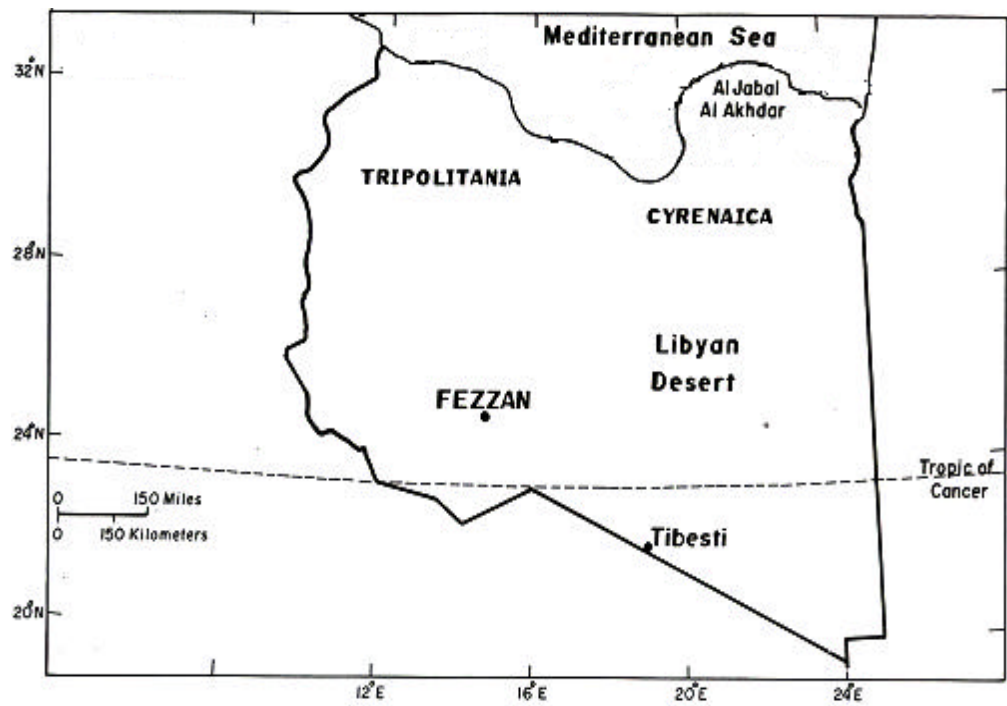


Figure 2-1: The study area of the Al Jabal Al Akhdar region in Cyrenaica, Libya

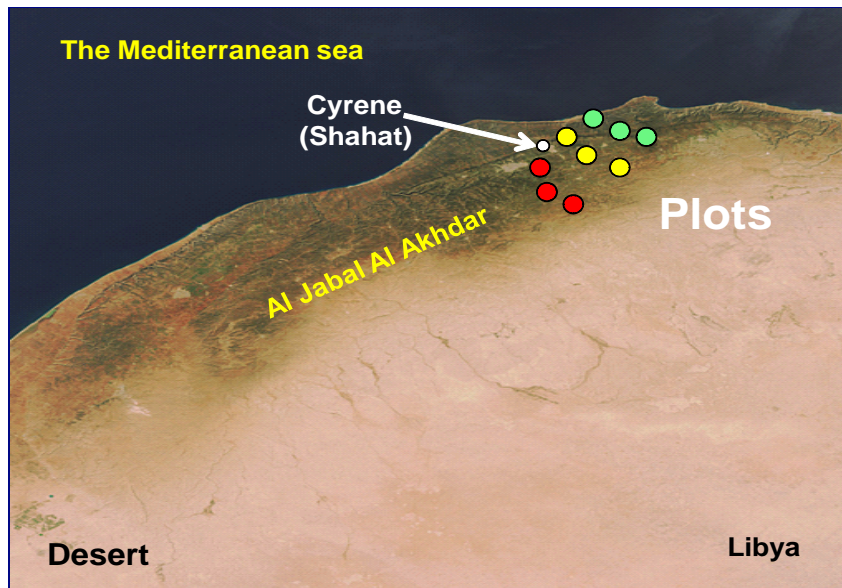


Figure 2-2: Study plots at three different elevations (● high, ● middle, and ● low) of Al Jabal Al Akhdar Mountain. High- (1-3), middle- (4-6) and low-elevation (7-9) plots are indicated.

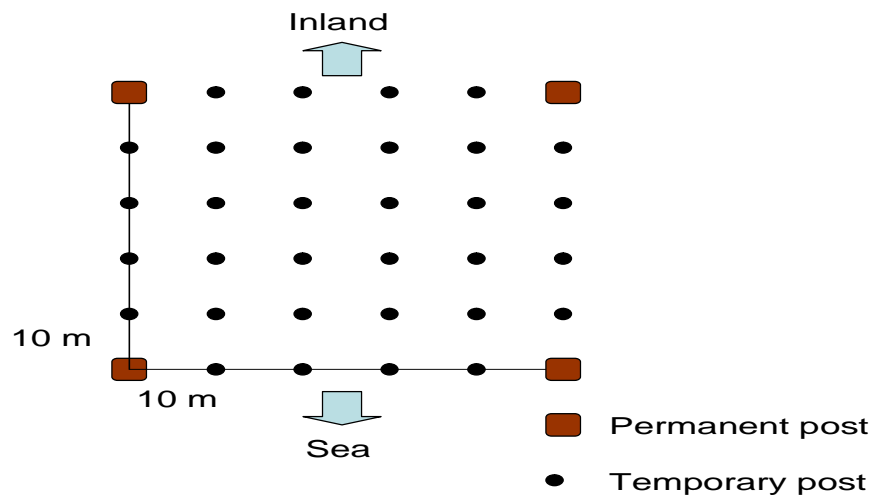


Figure 2-3 Pattern of permanent and temporary posts established each plot in order to map each plant within each study plot

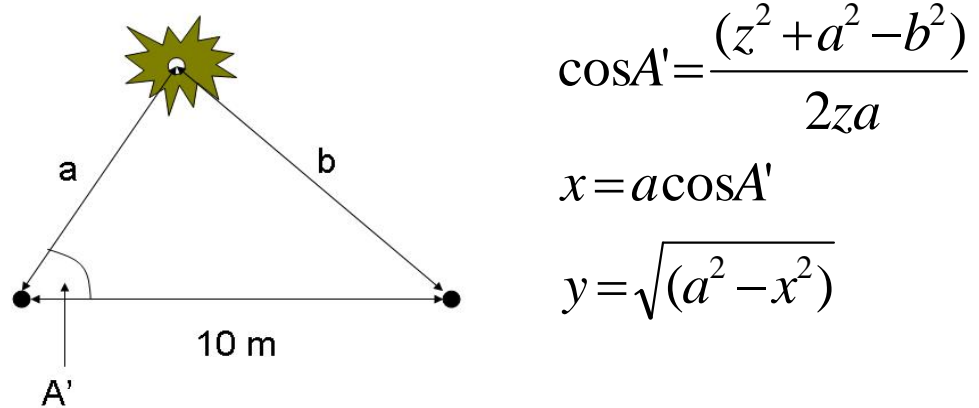


Figure 2-4 : Calculating the position of each mapped shrub within the plot from bearings and distances from the two nearest seaward posts Here, $z = 10$, and a , b and the angle A' are measured. The xy coordinates from the reference post of the subplot are then calculated as shown. XY coordinates can then be calculated with reference to the bottom-left permanent post of the plot.

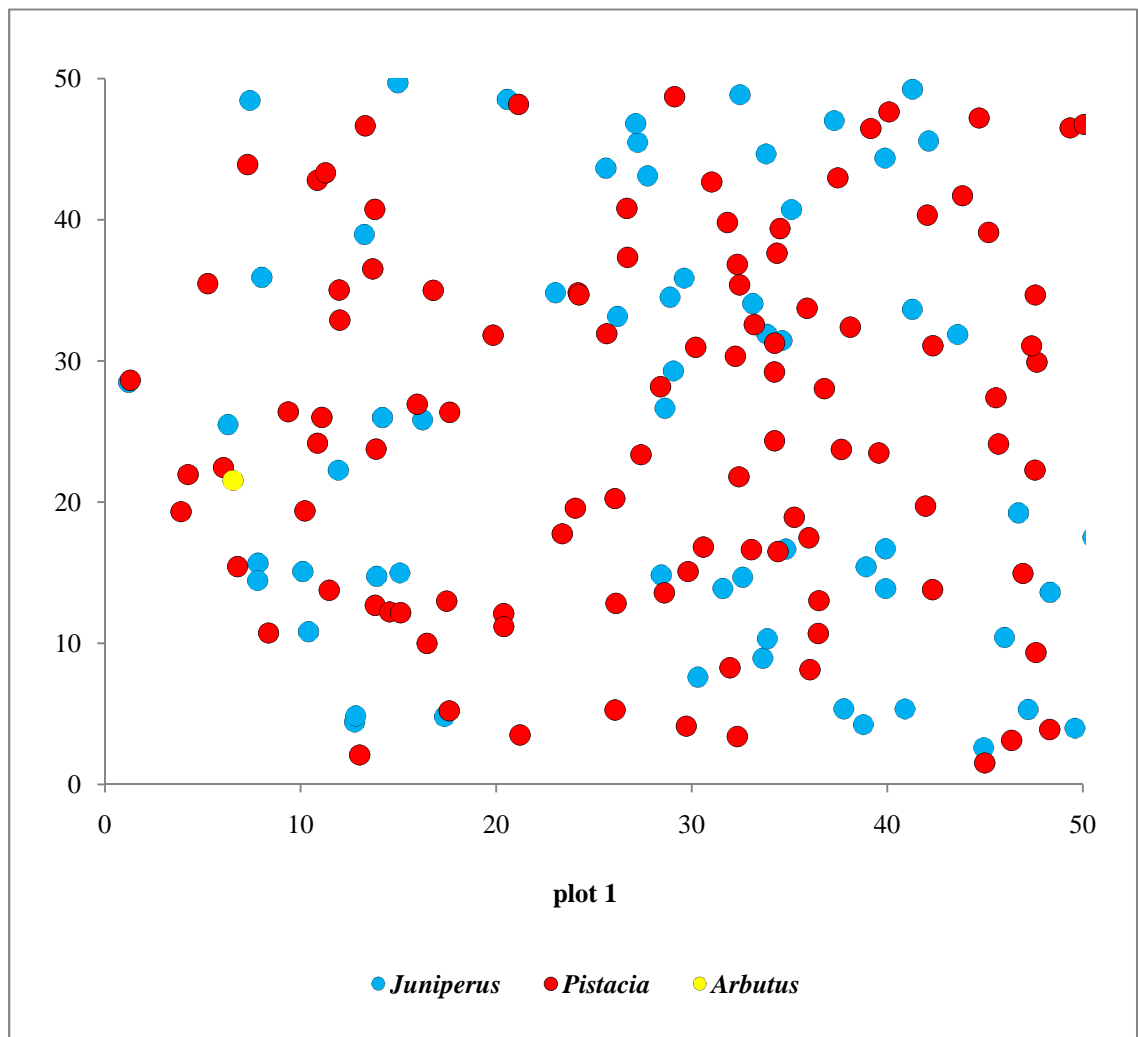


Figure 2-5: Mapped positions of trees in plot 1 (high elevation)

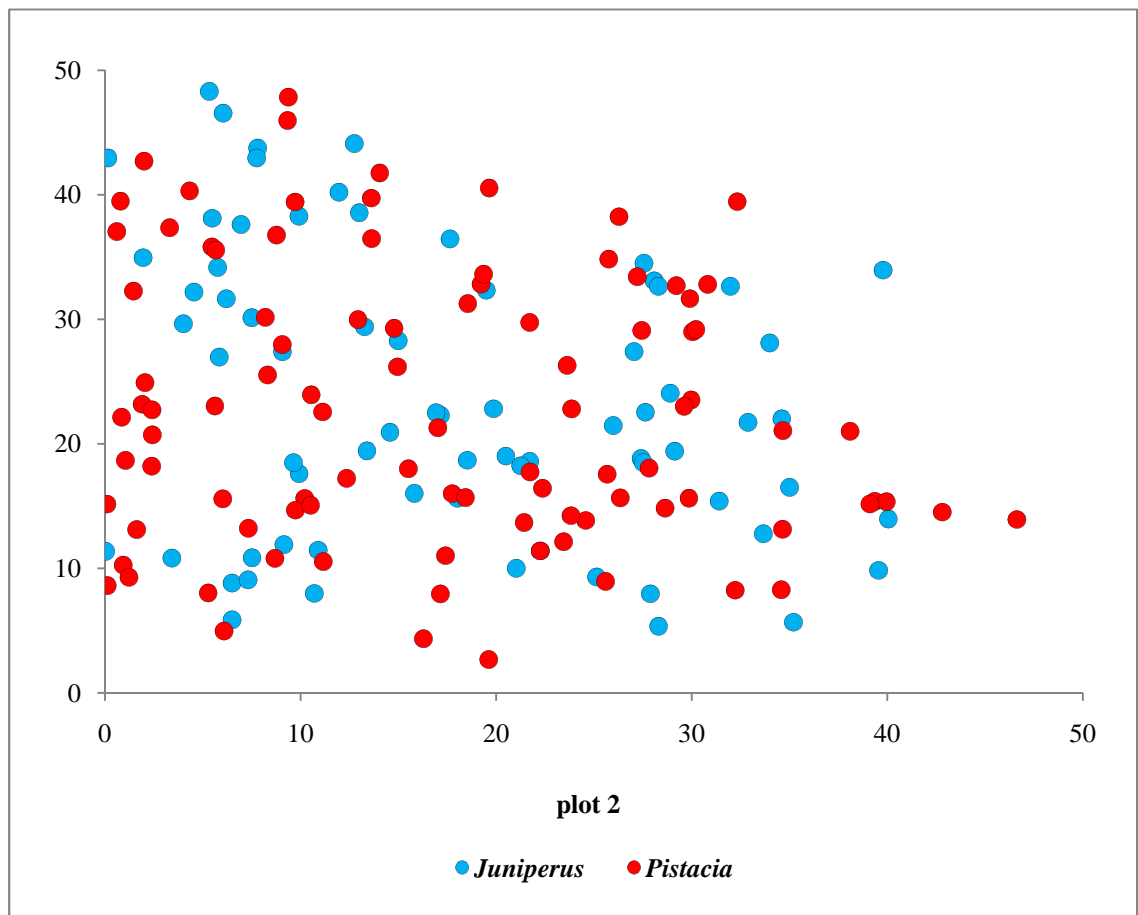


Figure 2-6: Mapped positions of trees in plot 2 (high elevation)

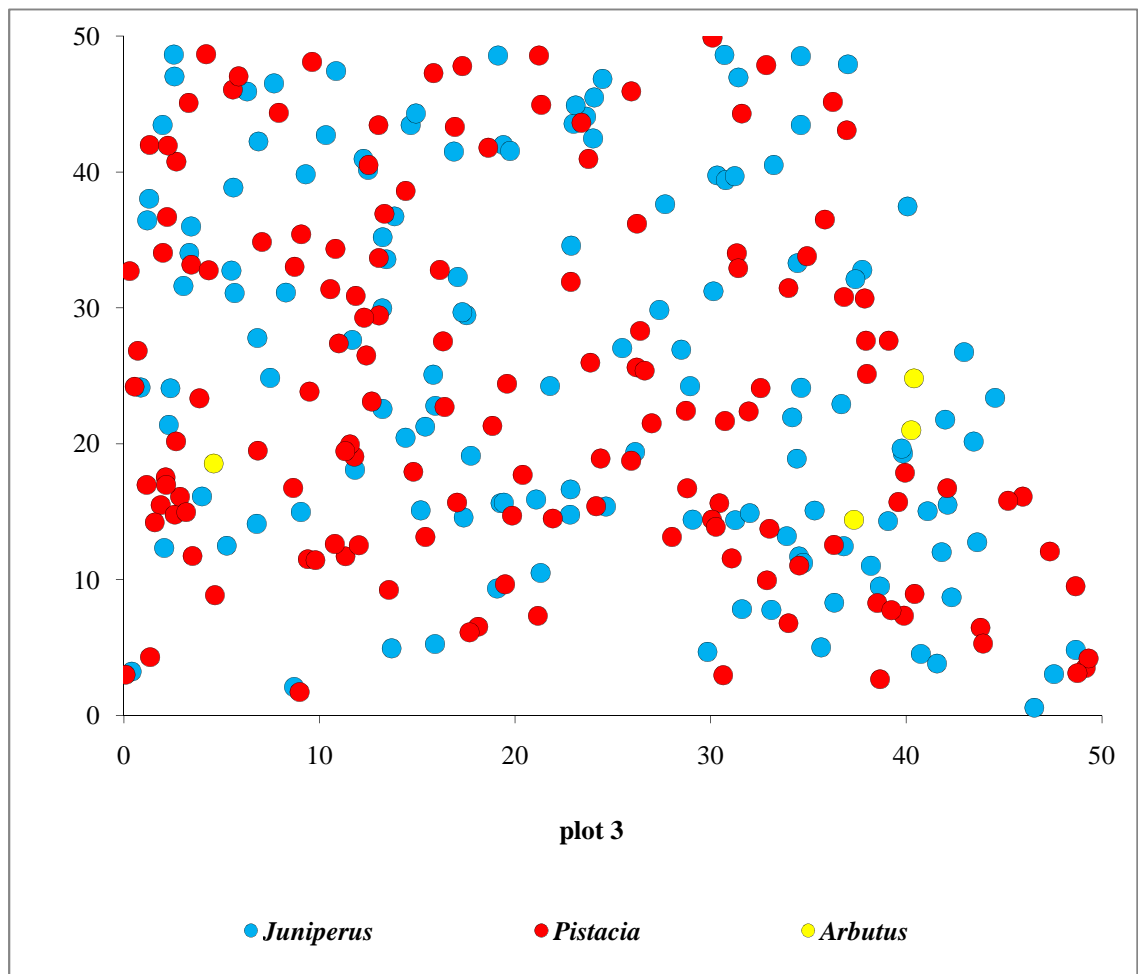


Figure 2-7: Mapped positions of trees in plot 3 (high elevation)

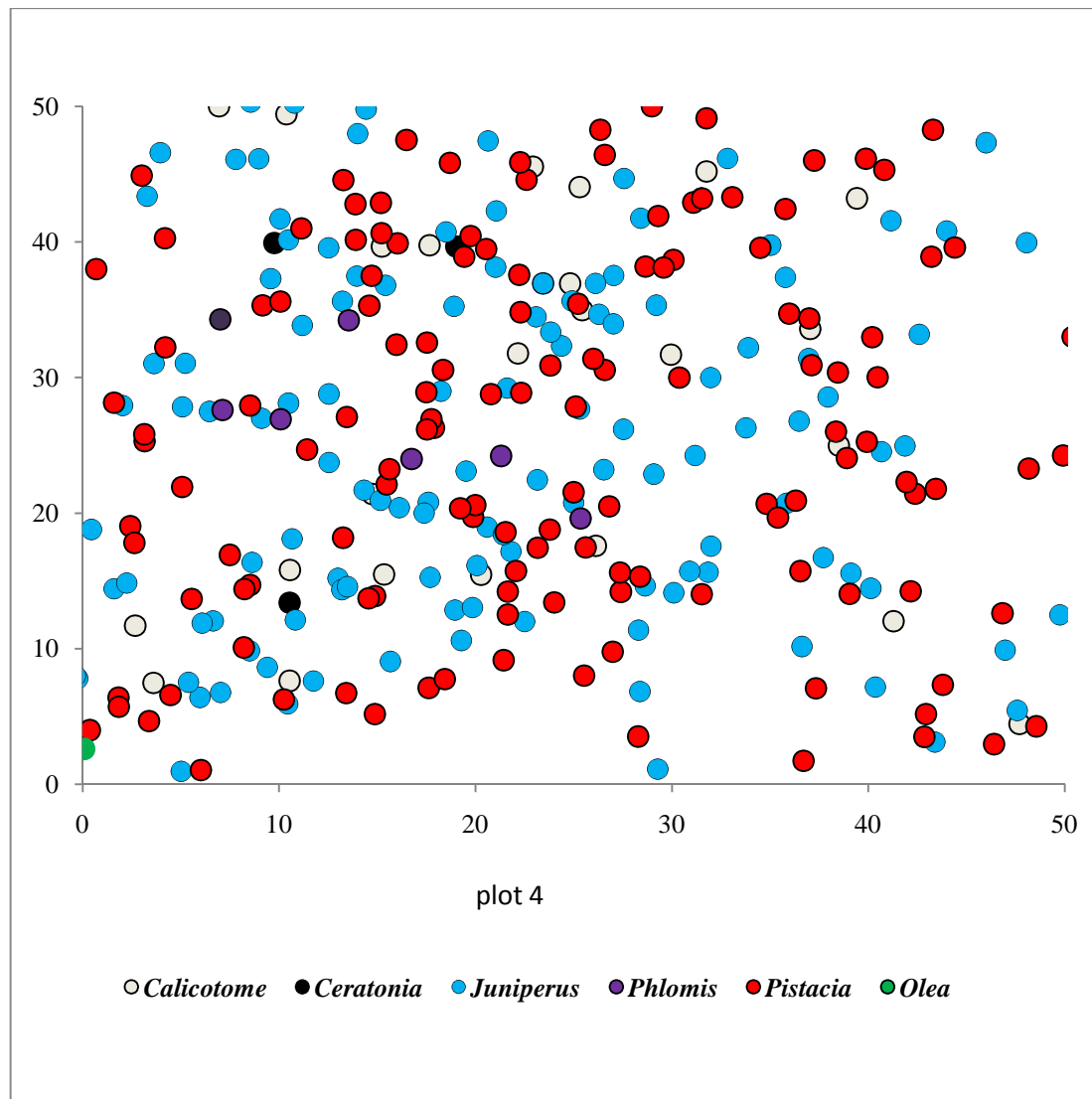


Figure 2-8: Mapped positions of trees in plot 4 (middle elevation)

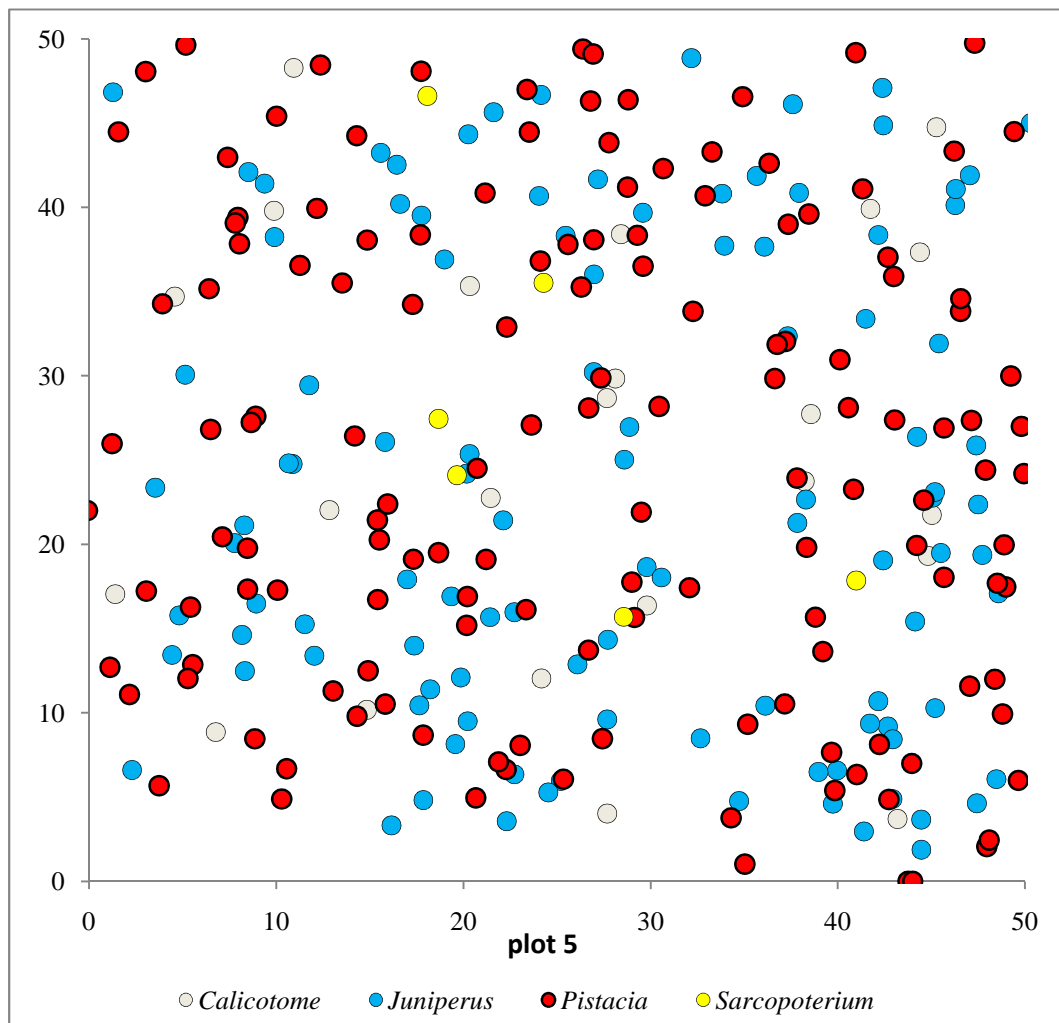


Figure 2-9: Mapped positions of trees in plot 5 (middle elevation)

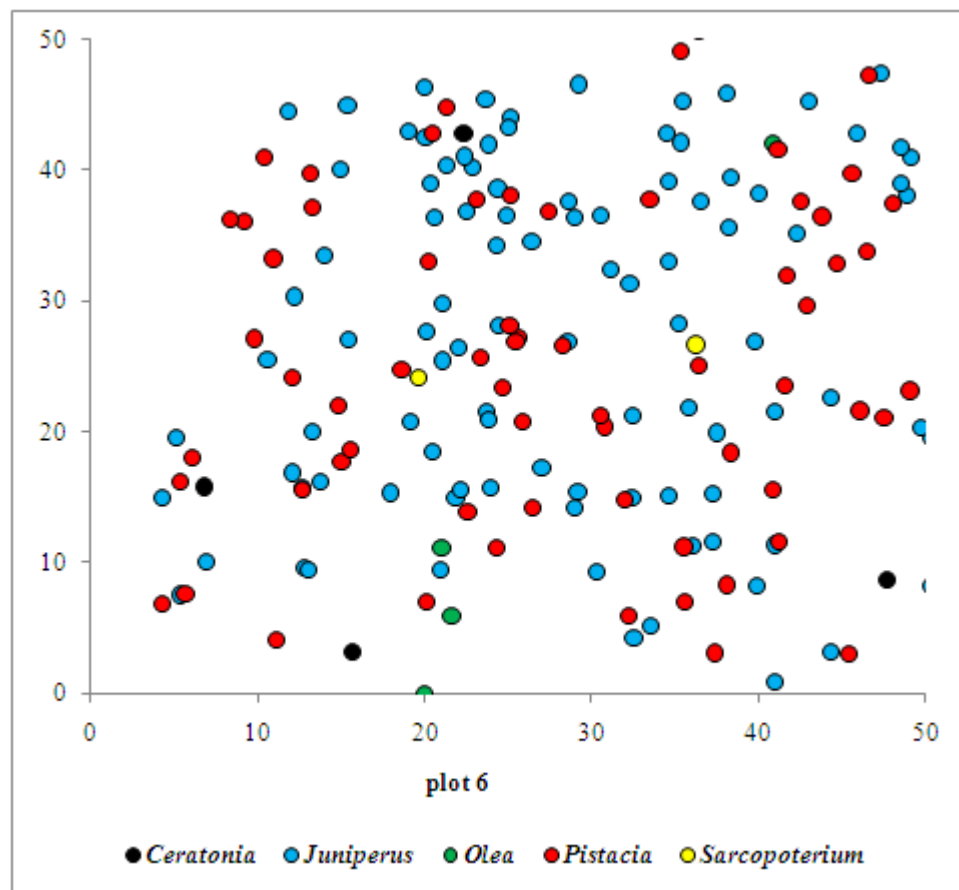


Figure 2-10: Mapped positions of trees in plot 6 (middle elevation)

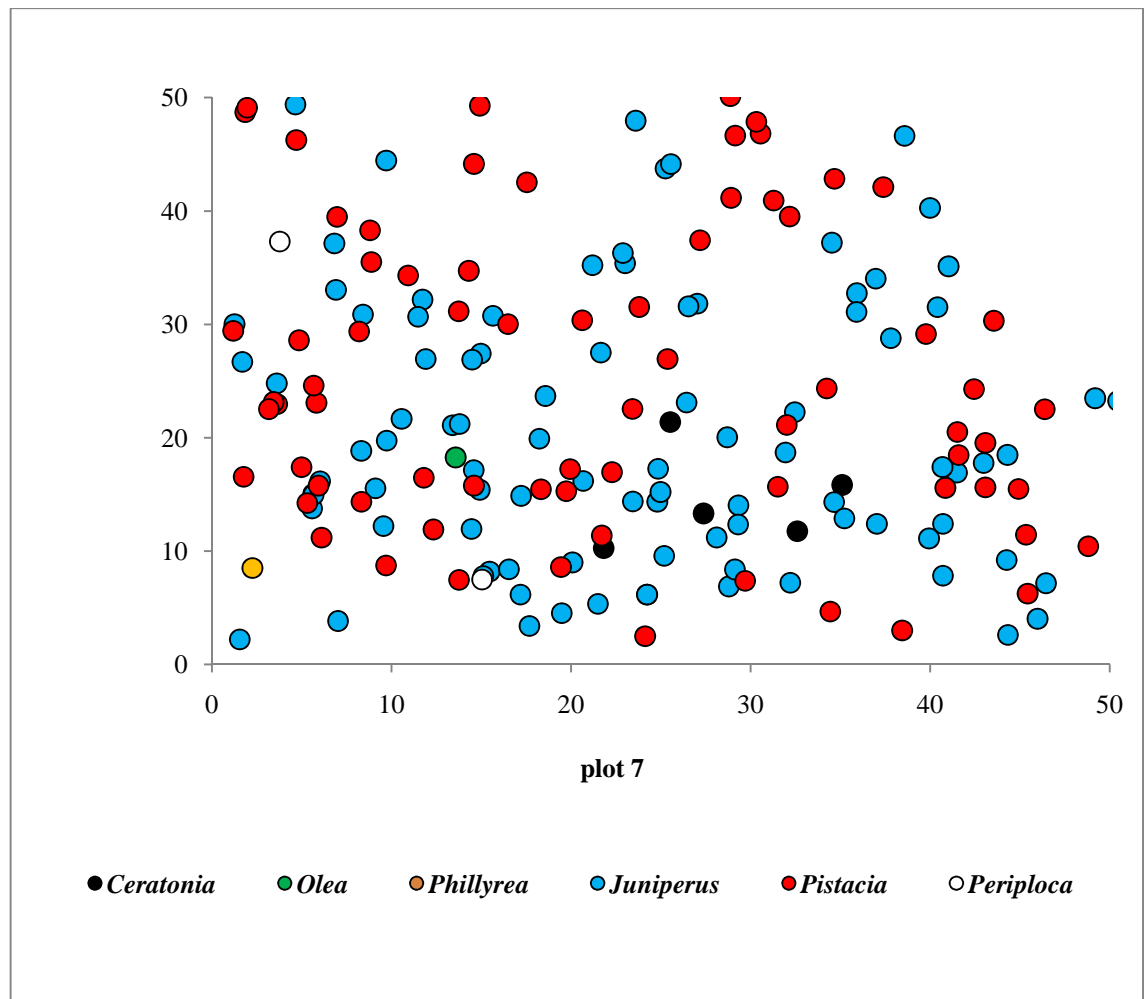


Figure 2-11: Mapped positions of trees in plot 7 (low elevation)

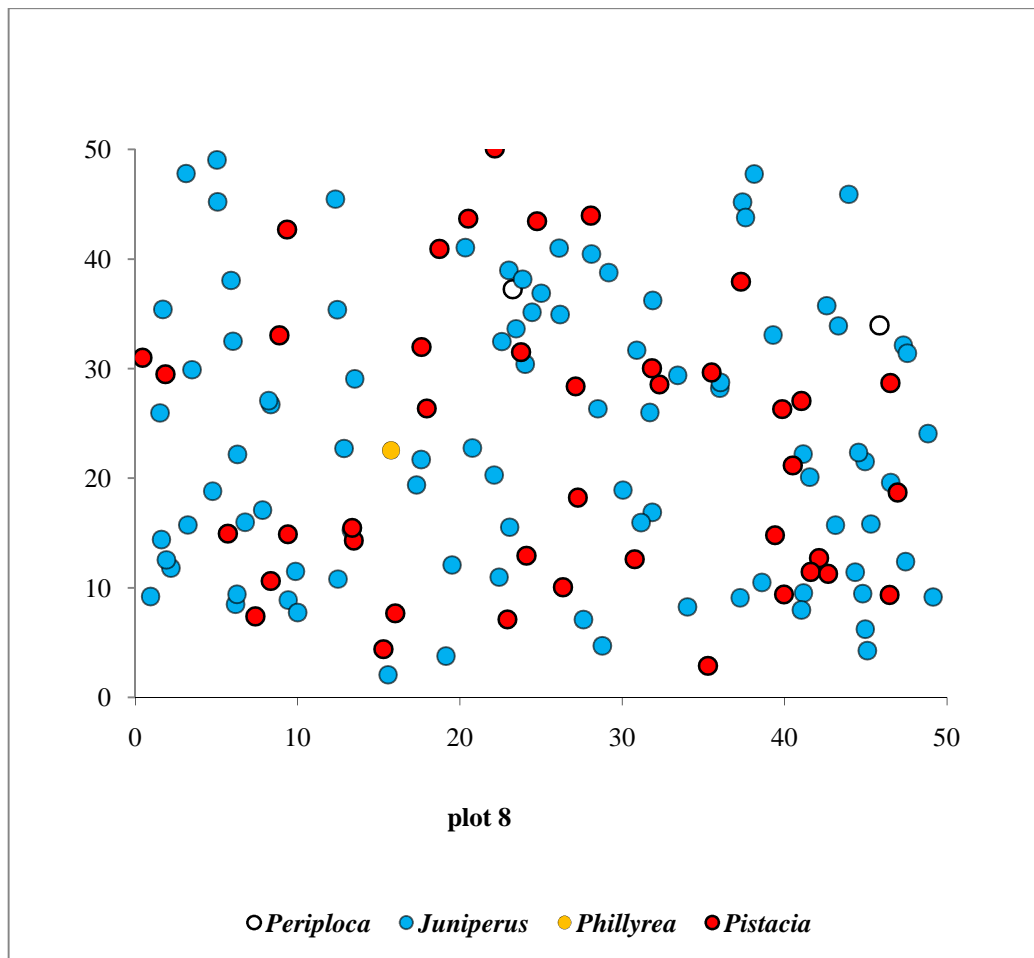


Figure 2-12: Mapped positions of trees in plot 8 (low elevation)

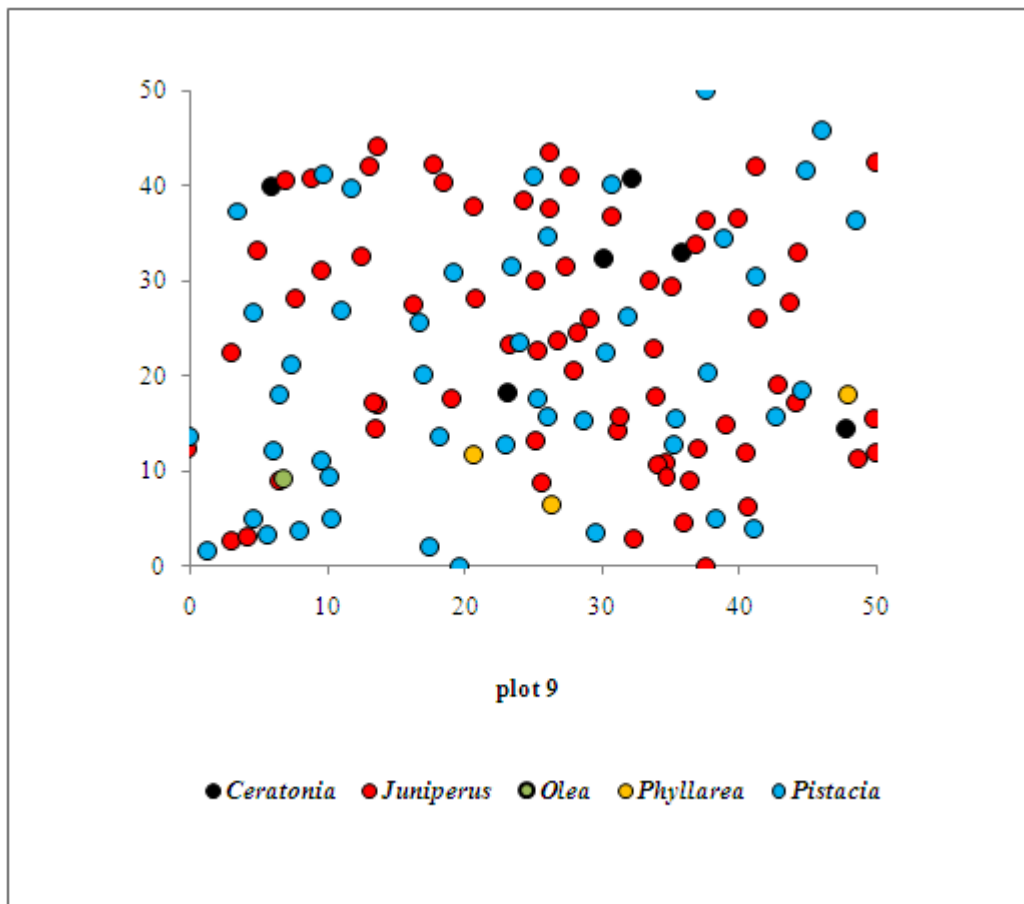


Figure 2-13: Mapped positions of trees in plot 9 (low elevation)

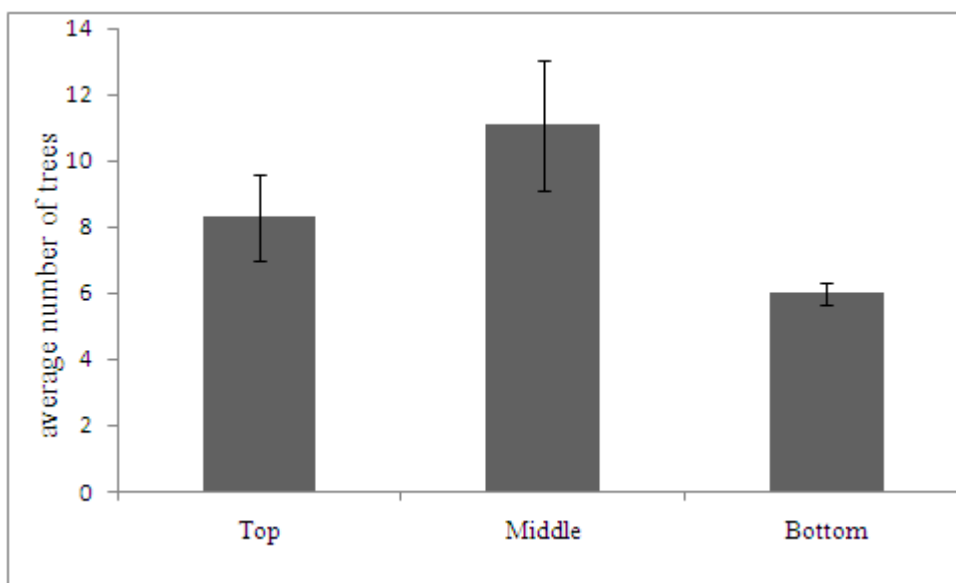


Figure 2-14: The average number of plants (trees/shrubs) per ha (sub-plot 10 x 10 m) at the three elevation levels (\pm s.e.)

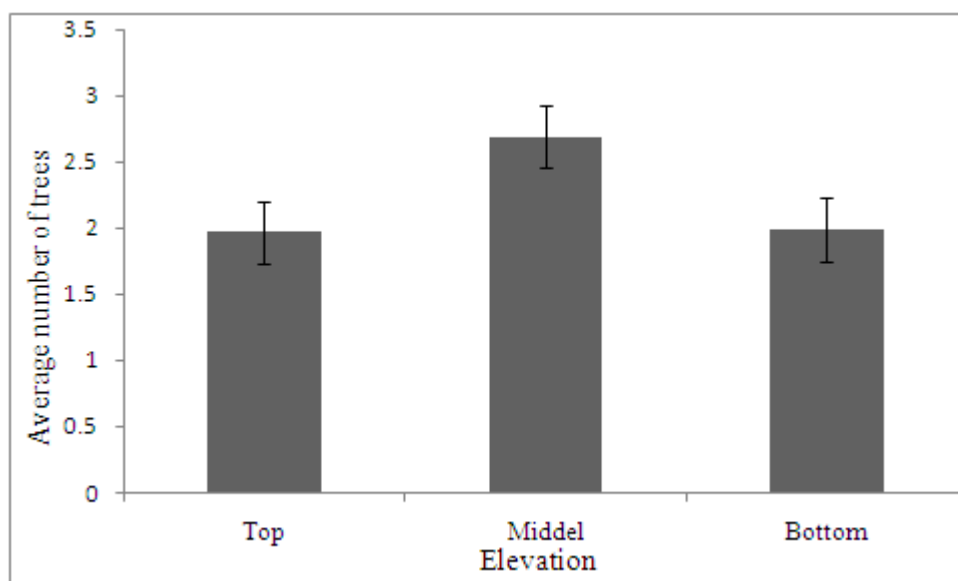


Figure 2-15: The average species richness of trees and shrubs per ha (sub-plot 10 x 10 m) at the three elevation levels (\pm s.e.)

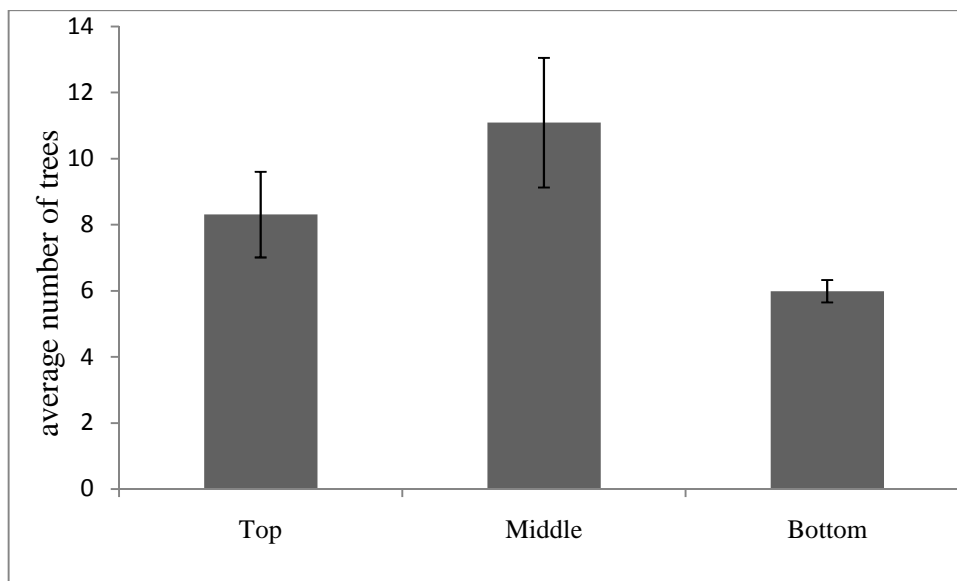


Figure 2-16: The average number of *Juniperus phoenicea* per ha (sub-plot 10 x 10 m) at the three elevation levels (\pm s.e.)

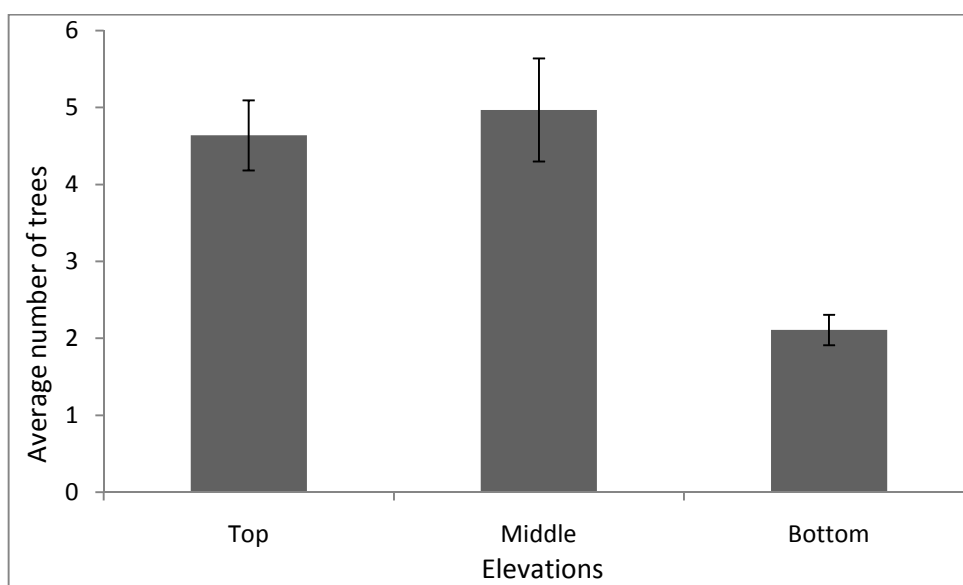


Figure 2-17: The average number of *Pistacia atlantica* per ha (sub-plot 10 x 10 m) at the three elevation levels (\pm s.e.)

Family	Species
Anacardiaceae	<i>Pistacia atlantica</i> Desf.
Asclepiadaceae	<i>Periploca laevigata</i> Aiton
Cupressaceae	<i>Juniperus phoenicea</i> L.
Ericaceae	<i>Arbutus pavarii</i> Pamp.
Labiatae	<i>Phlomis floccosa</i> D.Don
Leguminosae	<i>Calicotome villosa</i> (Poir.)
Leguminosae	<i>Ceratonía siliqua</i> L.
Oleaceae	<i>Olea europaea</i> L.
Oleaceae	<i>Phillyrea angustifolia</i> L.
Rosaceae	<i>Sarcopoterium spinosum</i> (L.) Spach

Table 2-1: The species recorded in the plots

Elevation	Plot	<i>Juniperus phoenicea</i>		<i>Pistacia atlantica</i>		<i>Arbutus pavarii</i>	<i>Ceratonía siliqua</i>	<i>Olea europaea</i>	<i>Calicotome villosa</i>	<i>Periploca laevigata</i>	<i>Phlomis floccosa</i>	<i>Sarcopoterium spinosum</i>	<i>Phillyrea angustifolia</i>
		N	Size (m ³)	N	Size (m ³)	N	N	N	N	N	N	N	N
Top	1	66	4.6 ± 0.6	108	2.7 ± 0.4	1	0	0	0	0	0	0	0
	2	72	4.3 ± 0.5	96	3.4 ± 0.4	0	0	0	0	0	0	0	0
	3	13 1	2.6 ± 0.2	141	1.2 ± 0.1	4	0	0	0	0	0	0	0
Middle	4	13 1	2.4 ± 0.2	146	2.5 ± 0.8	0	4	2	26	0	4	0	0
	5	12 0	2.3 ± 0.2	156	2.3 ± 0.3	0	0	0	25	0	0	2	0
	6	10 7	4.2 ± 0.5	80	2.4 ± 0.5	0	4	5	0	0	0	6	0
Bottom	7	95	9.2 ± 1.0	73	3.7 ± 0.4	0	5	1	0	2	0	0	1
	8	92	7.4 ± 0.8	42	3.8 ± 0.6	0	0	0	0	2	0	0	1
	9	70	3.8 ± 0.4	50	2.4 ± 0.4	0	7	1	0	0	0	0	4

Table 2-2: The number and size of *Juniperus phoenicea* and *Pistacia atlantica* shrubs and the number of other shrubs at top, mid and bottom elevations

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
plot(level)	18.369	6	3.062	6.06	.000
level	31.119	2	15.559	5.08	.050
Error	109.157	216	.505		

Table 2-3: Differences among elevation levels in tree/shrub density per ha, treating each subplot as a replicate. This analysis is derived from a GLM with normal errors. The test for level differences involves plot (level) as the error term, and has 2 and 6 df.

Source	Type III		
	Wald Chi-Square	df	Sig.
(Intercept)	1780.79	1	.000
plot(level)	18.96	6	.004
level	61.38	2	.000

Table2-4: Differences among elevation levels in species richness of trees and shrubs per ha, treating each subplot as a replicate. This analysis is derived from a GLM with quasiPoisson errors (because of underdispersion)

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
plot(level)	8.228	6	1.371	2.38	.030
level	4.160	2	2.080	1.52	.292
Error	124.537	216	.577		

Table 2-5: Differences among elevation levels in the number of *Juniperus phoenicea* per ha, treating each subplot as a replicate. This analysis is derived from a GLM with normal errors. The test for level differences involves plot (level) as the error term, and has 2 and 6 df.

Source	Type III Sum of Squares	df	Mean Square	F	Sig.
plot(level)	12.644	6	2.107	5.99	.000
level	37.500	2	18.750	8.90	.013
Error	75.932	216	.352		

Table 2-6: Differences among elevation levels in the number of *Pistacia atlantica* per ha, treating each subplot as a replicate. This analysis is derived from a GLM with normal errors. The test for level differences involves plot (level) as the error term, and has 2 and 6 df.

Chapter 3: The insect associated with *Juniperus* and *Pistacia* in Al Jabal Al-Akhdar

3.1 Introduction

The first zoological expedition made to Libya was probably that of Friederich Gerhard Rohlfs between December 1878 and October 1879. Rohlfs made several expeditions in the 'Libyan deserts' of Egypt and Libya, and was unusual in always taking scientists with him. On one particular expedition south from Tripolitania and Cyrenaica to the oasis of Kufra, he collected many zoological specimens, but he was very unlucky because almost all the specimens were damaged when the camp was attacked at the lakes of Kebabo in Kufra (Rohlfs 1881). Soon afterwards (in September 1879) a number of grasshoppers were collected from Tripoli and its surroundings by an Italian marine expedition. These represent the first Libyan specimens preserved in a museum, deposited in the Museo Civico di Storia naturale in Genoa, Italy (Massa 2009).

In 1881 under the auspices of the Societa d'Esplorazione of Milan, Giuseppe Haimann visited Cyrenaica with his wife in order to bring back zoological, botanical and archaeological specimens. The scientific investigation of Libya practically started after the Italian colonization following Italy's success in the war with the Ottoman Empire in 1911-2. Collections were made throughout the second decade of the 20th century, including in the coastal districts of Tripolitania and Cyrenaica. Many of the specimens collected in these districts by entomologists and agronomists such as Krüger, remained unpublished. Several years later, zoological expeditions took place under Italian scientific institutions to Al Jaghbub in 1926-27, Kufrah in 1931, Fazzan in 1931 and 1934, and Tassili in 1936; the results were partially

published by Zavattari (1934), who summarised all the earlier information about the Libyan fauna (Massa et al. 2004).

Even in well-known groups of insects, knowledge of the Libyan fauna remains poor, with many new records in every sustained ecological study. For example, Lepidopteran records are scattered and scanty. The butterflies of the Middle East are well served by a series of books by Torben Larsen (eg Larsen 1990) and those of the Maghreb by Tennent (1996), but there is no such guide for Libya. Damiano (1961) listed 146 species of Lepidoptera from Libya; Ahmed (1978) added three species; lists of many species of moth caught in light traps were given by Hessein (1981) for Tripoli, and El-Ghariani (1992) for Cyrenaica (the El-Beida area and Benghazi), 15 of which were new to Libya. El-Maghrabi & Amin (2007) summarised the 66 species of Lepidoptera recorded from El-Beida from March to December 2000 in a range of localities, 26 of which were new to Libya.

Juniper is well-known in the UK for having a specialized insect fauna (Ward 1971), but the species richness of its associated insects is not particularly low, given its small distributional area in the UK (Kennedy & Southwood 1984). Rather little is known about the insects associated with the *Juniperus* species of the Mediterranean region. Roques et al. (1984) looked at insects associated with the cones and seeds of three *Juniperus* species (*oxycedrus*, *phoenicea* and *thurifera*) in France. Of the nine insect species found feeding in the reproductive structures (one eriophyid mite *Trisetacus quadrisetus*, one weevil *Nanodiscus transversus*, two chalcid [Torymidae] wasps of the genus *Megastigmus* and four moths: two species of *Blastotere* [Plutellidae], *Brachyacma oxycedrella* [Gelechiidae], and two species of *Pammene* [Tortricidae]), six were recorded from *Juniperus phoenicea* (only the mite, one

Megastigmus and one *Pammene* species were lacking). *Nanodiscus transversus* and *Brachyacma oxycedrella* are known to occur in Algeria (Roques et al. 1984). Morphologically very similar but taxonomically unrelated species of weevils (*Anthonomus* spp) from juniper in the USA feed in the fungal galls of *Gymnosporangium* (Clark & Burke 2010), but this feeding niche is not known from the Mediterranean. A lygaeid bug *Orsillus depressus* (Rouault et al. 2005) feeds and oviposits on many Cupressaceae including most species of *Juniperus*.

Even less seems to be known about insects on *Pistacia* species in the Mediterranean, apart from 15 species of galling aphids (Fordinae: Pemphigidae) and their associated food webs (Wool & Mannheim 1986, Wool & Burstein 1991, Wool 1995) and seed predators (Traveset 1993). Aphid galls on *Pistacia* are often occupied by the kleptoparasite *Palumbina guerrini* (Stainton) (Gelechiidae) which eats the gall tissue (Sattler 1982). The leaves of *Pistacia* in the eastern Mediterranean are often attacked by *Thaumetopoea solitaria* caterpillars (Lepidoptera: Thaumetopoeidae) but these herbivores avoid any aphid galls (Martinez 2010).

The aim of this chapter is: first, to identify insects collected on *Juniperus phoenicea* and *Pistacia lentiscus* in the Al Jabal Al Akhdar plots; second, to establish which of these insects are herbivores on the plants; and third, to measure and establish differences in the types of insect herbivory among the top, middle and bottom elevations sampled.

3.2 Method

All nine plots at three elevations in the Al Jabal Al Akhdar region (see Chapter 2) were visited in random order over two months between March and April 2008, and again between May and June 2009; in 2008, we sampled randomly chosen focal trees of *Juniperus* and *Pistacia* (n=5 of each species per plot, 90 in total), while in 2009 we sampled every mapped tree or shrub in the plot for insects. We collected leaf and insects samples from every tree and shrub.

Insect damage to leaves and insect galls were sampled from each plant in the following manner. If the selected plant was a large tree or shrub, then all the main branches were numbered and one picked at random; this process was repeated for sub-branches, sub-sub-branches and twigs of the chosen branch, until we had selected a small branch with a set of twigs and leaves. If there were galls or insects on the twigs, a twig was chosen at random; if on the leaves, one leaf was chosen at random. We noted whether the chosen twig/leaf had any insect damage; if it had, it was picked and stored separately in a vial. This process was repeated until we had filled 50 vials or sampled 1000 leaves. If the selected plant was a small shrub, we used random numbers to sample leaves directly, again repeating until we had filled 50 vials or sampled either 1000 leaves or all the leaves of the shrub, whichever was achieved first. Most galls were caused by aphids (Homoptera: Pemphiginae: Fordini), with *Forda riccobonii* found at all sites.

For collecting free-living insects, the methods used were sweep nets and beating. Collected specimens were killed by freezing, then pinned, labelled and subsequently identified at the Natural History Museum in London with the kind cooperation of Max Barclay, Curator of Beetles. We selected elevations in random order, but visited

one plot in each until all had been visited, and then started again. Plots within elevations were visited in random order, and we tried to complete sampling of a single plot within a few consecutive days. In 2008 we sampled the marked trees used for assaying leaf chemistry, thus sampling five of each species per plot, each originally chosen at random within the plot subject to being a minimum of 5 m from the edge. In 2009 we sampled every mapped tree or shrub in the plot for insects.

The strategy was first to walk around the tree/shrub and observe what insects were present, catching those reachable without disturbance. Then we worked through the foliage by hand, starting from the top, thoroughly and systematically turning over leaves and branches. Initially until the range had been fully appreciated, all insects were collected from each individual tree/shrub, and after that we counted the number of each type. It took more than one day to sample five plants. Every individual insect was given an identification code that tracked when, where and on which plant it was collected.

Some individual insects were maintained alive to ascertain whether the species was really a herbivore of *Juniperus* or *Pistacia*. We placed a single insect in a Petri dish with some cut pieces of uneaten leaf material and a wad of wet tissue. The insects were checked periodically, and after 48 hours we noted which ones have or have not fed. All were then frozen and pinned. The distinction between true herbivores and other insects is made clear in the results section.

The assessment of the numbers of various insect pest species was done by carefully examining the selected host plants; leaf by leaf and turning of leaves as well to collect any insects from the under-surface of the leaves. The insect herbivores collected from each plot were identified, counted and recorded. Percentage leaf

damage was based on the total number of leaves damaged in sample divided by the total number of leaves in the sample. (Ogunjobi et al 2005).

This study focuses on the insect herbivores that feed on the leaves of *Pistacia* and *Juniperus*, and some that attack the trunk, if found. There are insect herbivores that were not studied for lack of time, such as those that feed on seeds, fruits, roots or that live under the bark.

The data (numbers of species, numbers of individuals) were analysed with a consistent set of predictors in the following order: *species* (*Juniperus/Pistacia*), *level*, *plot-within-level*, the *species*level* interaction and plant *surface area* (covariate). Where appropriate, we used a generalised linear model because sometimes general linear models with normal errors did not have homogeneous variances, and this was not correctable by transformation (log or sqrt). In such cases we used a generalised linear model with Poisson errors; if a model was over-dispersed, we used quasi-Poisson errors using the scaled deviance as the scale parameter.

3.3 Results

The insects collected from *Juniperus phoenicea* and *Pistacia lentiscus* and identified from specimens are listed below. I photographed many of the insect specimens with the help of the Imaging Division in the School of Biology. There are some insect specimens without photos. Different specialists kindly helped with the identification, as follows:

¹ = determined by Judith Marshall (BMNH)

² = determined by Max Barclay (BMNH)

³ = determined by Mick Webb (BMNH)

⁴ = determined by R.G. Booth (BMNH)

⁵ = determined by Kevin R Tuck (BMNH)

⁶ = determined by Thomas J. Simonsen (BMNH)

JCR= (Jean-Claude Ringenbach)

Herbivore insects

Orthoptera

Caelifera: Acridoidea: Pamphogidae

Oedopoda caerulescens (L., 1758) ¹ (Figure3-9)

The adults were on *Pistacia* in the high-elevation plots between May and June 2009. The distribution ranges from North Africa and the Canary Islands in the south up to Central Europe in the north and southwest Asia, Mongolia and China (Appelt & Poethke 1997); according to Damiano (1961), it is polyphagous insect not specialized at all to *Pistacia*.

Paracinipe (Acinipe) orientalis (Werner 1908) ¹ (Figure 3- 10)

The adults were on *Juniperus* shrubs in mid-elevation plots, between May and June 2009. This seems to be the most widespread species in Libya, and has been recorded from Cyrenaica in the 1920s (Massa 2009). The genus *Paracinipe* is distributed in northwest Africa (from Morocco to Tunisia), Sardinia and Sicily.

Scintharista notabilis (Walker 1870) ¹

The adults were on *Pistacia* shrubs in mid-elevation plots between May and June 2009. The species occurs with several subspecies from the Canary Islands to northwest India; one subspecies is confined to Arabia (Ingrisch 1999).

Acridoidea: Acrididae

Tmetonota rugosa (Stål, 1873)¹

This very large species apparently belongs to the genus *Tmetonota*. This genus contains seven species, most of which have a southern African distribution. The species is not listed in Massa (2009) as having been recorded previously in Libya. May, June 2008

Anacridium aegyptium (Linnaeus, 1764)¹ (Figure 3-11)

The adults were on *Pistacia* shrubs in the highest-elevation plots between May and June 2009. Apparently very widespread in Libya (Massa 2009), this is a common species around the Mediterranean.

Ensifera: Tettigoniidae: Bradyporinae (Ephippigerinae)

Steropleurus spp

We observed both adults and nymphs feeding on *Juniperus* shrubs at all elevations between May and June 2009. Massa (2009) lists three species in Libya: *innocentii* Finot & Bonnet 1885, *filenorum* Massa 1998 and *ientilei* Fontana & Massa 2008; *filenorum* is widely recorded from Cyrenaica, unlike the other two.

Tylopsis peneri (Ragge 1974)

Both adults and nymphs were feeding on *Pistacia* shrubs, in the mid- and top-elevation plots between May and June 2009.

Afromeconema felicis Massa, 1997

Massa (2009) stated that only four females of this species were known, with the male still unknown. However, we found adult females on *Juniperus* at all elevations between May and June 2009.

Coleoptera

Bostrichidae:

Xylomeira sp (Figure 3- 33)

Both adults and larvae were collected from *Pistacia* at the bottom elevation between May and June 2009. Species of Bostrichidae attack living trees and dead wood (M. Barclay, pers.comm).

Buprestidae: Agrilinae

Agrilus grandiceps cyrenaicus Curletti, 2005

The adults were collected on *Juniperus* from all elevations between May and June 2009. The subspecies was first collected and described from Wadi Kouf in Al Jabal Al Akhdar: other subspecies occur widely in the Mediterranean area (ssp *hemiphanes*) and in Cyprus (ssp *grandiceps*) (Curletti 2005). Ringenbach (2005) considers its host plant to be *Quercus coccifera* and perhaps *Pistacia lentiscus*, but this judgment probably just stems from the fact that the insects were trapped on these plants using sticky traps.

Agrilus (*Xeragrilus*) sp ² (Figure 3- 15)

The adults were collected on *Juniperus* from mid-elevation plots between May and June 2009. Buprestidae are well-known wood-boring beetles from North Africa; this subgenus is known to have representatives in the Palaearctic steppes associated with *Artemisia* (Konstantinov et al 2009).

Buprestidae: Julodinae

Julodis aequinoctialis harterti Théry 1930 (Figure 3-24)

We collected adults of this species feeding on *Juniperus* from all elevations between May and June 2009. Already known from Cyrenaica, it also found in Morocco.

Cerambycidae: Cerambycinae

Purpuricenus desfontainii desfontainii (Fabricius, 1792) (Figure 3-21)

Both larvae and adults were collected from twigs of *Pistacia* at all elevations. This is a polyphagous species on deciduous trees and shrubs (e.g. *Quercus ilex*, *Pistacia lentiscus*, *Ceratonia silica*, *Ziziphus*), and Sama et al. (2005) have already noted the larvae from dead and dying twigs of *Pistacia* in Cyrenaica. It is distributed across North Africa to the eastern Mediterranean; the type subspecies appears throughout the Maghreb from Morocco to Libya (Sama et al. 2005) April 2008

Stromatium unicolor (Olivier, 1795)

The larvae and adults were collected from *Juniperus* sp at all elevations between May and June 2009. This is a polyphagous insect feeding in dead dry wood of various trees, preferring deciduous trees (M. Barclay, pers.comm.), and distributed across the entire Mediterranean area and beyond to Iran and the Caucasus. This genus was not listed by Sama et al (2005) and hence has not been recorded from Libya before.

Icosium tomentosum tomentosum Lucas, 1854 (Figure 3-22)

Both larvae and adults were collected from *Pistacia* and *Juniperus* twigs, and found on the bottom and top elevations between May and June 2009. It is known to feed on other Cupressaceae such as *Cupressus* and *Thuja*, and is found right across the Mediterranean in two subspecies (Sama et al. 2005). In 2005 there had only been a single specimen recorded from Libya, but this was reared from a larva found in a *Cupressus* tree from Wadi bel Gadir near Shahat, very close to the plots of this study (Sama et al. 2005).

Cerambycidae: Lamiinae: Pteropliini

Niphona picticornis Mulsant, 1839 (Figure 3-23)

Both larvae and adults were collected from *Pistacia* twigs at the top elevation plots between May and June 2008. This is a relatively common and a broadly polyphagous species. The larvae are known to feed in dead branches and trunks of many shrubs and trees of the maquis vegetation: *Ficus*, *Spartium*, *Castanea*,

Ulmus, *Morus*, *Punica*, *Quercus*, *Sambucus*, *Laurus*, *Cercis*, *Pistacia* and *Rhamnus*. It is distributed along the coastal region of the whole Mediterranean (Sama et al. 2005).

Cerambycidae: Prioninae

Agapanthia annularis (Olivier 1795) (Figure 3- 12)

Adults appear during the spring season from February to April. This common species is distributed from in Spain, Portugal, and Morocco to Libya (Sama et al. 2005). We found it on *Juniperus* sp on lowest elevation. May, June 2008

Cerambyx nodulosus (Germar, 1817)

Adults were collected from *Juniperus* sp from the top elevation between May and June 2008. This is a polyphagous species in fruit and ornamental trees and bushes. It is distributed from Italy to Syria and the Caucasus (Özdikmen & Turgut 2009). Not recorded as Libyan by Sama et al. (2005), so presumably this is the first record for Libya.

Hesperophanes sericeus (Fabricius 1787) (Figure 3-19)

Larvae and adults are polyphagous on dead or dry trees and shrubs such as *Pistacia* and *Quercus* On lowest and middle elevation. It is distributed widely Iberia and North Africa to the Caucasus and Iraq (Zavattari 1934), and was recorded from Cyrenaica (Benghazi) in the early 1930s, but apparently not since (Sama et al. 2005). May and June 2008, 2009

Macrotoma palmata (Fabricius, 1792) (Figure 3-13)

We found on mid elevations plots between May and June 2008,9 on *Juniperus* sp. Both larvae and adults are known to feed on *Acacia tortilis*, *A. nilotica*, *A. scorpioides*, *Morus alba* and *Albizzia lebbek*. The species is widely distributed in Africa, from Egypt to the Cape, and Abyssinia to Senegal: in North Africa it is known from Mauritania, Morocco, Algeria, and Libya, and stretches further to Saudi Arabia and Yemen (Sama et al. 2005).

Phoracantha semipunctata (Fabricius 1775) (Figure 3-18)

We found on *Juniperus* sp but it did not eat it. A pest introduced from Australia, both larvae and adults feed on *Eucalyptus* trees. It is widely distributed across the entire Mediterranean region including North Africa (Sama et al. 2005). May and June 2008

Prinobius myardi (Mulsant 1842) (Figure 3-14)

We found on *Juniperus* sp but it did not eat it. This species is polyphagous on many trees, and widely distributed across the entire Mediterranean region including North Africa, and beyond to Iran. In Cyrenaica it has been recorded feeding on *Acacia*, *Ceratonia siliqua*, *Olea* and *Ficus*, and is known to be widespread in Al Jabal al Akhdar in Libya (Sama et al. 2005). May and June 2008, 2009

Labidostomis taxicornis (Fabricius 1792)²

The adults were collected on *Pistacia* from the top elevation. This species has been recorded from Morocco, and is also found in southern Europe.

Stylosomus ericeti Suffrian 1851² (Figure 3-27)

Adults were collected from *Pistacia* in mid-elevation plots. The genus of 28 species is mainly from the western Palaearctic; this species is also found in Spain and Cyprus, but to our knowledge has never been recorded from Libya before.

Tituboea biguttata (Olivier 1791) ²

The adults were collected on *Juniperus* from the top elevation plots. The species is also distributed in southern Europe and North Africa.

Coccinellidae: Chilocorinae

Exochomus sp ⁴ (Figure 3-28)

The adults were collected on *Juniperus* from the top elevation plots between May and June 2008. This is a predatory species.

Pharoscymnus sp ⁴ (Figure 3-30)

Adults were collected on *Juniperus* from the top and bottom elevations between May and June 2009. This is a predatory species.

Nephus sp ⁴ (Figure 3-29)

Adults were collected from *Juniperus* from the middle and top elevations between May and June 2009. This is a predatory species.

Curculionidae: Nanophyinae

Nanodiscus transversus (Aubé, 1850) ² (Figure 3-20)

We collected adults of this genus feeding on *Juniperus* at all elevations between May and June 2009, and also on *Cupressus* sp. The larvae feed in the juniper cones (Roques et al. 1984). The species has a Mediterranean distribution, and is known from Algeria.

Curculionidae: Entiminae

Phyllobius (Ectomogaster) festae F. Solari, 1925 (Figure 3-25)

Adults were collected on *Juniperus* from all elevations between May and June 2008.09. Ringenbach (2005) collected this species in the nearby Wadi Kouf by beating *Quercus coccifera*.

Polydrusus (Conocetus) festae F. Solari, 1925 (Figure 3-16)

We collected adults on *Juniperus* from all elevations between May and June 2009. This species was first collected from Cyrenaica, and has been collected from *Pistacia lentiscus* (Ringenbach 2005).

Calostrophus ringenbachii Pelletier, 2006 (Figure 3-26)

The adults were collected on *Juniperus* from all elevations between May and June 2009. When first collected in 2003-4 from Al Jabal AlAkhdar, it was found on *Pistacia lentiscus*, *Quercus coccifera* and *Arbutus pavarii* (Pelletier et al. 2006), so it is interesting that we did not find it on these plants in our study plots.

Curculionidae: Lixinae

Lixus (Epimeces) scolopax Boheman, 1836

We collected adults of this genus feeding on *Pistacia* from all elevations between May and June 2009. It was collected from Libya for the first time in 2003 (Ringenbach 2005); elsewhere it is known from France and Algeria.

Curculionidae: Brachycerinae

Scythropus raffrayi Desbrochers des Loges 1871 ²

Adults were collected on *Juniperus* from all elevations between May and June 2009, and it was also found on *Cupressus*. This species only occurs in North Africa.

Melyridae: Dasytinae

Psilothrix sp ²

Adults were collected on *Pistacia* sp from the bottom elevation. The genus has a wide distribution in Europe and North Africa.

Dasytes sp (Figure 3-34)

Adults collected on *Pistacia* from mid-elevation plots between May and June 2009. Beetles of this family usually eat flowers (M.Barclay, pers.comm).

Dasytinae sp (Figure 3-35)

Adults collected on *Juniperus* from the bottom elevation between May and June 2009. Beetles of this family usually eat flowers (M.Barclay, pers.comm).

Melyridae: Malachiinae

Malachius bipustulatus (L, 1758)

Adults were collected on *Pistacia* from mid-elevation plots between May and June 2009. This species is also found in North America, Europe, western Asia and the Middle East. Beetles of this family usually eat flowers.

Malaciinae sp (Figure 3-36)

Adults collected on *Juniperus* from the bottom elevation plots between May and June 2009. Beetles of this family usually eat flowers.

Meloidae: Lyttini

Alosimus mendax (Fairmaire 1876)

Adults were collected on *Pistacia* from mid-elevation plots in May and June 2009. This is a west Mediterranean species, distributed in Algeria, Tunisia and Libya (Bologna 2009); within Libya it has only previously been recorded from Tripolitania, and thus this is the first record for Cyrenaica.

Alosimus viridissimus (Lucas, 1849)

Adults were collected on *Pistacia* from the bottom elevation plots in May and June 2009. This species is endemic to the Maghreb, and also occurs in Morocco, Algeria and Tunisia (Bologna 2009). This appears to be the first record for Cyrenaica.

Scarabaeidae: Aphodiinae

Aphodius sp.² (Figure 3-32)

Adults were collected from *Pistacia* from the top elevation plots between May and June 2009. The larvae eat dung (M. Barclay, pers.comm).

Hemiptera

Cicadellidae

Cicadellidae sp

Adults were collected on *Juniperus* from all elevations between May and June 2009.

Cicadidae

Cicada barbara (Stål 1866)³ (Figure 3-43)

Adults were collected on *Juniperus* from all elevations between May and June 2009. One of two species recorded in Libya, this is a common species known from SW Europe and North Africa to Libya (Quartau & Simões 2005)

Cicada sp³

Adults were collected on *Juniperus* from the top elevation plots between May and June 2009.

Psalmocharias sp³ (Figure 3-42)

Adults were collected on *Juniperus* from the top elevation plots between May and June 2009. There are at least two North African species of this genus (Schedl 1999).

Pentatomidae: Pentatominae: Sciocorini

Sciocoris sp³ (Figure 3-45)

Adults and nymphs were collected on *Juniperus* from all elevations between May and June 2009. Species of this genus feed from many plant species, but as far as we know it has never been recorded from *Juniperus* before, or indeed from any Cupressaceae or any tree/shrub (Rider 2011).

Eurydema sp² (Figure 3-47)

Adults were collected on *Pistacia* from top elevation plots between May and June 2008, 09. Species of this genus feed on many plant species, mostly Brassicaceae, and as far as we know have never been recorded from *Pistacia* before, nor from any Cupressaceae (Rider 2011).

Scutelleridae: Odontotarsinae

Odontotarsus caudatus (Burmeister 1835)³ (Figure 3-44)

Adults and nymphs were collected on *Juniperus* from mid-elevation plots between May and June 2009. This species has only been recorded on species of Asteraceae before (Rider 2011).

Lepidoptera

Noctuidae

Autographa gamma (Linnaeus 1758) (Figure 3-41)

Adults were collected on *Pistacia* from the bottom elevation plots between May and June 2009. Very common at garden flowers, the larva eats a wide range of herbaceous plants, and can be a pest of some plants (Chinery 1986). It occurs throughout much of Europe, Asia, and northern Africa (Venette et al. 2003).

Tortricidae

Cirriphora pharaonana Kollar 1858⁵ (Figure 3-38)

Adults were collected on *Juniperus* from all elevations between May and June 2009. The larvae have been recorded on *Tamarix*, feeding on the blister galls of *Eriophyes* mites (Gerling et al. 1976). We know of no records of the genus on *Juniperus*.

Tineidae

Edosa lardatella Lederer 1858 ⁵ (Figure 3-39)

Adults were collected on *Juniperus* from all elevations between May and June 2009. The *E. lardatella* species-group comprises about a dozen species that all look much the same externally: they all have white forewings and yellowish-orange head vestiture. To identify them with certainty requires high quality material and may involve the need to dissect genitalia. Very little is known of the life-histories of *Edosa* species. We are not aware of any association of any species of the genus with *Juniperus*.

Crambidae: Schoenobiini

Donacaula cf. *forficella* (Thunberg 1794) ⁶ (Figure 3-40)

Adults were collected on *Pistacia* and *Juniperus* from the top elevation plots between May and June 2009. Although it looks quite like this species, it is very likely not on distributional grounds since *forficella* are recorded from northwest Europe, Latvia and Romania. It may be a schoenobiine, and possibly a species of *Donacaula*, but the identification is not 100% certain. To our knowledge no *Donacaula* species has been associated with *Juniperus*.

Chrysomelidae: Chrysomelinae

Timarcha sp

We collected this species feeding on *Pistacia* at the bottom elevation in May and June 2009. Popularly called the ‘bloody-nosed beetles’ (Europe) or

‘strawberry leaf beetles’ (USA), the genus consists of more than 100 species, all flightless herbivores feeding mostly on herbaceous Rubiaceae and/or Plantaginaceae. As far as we know, none has ever been recorded from Anacardiaceae (*Pistacia* family). They are known from Libya; the genus has a disjunction distribution in Europe and North Africa, with two species in western North America (Gomez-Zurita 2004)

Chrysomelidae: Cryptocephalinae

Clytra sp²

Adults were collected on *Juniperus* and *Pistacia* at the top elevation plots between May and June 2009. Several species of *Clytra* are known to feed on the leaves of *Pistacia* in Greece (Mourikis et al. 2004).

Lachnaia cerealis Olivier 1808²

Adults were collected from *Pistacia* sp at the bottom elevation between May and June 2009. This species is also found in Algeria (M. Barclay, pers. comm.). The genus consists of about 22 species, most of which have a western Mediterranean distribution.

Non- herbivores

Tenebrionidae: Tentyriini

Tentyria ocularis calcophila Koch 1937

Adults were collected on *Pistacia* from the bottom elevation plots between May and June 2009. The species is known from Cyrenaica and Tripolitania (Ringenbach 2005).

Phalacridae

Olibrus sp² (Figure 3-31)

Adults were collected on *Pistacia* from the mid-elevation plots between May and June 2009. The species may be fungivorous (M. Barclay, pers.comm).

Scarabaeidae: Cetoniinae

Tropinata squalida pilosa Brullé, 1832

Adults were collected from all elevations between May and June 2009. This is a species of the Mediterranean Basin and North Africa (Zavattari 1934); adults feed from flowers, while the larvae feed on the roots of herbaceous plants.

Tropinota sp²

Adults were collected from the bottom elevation plots between May and June 2009.

Coreidae: Pseudophloeinae

Anoplocerus sp (Kiritshenko, 1926)³

Adults and nymphs were collected on *Pistacia* in the bottom elevation plots between May and June 2009. *A.elevatus* has been found in juniper forest in Spain (Ribes et al. 1997).

Bothrostethus sp³

Adults were collected on *Pistacia* from the top elevation plots between May and June 2009. The widespread *B.annulipes* is found in Egypt, where its host plant is said to be *Bromus* and other Fabaceae (El Hamouly et al. 2010).

Issidae

Latilica sp³

Adults and nymphs were collected on *Juniperus* from all elevations between May and June 2009. There are a number of species known from the Mediterranean.

Lygaeidae: Orsillinae

Orsillus sp³

Adults were collected on *Juniperus* from all elevations between May and June 2009. Two species of cypress seed bug, *O.maculatus* and *O.depressus*, are known to attack cones of Cupressaceae in the Mediterranean, ovipositing either in partly open cones, or in the exit holes of the cypress seed chalcid *Megastigmus* (Torymidae) (Rouault et al. 2005).

Unidentified moth

Adults were collected on *Pistacia* from mid-elevation plots between May and June 2009.

Analysis

*a) data on marked individual trees of **Pistacia** and **Juniperus** (2008 sampling)*

For the 45 marked trees of each of the two major species, the number of species of insects on *Juniperus* were greater than on *Pistacia* at all sites (Figure 3-1), a highly significant effect (Table 3-1). There were no overall differences in species richness with elevation, but there was a significant species * level interaction (Table 3-1), illustrated in (Figure 3-1): for *Juniperus*, the middle level had the highest species richness, whereas for *Pistacia*, this had the lowest species richness. The effects of elevation were relatively small (see Figure 3-1). There was no effect of plant surface area on species richness.

Overall insect densities on *Pistacia* were much lower than on *Juniperus* (Figure 3-2, Table 3-2). There were significant overall effects of elevation, as well as a significant species * level interaction (Table 3-2). The mid-elevation plots had greater numbers of insects, but this effect was much more marked for *Juniper* than for *Pistacia* (Figure 3-2). Again there was no effect of plant surface area.

The type of herbivory on *Pistacia* differed among sites; there was relatively little damage to leaves, but most of the damage that was present was on young leaves, caused by grasshoppers (Acrididae) with some contribution from Lepidoptera and Homoptera. Most galls were caused by aphids, found at all sites. There were no leaf mines. Although there was large inter-plot variation, damage to leaves increased slightly but significantly with elevation (Figure 3-3, Figure 3-8, and Table 3-3). Spot damage to leaves was lowest at the bottom elevational level (mean of 5.5 ± 0.9 % of leaf damaged by spots), with similar levels at middle (7.8 ± 0.9) and top levels ($7.5 \pm$

0.9), but these differences were not significant (glm with normal errors, $\chi^2 = 3.9$, df = 2, $p = 0.34$). Gall damage to leaves was similarly highest in the middle elevation (Figure 3-4).

Juniperus phoenicea does not have leaves that can be assessed for damage, and hence the proceeding analyses done for *Pistacia* could not be carried out for this plant. The commonest damage was caused by bush-crickets (Tettigoniidae) and the moth *Apatele* sp (Noctuidae). In the field it seemed that *Pistacia* growing near *Juniperus* tended to receive more damage than those that grew alone. There were no leaf mines on *Juniperus*.

(Table 3-4) gives the commonest species collected from the marked individuals of *Juniperus* and *Pistacia* during the sampling period.

(b) data from all trees/shrubs in every plot (2009 sampling)

To analyse species richness on plants, we used a quasiPoisson error structure because the residuals were non-normal (Shapiro-Wilk 0.996, df=1890, $p < 0.01$). Surprisingly, there were no significant differences among plant species in the number of insect species per plant (Table 3-6), but there was an increase with elevation (Figure 3-5). Restricting the data to the two focal species, there were no significant differences in insect species richness overall between *Pistacia* and *Juniperus* ($\chi^2 = 0.05$, df = 1, n.s.), nor any species * level interaction (Figure 3-6; $\chi^2 = 2.49$, df = 2, n.s.).

The overall numbers of individual insects per plant was analysed with a glm with normal errors. The mean values are given in (Table 3-7). Again there were no significant differences among plant species (Table 3-8), but significant differences

overall among the three levels, with numbers increasing from bottom to top elevations (Table 3-5). Restricting the analysis to the two focal plant species, there were no overall differences ($\chi^2 = 0.22$, $df = 1$, n.s.) but there was a significant species * level interaction (Figure 3-7); $\chi^2 = 6.75$, $df = 2$, $p < 0.05$).

The overall numbers of the commonest species on *Juniperus*, *Xylomeira* sp, were analysed with a Glm with normal errors. For 2008 data, there were significant differences among levels (Wald $\chi^2_2 = 75.4$, $p < 0.001$) with mid-elevation plots having the highest densities. For 2009 data, there were again significant differences among the three levels (Table 3-9), with numbers per tree increasing from the bottom (2.38 ± 0.18) to top (3.39 ± 0.18) elevations.

The overall numbers of the commonest species on *Pistacia*, *Oedopoda caerulescens* were also analysed with a Glm with normal errors. For 2008 data, there were significant differences among levels (Wald $\chi^2_2 = 12.5$, $p < 0.01$), with the greatest densities at the top elevations. For 2009 data, there were no significant differences among levels (Table 3-10), but the mean values decreased from bottom (1.48 ± 0.06) to top (1.31 ± 0.06) elevations.

In terms of the types of insect damage on *Pistacia* leaves (Figure 3-8), there were few if any differences with altitude in the distributions of some functional groups of insect herbivores. In GLMs with normal errors, damage from chewers showed no differences among levels ($\chi^2 = 3.91$, $df = 2$, ns) and neither did spot damage ($\chi^2 = 2.11$, $df = 2$, ns). In contrast, there were clear altitude effects on the damage made by gallers (non-parametric KW = 22.1, $df = 2$, $p < 0.001$) and miners (KW = 29.1, $df = 2$, $p < 0.001$).

3.4 Discussion

The commonest species on *Juniperus* in both years of sampling was *Xylomeira* sp. (Bostrichidae), a species that attacks live and dead wood. This might be a significant herbivore that could affect the survival and life-history of juniper in Al Jabal Al Akhdar. In fact, nearly all the commonest species on juniper were beetles, including many wood-boring species, such as *Agrilus* (*Xeragrilus*) sp. (Buprestidae) and *Purpuricenus desfontainii* (Cerambycidae). Clearly juniper is a major resource for beetles in this area.

On *Pistacia*, on the other hand, the commonest species varied between years, with Orthoptera heading the list in the first sampling year: *Paracinipe* (*Acinipe*) *orientalis*, *Oedopoda caerulea* and *Scintharista notabilis*. A set of wood-boring beetles were commonest in the second year of sampling, some of which were the same as those on juniper. This variability may indicate that the quality of *Pistacia* as a host varies among years, but we do not really have any real indication as to its basis.

Geography is clearly one of the major influences on the distribution of the insect herbivore fauna of Al Jabal Al Akhdar in the Mediterranean ecosystem. The number of recorded species broadly increases with elevation, while middle elevations had the greatest overall insect abundances. Insect damage to plants also increased with elevation. In contrast, the two commonest species had their greatest abundances at the highest (*Xylomeira*) and the lowest (*Oedopoda*) elevations. Herbivore pressure has usually been found to be higher at lower elevations, from studies of insect herbivore abundance along elevational gradients in both tropical and temperate zones (Fernandes & Price, 1988). We have no data on the relative frequencies of mammalian herbivores in the study area.

These patterns might well also reflect differences in abiotic conditions with elevation. Explaining patterns in insect herbivore pressure with elevation was not the focus of this thesis, and therefore no special sampling of possible explanatory variables was undertaken. With rising elevation, there is an increase in radiation intensity, soils are usually more exposed and shallower, and have reduced nutrient accessibility and lower moisture-retaining ability (Sarmiento, 1986). Thus there might be a gradient of decreasing water and/or nutrient accessibility, coupled with increasing photosynthesis: plants at higher elevations should create an excess of carbon (Mattson, 1980) and hence the concentration of secondary compounds containing carbon, such as tannins, should rise at higher elevations (Bryant et al. 1983; Sarmiento, 1986).

The relative abundances of the two trees were broadly similar in most sites (see Chapter 2). Geographically widespread species of plant tend to have more species of insects feeding on them than similar but less widespread species, a fact first recognized by Southwood (1984). There are three general mechanisms thought to underlie the species-area relationship between insects and their host plants. Widespread species of plants occur in more habitats and over a broader range of climatic regions than uncommon plants. Therefore diverse species of insects are found in different parts of the ranges of widespread plants. Secondly, widespread plants present more conspicuous ‘targets’ for colonizing organisms. Thirdly, small populations on plants with limited ranges may be more prone to extinction.

Herbivorous insect diversity is also impacted by plant architecture, the size, growth form and diversity of feeding niches on the plant. Therefore, area for area, trees have more herbivores species than bushes, which in turn have more than herbs.

Several other plant traits are known to affect the diversity of insect herbivores: for example, plant biochemistry, taxonomic affinity and local richness.

If herbivore pressure really does increase with elevation in Al Jabal Al Akhtar, then we might predict that defence levels might mirror it. Thus we might predict increasing levels of tannin with elevation. This is the subject of the next chapter.

3.5 References

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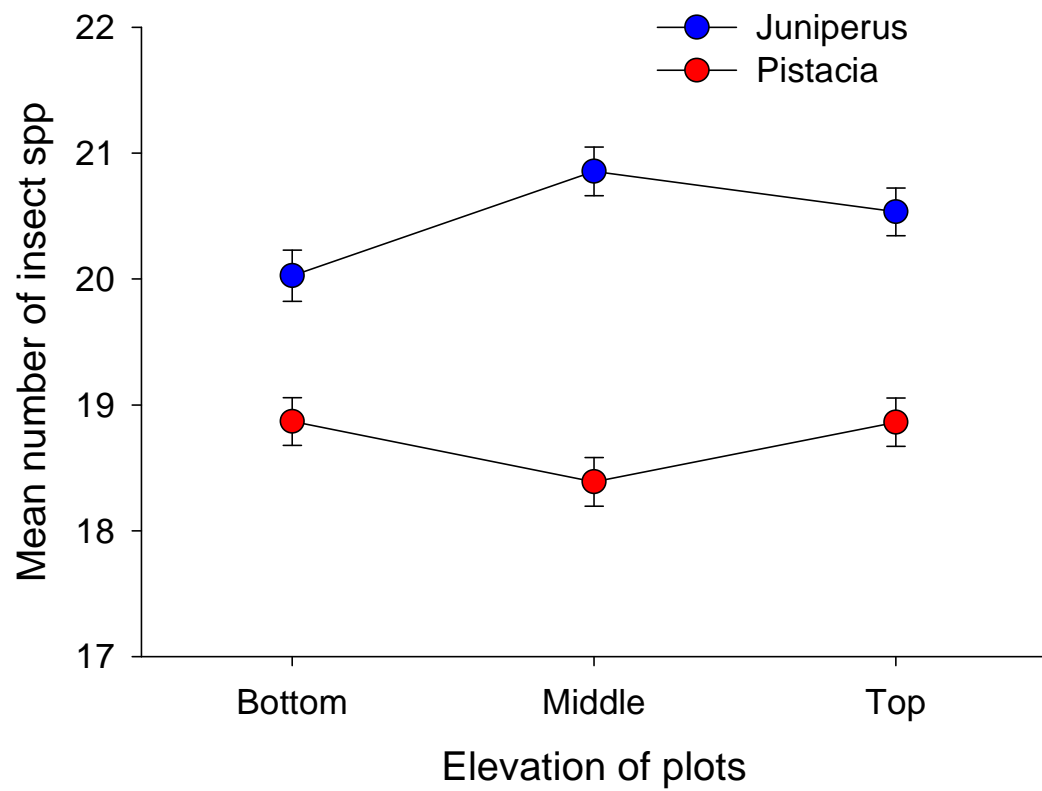


Figure 3-1: Average number (\pm s.e.) of insect species discovered on marked trees of *Juniperus* and *Pistacia* in permanent plots at three different elevations in the Al Jabal Al Akhdar study site

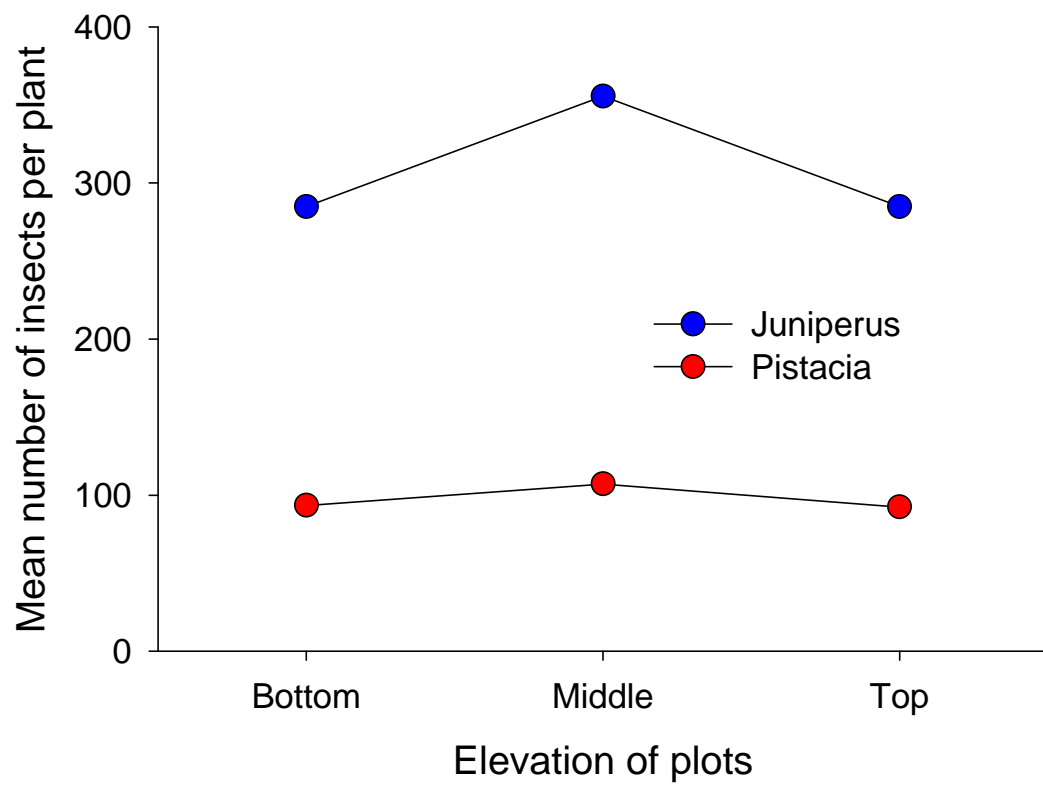


Figure 3-2: Average number (\pm s.e.) of individual insects discovered on marked trees of *Juniperus* and *Pistacia* in permanent plots at three different elevations in the Al Jabal Al Akhdar study site. The standard errors are too small to be seen.

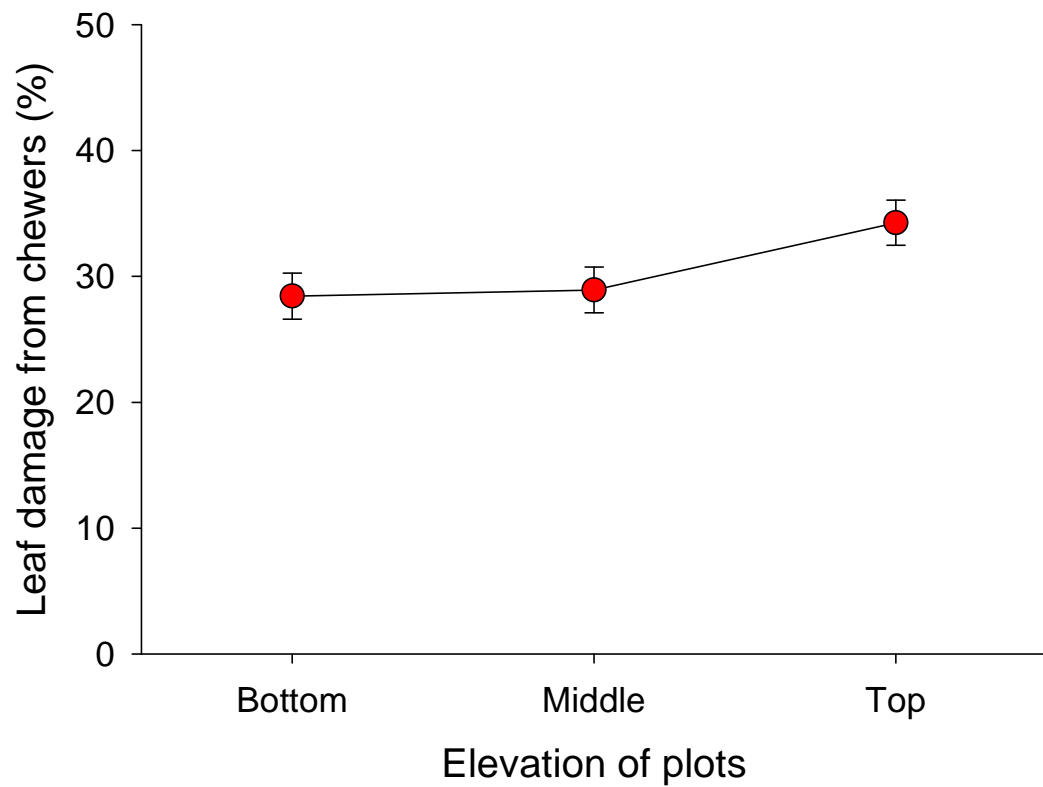


Figure 3-3: Average damage (\pm s.e.) from chewing insects (%) to leaves on marked trees of *Pistacia* in permanent plots at three different elevations in the Al Jabal Al Akhdar study site. the differences are significant (see Table 3.3)

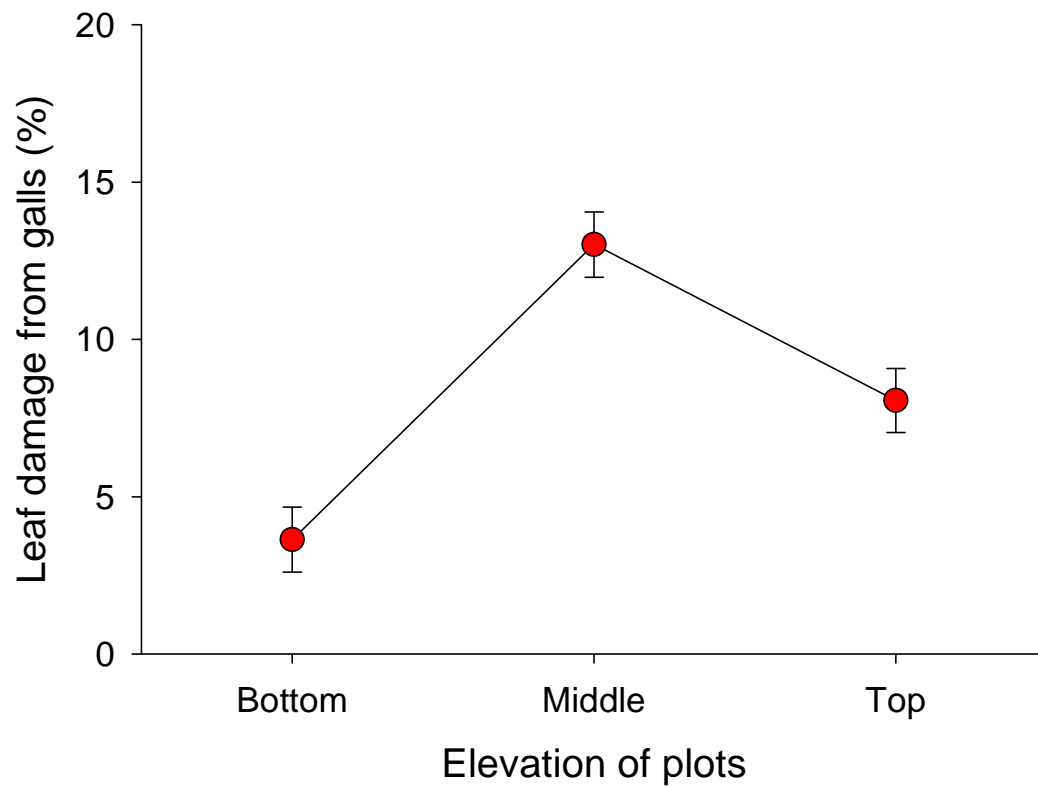


Figure 3-4: Average damage (%) (\pm s.e.) to leaves from insect galls on marked trees of *Pistacia* in permanent plots at three different elevations in the Al Jabal Al Akhdar study site. The differences are probably significant, but the data were not normal and could not be normalised by any transformation. Under ArcSine transformation, for example, there were differences among levels ($\chi^2 = 50.0$, $df = 2$, $p < 0.001$) but the residuals were still non-normal (Shapiro-Wilk = 0.931, $df = 45$, $p = 0.011$).

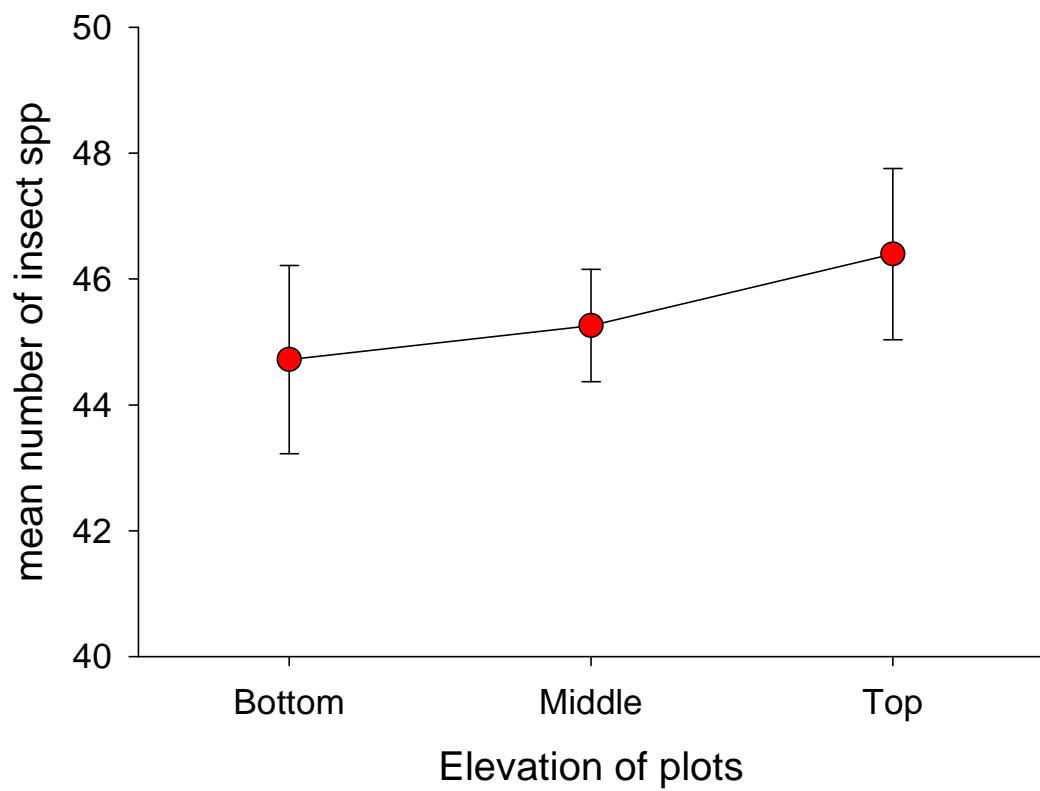


Figure 3-5: Average number (\pm s.e.) of insect species discovered on trees/shrubs in permanent plots at three different elevations in the Al Jabal Al Akhdar study site (2009 data). The differences are highly significant (Table 3.6)

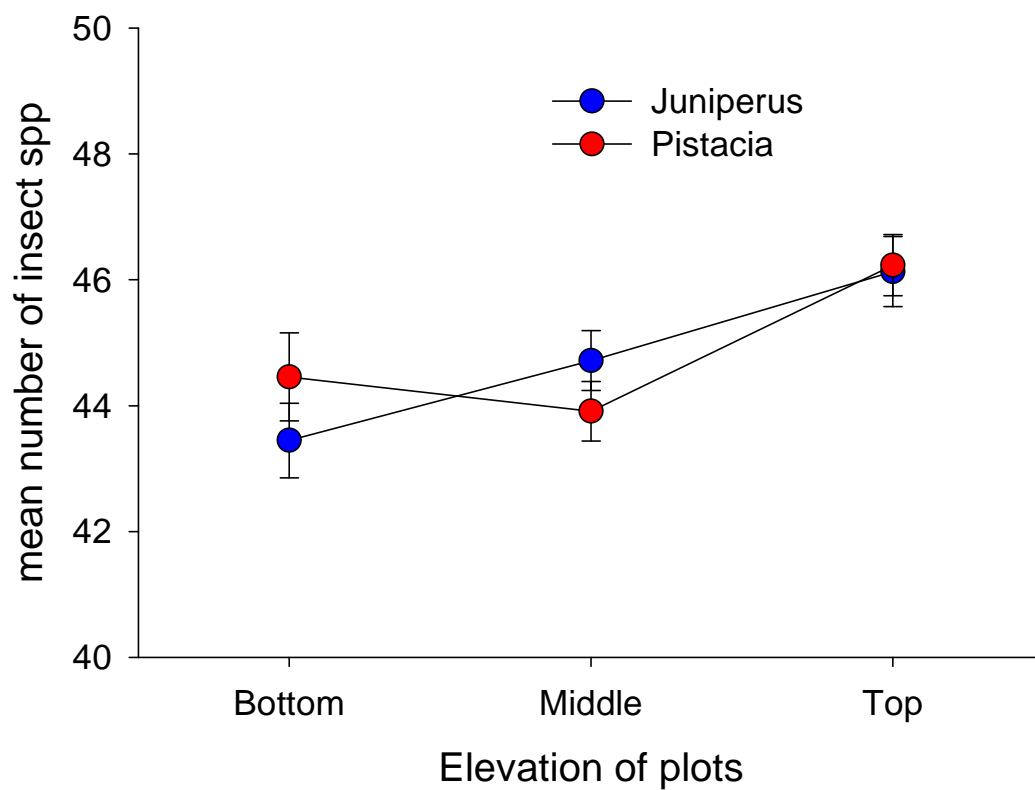


Figure 3-6: Average number (\pm s.e.) of insect species discovered on trees of *Juniperus* and *Pistacia* in permanent plots at three different elevations in the Al Jabal Al Akhdar study site (2009 data).

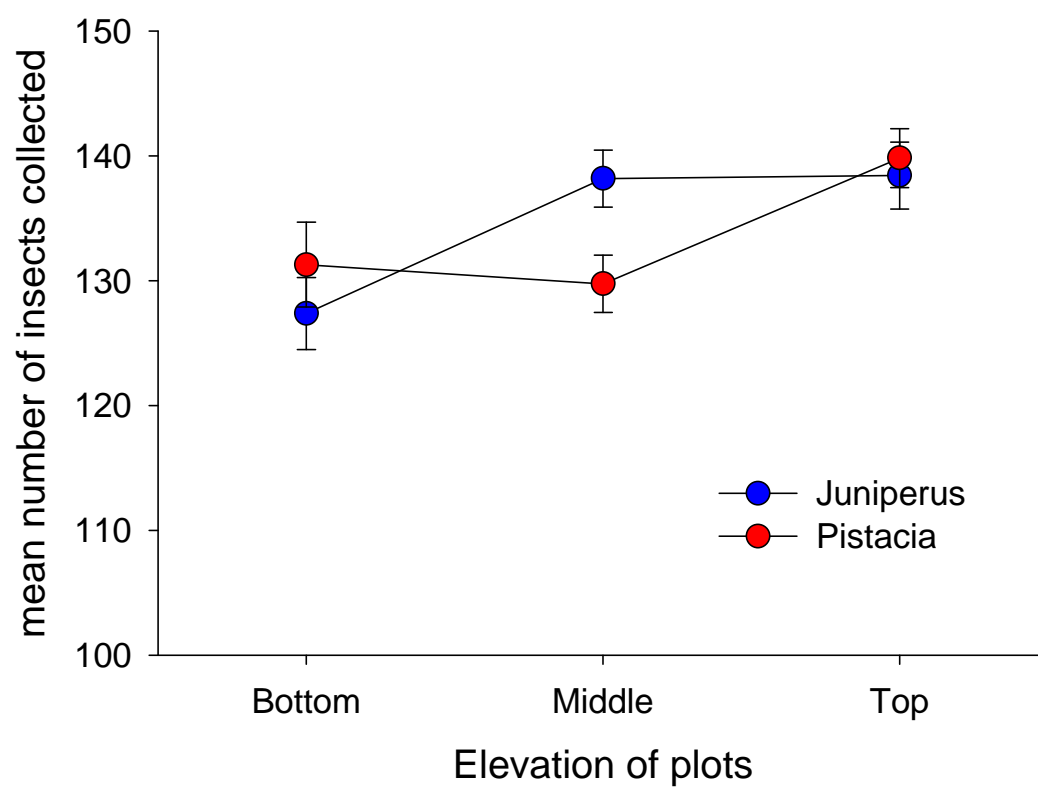


Figure 3-7: Average number (\pm s.e.) of individual insects discovered on trees of *Juniperus* and *Pistacia* in permanent plots at three different elevations in the Al Jabal Al Akhdar study site (2009 data).

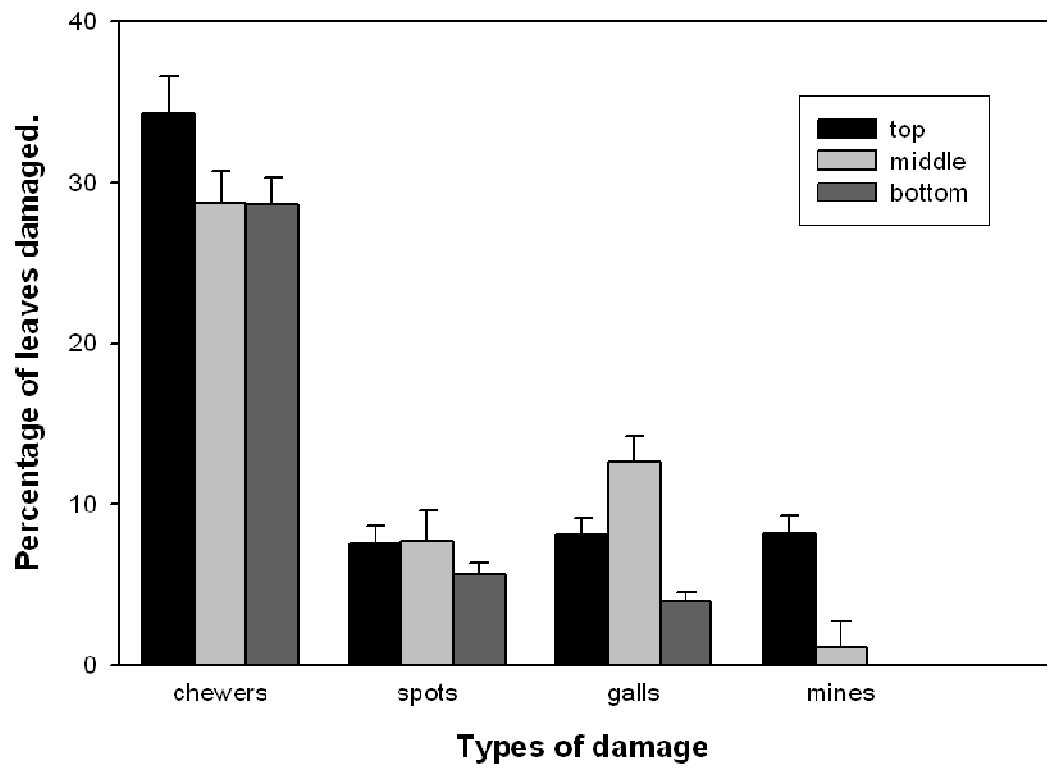


Figure 3-8: Patterns of insect damage on *Pistacia* leaves with elevation. Means (\pm s.e.)

3.6 Photos of insects



Figure 3-9 *Oedopoda caerulescens*



Figure 3-10 *Paracinipe (Acinipe) orientalis*♀



Figure 3-11 *Anacridium aegyptium*



Figure3- 12 *Agapanthia annular*
JCR 02/04/2005



Figure 3-13 *Macrotoma palmate*

JCR 02/04/2005



Figure 3-14 *Prinobius myardi*

JCR 02/04/2005

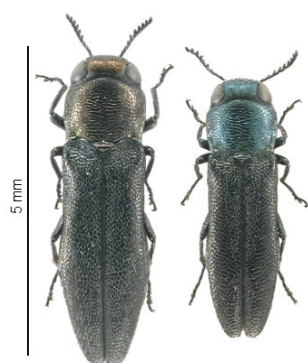


Figure3-15 *Agrilus grandiceps cyrenaicus*

JCR 02/04/2005

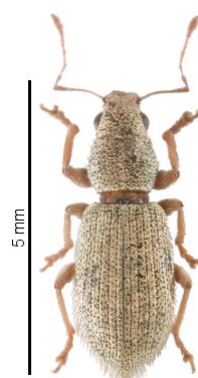


Figure 3-16 *Polydrusus (Conocetus) festae*

JCR 02/04/2005



Figure 3-17 *Prionobius myardi*

JCR 02/04/2005



Figure 3-18 *Phoracantha semipunctata*

JCR 02/04/2005



Figure3-19 *Hesperophanes sericeus*

JCR 02/04/2005



Figure 3-20 *Nanodiscus transversus*

JCR 02/04/2005

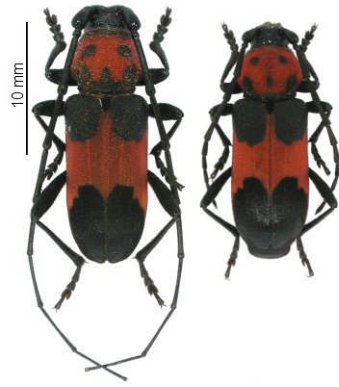


Figure3-21 *Purpuricenus desfontainii desfontainii*

JCR 02/04/2005



Figure3-22 *Icosium tomentosum*

JCR 02/04/2005

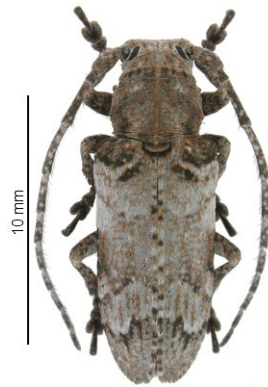


Figure3-23 *Niphona picticornis*

JCR 02/04/2005



Figure3-24 *Julodis aequinoctialis harterti*

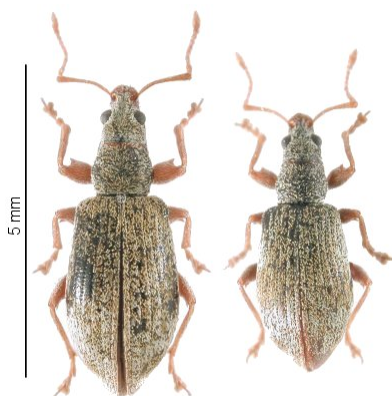


Figure3-25 *Phyllobius (Ectomogaster) festae*

JCR 02/04/2005



Figure3-26 *Caulostrophus ringenbachi*

JCR 02/04/2005



Figure3-27 *Stylosomus erigeti*



Figure 3-28 *Exochomus* sp

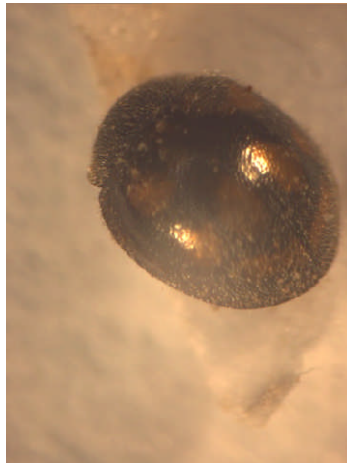


Figure3-29 *Nephus* sp



Figure 3-30 *Pharoscymnus* sp



Figure3- 31 *Olibrus* sp



Figure 3-32 *Aphodius* sp



Figure 3-33 *Xylomeira* sp



Figure 3-34 *Dasytes* sp



Figure 3-35 Dasytinae 1



Figure 3-36 Malachiinae 1



Figure 3-37 *Cirriphora pharaonana*



Figure 3- 38 *Edosa lardatella*



Figure 3-39 *Donacula forficella*



Figure 3-40 *Autographa gamma*



Figure 3-41 *Psalmocharias* sp



Figure 3-42 *Cicada barbara*



Figure3- 43 *Odontotarsus caudatus*



Figure3- 44 *Sciocoris* sp



Figure 3-45 *Eurydema* sp

Source	Type III		
	Wald Chi-Square	df	Sig.
species	124.965	1	<.001
level	1.700	2	.427
level * species	11.699	2	.003
plot(level)	17.568	6	.007
surface area	.132	1	.717

Table 3-1: Analysis of the number of insect species discovered on marked trees of *Juniperus* and *Pistacia* in permanent plots at three different elevations in the Al Jabal Al Akhdar study site. The model was fitted with normal errors.

Source	Type III		
	Wald Chi-Square	df	Sig.
species	5513.225	1	<.001
level	190.074	2	<.001
level * species	89.855	2	<.001
plot(level)	58.124	6	<.001
surface area	.163	1	.686

Table 3-2: Analysis of the number of individual insects discovered on marked trees of *Juniperus* and *Pistacia* in permanent plots at three different elevations in the Al Jabal Al Akhdar study site. The model was fitted with normal errors.

Source	Type III		
	Wald Chi-Square	df	Sig.
level	6.516	2	.038
plot(level)	26.522	6	<.001
surface area	.296	1	.586

Table 3-3: Analysis of the % leaf damage on leaves of marked *Pistacia* trees in permanent plots at three different elevations in the Al Jabal Al Akhdar study site. The model was fitted with normal errors, based on analysis of residuals.

INSECT SPECIES	<i>Juniperus</i>	Mean	±SEM	<i>Pistacia</i>	Mean	±SEM
<i>Xylomeira</i> sp	1328	1.50	0.083	90	0.101	0.026
<i>Agrilus (Xeragrilus)</i> sp	1168	1.32	0.076	62	0.069	0.026
<i>Anthia (Termophilum)</i> venator	1117	1.26	0.071	50	0.056	0.025
<i>Tropinota squalida pilosa</i>	1161	1.31	0.066	62	0.069	0.023
<i>Purpuricenus desfontainii desfontainii</i>	1240	1.40	0.057	116	0.130	0.020
<i>Niphona picticornis</i>	870	0.98	0.036	100	0.112	0.018
<i>Stromatium unicolor</i>	680	0.77	0.028	140	0.158	0.015
<i>Oedopoda caerulea</i>	434	0.49	0.026	341	0.384	0.011
<i>Paracnipe (Acnipe)</i> orientalis	402	0.45	0.027	373	0.420	0.011
<i>Scintharista notabilis</i>	360	0.41	0.028	337	0.380	0.009
<i>Autographa gamma</i>	361	0.42	0.026	325	0.366	0.008
<i>Vanessa cardui</i>	399	0.45	0.024	303	0.341	0.007
<i>Maniola</i> sp	383	0.43	0.023	310	0.349	0.007
<i>Cicadellidae: leaf hopper</i>	436	0.49	0.017	263	0.296	0.006
<i>Cicada barbara</i>	520	0.58	0.011	250	0.282	0.006
<i>Psalmocharias</i> sp	540	0.61	0.009	291	0.328	0.007
<i>Anoplocerus</i> sp	624	0.71	0.004	269	0.303	0.005
<i>Bothrostethus</i> sp	621	0.70	0.005	254	0.286	0.004
<i>Latilica</i> sp	590	0.66	0.006	217	0.244	0.003
<i>Orsillus</i> sp	627	0.71	0.083	234	0.264	0.026

Table 3-4: The commonest insect species collected in association with the marked trees/shrubs in the study plots. 45 individual plants of each species were sampled by sweeping and beating. Some species were clearly not herbivores of either plant.

level	species	Mean	Std. Error	N
Bottom	<i>Ceratonia</i>	43.635	2.597	12
	<i>Juniperus</i>	43.446	.594	257
	<i>Olea</i>	46.665	6.307	2
	<i>Periploca</i>	46.010	4.467	4
	<i>Phillyrea</i>	44.102	3.650	6
	<i>Pistacia</i>	44.457	.700	165
Middle	<i>Calicotome</i>	45.872	1.233	54
	<i>Ceratonia</i>	48.216	2.979	9
	<i>Juniperus</i>	44.716	.474	358
	<i>Olea</i>	41.592	3.452	7
	<i>Phlomis</i>	44.410	3.398	7
	<i>Pistacia</i>	43.912	.472	382
	<i>Sarcopoterium</i>	48.106	2.238	16
Top	<i>Arbutus</i>	46.826	4.000	5
	<i>Juniperus</i>	46.131	.555	269
	<i>Pistacia</i>	46.230	.485	345

Table 3-5: The mean number of insect species collected in association with the trees/shrubs in the study plots, sampled by sweeping and beating in 2009.

Tests of Model Effects			
Source	Type III		
	Wald Chi-Square	df	Sig.
species	4.282	9	.892
level	19.816	2	<.001
level * species	4.091	4	.394
plot(level)	116.672	6	<.001
surface area	.022	1	.881

Table 3-6: Analysis of the mean number of insect species collected in association with the trees/shrubs in the study plots.

level	species	Mean	Std. Error
Bottom	<i>Ceratonia</i>	115.280	12.521
	<i>Juniperus</i>	127.564	2.864
	<i>Olea</i>	126.411	30.407
	<i>Periploca</i>	122.230	21.537
	<i>Phillyrea</i>	134.835	17.596
	<i>Pistacia</i>	131.282	3.376
Middle	<i>Calicotome</i>	134.260	5.945
	<i>Ceratonia</i>	147.037	14.360
	<i>Juniperus</i>	138.107	2.285
	<i>Olea</i>	120.709	16.641
	<i>Phlomis</i>	116.954	16.382
	<i>Pistacia</i>	129.604	2.276
	<i>Sarcopoterium</i>	142.339	10.790
Top	<i>Arbutus</i>	143.004	19.283
	<i>Juniperus</i>	138.400	2.674
	<i>Pistacia</i>	139.745	2.337

Table 3-7: The mean number of individual insects collected in association with the trees/shrubs in the study plots, sampled by sweeping and beating in 2009.

Tests of Model Effects			
Source	Type III		
	Wald Chi-Square	df	Sig.
species	2.460	9	.982
level	11.497	2	.003
level * species	8.735	4	.068
plot(level)	84.635	6	.000
surface area	.289	1	.591

Table 3-8: Analysis of the mean number of individual insects collected in association with the trees/shrubs in the study plots.

Tests of Model Effects

Source	Type III		
	Wald Chi-Square	df	Sig.
(Intercept)	.452	1	.501
level	8.293	2	.016
plot#(level)	26.115	6	.000
No. individuals on the nearest conspecifics	6.438	1	.011
No. individual on the nearest allospecific	30.741	1	<.001
No. individual on all <i>Juniperus</i> within 2 m	5.744	1	.017

Table 3-9: Analysis of the mean number of *Xylomeira* sp collected in association with the *Juniperus* sp in the study plots.

Tests of Model Effects

Source	Type III		
	Wald Chi-Square	df	Sig.
(Intercept)	2.805	1	.094
level	10.702	2	.005
plot#(level)	43.008	6	<.001
No. individuals on the nearest conspecifics	9.493	1	.002
No. individual on the nearest allospecific	35.968	1	<.001
No. individual on all <i>Juniperus</i> within 2 m	17.542	1	<.001

Table 3-10: Analysis of the mean number of *Oedopoda caerulescens* collected in association with the *Juniperus* sp in the study plots.

Chapter 4: Chemical Defences of *Juniperus* and *Pistacia* against Herbivores

4.1 Introduction

In the general introduction I introduced the effects and evolution of chemical defences. In this chapter I measure levels of one type of chemical defence, the tannins. The prediction from the pattern of herbivore pressure of the previous chapter is that tannin levels should increase with elevation, mirroring attack by herbivores.

Plants in natural populations are frequently attacked by a huge number of natural enemies, comprising both pathogens and herbivores at all periods of their life cycles. Pest stress can be devastating, as demonstrated by the epidemics of insects and diseases in farmed crops, and in the success of weed control by biological control. Outbreaks or episodes of intensive herbivory may lead straight to the local extinction of a plant species, or weaken plants and make them vulnerable to subsequent ecological pressures. The lowering of photosynthetic ability due to disease or partial defoliation reduces resistance to biotic stresses or to infection by other pests, and decreases competitive capacity (Levin 1976).

Mediterranean climate regions experience reducing rainfall with decreasing latitude towards the equator, and there is a gradual change in vegetation toward plants that are adapted to semi-arid and desert conditions (Dallman 1998). The local vegetation of Mediterranean lands is adapted to survive long, hot summer droughts and prolonged wet periods in winter (Martinez 2002). As a result, the amount of energy allocated by Mediterranean plants to growth and maintenance is quite high.

Low resource accessibility may either be a cue for a plastic response that leads to, or a selection pressure for the evolution of, a greater investment in protection against biotic agents, that is shown by greater investment in defensive chemicals such as phenols, alkaloids, etc. Lack of water might also be involved; perhaps by restricting growth rate, this might also lead to the accumulation of defence compounds for greater resistance against natural enemies. Therefore, the cost of producing chemical defences might be predicted to be higher in Mediterranean species than in those living in more favourable biomes. Mediterranean habitats vary in a number of characteristics; if these include nutrient and water availability, we might predict differences in the chemical defences of Mediterranean plant species. Thus plants at different elevations should invest different amounts in defence because of the availability of water and nutrients (Martinez et al. 2002).

Plants are dynamic systems that show qualitative and quantitative chemical and structural variation over space and time as a result of genetics and changes related to growth, reproduction, senescence, climate and consumers (Crawley 1983; Hartley & Jones 1997). Herbivory can induce chemical and structural changes in plants which decrease the risk of further herbivore attack (Karban & Baldwin 1997). Chemical changes often involve an increase in secondary metabolites that act as feeding deterrents, reduce plant digestibility or are toxic (Karban & Myers 1989). Morphological changes following herbivory include the development of thorns and hairs, and the modification of leaf size and branch density (Massei et al. 2000).

The theory of optimal plant defence suggests that plants ought to have maximal investment in defences when and where herbivory is most probable to arise (Coley et al. 1985; Bryant et al. 1991). Thus the new, highly nutritious leaves that are the main objective for herbivore foraging ought to have higher concentrations of chemical

defences than elderly leaves (Bryant et al. 1991; Massey et al. 2000). In winter, when browsing is more intensive due to the poor availability of annual plants (Bruno & Apollonio 1991; Johnson et al. 1995), leaves of evergreen plants must have higher levels of chemical defences than in spring (Massey et al. 2000). In the Mediterranean, evergreen species tend to be associated with specialist herbivores (Chabot & Hicks 1982; Kikuzawa 1991).

Mediterranean plants have experienced high levels of herbivory by wild and domesticated ungulates over thousands of years (Massey et al. 2000); we might expect therefore the existence of evolved interactions with mammals, but actually very little attention has been paid to plant-mammal interactions in Mediterranean ecosystems. There are various bits of evidence about both natural levels and herbivore-induced variation in secondary compounds in Mediterranean plants (Massey et al. 2000), and Perevolotsky (1994) thought that the levels of tannins were constitutive rather than induced by browsing. Evergreen sclerophylls predominate (Dafni 1991; Meletiou-Christou et al. 1994), characterized by slow growth, slow leaf turnover, and high concentrations of carbon-based secondary compounds such as phenolics and tannins (Bryant et al. 1992; Massey et al. 2000). Phenolic concentrations were consistently higher in winter than in spring, and in new rather than old leaves, just when browsing is more probable (Glyphis & Puttick 1988; Massey et al. 2000). These compounds reduce the palatability of plants to mammalian herbivores, but there is no evidence that they are induced by browsing (Massey et al. 2000; Rohner & Ward 1997), although regrowth foliage following browsing may have decreased levels of phenolics in other ecosystems (Bryant et al. 1991, 1992). In addition to changes in chemical composition, browsed plants might have altered shoot or leaf sizes compared to undamaged plants (Massey et al. 2000; Danell et al.

1994). Plant species in the same community frequently vary greatly in their survival rates, presumably due to differences in their specific group of traits for coping with herbivory and other environmental conditions (Metz et al. 2010). Herbivory varies in its effects between seasons and locations (Rohner & Ward 1997).

Glyphis & Puttick (1988) showed that levels of phenolic compounds in shrub leaves are higher in winter than spring, and in new than older leaves, in only half of the species they checked. In contrast, Dement & Mooney (1974) found lower concentrations of carbon-based compounds in new than in older leaves. Mediterranean woody evergreens should have strong constitutive defences relative to deciduous species because they store more nutrients and carbon in their leaves, and hence herbivory is more damaging to them (Herms & Mattson 1992; Bryant et al. 1992). The leaves of *Quercus* spp. are high in phenolic compounds (Wold & Marquis 1997; Massei et al. 2000), but Massei et al. (2000) established that *Pistacia* had still higher concentration of phenolics, while *Olea* and *Phillyrea* had much lower concentrations. Ungulates attacked *Olea* and *Phillyrea* more heavily than *Quercus* and *Pistacia* (Massei et al. 2000).

Ungulate preferences among plants may be affected by a variety of other factors, including accessibility and convenience. Moreover, the levels of phenolics and other secondary metabolism are not the only chemical factors affecting ungulate preferences (Owen-Smith et al. 1993; Tixier et al. 1997). Invertebrate herbivores have been studied less in the Mediterranean context. An interesting experiment on *Poecilimon* (a phaneropterine cricket) feeding on *Arbutus* spp was carried out by Kouki & Manetas (2002). The soft young leaves were very high in phenolics and gallotannins, and crickets preferred the tough old leaves. As leaves aged, the concentration of these defensive chemicals reduced, the leaves became tough, and

the crickets switched to preferring younger leaves. Thus in this plant, leaf toughness was less important than leaf chemistry to the main herbivore.

The current study was carried out in the Al Jabal Al Akhdar region of Libya that consists of a high density of evergreen species of shrub and trees. We analysed variation among individual plants, among plots, and among elevations in the phenol levels of the two dominant species. The prediction is that there will be increases in phenol concentrations in *Juniperus* and *Pistacia* with elevation.

After testing this prediction, I will go on (in Chapter 5) to test the impact that the local neighbourhood of plants and plant chemistry (in the form of tannin levels) have on the density and distribution of insect herbivores on *Pistacia atlantica* and *Juniperus phoenicea*.

4.2 Materials and method

The study was carried in out in the Al Jabal Al Akhdar North Eastern of Libya (32°49'N; 21°51'E). The climate is Mediterranean: mean temperatures vary between 25 °C in February and 33 °C in July and rainfall ranges between 520 and 600 mm per year (Faituri 2001). The study area is dominated by *Juniperus phoenicea* and *Pistacia atlantica*, the two species selected for study. Five plants of each species were selected randomly and marked permanently in each of nine plots at three different elevations (bottom 0-200 m, middle 200-400 m, and top 400-880 m above sea level). At each elevation, three plots were placed randomly subject to the proviso that each was more than 1 km away from the others. Ten fresh leaves per shrub/tree were collected in May-June 2008 from each marked plant: *Juniperus* ($n = 45$) and

Pistacia ($n = 45$). Leaves were oven-dried at 60 °C and milled at Omar Al Mukhtar University laboratory (Libya) before analysis in Nottingham (UK).

Leaf samples were analysed for total phenolics measured by detecting phenolate ions. Tannic acid was used as a standard and extraction of dried leaf material was carried out using 50% aqueous methanol. Phenolic content was expressed as % dry weight (calculated as mg tannic acid equivalents per mg dry leaf mass $\times 100$). The phenolic assay was conducted according to the Folin-Ciocalteu method (Waterman & Mole, 1994). The protocol followed for this study was as follows. A standardised phenolic solution was prepared with 10 ± 0.3 mg tannic acid in 100 ml water. The precise amount used was recorded for use in later calculations. A 'blank' 4.2 ml cuvette was prepared with 3 ml DI water. A series of cuvettes were prepared with 50-450 μ l tannic acid solution in 50 μ l increments and made up to 3 ml with DI water, and used to produce a standard line of tannic acid concentration by regression. For each sample to be analysed, 15 ± 0.3 mg of ground leaf material was taken and the precise amount used recorded. Each sample was shaken with 10 ml of 50% methanol solution and phenolics extracted by standing in a sealed boiling tube in a water bath at 80°C for 30 minutes. Samples were immediately transferred to centrifuge tubes and spun at 3000 rpm for 15 mins, and 100 μ l of each sample was placed in two cuvettes. These were made up to 3 ml with DI water and 0.25 ml saturated CaCO_3 solution and 0.25 ml Folin-Ciocalteu reagents added to each cuvette. The cuvettes were covered in parafilm and mixed, then left to stand. After 1 hr the absorbance at 760 nm was measured for each cuvette against the blank prepared earlier. The mean value between the two replicates of each sample was taken. If the difference in absorbance between the two samples exceeded 0.05 the entire run was repeated, although in practice the two values were usually very close.

The percentage dry mass phenolic (%DM) in each sample was calculated by comparison with the standard line according to the formula:

$$\%DM = \frac{TA}{W} \times \left[\frac{A760 - c}{m} \right]$$

Where TA is the amount of tannic acid used in mg, W is the initial sample mass in mg, A760 is the mean absorbance for each sample and m and c are the gradient and y-intercept of the standard line. Standard lines were produced at the start and end of each day to confirm the consistency of measurements. Results are presented here as the parts per million of leaf dry weight that is made up of phenolics (or, more strictly, tannic acid equivalents).

We analyzed the phenolic content in the two species using GLM implemented in SPSS16. The design of the analysis was very similar to that of Chapter 3, i.e. *species + level + plot (level) + species*level + plant volume*. The data were not normal (Shapiro-Wilk = 0.955, df = 90, P = 0.001), and no transformation would normalise them. However, results were similar whatever the transformation, and hence we believe them to be robust to the deviations from normality.

4.3 Results

The two species differed greatly in their levels of phenols: *Pistacia* had the highest concentrations (overall 2308 ± 43 ppm, n = 45), with *Juniperus* quite a bit lower (1516 ± 43 , n = 45). Overall levels of phenols were highest in plants from the middle and lowest in those from the bottom elevational level (Figure 4-1): this effect of level was significant, but there was no species * level interaction, showing that the two species showed the same pattern with elevation (Table 4-1). There were

substantial differences among plots within levels, but no effect of plant volume (Table 4-1).

4.4 Discussion

The vegetation of these Mediterranean plant communities usually consists of 20–25 shrub species, but typically only six or seven species are dominant (e.g. *Quercus ilex*, *Erica multiflora*, *Arbutus unedo*, *Juniperus phoenicea*, *Viburnum tinus*, *Pistacia lentiscus* and *Hedera helix*: Rogosic et al., 2006). Utilization of Mediterranean shrubs by herbivores is thought to be limited by secondary compounds such as tannins, terpenes, and saponins (Perevolotsky et al., 1993).

The pattern of tannin concentration with elevation was the same in both species, peaking at mid-elevations. Unlike our prediction, this does not follow the pattern of insect damage (Chapter 3, highest at top elevations), but it does follow the pattern of insect herbivore density (Chapter 3). The study was not designed to test such a relationship, and with $n=3$ elevations it is not possible to say very much about the likelihood of tannin concentrations reflecting herbivore densities.

There was a clear difference in tannin concentration between *Juniperus* (low) and *Pistacia* (high), consistent across all plots and elevations. Although there may indeed be spatial and/or temporal differences in the defensive chemistry of plants in response to the risk of herbivory (Coley et al. 1985; Wold & Marquis 1997), attempts to document such effects in Mediterranean areas have not been very successful (e.g. Glyphis & Puttick 1988; Dement & Mooney 1974). Perevolotsky (1994) could not find any seasonal or age-related differences in the concentration of tannins in a variety of Mediterranean species, including *Pistacia*, *Phillyrea* and *Quercus*. Dafni (1991) and Perevolotsky (1994) thought that constitutive were more likely than

inducible defences in the Mediterranean. Although our study was not designed explicitly to test this, we observed a constancy of defence chemical differences for these two Mediterranean plants over a wide range of environmental conditions.

Mediterranean woody evergreens are predicted to have strong primary (constitutive) defences because, in contrast to deciduous woody plants, they keep more nutrients and carbon in their leaves: herbivory is therefore more damaging to evergreens than to deciduous plants (Herms & Mattson 1992; Bryant et al. 1992). Several studies have shown that the leaves of *Quercus* spp. are high in phenolic compounds (see Wold & Marquis 1997), and we found that *Pistacia* had even higher concentrations of phenolics than reported for *Quercus*, whilst *Juniperus* had much lower concentrations. Some studies seem to show that nitrogen content varies with season and leaf age but not among species (Dement & Mooney 1974; Meletioui-Christou et al. 1994).

Concentrated study of the defence chemistry of Mediterranean plants is clearly necessary before generalizations can be made. It is surprising that such studies have not been carried out already. What we do know is that phenolic compounds are widely distributed in many species of plants, where they play a role in protection from predation, and perhaps also in growth regulation (Bate-Smith & Swain 1962). Our study was not designed specifically to look at the role of species differences in defence chemistry, but to concentrate on the possibility of neighbourhood effects. We turn to these in the next chapter.

4.5 References

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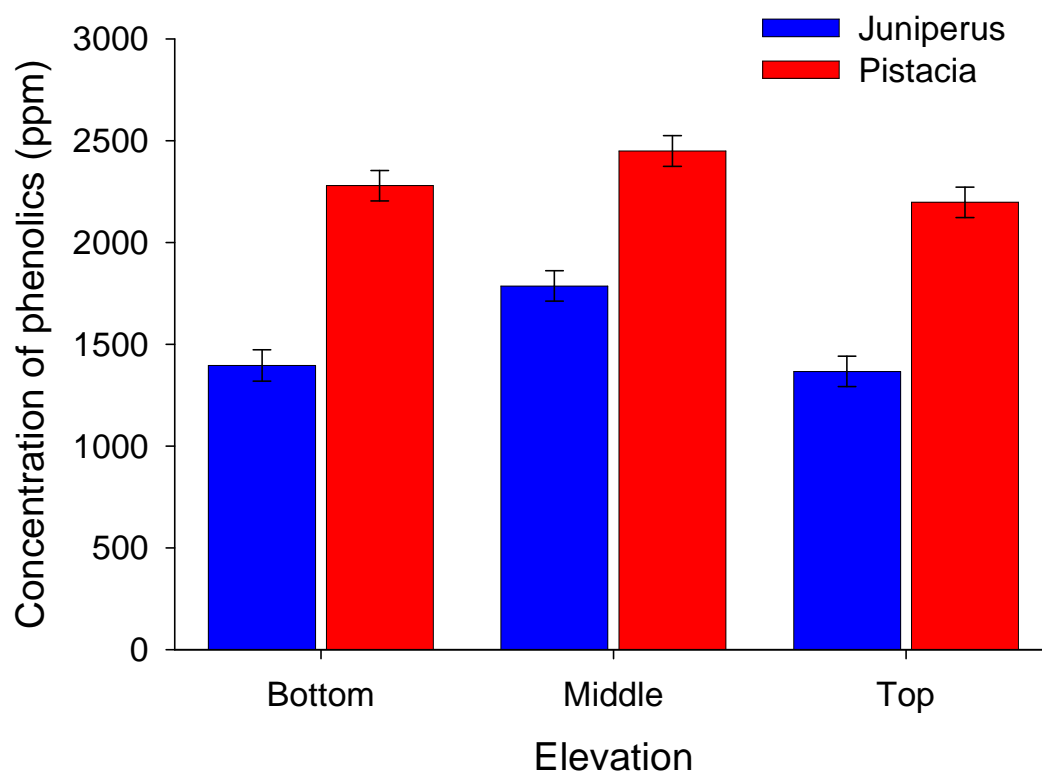


Figure 4-1: Phenolic content (parts per million) of leaves of *Juniperus phoenicea* and *Pistacia atlantica* shrubs/trees in the Al Jabal Al Akhdar region of Libya at three different elevational levels Means (\pm s.e.)

Tests of Model Effects			
Source	Type III		
	Wald Chi-Square	df	Sig.
species	165.616	1	<.001
level	21.924	2	<.001
plot(level)	33.589	6	<.001
level * species	2.371	2	.306
plant volume	.002	1	.960

Table 4-1: Analysis of variation in the total phenolic content of *Juniperus* and *Pistacia* at three elevational levels in the Al Jabal Al Akhdar region of Libya

Chapter 5: The effect of the local plant neighbourhood on insect herbivory

5.1 Introduction

Neighbouring plants in the locale of an individual plant may help or hinder it in the task of defence against herbivores, depending on their levels of defensive chemistry and their interactions with potential herbivores. This ‘associational resistance’, the role of nearby plants in the probability that an individual plant will be discovered or be susceptible to herbivory, was already a topic for study in the 1960s (e.g. Root and colleagues: Barbosa et al. 2007). Atsatt & O’Dowd (1976) argued that associational resistance was all about the right kind of local plants, because not all plant species change the rates of discovery by and/or susceptibility to herbivores. Neighbouring plants conferring associational resistance can be conspecifics that differ in some relevant property, or individuals of dissimilar species (Atsatt & O’Dowd 1976; Wada et al. 2000; Karban & Maron 2002; Barbosa et al. 2009). Associational resistance and susceptibility have been confirmed in terrestrial plants grazed by vertebrate (e.g. voles and hares: Hjältén et al. 1993) and invertebrate herbivores (e.g. pest Diptera: Finch et al. 2003), and also in marine grazing systems such as algae, epiphytes and phytoplankton (Barbosa et al. 2009). Associational resistance is clearly an important ecological interaction, as may be associational susceptibility, although this has been less often described. Much of what is outlined here comes from an excellent recent review by Barbosa et al. (2009).

The effects of association form part of the lexicon of terms for basic ecological associations, and can in some sense be regarded as special cases of more general terms. Associational resistance and susceptibility are similar to facilitation

and epibiosis. In associational resistance, the result of the interaction is positive for a study plant in that it escapes discovery or some damage by herbivores; the result is negative in associational susceptibility (Barbosa et al. 2009). Epibiosis is where one (epiphytic) creature survives connected to another, advantaged or damaged as influenced by the substrate species (Wahl & Hay 1995). Facilitation is an association among species advantageous to at least one of the species concerned (Stachowicz 2001). Consequently, some facilitation interactions can be explained as associational resistance if the advantage involves release from herbivory (Callaway 1995); and some epibiotic interactions can be interpreted in the same way (Wahl & Hay 1995). Like any indirect interaction, associational resistance and susceptibility depend on differential responses of herbivores or their natural enemies to plant characteristics as modified by a neighbouring plant (Barbosa et al. 2009).

We need to understand the mechanisms underlying associational resistance and susceptibility, but at the moment most of our data are anecdotal. The few tests of mechanisms do not permit a meta-analysis (Barbosa et al. 2009). The mechanisms may be abiotic or biotic.

Obviously, there are lots of abiotic factors affecting the probability of discovery by herbivore, or plant susceptibility to herbivores. Neighbouring plants compete for access to soil macronutrients, and the outcome of this competition is likely to affect the pool of nutrients to be allocated to major functions such as defence or growth, with implications for herbivores. For instance, defoliation of *Trifolium repens* increases nitrogen transfer to neighbouring ryegrass *Lolium perenne* (Ayres et al. 2007), and growing near nitrogen-fixing legume plants in general probably improves access to this critical nutrient even if only after the death of the legume (Van Ruijven & Berendse 2005). Herbivory itself can increase soil quality,

for example by frass deposition (Frost & Hunter 2007), leading to changes the defences of neighbours. Even alterations in microclimate caused by neighbouring plants can impact the behaviour (e.g. ability to discover hosts) and ecology (e.g. egg loads and survival) of herbivores and their natural enemies. Each or all of these factors could affect associational resistance or susceptibility (Barbosa et al. 2009).

Biotic mechanisms comprise (Barbosa et al. 2009) a set of influences on herbivores finding and attacking a focal plant (or indeed their natural enemies): (a) the traits of neighbouring plants; (b) plant-plant competition; and (c) simple differences in the size and relative abundances of plant species. By far the majority of studies have concentrated on the role of plant traits.

(a) Plant traits

A key role of neighbouring plants in generating associational resistance is probably their ability to disguise (Perrin & Phillips 1978), confuse, or obstruct herbivores in detecting and finding particular hostplants (Finch et al. 2003). Avoiding discovery by herbivores is a very efficient mechanism of associational resistance, occurring via pre-contact signals such as visual appearance or plant volatiles (Toth 2007), or post-contact dispersal reactions caused by detecting deterrents or anti-feedants on neighbouring plants.

Optical signals from neighbouring plants are known to be capable of interrupting colonization by herbivores. The physical structure of neighbouring plants can shield focal plants from view, interfere with foraging responses (Holmes & Barrett 1997) or repel herbivores (Marquis et al. 2002). *Diabrotica balteata* beetles tend to emigrate more from monocultures that include a non-host plant; pure

monocultures encourage area-restricted foraging, making herbivores less likely to depart a patch (Holmes & Barrett 1997). Leaf colour of neighbours disturbs host finding by the cabbage root fly *Erioischia brassicae* (Finch et al. 2003). Chemical volatiles also act pre-contact to affect discovery rates because they can act as indicators of toxins, even at very low concentrations (Nauen 1995). Such mechanisms repelling herbivores could lead to long- or short-distance dispersal, impacting either distant or neighbouring plants respectively (Potting et al. 2005).

Neighbouring plants can also work as decoys to attract and hold herbivores (Holmes & Barrett 1997), decreasing colonization and feeding on focal plants, enabling vulnerable stages to escape. This concept is well known in agriculture: for example, sorghum trap-crops interrupt stinkbug colonization of cotton fields (Tillman 2006). Decoy plants can lead to associational susceptibility of nearby focal plants: for instance, *Opuntia corallicola* growing near *Opuntia stricta* gets attacked and killed by *Cactoblastis cactorum* (Stiling et al. 2004).

We know that herbivore damage can create volatiles, mainly methyl jasmonate, that activate defences in neighbouring plants, conspecific or allospecific (Barbosa et al. 2009). Though controversial (Dicke et al. 2003), plants clearly eavesdrop on one another, an efficient mechanism of associational resistance via its effects on the behaviour and survival of herbivores (Karban & Maron 2002) and their natural enemies. For instance, undamaged wheat saplings attract aphids, while odours released from aphid-infested wheat seedlings keep away aphids, benefitting adjacent plants (Quiroz et al. 1997). Volatiles of neighbouring plants may (Engelberth et al. 2004) or may not (Paschold et al. 2006) prime or cause increases in defence chemicals in focal plants: in *Artemisia tridentata*, jasmonates are not enough to increase chemical defences, but prime them so that they respond to damage more

quickly. This can happen in both conspecific (Heil & Kost 2006) and unrelated plants (Kessler et al. 2006), but only over short distances, such as a few metres (Dolch & Tschardt 2000) to less than a metre (Karban et al. 2006), especially short for allospecific plants (Karban 2001; Karban et al. 2004, 2006). Indirect defences are also implicated in this kind of response: for example, volatiles resembling those from herbivore-infested bean plants led to an increase in floral nectar, a supplement for natural enemies such as ants (Kost & Heil 2006). Volatiles from maize plants attract the herbivore parasitoid *Cotesia marginiventris* (Ton et al. 2007). Relatively little work has been done on associational resistance or susceptibility caused by the attraction of natural enemies to volatiles of damaged or undamaged neighbouring plants (Barbosa et al. 2009).

Inter-plant communication is not just via airborne signals: underground linkages may also be involved, either physically (such as in plant-parasitic plants: Adler 2000; Marvier 1998; Schädler et al. 2005) or via endophytic mycotoxins (Lehtonen et al. 2005) or root exudates (Barbosa et al. 2009).

(b) Plant competition

Competition for resources between neighbouring plants is a key determinant of survival, growth, and size, acting through resource availability and allocation. Consequently it may also affect herbivory and associational resistance or susceptibility (Agrawal 2004, Agrawal et al. 2006) by changing the quantity or quality of anti-herbivore defences and associated chemicals (Barbosa et al. 2009). However, there are vanishingly few demonstrations that competition leads to associational resistance. It is easy to demonstrate effects of competition on some plant traits, clearly, but the causal links to herbivore resistance have not been made.

It is plausible: increasing light reaching a plant (as caused by herbivory on neighbours) is known to improve levels of cardenolide defences in milkweed *Asclepias syriaca* (Agrawal & Van Zandt 2003).

Obviously, more experimental proof is required to address the conditions below which plant competition guides to associational resistance and associational susceptibility, if indeed it does. Herbivory and competition may be indivisible and interacting forces. Sorting out cause and effect relationships may be a daunting task (Hambäck & Beckerman 2003). Predicting whether an association among competing plants will result in associational resistance and associational susceptibility is a complex and specific phenomenon, based on the study plant and neighbouring plant species, the herbivore, and existing biotic and abiotic circumstances (Barbosa et al. 2009).

(c) Size and relative abundance

Simple differences in relative abundances of plants and herbivores might also form a mechanism driving associational resistance and susceptibility, which may have less to do with the traits of neighbours than their density. The greatest predictor of egg load of the weevil *Rhinocyllus conicus*, on *Cirsium undulatum* was the flowerhead density of the native *Cirsium canescens*, with egg load decreasing as the density of *Cirsium canescens* increased (Russell & Louda 2005). Plant species differ in size, and the number of herbivores is probably related to plant biomass or the occurrence of large food reserves. Thus associational resistance or susceptibility may be most influenced simply by the density and size of surrounding plants.

The aim of this final part of the study is to test whether there are any detectable effects of the local plant neighbourhood on insect herbivore pressure on focal plants.

To do this, we use GLMs with the response variable being the number of herbivore individuals/species on a plant, and predictors reflecting the chemical defence (tannins), composition and distances of neighbouring plants. We test each species (*Juniperus*, *Pistacia*) separately.

5.2 Method

The methods are given in full in Chapters 2-4. Briefly, the study area was the Al Jabal Al Akhdar (“Green Mountain”) region situated directly behind the coastal strip in the north-eastern region of Cyrenaica in Libya. We chose three plots in each of the three layers of elevation going from the coast to the high mountains: the coastal strip (“bottom”, 0 - 200 m); “middle” (200 - 400 m); and the high mountains (“top”, 400 - 880 m). In each 50 by 50 m plot, we mapped the positions of the centres of every *Juniperus* and *Pistacia* plant, plus other trees and shrubs of all species within the plot (see Chapter 2), recording various aspects of size and shape of each plant. For five randomly chosen *Pistacia* and *Juniperus* from each of the 9 plots (90 in total), we collected leaf samples and determined the concentration of phenols.

Insects were collected by walking around the tree/shrub and observing what insects were present, catching those reachable without disturbance. Then we worked through the foliage by hand, starting from the top, thoroughly and systematically turning over leaves and branches, recording the number and type of all those found.

The data were analyzed using Generalized Linear Models in SPSS 18. We checked the distribution of the residuals for normality using the Shapiro-Wilk test. The response (dependent) variables were (a) the total number of herbivorous insects

on a plant of *Pistacia atlantica* or *Juniperus phoenicea* in the main year of insect sampling (year 2); (b) the number of herbivore species on a plant; and (c) the number of insects and insect species in year 1 of sampling (which was only available for the 90 focal trees); and (d) the same set but using tannin concentration as an extra predictor. The residuals of all final models were normally distributed, and therefore we used normal errors.

Each of the target plant species (*Pistacia*, *Juniperus*) was analysed separately to avoid complicated interactions with species. The predictors consisted of the following (in order of entry): the factors *level* (top, middle, bottom) and *plot* (nested within level); and the covariates of plant volume (m^3), the number of plants within 1 metre, the number of plants within 2 metres, the distance to the nearest conspecific plant, the number of herbivores and species on the nearest conspecific plant, the number of herbivores and species on the nearest allospecific plant, and the number of herbivores on plants within 1, 2, 3, 4 and 5 metres. When the number of species was being analysed, covariates of the number of herbivores within particular distances from the focal plant were replaced by the number of herbivore species within those distances. Covariate predictors were dropped in sequence from the full model according to the value of the Wald statistic, choosing the lowest, until only significant terms remained: the minimal sufficient model. The factors (*level*, *plot*) were always retained as predictors of all models.

In the case of (c), analysing the insects on focal plants collected in the first year of sampling, we did not have any data on the herbivores of non-focal plants. We therefore used the data from the subsequent year instead as a proxy for the missing data. This relies on the assumption that differences in insect loads remain stable through time, which is probably not true: there was no positive correlation between

the numbers of individuals in the two years of sampling (*Pistacia*, $r = 0.206$, ns; *Juniperus*, $r = 0.069$, ns: both, $n = 45$), nor the number of species ($r = -0.298$ and -0.042 , respectively; both ns, $n = 45$).

For a final set of analyses (d), tannin concentration was added to the minimal sufficient model of (c). If added as the first covariate of any model, it was never retained in the final model.

5.3 Results

(a) Numbers of individual insects per plant

The number of individual insects per plant was affected by the neighbourhood in both *Pistacia* Table 5-1 and *Juniperus* Table 5-2), but by elevation in neither. In the case of *Pistacia*, the number of individual insects on the nearest conspecific (Figure 5-1) and allospecific (Figure 5-2), distance to the nearest conspecific (Figure 5-3), insect density on plants within 2 m (Figure 5-4), and species richness on the nearest conspecific (Figure 5-5) and allospecific plant (Figure 5-6) were all positively related to the number of individual insects on a particular plant. There were negative relationships as well, with insect density on both other *Pistacia* (not shown) and *Juniperus* (Figure 5-7) plants within 2 m. The positive relationship with distance to conspecifics is important because it means that having near neighbours reduces the numbers of insects on a focal plant, and the same is true of the negative relationships with insect densities of neighbours.

For *Juniperus* the final minimal sufficient model (Table 5-2) was much simpler, and all significant relationships were with numbers of insects on neighbours

and were positive - the number on the nearest conspecific (Figure 5-8) and allospecific (Figure 5-9) plants. For comparison with *Pistacia*, we show the equivalent plots (Figure 5-10) to (Figure 5-13) of distance to nearest conspecific (Figure 5-10), number of insects on all plants within 2 m (Figure 5-11), herbivore species richness on the nearest conspecific (Figure 5-12) and allospecific (Figure 5-13)

(b) The number of herbivore species

Overall (Table 5-3) there were significant differences among levels, but not between species, nor was there a significant species*level interaction. For *Pistacia*, there were five significant covariates in the minimal sufficient model explaining the number of herbivore species (Table 5-4), as well as a significant effect of elevation. There were no distance effects. The clearest effects were both positive: the number of species on the nearest conspecific (Figure 5-14), and on the nearest allospecific (not plotted). The only negative effect was that of the number of individuals on the nearest allospecific plant.

For *Juniperus*, again there were five significant covariates in the final minimal sufficient model (Table 5-5), but no significant effect of elevation. The same positive effect of the number of species on the nearest conspecific was evident (Figure 5-15), but this time there were two negative effects for the number of individual insects on the nearest conspecific, and the number of species on all junipers within 2 metres.

(c & d) *herbivores in the first year of sampling*

There were no significant correlations between tannin concentrations and the number of herbivore species or individuals for *Pistacia* (Figure 5-16) & (Figure 5-17). For *Juniperus*, there was no significant correlation between tannin concentration and the number of herbivore species (Figure 5-18), but there was a highly significant positive correlation between the number of individual insects and tannin concentration (Figure 5-19).

Assuming the validity of using the insect data from the second year of sampling as predictors of insects sampled in the first year (for which there is contrary evidence: see Methods), we ran models trying to explain the number of individuals and species of herbivores on both target plants.

For the number of insects on *Pistacia*, there were three significant covariates in the final minimal sufficient model (Table 5-6), two of which are potentially important, as well as an effect of elevation. There was a positive effect of distance to the nearest plant of a different species, and a negative effect of the local density of *Pistacia* insects. There was no evidence of any extra effect of tannins when added to the final model.

The model for *Juniperus* (Table 5-7) was much simpler, retaining only the negative effect of local insect density on juniper, as well as an effect of elevation. There was a significant positive effect of tannin concentration when added to the final model.

When trying to explain the number of herbivore species, for *Pistacia* (Table 5-8) the model retained five covariates included two negative effects, that of the species richness of the nearest conspecific, and of local species richness on all plants within 2 metres. There was a highly significant effect of elevation. Tannin

concentration had no extra significant effect. For *Juniperus* (Table 5-9), seven covariates were retained in the final model, three of which were negative effects, and in addition there was a positive effect of distance to the nearest plant of a different species. There was also a significant effect of elevation. Tannin concentration made no extra significant effect to this model.

(e) *the commonest species on each focal plant*

The overall numbers of individual *Xylomeira* sp per *Juniperus* plant were analysed with a Glm with normal errors, simplified to the minimum sufficient model (Table 5-10). There was positive significant effect on the numbers of *Xylomeira* sp of elevation, the species richness on the nearest *Juniperus*, the number of insects on all *Juniperus* within 2 m, and the number of insects on the nearest allospecific plant.

The overall numbers of individual *Oedopoda caerulescens* per *Pistacia* plant was analysed in the same way (Table 5-11). Again there were significant positive effects on the numbers of *Oedopoda* of elevation, the species richness on the nearest *Pistacia* and on the nearest allospecific, and of the number of insects on all *Pistacia* within 2 m. There was also a significant negative effect of the number of individuals on the nearest allospecific.

5.4 Discussion

According to our results, apart from random variation among plots within elevations (nearly always the largest contributor to explaining the variation), four main factors affect the density and species richness of insect herbivores on individual plants of *Juniperus* or *Pistacia*: elevation, and the distance to, the number and richness of insects on the nearest plant of the same species. Sometimes the number of

insects on either conspecific or allospecific plants within a certain distance away also is a significant predictor, and this distance always seems to be 2 metres. For Juniper, tannin concentration also has an effect, but the coefficient is positive rather than negative. Thus we have no evidence for associational resistance. Nearly all of these effects are weak predictors of the response variable, accounting for very little of the variation.

The really interesting relationships are the positive distance effect, and the ones with negative coefficients, where increasing herbivore numbers/species on adjacent plants is associated with decreasing numbers/species on the focal plant. These indicate neighbourhood effects via associational avoidance, where a focal plant is protected by surrounding plants. There are several such relationships. For herbivore density on *Pistacia* plants, there are negative relationships with herbivore density on both *Juniperus* and *Pistacia* within 2 m. For herbivore species richness, both *Juniperus* and *Pistacia* show such negative relationships. For *Pistacia*, there is a negative relationship with the herbivore density on the nearest plant of a different species. For *Juniperus*, there are negative relationships with the herbivore density on the nearest plant of the same species, and on the number of herbivore species on all junipers within 2 m. The positive relationship for *Pistacia* with distance to conspecifics is also important because it means that having near neighbours reduces the numbers of insects on a focal plant.

Even with the smaller sample sizes of the first year of sampling, there are negative relationships. For *Pistacia*, the herbivore density is negatively related to the herbivore density on *Pistacia* within 4 m; and for *Juniperus*, with the herbivore density on all *Juniperus* within 1 m. Herbivore species richness in *Pistacia* is

negatively related to the species richness on the nearest conspecific, and on all shrubs within 2 m. In *Juniperus*, species richness is negatively related to herbivore density on the nearest conspecific, species richness on the nearest allospecific, and on all shrubs within 3 m. Similar patterns were seen in the analysis of the two commonest herbivores.

Thus we conclude that there is a weak but consistent signal of associational avoidance in these data. Although weak effects, individual plants gain by close spatial association with other plants, both conspecifics and allospecific, reducing their herbivore burdens. There is nothing comparable in the literature for Mediterranean systems, and therefore it is not possible to discuss this result relative to the findings of others in similar circumstances. We therefore discuss more generally our results.

Consistently the largest effects in our data stem from (a) the effect of elevation and/or variation among plots within elevations; and (b) the number of herbivore individuals and/or species on the nearest allospecific, always a positive effect. Thus although there is a signal of associational avoidance, the largest neighbourhood effect in our data is one of associational susceptibility. Individual plants growing near to other species suffer more when the latter's herbivore loads are high. The mechanism for this effect is unknown, and the next step is to address it experimentally via placing potted plants in specific locations to see what herbivore loads result.

Differences in herbivore densities with elevation and among plots may be attributable to non-uniform distribution of herbivores in the landscape, which can cause variation in the impact of neighbourhood effects. Baraza et al. (2006) found that barberry did not protect effectively maple shrubs located beneath them when the

herbivore pressure was high, since animals were forced to eat the less palatable plants (e.g. at the end of drought): spiny but palatable shrubs such as hawthorn and sloe provided protection only when herbivory pressure was low. Defence effectiveness depends on herbivore plant selection and the abundance of other feeding resources: no physical defence was completely successful. This resembles the concept of associational avoidance (Milchunas & Noy-Meir 2002).

Herbivore pressure changes the degree of protection by other plants. With high herbivore pressure, only very unpalatable shrubs can protect palatable ones, while for unpalatable shrubs the probability of attack tends to rise when growing near palatable shrubs. In contrast, with a low herbivore pressure, shrubs of middle palatability can protect palatable shrubs from herbivores, while the protective role of unpalatable shrubs rises, and unpalatable shrubs are safe in any microhabitat. With intermediate herbivore pressures, palatable plants have reduced attack when growing under plants of middle or low palatability, while unpalatable plants will experience damage when growing under palatable plants. Thus the quality of a microhabitat for recruitment is dependent on the degree of herbivore pressure, together with the palatability of surrounding plants. As a result, the landscape can alter from being, at a low herbivory level, a high-quality matrix for recruitment, to becoming, at high herbivore levels, a low-quality matrix where only unpalatable shrubs can recruit.

The high shrub variety in Mediterranean ecosystems can encourage the appearance of dependable associational avoidance for palatable shrubs, as occurred with both shrubs of this study. This protection of understory diversity in Mediterranean woodlands can be important, especially when herbivore pressure is moderate or high (Callaway 1995, Rousset & Lepart 1999, Meiners & Martinkovic 2002, García & Obeso 2003), in regeneration processes. Nurse plants give protection

from browsing, raising plant diversity by harbouring sensitive species (Milchunas & Noy-Meir 2002, Rebollo et al. 2002). From the point of view of saplings, the neighbourhood effect conditions regeneration capability depending on the vegetation matrix and its spatial distribution. Open gaps preferentially permit regeneration of less palatable species, while palatable species regenerate only in patches having a rich cover of less palatable shrubs (Rousset & Lepart 1999). High herbivore pressure favours coexistence of different tree species in different patches of the same plot (Jeltsch et al. 1996). From the viewpoint of herbivores, the neighbourhood effect determines the significance that one plant has in its diet in comparison with other species, impacting how the herbivore can filter the plant-species composition (Baraza et al. 2006).

Biotic interactions are major drivers of the structure and dynamics of plant communities (Lortie et al. 2004). Competition has traditionally been considered as the major interaction that structures plant communities (Grime 1974). However, an increasing number of studies have also emphasized the importance of facilitative or positive interactions (Bertness & Callaway 1994; Callaway 1995; Brooker et al. 2008). The net balance of positive and negative interactions determines community organization and composition (Callaway & Walker 1997; Holmgren, Scheffer & Huston 1997; Brooker et al. 2008). This balance is context-dependent, varying in response to abiotic stress, disturbance, life cycle stage, species identity and interactions with other neighbours (Callaway 2007).

Facilitation can be direct, i.e. one species increases the performance of a second species by ameliorating the abiotic environment (Callaway 1995; Pugnaire, Haase & Puigdefabregas 1996a; Pugnaire, Armas & Valladares 2004). Facilitation may also be indirect, i.e. a third species mediates interactions between the nurse species and

the target species. Thus, some plants protect other plants from herbivores, attract pollinators, concentrate propagules of other species, or enhance mycorrhizae and soil microbial activity (Callaway 1995, 2007). The occurrence of a third plant species may also convert the competition between two species into indirect facilitation via suppression of a shared competitor (Miller 1994; Levine 1999; Callaway & Pennings 2000). Indirect facilitation mediated by a third competitor mainly occurs in systems in which the three species compete for different resources, or use different mechanisms to acquire them (Levine 1999; Siemann & Rogers 2003; Kunstler et al. 2006).

In contrast to direct facilitation and competition, indirect facilitation has received less attention. Most studies assessing indirect facilitation have focused on interactions between different trophic levels, such as those analysing defence against herbivory (Boulant et al. 2008; Gómez-Aparicio et al. 2008; Anthelme & Michalet 2009) or concentration of propagules of other species (Verdú & García-Fayos 2003; Aerts et al. 2006).

Experimental field studies investigating indirect facilitation within the same trophic level are much less frequent (Brooker et al. 2008). Most studies of indirect facilitation among plants have been performed in productive or moderately productive environments; some have documented indirect facilitation (Levine 1999; Callaway & Pennings 2000; Siemann & Rogers 2003; Kunstler et al. 2006), while others failed to detect this process (Pagès & Michalet 2003; Pagès et al. 2003). To our knowledge, no experimental field study to date has explored the importance of indirect facilitation in semi-arid or arid systems.

Direct facilitation is a recognized recruitment mechanism for plants in Mediterranean environments (Verdú & García-Fayos 2003; Gómez-Aparicio, Gómez

& Zamora 2005a), but the importance of indirect facilitation remains to be assessed in this system. *Retama sphaerocarpa* is one of the most-studied Mediterranean nurse species. It promotes the development of a diverse herbaceous community due to changes in microclimate and soil fertility under its canopy (Pugnaire et al. 1996b; Pugnaire, Armas & Valladares 2004). Although herbs compete with the seedlings of woody species and impair woodland regeneration (Nambiar & Zed 1980; Rey Benayas et al. 2005), mid- and late-succession woody species such as *Quercus ilex* develop under the *Retama* canopy (Tovar 2009). Several studies have demonstrated the direct facilitative effect of *Retama* on the plant community developed under its canopy, but no study has assessed its potential indirect facilitative effect (Pugnaire, Haase & Puigdefábregas 1996a; Pugnaire et al. 1996b; Rodríguez-Echeverría & Pérez-Fernández 2003; Pugnaire, Armas & Valladares 2004).

Mediterranean ecosystems comprise an excellent model system to examine the causes of neighbourhood effects, since the extremely high diversity and heterogeneity remains at small spatial scales (Baraza et al 2006). There is a huge diversity of shrubs intermingled with trees within one small area, producing an array of interactions. The regeneration niche might be one productive arena of study in such systems. Many woody species naturally regenerate beneath pre-established plants (Baraza et al 2006), and there are clear impacts of herbivores within small scales (< 3m).

5.5 References

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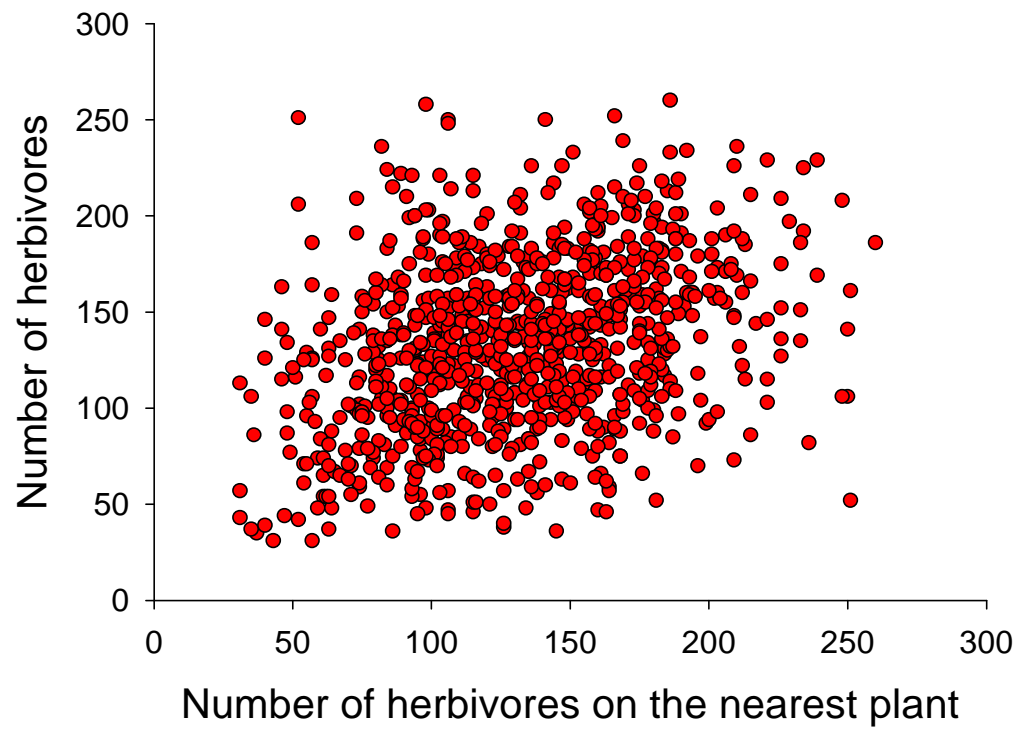


Figure 5-1: Number of insects recorded on a *Pistacia* plant as a function of the number on the nearest *Pistacia* plant

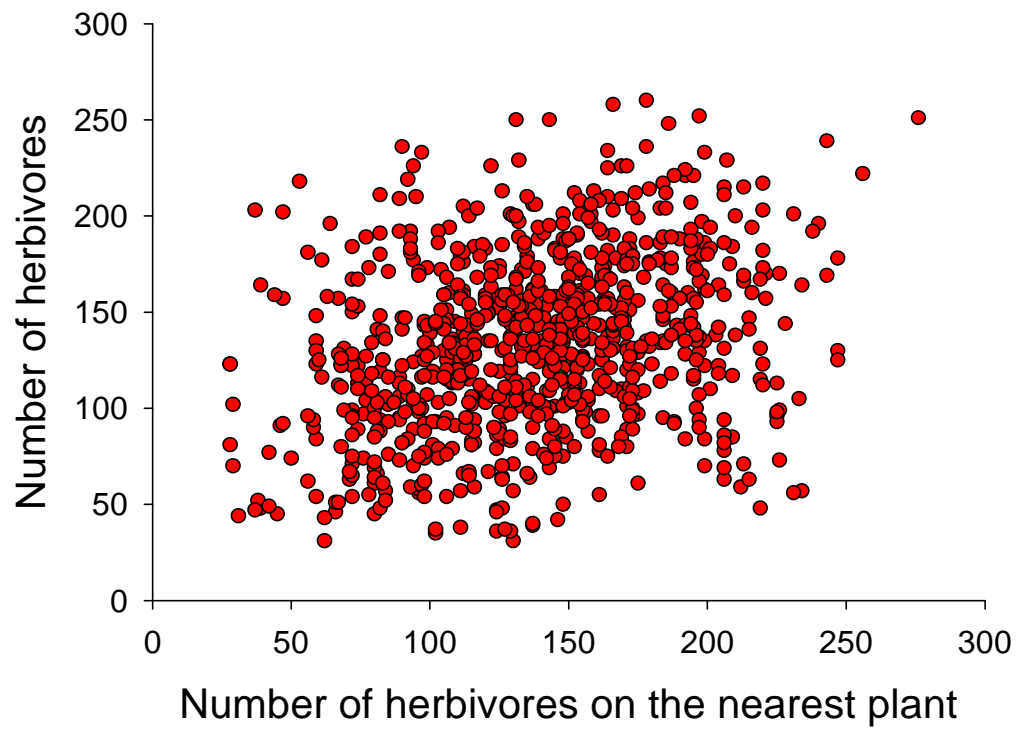


Figure 5-2: Number of insects recorded on a *Pistacia* plant as a function of the number on the nearest plant of a different species

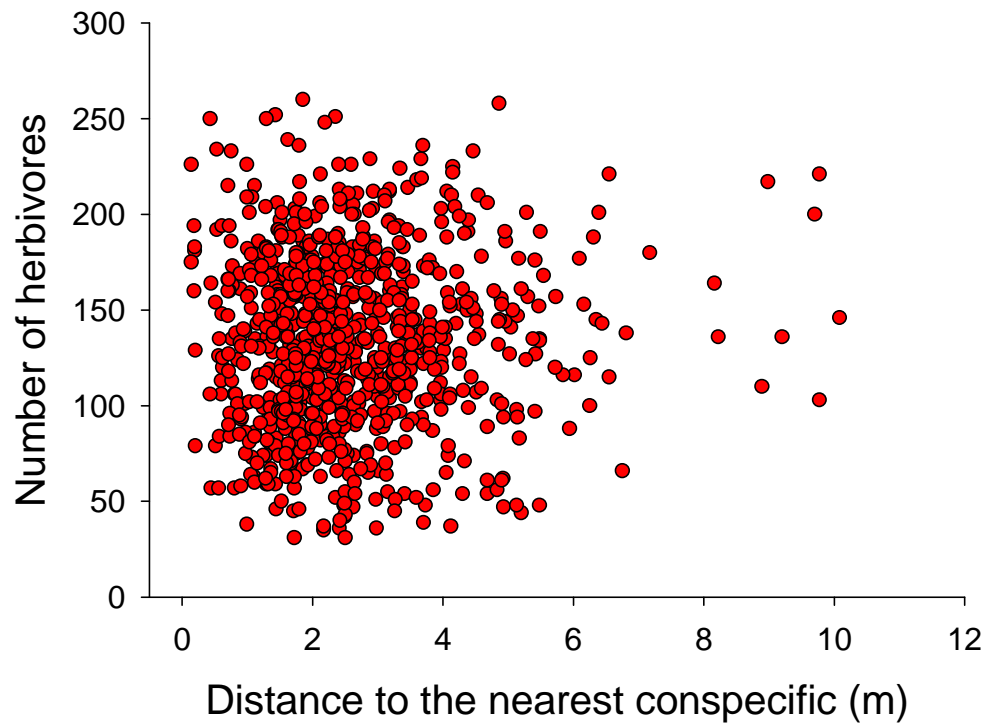


Figure 5-3: Number of insects recorded on a *Pistacia* plant as a function of the distance to the nearest *Pistacia* plant

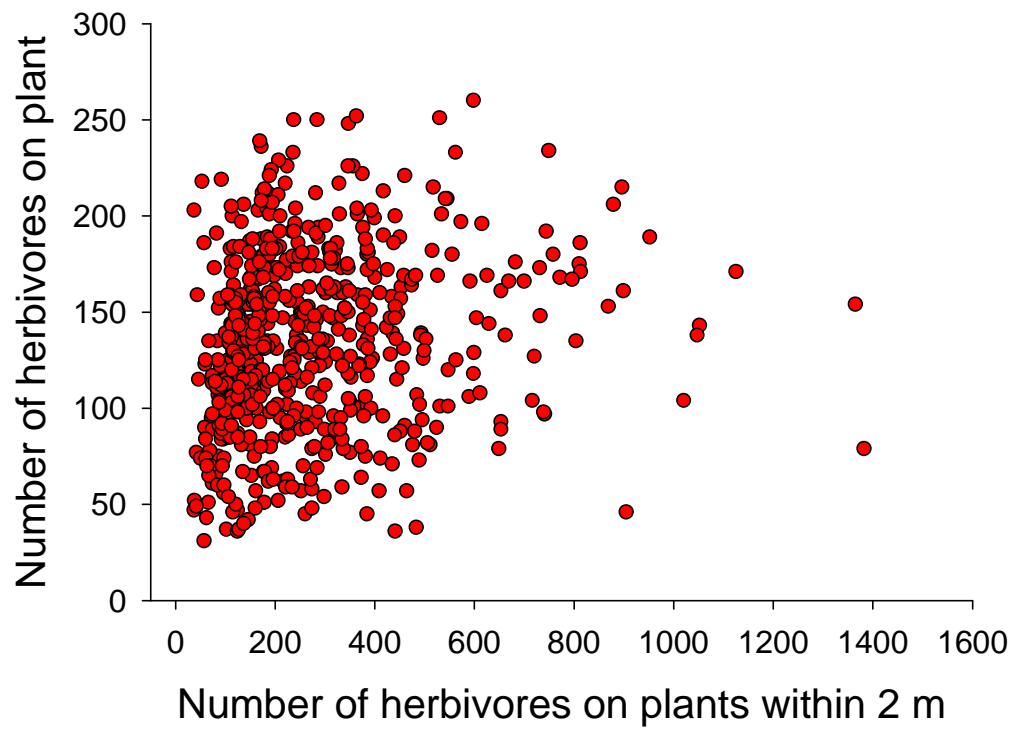


Figure 5-4: Number of insects recorded on a *Pistacia* plant as a function of the number of insects on all plants within 2 m

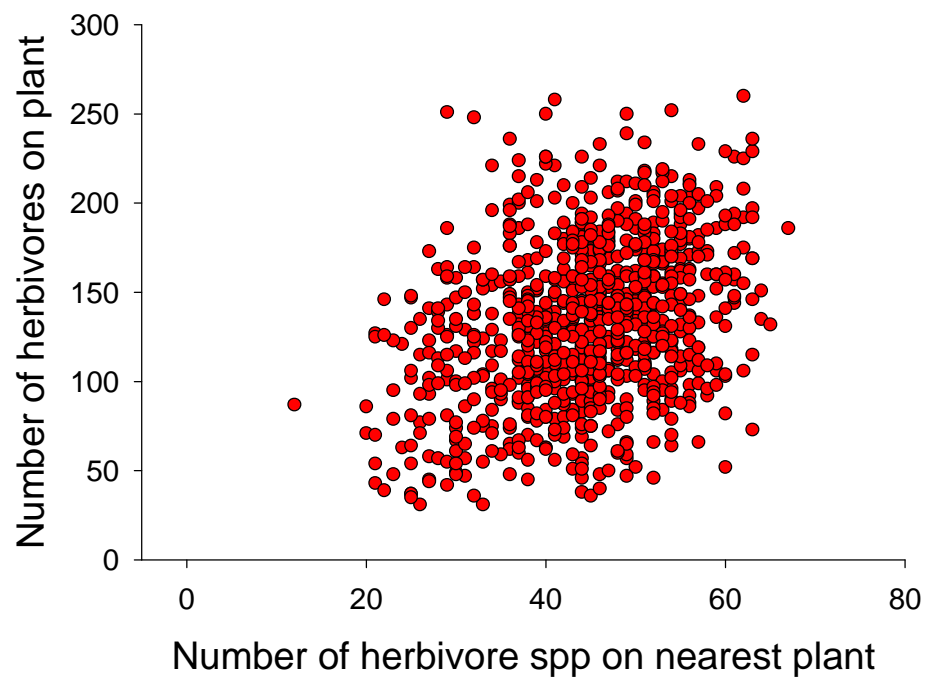


Figure 5-5: Number of insects recorded on a *Pistacia* plant as a function of the number of herbivore species on the nearest *Pistacia* plant

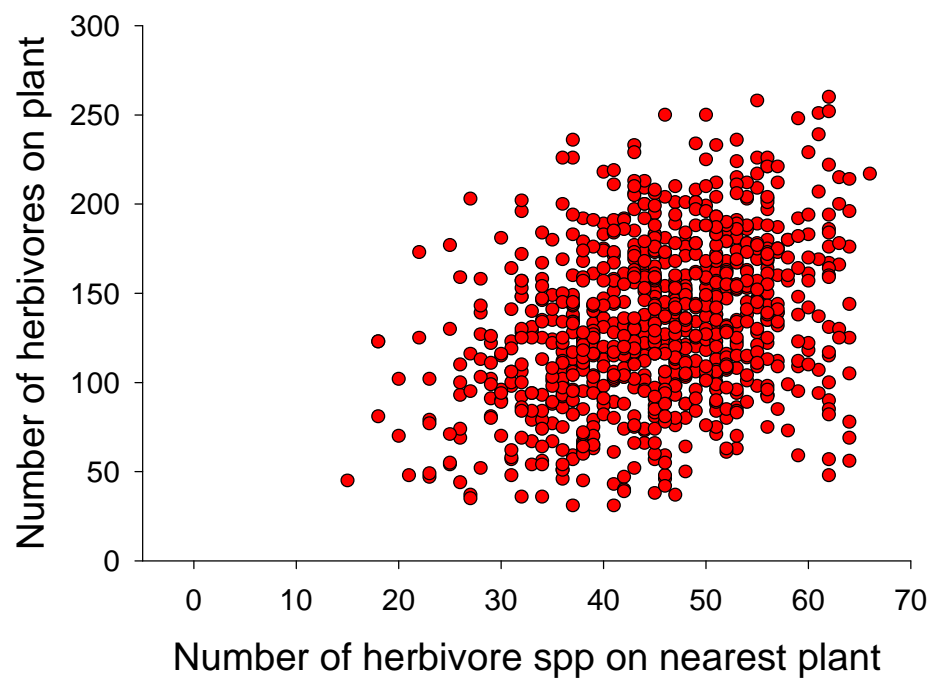


Figure 5-6: Number of insects recorded on a *Pistacia* plant as a function of the number of herbivore species on the nearest plant of a different species

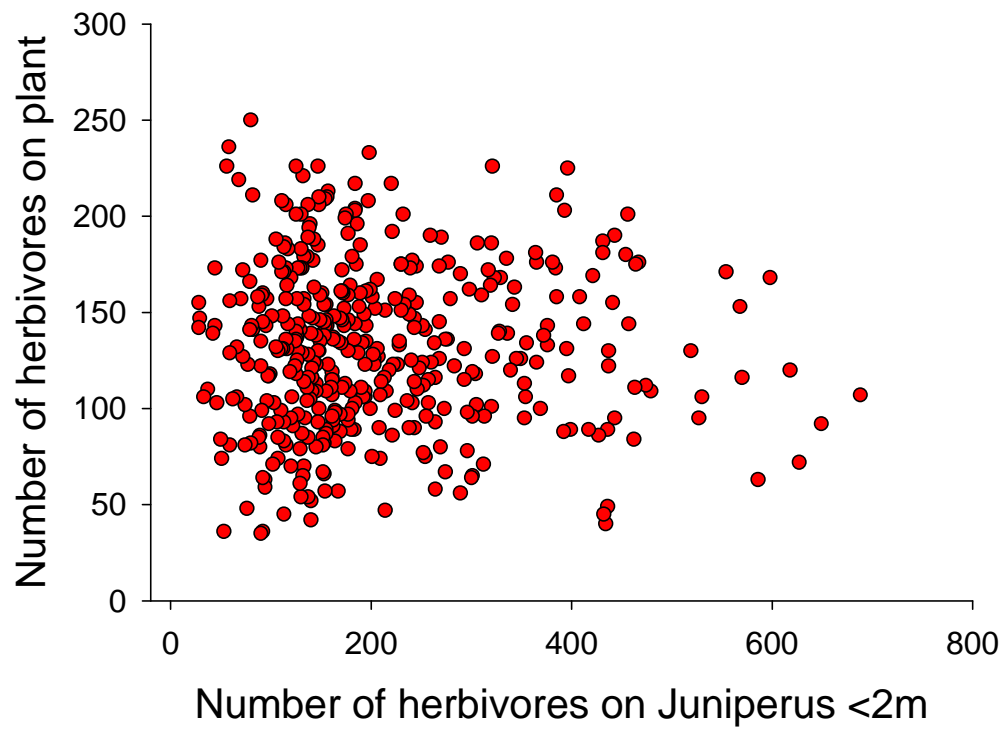


Figure 5-7: Number of insects recorded on a *Pistacia* plant as a function of the number of herbivores on all *Juniperus* plants within 2 m

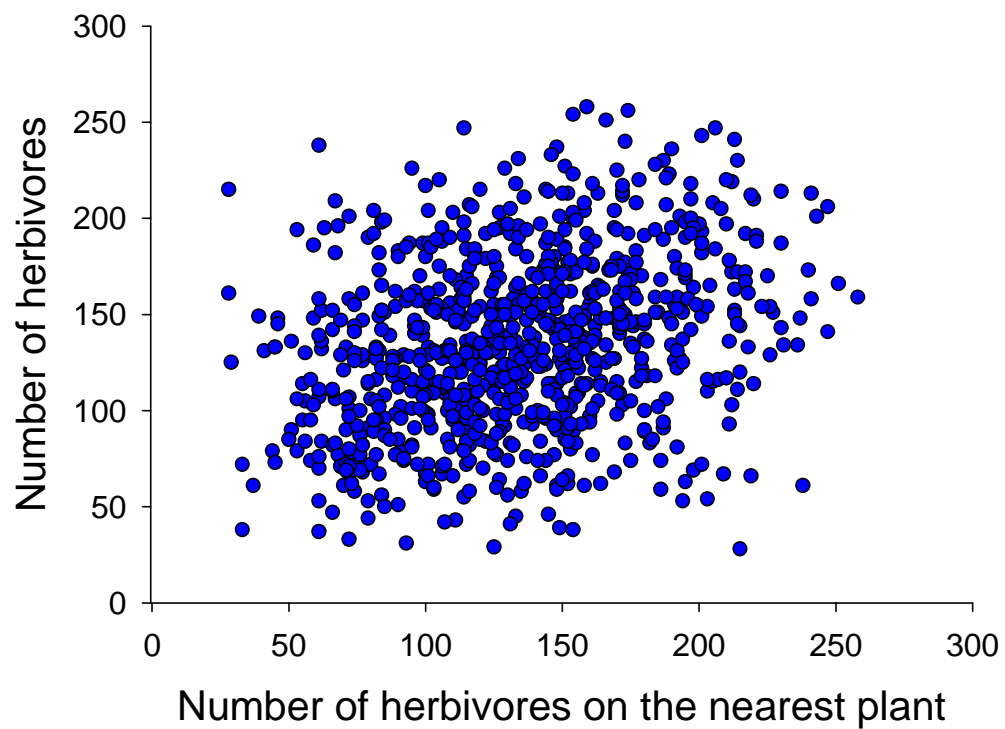


Figure 5-8: Number of insects recorded on a *Juniperus* plant as a function of the number on the nearest *Juniperus* plant

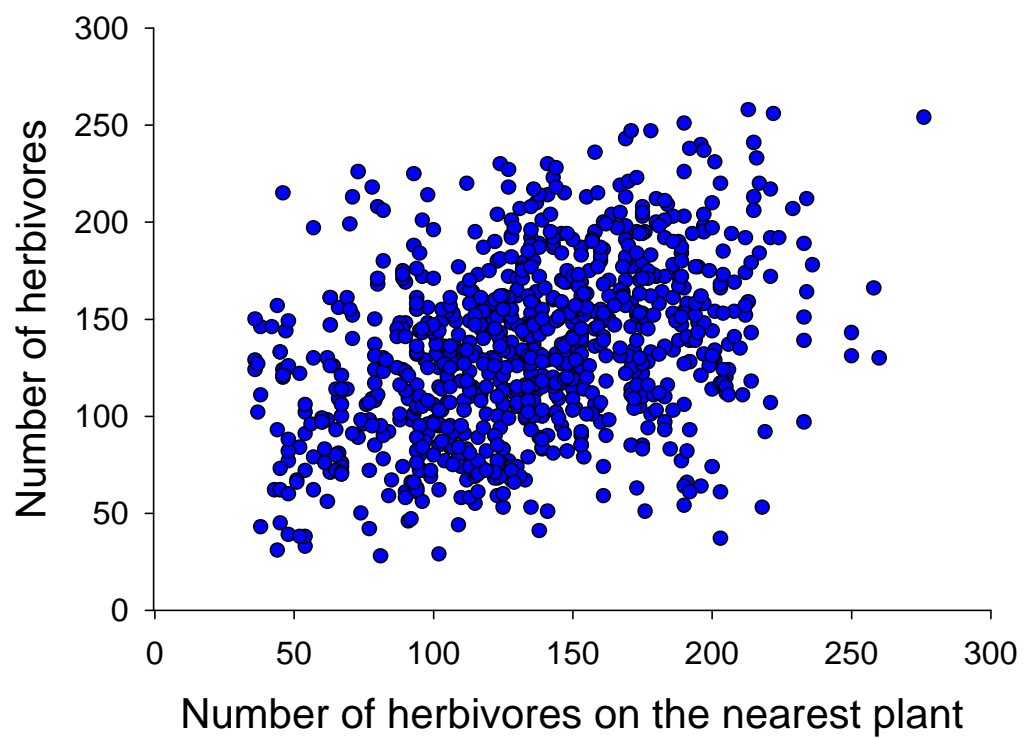


Figure 5-9: Number of insects recorded on a *Juniperus* plant as a function of the number on the nearest plant of a different species

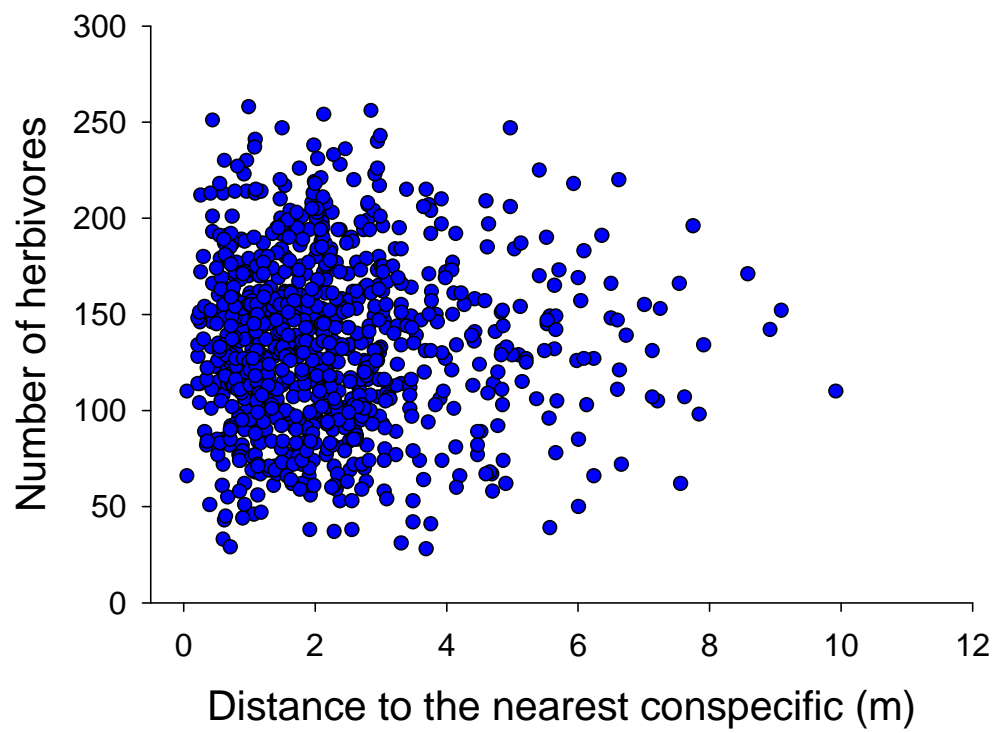


Figure 5-10: Number of insects recorded on a *Juniperus* plant as a function of the distance to the nearest *Juniperus* plant

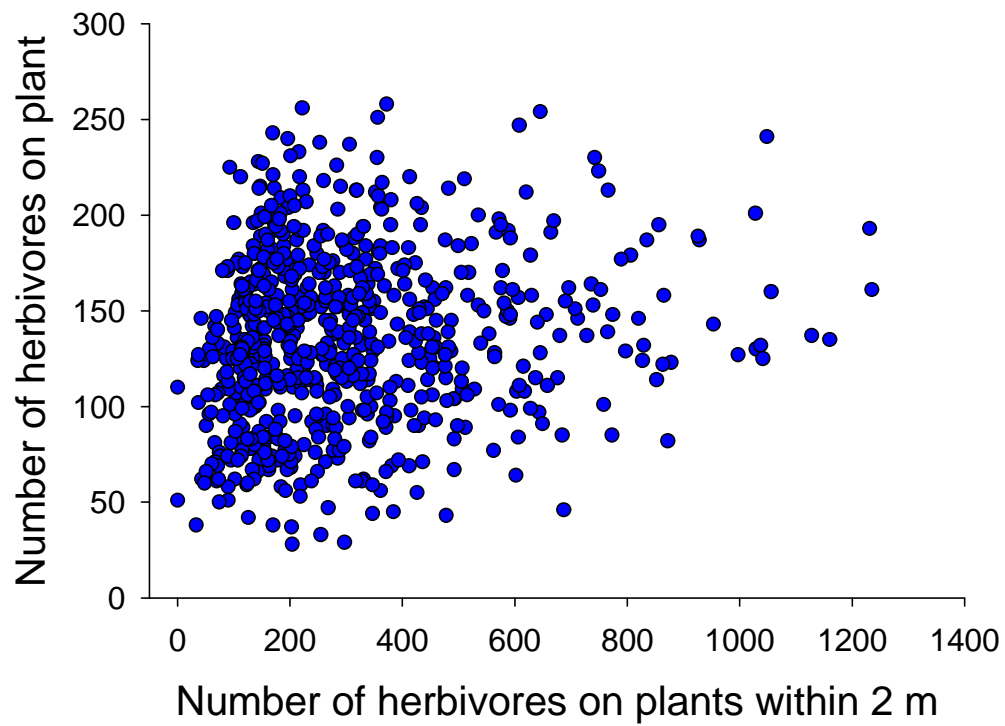


Figure 5-11: Number of insects recorded on a *Juniperus* plant as a function of the number of insects on all plants within 2 m

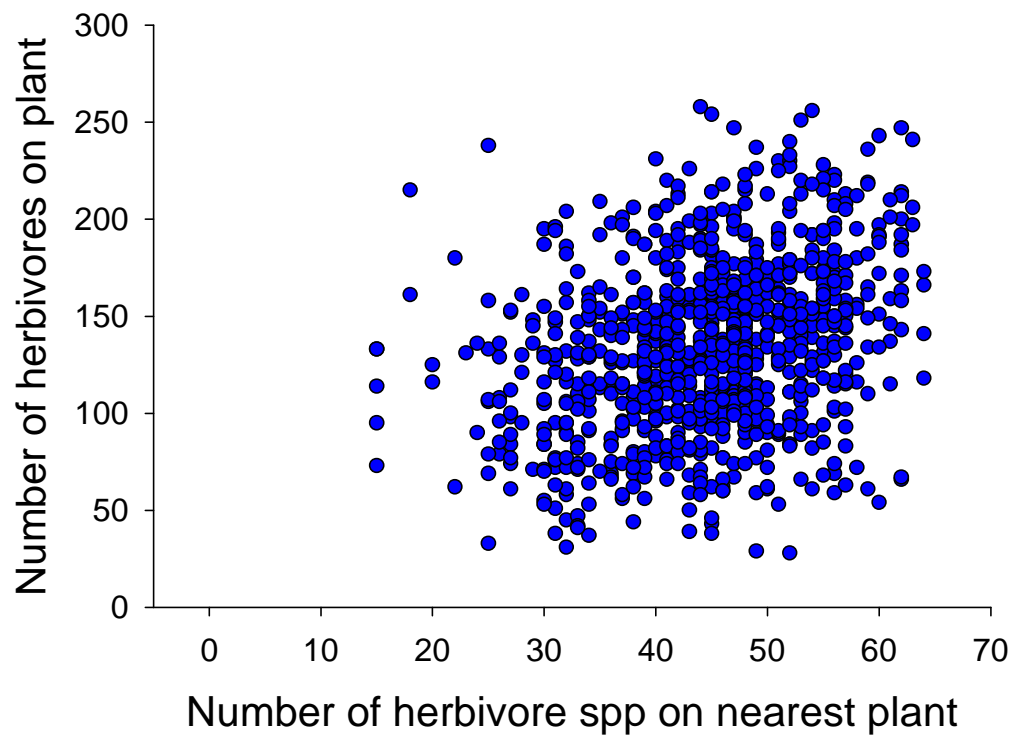


Figure 5-12: Number of insects recorded on a *Juniperus* plant as a function of the number of herbivore species on the nearest *Juniperus* plant

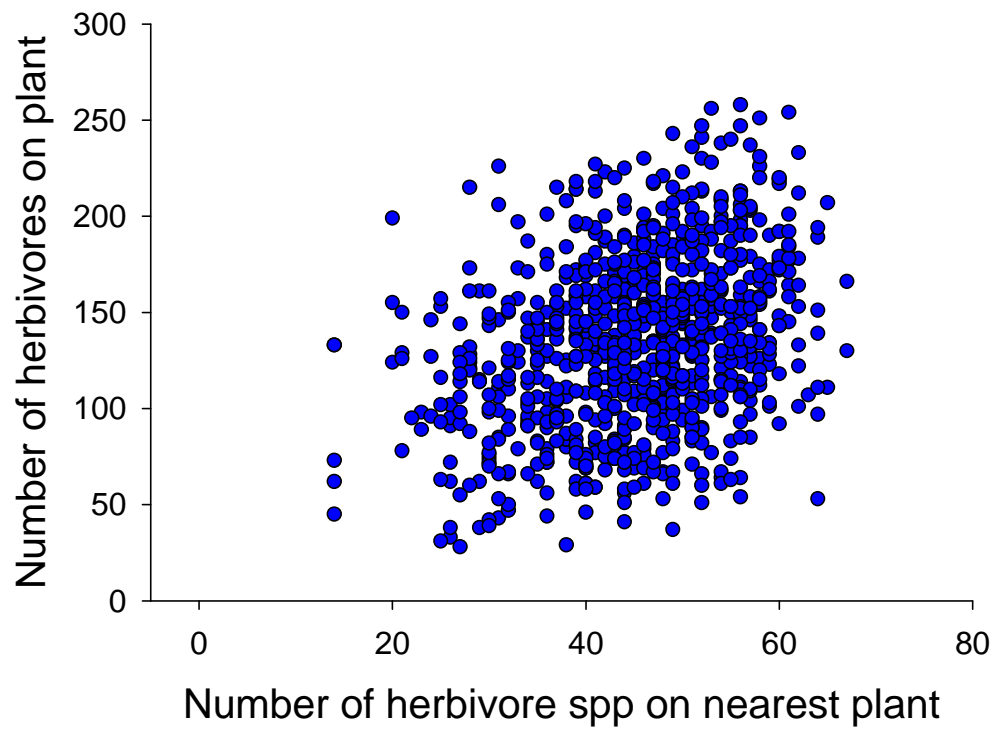


Figure 5-13: Number of insects recorded on a *Juniperus* plant as a function of the number of herbivore species on the nearest plant of a different species

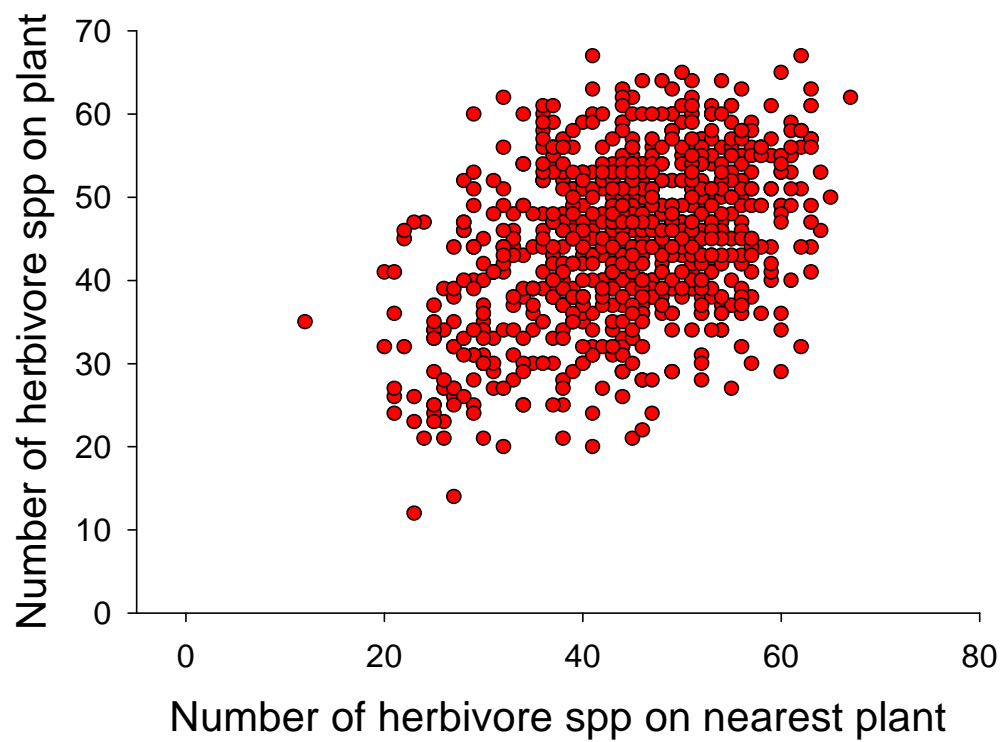


Figure 5-14: Number of herbivore species recorded on a *Pistacia* plant as a function of the number of species on the nearest *Pistacia* plant

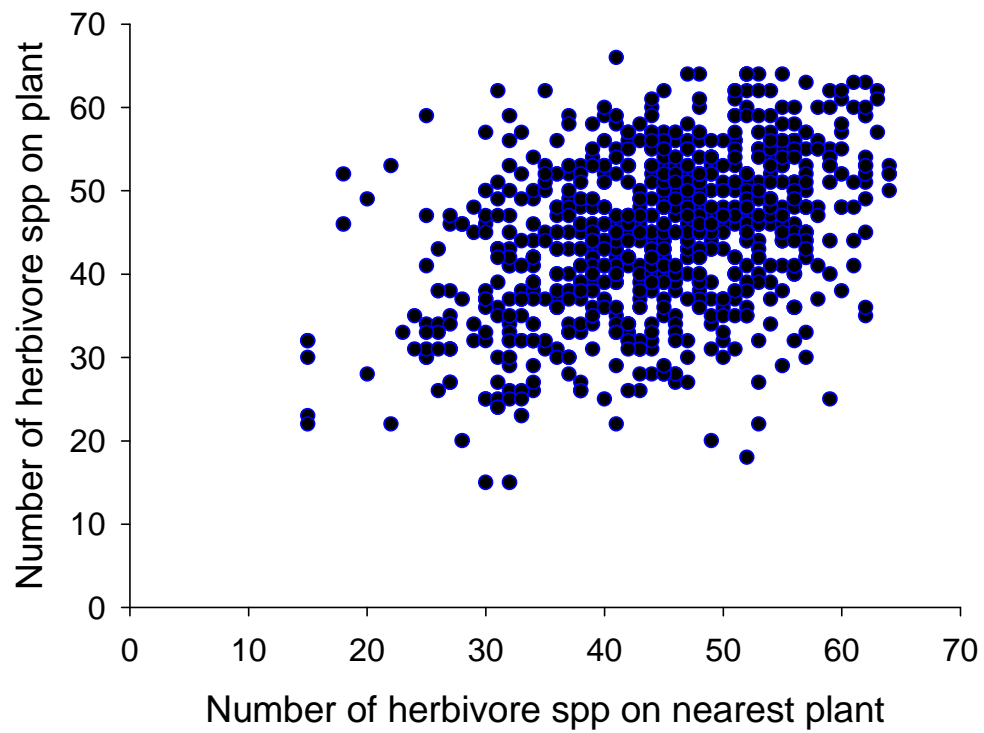


Figure 5-15: Number of herbivore species recorded on a *Juniperus* plant as a function of the number of species on the nearest *Juniperus* plant

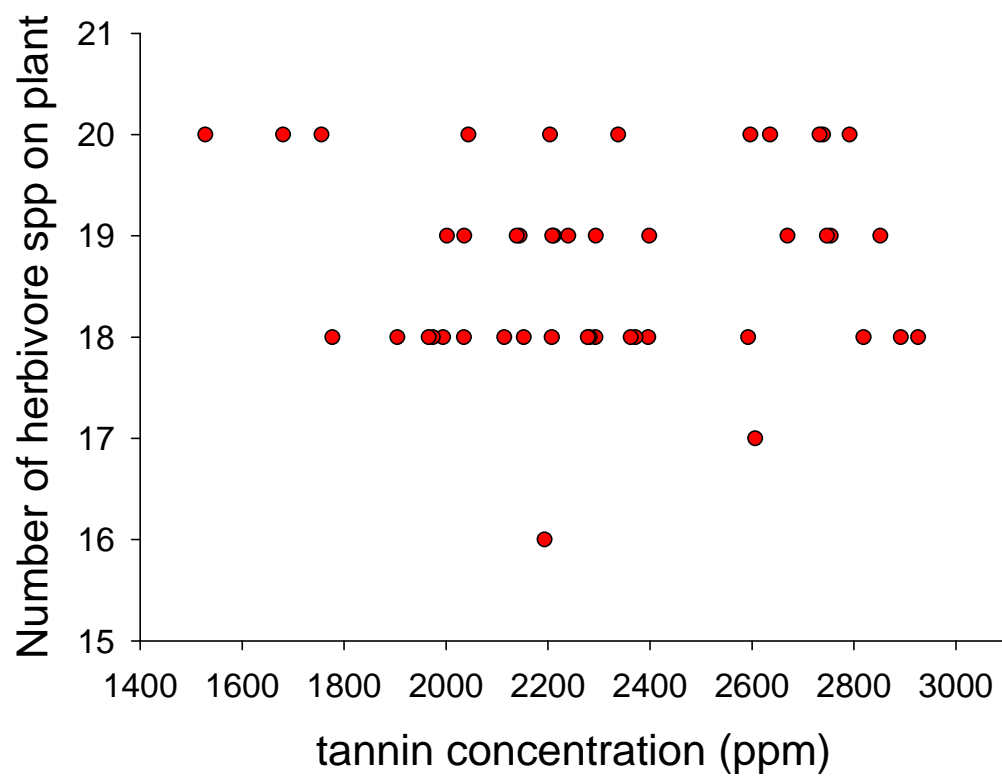


Figure 5-16: Number of herbivore species recorded on a *Pistacia* plant as a function of the measured tannin concentration in the leaves ($r = -0.005$, $n=45$, ns).

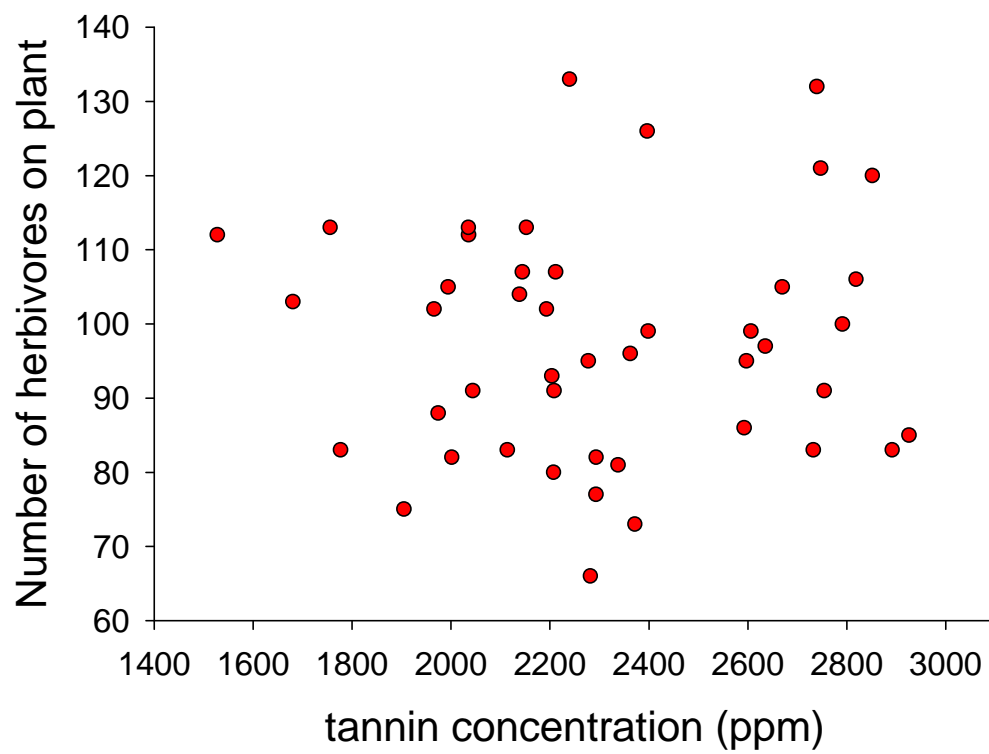


Figure 5-17: Number of herbivorous insects recorded on a *Pistacia* plant as a function of the measured tannin concentration in the leaves ($r = 0.027$, $n=45$, ns).

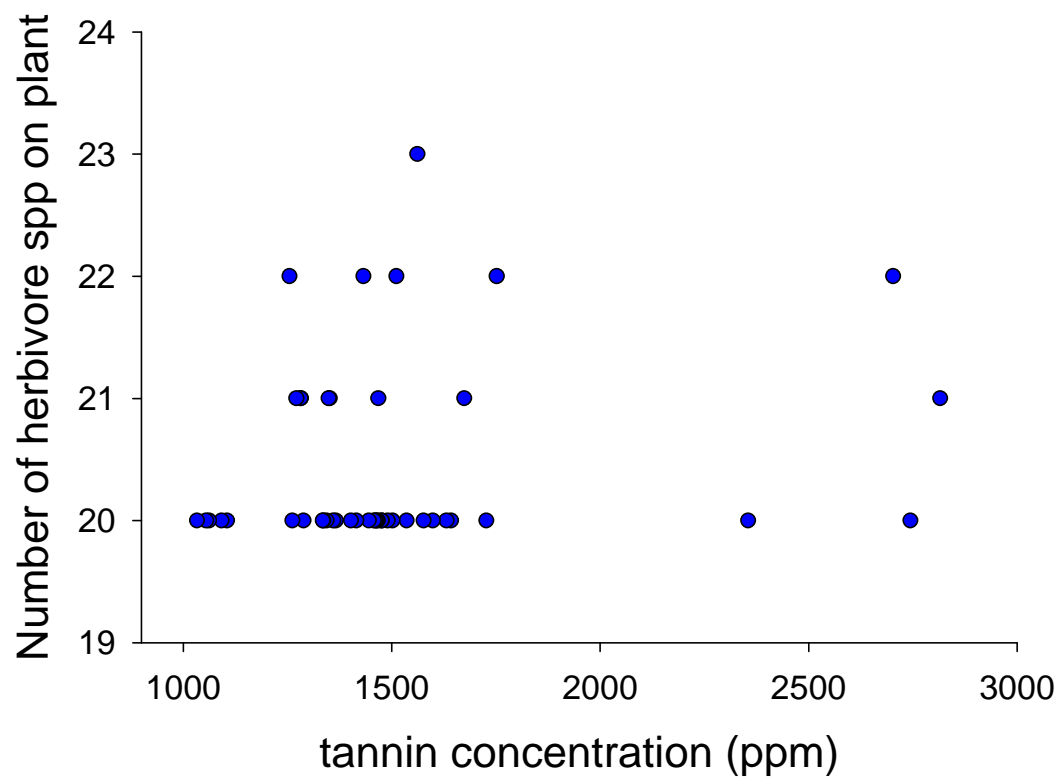


Figure 5-18: Number of herbivore species recorded on a *Juniperus* plant as a function of the measured tannin concentration ($r = 0.122$, $n=45$, ns).

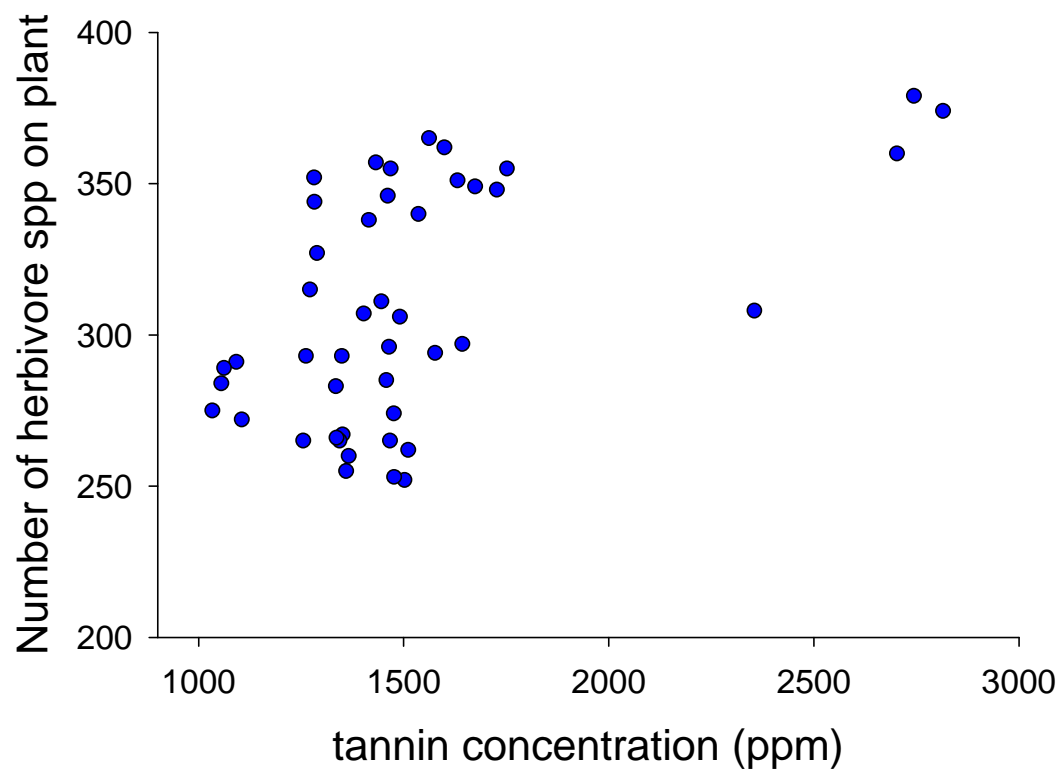


Figure 5-19: Number of herbivorous insects recorded on a *Juniperus* plant as a function of the measured tannin concentration ($r = 0.468$, $n=45$, $P = 0.001$).

Source	coefficient	s.e.	Wald χ^2	d f	Sig.
level			5.560	2	.062
plot(level)			13.669	6	.034
Distance to the nearest conspecific	2.874	1.2175	5.574	1	.018
No. individuals on the nearest conspecific	.134	.0588	5.229	1	.022
No. species on the nearest conspecific	.698	.2782	6.294	1	.012
No. individuals on the nearest allospecific	.227	.0314	52.122	1	.000
No. individuals on <i>Juniperus</i> within 2 m	-.072	.0324	4.950	1	.026
No. individuals on <i>Pistacia</i> within 2 m	-.067	.0333	4.097	1	.043
No. individuals on any shrub/tree within 2 m	.087	.0296	8.668	1	.003

Table 5-1: Minimal sufficient model analysing the number of herbivorous insects (response) on *Pistacia* in the study plots at three elevations (*level*) in Al Jabal Al Akhdar. The analysis used the Generalized Linear Model in SPSS18 with normal errors, with factors of level and plot nested within level; all other predictors were covariates.

Source	coefficient	s.e.	Wald χ^2	df	Sig.
level			3.710	2	.156
plot(level)			7.708	6	.260
No. individuals on the nearest conspecific	.211	.0318	43.890	1	<.001
No. individuals on the nearest allospecific	.314	.0324	93.891	1	<.001

Table 5-2: Minimal sufficient model analysing the number of herbivorous insects (response) on *Juniperus* in the study plots at three elevations (*level*) in Al Jabal Al Akhdar. The analysis used the Generalized Linear Model in SPSS18 with normal errors, with factors of level and plot nested within level; all other predictors were covariates.

Tests of Model Effects

Source	Type III		
	Wald Chi-Square	df	Sig.
(Intercept)	545601.026	1	<.001
plot(level)	121.042	6	<.001
level	20.106	2	<.001
species	.034	1	.854
species * level	2.473	2	.290

Table 5-3: Minimal sufficient model analysing the number of herbivorous insects (response) on all plants species in the study plots at nine elevations (plot (*level*), *level*, *species*, *species * level*) in Al Jabal Al Akhdar. The analysis used the Generalized Linear Model in SPSS16 with normal errors, with factors of level and plot nested within level; all other predictors were covariates.

Source	coefficient	s.e.	Wald χ^2	d f	Sig.
level			9.502	2	.009
plot(level)			12.361	6	.054
No. species on the nearest conspecific	.283	.0302	88.093	1	<.001
No. individuals on the nearest allospecific	-.036	.0116	9.414	1	.002
No. species on the nearest allospecific	.466	.0571	66.430	1	<.001
No. species on all <i>Juniperus</i> within 2 m	.023	.0102	5.059	1	.024
No. species on all minor plant spp within 2 m	.030	.0137	4.622	1	.032

Table 5-4: Minimal sufficient model analysing the number of herbivore species on *Pistacia* plants (response variable) in the study plots at three elevations (*level*) in Al Jabal Al Akhdar. The analysis used the Generalized Linear Model in SPSS18 with normal errors, with factors of level and plot nested within level; all other predictors were covariates.

Source	coefficient	s.e.	Wald χ^2	df	Sig.
level			3.710	2	.156
plot(level)			7.708	6	.260
No. individuals on the nearest conspecific	-.037	.0115	10.280	1	.001
No. species on the nearest conspecific	.459	.0562	66.847	1	<.001
No. individuals on the nearest allospecific	.055	.0064	72.244	1	<.001
No. species on all <i>Juniperus</i> within 2 m	-.027	.0132	4.299	1	.038
No. species on all shrubs/tree within 2 m	.048	.0150	10.482	1	.001

Table 5-5: Minimal sufficient model analysing the number of herbivore species on *Juniperus* plants (response variable) in the study plots at three elevations (*level*) in Al Jabal Al Akhdar. The analysis used the Generalized Linear Model in SPSS18 with normal errors, with factors of level and plot nested within level; all other predictors were covariates.

Source	coefficient	s.e.	Wald χ^2	df	Sig.
level			10.207	2	.006
plot(level)			18.632	6	.005
Distance to the nearest allospecific	2.447	1.3877	3.109	1	.078
No. herbivores on all <i>Pistacia</i> within 4 m	-.025	.0113	4.885	1	.027
No. herbivores on all shrubs/trees within 5 m	.014	.0044	9.473	1	.002
(tannin concentration)	.000	.0063	0.000	1	.997

Table 5-6: Minimal sufficient model analysing the number of insect herbivores collected in the first year of sampling from focal *Pistacia* plants (n=45) (response variable) in the study plots at three elevations (*level*) in Al Jabal Al Akhdar. The analysis used the Generalized Linear Model in SPSS18 with normal errors, with factors of level and plot nested within level; all other predictors were covariates (note that data for faunal covariates derived from the subsequent year). To the minimal sufficient model was then added a further predictor, the concentration of tannins (in brackets), and the details recorded

Source	coefficient	s.e.	Wald χ^2	df	Sig.
level			595.333	2	<.001
plot(level)			161.977	6	<.001
No. herbivores all <i>Juniperus</i> within 1 m	-.046	.0251	3.350	1	.067
(tannin concentration)	.010	.0044	5.534	1	.019

Table 5-7: Minimal sufficient model analysing the number of insect herbivores collected in the first year of sampling from focal *Juniperus* plants (n=45) (response variable) in the study plots at three elevations (*level*) in Al Jabal Al Akhdar. The analysis used the Generalized Linear Model in SPSS18 with normal errors, with factors of level and plot nested within level; all other predictors were covariates (note that data for faunal covariates derived from the subsequent year). To the minimal sufficient model was then added a further predictor, the concentration of tannins (in brackets), and the details recorded

Source	coefficient	s.e.	Wald χ^2	df	Sig.
level			13.828	2	.001
plot(level)			41.409	6	<.001
No. species on the nearest conspecific	-.034	.0160	4.558	1	.033
No. species on all <i>Juniperus</i> within 2 m	.019	.0087	4.646	1	.031
No. species on all <i>Pistacia</i> within 2 m	.023	.0089	6.372	1	.012
No. species on all minor plant species within 3 m	.024	.0083	8.038	1	.005
No. species on all shrubs/trees within 2 m	-.022	.0104	4.618	1	.032
(tannin concentration)	.000	.0004	.054	1	.815

Table 5-8: Minimal sufficient model analysing the number of herbivore species collected in the first year of sampling from focal *Pistacia* plants (n=45) (response) in the study plots at three elevations (*level*) in Al Jabal Al Akhdar. The analysis used the Generalized Linear Model in SPSS18 with normal errors, with factors of level and plot nested within level; all other predictors were covariates (note that data for faunal covariates derived from the subsequent year). To the minimal sufficient model was then added a further predictor, the concentration of tannins (in brackets), and the details recorded

Source	coefficient	s.e.	Wald χ^2	df	Sig.
level			39.806	2	<.001
plot(level)			26.224	6	<.001
plant volume	.036	.0180	3.908	1	.048
No. individuals on the nearest conspecific	-.013	.0022	32.191	1	.000
Distance of the nearest allospecific	.143	.0735	3.802	1	.051
No. species on the nearest allospecific	-.015	.0088	2.827	1	.093
No. species on all <i>Pistacia</i> within 3 m	.015	.0052	8.183	1	.004
No. species on all shrubs/trees within 3 m	-.013	.0059	4.715	1	.030
No. species on all shrubs/trees within 5 m	.057	.0206	7.785	1	.005
(tannin concentration)	.000	.0003	1.691	1	.193

Table 5-9: Minimal sufficient model analysing the number of herbivore species collected in the first year of sampling from focal *Juniperus* plants (n=45) (response) in the study plots at three elevations (*level*) in Al Jabal Al Akhdar. The analysis used the Generalized Linear Model in SPSS18 with normal errors, with factors of level and plot nested within level; all other predictors were covariates (note that data for faunal covariates derived from the subsequent year). To the minimal sufficient model was then added a further predictor, the concentration of tannins (in brackets), and the details recorded

Source	coefficient	s.e.	Wald χ^2	df	Sig.
(Intercept)			.452	1	.501
level			8.293	2	.016
plot#(level)			26.115	6	<.001
No. species on the nearest conspecific	0.028	0.011	6.438	1	.011
No. individuals on the nearest allospecific	0.013	0.002	30.741	1	<.001
No. individuals on all <i>Juniperus</i> within 2 m	0.001	0.0005	5.744	1	.017

Table 5-10: Analysis of the mean number of *Xylomeira* sp collected in association with *Juniperus* in the study plots. (GLM with normal errors).

Source	coefficient	s.e.	Wald χ^2	df	Sig.
(Intercept)			14.358	1	<.001
level			8.589	2	.014
plot#(level)			42.802	6	<.001
No. species on the nearest conspecific	0.019	0.004	18.505	1	<.001
No. species on the nearest allospecific	0.050	0.008	37.585	1	<.001
No. individuals on the nearest allospecific	-0.007	0.0017	15.472	1	<.001
No. individuals on all <i>Pistacia</i> within 2 m	0.001	0.0002	20.877	1	<.001

| Table 5-11: Analysis of the mean number of *Oedopoda caerulescens* collected in association with *Pistacia* in the study plots (GLM with normal errors).

Chapter 6: Conclusion

This thesis set out to test whether there were neighbourhood effects on insect herbivory among the plants of the Al Jabal Al Akhdar region of Libya. Having chosen to concentrate on the two main species of three study sites, *Juniperus phoenicea* and *Pistacia atlantica*, nine plots were mapped in detail and the insect herbivores sampled from focal plants (2008), and then from all plants (2009). Leaves were sampled for chemical analysis of their phenol (tannin) content.

Pistacia and *Juniperus* dominated all plots, but were less frequent at the bottom elevation, especially *Pistacia*. There were a number of other shrubs growing in the plots, but only *Calicotome villosa* was at all frequent, and only in mid-elevation plots: all others were rare. Plant size was generally highest at the lowest elevation in both species. Middle-elevation plots were clearly the most diverse and the most densest.

The density of plants varied significantly among levels, with the highest densities in the middle plots, and the lowest in the bottom coastal plots. The number of species per ha also varied among levels, again with middle-level plots containing the greatest species richness.

The numbers of *Juniperus* varied a lot among plots within levels; although the highest mean number occurred in the middle-elevation plots, the differences among levels were not significant. The numbers of *Pistacia* was less variable among plots within levels, and bottom-elevation plots were clearly significantly less dense than

mid- or top-elevation plots.

The commonest species on *Juniperus* in both years of sampling was *Xylomeira* sp. (Bostrichidae), a species that attacks live and dead wood. This might be a significant herbivore that could affect the survival and life-history of juniper in Al Jabal Al Akhdar. On *Pistacia*, on the other hand, the commonest species varied between years, with Orthoptera heading the list in the first sampling year: *Paracinipe* (*Acinipe*) *orientalis*, *Oedopoda caerulea* and *Scintharista notabilis*.

Geography is clearly one of the major influences on the distribution of the insect herbivore fauna of Al Jabal Al Akhdar in the Mediterranean ecosystem. The number of recorded species broadly increases with elevation, while middle elevations had the greatest insect abundances. Insect damage to plants also increased with elevation. Relative abundances of the two trees were broadly similar in most sites (see Chapter 2).

According to our results, four main factors affect the density and species richness of insect herbivores on individual plants of *Juniperus* or *Pistacia*: elevation, and the distance to, the number and richness of insects on the nearest plant of the same species. The number of insects on either conspecific or allospecific plants within a certain distance away also is a significant predictor, and this distance always seems to be 2 metres. For Juniper, tannin concentration also has an effect, but the coefficient is positive rather than negative. Thus we have no evidence for associational resistance. The largest effects are those of elevation and the positive impact of the herbivore load on the nearest allospecific - indicating associational susceptibility.

The interesting effects are the positive distance relationship, and the ones with negative coefficients, where increasing herbivore numbers/species on adjacent plants

is associated with decreasing numbers/species on the focal plant. These indicate neighbourhood effects via associational avoidance, where a focal plant is protected by surrounding plants. There are several such relationships. For herbivore density on *Pistacia* plants, there are negative relationships with herbivore density on both *Juniperus* and *Pistacia* within 2 m. For herbivore species richness, both *Juniperus* and *Pistacia* show such negative relationships. For *Pistacia*, there is a negative relationship with herbivore density on the nearest plant of a different species. For *Juniperus*, there are negative relationships with the herbivore density on the nearest plant of the same species, and on the number of herbivore species on all juniper within 2 m. The positive relationship for *Pistacia* with distance to conspecifics is also important because it means that having near neighbours reduces the numbers of insects on a focal plant.

Thus there is a weak signal of associational avoidance in these data. Individual plants gain slightly by close spatial association with other plants, both conspecifics and allospecific, reducing their herbivore burdens.

The high shrub variety in Mediterranean ecosystems may encourage the appearance of dependable associational avoidance for palatable shrubs, as occurred with both shrubs of this study. Protection of understory diversity in Mediterranean woodlands is therefore important, especially when herbivore pressure is moderate or high.

Thus in the plant communities of Al Jabal Al Akhdar, associational avoidance and associational susceptibility appear to be the major mechanisms of neighbourhood effects, rather than associational resistance.