# *Acacia victoriae* and its Association with Sugary-Exudate Producing Herbivorous Insects and Trailing Dominant Ants in Semi-arid Australia

## Nyree Weichel

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School of Earth and Environmental Sciences
University of Adelaide, South Australia

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**Cover image:** Small black *Iridomyrmex* sp. attends *Sextius* sp. nymphs on *Acacia victoriae*, 2010. Photo N. Weichel.

## Contents

# Declaration

I, Nyree Weichel, certify that this work contains no material which has been accepted for the award of any other degree or diploma in any university or other tertiary institution and, to the best of my knowledge and belief, contains no material previously published or written by another person, except where due reference has been made in the text.

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# Thesis Abstract

Associations between ants, sap-sucking herbivores, and plants are often mutualistic, but abiotic and biotic conditions can change the magnitude of costs and benefits to partners. This is sometimes sufficient to tip the outcome for at least one partner to neutral or negative. Little is understood about this conditionality of outcomes. Other arthropods on the plants are likely to affect outcomes for partners, but little is known about broader effects of these associations on the arthropod community. Consistent differences in environmental conditions might bring about differences in function of these associations, so there may be a tendency for outcomes for partners to depend on biome, for which there is some evidence. Associations where plants provide sugary exudate (SE) to ants either directly or through supporting populations of herbivorous insects are widespread and common in Australia on the two most common genera *Acacia* and *Eucalyptus*, yet very few studies of this have been done. Due to the unique suite of environmental conditions on the Austral continent such as the prevalence of aridity, these types of associations may be particularly adaptive for woody plants. *Acacia victoriae* is the second most widespread acacia in Australia. A membracid of a genus which is widely distributed across Australia, *Sextius* sp., frequently colonizes it in semi-arid southern Australia, as do various Margarodidae and lycaenid larvae. Very aggressive dominant small black ants of the genus *Iridomyrmex* (SBIs) trail among the shrubs to attend these sugary-exudate producing herbivores (SEPHs) on them. Spatial distribution of association partners can provide clues not only to likely effects partners have on each other but to how the associations might affect other sectors of the natural community. On gridded sites, *A. victoriae* shrubs, SEPHs on them, and nests of SBIs were mapped and their spatial distribution analysed. A field experiment and field survey examined effects of different densities of SEPHs/*Sextius* sp. on *A. victoriae* growth and

reproductive success and associated arthropod assemblages and investigated factors determining densities of SEPHs on the shrubs. The field survey also examined effects of slope-aspect, as an aridity gradient, on soil parameters, shrub growth and reproductive success, SEPHs attended by *Iridomyrmex*, and other arthropods on shrubs. The study found that *A. victoriae* clustering on sites decreased with size of shrub, and SEPH populations were positively associated with SBI populations both on individual shrubs and at the scale of 15 m x 15 m. SEPH/*Sextius* presence on *A. victoriae* depended on SBI presence, and SBI numbers on stems depended on SEPH numbers more than on stem size. *Sextius* density was linked to nutrient levels of soil near shrubs and shrub leaves and to shrub growth rate. *A. victoriae* growth rate was in turn reduced by exclusion of *Sextius* on stems, and on shrubs with naturally high SEPH/*Sextius* densities, growth and seed production was higher, folivory was lower, and assemblages of small arthropods were different. Parasitoid wasp densities were strongly affected by SEPH/*Sextius* densities and there was some evidence that wasp densities regulated SEPH/*Sextius* densities in turn. Indeed, parasitoid wasps and SEPH/*Sextius* may strongly regulate densities of each other and through that regulate densities of other small arthropods on the shrubs. Dynamics of both potentially influential insect groups may be affected by rainfall and insolation. Slope-aspect affected most parameters considered. Results were consistent with the possibility of favourability of semi-arid conditions for the shrub-SEPH-SBI interaction. This study is the first to investigate the association between these taxa and warrants further research into the dynamics of the association, favourability for the association of arid conditions, and how the association affects the broader community.

# Chapter 1:

# Introduction

Tritrophic food-for-protection associations between ants, sap-sucking hemipterans, and plants, including facultative ones, are usually beneficial to the plants (Styrsky and Eubanks 2007) and can be beneficial to the sap-suckers (Delabie 2001). However, fitness costs and benefits to plants and sap-suckers can depend on the species and abundance of partners and will also depend on abiotic and biotic conditions (Bronstein 1998). For example, the abundance and types of folivorous arthropods on the plant that are susceptible to deterrence by the ants will affect the total cost of herbivory in terms of photosynthate and nutrients allocated to repairing and replacing the biomass. These costs may be higher or lower than the costs of photosynthate and nutrients in sap taken by the sap-sucker populations attracting the ants onto the plant. Abundance and type of predatory arthropods that are susceptible to deterrence by the ants could also affect the survival or reproduction of the sap-suckers. Insolation, rainfall, and soil nutrients could also affect amount and type of resources available to produce defensive compounds (Hare 2011) in the case that the tritrophic interaction is affected by these . This could affect the balance of costs and benefits of the association to a partner species according to spatial and temporal variation in these abiotic and biotic conditions. Thus, the same interaction might be mutualistic, neutral, or antagonistic in its effects on the same partner species over time or across space (Bronstein 1994). Outcomes to partners of associations which are facultative or in which a third species is involved should be more variable than those with only two species (Bronstein 1998), as should those in particularly variable environments. Little is yet understood about how variation in abiotic and biotic environment affects outcomes of these associations for partner species.

Consistent differences in environmental conditions might bring about differences in function of these associations. However, studies of outcomes for partners across environmental gradients are rare. A meta-analysis found that ant removal from plants providing food for ants, sometimes via hemipterans feeding on them, in facultative associations increased herbivory and decreased reproduction more in studies in tropical areas than temperate (Rosumek et al 2009). Another meta-analysis found that increases in measures of plant vigour were found to have a stronger effect on increases in some functional groups of herbivorous insects including sap-suckers in tropical than temperate locations (Cornelissen et al 2008). However, effects of biome have rarely been considered. A survey along a geographic gradient in Central America found that temporal changes of temperature and rainfall over one year were implicated in number of ant-plant and ant-sap-sucker-plant food-for-protection associations (Rico-Gray et al 1998). These changes appeared to have different effects in different biome types. Indeed, effects of typical arid land conditions such as low soil moisture and nutrient levels and high light levels and temperature have rarely been linked to the function and abundance of these types of associations.

Bottom-up effects of abiotic factors including insolation, rainfall, and soil parameters on ant- sap-sucker-plant associations have been investigated very rarely, although these variables can vary with biome and might influence densities of partners in the associations. Together, soil moisture, light levels, and temperature regulate photosynthesis in the arid zone, which contribute to determining the amount of photosynthate that might be used by sap-suckers with negligible detriment to the plant.  Soil nutrient concentrations and uptake by the plant due to moisture levels may affect not only plant performance but also plant chemistry, which could regulate the species and density of arthropods on plants (Ode 2006, Hare 2011).  Soil nutrient concentrations, such as that of nitrogen which is used in many plant defence chemicals, are characteristically low in the arid zone and particularly in Australia, while high insolation makes photosynthesis cheap when moisture is not limiting. This has been postulated as a reason for ant-sap-sucker -plant associations being common and widespread in the Australian arid zone (Stafford Smith and Morton 1990, Orians and Milewski 2007).  Similar associations that ants, usually of dominant species, have with lycaenid larvae are also common in Australia (Eastwood and Fraser 1999).  However, despite their abundance, very little is known about these associations on the driest warm continent.

As to their ecological consequences, very little is known about the broader effects of these associations on whole arthropod communities (Styrsky and Eubanks 2007).  Species of ants that tend sap-suckers tend to be behaviourally dominant and highly abundant (Davidson et al 2003) and could have strong effects on arthropod communities both on host plants of the sap-sucking hemipterans and on the ground if they nest there.  Spatial distribution of partner species might give some clues not only to how the abiotic environment influences them, but to how these associations might affect the broader ecosystem.  Also, species involved in interactions where they have positive effects on each other are likely to have positive spatial association at some spatial scale (Perry 1995).

In Australia, horned treehoppers (Hemiptera:  Membracidae) of the most common membracid genus, *Sextius*, are found in all states and territories, on many species of the woody perennial genus *Acacia* (Day 1999). Behaviourally dominant and highly abundant ants of the genus *Iridomyrmex* are the most common tenders of such sugary-exudate producing sap-suckers, consuming their sugary exudates (Greenslade 1978). *Acacia victoriae* is one plant that hosts *Sextius* and is widespread, being the second most common species of the abundant Australian genus *Acacia*, by one measure of distribution (Fox 1987). The widespread distribution of all these taxa and their high abundance makes them a potentially important influence in many ecosystems on the Australian continent. In semi-arid areas of the Flinders Ranges of southern Australia, populations of sugary-exudate producing herbivores (SEPHs) inhabit *Acacia victoriae* shrubs, between which stretch vast networks of interconnected trails of small black *Iridomyrmex* ants (SBIs) consuming the sugary-exudates from the herbivores. The SEPHs are comprised not only of sap-sucking hemipterans from several species in the family Margarodidae (Sternorrhyncha) and an unidentified species in the family Membracidae (Auchenorrhyncha), *Sextius* sp., but also larvae of a species of the family Lycaenidae (Lepidoptera), *Jalmenus icilius*. The tritrophic association between these three groups is facultative on the part of the ants and the shrubs and is putatively a mutualistic one.

This project addressed five main aims related to this tritrophic association in semi-arid southern Australia and its shrub and SEPH partners at sites in the southern Australian location described above. These were to:

1. Determine factors involved in spatial distribution of *Acacia victoriae* in semi-arid Australian shrublands. (Chapter 2).

2. Determine factors involved in spatial distribution of SEPHs and SBIs and how they relate to each other. (Chapter 3).
3. Establish factors involved in determining densities of SEPHs on *A. victoriae* at individual and site scale. (Chapter 4)
4. Establish how *A. victoriae* and *Sextius* sp. might benefit from the association with SBIs. (Chapter 4)
5. Identify how factors characteristic of aridity affect distribution of the association and its effects on shrubs. (Chapter 5)

These aims describe the topic of each chapter in this thesis, starting with chapter 2 covering aim 1 and so on, except in the case of Chapter 4 which covers aims 3 and 4 because they are interconnected. Chapter 6 will link these chapters together and report conclusions of the project.

# Chapter 2:

# Small Scale Spatial Distribution of Shrub *Acacia victoriae* in Degraded Semi-Arid Australian Rangelands

## Abstract

Spatial distribution of Australian woody perennial *Acacia victoriae* in a semi-arid environment was investigated for evidence of intraspecific competition and clustering, including influences of seedling facilitation and soil resource heterogeneity. This species is one of the most common acacias in Australia and sometimes comprises the dominant canopy in semi-arid and arid systems. Seedling density in the area of influence of roots around live and dead conspecifics was compared with that in bare patches with no influence of woody perennials to reveal any evidence of facilitation; soil parameters on and beside dead shrub patches were also compared. Nearest neighbour groups of shrubs were used to find any distribution patterns connoting competitive effects, while size clustering of neighbour groups and correlation of size with soil parameters were used to identify effects connoting soil resource heterogeneity. Ripley's K function was used to determine clustering tendencies across whole sites. Seedling density was moderately higher near live conspecifics than in bare patches but substantially higher near large dead conspecifics than near live ones. Nearest neighbour patterns suggested both intraspecific competition and influence of patch resource heterogeneity, but top-soil resources were likely to be affected by large abundances of invasive weeds. Ripley's K function showed clustering at scales similar to those of neighbour groups. Clustering of mature shrubs across sites could be due partly to seedling facilitation leading to establishment of shrubs near conspecifics, while competition limits their density as they grow. Clustering is also probably in part due to variation in soil resources in different patches but clustering in size could have been enhanced by pulsed growth and intermittent establishment during periods of high moisture availability, which was not studied. These patterns are similar to those found for woody perennials in arid areas on other continents where initial clustering of seedlings reduces as they

grow. This semi-arid Australian example of such spatial patterns warrants further investigation including manipulative experiments to verify causes of these patterns.

## Introduction

Shrubs are the dominant life form in many arid environments (Schenk and Jackson 2002), so interactions and processes regulating their distribution are a major factor in arid zone community structure and function (Scholes and Archer 1997). Substantial areas of arid lands worldwide are degraded due to present or past stocking practises and understanding factors driving spatial distribution of vegetation is necessary for management and rehabilitation. One example of where this understanding is important for management is the recent trend towards increases in woody plant biomass in arid grasslands globally that have produced substantial changes to vegetation structure (Jeltsch et al 1997). Increased atmospheric $CO_2$ and temperatures due to climate change may already be augmenting these changes in shrub biomass (Hughes 2003), increasing the importance of knowledge of spatial distribution processes for rehabilitation/management of arid ecosystems as climatic conditions change in the near future. The natural processes that influence structure of woody perennial populations in xeric savannas and shrublands appear to differ from those in mesic environments. In arid environments the key factors are more severe water and nutrient restriction, unlimited light availability, and therefore acting as a stressor by leading to photo-inhibition (rather than plants competing for it), and heat stress. Therefore, information on spatial distribution of arid land vegetation cannot be derived solely from the more thoroughly studied mesic systems and will likely require the development of arid-specific models which include these factors.

Abiotic conditions are suspected to be of more importance to vegetation (relative to biotic processes) in arid areas than in mesic ones because of high density-independent mortality, particularly before recruitment, due to insufficient resources (Grime 1973, 1979; but see Chesson et al 2004). This mortality can mean competition is reduced because individuals are on average further from each other and root systems overlap less. Generally, low availability of some resources mean plant populations and communities cannot support large populations of herbivores and therefore herbivory is not thought to often limit vegetation (Crawford 1986). Additionally, fire occurs more commonly in arid zones due to high temperatures and low moisture levels and can regulate spatial distribution of woody perennials (Skarpe 1991). However, the different resource types are not necessarily independent; for example, abiotic conditions tend to be modified by biotic interactions such as when differing combustibility of plant species and plant individuals alters the soil around them. In any biome, soil modification by particular plant species can result in better conditions for smaller plants to establish (Scholes and Archer 1997, Facelli and Brock 2000, Callaway 2007). Soil resources are characteristically low in the arid zone , so creation of patches of higher soil resource concentrations by larger plants with larger root systems is likely to be of particular importance in arid areas. The characteristically high heterogeneity of soil in the arid zone (Crawford and Gosz 1982) appears to be enhanced by resource concentration by larger plants (Facelli and Brock 2000) which could further increase clustering of vegetation. Spatial heterogeneity in soil characteristics plays a key role in structuring vegetation in arid lands (Aguiar and Sala 1999). Temporal heterogeneity in moisture levels means soil resource concentrations are only likely to be important during resource pulses during which the majority of growth and competition probably occurs. Spatial and temporal heterogeneity of rainfall and spatial heterogeneity of soil in arid Australia exceed that of other areas of similar aridity (Stafford Smith and Morton 1990) and might shift predominating determinants of woody plant spatial distribution, resulting in different distribution characteristics from those on other continents.

Along with heterogeneity in soil resource levels, clustering of shrubs can also be caused by clonal reproduction, seed dispersal, topography, facilitation, germination patterns, or disturbance such as fire and pathogens (Scholes and Archer 1997). Harvesters collect seeds in their nests for food, a certain proportion of which may germinate, and in Australia the majority of seed harvesters are ants (Hughes and Westoby 1990). Shrub seeds, which tend to be larger than those of smaller plants, can accumulate in depressions, at the bottom of slopes, under the parent plant, or under plants on which birds perch and drop faeces and plant growth is affected by quantity and structure of rocks and stones in the soil. Seedling facilitation by conspecifics can create clustering of seedlings and shrubs in both mesic and xeric zones, and this could have a greater effect on spatial distribution in arid lands if the effect of facilitation is stronger as is thought. Related to this, if several seedlings near a conspecific sometimes germinate simultaneously, establish, and grow, it could produce patches with individuals of more similar size than expected from a random size distribution. Spatial heterogeneity of soil nutrients can create areas of concentrated vegetation alternating with sparse vegetation which affects growth of the shrub for its entire lifetime (Facelli and Brock 2000). Fire is a common disturbance in arid regions, but it can have different effects on spatial distribution of plants including clustering depending on conditions and intensity of fire (Skarpe 1991) and response of the seed bank which could reflect previous patterns of resource heterogeneity. All these mechanisms are possible candidates for producing clustering that can operate at various life stages of plants.

The relative importance of competition, the various mechanisms causing clustering of spatial distribution of woody perennials in arid zones, and how these factors differ between arid and mesic areas are not fully understood. Density of woody perennials can have a strong impact on community structure and function in semi-arid grasslands and shrublands (Scholes and Archer 1997) so these could be affected by intraspecific competition between woody perennials. A relatively dispersed distribution of larger shrub conspecifics is thought to reflect competition in arid shrublands and savannas of southern Africa (Skarpe 1991, Carrick 2003, Wiegand et al 2005), the Mediterranean area (Haase et al 1996), South America (Gutierrez and Fuentes 1979), North America (Yeaton and Cody 1976), and Australia (Cody 1991). Although many studies have recorded importance of competition between shrubs in arid savannas elsewhere, very few studies have adequately recorded signs of competition between woody perennials of arid Australia, including acacias (but see Cody 1991, Reid et al 1992). However, two chenopod shrub species in an arid Australian location did not exhibit such patterns (Facelli and Springbett 2009) so shrub species or other environmental factors can change the pattern.

Competition and facilitation by mature shrubs can produce different effects on different life stages of plants (Miriti 2006, Callaway 2007). Generally, seedlings compete with mature conspecifics for the same resources including soil nutrients and light. However, in the arid zone under the canopy of the mature plant, reductions of excessive ambient heat and light and resulting increases in humidity can decrease stresses for seedlings, which can override competition for soil resources (Callaway 2007). Moreover, soil in arid lands is characteristically dry and low in nutrients, which increases stress on seedlings. Hydraulic lift can occur in mature plants increasing soil moisture (Caldwell and Richards 1989) and accumulation of plant litter and sometimes of dung from sheltering animals which can increase soil nutrients (Scholes 1990, Facelli and Picket 1991) creating further benefits for seedlings and seeds can accumulate under canopies from droppings of birds perched on them. Thus facilitation of seedlings by larger conspecifics is thought to have a greater influence on plant distribution relative to competition and be more common in arid areas than in mesic areas (Callaway 2007). Moreover, in arid lands because competition is thought to have weaker effects than in mesic, some degree of seedling clustering can remain throughout the life of shrubs (Skarpe 1991).

On the Australian continent, shrub-lands and low woodlands cover about 40% of land area and about 60% of its arid lands (Moore 1973) making them important vegetation types. Within these and other habitat types, sclerophyllous Eucalyptus and Acacia are the dominant woody genera in most parts of arid Australia. The Australian endemic shrub *Acacia victoriae* occurs in all states, mainly in dry areas (Fowler and Fox 1995), being the second most widespread Acacia throughout the continent (Fox 1987). It inhabits a wide latitudinal range, growing in areas with rainfall from 100 to 1000mm per annum, which allows its growth, mortality, and reproductive output to be compared over a wide range of climatic conditions. This makes it an important species for the ecology of arid Australia.

The study examines via non-manipulative correlational survey methods:

1. Spatial distribution connoting intraspecific competition in mature *Acacia victoriae*.

2. Spatial distribution connoting clustering due to heterogeneity in soil resources in mature *Acacia victoriae*.
3. Spatial distribution of seedlings of *Acacia victoriae* that connotes facilitation by live or dead shrubs.

## Methods

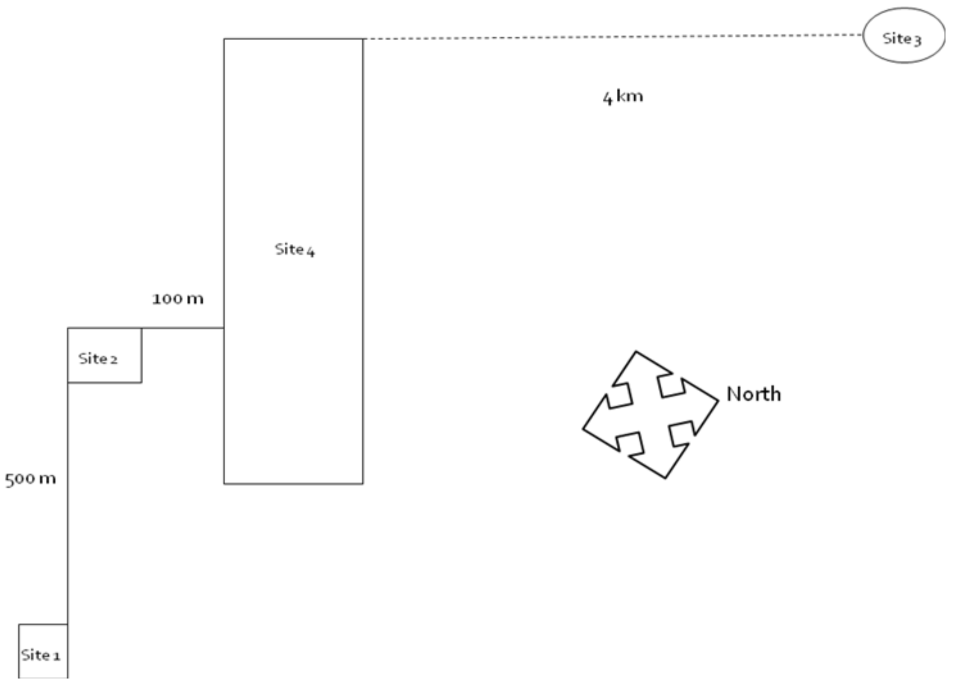


Figure 1: Survey sites used in this study

This study was conducted in the Flinders Ranges National Park, 500 km north of Adelaide, South Australia, on a previously heavily grazed former sheep station, currently under a rehabilitation program. At an elevation of 550m , mean annual rainfall is 321mm, with median 324mm and coefficient of variation 31%, with a mean 66% rain falling in the colder months from May to October, from records which span from 1985 to 2009. Mean annual areal potential evaporation is 1200-1300mm (Bureau of Meteorology), giving an aridity index $AI_U$ of 0.25-0.27 (UNEP 1992). There was 199 mm rainfall in the 12 months before the study took place, which was less than the mean minus the standard deviation of rainfall for that time period, with only 37mm falling in the three months beforehand, which was about half the spring median of 72mm. The predominant soil type is calcareous loam. Still severely degraded and supporting huge rabbit populations, understory vegetation is sparse and dominated by the exotic invasive species *Carrichtera annua*, *Asphodelus fistulosus*, *Echium plantagineum*, and *Marrubium vulgare*, with *A. victoriae* as the dominant canopy in many areas. In the semi-arid Flinders Ranges of southern Australia, *A. victoriae* was present at 27% of survey sites and constituted the dominant canopy in many (Brandle 2001), and might be particularly common in areas degraded by agricultural grazing. Reproduction is by germination from a seed with a relatively large elaiosome attached which aids its dispersal by ants such as *Rhytiponera* and *Iridomyrmex purpureus*, although it is likely that this is not species specific (Davidson and Morton 1984). *Rhytidoponera* sp. of over 10mm are in high abundance at these sites (pers. obs.). Growth and reproductive period of *A. victoriae* are seasonal, making it more predictable than fully arid-adapted species, which gives it advantages for some studies. The seeds would be too large to be dispersed by any but the largest birds such as emus.

Sites 1 and 2 were selected for the study because they were relatively flat, of sufficient size, almost all woody plants were *A. victoriae*, and they also met conditions for a simultaneous study on insect-plant interactions (see Chapter 3). On site 1 selected in December 2008, grid of 55m x 55m with 5m x 5m quadrats was constructed with stakes driven into the ground. On site 2, 500m away, selected in January 2009 a similar grid of 45m x 85m was constructed. Disturbed areas where explosives had destroyed rabbit warrens were avoided as were washes and large areas of surface rock. Very few shrubs of other species were present on sites and their root canopies did not intersect with those of shrubs used in the study. *A. fistulosus* and *C. annua* are abundant, particularly in spring. Shrub location on the grid was mapped according to quadrat and distance from nearest quadrat edges. Shrub height, h, maximum canopy width, $w_{max}$, canopy width perpendicular to the maximum, $w_{perp}$, and stem circumference at the base below the first branch were recorded. The study only considered adult shrubs; those less than 0.30 m high were ignored since they were pre-reproductive (pers. obs.).

To compare effects of dead shrubs, mature shrubs, and available open spaces on *A. victoriae* germination, a survey on site 4, an area of approximately 800m x 200m approximately 100m north of site 2, was conducted in October 2010. The area was reasonably flat and of similar soil type, but there would be some drainage on and off it, and washes were avoided. Ten live *A. victoriae* of canopy diameter at least 2m were haphazardly selected so that a circle of radius one canopy diameter around them did not intersect with a circle of radius two canopy diameters of any other live or dead woody plants around the other shrubs. Shrubs under which at least 50% of soil surface was covered with stones were avoided since this could inhibit seedling growth. Most live *A. victoriae* at the site had either dead conspecifics or live shrubs of other species too close to use them in the survey. Seedlings were counted within one canopy diameter of the base of each shrub, in a circle divided by the four compass points to avoid double counting. Where dense stands of invasives, mainly *A. fistulosus* , potentially obscured seedlings, care was taken to look amongst them and when necessary they were pulled out to facilitate counting. Ten dead *A. victoriae* of estimated canopy diameter of at least 2.5m were located. The canopy was estimated as slightly larger than live shrubs to estimate the effect of the higher likelihood of death of senescent shrubs, whose branches spread without increase in biomass. Any other shrub was at least two of its canopy diameters from within one canopy diameter of the dead shrub. Dead shrubs surveyed had some of the stem still in the ground even if it was broken off, at least 50% of the outer bark remaining but separated from the stem, and at least 50% of third order twigs of diameter 3-5mm remaining. Almost all dead shrubs had bark which had all separated from the stem. This was done to limit time since death in case effects of their root system on the soil had gone. Almost all shrubs were estimated to have been dead for at least eight years. Canopy diameter of dead shrubs was estimated from standing or fallen limbs. Canopy diameter error was estimated as slightly larger than measured to prevent wrongly increasing seedling density. It was difficult to find dead shrubs with live shrubs or dead shrubs or trees of other species a sufficient distance away as required by methodology. Another treatment of dead with alive shrubs were selected using the same methodology. Within the circle of radius one canopy diameter of the central dead shrub were at least one live mature *A. victoriae* of canopy diameter at least 1m or multiple live shrubs totalling at least the same volume. Only eight replicates were found. Ten *Rhagodia parabolica* at least 2m in diameter with both live and dead *A. victoriae* nearby were chosen haphazardly as a comparison. As a control treatment, ten rectangular areas were located of width and length of at least 5m with woody plants at least one canopy diameter away. These were called bare patches. Since they had very low seedling density, their area was allowed to be larger than that of the other patches if possible to not underestimate number of seedlings. It was difficult to find bare patches of sufficient size amongst areas where *A. victoriae* were, so most bare patches were at the edge of these areas.

No obvious accumulation of seeds under shrub canopies was seen over the several months of the study (pers. obs.). Seed pod covers with some seeds still attached seeds are scattered across the ground, not just under shrub canopies, after seeds mature (pers. obs.), presumably by wind, water, and possibly ants. *Rhytidoponera* sp., a large species which could carry the large seeds of *A. victoriae*, are in high abundance on these study sites (pers. obs.) which is consistent with preference of the genus for heavily degraded sites. Therefore a large proportion of seeds would be dispersed from under shrub canopies. Longevity of these seeds is estimated at ten to twenty years.

The shape of *A. victoriae* shrubs is irregular and varies substantially amongst individuals, reducing the accuracy of any formula for estimating biomass. Shrub volume was approximated by an inverted cone with oval base more closely than any other geometric shape (figure 1). Shrub height, h, had a regression relationship with mean canopy width, w, that was only moderately strong ($\ln(h + 1)$, $\ln(w + 1)$, $n = 225$, $p = 0.0001$, $r^2 = 0.746$), so both h and w must be used to estimate shrub biomass. For this study, shrub volume, v, was estimated from height, h, and both perpendicular widths, $w_{max}$ and $w_{perp}$, as the volume of a cone with oval base, $\pi/3\ h\ (w_{max}/2)\ (w_{perp}/2)$.

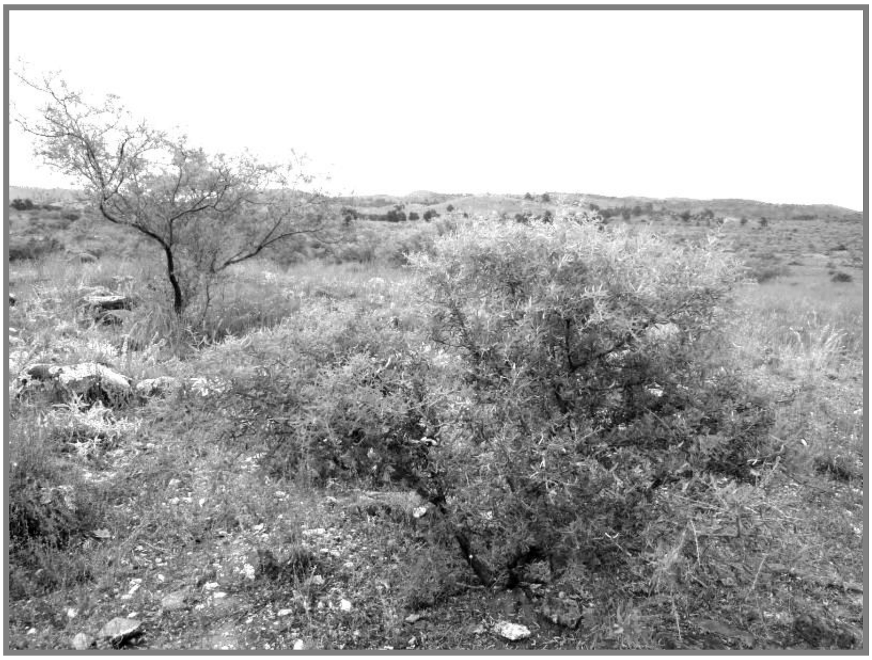


Figure 1: Two *Acacia victoriae* individuals, 2011. (Photo N. Weichel.)

Other studies have used different estimates of volume as a substitute for biomass which correlated reasonably closely with the method used in this study. One study of this species in arid NSW (Grice et al 1994) found that height + mean width, S, correlated very closely with wet weight, W, of 32 *A. victoriae* of different sizes in the range S = 0 to over 6 m ($\log_{10}(S + 1)$,$\log_{10}(W + 1)$, $n = 32$, $r^2 = 0.94$). Another study used height x width, HW, to estimate biomass (Reid and Stafford Smith 2000). Volume, v, of all shrubs on sites 1 and 2 pooled correlated strongly with S (SPSS, $\ln(\sqrt[3]{v})$, $\ln(S)$, $n = 225$, $p = 0.0001$, $r^2 = 0.821$) and with HW (SPSS, $\ln(\sqrt[3]{v} + 1)$, $\ln(\sqrt{HW} + 1)$, $n = 225$, $= 0.0001$, $r^2 = 0.803$). Total radius, $R_{tot}$, of combined shrub stems was calculated from the summed area of stems on shrubs and correlated strongly with S (SPSS, $\ln(R_{tot} + 1)$, $\ln(S + 1)$,$n = 99$, $p = 0.0001$, $r^2 = 0.862$), with volume (SPSS, $\ln(R_{tot} + 1)$, $\ln(\sqrt[3]{v} + 1)$, $n = 99$, $p = 0.0001$, $r^2 = 0.892$), and with HW (SPSS, $\ln(R_{tot} + 1)$, $\ln(\sqrt{HW} + 1)$, $n = 99$, $p = 0.0001$, $r^2 = 0.874$). Since shrub volumes were used to calculate insect densities in another study (Chapters 3, 4, 5), volume was preferred over S or HW.

For neighbour group analyses, soil was taken with a 5cm long core in samples of approximately 50g from neighbour groups one canopy diameter from each shrub in the directions of the four compass points. Soil from each patch was pooled for analyses. For the seedling density survey, soil was sampled inside and outside the estimated zone of influence of shrub roots of dead shrubs. The top 5cm of soil was taken in approximately 50g lots one metre from the stem base directly to the north, east, south, and west and then pooled. Soil was also sampled in the same way two estimated canopy diameter widths from the shrub stem base. All pooled soil samples were analyzed for concentrations of total nitrogen, $N_{total}$ (%), available phosphorous, $P_{available}$(mg/kg), potassium, K(mg/kg), organic carbon, $C_{organic}$(%), and sulphur, S (mg/kg), and for electrical conductivity, EC (dS/m) and pH (using the $CaCl_2$ method) at CSBP Laboratories in Bibra Lake, Western Australia.

Nearest neighbour analysis using multiple neighbours is an established method for determining strength of competitive effects that regulate age or biomass and spatial distribution of woody perennials even when competition does not increase mortality that does not involve lengthy experiments. Positive correlation between volume, usually used as a surrogate for biomass, and distance indicates that larger shrubs are on average further from each other, caused by competition or density independent mortality that are inversely proportional to shrub size. A shrub is randomly selected as the target with its nearest neighbours as the neighbour group. Use of only one neighbour underestimates influence of large shrubs further away which have more influence on the target shrub due to their size, so using more than one neighbour provides more statistical robustness (Shackleton 2002). Also, using more neighbours may increase the chance that there are shrubs in the neighbour group whose roots overlap because the next closest shrub to the target might be closer to one of the non-target shrubs in the neighbour group. This will increase detection of any existing competitive effects. This is particularly important in cases where root system diameter of shrubs is not as large as expected, and often little is known about the size of the shrub root system.

For neighbour analyses, target shrubs were selected randomly from shrub maps until there were no unselected groups of five nearest neighbours. A maximum of one neighbour shrub in a group was allowed to overlap with a neighbour shrub of another group. No shrubs of other species were within two canopy diameters of two canopy diameters of any target or neighbour shrub. Nearest neighbour analyses can be used to differentiate between intensity and importance of competitive effects on spatial distribution (Welden and Slauson 1986). The higher the $r^2$ value, the more important any existing competitive effects are to the spatial distribution of the species on that site. The slope indicates strength (intensity) of the relationship (Welden and Slauson 1986). The stronger the relationship, the more distance on average will be required between shrubs to be able to reach the same total volume and the higher the slope will be.

Age of *A. victoriae* does not correlate well with size (Grice et al 1994) and there is no other way to determine shrub age, so shrub age was not considered here. Small individuals less than 30cm high can remain at the same size for several years without growing (G. Mütze, pers. comm.). There is no information available on structure of roots of *A. victoriae*, making estimation of root diameter difficult; the rockiness of the soil precluded the possibility of adequate measurement. For the purposes of this study, diameter of area of influence of roots was estimated as two canopy diameters of the individual.

Clustering of plants can be detected using the univariate Ripley's K function. The pattern of points representing plant location on a grid is analysed by randomly placing circles of radius less than and equal to the selected radius around randomly selected points on the map and counting the number of points representing plant location within each circle. From the distributions of numbers of points representing plant location within circles of different radii, Monte Carlo simulations are used to construct confidence envelopes for comparing the existing pattern with the null hypothesis of complete spatial randomness (Ripley 1976).

Positive correlation between target shrub size and summed size of neighbour shrubs indicates that on average nearest neighbours are more similar in size to the target than would be expected from a random size distribution. For a random size distribution, slope would be zero indicating there is no size correlation between a randomly chosen shrub and those nearest to it. Over a number of neighbour groups, this means the size of a shrub is not linked to the size of shrubs nearest it. The reasons for this could include that on average shrubs near each other germinate and establish at more similar times than random (growing fast at times of unusually high rainfall and growing relatively little in between) or that resources within a neighbour group have a similar influence on neighbours and that all shrubs in the group grow at a rate relative to shared resource quality. As the slope approaches four, the maximum possible, on average a shrub becomes more similar in size to the other shrubs nearest it, so the stronger the effect gets. The closer the slope is to to four, the closer in time nearby individuals germinate. This would be more likely if seedlings germinate near the parent shrub at the same time due to infrequent high rainfall and establish. The parent shrub which would be larger than these shrubs would be nearby until it dies, which would reduce size uniformity of groups of shrubs that are near each other. A slope of four would never occur because even if nearby shrubs germinated at the same time, they would not necessarily be in the same neighbour group. Variation in the pattern might be caused by different intrinsic growth rates of shrub individuals or different intrinsic susceptibility to herbivory. The further from four the slope is, the more different on average across the site are the exact times when germination or growth of different shrubs occurred. A lower slope nearer zero, indicating more variance of shrub size in the same location amongst groups of nearest neighbours, suggests predominant influence of variable resource quality among patches, perhaps with pulsed growth. Area of neighbour groups differs and soil heterogeneity increases with distance, which adds variability to any underlying pattern.

### Analyses

Primer-E 6.0 with PERMANOVA+ (Anderson 2001) with Euclidean distances was used for all factor analyses and associated tests of dispersion. Permanova tests were used for all factor analyses. PermDisp was used to check whether data sets used for factor analyses were evenly dispersed with respect to factors. SPSS V17.0 – 19.0 (SPSS 2011) was used for all regressions and correlations. *Programita* (Wiegand and Moloney 2004) was used to determine degree and scale of aggregation on each site by Ripley's K function.

A relationship between shrub volume per area of neighbour groups and soil parameters might reveal how much spatial heterogeneity in soil parameters might influence spatial distribution of shrubs. Soil parameters of neighbour patches, s, were transformed to natural logarithms, $\ln(s + 1)$, and regressed against total neighbour group shrub volume, $V_5$, per summed four distances between central shrub and each of four neighbours, $D_4$ transformed to approximate shrub volume per area and then to natural logarithms: $\ln(\sqrt{V_5} / D_4 + 1)$. Distance was used as surrogate for area of neighbour groups since area of shrub roots is unknown and was not calculated for the neighbour groups.

To look for differences in seedling density near live and dead shrubs, seedlings per root system area, S, was tested in single factor analyses after transformation to natural logarithms, ($\ln(S + 1)$). Root system area of each shrub was calculated using the canopy diameter as the radius of a circle around the shrub stem in the ground.

Soil parameters of dead shrubs were transformed to natural logarithms, outliers removed, and one-factor analysis tested to compare soil samples near shrubs and samples from two canopy diameters away.

Using the nearest neighbour method, a regression relationship between summed distances between neighbour shrubs and total shrub volume was sought. All ten distances between each pair of neighbour shrubs were summed, $D_{10}$, and transformed to square roots and then transformed to natural logarithms ($\ln(\sqrt{D_{10}} + 1)$). Against these values were regressed natural logarithms of cubic root of total volume, $V_5$, of all shrubs in a neighbour group ($\ln(\sqrt[3]{V_5} + 1)$). This was done for each site and for datasets from both sites pooled. Although distance between shrubs may approach zero, for example if seedlings in a clump of emu dung or shallow ant nest germinated together, intercept suppression was not used since the relationship might change over the unmeasured range of values.

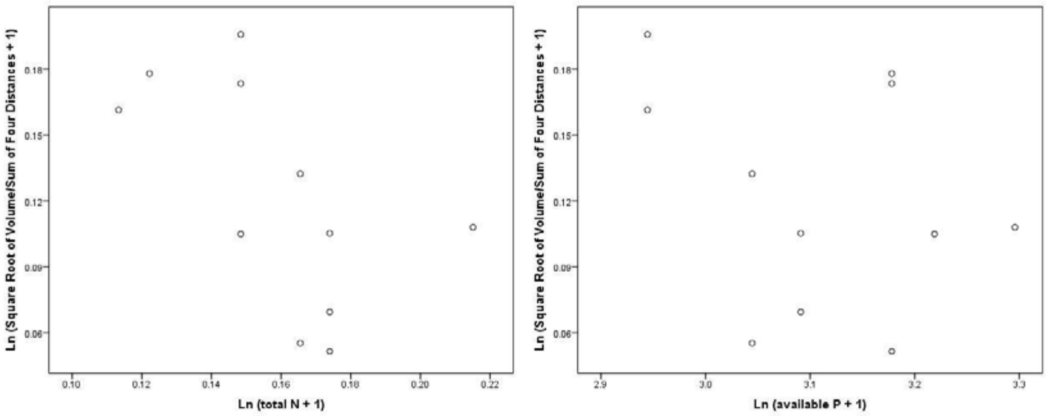
Ripley's K function was used to ascertain whether there was clustering of shrubs on whole sites using points on the grid maps.

To find the level of uniformity in size of shrubs in patches of relatively similar size, the volume of the four nearest neighbours, $V_4$, was regressed against volume of the target shrub, $v_t$. Shrub volumes were transformed to cubic roots before analysis to make the relationship linear. Neighbour group patches are different sizes and competition effects could be less where more shrub biomass is further away from other shrubs in the group. Therefore to correct for the distance per volume, volume of four neighbours ($^3\sqrt{}V_4$) was then divided by the linearized distance per volume of shrubs, $\ln(\sqrt{}D_{10}+ 1) / \ln(^3\sqrt{}V_5 + 1)$, and correlated against target shrub volume, $\sqrt{}^3v_t$.

# Results

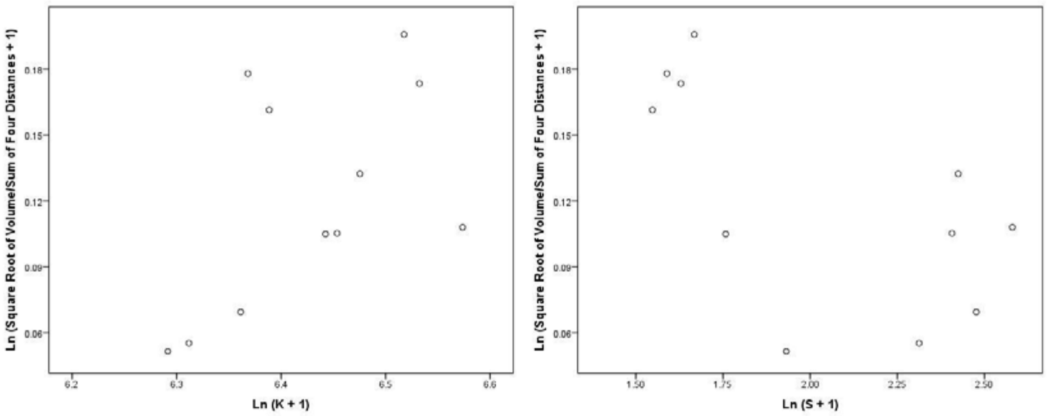
## Relationship of soil parameters with shrub volume per area at neighbour group scale

There were regression relationships for some soil parameters with total volume per distance of neighbour groups, $\ln(( \sqrt{}V_5 / D_4 + 1)$ (graph 1, table 1). The strongest regression of total volume per distance was with potassium, K, and total nitrogen, $N_{total}$, combined ($\ln(( \sqrt{}V_5 / D_4 + 1)$, $\ln(N_{total} + 1)$, $\ln(K + 1)$, $n = 11$, $p < 0.0001$, $r^2 = +0.882$, adj. $r^2 = 0.852$; K: $0.420 \pm 0.070$, $p < 0.0001$; $N_{total}$: $b = -1.448 \pm 0.231$, $p < 0.0001$). The influential point in the top left corner of graphs 1g and 1h was removed in the regressions with pH (table 1). There was no relationship between volume per distance and either $P_{available}$ or $C_{organic}$.



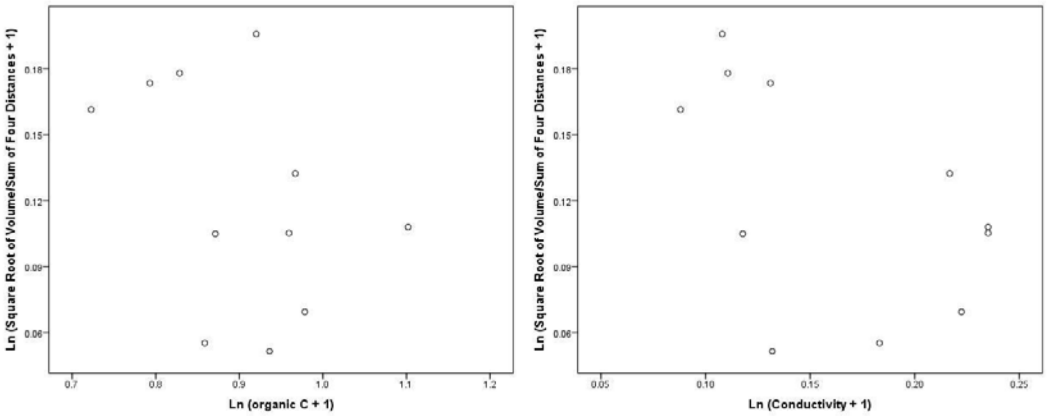
(a) Nitrogen Δ

(b) Phosphorous



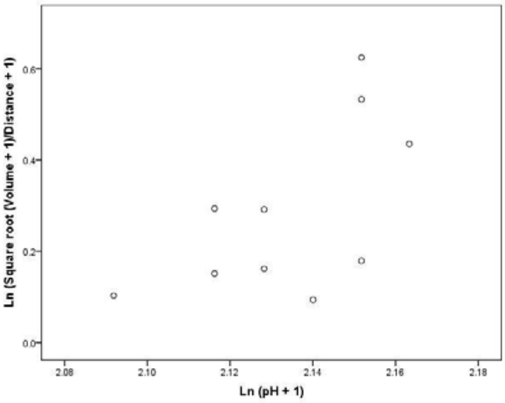
(c) Potassium Δ

(d) Sulphur *



(e) Organic Carbon

(f) Conductivity



(g) pH $^{\Delta}$

Graph 1: Soil parameters, $\ln(s + 1)$, against volume per distance $\ln((\sqrt{V_5} / D_4 + 1)$

N.B. In graphs, $^{\Delta}$ indicates marginal significance, $p \leq 0.08$; * indicates $p \leq 0.05$; and ** indicates $p \leq 0.005$

Table 1: Regression of soil parameters, $\ln(s + 1)$, against volume per distance, $\ln((\sqrt{V_5} / D_4 + 1)$

| n = 11 | | $N_{total}$ & K combined | $N_{total}$ | K | S | pH<br>(n = 10) |
|---|---|---|---|---|---|---|
| Volume per distance | $r^2$ | 0.882<br>(N negative $p < 0.001$,<br>K positive $p < 0.001$) | -0.343 | +0.302 | -0.399 | +0.364 |
| | p | <**0.0001** | *0.058* | *0.080* | **0.037** | *0.065* |

N.B. In tables, **bold type** indicates statistical significance and *italics* indicate marginal significance.

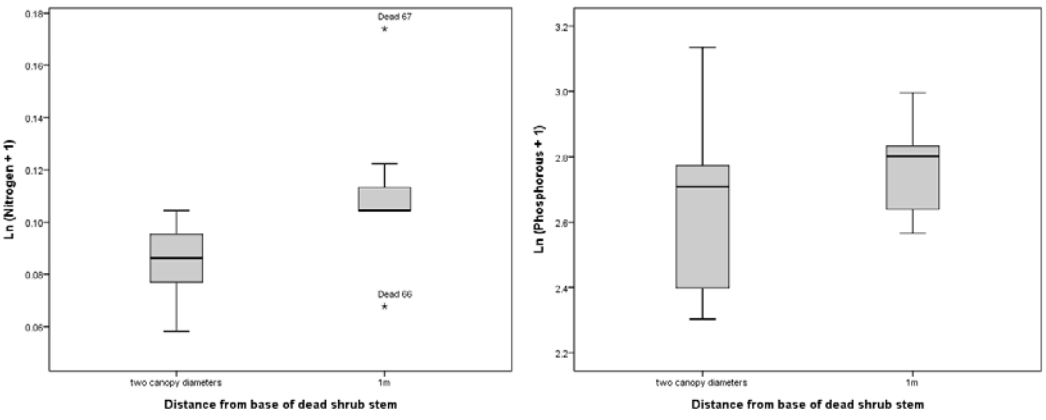
Table 2: Auto-correlating soil variables in neighbour groups (ln(s + 1), Pearson correlation)

| n = 11 | | S | $C_{organic}$ | EC |
|---|---|---|---|---|
| $N_{total}$ | r | +0.839 | +0.908 | +0.807 |
| | p | **0.001** | **<0.001** | **0.003** |
| S | r | | +0.801 | +0.972 |
| | p | | **0.003** | **<0.001** |
| $C_{organic}$ | r | | | +0.775 |
| | p | | | **0.005** |

## Facilitation of seedlings by mature and dead conspecifics

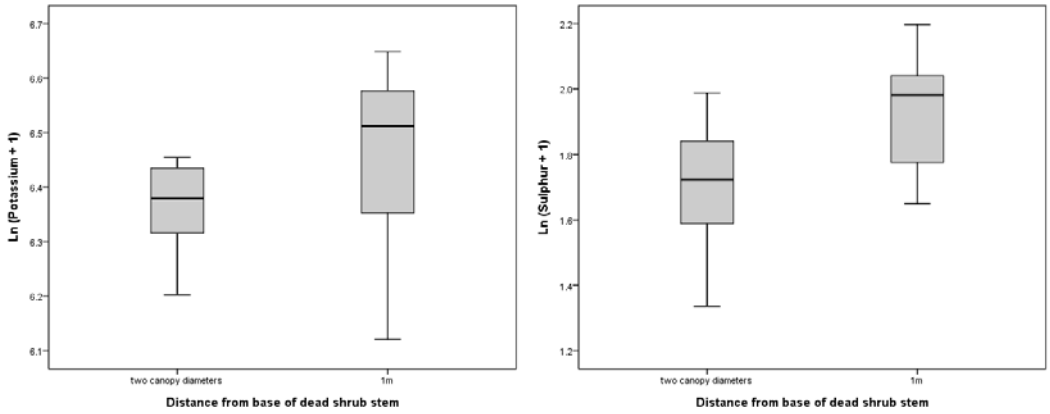
Seedling density, ln(S + 1), in the area of influence of roots of live shrubs was higher than in bare areas (n = 17 (live shrubs), 10 (patches); p = 0.045). Density of seedlings near only dead *A. victoriae* was not different from density near combinations of dead and live *A. victoriae* (n = 10 (dead only), 8 (dead and alive); p = 0.591), so data for dead shrubs were pooled and seedling density near all dead shrubs was higher than near live shrubs (n = 17 (live shrubs), 18 (dead shrubs); p = 0.0001) or bare patches (n = 10, p = 0.0001). Density near saltbushes *R. parabolica* with both live and dead *A. victoriae* was higher than near live *A. victoriae* alone (n = 9 (saltbushes), 17 (live); p = 0.015) but lower than near dead *A. victoriae*, of marginal significance (n = 9 (dead shrubs); p = 0.060). Seedling density was dispersed differently amongst bare patches than dead shrub patches (PermDisp, p = 0.0055) or live shrub patches (PermDisp, p = 0.0260). There was no relationship between seedling density near live shrubs and shrub volume. Average seedling density around dead shrubs only was 0.89 $m^{-2}$, around dead with live shrubs 0.98 $m^{-2}$, around live shrubs only 0.12 $m^{-2}$, around saltbushes 0.39 $m^{-2}$, and in bare areas 0.02 $m^{-2}$.

Parameters of soil taken next to the stem of a dead shrub differed from those in soil taken two canopy diameters away (graph 2). Next to dead shrubs sulphur at 6 mg/kg ($p = 0.0077$, $n = 10$) and total nitrogen at 0.117 % ($n = 10$, $p = 0.0091$) concentrations were both on average 34% higher and organic carbon at 0.979% was 50 % higher ($p = 0.0002$, $n = 10$) than two canopy diameters away. Potassium concentration at 648.2 mg/kg did not differ between positions ($n = 10$, $p = 0.0988$). In a different study, parameters of soil taken under the canopy of different live shrubs of at least 2 metres in canopy diameter to the north, N, and south, S, also differed from those in soil taken two canopy diameters away from the stem base (unpublished results). After the removal of one outlier, sulphur levels were 36 % higher in N than Off ($n = 9$, $p = 0.00017$) and 25% higher in S than Off ($n = 9$, $p = 0.0034$). Total nitrogen was on average 31% higher in both N than Off ($n = 9$, $p = 0.0108$) and in S than Off ($n = 9$, $p = 0.0109$) after one outlier was removed. Organic carbon levels were 22% higher in S than Off ($n = 10$, $p = 0.018$). Potassium levels were 18% higher in N than Off ($n = 10$, $p = 0.0004$) and possibly 8% higher in S than Off, although this was of very marginal significance ($n = 10$, $p = 0.085$). The pH of $H_2O$ was 1% higher in N than Off after removal of the same outlier as for total nitrogen ($n = 9$, $p = 0.0181$).



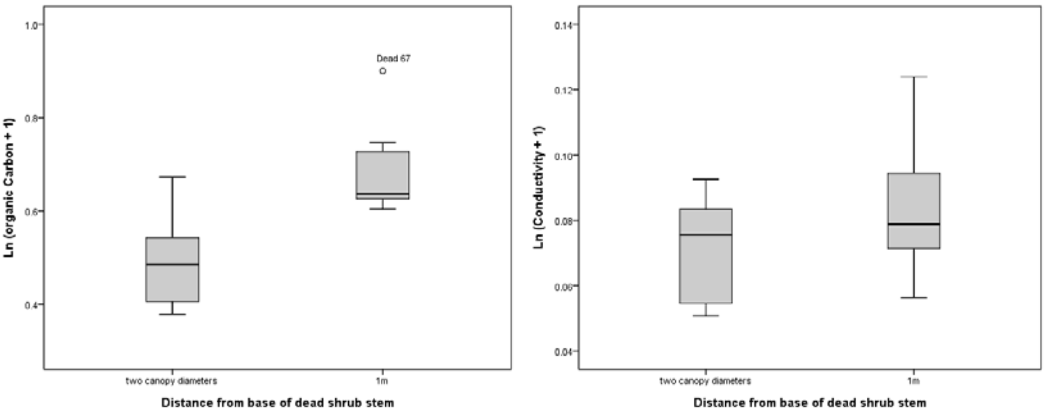
(a) Total nitrogen *

(b) Available phosphorous



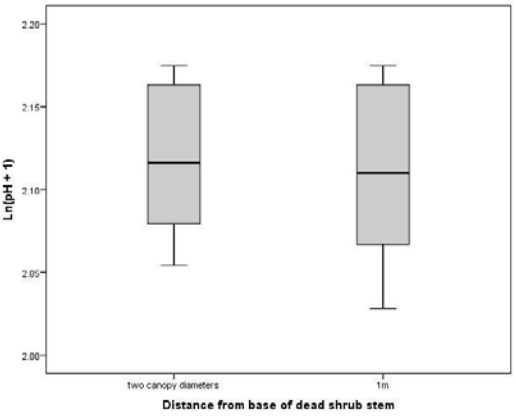
(c) Potassium

(d) Sulphur *



(e) Organic carbon **

(f) Electrical conductivity

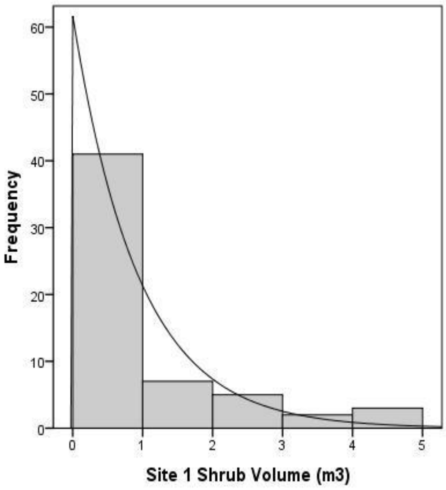


(g) pH

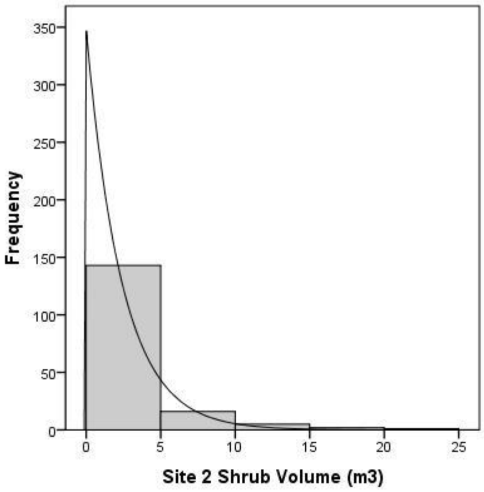
Graph 2: Comparison of soil parameters, ln(s = 1), near (1m from base of stems of) dead shrubs and two canopy diameters away

## Shrub demographics

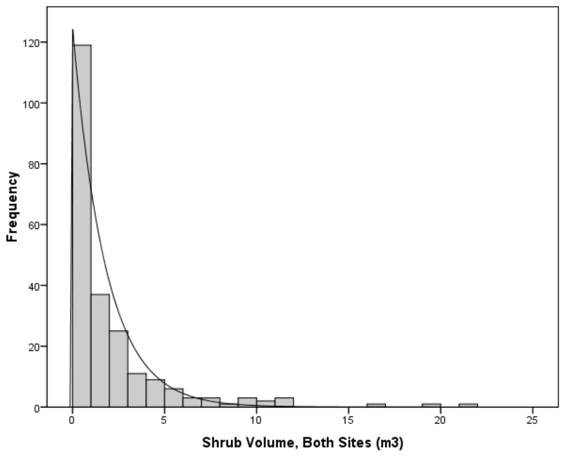
The two sites differed somewhat in demographics, showing slightly different patterns of spatial distribution. Mean sizes of all shrubs surveyed, v, were different on sites 1 and 2 (table 3) ($n = 169$: $^{3}\sqrt{v}$, $p = 0.0001$; $\ln(^{3}\sqrt{v})$, $p = 0.0002$), with site 2 shrubs almost twice as large on average as those on site 1. However, a negative exponential distribution of shrub volume was apparent on both sites (graphs 3 and 4) and from combined sites (graph 5) and neighbour group data (graph 6). Mean total volume of neighbour group shrubs on site 2 was three times that on site 1 (Permanova, $n = 9/23$, $p = 0.0008$) and range of values on site 2 was much wider than on site 1 (table 1). Summed distances between pairs of shrubs in groups did not differ between sites (Permanova, $n = 9/23$, $p = 0.391$).

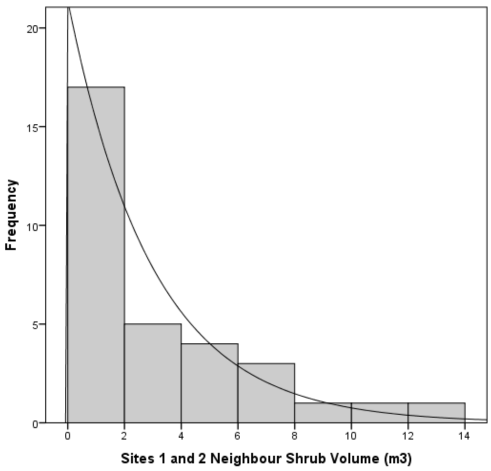


Graph 3: Volume frequency distribution of shrubs on site 1



Graph 4: Volume frequency distribution of shrubs on site 2





Graph 5: Frequencies of Shrub Volume, both sites n = 227

Graph 6: Volume frequency distribution of all neighbour shrubs (n = 32)

Table 3: Demographics of all individual *A. victoriae* on the two sites

| Site | Area ($m^2$) | Canopy area ($m^2$) | | | | Shrub volume ($m^3$) | | | | Shrubs per square metre ($m^{-2}$) | Canopy per area ($m^{-2}$) | Volume per area ($m^3m^{-2}$) |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Mean | n | Min | Max | Mean | n | Min | Max | Mean | Mean | Mean |
| 1 | 3025 | 1.87 | 58 | 0.10 | 6.49 | 0.94 | 58 | 0.015 | 4.62 | 0.019 | 0.075 | 0.018 |
| 2 | 5735 | 3.24 | 169 | 0.09 | 20.42 | 2.31 | 169 | 0.009 | 21.37 | 0.029 | 0.130 | 0.092 |
| Pooled | | 2.89 | 227 | | | 1.96 | 227 | | | | | |

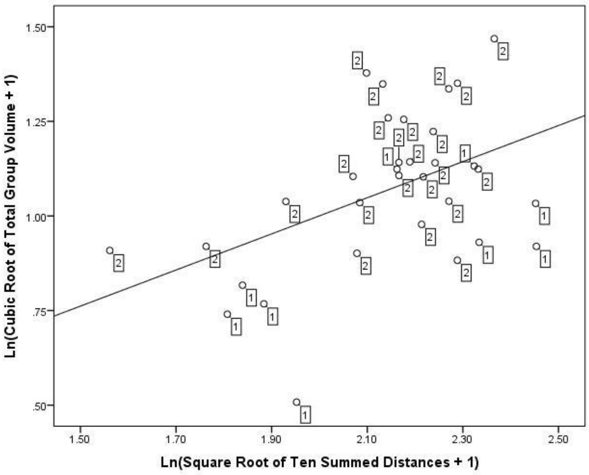
On site 1, mean distance between target shrub and furthest neighbour was 6.6m, mean distance between neighbours was 5.3m, maximum distance between neighbours was 10.5m, and mean canopy diameter of all shrubs was 1.54m. On site 2, mean distance between target shrub and furthest neighbour was 6.0m, mean distance between neighbours 4.2m, maximum distance between neighbours 10.5m, and mean canopy diameter of all shrubs 2.04m. Therefore on average a circle of radius two canopy diameters around a shrub estimating the root system area will overlap with a similarly derived circle around at least half of the other neighbour shrubs in the group.

## Volume-distance regression suggesting intraspecific competition

Total neighbour group shrub volume, $V_5$, had a regression relationship with summed distances between all ten pairs of shrubs in the group, $D_{10}$ ($x = \ln(\sqrt{D_{10}} + 1)$, $y = \ln(\sqrt[3]{V_5} + 1)$, SPSS, $n = 32$, $p = 0.007$, $r^2 = 0.221$, adj. $r^2 = 0.195$, slope $0.477 \pm 0.164$, constant $0.046 \pm 0.352$) (graph 7). Site 1 neighbour shrub groups were smaller than those on site 2 (table 4). Groups of shrubs smaller than those included in the study were observed much closer together than shrubs in the study and groups of seedlings were sometimes in small groups of up to six with individuals less than 10cm from each other (pers. obs.)

Table 3: Demographics of *A. victoriae* neighbour groups on the two sites

| | | Total volume ($m^3$) | | | Summed distances (m) | | |
|---|---|---|---|---|---|---|---|
| | n | Mean | Min | Max | Mean | Min | Max |
| Site | | 0.0008 ($\ln(\sqrt[3]{V})$ | | | 0.4365 ($\ln\sqrt{D_{10}}$) | | |
| 1 | 9 | 4.04 | 0.29 | 9.28 | 22.58 | 8.13 | 35.01 |
| 2 | 23 | 11.99 | 2.85 | 37.34 | 18.92 | 5.32 | 31.13 |
| Pooled | 32 | 9.75 | 0.29 | 37.34 | 19.95 | 5.32 | 35.01 |



Graph 7: Total neighbour group shrub volume ($\ln(\sqrt[3]{V_5} + 1)$) against ten summed distances ($\ln(\sqrt{D_{10}} + 1)$)

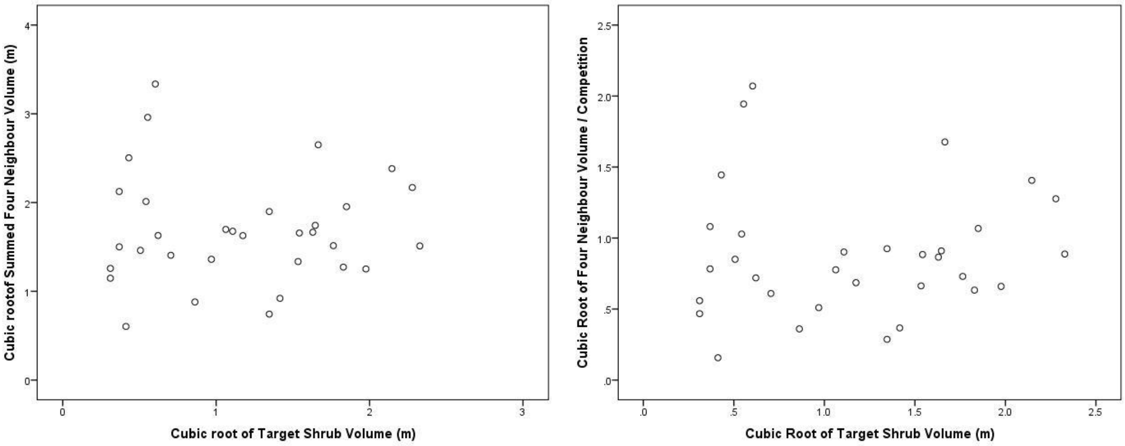
## Clustering of shrubs

Spatial pattern of shrubs showed clustering at scales up to 28m on site 1 (Ripley's, 99 simulations, n = 58, cell size 1m, $p \leq 0.05$, point size 0.2m, $C_g$= 0.1, $C_L$= 18, cluster size 8.57 m to 41m) and also on site 2 (Ripley's, 99 simulations, n = 145, cell size 1m, $p \leq 0.05$, point size 0.2m, $C_g$= 0.2, $C_L$= 18, cluster size 5.2m to 48 m). This is a similar scale to neighbour shrub groups.

## Pattern of distribution of shrub size

Two different patterns of target shrub with summed neighbour volume occurred. For the eleven target shrubs of volume 0.24$m^3$ or less, there was no relationship between target and neighbour volume. For larger target shrubs, there was a positive regression for summed neighbour volume, $V_4$, with target volume, $v_t$, although significance was marginal ($\sqrt[3]{V_4}$, $\sqrt[3]{v_t}$, n = 21, p = 0.071, $r^2$ = 0.161, adj. $r^2$ = 0.117, slope 0.416 ± 0.218, constant 0.949 ±

0.347). Uniformity of shrub size would be represented by a slope of 1.59 since shrub volume is transformed to cubic roots. When $^3\sqrt{\Sigma} V_4$ is divided by $(\ln(\sqrt{D_{10}} + 1) / \ln(^3\sqrt{V_5} + 1))$ to correct for competition effects caused by different distances between shrubs in a group, variation in the relationship with target shrub volume reduces ($^3\sqrt{V}$, n = 21, p = 0.042, $r^2$ = 0.279, adj. $r^2$ = 0.241, slope 0.398 ± 0.147, constant 0.203 ± 0.234). Uniformity of shrub size due to resource quality in this relationship would be represented by a slope of 0.76 since the slope of the competition graph is 0.477.



(a) $^{\Delta}$ (b) *

Graph 8: (a) Target shrub volume ($^3\sqrt{v_t}$) against summed volume of four neighbours ($^3\sqrt{V_4}$) (b) Target shrub volume ($^3\sqrt{v_t}$) against summed volume of four neighbours ($^3\sqrt{V_4}$) divided by summed distances to target per total volume $(\ln(\sqrt{D_{10}} + 1) / \ln(^3\sqrt{V_5} + 1))$

$^{\Delta}$ indicates marginal significance

# Discussion

Spatial distribution of *Acacia victoriae* in nearest neighbour groups in degraded semi-arid Australian lands points both to effects of intra-specific competition and heterogeneity of soil resources and is probably influenced by seedling facilitation by mature live or dead conspecifics. Parameters of top soil near dead mature shrubs were different from those outside the zone of influence of the roots and seedling density was higher near dead shrubs than near live shrubs. Around both live and dead shrubs, seedling density was higher than in bare patches without woody perennials. Regression of distance between shrubs and shrub size suggested that competitive effects were moderate on these sites. Soil resources also appeared to influence density of mature shrubs. The clustered pattern observed in shrubs and the tendency for non-random similarity of shrub size in a patch probably reflect multiple parameters such as heterogeneity of resources combined with pulsed growth or underground differences along with seedling facilitation early in life.

Shrub volume per area (using summed distances between shrubs as a surrogate for area) was strongly correlated with some soil parameters. Due to autocorrelations, it is unclear which soil parameter has the strongest relationship with shrub volume per area. The reason for the negative relationship of shrub volume per area with soil total nitrogen and possibly sulphur is not known. There appears to be two isoclines with a positive regression relationship between sulphur and shrub volume per area (figure 1d). Sulphur can be the second most limiting nutrient after nitrogen in some semi-arid grasslands (Brady and Weil 2008), legumes requiring particularly large quantities of it (Price 2006), so a positive correlation would be expected. Since *A. victoriae* roots are likely to be deep and nutrients are typically in the top 5cm of soil, the large abundances of nutrient demanding invasive species such as *A. fistulosis* and *E. plantagineum* growing under their canopies might deplete soil nitrogen or possibly sulphur. The shrubs might facilitate growth of these invasive species (Facelli and Brock 2000), and if so they might be responsible for use of more increased top soil nutrients in locations with higher shrub density per area. Negative regression with nitrogen might otherwise indicate some unknown indirect effects. Positive correlation of shrub volume with pH probably reflects shrub preference for more alkaline soils. If shrubs have very long roots, they may cause particular minerals such as calcium from deep water to accumulate in the soil which might reach top-soil through bioturbation of soil invertebrates. The positive regression with potassium might reflect its importance to the ability of the plant to withstand effects of drought (Price 2006) since shrubs may need large quantities of it to grow faster or survive longer. Also, potassium levels can be negatively correlated with induced responses to plant herbivory (Amtmann et al 2008, Troufflard et al 2010), and costs of plant defence might reduce shrub growth over a long period (Herms and Mattson 1992). However, potassium deficiency can also enable increases in populations of phloem-feeders on leguminous plants (Bruulsema et al 2010) so lower levels of it might possibly increase plant stress by any SEPHs that are parasitic to the plant and reduce growth. Since soil parameters correlate with shrub volume per distance, and these parameters can be important to plant growth, they should explain much of the variation in the volume against distance relationship. Field experiments are needed to determine effects of shrubs and other plants on soil and, conversely, effects of different soil parameters on shrubs including the depth at which shrub roots access soil resources.

Seedling density in the estimated zone of influence of roots of dead conspecifics was more than seven times higher than that for live shrubs and over forty times that of bare areas. The simplest explanation for why seedling density is higher under dead shrubs than live could be that in either case seed has fallen from the shrub and accumulated under the canopy and in the case of dead shrubs there is no intraspecific competition to limit seedlings. However, for a number of reasons this is likely to not be a strong factor causing the pattern. In fact, obvious accumulation of seeds under the parent was not observed, perhaps because the abundant weeds that might prevent their dispersal by wind (while seed pods are still attached) and by water start to die at approximately the same time as the seeds mature (pers. obs.). Also, there are large abundances on these sites of large *Rhytidoponera* that would disperse the large seeds as they drop to the ground (Davidson and Morton 1984). Additionally, the equally high density of seedlings in the estimated area of influence of roots of dead conspecifics with and without influence of live conspecifics also suggests that competition with live conspecifics may not be the driver of this pattern.

There are a number of factors that might explain this pattern. Perhaps seedlings germinate more in high light levels, so dead shrubs without large live ones nearby casting shade for much of the day confer greater advantage. In a spring of such abnormally high rainfall, dehydration would constitute less of a threat to seedling survival, so perhaps shade from live shrubs after germination was not important. However, effects of live shrubs would probably have been lower for mixed dead and live shrubs than in the alive-only treatment, because in this treatment live shrubs were either further from seedlings or smaller. Distance of seedlings from dead and live mature shrubs and canopy cover above them were not recorded, so the strength of effect of each is not discernible. Seedling density near only live shrubs may be lower than those containing only dead ones due to indirect effects. For example, live shrubs might facilitate plants (such as abundant weed species) which compete with the small shrub seedlings through alterations to light levels and humidity. However, densities of these grasses and forbs were in some cases high near dead shrubs also.

Concentrations of some soil parameters including total nitrogen are higher under live and dead shrubs, so this is likely to be an important mechanism of facilitation. Also, under dead shrubs there may have been a higher concentration of organic carbon than under live shrubs and possibly a larger concentration of sulphur. Organic carbon of soil is usually closely correlated with organic matter content and this increases moisture-holding capacity, which is probably important to seedlings. Differences in total nitrogen and organic carbon concentrations near and far from dead shrubs follow similar patterns to those near dead *A. papyrocarpa* trees in arid southern Australia (Facelli and Brock 2000), but in contrast in the current study phosphorous was not found to be affected, whereas potassium was. Thus, influence of soil parameters on spatial distribution of shrubs appears likely in these areas of high shrub density. It is possible that the bare patches have fundamentally different soil attributes that are unfavourable for *A. victoriae* growth, which could be why most were only at the edges of large patches of *A. victoriae* which may be near maximum possible density. Perhaps *A. victoriae* gradually spreads into new areas when it grows near mature conspecifics on the edge of dense stands. Isolation of factors related to shrub presence and its effects on seedling germination is difficult in a field survey and controlled experiments are necessary to establish both effects of *A. victoriae* on soil and effects of different soil on its seedlings. Large germination events for *A. victoriae* occur during periods of high rainfall, although seedling mortality rates can be 100% in the field (Grice and Westoby 1987) if required rainfall does not continue, so the year 2010 provided an unusual opportunity to record high emergence rates. Whether these shrubs facilitate later survival and establishment should also be determined to establish effects of such facilitation on shrub spatial distribution.

Spatial distribution of mature *A. victoriae* in this study included a tendency for increased distance between individuals with increased size. This suggests density-dependent effects of intraspecific competition (Welden and Slauson 1986). However, such distribution patterns can be caused by density-independent mortality, as for a phreatophytic perennial Asteraceae in arid North America, for which levels of deep soil moisture were thought to be shared across a whole site rather than competed for among neighbouring shrubs (Toft and Frazier 2003). In such a case, mortality is inversely related to age, probably due to advantages of having a larger root system, where on average there are larger spaces between the remaining increasingly less common larger shrubs. Experiments monitoring relative growth rate can determine effects of removing shrubs that could be competing (Shackleton 2002). One study of over 700 *A. victoriae* in arid NSW found that mortality was related to size of shrub: mortality was highest in the smallest and in the largest of seven size classes in the three years from 1978 to 1981, but from 1981 to 1992 shrub size was positively correlated with mortality rate (Grice et al 1994). In contrast, another study in NT found that the smallest plants were least likely to die and the intermediate size the most likely to die over a 3.5 year period from 1991 to 1994, with mortality rate of the largest shrubs falling between the two (Reid and Stafford Smith 2000). Therefore, demographics of *A. victoriae* vary widely by site and spatial distribution might be dominated by different factors in different places depending on environmental conditions, so density-independent mortality is possible for the sites used in the current study.

Over 20% of variation is explained by the pattern of volume to distance despite accuracy in the estimate of shrub size being difficult to determine without harvesting large numbers of shrubs. The method will never describe perfectly the pattern of influence of root competition on spatial distribution since there will not be a perfect correspondence of aboveground size and shape with root system size and shape, but it provides results in relatively little time. The importance of size-distance relationships between shrubs to spatial distribution on these sites is comparable to that inferred from studies of woody perennials in southern African and central American arid areas (Briones et al 1996, Wiegand et al 2005, Carrick 2003). Slope of one means that cubic root of total shrub volume is equal to square root of ten summed distances between all pairs of shrubs e.g. five 2m$^3$ shrubs will be separated by 3.2 m on average between each pair, but this is positively correlated with and sensitive to changes in size. Since the slope found in the current study was less than 0.5, meaning that less volume is present for the same distance of separation, the effect is stronger. However, since it only explains 20% of the difference this is likely not the strongest determinant of spatial distribution.

The two patterns in neighbour to target volume would likely be caused by shrubs under 0.24m$^3$ not being able to acquire sufficient resources to maintain growth. Shrubs of this size either die or sustain a reduction in growth rate for a number of years until conditions become more favourable. Shrubs of this size cannot grow larger unless they can acquire sufficient soil resources. Root systems of these small shrubs must be small enough in diameter and length to exist in gaps between those of larger competing shrubs when present. If these small shrubs stay at this size or die, their root systems are probably not large enough either in absolute or relative size to acquire resources from a sufficiently large volume of soil to grow larger. Perhaps heterogeneous distribution of moisture due to stoniness of soil makes larger roots necessary for the shrub to obtain sufficient quantities. If ground water is the limiting resource for which small shrub roots are too shallow, the critical size would vary with depth of groundwater in the location. Alternatively, there may be some critical change in ratio of biomass of roots to aboveground biomass which occurs around this critical size for the mature shrub to be able to obtain adequate resources. If pulsed growth in high rainfall years contributes substantially to total biomass, heterogeneous distribution of soil nutrients might confer advantage on larger root systems. More knowledge of root systems would enable these potential effects to be analyzed and perhaps provide further explanation, as might information on depth and location of ground water reserves, which was not available at the time of this study.

For shrubs larger than 0.24m$^3$, the positive regression relationship found indicates that there is substantially more uniformity in size of nearest neighbours than would be expected by a random size distribution. The slope of both regressions is a substantial proportion of the slope which indicates uniformity of neighbour shrub size in either case. This pattern suggests that shrub size is influenced by location, which would generally be due to resource quality. The key limiting resource might be soil water holding capacity or groundwater, and soil nutrients might be important in years with high moisture levels. Results suggest that there is a complex interaction between numerous variables. Resource quality might influence how competition affects size differences of shrubs in a given neighbour patch. Obviously, different individual competitive ability and different growth rates of different individuals would contribute to variation in the regression. In the arid zone, where amount and periodicity of rainfall is characteristically variable, stands of shrubs can consist of distinct age classes, which either recruit or grow at very high rates after long periods of relative dormancy (during dry periods) in unusually wet periods, which are large infrequent events (Harrington 1991). Survival and establishment of seedlings would depend on adequate follow-up rainfall in subsequent years, making lasting effects of large germination events even rarer than occurrences of years that are sufficiently wet for large germination events. If multiple seedlings near a large conspecific sometimes emerge simultaneously, grow, and establish, this could be one reason for nearest neighbour shrubs being more similar in size on average than would be expected from a random size distribution. If this effect was a major contributor to non-random size distribution, there would be distinct groups of shrubs by size in many neighbour groups, but the pattern of homogeneity of shrub size found here is weaker than would be expected by this effect alone, as indicated by the slope. Although size distribution of these shrubs is unlikely to be affected predominantly by simultaneous germination and establishment, it is nevertheless likely to contribute to the regression relationship. Indeed, germination of *A. victoriae* tends to occur mainly in years of high rainfall (Greg Mütze, pers. comm.). Pulsed growth during large rainfall events can also create groups of shrubs of similar size in the same place despite being different ages (Harrington 1991). Pulsed growth in high rainfall years and corresponding losses of biomass in intervening years might accentuate differences due to resource quality (and competition with facilitating conspecifics or other species), exerting moderately strong equalizing effects. Therefore resource quality, as suggested by the relationships between soil parameters and shrub volume, and pulsed growth influenced by juvenile facilitation by mature conspecifics in some neighbour groups are likely causes of this pattern of spatial distribution of shrub size.

Spatial distribution of individual shrubs across sites showed clustering at moderate scales using Ripley's K function. This is likely to result from influences of resource quality or facilitation of younger shrubs by conspecifics and probably both. The regression relationships of shrub volume per area with soil parameters might support this hypothesis since areas of neighbour groups were within the scale of the clustering pattern found. Additionally, since the deep underground structure of the soil at this site is unknown it is possible that large sub-surface rocks might affect the pattern as would groundwater availability. Soil resources might explain much of the variation in the regression of volume against distance that might be due to competition. Thus, the spatial distribution of these shrubs appears to be substantially influenced by both processes, which appear to result in increasing dispersion and clustering.

## Conclusion

The relative influence of each factor affecting spatial association indicated by the results here could depend heavily on abiotic and biotic conditions at the particular location or year.

There is substantial variation in spatial distribution of *A. victoriae* at different locations, for which other studies of the species provide some evidence as to contributing variables. Ascertaining degree of density-dependent or -independent growth and mortality by way of field experiments including shrub removal or separation of root systems would be useful for understanding dynamics of these semi-arid ecosystems. Also, if more were known about root system dimensions relative to dimensions of aboveground shrub biomass and relative to variation in soil resources, then area or volume of root system overlap with adjacent shrubs could be estimated slightly more accurately to select an optimal number of neighbours, reducing noise in the pattern. Such information might also enable explanation of the relationship between small target shrubs and their nearest neighbours. This would clarify dynamics of the transition from clustering of seedlings near conspecifics to repulsion if intra-specific competition is occurring. Manipulations of levels and timing of soil resources could ascertain effects of moisture dynamics and soil nutrient concentrations on competition, growth, mortality, and spatial distribution in the field.

Soil parameters suggest that *A. victoriae* which have been dead for a period of around 10 years create fertile patches which facilitate growth of smaller plants, as has been observed in other arid zone acacias (Facelli and Brock 2000). They are thus likely to have an important effect on rehabilitation of degraded rangelands over a substantial period of time. Seedlings of this shrub are probably facilitated by conspecifics, but the reason for seedling density being higher near dead shrubs than live ones should be examined further with manipulative experiments as should shrub effects on soil parameters and possible shrub facilitatation of other plant species. Although microsite resource quality has frequently been mentioned as a driver of spatial distribution of woody perennials in arid zones (Jeltsch et al 1998), few studies have measured its effects or investigated it concurrently with competition (but see Wiegand et al 2005). This study provides an Australian example of spatial distribution of a dominant shrub species in a semi-arid environment, with results suggesting seedling facilitation by mature conspecifics and a tendency for initial clustering which shifts towards a more random distribution due to intraspecific competition as shrubs establish and grow, as was found on other continents. More studies at different scales and manipulating parameters recording spatial distribution and dynamics of soil including how it is affected by biotic processes will improve our understanding of how these factors affect spatial distribution of vegetation.

# Chapter 3:

# Aspects of Spatial Association of Shrubs, Sugary-Exudate Producing Herbivores, and Attending Ants in Semi-arid Australia

## Abstract

Examining spatial associations between herbivorous producers of sugary exudates (SEPHs) on plants and ants that consume their sugary exudates (SE) can provide clues to how environmental conditions affect the association and to the impact of the association both on fitness of its partners and on the wider plant and arthropod community. In semi-arid Australia on individual *Acacia victoriae* shrubs, regressions of counts of trailing small black dominant *Iridomyrmex* ants (SBIs) and of their nests against numbers of *Sextius* sp. (Cicadelloidea: Membracidae) nymphs and adults, Margarodidae (Coccoidea), and lycaenid larvae (all SEPHs) were used to infer spatial association. On individual shrubs, there was a positive regression between SBI counts and SEPH numbers both at natural levels and after experimental SEPH removal from shrubs. Number of SEPHs on the shrub made a negative envelope regression for maximum distance of nearest SBI nest entrance to shrub. There was a positive regression between numbers of SBI nests in 15 m x 15 m quadrats and total SEPH counts whereas there was no regression when using 5 m x 5 m quadrats, revealing the spatial scale at which SEPH populations affect SBI nests. At small patch scale, regressions of SEPH density with soil parameters and the negative regression envelope of SEPH numbers with shrub volume density per area pointed to soil resources driving SEPH populations on shrubs, which suggests a substantial bottom-up influence. Influence of SEPH numbers on SBI numbers was much stronger than influence of shrub volume. This is the first study to quantitatively relate partners of this type of association in a natural system with native species. These preliminary findings indicate strong spatial association between the abundant small dominant trailing ants, SEPHs, and shrubs in this putatively mutualistic food-for-protection association in arid Australia.

This suggests that these interactions have importance for fitness of the partners and perhaps on the broader plant and arthropod communities. Such an abundant widespread interaction might affect the structure of communities over large areas of arid Australia.

## Introduction

Ants are highly abundant in arid Australia and thrive in hot environments (Greenslade 1978). The maximum known behavioural and numerical dominance of ants in natural arthropod communities occurs on tropical rainforest canopies, where they constitute up to 70% of arthropod biomass (Davidson 1997). Ants may be at their second highest abundance in arid lands such as arid Australia where they are particularly abundant and to which conditions they are well adapted (Stafford Smith and Morton 1990). In tropical canopies, the majority of ant biomass at a site is constituted of one to a few behaviourally dominant species which feed on large quantities of sugary exudates (SEs) from both plant extrafloral nectaries and excreta of sap-feeding hemipterans (Davidson 1997, Davidson et al 2003). A link between ant abundance and SE availability has been recorded in semi-arid shrub-lands (Nash et al 1998, 2000), tropical cerrados (Oliveira and Freitas 2004), sub-tropical and tropical plantations (Room 1971, Greenslade 1971, Samways 1983), and tropical rainforests (Blüthgen et al 2000, Blüthgen et al 2006) including those in Australia (Blüthgen et al 2004). There is also a strong concurrence of ant species that consume hemipteran excreta and those that consume sugary lycaenid larvae secretions when available (Eastwood and Fraser 1999). Species of ants that consume SE are behaviourally and numerically dominant in arid Australia too (Greenslade 1979, Morton 1993, Eastwood and Fraser 1999). Many biologists have observed that associations between ants and SE producing herviores (SEPHs) such as sap-sucking hemipterans and lycaenid larvae are very common, widespread, and abundant in arid Australia (Briese and Macauley 1977, Greenslade 1979), where dominant vegetation types are commonly *Acacia* and *Eucalyptus*, which contain many species bearing extrafloral nectaries or hosting SEPHs (Williams 1982). Indeed, the association of some Australian lycaenid larvae with specific attendant ant species appears to have produced unusual biological outcomes such as the use of ant nests by some *Ogyris* species of arid or semi-arid systems for part or all of their larval development (Braby and Douglas 2008). However, very little has been documented about these associations in arid environments or specifically in Australia.

There is a range of evidence indicating that SEs could be important to ecological dynamics of communities that include ants. Energy that ants obtain from large quantities of SEs could be used to increase activity rates of workers and to increase colony size (Davidson 1997) as has occurred in a laboratory setting (Pierce et al 1991, Helms and Vinson 2008), and this could increase the area over which ant activity rates are high and effects on other taxa due to SEs are strong. In fact, the tropical fire ant *Solenopsis invicta* obtained approximately 1/5th – 2/3rd their energy requirements from hemipteran honeydew in non-agricultural systems they had invaded in humid subtropical North America (Helms and Vinson 2002), suggesting that SE was a primary resource that facilitated their colonization. Ants in arid systems may also benefit directly from water consumption when feeding on SEs. However, ants require protein in addition to sugars, so a proportion of energy gained from hemipteran SEs would be used to attack other arthropods as prey as well as to defend SEPHs (Davidson 1997). Hemipteran SE alone was not sufficient to allow colonies of *S. invicta* grow in laboratory conditions but addition of arthropod prey facilitated colony growth (Helms and Vinson 2008). In addition, dominant ants in a laboratory setting can behave more aggressively towards other arthropods when SE is available (Grover et al 2008). However, where lycaenid larvae SEs are available, nitrogen might also be obtained since some lycaenid SEs contain amino acids (Baylis and Pierce 1991). Increased aggression due to sugar consumption could increase effects of such abundant aggressive predators on natural arthropod communities. Indeed, ants that consume SEs tend to reduce survival and abundance of non-SEPH arthropods on plants (Styrsky and Eubanks 2007, Rosumek et al 2009). This phenomenon is the basis for protective effects of attendant ants on both plant and on SEPHs. A meta-analysis (Rosumek et al 2009) found that ant removal resulted in predator abundance on plants doubling on average and an increase in herbivores of 50% on average. Therefore, aggressive dominant ants in this type of interaction are likely to have similar strong effects on arthropod communities with which they interact in arid Australia.

Landscape scale distribution of plant-SEPH-ant associations depends on distribution of SEPHs on individual host plants. The factors that regulate herbivore selection of hosts are numerous, complex, and interactive. Hemipterans and lycaenids sometimes select individual host plant for oviposition based on presence of ants (Morales 2002, Atsatt 1981), species of ants present (Fraser et al 2002), plant nutrient levels and availability (Powell et al 2006), plant secondary metabolites (Powell et al 2006, Atsatt 1981), and faster growth rate or amount of fresh growth (Fiedler 1996, Cornelissen et al 2008). Soil characteristics and rainfall also directly affect distribution of ant species (Greenslade and Halliday 1982, 1983, Greenslade 1987). Growth rate, nutrient levels, and secondary metabolites in plants can be affected by soil parameters. For example, phloem sap varies in composition over time at various scales, with ontogenetic phase, temperature, soil nutrient, and soil moisture levels (Douglas 2006, Amtmann et al 2008). Although hemipteran SE composition is not identical to that of sap (Douglas 2006), it nevertheless depends on constitution of phloem sap. In the case of lycaenids, sugary secretions can contain elevated levels of nitrogen concentrated from leaves consumed by the larvae (Baylis and Pierce 1991). Therefore spatial distribution of a SEPH species and the ants whose numbers are affected by it could also be modulated by variation in soil parameters, potentially over relatively small scales. In this way, the particularly heterogeneous soil characteristics and rainfall of arid Australia (Stafford Smith and Morton 1990) will be likely to affect distribution of partners in this association at various scales. Since ant-hemipteran-plant interactions, including facultative ones, have increased plant growth or reproductive output in most studies of natural systems (Styrsky and Eubanks 2007), the association might also affect host plant distribution, particularly if SEPH populations tend to remain on or return to the same plant individuals, influencing reproductive success of particular genotypes. In arid Australia, large populations of these ants consuming SEs over a large area might have strong impacts on plants containing SEPHs (Orians and Milewski 2007) and on arthropod assemblages both on the plants and on the ground where ants nest.

Spatial distribution of dominant ants can be driven by spatial distribution of sugar sources. For example, provision of sucrose facilitated invasion of remnant natural forest in mesic North America by dominant invasive Argentine ants *Linepithema humile*, and when the sucrose was removed, they retreated (Rowles and Silverman 2009). On plants, ants can select SEPHs based on aggregation size (Morales 2000, Pierce et al 1987), quantity of SE produced (Fischer et al 2001), type and concentration of sugar in the exudate (Cushman and

Addicott 1991), and other nutrients in the exudate (Cushman and Addicott 1991, Baylis and Pierce 1991). Depending on ant preferences, composition of SEs as determined by sap can vary with spatial variability in soil parameters. Close spatial association of interacting ant and SEPH populations can point to strong fitness benefits to these populations. For instance, most of the SE consumed by *S. invicta* in a humid subtropical North American location came from an invasive hemipteran with which it was spatially associated on a large scale (Helms and Vinson 2002) and ant population growth was found to follow hemipteran abundance but not *vice versa* (Helms et al 2011). Some degree of spatial association between the aggressive dominant ant genus *Iridomyrmex* and SEPHs has been demonstrated in Australia. Nests of one dominant SE-consuming *Iridomyrmex* species were distributed close to trees housing SEPHs in *Eucalyptus* woodlands (Wilgenburg and Elgar 2007). Nests of another dominant SE-consuming *Iridomyrmex* species were clustered at the base of shrubs with SEPHs in semi-arid *Acacia* shrub-lands (Clarke 2006). However, until now no study of SEPHs and SE-consuming ants has examined in detail the strength and scale of spatial association between a native ant species and native SEPHs in a natural environment.

This study aims to advance understanding of the widespread abundant ant-hemipteran-plant associations in Australia, which have received little attention, by examining the strength and scale of spatial association of partner populations with each other and with soil parameters. These associations are likely to strongly affect arid land arthropod and plant communities, particularly with the high aggression of Australian ants. At times of peak activity, over summer, the largest proportion of ants, which nest underground, can be observed. This study therefore records ant activity during summer. It investigates the following questions through non-manipulative survey methods and a manipulative field experiment on sites dominated by a common Acacia species *Acacia victoriae* inhabited by SEPH taxa, *Sextius* sp., various Margarodidae, and lycaenid larvae, with abundant trails of very aggressive small black dominant *Iridomyrmex* sp. (SBIs) in semi-arid southern Australia:

1. How are SEPHs distributed across the habitat with respect to distribution of soil parameters and *Acacia victoriae* shrubs?
2. How strongly are trailing dominant small black *Iridomyrmex* (SBI) populations correlated with sugary exudate producing herbivore (SEPH) populations on individual *A. victoriae* within the habitat?
3. How strongly are SBI populations correlated with SEPH populations on small patches within the habitat and at what unit scale?

# Methods

## Study site

This study was conducted in the Flinders Ranges National Park on a previously heavily grazed former sheep station, Oraparinna Station, (31.37° S, 138.72°, elevation 550 m) currently under a rehabilitation program. From 1985 to 2009, mean annual rainfall was 321 mm, with median 324 mm and coefficient of variation 31%, with a mean 66% rain falling in the colder months from May to October. Mean annual areal potential evaporation is 1200-1300mm (Bureau of Meteorology), giving an aridity index $AI_U$ of 0.25-0.27 (UNEP 1992). There were 199 mm rainfall in the 12 months before the study took place in 2008 December and 2009 January, which was less than the mean minus the standard deviation, and only 37 mm in the three months beforehand, which was about half the spring median of 72 mm. Therefore, conditions leading up to the study were significantly drier than the recorded means and could be seen as representative of drought conditions. The predominant soil type is calcareous loam. The vegetation is still severely degraded and the area supports a large rabbit population. The sparse overstorey is formed by *Acacia victoriae* (Whibley and Symon 1992) which shows a somewhat clumped distribution (Chapter 2). The understorey vegetation is sparse and dominated by the exotic invasive species *Carrichtera annua*, *Asphodelus fistulosus*, *Echium plantagineum*, and *Marrubium vulgare*.

Sites 1 and 2 were selected to study effects of SEs on SBI trails and nests because they were relatively flat, of sufficient size, and dominated by *A. victoriae* supporting substantial populations of SEPHs with large numbers of SBIs trailing amongst them, with few other shrub species and similar ground cover. Site 3 was selected 4.5 km away from sites 1 and 2 in September 2010 for similar reasons. On site 1, a grid of 55 m x 55 m with quadrats of 5 m x 5 m was constructed in December 2008. In January 2009, 500 m away on site 2, a similar grid of 45 m x 85 m was constructed. Disturbed areas where explosives had been used to destroy rabbit warrens were avoided, as were washes and creek beds and areas of dense surface rock. Shrubs were mapped on the grids (see Chapter 2) and the numbers of SEPHs on shrubs were recorded for separate stems.

## Species

In the semi-arid Flinders Ranges of southern Australia, *A. victoriae* is present at 27% of sites surveyed and constitutes the dominant canopy in many (Brandle 2001), and appears to be particularly common in areas degraded through grazing. Many of these shrubs support populations of SEPHs: green treehoppers (*Sextius* sp.), lycaenid (*Jalmenus icilius)* larvae, and unidentified grey, red, black, and green species of scale insects (Margarodidae). Between such shrubs stretch trails of SBIs, linking their nests to food sources.

SBIs were primarily of the *Iridomyrmex rufoniger* complex (Shattuck 1999), although others may have belonged to other distinct morphospecies of *Iridomyrmex*. SBIs move distinctly faster with more erratic changes in direction than other ants, particularly when ground temperatures are high. SBIs were identifiable by their colour, size, and activity patterns within long interconnected trails. SBIs were seen behaving aggressively towards ants of other species that ventured onto stems with SEPHs, including larger *Iridomyrmex* species. Therefore for the purposes of this study, SBIs were classified as all approximately black ants of approximately 4 mm in length travelling along sufficiently long and interconnected trails on the ground or on *A. victoriae* stems. These ants are omnivorous, fiercely predatory, and nest in soil (Greenslade 1978). SBI activity increases with increased temperature up to a threshold. Trailing activity of *Iridomyrmex purpureus viridiaeneus*, a larger black species, is restricted to times when soil temperature is between approximately 12°C and 45°C in a semi-arid location (Greenaway 1981). Similarly, SBIs appear to start activity in mornings once soil surface temperatures reach approximately 12°C and return to nests when temperatures exceed approximately 42°C (pers. obs.).

## Sampling procedures

Groups of five nearest neighbour shrubs were chosen from shrub grid maps of sites 1 and 2, selecting the central shrub randomly until no more groups remained. One outer shrub in a group was allowed to be an outer shrub of another group. Just outside each neighbour group, soil in 50 g cores was sampled from four compass points one canopy diameter from the nearest shrub and was pooled. All pooled soil samples were analyzed for concentrations of total nitrogen, $N_{total}$ (%), available phosphorous, $P_{available}$ (mg/kg), organic carbon, $C_{organic}$ (%), potassium, K (mg/kg), sulphur, S (mg/kg), and for conductivity, EC (dS/m) and pH (using the $CaCl_2$ method) by CSBP Laboratories in Bibra Lake, Western Australia.

On 75 shrubs on sites 1 and 2 in December 2008 and January 2009, single stems were selected for good visibility of SBI trails leading up them. At site3 in December 2010, 8 shrubs with high SEPH densities were located. After counting SEPHs on single stems and looking for other insects and fungus as sources of food on the stem, soil surface temperatures were measured in four places near the shrub and averaged. SBIs running up each stem were counted for a 4 minute period during mornings while average soil surface temperatures around shrubs were between 26°C and 38°C. Since SBI activity depends on ground temperature and some trails may have different activity rates in mornings and afternoons (pers. obs.), this was done to minimize variation in SBI densities. At site 3, tangletrap was applied to all stems to prevent SBIs from accessing them, to get rid of SEPHs. After most SEPHs had gone a few weeks later, SBI trails still passed directly under the shrubs. Tangletrap was removed and stems were left for four or five days to let SBIs on them reach stable densities, and then SBIs running up stems were again counted in the same way.

SBI activity during daylight peaked in the last 1.5-2 hours before sunset once soil surface temperatures had dropped below the activity threshold (pers. obs.), so nests and trails were mapped during this time to minimize variation due to time of day. Nests of *L. humile* are shallow chambers linked by passages in the top 30-40 cm of soil (Heller 2004) and SBI nests are likely to be of similar structure to those of this closely related species. Surface area, a measurement of size, could predict 92% of *L. humile* worker population per nest (Heller 2004), and similarly, number of nest entrances is likely to be strongly correlated with nest size and worker population per nest. For each *A. victoriae* on site 1, the nearest active SBI nest entrance, with SBIs running into or out of it, was located and its distance to base of shrub measured. Additionally, to estimate SBI abundance in quadrats active nest entrances were counted during times of peak activity to identify the SBIs. Nest entrances were searched for exhaustively until no more could be found and their position in the grid recorded. Locations of SBI trails could vary between morning and evening and were extremely difficult to map with certainty unless activity was high. To record SBI activity within quadrats, at times of peak activity SBI trails were mapped if flow rate past a fixed point was at least 20 SBIs $\text{min}^{-1}$. Since nests are linked by trails and reflect a somewhat permanent distribution, they were used in quadrat analyses in preference to trails.

## Data analyses

All linear regressions were performed using SPSS software (Versions 17.0 – 19.0, SPSS Inc.). Blossom software (Cade and Richards 2005) was used for quantile regressions and asymptotic p was reported. Primer-E 6.0 with PERMANOVA+ (Anderson 2001) with Euclidean distances was used for factor analyses.

To determine if there was a relationship between number of SEPHs on a shrub and number of SBIs foraging on the same shrub, linear regressions were performed. Regressions were done of numbers of SBIs running up stems, a, against numbers of SEPHs on stems, S, and against volume of stems, v. Regression of numbers of SEPHs on stems, S, against stem volume, v, was also done to check for autocorrelation. SBI numbers were transformed by square roots, $\sqrt{a}$, since the circumference of stem base, which limits their numbers, is linear, stem volume was transformed to cubic roots, $\sqrt[3]{v}$, and SEPH numbers were transformed to cubic roots, $\sqrt[3]{S}$, to account for stem volume. For the SEPH removal experiment, numbers of SEPHs, $\sqrt[3]{S}$, and numbers of SBIs, $\sqrt{a}$, before and after SEPH removal were compared separately in a one-factor factor analysis. Data obtained before and after removal were pooled and number of SEPHs, $\sqrt[3]{S}$, was regressed against number of SBIs, $\sqrt{a}$. Regression of SBI count per stem circumference, c (cm) ($\sqrt{(a/c)}$) against number of SEPHs per volume of shrub ($m^{-3}$) ($\sqrt[3]{(S/v)}$) was also done to test for limitation of stem circumference on SBI numbers.

To examine association of SBI populations with *A. victoriae* volume and with SEPHs independent of autocorrelation with shrub volume, quantile regression and linear regression tested the relationship between distance to shrub of nearest SBI nest, d, transformed to square roots before analysis, $\sqrt{d}$, and number of SEPHs on the shrub, $\sqrt[3]{S}$, separately from shrub volume, $\sqrt[3]{v}$.

Relationships between soil parameters in neighbour groups were sought using linear regressions of each soil parameter, x, transformed to ln(s + 1), against neighbour group average SEPH density, total number of SEPHs on all shrubs in neighbour group ($S_5$) over total volume of shrubs in neighbour group ($V_5$), $S_5/V_5$, transformed to logarithms, $\ln(S_5/V_5 + 1)$.

To detect any relationship between shrub volume and SEPH count in groups of nearest neighbour shrubs, regressions were done for both total SEPH count of the neighbour group, $S_5$ ($\ln(S_5 + 1)$), and for SEPH density, $S_5$ per total neighbour group shrub volume, $V_5$ ( $\ln(S_5 / V_5 + 1)$ )), against each soil parameter, ln(s + 1). Additional regression was done for total number of SEPHs, $\ln(\sqrt[3]{S_5} + 1)$, against total neighbour group shrub volume, $V_5$, per sum of four distances between target and neighbour shrubs in each nearest neighbour group, $D_4$ ($\ln(\sqrt{V_5} / D_4 + 1)$ with summed distances between shrubs as a proxy for area, so this represents shrub volume per area. A LAD quantile regression in Blossom was done for this analysis also. To determine whether any relationship between these parameters was related to distance between shrubs, regression was done of total neighbour group SEPH count, $S_5$, with summed four distances between target and neighbour shrubs, $D_4$.

The relationship between number of SEPHs or shrub volume in an area and number of SBI nest entrances, N, as an estimate of population and the spatial scale at which a clear relationship exists was determined through regression at two spatial scales. From the gridded maps of ant trails and nests, the spatial association of number of SBI nest entrances, N ($\ln(\sqrt{N} + 1)$) with total numbers of SEPHs on shrubs, $S_{total}$ ($\ln(\sqrt[3]{S_{total}} + 1)$) and total shrub volume, $V_{total}$ ($\ln(\sqrt[3]{V_{total}} + 1)$) in each quadrat was analyzed at two scales using the most appropriate data transformations:  first, single quadrats, and second, 9 of these adjacent single quadrats forming 15 m x 15 m squares.  At both scales, there was no overlap of quadrats and there were no gaps between quadrats.  In the case of the 9 quadrat squares, only single quadrats at the edges of sites were unused, since the number across was not a multiple of three.  For quadrats at both these scales, regressions were done for numbers of SBI nests in quadrats against total SEPH numbers, numbers of SBI nests in quadrats against total shrub volume, and total shrub volume against total SEPH count (to check for autocorrelation).  A three-dimensional regression model linking SBI nests to SEPH counts and shrub volume in each quadrat was attempted.  Despite non-independence of sample units, regressions were used since statistical methods suited to spatial analysis required more precise measurement of location of ant nests than was available.  Although there is a good degree of confidence regarding patterns found, there is less certainty regarding their magnitude due to the non-independence of sample units.

# Results

## SEPH presence on shrubs and relation to other food sources

SEPHs were present (more than 3 individuals) on 42% of *A. victoriae* shrubs surveyed. *Sextius* sp. always constituted the majority, comprising 87% of SEPHs (table 1).

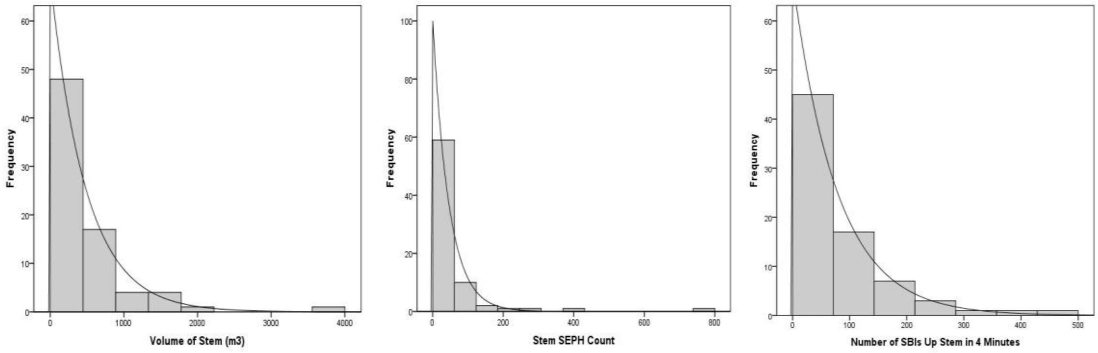
Table 1: Proportions of shrubs with the various SEPHs present at sites: all shrubs (All*) and SEPH-present shrubs (Present$^{\Delta}$) only

| | | **Group of SEPHs:** | **Combined SEPHs (%)** | **Treehoppers (*Sextius* sp.) (%)** | | **Lycaenid larvae (%)** | | **Red Scale Insects (%)** | | **Grey Scale Insects (%)** | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | **All shrubs or only SEPH-present shrubs:** | Present | All* | Present$^{\Delta}$ | All* | Present$^{\Delta}$ | All* | Present$^{\Delta}$ | All* | Present$^{\Delta}$ |
| | Number of shrubs | | | | | | | | | | |
| **Site 1** | 58 | | 74 | 67 | 91 | 22 | 30 | 24 | 32 | 24 | 32 |
| **Site 2** | 101 | | 22 | 19 | 86 | 6 | 27 | 7 | 32 | 1 | 5 |
| **Pooled** | 159 | | 42 | 30 | 87 | 12 | 29 | 13 | 31 | 9 | 21 |

SEPHs were not the only abundant food source observed to be used by SBIs. SBIs on some occasions were seen forming dense and highly active trails to small holes in the ground away from which they carried termites along trails. It appeared that quadrats where there had been termites had many more SBI nests and trails than expected from SEPH populations (pers. obs.). However, termite activity seemed highly variable as it was not observed in the same location on different days during the study; therefore the effect of termite presence was not assessed as part of the statistical analyses.

## Correlation of SBI and SEPH numbers on shrub stems: natural levels

On the shrubs in this study, only rarely was a non-SBI ant observed running up an *A. victoriae* stem. Occasional *I. lividus* ventured up stems but were not included in counts since their numbers were negligible, they could have different effects from SBIs on ecological patterns being analyzed, and SBIs chase them away promptly once detected. SBIs were observed lingering near and assessing SEPH nymphs and adults (occurring alone or in groups) with sensory structures such as palps and antennae, sometimes touching them with open mandibles. Numbers of both SBIs and SEPHs on shrubs fit a negative exponential distribution and stem volumes came close to such a distribution (Figure 1). Regression of SBI counts ($\sqrt{a}$) and SEPH counts ($\sqrt[3]{S}$) yielded a moderately strong positive relationship ($n = 74$, $r^2 = +0.516$, adj. $r^2 = 0.509$, $p < 0.0001$, slope = 1.587 ± 0.180) (Figure 2). The highest densities of SBIs occurred on shrub stems. Regression of SBI count per stem circumference ($\sqrt{(a/c)}$) against number of SEPHs per volume of shrub ($\sqrt[3]{(S/v)}$) gave a moderately strong positive relationship ($n = 74$, $r^2 = +0.509$, adj. $r^2 = +0.503$, $p < 0.0001$, slope = 27.093 ± 3.134, constant = 10.731±1.593) (Figure 3).



(a) (b) (c)

Figure 1: Frequency distributions for SBI counts on *A. victoriae* stems for (a) estimated stem volume, (b) SEPH numbers, and (c) SBI numbers

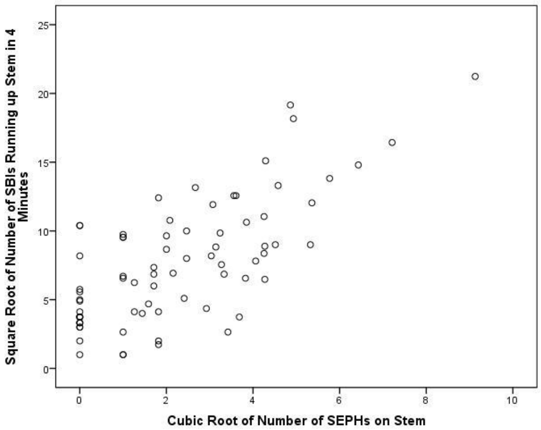


Figure 3 : Numbers of ants running up stems ($\sqrt{a}$) against SEPH numbers on stems ($\sqrt[3]{S}$)

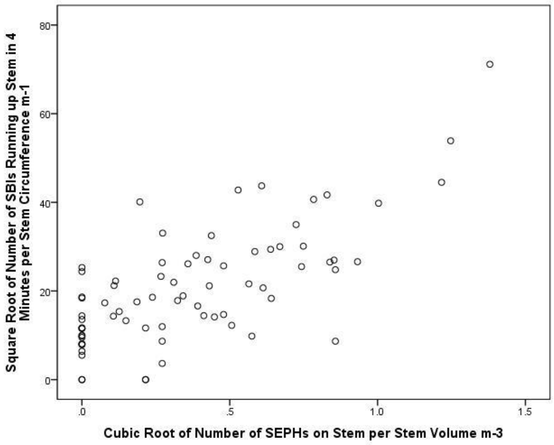


Figure 2: Numbers of ants running up stems per circumference ($\sqrt{a/c}$) against SEPH numbers on stems per volume ($\sqrt[3]{S/v}$)

Stem volume ($\sqrt[3]{v}$) correlated positively but very weakly with SBI counts ($\sqrt{a}$) ($\ln(\sqrt[3]{v} + 1)$, $\ln(\sqrt{a} + 1)$, $n = 72$, $r^2 = +0.098$, adj. $r^2 = +0.085$, $p = 0.007$, slope = 0.073 ± 0.026, constant = 1.753 ± 0.104). Only comparatively small numbers of other arthropods were seen on shrubs during counts of ants or SEPHs. It was rare to see SBIs carrying a SEPH and they were never observed to be aggressive towards SEPHs with which they were interacting. There were a few late developing flowers on some of the shrubs, but usually an ant remained on a flower only briefly unless there was a lycaenid larva on it, suggesting that SEs were far more important a food resource than the flowers (which are not rich in nectar). No other obvious consistent food resources for SBIs were identified in substantial quantities on *A. victoriae*, and SBIs did not appear to visit nectaries often or to linger at those they investigated.

Stem volume ($\sqrt[3]{v}$) did not correlate with SEPH counts ($\sqrt[3]{S}$) ($n = 72$, $r^2 = 0.003$, $p = 0.632$, slope = 0.123 ± 0.256, constant = 0.736 ± 0.524) so autocorrelation does not need to be considered.

The three dimensional model with SEPH count ($\sqrt[3]{S}$) and stem volume ($\sqrt[3]{v}$) explained less variability in SBI numbers ($\sqrt{a}$) than SEPH count alone ($n = 72$, $r^2 = 0.484$, $p < 0.0001$; stem volume $p = 0.002$, slope = 0.484 ± 0.151; SEPH $p < 0.0001$, slope = 0.507 ± 0.071; constant = 0.545 ± 0.314).

## Correlation of SBI and SEPH numbers on shrub stems: experimental removal of SEPHs

There were still high densities of SEPHs on other shrubs and high activity of SBIs around site 3 after removal of SEPHs from experiment shrubs. There was a large difference in numbers of both SEPHs ($\sqrt[3]{S}$) and SBIs ($\sqrt{a}$) on site 3 target shrubs, when compared before and after SEPH removal ($n = 30$, $p = 0.0001$ for both variables) (Figure 4). Numbers of SEPHs ($\sqrt[3]{S}$) and SBIs ($\sqrt{a}$) correlated moderately positively for pooled counts before and after SEPH removal ($n = 29$, $r^2 = +0.653$, $p = 0.0001$). Temperatures were approximately the same during counting of both before and after treatments, so lower SBI numbers on shrubs after removal of SEPHs were not due to temperature (Figure 5).

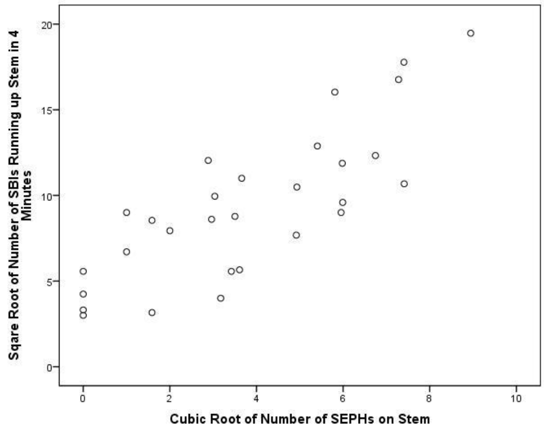


Figure 4: Numbers of ants running up stems ($\sqrt{a}$) against SEPH numbers on stems ($\sqrt[3]{S}$)

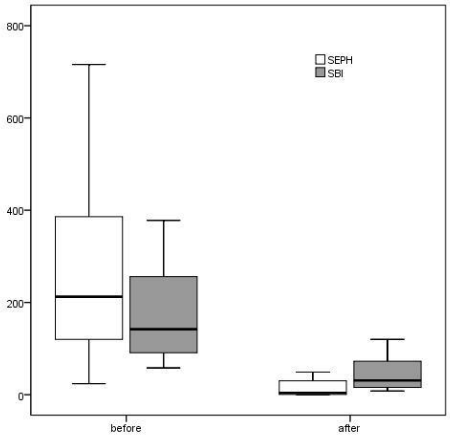
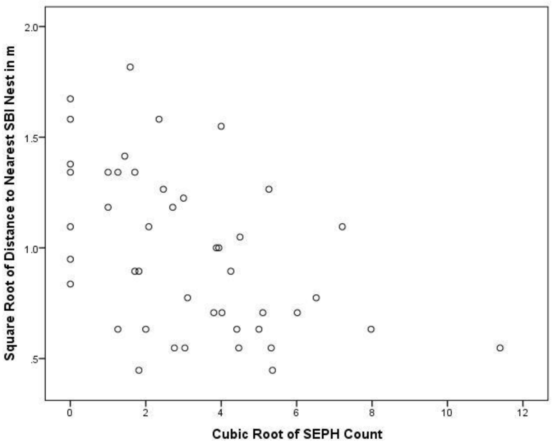


Figure 5: Numbers of SEPHs ($\sqrt[3]{S}$) and SBIs ($\sqrt{a}$) on stems before and after SEPH removal

## Correlation of distance of nearest SBI nest entrance to individual shrubs with SEPH count and shrub volume

SBI nest entrances were often under plant litter scattered beneath *A. victoriae* shrubs, amongst clumps of long *A. fistulosis* which were often dense under *A. victoriae*, under low shrub branches, or between or under rocks. The closer to a shrub a nest entrance was, the more likely the nest entrance was to be obscured, and at times nest entrances were not accessible for counting. This would have biased nest entrance counts towards being underestimated when occurring nearer to shrubs. After removal of one outlier, SEPH count ($^3\sqrt{S}$) on site 1 shrubs formed a negative envelope regression for distance from shrubs to nearest SBI nest entrance ($\sqrt{d}$) which increased in strength up to the 0.9th quantile (Figure 6a, table 2). There was a weak negative regression between the two variables ($n = 45$, $r^2 = -0.239$, adj. $r^2 = -0.221$, $p = 0.001$, slope = $-0.074 \pm 0.020$, constant = $1.219 \pm 0.079$). There was also a weak negative regression for distance to nearest SBI nest entrance and shrub volume ($n = 45$, $r^2 = -0.113$, adj. $r^2 = -0.093$, $p = 0.024$, slope = $-0.305 \pm 0.130$, constant = $1.240 \pm 0.119$) (Figure 6b) but they were not correlated for shrubs with no SEPHs (Figure 6c). Shrub SEPH count ($^3\sqrt{S}$) and shrub volume ($^3\sqrt{v}$) correlated moderately positively for all shrubs ($n = 45$, $r^2 = +0.447$, adj. $r^2 = +0.437$, $p < 0.0001$) (figure 6d).



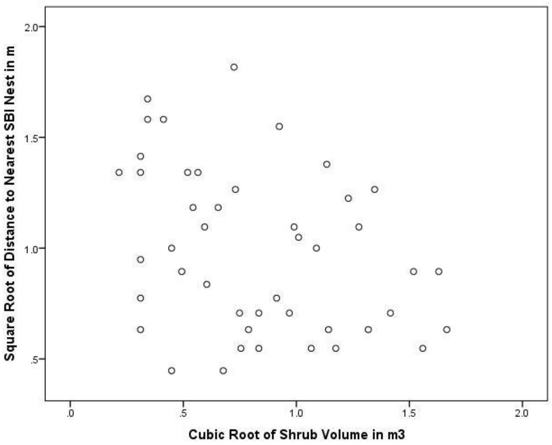
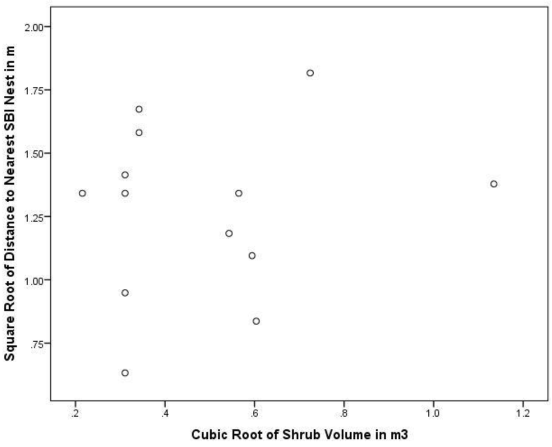


Figure 6a: Distance to nearest SBI nest entrance ($\sqrt{d}$) against SEPH count on shrub ($^{3}\sqrt{S}$)

Figure 6b: Distance to nearest SBI nest ($\sqrt{d}$) against volume of shrubs ($^{3}\sqrt{v}$)



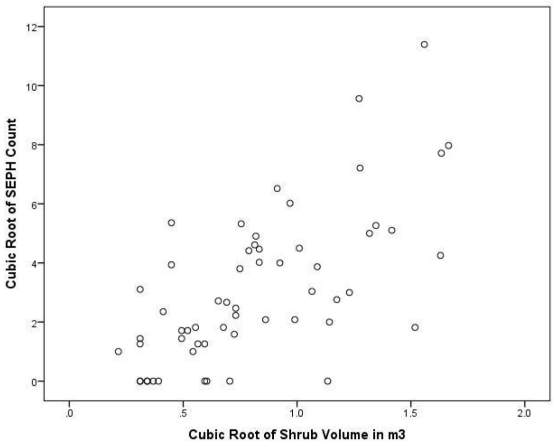


Figure 6c: Distance to nearest SBI nest entrance ($\sqrt{d}$) against volume of shrubs ($^{3}\sqrt{v}$) with <5 SEPHs

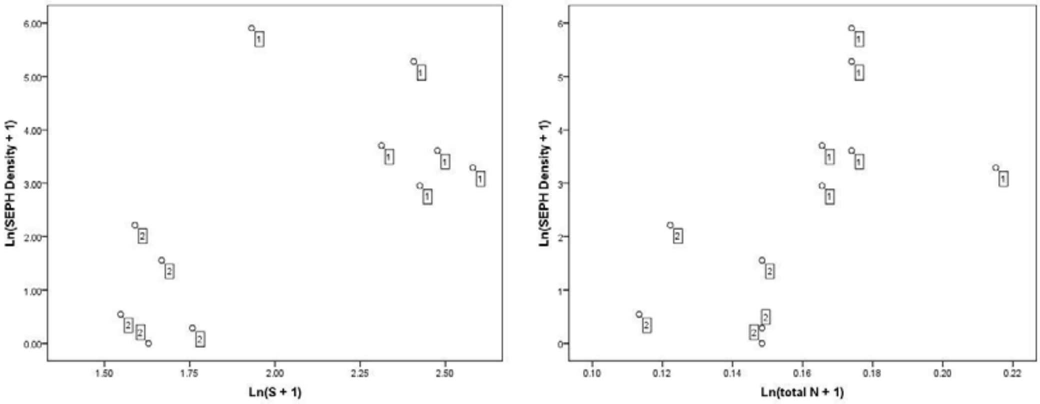
Figure 6d: SEPH count on shrub ($^{3}\sqrt{S}$) against volume of shrub ($^{3}\sqrt{v}$)

Table 2: LAD envelope regression for distance from shrub to nearest SBI nest entrance ($\sqrt{d}$) (y-variable) against SEPH count on shrub ($^{3}\sqrt{S}$) (x-variable)

| n = 44 | Quantile: | | | | |
|---|---|---|---|---|---|
| | 0.5th | 0.6th | 0.7th | 0.8th | 0.9th |
| r | 0.5000 | 0.5355 | 0.6086 | 0.7133 | 0.8713 |
| p | **0.0191** | **0.0157** | **0.0050** | **0.0073** | **0.0065** |
| slope | -0.1065 | -0.0881 | -0.0963 | -0.1114 | -0.0824 |
| constant | 0.3416 | 0.3784 | 0.4630 | 0.5811 | 0.6733 |

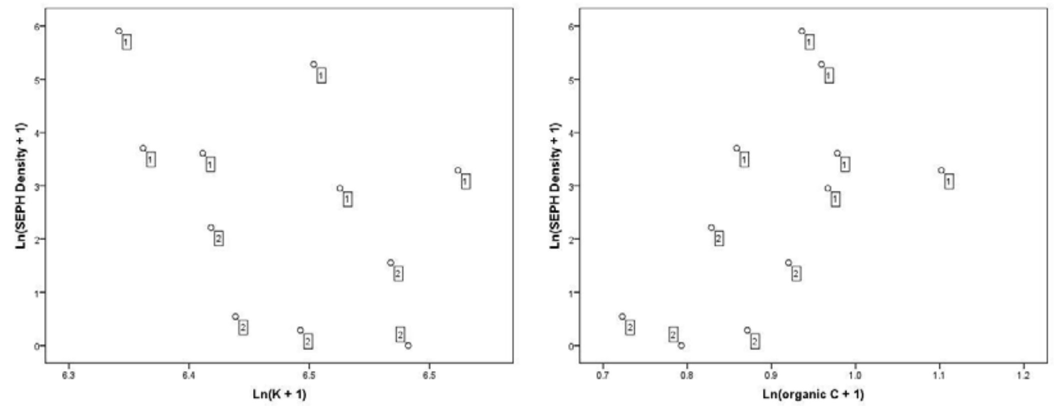
## Correlation of SEPH density with soil parameters on patches of neighbour groups

Total SEPH densities had stronger regressions with soil parameters than total SEPH counts did on *A. victoriae* within neighbour groups. A number of soil parameters (ln(s + 1)) had regressions with SEPH density (ln($S_5/V_5$ + 1)), either alone or in combination (Figure 7) (table 3). Regressions of SEPH densities for neighbour groups were strongest with nitrogen and potassium combined (negatively with potassium, p = 0.013; positively with nitrogen, p = 0.005). All other soil parameters with a significant regression with SEPH density except potassium correlated with nitrogen (Chapter 2). Phosphorous was not correlated to SEPH count or density (n = 11, p = 0.743) and neither was pH (n = 11, p = 0.346), or to any other soil parameter (Chapter 2). In the graph for sulphur and perhaps electrical conductivity it appears that there are two isoclines, relating to separate sites, which show individually negative correlations with SEPH density. All soil nitrogen concentrations on site 1 were higher than all on site 2 and the same was true for sulphur. Since there are autocorrelations between all of the soil parameters that correlated with SEPH density except potassium, these soil parameters were considered together.



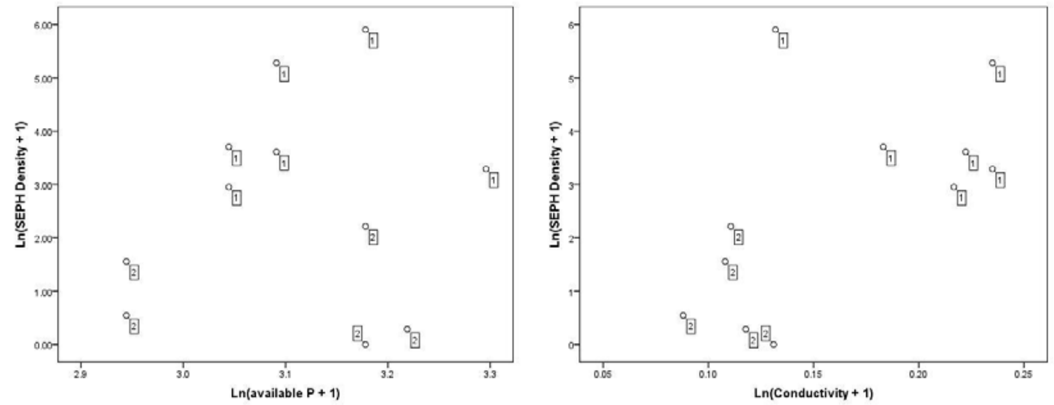
(a) Sulphur *

(b) Total nitrogen Δ



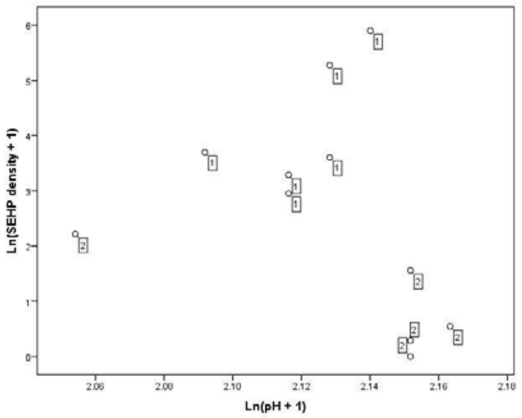
(c) Potassium

(d) Organic carbon $^{\Delta}$



(e) Available phosphorous

(f) Electrical conductivity $^{\Delta}$



(g) pH

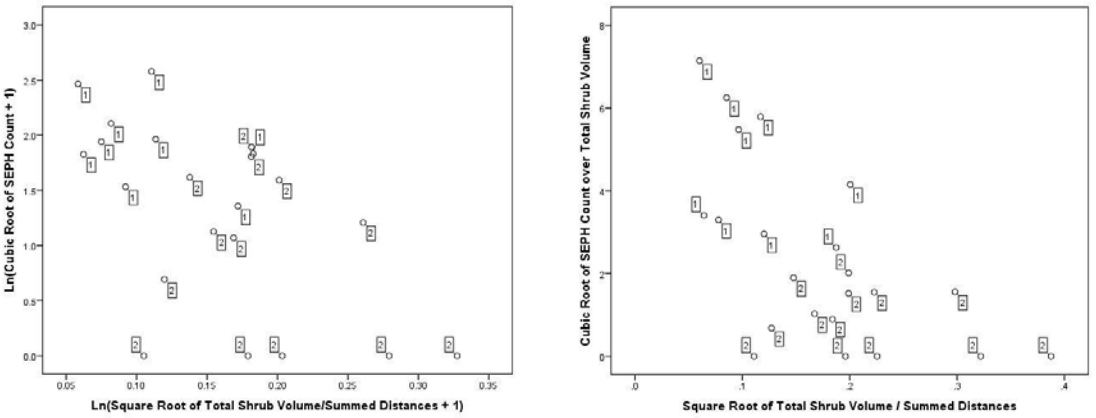
Figure 7: Neighbour group soil parameters (ln(s + 1)) against total SEPH count per total volume (ln(($\sqrt{(S_5 / V_5)}$ + 1))

Table 3: Correlations between neighbour group soil parameters ($\ln(s + 1)$) against total SEPH count per total volume ($\ln((\sqrt{(S_5 / V_5)} + 1)$)

| n = 11 | | N & K | K | N | S | E.C. | $C_{organic}$ |
|---|---|---|---|---|---|---|---|
| SEPH density | $r^2$ | 0.715<br>(N positive, p = 0.005,<br>K negative, p = 0.013) | -0.175 | *+0.355* | +0.414 | *+0.349* | *+0.342* |
| | p | **0.007** | 0.201 | ***0.053*** | **0.033** | ***0.056*** | ***0.059*** |

## Correlation of SEPH count with shrub volume per area density on patches

SEPH count on groups of neighbour shrubs ($\ln(\sqrt[3]{S_5} + 1)$) had a negative regression relationship with volume per summed distances of neighbour groups for pooled sites ($\ln( \sqrt{V_5} / D_4 + 1)$ (Figure 8a) (n = 22, $r^2 = +0.336$, p = 0.005), however residuals on site 1 were generally higher than on site 2, indicating some influential factor that differed between sites. The relationship between SEPH count per shrub volume, the weighted average SEPH density for neighbour groups, and summed distances between shrubs was similar (n = 22, $r^2 = +0.395$, adj. $r^2 = +0.365$, p = 0.002) (Figure 8b). Total SEPH count did not correlate at all with total shrub volume. Volume per distance ($\ln( \sqrt{V_5} / D_4 + 1)$ made a negative envelope for SEPH count ($\ln(\sqrt[3]{S_5} + 1)$) which was increased in strength of pattern and effect up to the 0.8th quantile, although the relationship at the 0.5th and 0.7th quantiles was of marginal significance (table 4). Total neighbour group SEPH count had a positive regression with summed distances between pairs of shrubs (n = 22, p = 0.010, $r^2 = +0.289$, adj. $r^2 = +0.253$) (figure 9).



(a) (b)

Figure 8: (a) Neighbour group total SEPH count $(\ln(\sqrt[3]{S_5} + 1))$against total group shrub volume over summed distances between pairs of shrubs $(\ln( \sqrt{V_5} / D_4 + 1)$ and (b) Neighbour group total SEPH count per total volume $(\ln(\sqrt[3]{(S_5/ V_5} + 1))$ against total group shrub volume over summed distances between pairs of shrubs $(\ln( \sqrt{\Sigma V_5} / \Sigma D_4 + 1)$

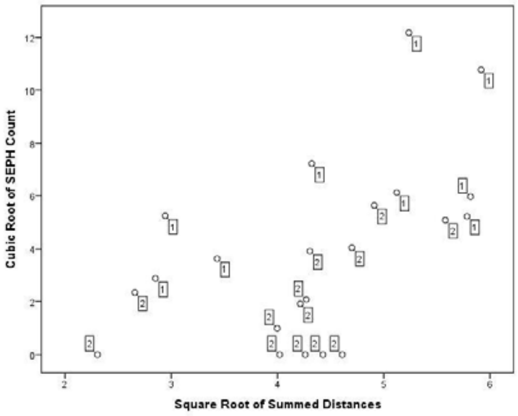


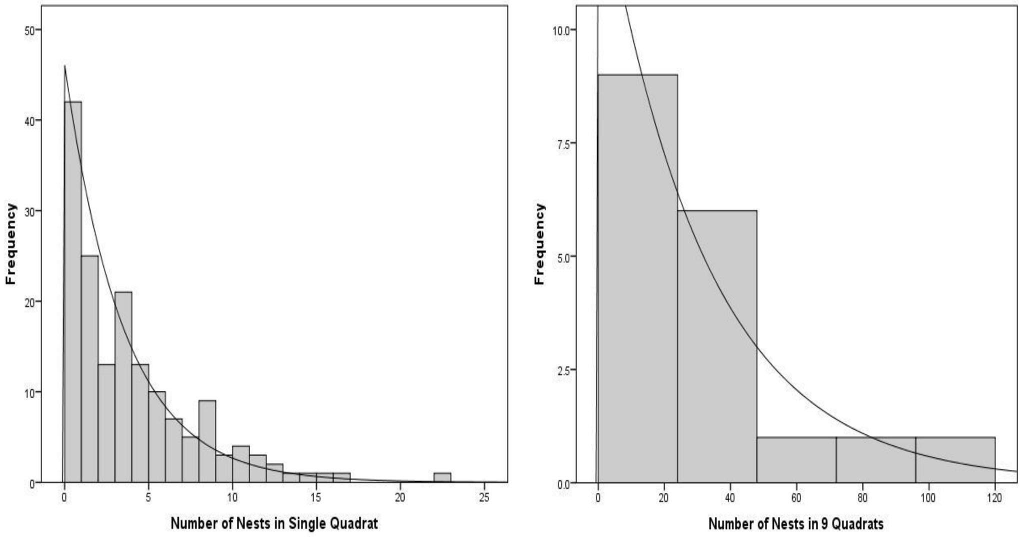
Figure 9: Neighbour group total SEPH count $(\sqrt[3]{S_5} + 1)$ against summed distances between pairs of shrubs $(\sqrt[3]{D_4})$

Table 4: LAD quantile regression of SEPH count $(\ln(\sqrt[3]{S_5} + 1))$ for volume over summed distances $(\ln( \sqrt{V_5} / D_4 + 1)$ of neighbour groups

| n = 22 | Quantile: | | | | |
|---|---|---|---|---|---|
| | 0.5th | 0.6th | 0.7th | 0.8th | 0.9th |
| r | 0.5000 | 0.5424 | 0.6017 | 0.7145 | 0.8586 |
| | *0.0721* | **0.0341** | *0.0591* | **0.0350** | 0.1365 |
| p | -20.3825 | -26.4942 | -25.5056 | -31.9331 | -37.6808 |
| | 6.9060 | 8.9767 | 9.0396 | 10.8195 | 73.4898 |
| slope | | | | | |
| constant | | | | | |

## Correlation of SBI nest entrance counts with SEPH counts and total shrub volume in quadrats

Correlation of numbers of SBI nests ($\ln(\sqrt{N} + 1)$) with numbers of SEPHs ($\ln(\sqrt[3]{S_{total}} + 1)$) or shrub volume ($\ln(\sqrt[3]{V_{total}} + 1)$) in quadrats depended on unit scale, but distribution of both approximated the exponential (figure 10).



(a) (b)

Figure 10: Frequency distribution of number of SBI nests in (a) single quadrats and (b) 9 adjacent quadrats

In single quadrats, there was virtually no relationship between numbers of SBI nest entrances and SEPHs ($n = 161$, $r^2 = 0.064$, $p = 0.001$) (Figure 11). Mean number of SBI nests in single quadrats was 3.25 ($n = 162$), with minimum 0 and maximum 22. For nine adjacent quadrats pooled, correlation of SBI nest entrance counts with SEPH counts was positive and moderately strong ($n = 18$, $r^2 = +0.454$, $p = 0.002$, slope = 0.472 ± 0.130, constant = 0.946 ± 0.233) (Figure 12). Average number of SBI nests in 9 adjacent quadrats was 31.61, with minimum 0 and maximum 101 ($n = 18$).

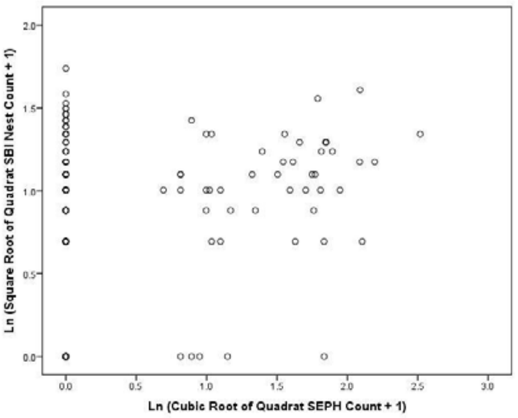


Figure 11: Numbers of SBI nests ($\ln(\sqrt{N} + 1)$) against SEPHs ($\ln(\sqrt[3]{S_{total}} + 1)$) in single quadrats

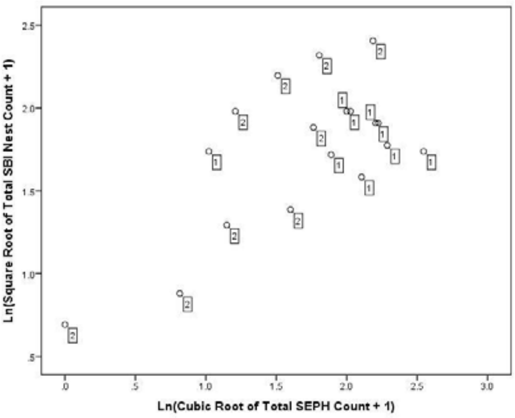


Figure 12: Numbers of SBI nests ($\ln(\sqrt{N} + 1)$) against SEPHs ($\ln(\sqrt[3]{S_{total}} + 1)$) for 9 pooled quadrats

In single quadrats with the same influential point removed, regression of shrub volume with SBI nest entrances showed no relationship ($n = 161$, $r^2 = 0.009$, $p = 0.223$). Regression of pooled number of SBI nests in 9 adjacent quadrats showed no relationship with shrub volume after removal of one outlier ($n = 18$, $r^2 = 0.091$, $p = 0.238$).

Total shrub volume and SEPH numbers in single quadrats were moderately positively correlated ($n = 161$, $r^2 = 0.409$, $p = 0.0001$). There was however no relationship when considering 9 adjacent quadrats, even with an outlier corresponding to shrub 57 removed ($n = 17$, $r^2 = 0$, $p = 0.978$) so autocorrelation need not be considered.

A three-dimensional model of SBI nests ($\sqrt{N}$) against SEPH counts ($\ln(\sqrt[3]{S_{total}} + 1)$) and shrub volume ($\ln(\sqrt[3]{V_{total}} + 1)$) explained a slightly larger proportion of the variation than either variable alone after one outlier was removed for nine adjacent quadrats ($n = 17$, $r^2 = 0.508$, $p = 0.007$; for SEPH, $p = 0.014$; for Volume, $p = 0.026$).

## Discussion

This study has found a strong positive spatial association between SBIs and SEPHs on shrubs which is far in excess of the weaker positive association of SBIs and their nests with shrubs. This positive association was found at the scale of individual shrub stems, where SEPH populations were much more strongly correlated with SBI activity on shrubs than was stem volume, and this appeared to be causative when SEPH populations were removed from different shrubs. SBI nest entrance placement with respect to shrubs also reflected SEPH populations and not shrub size at the scale of single shrubs, showing the strong influence of SEPH populations on SBI populations. Additionally, SBI nest entrance density was positively associated with SEPH populations at the scale of 15m x 15m quadrats. Shrub volume appeared to have little influence on SBI populations. There is also spatial association between SEPH populations and both soil parameters and shrub density, which suggests bottom-up influence of the SBI-SEPH-shrub association.

In this study, numbers of SBIs running up single *A. victoriae* stems had a moderately strong and positive regression relationship with numbers of SEPHs on stems, whereas correlation of SBI numbers with stem volume was very weak. This provides strong evidence that what draws SBIs to shrubs is mainly number of SEPHs rather than other populations or physiological characteristics proportional to stem volume. The similar regression relationship when numbers of SBIs were proportional to stem circumference suggests that numbers of SBIs on shrubs are not limited by stem circumference. A linear relationship between numbers of SEPHs on woody perennials and numbers of ants tending them was found in another arid environment in the Judean Desert, Israel, with approximately 30 aphids per ant on poplars (Degen et al 1986). This begins to suggest generality of the relationship in similar environments in that SE resources proportionate to the SEPH population are key factors regulating ant activity levels on woody perennials. Removal of high densities of SEPHs from *A. victoriae* shrubs resulted in decreased numbers of SBIs trailing up them in proportion to numbers removed. This indicates that SEPH numbers were the primary driver of SBI activity on shrubs in this study, at least during a time of peak activity of SBIs. If numbers of SEPHs have such a strong effect on numbers of these predators, numbers of SEPHs are likely to affect other processes on shrubs and on the ground such as SBI predation or deterrence of other arthropods.

Limitation of distance of nearest SBI nest entrance to shrubs by increasing shrub SEPH counts concurs with data from a different study on a nearby site at three shrubs with high SEPH densities, where SBI nest entrance density was inversely proportional to distance to shrub up to distances of a few metres (Clarke 2006). SEPH population strongly influenced SBI nest entrance placement, so SBIs might compete for space near a shrub more intensely, the larger the SEPH population is on a shrub. Since in the current study, distance of nearest SBI nest entrance to shrub was only very weakly negatively correlated with shrub volume and shrub volume was more strongly positively correlated with SEPH counts but not with distance to nearest SBI nest entrance for shrubs with very few SEPHs, it appears that shrub volume is not very important to distance of nearest nest entrance to the shrub. These results show that SBI populations are distributed in relation to SEPH population and that this strongly influences their abundance at small scales. Variation in this pattern which is not explained by SEPH correlation with distance to nearest nest entrance suggests there might be other reasons for SBIs nesting closer to a particular shrub. Nests of *L. humile* in North America were usually within 2-4m of woody perennials but in a laboratory experiment, humidity was a more important determinant of nest location for closely related *L. humile* than food (Heller et al 2006). SBIs that nest directly under shrubs may be able to forage for longer periods of the day when temperatures are too high to travel across unshaded soil surfaces (pers. obs.). The advantages of building nests under shrubs may include higher moisture levels, lower temperatures, and higher levels of organic matter underneath. Through these conditions, under shrubs SBIs and brood in particular might experience less dehydration, soil structure may facilitate nest construction and maintenance, and soil may have other beneficial qualities such as superior aeration from moister soil with more organic matter content, providing more oxygen. In addition, important protein resources for SBIs such as fossorial invertebrates might exist in the soil under shrubs, which are possibly related to effects of honeydew dropping on soil below.

Correlations between soil parameters and neighbour group SEPH density on *A. victoriae* neighbour patches suggest that soil influences spatial distribution of the association. Lack of correlation of SEPH count and shrub volume at the scale of 15m x 15m, a similar scale, suggests this is not due to soil effects on shrub volume. Parameters of soil around shrub roots affect leaf nutrient concentrations, different levels of which are associated with different *Sextius* densities on nearby sites (Chapter 4), suggesting a link between soil, shrub nutrient concentrations, and SEPH abundances. Concentration of soil phosphorous has been linked to abundance of an insect herbivore in another desert system (Schade et al

2003). Neighbour group SEPH density has positive regression relationships with total nitrogen, organic carbon, sulphur, and electrical conductivity, which all correlate with each other. Therefore one nutrient might have a strong limiting influence on SEPH density in these conditions, particularly if it is a limiting factor on shrub fitness. Although the nutrient that is most commonly limiting for sap-suckers is thought to be nitrogen (Mattson 1980), it is unclear which soil parameter has the strongest relationship with SEPH density. There are many unknown factors to how soil nutrients might affect plant physiology related to insect deterrence. Although sulphur correlates positively with neighbour group SEPH density, it appears that with more replicates, the graph for sulphur might show site-specific isoclines of a negative correlation as might conductivity. Organic carbon in the soil probably reflects accumulation of organic carbon by vegetation (Facelli and Brock 2000) which may correlate with age of patch. The negative envelope created for total neighbour group SEPH count by total shrub volume per summed distances between shrubs and the two other relationships found between related parameters suggest that shrubs undergoing less competition for soil resources with conspecifics have higher maximum possible SEPH count or density. Sign of correlations of soil nutrients nitrogen, potassium, and sulphur with SEPHs is opposite that of correlation with volume per distance (see Chapter 2) which is consistent with the pattern found of higher SEPH densities occurring with lower shrub densities. After a year of rainfall one standard deviation below the mean, this might be because shrubs in less fertile patches which would be smaller were subjected to greater water stress, which elevated levels of nitrogen in sap, which many sap-suckers prefer. Alternatively, it may be due to indirect effects. Manipulative experiments could reveal causative factors and identify the most important parameter.

Potassium tends to affect herbivore-induced plant volatiles (HIPVs), but causal relationships between HIPVs and overall consequences for plants are not yet known (Amtmann et al 2008). The phytohormone jasmonic acid (JA) is usually involved in defence against attacks by herbivores, while salicylic acid (SA) is involved in defence against pathogen attack. Since phloem-feeders usually only slightly damage cell tissue, SA-activated defences are more important than JA-activated ones, but no studies were found on nutrient effects on SA. Limited potassium supply can increase concentrations of sugars and amino acids in the plant, which might increase attractiveness and of the plant to herbivores including SEPHs, but this tends to be counteracted by increased defence (Amtmann et al 2008). However, since the JA pathway tends to inhibit the SA pathway (Amtmann et al 2008), defence against sap-suckers might possibly be reduced, causing higher SEPH densities with lower soil potassium. Alternatively, if the same shrubs host large populations of SEPHs for several years, they may deplete the area around the shrub of potassium, since there tends to be large quantities of it in sap (Dinant et al 2010). Perhaps SEPHs could exert similar effects on other soil nutrients with which they are negatively correlated such as sulphur where they occur in high densities in repeated years. Individual shrub levels of nutrients related to SEPH population are dealt with in another paper (Chapter 4) and relationships may vary with rainfall in the preceding period.

There is a close correlation in amino acid composition between phloem-feeder SE and phloem sap (Weibull et al 1990 in Yao and Akimoto 2002), and ants can be attracted in larger numbers to SE of higher nutritional quality (Völkl et al 1999, Woodring et al 2004). Therefore nutrient concentrations in sap may affect number of SBIs tending phloem-feeders, potentially altering association dynamics. This might reduce or increase growth depending on environmental conditions and species of SEPHs, since some SEPHs might be parasitic on shrubs some or all of the time. SBIs might even tend *Sextius* sp. only when on shrubs of sufficient nutritional quality, which could determine distribution of the association since *Sextius* adults leave soon after SBI removal (chapter 4). Although the role of soil nutrients in plant defence may be important (Orians and Milewski 2007), experiments manipulating soil nutrients and monitoring effects on SEPH density and plant chemistry are needed to determine specific causation.

Positive correlation of numbers of SBI nest entrances with total SEPH numbers on shrubs in 15m x 15m squares of 9 quadrats is reasonably strong and suggested spatial association between SEPH and SBI populations. The different soil parameter values on sites 1 and 2 affected SEPH densities. This could explain the different pattern of SBI nests to SEPH numbers between the two sites. The range of concentrations of sulphur and potassium is greater on site 1, which may have added variation to resource distribution for SBIs and perhaps explained the lack of pattern for SEPH count against nest entrance count on site 1 alone. Since single quadrat shrub volume correlates positively with shrub SEPH count, an intuitive conclusion to draw is that some of the influence of shrub volume on SBI nest entrance numbers must be that larger shrub volume hosts more SEPHs. Also, SEPH numbers and shrub volume as independent variables correlate slightly more strongly with nest entrance numbers than either alone, showing some importance of shrub volume. However in the current study, lack of correlation of nest entrance numbers with total shrub volume suggested greater importance of SEPH numbers than shrub volume to SBI nest entrance distribution at unit scale of 15m x 15m.

SEPH numbers appear to influence SBI numbers at unit scales larger than single quadrats. There was no correlation between total SEPH numbers and number of SBI nest entrances at unit scale of 5m x 5m, whereas there was positive correlation at 15m x 15m. There were not enough quadrats in this study to pool data from larger unit areas than 15m x 15m. It is possible that spatial distribution or nutritional composition of SEs alters the distances over which SBIs travel to forage, and SBI capability to travel might limit this, in which case the optimal scale on which to determine a pattern of spatial distribution might be a function of SEPH density. This is an alternative explanation for the lack of pattern of nest entrance count and SEPH count on site 1, where SEPH densities were higher. Since a pattern linking SEPH and SBI abundances was found at unit scale of greater than 5m x 5m, SBIs probably travel on average more than 5m and less than 15m along trails between nests and shrubs at the study sites.

SBI activity on shrubs is much lower when SEPH populations and other resources are low according to a seasonal cycle which varies from year to year (pers. obs.), probably through influences of variation in temperature, insolation, and soil water availability. Nests of *L. humile* in North America on average were located in relatively cooler positions in summer than in winter (Heller et al 2006). SBIs might move nest entrances and perhaps nests quickly and distribute them according to a seasonal cycle in response to seasonally available resources. One study of two *Iridomyrmex* species in mesic NSW found that the mean period for which a nest entrance of *I. darwinius* and *Iridomyrmex* species A remained in use was 1.5 and 2 months respectively, with very few remaining continually in use for over 3 and 6 months respectively (Hughes 1991), and it was deemed that nest structure probably altered with movement of entrances. Over a 3 month period around 30% of closed nest entrances of both ant species reopened. SBI colonies are likely to behave in a similar way, moving nest entrances and probably nests back and forth according to the seasonal cycle. SEPH spatial distribution may change substantially over shorter timescales due to events such as rainfall regulating sap quality and quantity and presence of pathogens or predators, and over longer timescales such as soil resource removal by runoff or vegetation sequestration. Manipulating distribution and perhaps quality of SEs and distribution of other seasonal effects on nest suitability such as light and soil moisture levels might be used to reveal spatial and temporal scales on which SE affects SBI nest distribution.

It is possible that SEs are not the only important source of food for SBIs on shrubs, but other resources would occur only sporadically with SBIs accessing them opportunistically, since biotic temporal and spatial variability is high. SBIs use SE for energy, but they also require sufficient nitrogen to produce brood and therefore this element may be limiting for the SBI potential to create and maintain large populations. However, provisions of aphid honeydew to laboratory populations of hemipteran-tending *S. invicta* increased mass of workers and brood, while amino acids had no positive effect (Wilder et al 2011), so this cannot be assumed. While shrubs are in flower, SBIs might collect pollen to obtain nitrogen. Different quantities and nutritional quality of SEs from different SEPHs may influence SBI behaviour reflected in preference for certain SEPH species. Sources of protein such as other arthropods susceptible to SBI predation might preferentially inhabit areas that are also frequented by SBIs. One study of an *Iridomyrmex* species found that nest entrances were clustered around both termite mounds and trees housing SEPHs (McIver 1991). Termites in the present study also strongly influenced trails in quadrats for at least short periods of time and may be important to SBIs in combination with SE.

Colonies of a dominant ant *S. invicta* grew about 50% larger in a greenhouse when SE was made available than when provided with arthropod prey alone, but shrank when SE was available but arthropod prey was removed (Helms and Vinson 2008). In another laboratory study, in a population of *L. humile*, worker survival and fat levels increased when honeydew was available and lack of honeydew meant no brood were produced, but high prey availability with honeydew increased worker survival more than honeydew alone (Grover et al 2007). Termites are one of the most abundant members of the fauna in the arid zone (Morton 1993) and ants often appear to build nests near them (pers. obs.), but patterns of ants consuming termites are unknown, so this should be studied in relation to effects of SEs on dominant ants in arid Australia. Since SE may fuel collection of more protein for dominant ants (Davidson 1997), SE and protein may generally facilitate growth of colonies in combination.

## Conclusion

This study is the first to quantitatively document spatial distribution of populations of native dominant ants, *Acacia* shrubs, and predominantly native SEPHs in a putatively mutualistic food-for-protection association in a natural, if degraded, system. Evidence for strong spatial association between abundant SBIs, SEPHs, and shrubs has been found and for the importance of SE to dominant ants in arid Australia. Manipulations of SE levels should be done to assess this finding over the spatial scale of a site, such as one hectare, with sufficient site replication to elucidate further how dominant ant colony structure and activity respond to different distributions of SEs. For example, what is the relationship between SEPH abundance and SBI colony size? Other food resources might be needed to complement effects of SE, and these should be incorporated into studies. Under influences of climate change, it is predicted that rises in temperatures and $CO_2$ levels will cause increases in arid zone woody plants (Hughes 2003) but numbers of most insects except phloem-feeders may be negatively affected (Hughes and Bazzazz 2001). If woody plants increase in biomass, nitrogen limitation on growth might increase, and then carbon-based plant defences may become even more relatively important. Numbers of ants, which are thermophilic, could increase with temperature in cooler areas. Climate change might alter abundances of partners in this association and the dynamics with which they interact.

This study strongly suggests that SEPHs have substantial impacts on the distribution of dominant ants which have strong effects on structure of local ant communities over vast areas. Increased aggression and activity of dominant ants due to increased availability of SEs would likely multiply the strength of their effects on arthropod and plant communities. Indeed, arthropod biomass in the arid zone can be up to nine times that of vertebrates (Polis 1990) so in such a biome, it is likely that strong spatial association between dominant vegetation and dominant invertebrate predators has a substantial impact. This study provides information about spatial scales at which effects of SEPHs on dominant ant colonies take place. Little is known about how ant community structure affects other flora and fauna in the community. Further studies should ascertain at relevant scales how large numbers of dominant ants affect other organisms with which they interact.

Soil nutrients also appeared to regulate SEPH populations on shrubs at the spatial scale of neighbour groups and thus through SEPHs may be a strong driver of SBI colony size and activity at the unit scale of 15m x 15m. In this way, bottom-up processes may strongly determine abundance of dominant ants and regulate their behaviour, which through levels of competition and predation by these dominant ants would be likely to produce broader impacts on the whole ecosystem. However, more research including the use of nutrient manipulation is required to determine how soil concentration of specific nutrients and shrub density regulate SEPH populations on shrubs or whether shrub or microorganism processes regulate shrub nutrient use more strongly. Moisture levels are likely to influence these effects, although there may be a lag time to see the outcomes in insect populations. Manipulative studies need to isolate soil parameters to ascertain causation in these relationships and trophic links to ants. In arid Australia the high heterogeneity of such abiotic factors that control bottom-up processes might create distinctive spatial variation that is reflected in populations of organisms at higher trophic levels such as those studied here. Arid Australian ecosystems constitute a useful model system to study spatial and temporal scales over which community patterns are regulated by bottom-up processes in systems where water is limiting. This may have particular relevance for research into the effects of climate change on biological processes and adaptation.

# Chapter 4:

# Host Preference and Partner Outcomes in an Association between Shrubs, Sugary-Exudate Producing Herbivores, and Attending Ants in Semi-arid Australia

## Abstract

This study investigated factors determining density of sugary-exudate producing herbivores (SEPHs) attended by ants on *Acacia victoriae* shrubs, including the treehopper *Sextius* sp., and it investigated effects of their association with small black trailing *Iridomyrmex* ants (SBIs) on the shrubs and on survival of *Sextius* sp. nymphs. A field experiment contrasted different SEPH levels using high and low natural densities of *Sextius* on individual shrubs with a Tangle-Trap treatment excluding SBIs and SEPHs. Soil parameters, leaf nutrients, shrub growth rate, seed production, folivory rates, and assemblages of small arthropods on shrubs were recorded. A field survey correlated natural SEPH densities of individual shrubs with shrub growth rate before and after the growth period, seed production with and without bags excluding seed-consuming insects, and assemblages of small arthropods. A small field experiment tested effects on *Sextius* nymph populations of excluding all crawling predators including SBIs both with and without further exclusion of flying and jumping predators. It is unclear which small arthropods SBIs deter on shrubs since assemblages vary over time, but it is likely that parasitoid wasps and SEPHs affect densities of each other. High *Sextius* levels were correlated with low concentrations of soil sulphur or electrical conductivity near shrubs, levels of specific shrub nutrients, high shrub growth rate, and their presence depended on SBI presence on stems. At the scale of approximately 20m x 20m, soil parameters were correlated with average SEPH density of shrubs and with shrub growth and densities of other small arthropods. It was difficult to ascertain whether natural SEPH/*Sextius* density on shrubs affected shrub growth rate, but exclusion of *Sextius*, SBIs, and other crawling insects using Tangle-Trap reduced growth rate of stems with high densities. On shrubs with high *Sextius* density, growth rate was higher, seed production was higher, folivory rates were lower, and assemblages of small arthropods were different, particularly densities of wasps of 1mm in length and less which would be mostly egg parasitoids.

Other phloem feeders may be limited by SEPH densities and may provide resources for higher densities of parasitoid wasps in the absence of high SEPH densities. This study pinpoints variables that warrant further study in terms of affecting the dynamics of this tritrophic system.

## Introduction

Tritrophic food-for-protection interactions involving ants and sugary-exudate (SE) producing sap-sucking herbivorous insects on plants, including those that are facultative, increase plant performance in about three quarters of studies of natural systems (Styrsky and Eubanks 2007). Ants consume the sugary exudates produced in most cases by sap-sucking Auchenorrhyncha or Sternorrhyncha as a waste product, and in return usually reduce overall herbivory on plants and predation or parasitism on the sap-suckers. Ant attendance can provide other benefits such as prevention of sap-suckers drowning in exudate or plant infection by moulds that grow on accumulated exudate (Delabie 2001) or increases in hemipteran reproductive and developmental rates due to increased feeding rates (Stadler and Dixon 1999). Similar interactions between ants and lycaenid larvae can be beneficial to lycaenid survival (Eastwood and Fraser 1999) although effects on the plant have not been tested. However, benefits can vary with species of partners (Bronstein 1998, Stadler and Dixon 1999). Benefits to a partner can be highly variable, depending on abiotic and biotic environmental conditions, so outcomes of this type of interaction even for the same species in the same environment might range from positive to neutral to negative (Bronstein 1994, 1998). Even intraspecific genetic plant variation can affect whether outcomes for a partner of a given ant-aphid-plant interaction are antagonistic, neutral, or mutualistic (Mooney and Agrawal 2008) so variation in plant characteristics which might be influenced by the abiotic environment, such as sap nutrient concentration, might strongly affect this type of association. However, studies of outcomes across environmental gradients are rare. Suites of environmental gradients typify different biomes. A recent meta-analysis of the general role of ants in protection of plants found that ant removal resulted in a doubling of herbivore damage and a 50% increase in herbivore abundances on plants in mesic areas, but these effects were three times higher on tropical than mesic plants (Rosumek et al 2009) suggesting strong effects of biome type. The trophic complexity of tritrophic interactions might result in a particularly wide range of net outcomes for partners in environments with high temporal and spatial variability such as arid zones. However, very few studies have examined outcomes of ant-exudate-producer-plant associations in arid systems or along gradients relevant to aridity, such as moisture and nutrient availability and insolation.

Most studies show that ant presence on plants due to attending hemipterans increases plant fitness and this happens through reducing densities of insect herbivores (Styrsky and Eubanks 2007, Rosumek et al 2009), although some studies have found no effect or negative effects. In some cases, they might have different effects on one measure of plant fitness such as vegetative growth from on another measure such as seed production. Where only one ant species takes part in an association either with extra-floral nectaries or with attended hemipterans, reductions of herbivory tend to be stronger than when several ant species are involved (Rosumek et al 2009). Few studies have focussed on effects of this type of mutualism on whole arthropod communities on plants (Styrsky and Eubanks 2007, Grover et al 2008). Removal of ants generally results in higher herbivory, greater herbivore abundance, more predators, fewer attended sap-suckers, lower seed production, and lower plant biomass and leaf production (Rosumek et al 2009). Little information is available on effects on plants of associations of ants with lycaenid larvae.

The plant vigour hypothesis (PVH) predicts that herbivorous insects preferentially oviposit on plants or plant parts which are growing faster and that they will perform better on these (Price 1991). A meta-analysis found that sap-suckers tend to prefer faster growing plants to a greater degree than other insect guilds (Cornelissen et al 2008). PVH does not specifically propose mechanisms, which could include not only increased quantity and quality of food resources including higher osmotic potential, lack of defensive compounds, or lower abscission rates, but also decreased time and energy expenditure in oviposition (Cornelissen et al 2008). In harsh environments, the ability to locate vegetation with such qualities might be particularly important. The plant stress hypothesis (PSH), which may or may not contradict the PVH, states that insects prefer and perform better on plants under stress, due to increased nitrogen content and decreased production of defensive chemicals (Huberty and Denno 2004). This was particularly expected for phloem-feeders since concentration of allelochemicals in vascular tissue can be reduced under stress. However, a meta-analysis showed that phloem-feeders survive less on continuously water-stressed plants but can perform better on intermittently stressed plants (Huberty and Denno 2004). Intermittent stress may favour phloem-feeder populations in semi-arid environments where stress levels are likely to fluctuate substantially across time and space. Host plant selection is also influenced by plant nutrient composition or ratio of nutrients to toxins (Lambdon and Hassall 2005) which in arid environments might have relatively high temporal variability due to moisture and high spatial variability due to highly heterogeneous soils.

Insects have greater negative impacts on plant biomass than mammalian herbivores, in unmanaged systems (Bigger and Marvier 1998), so insects are important in controlling plant community structure.  In studies included in a review, plant population growth was decreased more by native insects than by native mammals (Maron and Crone 2006) so insects might indirectly influence the whole community in important ways.  The two most common types of herbivory, sap-feeding and folivory, have different effects on the host plant.  Removing sap can remove carbon sources such as sugar and nutrients, but removal of leaf tissue reduces photosynthetic capacity and this effect requires much more time to be overcome.  Although insect activity can vary substantially within a particular month among years and among months in the same season in semi-arid systems (Palmer 2010), and therefore herbivory can vary similarly, costs of losing leaf tissue to herbivores in conditions of resource limitation might consistently outweigh costs of providing readily produced sap.  Despite possessing very tough leaves adapted to deter herbivores, insect defoliation appeared to have a more severe impact on Australian eucalypts than it has on trees of other continents (Lowman and Heatwole 1992).  Therefore, arthropod herbivory might have the potential for substantial effects on Australian sclerophyllous plants which might necessitate high defense levels at some times.  Low nutrient levels of Australian sclerophylls might lead to different dynamics of herbivory in Australia (Peeters 2002) or the arid zone in general.

Associations between ants, SEPHs, and *Acacia* or *Eucalyptus* shrubs or trees, are common and widespread in Australia, the majority of which is arid.  These interactions can have strong cascading effects on ecosystems in mesic regions (Wimp and Whitham 2001, Eubanks and Styrsky 2006) and may have similar effects in arid Australia. In fact, low nutrient levels in arid Australian soils are suspected to make ant-SEPH food-for-protection interactions on plants particularly common since carbon is relatively abundant (Stafford Smith and Morton 1990) and plant defence tends to be limited either by carbon or by nutrients such as nitrogen (Orians and Milewski 2007). This may even contribute to fire regimes by decreasing herbivory, thereby increasing available fuel and as a result raising intensity of fires (Orians and Milewski 2007). Despite this, studies on partner outcomes of this interaction type in Australian arid areas are virtually unknown.

In Australia, horned treehoppers (Hemiptera: Auchenorrhyncha: Membracidae) of the genus *Sextius* inhabit many species of the woody perennial genus *Acacia* (Cookson and New 1980, Day 1999) where they are attended by ants consuming their SE. These ants are most often of the behaviourally dominant and highly abundant Australian genus *Iridomyrmex* (Greenslade 1978). Both ants and *Acacia*s are particularly common in semi-arid to arid and dry tropical areas of Australia (Greenslade 1978, Whibley and Symon 1992) and *Sextius* is widespread throughout Australia where it is the most common membracid (Day 1999). The three most common species of *Acacia* in Australia by one measure of distribution, in descending order, are *Acacia ligulata*, *Acacia victoriae,* and *Acacia aneura* (Fox 1987). The last two species have large populations of SEPHs on them in at least some environments. In Australia, the diversity of species involved in this type of interaction and the high level of aggression in the ants (Greenslade 1978) suggest that this type of association might exert a strong influence on partner populations across the continent.

This study addressed three main questions concerning the association between *A. victoriae*, total SEPHs or *Sextius* on shrubs, and small black *Iridomyrmex* (SBIs) that often attend them:

1. How do SBI presence, *A. victoriae* growth rate, concentrations of shrub leaf nutrients, and soil parameters near shrubs influence SEPH/*Sextius* populations?
2. How do *A. victoriae* growth rate, folivory of shrubs , shrub reproductive success, and assemblages of small arthropods on shrubs differ with SEPH/*Sextius* density?
3. How are growth and survival of *Sextius* populations on shrubs influenced by SBIs?

## Methods

### Site

This study was conducted in the Flinders Ranges National Park on a previously heavily grazed former sheep station, currently undergoing a rehabilitation program. Mean annual rainfall is 321mm, with median 324mm and coefficient of variation 31%, with a mean 66% rain falling in the colder months from May to October (records between 1985 and 2009). Mean annual potential evaporation is 1200-1300mm (Bureau of Meteorology), giving an aridity index $AI_U$ (UNEP 1992) of 0.25-0.27. A total 325 mm rain fell in the 12 months before the study began in December 2009, which is approximately the mean. Spring rainfall in the three months beforehand was 137mm, which was about 50% more than the spring mean of 92mm. From December 2009 to November 2010 inclusive, a total of 564mm rain fell, which was higher than any 12-month period at the same time in the previous 25 years of records and well over 2 standard deviations above the mean. Spring rainfall for 2010 was 202.7mm, over twice that of the mean. The predominant soil type is calcareous loam. Still severely degraded and supporting huge rabbit populations, understory vegetation is sparse and dominated by the exotic invasive species *Carrichtera annua*, *Asphodelus fistulosus*, and *Marrubium vulgare*, with *A. victoriae* as the dominant canopy in many areas. In the semi-arid Flinders Ranges of southern Australia, *A. victoriae* was present at 27% of sites surveyed and constitutes the dominant canopy in many (Brandle 2001), and might be particularly common where range condition is poor. Reproduction is by germination from a seed with an elaiosome attached which aids its dispersal by ants (Davidson and Morton 1984). Its growth and reproductive period are seasonal, making it more predictable than fully arid-adapted species, which gives it advantages as a study species. Many of these shrubs support populations of SEPHs: green horned treehoppers *Sextius* sp., larvae *of Jalmenus icilius* (Lycaenidae), and grey and red scale insects (Margarodidae). *Sextius* sp. feeds on both new and old growth and emerges in spring, earlier in warmer seasons. A different species which was not found on these sites, *S. virescens*, makes a slit into young branches into which it oviposits eggs, and the process for a whole batch of eggs can last around one hour (Cookson and New 1980). Between these shrubs, vast networks of interconnected trails of small black *Iridomyrmex* sp. (SBIs) stretch. SBIs were primarily of the *Iridomyrmex rufoniger* complex (Shattuck 1999), although others may have belonged to other distinct morphospecies of *Iridomyrmex*. Although only around 4mm in length, these highly aggressive ants that occur in huge colonies sometimes can compete successfully with other *Iridomyrmex* species about eight times their size (pers. obs.). On warm nights in summer, these ants continue to trail to shrubs and attend SEPHs, depending on the temperature (pers. obs.).

## Set-up of experiment and survey

The study included a main field experiment for the purposes of controlling the most important factors and a field survey for the purpose of inclusion of a range of natural conditions.  The field survey recorded soil parameters (see below), shrub growth, seed production, SEPH populations, and small arthropod assemblages on shrubs between two periods of peak insect activity to test effects over a wider spatial scale than the more controlled experiment, with the aim of incorporating more environmental heterogeneity.  A large field experiment examined differences in soil parameters near shrubs, leaf nutrients, shrub growth, folivory, seed production, SEPH populations, and small arthropod assemblages on shrubs.  SEPH populations were manipulated on experiment shrub stems by removing those that were not *Sextius* and removing all SEPHs on one stem of each shrub between two periods of peak insect activity to test effects on shrub growth, seed production, and insect assemblages.  Since other non-*Sextius* SEPH densities comprised a large proportion of total SEPHs in the survey, are high on some shrubs with no *Sextius*, have a similar effect on SBIs, and shrubs with high SEPH levels were limited in number, SEPH levels were used in the study rather than *Sextius* levels.  A small field experiment manipulated SBI and other arthropod access to *Sextius* nymphs over the estimated period it takes to go through one instar, which would be approximately seven to ten days based on other membracids (Linares et al 2010), to test effects on nymph survival.  Behavioural trials of a common chrysomelid, *Monolepta* nr *divisa* (subfamily Gelerucinae, tribe Galerucini, R. Glatz) tested SBI effects on beetle activity on twigs with *Sextius* present or absent.  Although *A. victoriae* possesses extra-floral nectaries, these did not attract much attention from ants.  Sometimes SBIs would pause briefly at nectaries when SEPHs were at low density on a branch, but effect of nectaries on SBI behaviour was considered to be negligible.

Sites for the field experiment were chosen that were relatively flat, had large populations of SBIs, and had high *Sextius* populations on *A. victoriae* that had sufficient space for a person to move around them. Selection of shrubs began as soon as the majority of *Sextius* emergence had occurred: in 2010 November for the field survey and in 2010 December for the field experiments. Shrubs for the field experiments were selected that:

- were of sufficient size to reproduce and not too large to assess SEPH populations on;
- had branches that were not too entangled to count SEPHs on;
- looked healthy enough to survive for the duration of the study;
- had at least two or three stems that were separated enough to prevent ants crawling from one to the other above the level of the exclusion treatment (using Tangle-Trap ®, Ladd Research), and to which Tangle-Trap would be easy to apply at an angle to the ground sufficient to prevent small birds from perching on them;
- were on the same site other selected shrubs allowing for replication.

The three experiment sites were distributed along a curve of approximately 8.5km at elevations of approximately 500 - 530 m (figure 1).

For the survey, three sites were selected that were distributed along a curve of approximately 7km at elevations of 500 to 560 m. In the survey, slope-aspect was incorporated into the four sites for another study (chapter 5), each slope-aspect-site combination being referred to as a sub-site. Sites were selected to cover the widest area practicable to not limit environmental variation within a given number of replicates. Flats selected were the lowest areas to which water would drain from the surface of the slope of the same site, which were mainly creek beds and adjacent areas, and therefore soil properties may have been different from other low areas. A fourth site was added in late September/October 2010 after results indicated that the level of observed variability required further replication. Shrubs for the survey were selected that:

- were within a territory dominated by an SBI colony
- were of sufficient size to reproduce i.e. at least 30cm tall;
- looked healthy enough to survive for the duration of the study;
- occurred on slopes at least two average shrub heights above flats and at least one average shrub height below the hilltop on south-facing slopes;
- were within the compass points N and NW on north-facing slopes and within the compass points S and SE on SFS;
- on flats were where water would drain onto them from the adjacent slope, and the base of the stem was no higher than one average shrub height above the lowest point;

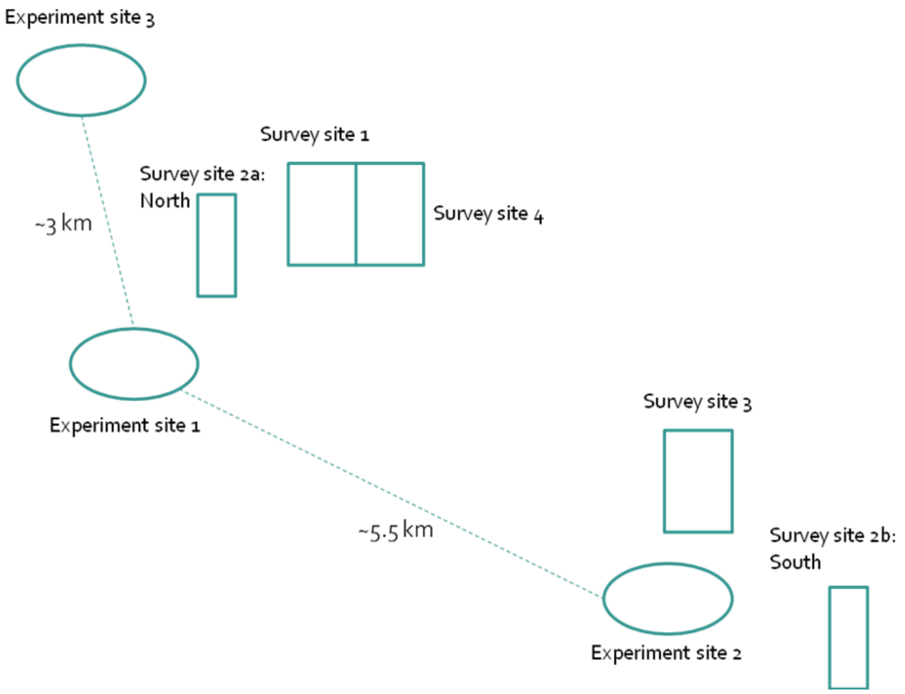


Figure 1: Map of field sites encompassing manipulated experiments and surveys

Nothing about *Sextius* dispersal behaviour was known at the outset of the experiment. Initially to study different *Sextius* densities in the main experiment, on shrubs with at least three stems inhabited by *Sextius*, *Sextius* was removed from one stem, while SBIs were prevented from accessing a second *Sextius*-inhabited stem by application of Tangle-Trap, and a third stem was left as a high-*Sextius* control or received *Sextius* additions. However, when *Sextius* were removed from one stem and adults placed on the high-*Sextius* treatment stem, adults flew back onto the removal stem within a few days, so separate individual shrubs had to be used with naturally different *Sextius* densities. However, when additions of *Sextius* to whole shrubs were attempted, few remained on the shrubs to which they were transferred. Most *Sextius* nymphs were killed by damage to stylets which had been inserted into twigs while feeding, whereas most adults were easy to remove without adverse effects on them. Interestingly, all *Sextius* were removed from three high-*Sextius* shrubs and the shrubs were not recolonized in the same season. Thus the final experiment treatments on shrubs were *Sextius* densities that were naturally low (≤ 20 $m^{-3}$) or high (≥ 80 $m^{-3}$), using on both an exclusion stem (using Tangle-Trap) and a control stem on the same shrub.

Stems undergoing exclusion (Tangle-Trap) treatment (hereafter referred to as "Tangle-Trap stems") were chosen for feasibility of application of tape and Tangle-Trap. Tangle-Trap has been used in a large number of studies measuring effects of ant exclusion on plants (Rosumek et al 2009) and does not damage the plant. Tangle-Trap was applied to plastic wrap on green adhesive tape at the base of the stem of each experiment shrub in January 2010. It is unlikely that other crawling insects stopped by Tangle-Trap would affect shrub growth, since they are in much smaller numbers or of much smaller size than SBIs or *Sextius*. It was not known what characteristics of shrub stems such as size, SEPH or *Sextius* density, or seed production might be influenced by growth rate, potentially biassing results. Individual shrub growth rate among stems varies over time and cannot be predicted from previous years (pers. obs.). Often one stem of a shrub was smaller than the other, so care was taken to balance the number of smaller and larger stems of the same shrub that received Tangle-Trap in case one or the other size would have different growth rates. Potential ant bridges from exclusion stems to other stems and to grasses and forbs growing underneath were removed at least monthly during periods of high insect activity and plant growth for the duration of the study as were SEPHs other than *Sextius*.

## Sampling

### Soil

To find relationships between soil parameters and measures of shrub performance or insect densities on shrubs, soil near shrubs was sampled. For the main experiment, samples of approximately 50g of the top 5cm of soil were taken once in September 2011 at a distance of two canopy diameters from experiment shrubs at points as widely distributed around a circular transect as possible, and not within two canopy diameters of any other shrub. This distance was chosen to assess soil characteristics not modified by the shrub. Samples for a shrub were pooled to give a total of 200g. For the survey, soil was sampled by choosing a point near the centre of each sub-site such that four points 5m apart on four rows 5m apart were centred upon it and no point was within two canopy diameters of any shrub. From each point, 50g was taken from the top 5cm of soil using a 5cm long core. Soil from one row at the same height of each sub-site was pooled, giving four samples per slope or flat. In correlations involving survey soil, sub-sites consisting of one slope-aspect on one site were used e.g. site 1 south-facing slope, site 2 north-facing slope, site 3 flat area. This provides the pattern for a slightly larger scale than that of individual shrubs. All soil samples were oven dried at 70°C for 3-4 days before being sifted through a 1mm mesh and analyzed for concentrations of total nitrogen, $N_{total}$ (%), available phosphorous, $P_{available}$(mg/kg), potassium, K(mg/kg), organic carbon, $C_{organic}$(%), and sulphur, S (mg/kg), and for electrical conductivity, EC (dS/m) and pH (using the $CaCl_2$ method) at CSBP Laboratories in Bibra Lake, Western Australia.

### Shrub nutrients

To assess nutrient concentrations in sap on which SEPHs might base host preference, experiment shrub leaves were collected. Sap that phloem-feeders consume transports large proportions of shrub nutrients to leaves, so leaves should be representative of the nutrients carried in sap. Approximately 1.5g leaves (attached to twigs) were collected from four randomly chosen positions on experiment shrubs in 2011 December to test for leaf nutrient differences according to *Sextius* level. Since all samples were taken from outer foliage, new growth was likely to be sampled on all shrubs. Leaf samples were sent to CSBP Laboratories for analysis of % calcium, magnesium, phosphorous, potassium, sodium, sulphur, total nitrogen, and carbon and for mg/kg boron, copper, iron, manganese, and zinc.

### Shrub performance and folivory

Since effects on non-SEPH herbivory of ants consuming SE are not necessarily represented proportionately in effects on plant performance (Chamberlain and Holland 2009), both folivory and plant performance were recorded in this study to find effects of SEPHs on the shrubs and to determine host preferences for *Sextius*. Plant performance was determined in two ways: by recording growth over a one year period and by recording seed output. To measure shrub growth over the allocated period, in the survey, in January 2010 10 to 30 green shoots (depending on shrub size) of diameter not larger than 3mm with no off-shoots located on the outer foliage at a height easy to reach were randomly selected, half the number of shoots being pruned and half not pruned. A dot of acrylic paint was put 5cm from the end of each shoot. All shoots from previous apex to current apex were measured and lengths of shoots branching off the same shoot were summed. Mean growth of shoots on each shrub was used in analyses. To measure growth on experiment shrubs, 10 shoots on each shrub stem were used in the same way. *Acacia victoriae* growth peaks in spring to early summer even in variable arid conditions (Maconochie 1973, Friedel et al 1994, pers. obs.), so growth of experiment shrubs was measured in December 2010 from 7th to 11th, 11 months after the experiment began and in mid-January 2011 for survey shrubs.

Shrub volume was used to calculate SEPH/*Sextius* densities and relative growth rate (RGR) of shrubs. For experiment analyses, circumference, C, at the base of each experiment shrub stem was measured in December 2009 to calculate volume of individual stems and radius, r, was calculated from $r = C/(2.\pi)$ assuming stems were circular. Stem volume, $V_{stem}$, was estimated from the formula of the regression between whole shrub volume, $V_{shrub}$, and total stem radius, r (Chapter 2): $V_{stem} = (28.633.r + 0.166)^3$. This was likely to be a better estimate of biomass and of total area of twigs covered by SEPHs, since shapes of single stems are irregular and twist around each other on the same shrub, making volume difficult to estimate using length and width. Additionally, variation in leaf and twig density of individual shrubs is very high (pers. obs.). For the survey, in November 2009, shrub dimensions were measured. The volume of a cone with oval base was calculated for each shrub from height, H, maximum canopy width, $W_{max}$, and canopy width perpendicular to that, $W_{perp}$: $1/3 \times \pi \times H \times W_{max}/2 \times W_{perp}/2$. Dimensions were re-measured and the volume recalculated at the beginning of the second summer SEPH survey in January 2011.

To find differences in folivory levels on shrubs containing different *Sextius* densities, thirty leaves were randomly selected from ten low-*Sextius* and ten high-*Sextius* experiment shrub stems. Attempts were made to include only shrubs with a rougly even spread of *Sextius* among stems since *Sextius* can fly from stem to stem. This was done at the end of peak insect activity and after a period of low activity, in 2010 in late January and in April, respectively. Each leaf was examined on both sides for evidence of coccoids, miners, gall-makers, and values were calculated for total leaf area lost (LAL) and total leaf area damaged (TAD). Marks that may have been made by fungal infections on leaves made vulnerable to attack by herbivory were included as herbivore damage. Marks were scored according to proportion of leaf area on both sides in classes of 0%, ≤ 1%, ≤ 5%, ≤ 10%, ≤ 20%, ≤ 30%, ≤ 40%, ≤ 60%, ≤ 80%, and ≤ 100%. Classes were based on ease of determination. In the case of TAD, since very few leaves had no damage, there was no 0% category.

To record reproductive success of each shrub, seed pod bunches were collected as soon as they were ripe but prior to dehiscence, to prevent seed dispersal. Numbers of seed pods vary greatly on different shrub individuals and some did not have seed pods on ten small branches intended for collection, so for adequate replication only shrubs with a minimum of ten pods were included in analyses. When developing seed pods were first observed on shrubs in November to December 2009, bags made from organza were tied around 10 bunches on each shrub on sites 1 and 2. This prevented seed loss through dispersal, enabled recording of intrinsic mass of seeds, and excluded insects which damage the seeds. Site 3 seed pods were not placed into bags. After harvesting in January 2010, numbers of initiated seeds in pods were counted, seeds were examined for weevil larvae or exit holes under a 10x magnification dissecting microscope, tested for viability by squeezing as hard as possible with tweezers, and seeds deemed viable were pooled and weighed for each shrub.

## Arthropod densities

If location of SEPHs on shrubs is predictable, SEPH density might be estimated from counts on proportions of the shrub less than the whole. To test for differences in treehopper location on shrubs with respect to the compass points, in October in spring of 2008 twenty shrubs were selected and at each compass point (NESW) two twigs were randomly selected. SEPHs were counted on the apical 30cm of twigs, where light levels would contrast more sharply than the more shaded interior.

*Sextius* adults and nymphs occupy small twigs on shrubs. *Sextius* numbers can be strongly aggregated among branches within shrubs, but at higher densities they may appear more dispersed. Since nymphs can move around branches and adults can fly or walk from one stem to another, not only is their density on a shrub difficult to estimate with any accuracy from counting only a proportion of branches, but distribution can change. Distribution can affect how well ants protect membracids they attend (Morales 2000). Therefore densities of the whole shrub were used in analyses for shrubs and stems before exclusion (Tangle-Trap) treatment. Also, effects of the association might vary through space on the shrub and through time.

Times chosen for counting SEPHs in each year were: soon after emergence, near the end of peak insect activity in summer, and at a time of low activity. In the experiment, numbers of *Sextius* on shrub stems were recorded at the beginning of the study before manipulations were applied in mid-summer in 2010 (11th - 18th January) and then in autumn 2010 (April 22nd), in spring 2010 (September 5th), in early summer 2010 (3rd - 12th December) once manipulations had had time to take effect, and finally in late summer 2011 (8th - 9th February). In the survey, all SEPHs were counted on whole shrubs from 7th - 14th November 2009 and from 4th – 28th February to March 2010. In the next season, they were counted in 2011 from 3rd - 7th January and from 13th – 15th February 2011. Since SBIs do not feed at first instar nymphs, which suffer high mortality, first instars were not included in densities. SEPH/*Sextius* density was calculated as number of *Sextius* over shrub or stem volume. High *Sextius* density was designated as at least 80m$^{-3}$ and low as 20m$^{-3}$ or less. Reproductive fitness of *Sextius* could not be measured since *Sextius* eggs were not found during the study. This was probably because *Sextius* oviposits eggs into twigs, after which the shrub repairs the wound.

To confirm SBI abundance at survey sub-sites, ten pitfall traps of 4.5cm diameter with saturated salt solution and a drop of detergent to break surface tension were spaced at 5m intervals along two transects 5m apart and left for 48 hours in December 2009.

To survey arthropod assemblages on shrubs with different SEPH densities, a non-destructive standardized method was required. On experiment shrub stems and survey shrubs, half a yellow sticky trap was hung from a small branch with a twig through the middle (to increase contact of crawling insects), on as similar a position as possible on each stem as possible with respect to solar exposure. Times of peak and low insect activity were used in the experiment: in January 2010 for 7 days, just before Tangle-Trap treatments were applied to experiment shrubs. The first sampling in January 2010 included survey shrubs. In February 2011, traps were present for 10 days on both control and Tangle-Trap experiment stems and due to the low observed arthropod activity, whole traps were used.

Small arthropods were assigned to the lowest taxonomic group possible without dissection. For analyses, wasps were divided among three classes: "minute" wasps which were 1mm in length or less, the majority of which are egg parasitoids or parasitoids of thrips larvae/pupae; those over 2mm were included in the class "predators" since most of the wasp species of this size caught on traps predatory or are parasitoids of larvae, which also kill their hosts, and are therefore equivalent to predators for the purposes of this study; the very small proportion that were between these lengths were put into the category "other" to reduce noise since there was not sufficient information to determine to which of the other size classes they belonged. "Predators" included all non-SBI arthropods whose diet is thought to consist of at least 50% faunal matter, except mirids or lygaeids, which were allocated their own class since individual species were not identifiable and have a range of different diets. The vast majority of "other" Auchenorrhyncha were cicadellids, along with other rarely caught derbids which do not appear to aggregate on *A. victoriae*. Thrips and psyllids were counted in their own separate classes. When psyllids and "other" Auchenorrhyncha are grouped together they are referred to as "other" phloem-feeders. Any other herbivore was assigned the class "other" herbivores, since the lack of taxonomic knowledge meant that feeding behaviour was not known. The very few other arthropods caught that did not fall within these groups were placed in the category "other".

## Insect deterrence behaviour

To observe deterrence by SBIs of small arthropods on shrubs, on 8th November 2011 a chrysomelid beetle *Monolepta* sp., which was in high abundance at one experiment site, was used in behavioural trials with SBIs. First, 20 different individuals were placed one at a time on one of five different twigs of an *A.victoriae* at least 20cm from ants or other predators. After their legs were settled on the twig they were released, and all stayed on the 30cm of the twig for at least 30 seconds. Second, 20 individuals were placed one at a time on one of five different twigs of an adjacent shrub with no *Sextius* at a point at which there was a flow of at least 15 SBIs per minute with no SEPHs. Each beetle was released after its legs were settled on the twig and an SBI was within 5mm. Whether they dropped or flew off the plant within 2s, 10s, or 20s, were attacked by at least one SBI within 1s, 10s, or 30s, or dropped onto or crawled to another part of the plant within 10s and remained on the plant for 30s, or remained on the 30cm of twig for 30s was recorded. Third, 20 individuals were placed one at a time on five different twigs of the first shrub individual at a point with the same minimum SBI flow and with *Sextius* within 10mm. Fourth, 20 individuals were placed one at a time on five different twigs of the same shrub at a point with the same minimum SBI flow and with *Sextius* 50mm away. The same data were recorded for these trials as for the twigs without SBIs.

A smaller field experiment tested effects of SBIs on *Sextius*. Four shrubs with four branches suitable for cages with at least 30 *Sextius* nymphs were located. In this case only, nymphs were used since they cannot fly away and sometimes only nymphs benefit from ant attendance (Cushman and Whitham 1989). Cages made from white curtain netting with mesh of approximately 1.5mm long and 1mm wide hexagons were applied to each branch, two receiving a long slit on either side to allow access of flying predators and spiders. Shading for nymphs was maximized in these cages. To one branch with a closed cage and one with an open cage on each shrub, duct tape was wound around the branch supporting the cage to which Tangle-Trap was applied to prevent ants accessing the nymphs from the branch and potential foliar bridges were eliminated. Tangle-Trap is hard to apply with great accuracy, however width was never more than 4cm and always at least 2cm. On these eight branches, cages were placed on branches without other branches directly overhead or two layers of caging material were pegged to the underside of branches above to prevent ants gaining access to cages from above. At the beginning of the experiment on 2nd December 2010 before cages were closed with wire, all *Sextius* adults and predators were removed and nymphs were counted. Counting was very difficult due to the intertwined and twisted nature of the twigs they occupy, since nymphs frequently occupy the fork of two twigs, and since nymphs move from one twig to another if disturbed. At the end of the experiment on 12th December 2010 after some fifth instar nymphs had metamorphosized to adults, adult *Sextius* moved very little, remaining on the twig, so it is improbable that any flew away. When cages were removed, *Sextius* adults and nymphs were counted.

### Analyses

The statistical program Primer-E 6.0 with PERMANOVA+ (Anderson 2001) with Euclidean distances was used for all factor analyses. For factor analyses, 9999 permutations were used of the PERMANOVA test with Type III partial squares. With only one factor, unrestricted permutation of raw data was used, while with two or more crossed factors permutation of residuals under the reduced model was used. PermDisp was used to test for homogeneity of dispersion in factor analyses for pairs of factor levels, which is very important for two-factor analyses. Number of permutations used was always 999 and dispersion of medians was used since analyses were univariate. For all linear correlations and linear and non-linear non-envelope regressions SPSS software (Versions 17.0 – 19.0, SPSS Inc.) was used. The Pearson correlation method was applied since parameters were transformed to maximize linearity. For regressions, normal residual frequency was optimized by considering histograms of residuals and homogeneous variance was optimized by considering the graph of residuals versus predicted values. For all envelope regression, least absolute deviation (LAD) quantile regression in the Blossom software (Cade and Richards 2005) was used and asymptotic p-value was reported.

To test for differences in experiment soil between *Sextius* levels, soil parameters were grouped by *Sextius* levels that were consistent from December in both 2009 and 2010, so that shrubs with *Sextius* densities lower than 20 $m^{-3}$ in both seasons were in the low-*Sextius* group and those with *Sextius* densities higher than 60 $m^{-3}$, but mostly over 100 $m^{-3}$, were in the high-*Sextius* group. For correlations of soil parameters with survey SEPH/*Sextius* densities, January 2011 densities were used that were counted at almost the same time growth was measured. Soil parameters, s, were transformed to square roots ($\sqrt{s}$).

To determine whether soil affected leaf nutrients, for experiment shrubs soil parameters, s, were correlated with leaf nutrients, L, using logarithms of both, $\ln(s + 1)$, $\ln(L + 1)$. To determine whether leaf nutrients varied with *Sextius* density, a one-factor PERMANOVA of logarithm-transformed values ($\ln(L + 1)$) was done.

Means of total length of new growth on each twig over the allocated time period (or growth rate) were used in analyses: stem means for the experiment and shrub averages for the survey. Within experiment shrub control stems, there was no regression relationship between growth and stem size ($\ln(g + 1)$, $\ln(v + 1)$, $n = 22$, $p = 0.573$; $\sqrt{g}$, $\sqrt[3]{v}$, $n = 22$, $p = 0.5655$), possibly because of the lower number of shrubs and the homogeneity of size of stem relative to the survey, so the shrub volume correction used for the survey was not necessary with experiment data. Also growth data was not heterogeneously dispersed on different *Sextius* levels or treatments, so raw growth values were used. To test effects of previous growth on SEPH/*Sextius* density in the survey, raw growth data were used. To test effect of different SEPH/*Sextius* densities on growth rate, g, in the survey, relative growth rate (RGR), growth relative to shrub volume, v, was used, $g/\sqrt[3]{v}$. In analyses, raw growth and RGR, g, were transformed by square roots ($\sqrt{g}$) since they are linear.

In regressions of raw growth and 2011 SEPH/*Sextius* densities where only shrubs whose SEPH/*Sextius* density had changed level between years were used, the levels low (below 20 $m^{-3}$) and high (above 60 $m^{-3}$) were used. Use of shrubs with changed densities was to take into account possible accumulated effects of SEPHs/*Sextius* density on shrub growth from the previous year. In 2011 *Sextius* growth regressions, total SEPH level was used as an indication of whether the level had changed on survey shrubs, since more SEPHs bring more

SBIs onto the shrub regardless of species even if they might have different effects on growth.

Since values of leaf damage of various types (d) were proportions of total leaf area, they were transformed using arcsine ($\sqrt{d}$) before analysis of difference between treatments. When a category contained less than 5% of samples, it was pooled with the next category exhibiting a similar pattern in box plots, or in the absence of one, the next larger category, or without one, in the next smaller category if their patterns were not distinctly different. The SEPH density on the entire shrub was used, since at least some SEPHs might fly from one stem to the other. SPSS was used to calculate the mean and standard error of the percentage of each leaf area category of each category of damage in each *Sextius* level.

Since *Sextius* were not always the only SEPHs on experiment shrubs during seed development before the experiment was started, the total SEPH level was used for correlations with seeds. For seeds from experiment shrubs, viable per initiated seeds and seeds eaten per initiated were calculated for individual seed pods. The mean of each parameter from each experiment shrub stem was used in analyses while for the survey, the sub-site mean of the shrub means was used.

Numbers of SEPHs at each compass point were tested in a one-factor PERMANOVA. In analyses, cubic root of SEPH/*Sextius* density, S, were used ($\sqrt[3]{S}$) since it was dependent on shrub volume.

For sticky trap analyses, square roots of taxon counts, a, were used (√a) since individuals occurred on an area of trap. The category "other" contained too many zeroes to be used in factor analyses. Although slope on sub-sites may affect SEPH/*Sextius* densities and other small arthropod numbers, there were insufficient replicates to use only shrubs on the flats for PERMANOVAs. One-factor PERMANOVAs were used for survey data, since effects of site and slope were not sought in this context. For envelope regression relationships between SEPHs/*Sextius* and "minute" wasps or "other" Auchenorrhyncha, to find the pattern determining the top of the envelope, the data were divied into sets of each cubic root value of SEPHs/*Sextius* $m^{-3}$ and the top 50% of the data, rounded up in cases of odd numbers, were selected. A non-linear regression was conducted on these data.

To determine effects of closed cages and Tangle-Trap on *Sextius* nymphs, the number of adult and nymph *Sextius* counted on the branch at the end of the experiment was divided by the number of nymphs counted at the beginning to provide a proportional estimate of population fluctuation. Since this number, x, was larger than one in many cases, it was divided by the maximum for the whole experiment to make possible transformation by arcsine (√x), to which the proportions were transformed. This gave the most normal distribution of data.

Whereas ordinary least squares regression minimizes the sum of the squared residuals to obtain estimates of predicted values, least absolute deviation (LAD) regression minimizes the sum of the absolute residuals (Cade and Richards 2005). In a LAD regression, a regression of the 0.9th quantile estimates a line such that 10% of the observations are greater and 90% less than the estimate (Cade et al 1999).

Although values of 0.05 or less indicate clear significance of statistical tests given appropriate data, since the 95% confidence parameter is essentially arbitrary, p-values of up to 0.08 are considered marginally significant.

# Results

## SEPH/*Sextius* densities on shrubs

### Soil effects on leaf nutrient concentrations, shrub growth, and SEPH/*Sextius* densities

There were weak regression relationships indicated for some leaf nutrient concentrations, L, with some parameters, s, of soil taken from just outside the zone of influence of experiment shrubs (see table 1 below). There were many auto-correlations among leaf nutrient concentrations (table 2).

Table 1: Regressions of leaf nutrient concentrations, l, with soil parameters, s. (Only significant relationships are indicated).

| n = 21 | | | x-variable, soil parameter, ln(s + 1) | | |
|---|---|---|---|---|---|
| | | | S | $C_{org}$ | EC |
| y-variable, leaf nutrient, ln(l + 1) | **Mg** | $r^2$ | +0.411 | +0.299 | +0.352 |
| | | adj. $r^2$ | +0.380 | +0.262 | +0.317 |
| | | p | **0.002** | **0.010** | **0.005** |
| | | slope | 0.310±0.036 | 0.637±0.224 | 0.761±0.237 |
| | | constant | 0.007±0.084 | -0.156±0.152 | 0.173±0.237 |
| | **Bo** | $r^2$ | | *+0.159* | |
| | | adj. $r^2$ | | *+0.114* | |
| | | p | | ***0.074*** | |
| | **C** | $r^2$ | | *+0.166* | |
| | | adj. $r^2$ | | *+0.122* | |
| | | p | | ***0.066*** | |
| | **Zn** | $r^2$ | | | -0.218 |
| | | adj. $r^2$ | | | -0.177 |
| | | p | | | **0.033** |
| | | slope | | | -1.1514±0.658 |
| | | constant | | | 2.402±0.140 |

(For all tables, values in **bold** are statistically significant and values in ***bold italics*** are marginally significant. )

Table 2: Auto-correlations of leaf nutrients, l (Pearson correlation). (Only significant relationships are indicated)

| n = 22 | | | x-variable, ln(l + 1) | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | Zn | P | S | K | Mn | Mg | Ca | Bo |
| y-variable, ln(l + 1) | S | $r^2$ | -0.391 | | | | | | | |
| | | p | ***0.072*** | | | | | | | |
| | K | $r^2$ | +0.560 | +0.823 | | | | | | |
| | | p | **0.007** | **<0.001** | | | | | | |
| | N | $r^2$ | | +0.587 | | | | | | |
| | | p | | **0.004** | | | | | | |
| | P | $r^2$ | +0.560 | | | | | | | |
| | | p | **0.007** | | | | | | | |
| | Mn | $r^2$ | -0.669 | -0.479 | +0.481 | -0.619 | | | | |
| | | p | **0.001** | **0.024** | **0.024** | **0.002** | | | | |
| | Mg | $r^2$ | -0.652 | -0.380 | +0.523 | -0.685 | +0.818 | | | |
| | | p | **0.001** | 0.081 | **0.012** | **<0.001** | **<0.001** | | | |
| | Cu | $r^2$ | +0.612 | +0.581 | | +0.524 | -0.580 | -0.416 | | |
| | | p | **0.002** | **0.005** | | **0.012** | **0.005** | ***0.054*** | | |
| | Ca | $r^2$ | -0.642 | -0.562 | | -0.623 | +0.533 | +0.559 | | |
| | | p | **0.001** | **0.006** | | **0.002** | **0.011** | **0.007** | | |
| | Bo | $r^2$ | -0.538 | -0.496 | | -0.712 | +0.746 | +0.750 | +0.803 | |
| | | p | **0.010** | **0.019** | | **<0.001** | **<0.001** | **<0.001** | **<0.001** | |
| | C | $r^2$ | +0.457 | +0.404 | -0.511 | +0.578 | -0.468 | -0.627 | -0.836 | -0.781 |
| | | p | **0.033** | **0.063** | **0.015** | **0.005** | **0.028** | **0.002** | **<0.001** | **<0.001** |

Sulphur concentration, s, of soil near low-*Sextius* shrubs at an average of 20.5 mg/kg was 3 times that of soil near high-*Sextius* shrubs (PERMANOVA, ln(s + 1), n = 10 (high-*Sextius*)/n = 11 (low-*Sextius*), p = 0.0559) (without removing an outlier, p = 0.0501, 6.39 times) and was differently dispersed between *Sextius* levels (PermDisp, ln(s + 1), n = 10 (high-*Sextius*)/n = 11 (low-*Sextius*), p = 0.0004). Electrical conductivity, EC, of soil around experiment shrubs, representing salinity levels, at an average of 0.258 dS/m near low-*Sextius* shrubs was 2.24 times that of high-*Sextius* shrubs (PERMANOVA, ln(EC + 1), n = 10(high-*Sextius*)/n = 11 (low-*Sextius*), p = 0.0498) and was differently dispersed between *Sextius* levels (PermDisp, ln(s + 1), n = 10 (high-*Sextius*)/n = 11 (low-*Sextius*), p = 0.027). There was no regression relationship of soil conductivity or sulphur level with *Sextius* density, but high *Sextius* density shrubs had values only in the lower end of the range. There were auto-correlations between experiment soil parameters (table 4).

There was no regression relationship indicated for any soil parameter with experiment shrub growth rate. Average raw growth, g, of all shrubs on survey sub-sites correlated negatively with potassium as did average volume-standardized growth on randomly chosen shrubs (table 7). Average survey sub-site volume, v, of randomly selected shrubs, correlated with particular single soil parameters (table 5). Some soil parameters auto-correlated (table 6). There was no regression relationship between November 2009 or January 2011 survey SEPH or *Sextius* density and volume of shrub.

Experiment shrub leaves contained differences amongst high- and low-*Sextius* shrubs in the proportions of some nutrients per leaf dry weight, L (PERMANOVA, ln(L + 1), n = 9, (high-*Sextius*)/n = 10 (low-*Sextius*), p = 0.0008) (table 3). There were, however, autocorrelations between many leaf nutrient levels (table 2).

Table 3: Leaf nutrient concentration quantities in high- compared to low-*Sextius* shrub leaves. (Only significant results are indicated. )

| n = 9 (high-*Sextius*)/ 10 (low-*Sextius*) | Bo mg/kg | C % | Ca % | Fe mg/kg | K % | Mg % | Mn mg/kg | P % | Zn mg/kg | P:C |
|---|---|---|---|---|---|---|---|---|---|---|
| Mean for high-*Sextius* | 25.6 | 44.77 | 1.61 | 44.465 | 0.631 | 0.19 | 25.61 | 0.119 | 9.46 | 0.0027 |
| Mean for low-*Sextius* | 63.9 | 43.18 | 3.21 | 49.405 | 0.421 | 0.47 | 51.21 | 0.085 | 6.76 | 0.0019 |
| High-*Sextius* concentration<br>Low-*Sextius* concentration | 0.4 | 1.0 | 0.5 | 0.9 | 1.5 | 0.4 | 0.5 | 1.4 | 1.4 | 1.4 |
| p-value (ln(L + 1)) | **0.0005** | **0.0072** | **0.0045** | **0.0039** | **0.0028** | **0.0028** | **0.0098** | **0.0010** | **0.0295** | **0.0037** |
| p-value of dispersion when different | | | ***0.076*** | | ***0.060*** | **0.030** | | | **0.045** | |

Table 4: Experiment soil parameter autocorrelations (square root of soil parameter, n = 27). (Only significant results are indicated. )

| n = 27 | | Total Nitrogen | Sulphur | pH of $H_2O$ | Conductivity |
|---|---|---|---|---|---|
| Potassium | $r^2$<br>p | | *-0.347*<br>***0.076*** | *-0.357*<br>***0.068*** | |
| Organic Carbon | r<br>p | +0.669<br>**< 0.001** | | | +0.386<br>**0.047** |
| Conductivity | $r^2$<br>p | | +0.695<br>**< 0.001** | | |
| pH of $CaCl_2$ | $r^2$<br>p | | | +0.901<br>**< 0.001** | |

Table 5: Survey soil parameter autocorrelations (square root of soil parameters, n = 48) (Pearson's). (Only significant results are indicated. )

| n = 48 | | Total Nitrogen | Sulphur | Conductivity | Potassium | Organic Carbon | pH of $H_2O$ |
|---|---|---|---|---|---|---|---|
| Phosphorous | r | | | | +0.368 | | |
| | p | | | | **0.010** | | |
| Sulphur | r | +0.569 | | | | | |
| | p | **<0.001** | | | | | |
| Organic Carbon | r | +0.699 | +0.734 | +0.689 | | | |
| | p | **< 0.001** | **<0.001** | **<0.001** | | | |
| Conductivity | r | +0.333 | +0.758 | | | | |
| | p | **0.021** | **<0.001** | | | | |
| pH of $H_2O$ | r | +0.381 | +0.312 | +0.471 | -0.384 | +0.317 | |
| | p | **0.008** | **0.031** | **<0.001** | **0.007** | **0.028** | |
| pH of $CaCl_2$ | r | +0.444 | +0.395 | +0.573 | -0.326 | +0.399 | +0.911 |
| | p | **0.002** | **0.005** | **<0.001** | **0.024** | **0.005** | **<0.001** |

Table 6: Survey sub-site average soil parameter autocorrelations (square root of soil parameters, n = 12) (Pearson's). (Only significant results are indicated. )

| n = 12 | | Total Nitrogen | Sulphur | Conductivity | Potassium | Organic Carbon | pH of $H_2O$ |
|---|---|---|---|---|---|---|---|
| Sulphur | r | +0.819 | | | | | |
| | p | **0.001** | | | | | |
| Organic Carbon | r | +0.880 | +0.821 | +0.692 | | | |
| | p | **< 0.001** | **<0.001** | **0.013** | | | |
| Conductivity | $r^2$ | +0.635 | +0.807 | | | | |
| | p | **0.026** | **0.002** | | | | |
| pH of $H_2O$ | $r^2$ | +0.667 | *+0.538* | +0.648 | -0.710 | | |
| | p | **0.018** | ***0.071*** | **0.023** | **0.010** | | |
| pH of $CaCl_2$ | $r^2$ | +0.674 | +0.649 | +0.838 | -0.692 | +0.598 | +0.916 |
| | p | **0.016** | **0.022** | **0.001** | **0.013** | **0.040** | **<0.001** |

There was virtually no regression relationship between 2009 *Sextius* densities and volume of experiment shrub ($\sqrt[3]{S}$, $\sqrt[3]{v}$, $n = 71$, $r^2 = 0.080$, adj. $r^2 = 0.053$, $p = 0.094$). On experiment shrub control stems, there was no regression relationship either for 2009 or 2010 December *Sextius* count ($\sqrt[3]{S}$) with any soil parameter ($\sqrt{s}$). Mean January 2011 SEPH and *Sextius* densities on sub-sites correlated with some single soil parameters (table 7).

Table 7: Regressions of survey sub-site mean growth (square root, n = 9) and SEPH densities (cubic root, n = 12) with single soil parameters (square root). (Only significant results are indicated. )

| | | Nitrogen | Potassium | Sulphur | Organic Carbon |
|---|---|---|---|---|---|
| Shrub volume, $\sqrt[3]{v}$<br>(n = 12) | $r^2$<br>adj. $r^2$<br>p | | | +0.424<br>+0.367<br>**0.022** | *+0.299*<br>*+0.229*<br>***0.066*** |
| Raw growth, $\sqrt{g}$<br>(n = 9) | $r^2$<br>adj. $r^2$<br>p | | -0.491<br>-0.418<br>**0.036** | | |
| Volume-standardized growth, $\sqrt{g}$<br>(n = 9) | $r^2$<br>adj. $r^2$<br>p | | -0.519<br>-0.450<br>**0.029** | | |
| 2011 January SEPH density, $\sqrt[3]{S}$<br>(n = 12) | $r^2$<br>adj. $r^2$<br>p | *-0.293*<br>*-0.222*<br>***0.069*** | | -0.390<br>-0.329<br>**0.030** | -0.405<br>-0.346<br>**0.026** |
| 2011 January *Sextius* density, $\sqrt[3]{S}$<br>(n = 12) | $r^2$<br>adj. $r^2$<br>p | | | *-0.294*<br>*-0.224*<br>***0.068*** | *-0.289*<br>*-0.218*<br>***0.071*** |

## SBI abundance on stems and effects on *Sextius*

SBIs were abundant at all survey sub-sites, with over 100 SBIs in each pitfall trap (apart from 3 pitfalls that dried up and were excluded). SBIs were always numerous and trailing to shrubs with SEPHs whenever ground temperature was between approximately 15 and 42°C. Trails were abundant from spring to autumn near all experiment shrubs and lead to all shrubs with moderate or higher levels of SEPHs. On high-*Sextius* Tangle-Trap stems after SBIs had jumped off four to five days after Tangle-Trap application, large amounts of honeydew accumulated on twigs. Then six to seven days after Tangle-Trap application, all adult *Sextius* had gone. By contrast adult *Sextius* took at least twice as long to disperse when Tangle-Trap was applied to the base of all stems of eight shrubs with high (over 80 $m^{-3}$) *Sextius* densities at a third nearby site in mid-summer, January 2011. In the spring/summer of 2010/2011 following application of Tangle-Trap, only negligible numbers of *Sextius* were found on experiment Tangle-Trap stems set up in January 2009. However, substantial numbers of grey scale and lycaenid caterpillars were found on some Tangle-Trap stems, sometimes months after Tangle-Trap application.

### *Sextius* distribution within shrubs

On shrubs tested for *Sextius* location with respect to compass point orientation, adults represented only 9.4% of the total *Sextius* population, so most individuals, being nymphs, were not as mobile. There was no difference in *Sextius* population according to compass point orientation (PERMANOVA, $\sqrt[3]{S}$, $n = 20$, $p = 0.6778$). Counts indicated that *Sextius* flew from the one Tangle-Trap stem to other stems when Tangle-Trap was applied to one only stem of each experimental shrub. Since their distribution patterns within shrubs are not known, their densities on the whole shrub were used in analyses for 2009 when SBIs had access to all stems.

### Proportion of *Sextius* and lycaenids of total SEPHs in survey

In December, 2009 82.1% of SEPHs on experiment shrubs were *Sextius* and 4.0% were lycaenid larvae. In November 2009, 89.6% of SEPHs on survey shrubs were *Sextius* and 71.5% of *Sextius* were nymphs. Only 3.3% were lycaenid larvae. In January 2011, 56.3% of survey SEPHs were *Sextius* and 3.5% were lycaenid larvae. In February 2011, 81.3% of survey SEPHs were *Sextius* and only 0.4% were lycaenid larvae.

### SEPH/*Sextius* within-season population change

Densities of *Sextius* remained high only from December to February in the 2010/2011 spring/summer. In 2010 September at the end of winter, there were high densities of *Sextius* on two high-*Sextius* shrub control stems, moderate densities (over 30 $m^{-3}$ and less than 80$m^{-3}$) on four, and negligible densities on other experiment shrubs. By 2011 January, there were moderate (over 30 $m^{-3}$) to very high (over 200 $m^{-3}$) levels on control stems of all experiment shrubs that originally had high-*Sextius* density (except one). There was one December low-*Sextius* shrub with a high density the following January, but three formerly high-*Sextius* shrubs displayed low densities in January. On 8th March 2011, there were still high densities of *Sextius* on some high-*Sextius* experiment shrub control stems. On 22nd April 2011, there were still moderate densities of *Sextius* observed on three formerly high-*Sextius* shrub control stems, but low densities (or no individuals) on other shrubs. Survey SEPH densities in 2011 January were positively moderately strongly correlated with those in February (SPSS, $\sqrt[3]{S}$, $n = 173$, $r^2 = 0.536$, adj. $r^2 = 0.533$, $p < 0.001$) after removal of one outlier and *Sextius* densities compared at the same times were slightly more strongly correlated (SPSS, $\sqrt[3]{S}$, $n = 172$, $r^2 = 0.651$, adj. $r^2 = 0.649$, $p < 0.001$) after removal of two outliers.

## SEPH/*Sextius* between-season population change

Experiment control stem *Sextius* densities, S, correlated positively and weakly from one year to the next (SPSS, $^3\sqrt{S}$, n = 35, $r^2$ = 0.241, adj. $r^2$ = 0.218, p = 0.003, constant = 1.033±0.970, slope = 0.704±0.218) (figure 2). On randomly selected survey shrubs that remained alive in both seasons, *Sextius* densities counted in 2009 November correlated positively with those counted in 2011 January (SPSS, $^3\sqrt{S}$, n = 121, $r^2$ = +0.160, p < 0.001, slope = 0.305±0.064, c = 0.692±0.147) and total SEPH numbers from the same counts correlated slightly more weakly (SPSS, $^3\sqrt{S}$, n = 121, $r^2$ = +0.132, p < 0.001, slope = 0.337±0.079, c = 1.102±0.201).

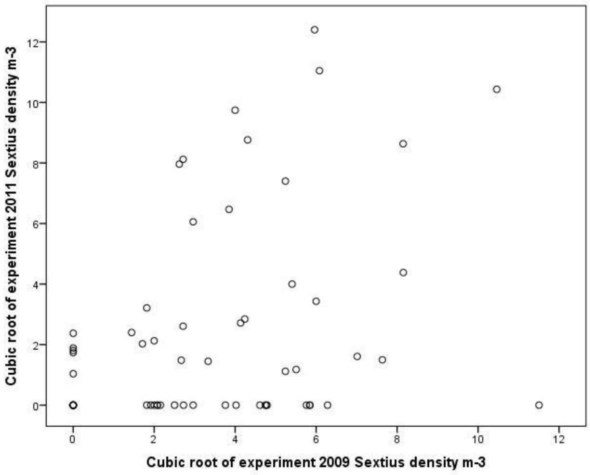


Figure 2: Experiment shrub control stem *Sextius* densities for summer 2011 versus summer 2009. (Only significant results are indicated. )

## SEPH/*Sextius* occurrence on shrubs with different growth rates

When growth of shrub shoots was recorded, care was taken to find every marked shoot, but approximately 5% could not be found. There was no difference in length of pruned and un-pruned shoots.

*Sextius* densities after growth of experiment shrub control stems

There was no relationship between shrub size and SEPH or *Sextius* density in experiment shrubs. On control stems with natural *Sextius* densities, on high-*Sextius* shrubs in December 2010 raw growth over the preceding 11 months, g (averaging 41.5cm per twig) averaged 3 times higher than growth on low-*Sextius* shrubs (PERMANOVA, $\sqrt{g}$, $n = 10$, $p = 0.0011$). One high-*Sextius* shrub was removed from all analyses involving data collected from December 2010 or later due to an unusually high density scale outbreak and unusually high defoliation of leaves on the Tangle-Trap stem which may have been caused by an orthopteran outbreak. When only shrubs that changed in *Sextius* level between seasons from low (below 20 $m^{-3}$) to high (above 60 $m^{-3}$) or vice versa were included in the analysis, no difference was found in growth between shrubs that had low and high *Sextius* densities in December 2010 (PERMANOVA, $\sqrt{g}$, $p = 0.6012$, $n = 3$). Raw growth on control stems correlated positively but weakly with December 2010 *Sextius* levels, S, in the experiment (SPSS, $n = 35$, $\sqrt[3]{S}$, $\sqrt{g}$: $r^2 = 0.380$, adj. $r^2 = 0.361$, $p < 0.001$, slope = 0.779±0.173, constant = -0.334±1.009) (figure 3). However, including only shrubs whose *Sextius* levels had changed either between below 20 $m^{-3}$ and above 60 $m^{-3}$ or by at least 5 times if over the maximum low density in both years, they correlated positively (SPSS, $\sqrt[3]{S}$, $\sqrt{g}$, $n = 11$: $r^2 = 0.395$, adj. $r^2 = 0.328$, $p = 0.038$) (figure 4).

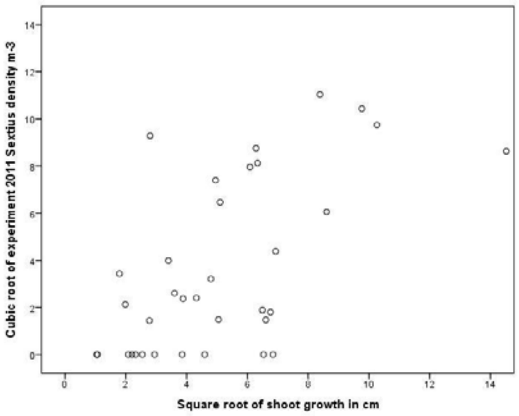


Figure 3: Summer 2011 *Sextius* density versus experiment experiment shrub growth (n = 35)

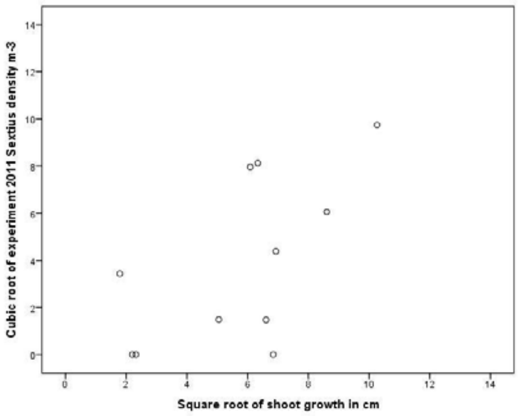
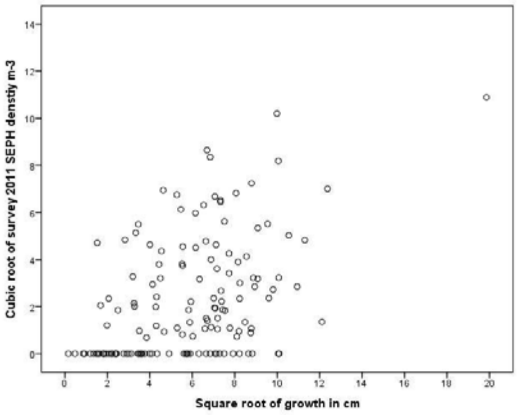


Figure 4: Summer 2011 *Sextius* density versus shrub growth for shrubs with substantial change in *Sextius* level (n = 11)

## SEPH/*Sextius* occurrence with different growth rates of shrub

### SEPH/*Sextius* densities after growth of survey shrubs

For all survey shrubs, 2011 *Sextius* and SEPH densities correlated positively and weakly with raw growth, which was measured at about the same time as SEPHs were counted; growth made envelope regressions for SEPH/*Sextius* densities (table 8; graphs 5,6) (SEPH: slope = 0.389±0.063, constant = 0.000±0.4112; *Sextius*: slope = 0.310±0.048, constant = -0.295±0.312). One outlier was removed which was far out of range of the other data (figures 5-8). Increasing slopes in envelope densities with larger quantiles strengthen evidence for the envelope relationship. Raw growth made an envelope for all 2011 SEPH densities and the relationship was very similar for all 2011 *Sextius* densities. When only using shrubs that occurrred on flats to remove effects of hillslope, the relationship was stronger in the case of *Sextius* densities (SEPH: slope = 0.421±0.075, constant = -0.330±0.478; *Sextius*: slope = 0.312±0.053, constant = -0.450±0.337). There were also very similar envelopes made by raw growth versus SEPHs and *Sextius* (table 8; graphs 7,8). For all survey shrubs that changed from high (60+ $m^{-3}$) to low (20- $m^{-3}$) SEPH densities or vice versa between seasons, raw growth, g, and early 2011 *Sextius* density, S, correlated positively but weakly (SEPH: slope = 0.320±0.140, constant = 2.127±0.963; *Sextius*: slope = 0.358±0.130, constant = 0.697±0.895) (table 8; graphs 9, 10).



All survey shrubs, summer 2011
Figure 5: SEPH density versus shrub growth

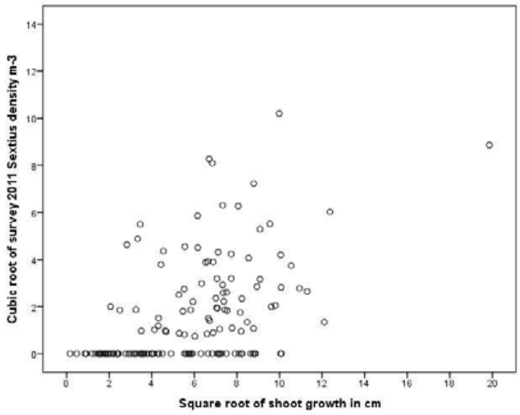
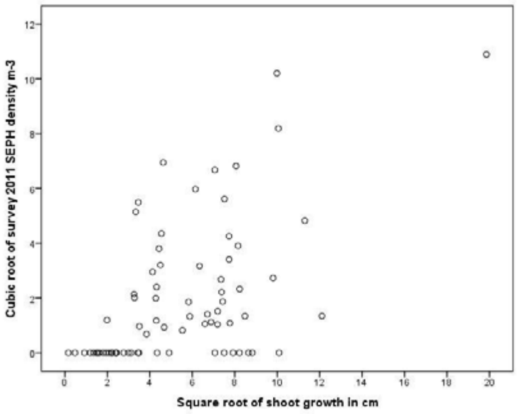


Figure 6: *Sextius* density versus shrub growth



Shrubs on flat sub-sites only, summer 2011
Figure 7: SEPH density versus shrub growth

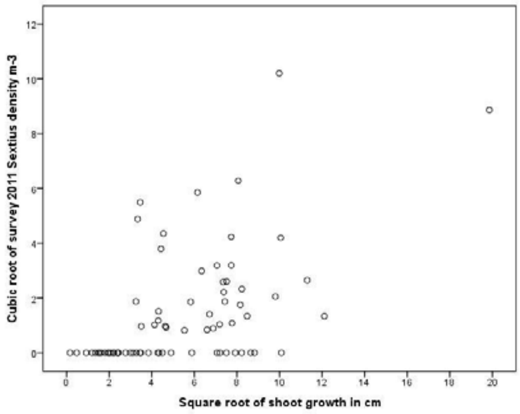
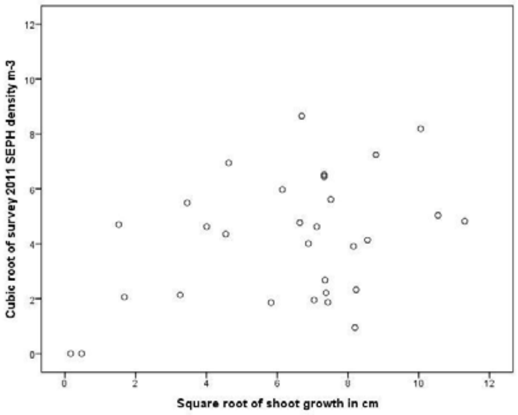


Figure 8: *Sextius* density versus shrub growth



Shrubs that changed SEPH level from all survey shrubs, summer 2011
Figure 9: SEPH density versus shrub growth

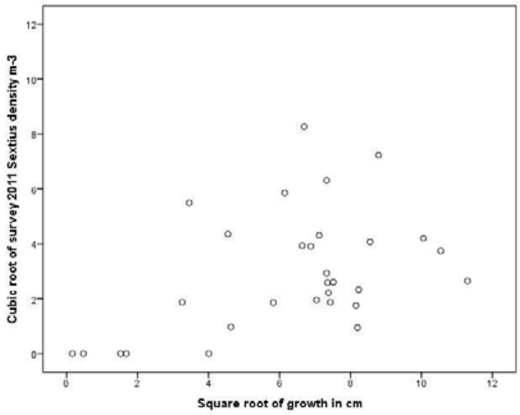


Figure 10: *Sextius* density versus shrub growth

Table 8: Linear and LAD quantile regressions of 2011 survey SEPH densities, S, and shrub raw growth, g, for different subsets of the data (Number in parentheses underneath the SEPH, e.g. Sextius (3), indicates numbers of outliers removed from analysis. )

| | | | x-variable: Raw growth, $\sqrt{g}$ | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | Quantile: | | | | |
| y-variable: SEPH density, $\sqrt[3]{S}$ | | | Linear | | 0.5th | 0.6th | 0.7th | 0.8th | 0.9th |
| All shrubs | SEPH | $r^2$ | 0.213 | r | 0.5000 | 0.4929 | 0.5838 | 0.7037 | 0.8757 |
| | (1) | adj. $r^2$ | 0.208 | | | | | | |
| (n = 143) | | p | **<0.001** | p | **<0.0001** | **<0.0001** | **0.0008** | **0.0021** | **0.0006** |
| | | | | slope | 0.3783 | 0.4773 | 0.5458 | 1.2385 | 3.7048 |
| | | | | constant | -0.5842 | -0.5722 | 0.0523 | 0.4860 | 0.4003 |
| | *Sextius* | $r^2$ | 0.230 | r | 0.5000 | 0.5026 | 0.5569 | 0.6724 | 0.8535 |
| | (3) | adj. $r^2$ | 0.224 | | | | | | |
| | | p | **<0.001** | p | **<0.0001** | **<0.0001** | **<0.0001** | **0.0007** | **0.0025** |
| | | | | slope | 0.3060 | 0.3855 | 0.4827 | 0.5043 | 0.6115 |
| | | | | constant | -0.5651 | -0.6283 | -0.7196 | -0.2123 | 0.7375 |
| Flats shrubs | SEPH | $r^2$ | 0.303 | r | 0.5000 | 0.4986 | 0.5779 | 0.7258 | 0.8611 |
| | | adj. $r^2$ | 0.294 | | | | | | |
| | | p | **<0.001** | p | **0.0002** | **0.0017** | **0.0014** | **0.0021** | **0.0008** |
| (n = 73) | | | | slope | 0.3308 | 0.4915 | 0.5715 | 0.7332 | 0.8911 |
| | | | | constant | -0.5108 | -0.7236 | -0.4572 | -0.2460 | -0.1446 |
| | *Sextius* | $r^2$ | 0.329 | r | 0.5000 | 0.4952 | 0.5509 | 0.6433 | 0.8162 |
| | (2) | adj. $r^2$ | 0.319 | | | | | | |
| | | p | **<0.001** | p | **<0.0001** | **0.0001** | **0.0002** | **0.0017** | **0.0317** |
| | | | | slope | 0.2761 | 0.3491 | 0.4414 | 0.4682 | 0.6047 |
| | | | | constant | -0.4977 | -0.5391 | -0.6580 | -0.4300 | -0.0981 |
| All shrubs that changed SEPH level | SEPH | $r^2$ | 0.157 | r | 0.5000 | 0.4800 | 0.5527 | 0.7121 | 0.8651 |
| | | adj. $r^2$ | 0.127 | | | | | | |
| | | p | **0.030** | p | 0.5259 | 0.1725 | 0.5033 | 0.3588 | 0.2223 |
| | *Sextius* | $r^2$ | 0.212 | r | 0.5000 | 0.4402 | 0.4440 | 0.5376 | 0.8424 |
| (n = 30) | | adj. $r^2$ | 0.184 | | | | | | |
| | | p | **0.011** | p | **0.0442** | 0.2320 | 0.5404 | 0.7958 | 0.4881 |

## SEPH/*Sextius*-SBI association effects on shrubs

### Soil effects on shrub reproductive success

Mean seed mass per experiment shrub, m, mean number of seeds initiated per pod, i, mean number of seeds eaten per pod, e, and mean number of viable seeds per pod, v, correlated with particular single soil parameters, s (table 9 ).

Table 9: Experiment seed parameter autocorrelation regressions (square root, n = 12)

| n = 12 | | | Eaten per pod | Viable per pod |
|---|---|---|---|---|
| | | | y-variable | |
| Initiated per pod | x-variable | $r^2$ | -0.526 | +0.699 |
| | | adj. $r^2$ | -0.479 | +0.669 |
| | | p | **0.008** | **0.001** |
| Eaten per pod | | $r^2$ | | -0.711 |
| | | adj. $r^2$ | | -0.682 |
| | | p | | **0.001** |

Eaten seeds per initiated on experiment shrubs, e/i, and viable seeds per initiated, v/i, correlated with particular soil parameters (table 10 ).

Table 10: Experiment direct seed parameter regressions with single soil variables

| n = 12 | | | Sulphur | Conductivity | pH (of $H_2O$) | pH (of $CaCl_2$) |
|---|---|---|---|---|---|---|
| | | | x-variable, √s | | | |
| Number of seeds initiated per pod, √i | y-variable | $r^2$ | -0.626 | -0.556 | -0.588 | -0.567 |
| | | adj. $r^2$ | -0.588 | -0.512 | -0.547 | -0.524 |
| | | p | **0.002** | **0.005** | **0.004** | **0.005** |
| Number of seeds eaten per pod, √e | | $r^2$ | +0.585 | +0.535 | +0.486 | +0.476 |
| | | adj. $r^2$ | +0.544 | +0.488 | +0.434 | +0.423 |
| | | p | **0.004** | **0.007** | **0.012** | **0.013** |
| Number of viable seeds per pod, √v | | $r^2$ | -0.568 | -0.670 | -0.539 | -0.480 |
| | | adj. $r^2$ | -0.525 | -0.637 | -0.493 | -0.428 |
| | | p | **0.005** | **0.001** | **0.007** | **0.012** |

Seed parameters initiated seeds, i, eaten seeds, e, and viable seeds, v of experiment shrub seeds correlated with each other (table 11 ). None of these seed parameters correlated with seed mass.

Table 11: Relative seed parameter regressions with single soil variables, experiment shrubs

| n = 12 | | | Sulphur | Conductivity | pH (of $H_2O$) | pH (of $CaCl_2$) |
|---|---|---|---|---|---|---|
| | | | x-variable, √s | | | |
| Number of seeds eaten per initiated<br>arcsine(√(e/i)) | y-variable | $r^2$ | +0.849 | +0.731 | +0.430 | +0.515 |
| | | adj. $r^2$ | +0.834 | +0.705 | +0.373 | +0.468 |
| | | p | **<0.001** | **<0.001** | **0.021** | **0.008** |
| | | slope | 0.070±0.009 | 1.693±0.325 | 4.022±1.464 | 3.649±1.117 |
| | | constant | -0.130±0.058 | -0.591±0.161 | -11.242±4.172 | -9.733±3.045 |
| Number of viable seeds per initiated<br>arcsine(√(v/i)) | | $r^2$ | -0.467 | -0.547 | -0.397 | -0.341 |
| | | adj. $r^2$ | -0.413 | -0.502 | -0.337 | -0.275 |
| | | p | **0.014** | **0.006** | **0.028** | **0.046** |
| | | slope | -0.062±0.021 | -1.736±0.499 | -4.584±1.785 | -3.517±1.545 |
| | | constant | 0.910±0.129 | 1.433±0.248 | 13.664±5.084 | 10.195±4.212 |

## Possible effects of natural SEPH/*Sextius* density on growth rate of shrubs

Growth of experiment shrub control stems with presence of low- versus high-*Sextius* level

In experiment shrubs, naturally high December 2009 *Sextius* densities were followed by total shoot growth that was on average 3 times longer than on low-*Sextius* shrubs when measured 11 months later (PERMANOVA, √g, n = 9 (high-*Sextius*)/10 (low-*Sextius*), p = 0.002). Regression of 2009 December *Sextius* densities on all experiment control stems showed a weak positive regression relationship with growth (SPSS, √g, $\sqrt[3]{S}$, n = 35: $r^2$ = 0.175, adj. $r^2$ = 0.150, p = 0.012, slope = 0.476±0.189, constant = 3.327±0.800) (figure 11). To compare with regressions of 2011 *Sextius* densities, only on shrubs whose *Sextius* level had changed between seasons, there was no difference (√g, n = 3, p = 0.693) and there was no regression relationship when also including shrubs whose *Sextius* density had changed by at least 5 times if over the maximum low density in both years (SPSS, $\sqrt{g}$, $\sqrt[3]{S}$, n = 11: $r^2$ = -0.088, adj. $r^2$ = -0.013, p = 0.375) (figure 12).

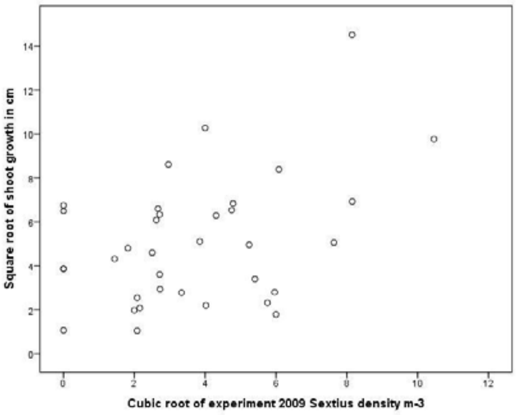


Figure 11: Summer 2009 *Sextius* density versus experiment experiment shrub growth (n = 35)

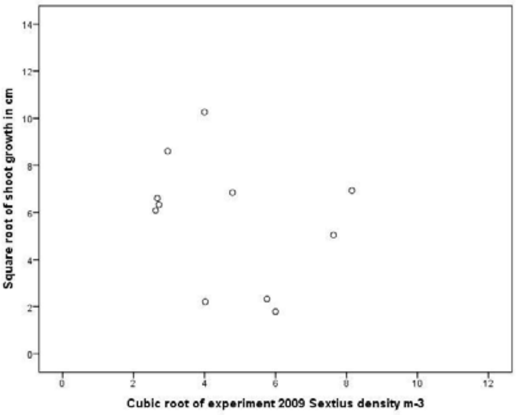
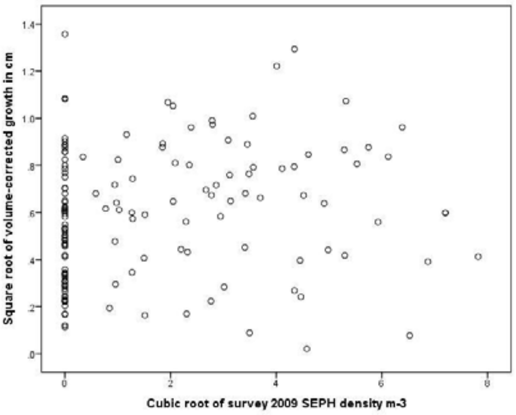
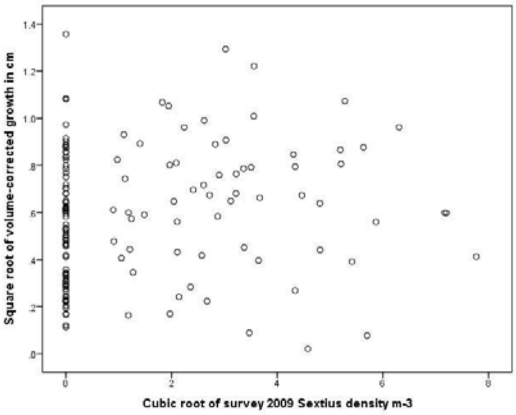


Figure 12: Summer 2009 *Sextius* density versus shrub growth for shrubs with substantial change in *Sextius* level (n = 11)

Growth of survey shrubs with presence of low- versus high-SEPH/*Sextius* level

For all shrubs in the survey, neither *Sextius* nor SEPH density, S, in late 2009 correlated with volume-corrected growth, g (SPSS, $\sqrt{g}$, $\sqrt[3]{S}$, n = 144; Sextius: $r^2$ = 0.021, adj. $r^2$ = 0.014, p = 0.083; SEPHs: $r^2$ = 0.027, adj. $r^2$ = 0.020, p = 0.049 ) (figure 13). Patterns for all shrubs occurring on flats were slightly stronger but similar. To compare with regressions of 2011 SEPH/*Sextius* densities, for only survey shrubs that changed from high (≥60 $m^{-3}$) to low (≥20 $m^{-3}$) SEPH densities or vice versa between years, volume-corrected growth did not correlate with either 2009 SEPH or *Sextius* density (SPSS, Sextius, $\sqrt{g}$, $\sqrt[3]{S}$, n = 30: $r^2$ = 0.010, adj. $r^2$ = -0.025, p = 0.600; SEPH, $\sqrt{g}$, $\sqrt[3]{S}$, n = 30: $r^2$ = 0.029, adj. $r^2$ = -0.025, p = 0.367) (figure 14).





All survey shrubs, summer 2009/2010, volume-corrected growth:
Figure 13: SEPH density versus shrub growth
Figure 14: *Sextius* density versus shrub growth

## Tangle-Trap effects on growth rate of shrubs

### Growth of experiment shrub Tangle-Trap versus control stems with low- and high-*Sextius* levels

Growth of shoots on control stems of high-*Sextius* experiment shrubs was on average 2.2 times that on Tangle-Trap stems, with one outlier removed (PERMANOVA, √g, n = 9, p = 0.0453). For shrubs that had consistently high or low *Sextius* densities in December of both 2009 and 2010, high-*Sextius* shrub shoots on control stems grew 2.9 times more than shoots on Tangle-Trap stems, but was not significant (PERMANOVA, √g, n = 6, p = 0.0873) (figure 15). For shrubs that changed from high- to low-*Sextius* over the two seasons, there was no difference in growth found between treatment levels (PERMANOVA, √g, n = 4, p = 0.4319). However, there was clearly no significant difference between control and Tangle-Trap stem growth for low-*Sextius* shrubs (PERMANOVA, √g, n = 12, p = 0.5977). Also, there was no significant difference found in growth between Tangle-Trap stems on high-*Sextius* and low-*Sextius* shrubs (PERMANOVA, √g, n = 9 (high-*Sextius*)/10 (low-*Sextius*), p = 0.2615).

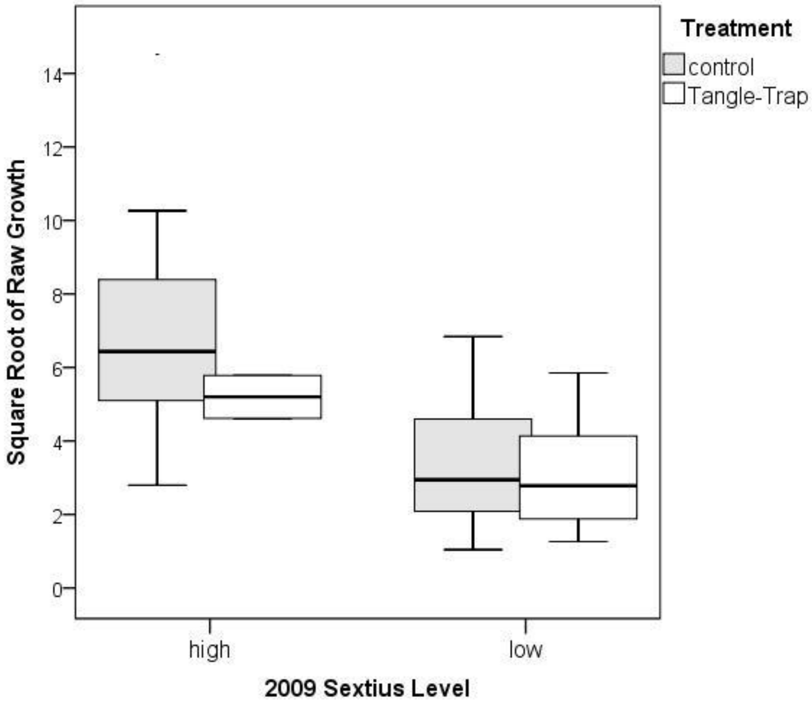


Figure 15: Raw growth, √g, of experiment shrub control and Tangle-Trap stems for summer 2009 *Sextius* levels (n = 9 (high-*Sextius*)/10 (low-*Sextius*))

## Folivory and *Sextius* density

### Folivory marks on experiment shrubs in January 2010 before application of Tangle-Trap treatment

Patrolling SBIs venture onto leaves at any sign of disturbance, moving erratically in different directions. Herbivory marks on leaves in January 2010 showed differences between low- and high-*Sextius* shrubs (tables 12-16; figures 16-20). There was a higher proportion of high-*Sextius* shrubs with ≤ 1% of leaf area damaged for all leaf damage types except galling (table 16; figure 20). High-*Sextius* shrubs lacked substantial damage in 13% more leaves than low-*Sextius* from LAL analysis and in 35% more from TAD analysis. The majority of leaf damage by all damage types covered a low proportion of total leaf area (tables 12-16).

A higher proportion of leaves on low-*Sextius* shrubs had LAL and TAD from 1 to just less than 10% of leaf area, comprising 22% and 46% of all leaves sampled, respectively (table 12, 13; figure 16, 17). In combination, there was no difference between shrubs with the two *Sextius* levels in proportions of leaves with over 10% LAL or over 10% TAD (n = 12, p = 0.617) so overall differences indicated that folivory levels were higher on low-*Sextius* shrubs and this was reflected in leaves with ≤ 10% of area damaged. Mining damage in the 91% of leaves where mining covered less than 60% of area was more common on low-*Sextius* shrubs (table 14; figure 18). For the 58% of leaves with coccoid marking on 1 to 5% of area, this type of damage was equally common in the two treatments, but leaves with coccoid damage ≥ 5% were more common in low-*Sextius* shrubs (table 15; figure 19). There was no difference in area of galling in shrubs of different *Sextius* levels (table 16; figure 20).

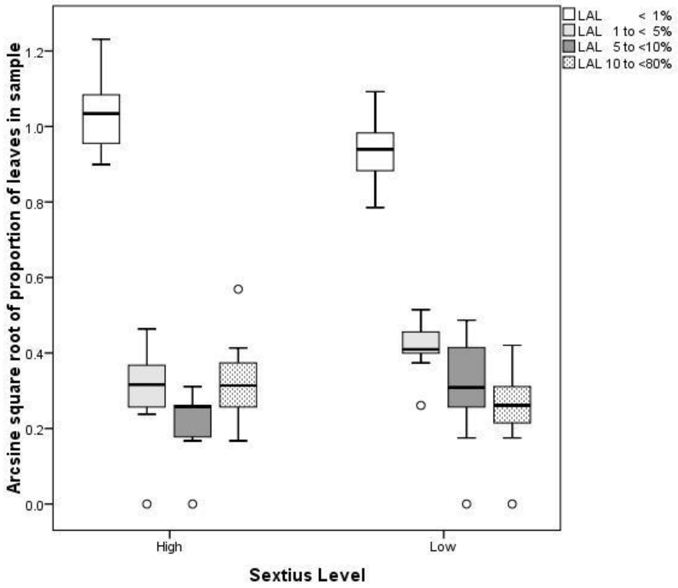


Figure 16: LAL on leaves of January 2010 experiment shrubs of high and low *Sextius* levels

Table 12: LAL on leaves of January 2010 experiment shrubs of high and low *Sextius* levels

| n = 10 (high *Sextius*)/12 (low *Sextius*) | p-value of categories | | **Treatment**<br>Mean % leaves of treatment sample in category ± standard error (1 d.p.) | | % high-*Sextius* leaves in this category / % low- (0 d.p.) |
|---|---|---|---|---|---|
| **Category** (mean leaf area in sample with this % of LAL) | p-value (difference between treatments, 3 d.p.) | % whole sample in category (to nearest 1%) | High *Sextius* | Low *Sextius* | |
| < 1% | **0.033** | 69 | 73.3 ± 2.6 | 65.0 ± 2.4 | 113 |
| 1 to < 5% | **0.006** | 14 | 10.1 ± 1.7 | 16.9 ± 1.3 | 60 |
| 5 to < 10% | *0.085* | 8 | 5.4 ± 0.9 | 10.7 ± 2 | 50 |
| 10 to < 80% | 0.175 | 9 | 11.2 ± 2.4 | 7.5 ± 1.4 | |

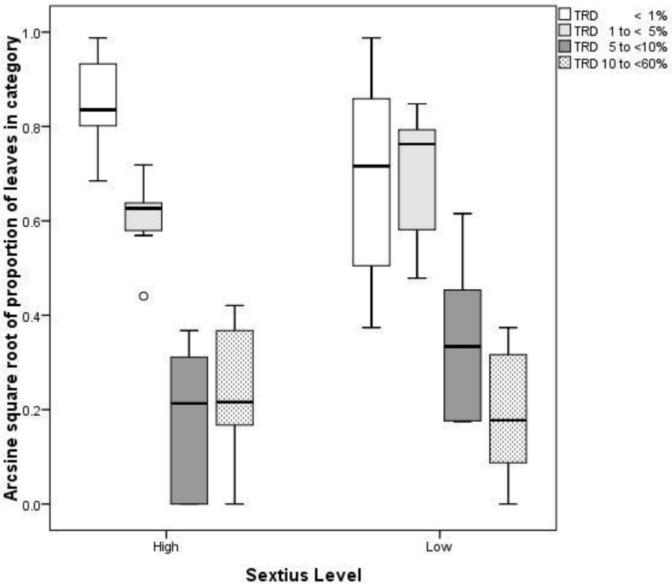


Figure 17: TAD on leaves of January 2010 experiment shrubs of high and low *Sextius* levels

Table 13: TAD on leaves of January 2010 experiment shrubs of high and low *Sextius* levels

| n = 10 (high *Sextius*)/12 (low *Sextius*) | p-value of categories | | **Treatment**<br>Mean % leaves of treatment sample in category ± standard error (1 d.p.) | | % high-*Sextius* leaves in this category / % low- (0 d.p.) |
|---|---|---|---|---|---|
| **Category** (mean leaf area in sample with this % of TAD) | p-value (difference between treatments, 3 d.p.) | % whole sample in category (to nearest 1%) | High *Sextius* | Low *Sextius* | |
| < 1% | **0.044** | 48 | 55.4 ± 3.2 | 41.0 ± 5.5 | 135 |
| 1 to < 5% | ***0.080*** | 38 | 33.1 ± 2.1 | 41.4 ± 3.6 | 80 |
| 5 to < 10% | **0.027** | 9 | 4.8 ± 1.5 | 12.1 ± 2.9 | 40 |
| 10 to < 60% | 0.660 | 6 | 6.7 ± 2.0 | 5.4 ± 1.4 | |

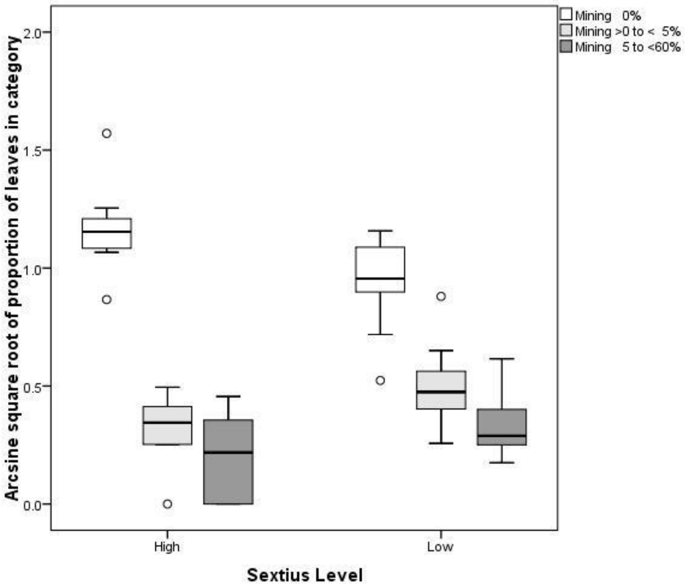


Figure 18: Mining on leaves of January 2010 experiment shrubs of high and low *Sextius* levels

Table 14: Mining on leaves of January 2010 experiment shrubs of high and low *Sextius* levels

| n = 10 (high *Sextius*)/12 (low *Sextius*) | p-value of categories | | **Treatment**<br>Mean % leaves of treatment sample in category ± standard error (1 d.p.) | | % high-*Sextius* leaves in this category / % low- (0 d.p.) |
|---|---|---|---|---|---|
| **Category** (mean leaf area in sample with this % of mining) | p-value (difference between treatments, 3 d.p.) | % whole sample in category (to nearest 1%) | High *Sextius* | Low *Sextius* | |
| 0% | **0.052** | 73 | 82.0 ± 3.4 | 64.8 ± 4.8 | 127 |
| >0 to < 5% | **0.014** | 18 | 11.3 ± 2.0 | 23.5 ± 4.2 | 48 |
| 5 to < 60% | **0.034** | 9 | 6.7 ± 2.3 | 11.7 ± 2.4 | 57 |

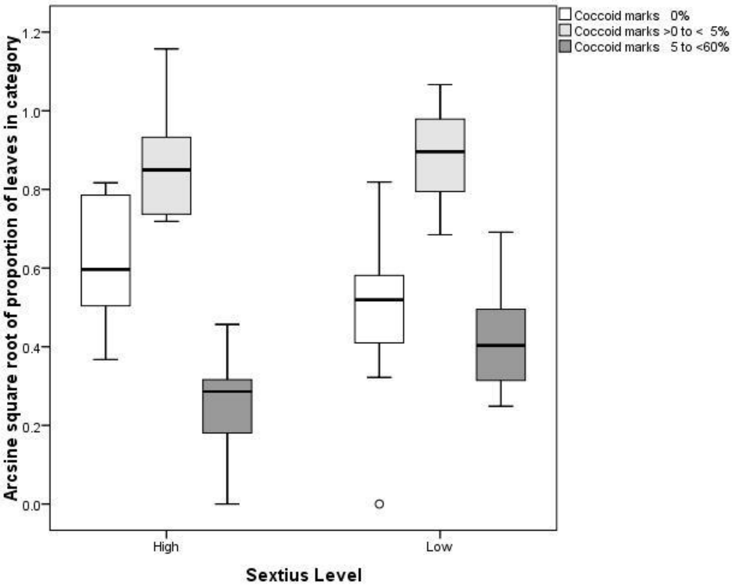


Figure 19: Coccoid markings on leaves of January 2010 experiment shrubs of high and low *Sextius* levels

Table 15: Coccoid markings on leaves of January 2010 experiment shrubs of high and low *Sextius* levels

| n = 10 (high *Sextius*)/12 (low *Sextius*) | p-value of categories | | **Treatment**<br>Mean % leaves of treatment sample in category ± standard error (1 d.p.) | | % high-*Sextius* leaves in this category / % low- (0 d.p.) |
|---|---|---|---|---|---|
| **Category** (mean leaf area in sample with this % of coccoid marking) | p-value (difference between treatments, 3 d.p.) | % whole sample in category (to nearest 1%) | High *Sextius* | Low *Sextius* | |
| 0% | ***0.068*** | 29 | 34.9 ± 4.6 | 23.4 ± 3.8 | 152 |
| >0 to < 5% | 0.678 | 58 | 57.0 ± 4.2 | 59.5 ± 3.6 | |
| 5 to < 60% | **0.013** | 13 | 8.1 ± 2.0 | 17.1 ± 2.9 | 47 |

Figure 20: Galling on leaves of January 2010 experiment shrubs of high and low *Sextius* levels

Table 16: Galling on leaves of January 2010 experiment shrubs of high and low *Sextius* levels

| n = 10 (high *Sextius*)/12 (low *Sextius*) | p-value of categories | | **Treatment**<br>Mean % leaves of treatment sample in category ± standard error (1 d.p.) | | % high-*Sextius* leaves in this category / % low- (0 d.p.) |
|---|---|---|---|---|---|
| **Category** (mean leaf area in sample with this % of galling) | p-value (difference between treatments, 3 d.p.) | % whole sample in category (to nearest 1%) | High *Sextius* | Low *Sextius* | |
| 0% | 0.515 | 86 | 82.1 ± 4.9 | 88.5 ± 2.1 | |
| >0 to < 5% | 0.521 | 14 | 17.9 ± 4.9 | 11.6 ± 2.1 | |

Folivory marks on leaves of experiment shrubs in autumn after application of Tangle-Trap treatment

Folivory marks on leaves in April 2010 showed differences between low- and high-*Sextius* shrubs on control stems and sometimes on Tangle-Trap stems, with similar relationships as before treatments were applied, but not between control and Tangle-Trap stems.

### Shrub reproductive success and *Sextius* density

Seed pods from experiment shrubs: insects not excluded by seed pod bags

Seed pods on shrubs developed in December 2009, when there were already substantial populations of *Sextius* and sometimes other SEPHs on them which may have affected seed development at all stages. *Sextius* comprised 82.2% SEPHs when shrubs were first counted in December2009. Including only experiment shrubs that had at least 10 seed pods, in the summer of 2009/2010 the number of seeds initiated per pod, i, compared between high-*Sextius* and low-*Sextius* shrubs was on average 35% higher on high-*Sextius* shrubs (as a proportion of low-*Sextius* levels) and this was marginally significant (PERMANOVA, √i, n =

8/7, p = 0.0568). Mean seed mass (m) did not differ between shrubs with different *Sextius* densities (ln(m + 1), n = 8/7, p = 0.2964, Type III (partial)).

In the first year, some shrubs did not produce over 10 seed pods and were not included in analyses, but all other experiment shrubs with the required *Sextius* densities were included. In the second year, shrubs had almost no seed pods, so effects of experimental manipulations on reproductive output could not be ascertained. Inside many seeds, a weevil larva was found, the contents indicated by a minute hole. On average 2.4% of seeds per pod (e) had been eaten and rendered nonviable on high-*Sextius* shrubs, but 38.0% had been eaten on low-*Sextius* shrubs which were different (PERMANOVA, √e, n = 8/7, p = 0.0054) and they were differently dispersed between *Sextius* levels (PermDisp, √e, n = 8/7, p = 0.075). Number of viable seeds per pod, v, on high-*Sextius* shrubs was on average 81.3% of seeds initiated in the same pod, which was 2.8 times as many viable seeds per pod as for low-*Sextius* shrubs (PERMANOVA, √v, n = 8/7, p = 0.0109). Average number of viable per initiated seeds per pod on high-*Sextius* shrubs was 240% of that on low-*Sextius* shrubs (PERMANOVA, arcsine√(v/i + 1), n = 10, p = 0.0002) and mean ratio of eaten seeds per initiated seeds per pod on high-*Sextius* shrubs was 5.1%that of low-*Sextius* shrubs (PERMANOVA, arcsine√(e/i + 1), n = 10, p = 0.0003). As a result, seed mass per pod on high-*Sextius* shrubs was on average approximately 3 times that occurring on low-*Sextius* shrubs.

Seed pods from survey shrubs: insects excluded versus insects not excluded by seed pod bags

On site 3 of the survey where seed pods did not receive bags, an average 2.6 times as many seeds per pod were viable on moderate- (over 40 to 60 $m^{-3}$) to high- (over 60 $m^{-3}$) SEPH shrubs than on low- (20 $m^{-3}$ or under) SEPH shrubs (PERMANOVA, $\sqrt{v}$, n = 6/14, p = 0.0108) and 159% of seeds were viable per those initiated on moderate to high- SEPH shrubs relative to low- SEPH shrubs (PERMANOVA, $\sqrt{(v/i)}$, n = 6/14, p = 0.0506). On these higher-SEPH shrubs, on average 35% more seeds had been eaten on high- SEPH shrubs (as a proportion of those on low- SEPH) (PERMANOVA, $\sqrt{e}$, n = 6/14, p = 0.0337) and 51% of seeds were eaten per initiated on moderate to high- SEPH shrubs relative to low- SEPH shrubs (PERMANOVA, $\sqrt{(e/i)}$, n = 6/14, p = 0.0324). This equates to a seed pod mass of on average around 70% more on moderate-to-high-SEPH shrubs. On sites 1 and 2 where seed pods received bags to prevent seed dispersal, there was no difference between moderate-to-high-SEPH and low-SEPH shrubs in seed mass (PERMANOVA, $\sqrt{m}$, n = 11 (moderate to high)/26 (low), p = 0.7242), number of seeds initiated per pod (PERMANOVA, $\sqrt{i}$ , n = 11 (moderate to high)/26 (low), p = 0.1378), viable seeds per pod (PERMANOVA, $\sqrt{v}$, n = 11/26, p = 0.5703), or number of seeds eaten per pod (PERMANOVA, $\sqrt{e}$, n = 11/26, p = 0.5043) which was on average 0.19 seeds per pod over all sub-sites.

### Relationship between soil properties and other small arthropods ocurring on shrubs

Experiment shrub small arthropods and soil

Some taxa caught on sticky traps placed on experiment shrubs correlated with some single soil parameters (table 17 ).

Table 17 : Regressions of densities of small arthropods on traps, a, with single soil parameters, s, associated with shrubs on experiment sites in January 2010. (Only significant results are shown.)

| n = 13 | | | Potassium | Conductivity | pH (of $H_2O$) |
|---|---|---|---|---|---|
| | | | x-variable, √s | | |
| Thrips | y-variable, √a | $r^2$ | | +0.357 | |
| | | adj. $r^2$ | | +0.299 | |
| | | p | | **0.031** | |
| "Other" Auchenorrhyncha | | $r^2$ | | | *+0.283* |
| | | adj. $r^2$ | | | *+0.217* |
| | | p | | | ***0.061*** |
| Psyllids | | $r^2$ | -0.407 | | |
| | | adj. $r^2$ | -0.353 | | |
| | | p | **0.019** | | |
| "Other" herbivores | | $r^2$ | | +0.307 | |
| | | adj. $r^2$ | | +0.244 | |
| | | p | | **0.049** | |

## Survey sub-site small arthropods and soil

Some survey sub-site mean soil parameter concentrations, s, correlated with mean numbers of arthropod taxa, a, in the survey of 2010 January (table 18 ).

Table 18: Regressions of average densities of single small arthropod groups, a, with average soil parameters, s, associated with shrubs on survey sub-sites in January 2010. (Only significant results are shown.)

| n = 9 | | | x-variable, √s | | | |
|---|---|---|---|---|---|---|
| | | | Total Nitrogen | Sulphur | Organic Carbon | Conductivity |
| y-variable, √a | "Minute" wasps | $r^2$ | +0.605 | +0.497 | +0.759 | +0.452 |
| | | adj. $r^2$ | +0.508 | +0.426 | +0.725 | +0.374 |
| | | p | **0.014** | **0.034** | **0.002** | **0.047** |
| | Predators | $r^2$ | +0.575 | +0.657 | | |
| | | adj. $r^2$ | +0.514 | +0.608 | | |
| | | p | **0.018** | **0.008** | | |
| | Mirid/lygaeids | $r^2$ | +0.767 | +0.494 | +0.452 | *+0.418* |
| | | adj. $r^2$ | +0.734 | +0.421 | +0.374 | *+0.335* |
| | | p | **0.002** | **0.035** | **0.047** | ***0.060*** |
| | Thrips | $r^2$ | +0.763 | +0.488 | +0.483 | |
| | | adj. $r^2$ | +0.729 | +0.415 | +0.410 | |
| | | p | **0.002** | **0.036** | **0.038** | |
| | "Other" Auchenorrhyncha | $r^2$ | | *+0.410* | | *+0.410* |
| | | adj. $r^2$ | | *+0.326* | | *+0.326* |
| | | p | | ***0.063*** | | ***0.063*** |
| | "Other" herbivores | $r^2$ | | | *+0.383* | +0.451 |
| | | adj. $r^2$ | | | *+0.295* | +0.373 |
| | | p | | | ***0.075*** | **0.047** |

Some summed taxa, a, correlated more strongly with soil parameters, s, than single taxa did (see table 19 below).

Table 19: Regressions of average densities of pooled taxa small arthropods, a, with average soil parameters, s, associated with shrubs on survey sub-sites in January 2010. (Only significant results are shown.)

| n = 9 | | | x-variable, $\sqrt{s}$ | | | |
|---|---|---|---|---|---|---|
| | | | Nitrogen | Organic Carbon | Conductivity | pH (of $CaCl_2$) |
| y-variable, $\sqrt{a}$ | All arthropods except "minute" wasps or SEPHs | $r^2$ | +0.583 | *+0.388* | +0.422 | *+0.358* |
| | | adj. $r^2$ | +0.524 | *+0.300* | +0.340 | *+0.267* |
| | | p | **0.017** | ***0.073*** | **0.058** | *0.089* |
| | All herbivores | $r^2$ | +0.546 | *+0.371* | *+0.412* | *+0.361* |
| | | adj. $r^2$ | +0.481 | *+0.282* | *+0.328* | *+0.270* |
| | | p | **0.023** | ***0.082*** | ***0.063*** | *0.087* |
| | Phloem-feeders | $r^2$ | | | *+0.392* | +0.425 |
| | | adj. $r^2$ | | | *+0.305* | +0.342 |
| | | p | | | ***0.071*** | **0.057** |

## SBI deterrent effects on small arthropods

In the predation trials with no ants present, the *Monolepta divisa* remained on the twig every time as long as it was released after its legs had settled on the twig, which it did readily. In the absence of the beetle, when fingers touched a twig causing disturbance, some SBIs would run over the fingers onto the hand or further, sometimes biting, while other SBIs would run towards the fingers and away alternately back and forth. The other results are recorded in table 20. When the beetle was manually placed on the twig, the collective response of the SBIs was first that some attacked the beetle while other SBIs ran away from the human fingers for about 40mm and then changed direction or crawled onto the human hand. The result of ants attacking the beetle was that it flew away within 30s unless stated otherwise in the table. Sometimes one ant attacked the beetle, gave up, and others took over, in which case only the attack of the first ant was recorded. When the time, t, it took either for the beetle to fly or drop off the twig was counted, either if SBIs attacked it or not, there was no difference between SBIs distant from *Sextius* and SBIs within 5mm of Sextius (√t, n = 20, p = 0.3631) (table 20), but SBIs 50mm from *Sextius* took more time to attack the beetle than 5mm away (√t, n = 20, p = 0.0004) or than with no Sextius (√t, n = 20, p = 0.0009), but in all cases the beetle flew away or dropped off the twig quicker than if there was no SBI present (√t, n = 20, p = 0.0001). If it only crawled away, it was counted as staying, but if it dropped onto another branch, it was counted as leaving.

Table 20: SBI and chrysomelid responses to proximity to each other on *A. victoriae* twigs

| Twig treatment (n = 20): | Ants, no SEPHs | Ant within 5mm, Sextius within 5mm | Ant within 5mm, Sextius within 50mm |
|---|---|---|---|
| Ant attacked beetle within 1s | 4 | 8 | |
| Ant attacked beetle within 10s | 1 | 3 | 6 |
| Ant attacked beetle within 20s | | 1 | 2 |
| Ant attacked beetle within 10s and pursued it until 30s | 1 | | |
| Beetle dropped off or flew away from plant without attack within 2s | 11 | 3 | 4 |
| Beetle dropped off or flew away from plant without attack within 10s | | 5 | |
| Beetle crawled away and remained on plant without attack for 30s | 2 | | 7 |
| Beetle dropped or flew onto another part of plant within 10 s and remained on plant without attack for 30s | 1 | | 1 |

## SEPH/*Sextius* densities and densities of other small arthropods on shrubs

*Sextius* and lycaenid caterpillar co-occurrence

At experiment sites in 2009, *Sextius* densities did not correlate with lycaenid caterpillar densities ($^3\sqrt{S}$, $^3\sqrt{C}$, n = 55: $r^2 = 0.079$, adj. $r^2 = 0.062$, $p = 0.037$) and neither did *Sextius* densities at survey sites in 2011 ($^3\sqrt{S}$, $^3\sqrt{C}$, n = 183: $r^2 = 0.076$, adj. $r^2 = 0.070$, $p < 0.001$).

"Other" small arthropod assemblages on experiment shrubs in January 2010 before application of Tangle-Trap treatment

On experiment shrubs in 2010 January, the middle of summer when activity is likely to peak, small arthropod assemblages on sticky traps differed among low- and high-*Sextius* experiment shrubs. Over all traps, 71.6% of individuals were minute wasps, thrips 10.5%, "other" Auchenorrhyncha 8.28%, psyllids 6.4%, mirid/lygaeids 2.1%, and other predators 1.15%. There were 170% minute wasps, a, on high-*Sextius* as a proportion of low-*Sextius* shrubs (PERMANOVA, $\sqrt{a}$, n = 7, p = 0.0123) (figure 21). There were fewer "other" phloem-feeding herbivores on high-*Sextius* shrubs. Only 42% as many "other" Auchenorrhyncha, a, were on high-*Sextius* shrubs compared with low (PERMANOVA, $\sqrt{a}$, n = 7, p = 0.0318) and 55% as many psyllids, a, were on high-*Sextius* shrubs compared with low (PERMANOVA, $\sqrt{a}$, n = 7, p = 0.0308). In combination, the difference of "other" Auchenorrhyncha and psyllids was clearer (PERMANOVA, $\sqrt{a}$, n = 7, p = 0.027). Predator numbers, a, did not differ between natural *Sextius* levels (PERMANOVA, $\sqrt{a}$, n = 7, p = 0.8541) and neither did thrips, a (PERMANOVA, $\sqrt{a}$, n = 7, p = 0.664) (although thrips were differently dispersed on *Sextius* levels (PermDisp, $\sqrt{a}$, n = 7, p = 0.066)) or mirid/lygaeids (PERMANOVA, $\sqrt{a}$, n = 7, p = 0.1809). Many *Mictis profana* (crusader bugs) and other phytophagous Heteroptera larger than those caught on traps were seen on shrubs. Also, many larvae were seen on foliage but larvae were rarely caught on traps. Psyllids seen on these shrubs were not seen attended by SBIs at any life stage.

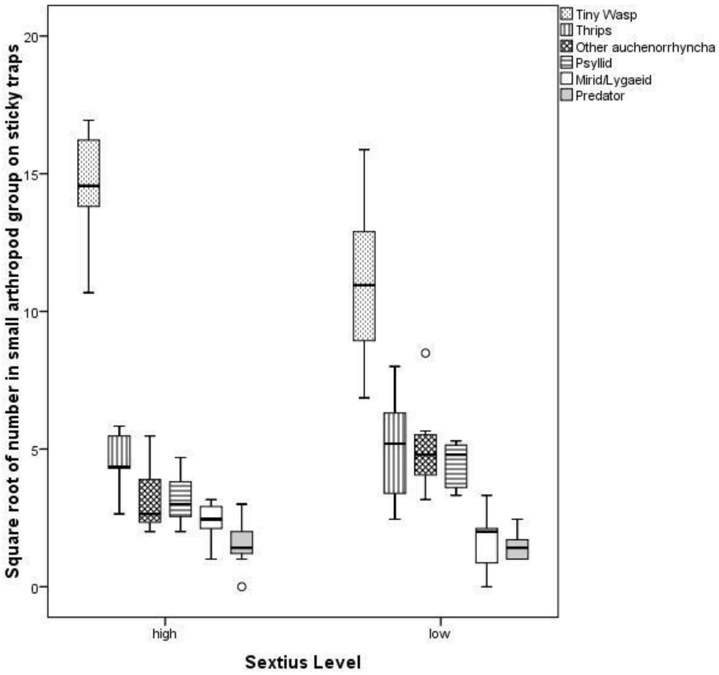


Figure 21: Numbers of small arthropods, s (√s), on sticky traps on experiment shrubs in January 2010

Numbers of minute wasps on experiment control stem sticky traps correlated positively with mirid/lygaeids (Pearson's, √w, √m, n = 14: r = +0.558, p = 0.038). Thrips correlated positively with "other" Auchenorrhyncha (Pearson's, √t, √c, n = 14: r = +0.539, p = 0.047) and marginally positively with psyllids (Pearson's, √t, √p, n = 14: r = +0.494, p = 0.073).

### "Other" small arthropod assemblages on experiment shrubs in February 2011, 13 months after application of Tangle-Trap treatment

In February 2011, there were fewer insects active than in January 2010: on high-*Sextius* 2011 control sticky traps, there were only 35% thrips, 64% minute wasps, 9.6% "other"

Auchenorrhyncha, 22% psyllids, 21% mirid/lygaeids, 14% “other” herbivores, and 246% predators as a proportion of those on January 2010 sticky traps despite February 2011 traps being twice the size. This was probably due to variation in weather conditions between years during the experiment, since traps had to be put out during a cool period. After treatments had been applied for 13 months during which there had been the second highest annual rainfall on record, there were twice as many minute wasps, w, on high-*Sextius* shrubs on control stems than on Tangle-Trap stems, of marginal significance (PERMANOVA, $\sqrt{w}$, $n = 7$, $p = 0.0566$) (figure 22) and within low-*Sextius* shrubs there were 1.64 times as many wasps on control stems, but this was rather marginal (PERMANOVA, $\sqrt{w}$, $n = 7$, $p = 0.0975$). On control stems, mirid/lygaeids, m, were higher on low-*Sextius* shrubs (PERMANOVA, $\sqrt{m}$, $n = 7$, $p = 0.0352$), other herbivores, h, were lower (PERMANOVA, $\sqrt{h}$, $n = 7$, $p = 0.0517$) and were differently dispersed by *Sextius* level (PermDisp, $\sqrt{h}$, $n = 7$, $p = 0.041$), and together there was a clearer significant difference between *Sextius* levels than for either alone (PERMANOVA, $\sqrt{m}$, $\sqrt{h}$, $n = 7$, $p = 0.0262$). “Other” Auchenorrhyncha were differently dispersed between *Sextius* levels (PermDisp, $\sqrt{c}$, $n = 7$, $p = 0.008$). On control stems, there was also a clearer significant difference between *Sextius* levels when numbers of mirid/lygaeids was combined with predators, p (PERMANOVA, $\sqrt{m}$, $\sqrt{p}$, $n = 7$, $p = 0.027$). When only Tangle-Trap treatment was considered and *Sextius* levels were pooled, minute wasp densities were clearly higher on control stems (PERMANOVA, $\sqrt{w}$, $n = 7$, $p = 0.0117$). Tangle-Trap affected some taxa only when low- and high-*Sextius* treatments were considered together. Differences by treatment in arthropods became clearer when wasps were combined with psyllids (PERMANOVA, $\sqrt{w}$, $n = 7$, $p = 0.0097$), with mirid/lygaeids

(PERMANOVA, √w, n = 7, p = 0.0107), and with other herbivores (PERMANOVA, √w, n = 7, p = 0.0101. No other group showed a difference associated with Tangle-Trap.

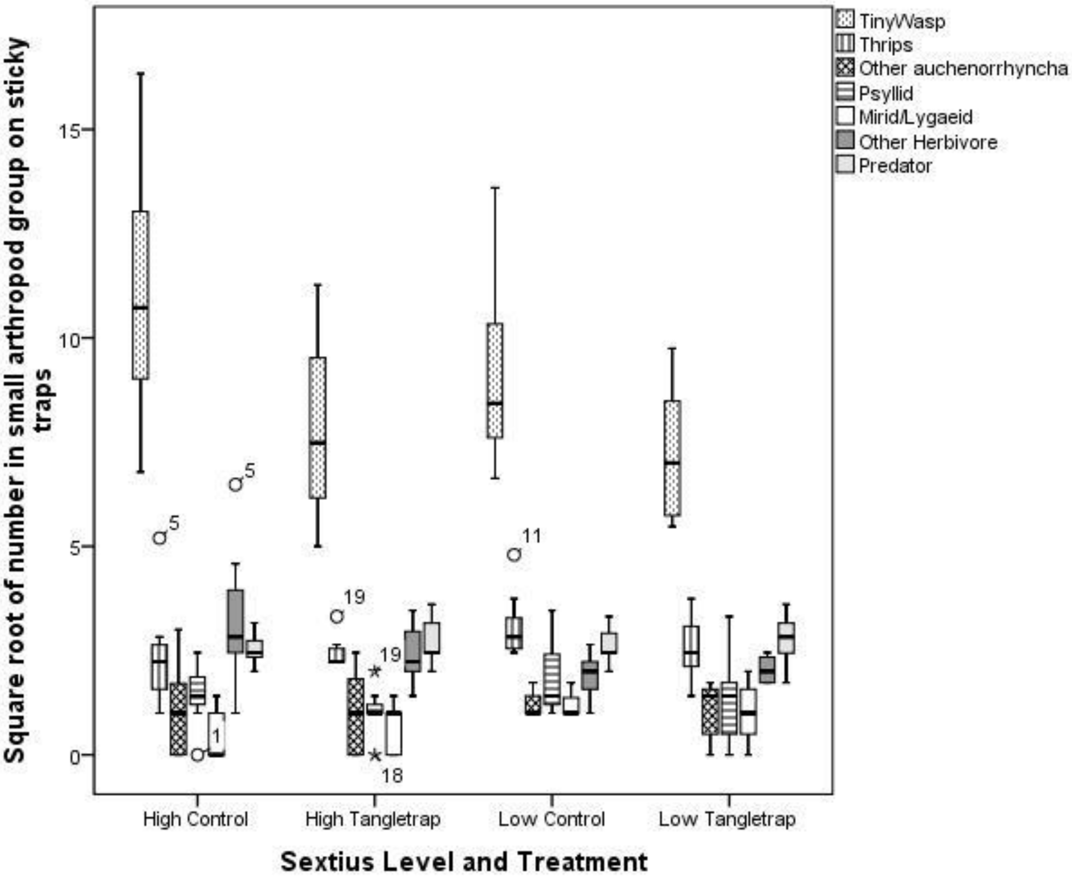


Figure 22: Numbers of small arthropods, s (√s), on sticky traps on experiment shrubs in February 2011

"Other" small arthropod assemblages on survey shrubs in January 2010

In the January 2010 survey, on flat sub-sites minute wasps comprised 56% of arthropod individuals, thrips 19%, psyllids 13%, "other" Auchenorrhyncha 7%, mirid/lygaeids 2%, non-SBI predators 2%, and other herbivores less than 1%. "Minute" wasps were differently dispersed between SEPH levels (PermDisp, √w, n = 13 (low-SEPH), 9 (high-SEPH), p = 0.072). Numbers of predators were 2.4 times more on high-SEPH shrubs than those on low-

SEPH shrubs, of marginal significance (PERMANOVA, √p, n = 13 (low-SEPH), 9 (high-SEPH), p = 0.061) (figure 23). There were 3.1 times as many "other" herbivores on low-SEPH as on high-SEPH shrubs (PERMANOVA, √h, n = 13 (low-SEPH), 9 (high-SEPH), p = 0.0741). However when these two were combined, there was a significant difference between the two treatments (PERMANOVA, √p, n = 13 (low-SEPH), 9 (high-SEPH), p = 0.0224). There were at least 200 SBIs in each pitfall trap.

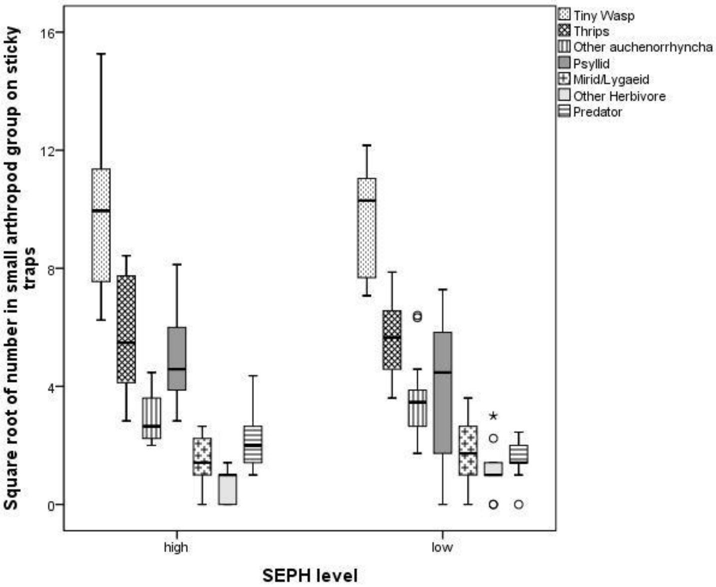
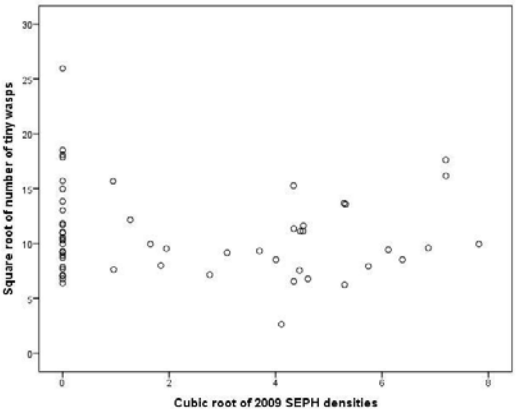


Figure 23: Numbers of small arthropods, s (√s), on sticky traps on survey flat sub-site shrubs in January 2010

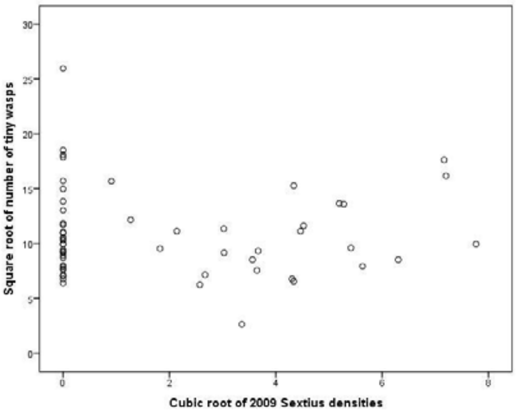
There were envelope regression relationships between SEPH densities and numbers of small arthropods caught on traps (table 21; figure 23). Using the top 50% of data, there was a quadratic relationship made by 2009 SEPH densities, S, for "minute" wasp densities, w (SPSS, $^3\sqrt{S}$, √w, n = 29, $r^2 = 0.213$, p = 0.045, constant = 14.909, a = -2.270 (x), b = 0.317 ($x^2$)) (figure 23a) and by 2009 *Sextius* densities, S, for "minute" wasp densities, w (SPSS, $^3\sqrt{S}$, √w, n = 29, $r^2 = 0.219$, p = 0.040, constant = 14.418, a = -2.268 (x), b = 0.371 ($x^2$)) (figure 23b). Differentiating the formula for "minute" wasp densities made by SEPH densities, this gives a minimum number of "minute" wasps at 3.58 SEPHs $m^{-3}$ (45.9 when cubed) or 3.06 *Sextius*

$m^{-3}$ (28.6 when cubed) on average. Using the top 50% of data, there was a quadratic relationship made by 2009 SEPH densities, S, for "other" Auchenorrhyncha numbers, c (SPSS, $^{3}\sqrt{S}$, $\sqrt{w}$, n = 30, $r^2 = 0.226$, p = 0.032, constant = 4.280, a = -0.634 (x), b = 0.086 ($x^2$)) (figure 23c) and by 2009 *Sextius* densities, S, for "other" Auchenorrhyncha densities, c (SPSS, $^{3}\sqrt{S}$, $\sqrt{w}$, n = 29, $r^2 = 0.330$, p = 0.005, constant = 4.205, a = -0.766 (x), b = 0.118 ($x^2$)) (figure 23d). This gives a minimum number of "other" Auchenorrhyncha at 3.56 SEPHs $m^{-3}$ (45.2 when cubed) or 3.25 (cubed 34.2) *Sextius* $m^{-3}$ on average.

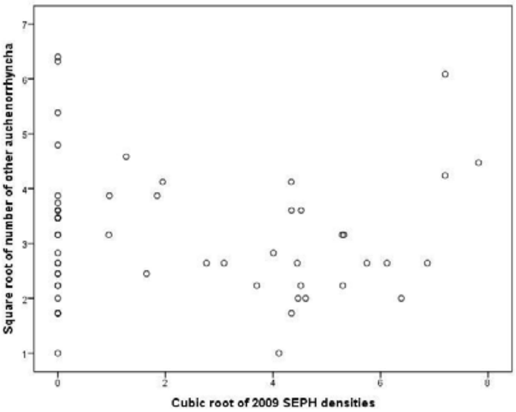
Some non-SEPH small arthropod taxa on sticky traps were correlated in the January 2010 survey (tables 22, 23; figure 23).



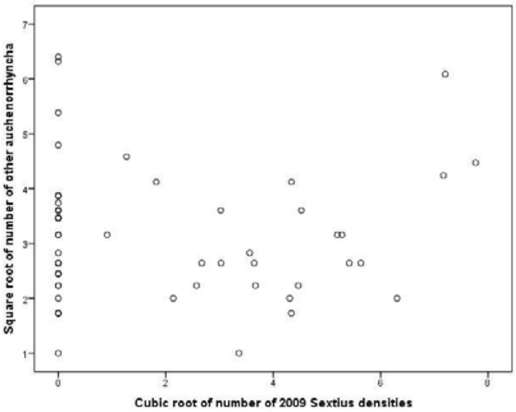
(a) Densities of 2009 SEPHs versus "minute" wasps



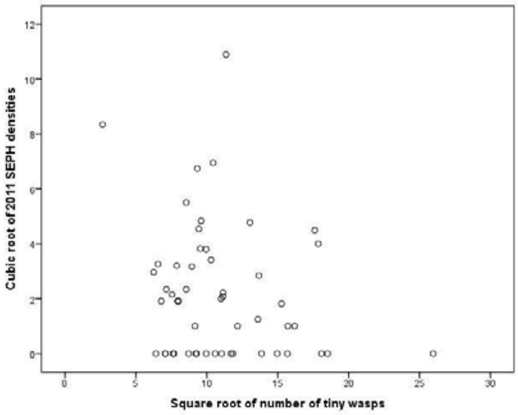
(b) Densities of 2009 *Sextius* versus "minute" wasps



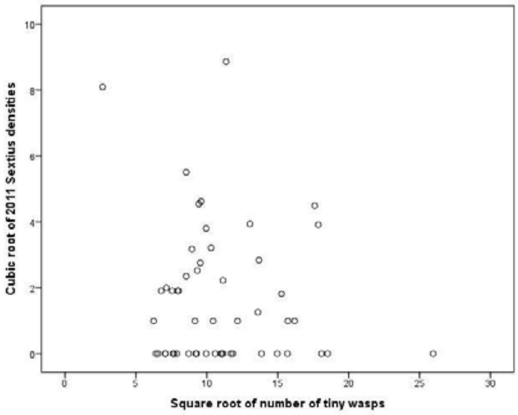
(c) Densities of 2009 SEPHs versus other Auchenorrhyncha



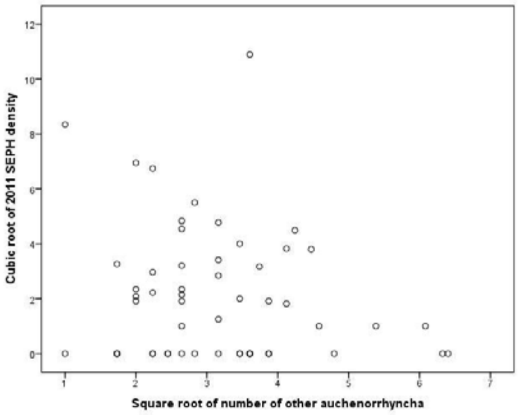
(d) Densities of 2009 *Sextius* versus other Auchenorrhyncha



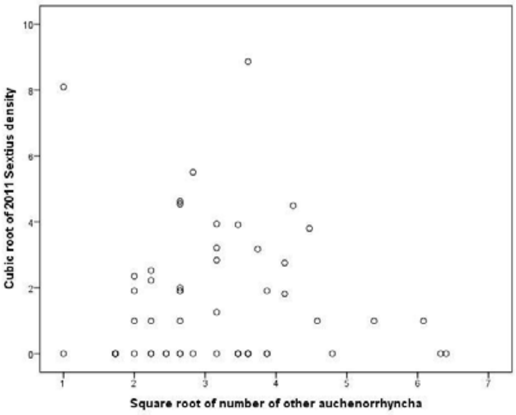
(e) Densities of "minute" wasps versus 2011 SEPHs



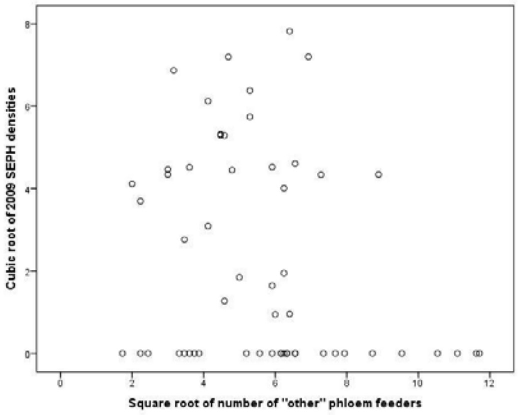
(f) Densities of "minute" wasps versus 2011 *Sextius*



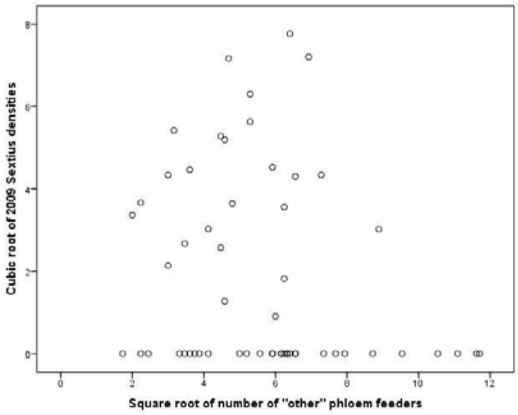
(g) Densities of other Auchenorrhyncha versus 2011 SEPHs



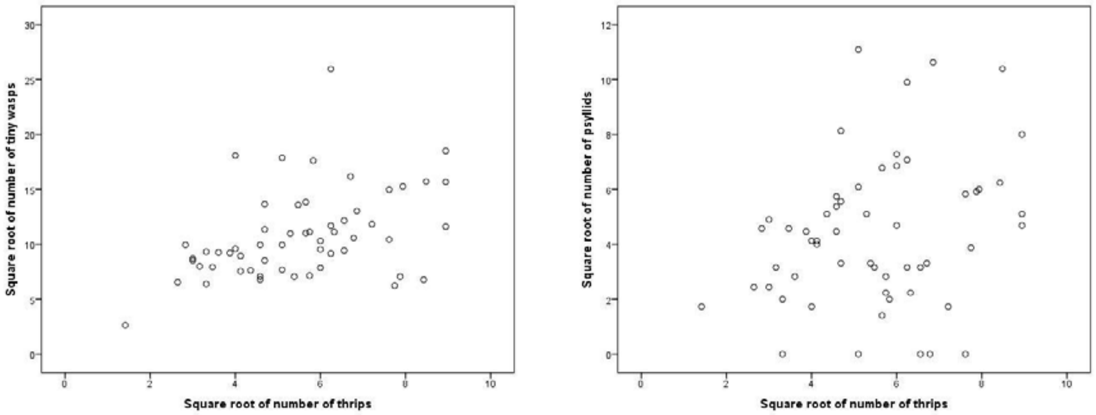
(h) Densities of other Auchenorrhyncha versus 2011 *Sextius*



(i) Densities of other phloem feeders versus 2009 SEPHs

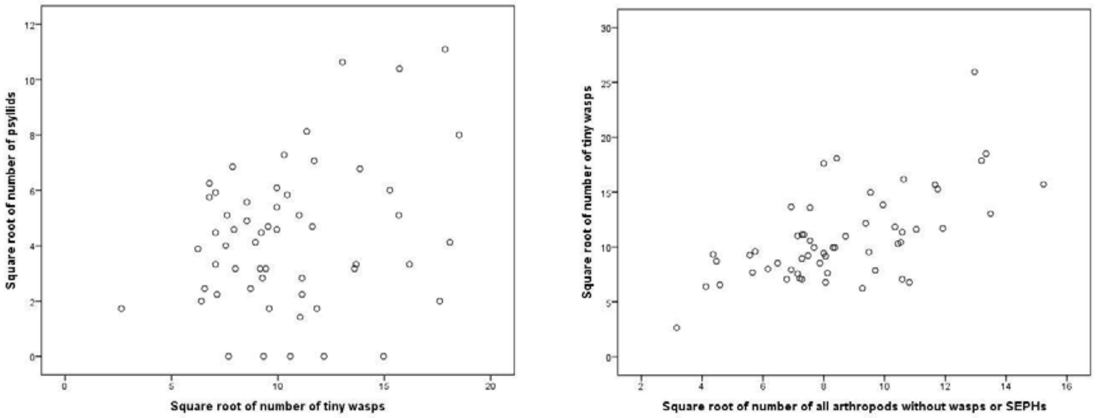


(j) Densities of other phloem feeders versus 2009 *Sextius*



(k) Densities of thrips versus "minute" wasps

(l) Densities of thrips versus psyllids



(m) Densities of "minute" wasps versus psyllids

(n) Densities of non-"minute" wasp non-SEPH small arthropods versus "minute" wasps

Figure 23: Envelope regressions identified for different arthropod taxa densities versus each other in January 2010 on survey shrubs

Table 21: Envelope regressions of densities of different arthropod taxa densities on survey shrubs in January 2010. (Only significant results are presented. The quantile with the strongest r value is presented where multiple quantiles were significant. )

| n = 55 (minus outliers noted) | | x-variable | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | "Minute" wasps ($\sqrt{w}$) | Thrips ($\sqrt{t}$) | 2009 SEPHs ($\sqrt[3]{S}$) | 2009 *Sextius* ($\sqrt[3]{S}$) | Phloem-feeders ($\sqrt{p}$) | "Other" Auchenorrhyncha ($\sqrt{c}$) |
| y-variable | "Minute" wasps ($\sqrt{w}$) | r | | 0.7128 | 0.5992 | 0.5992 | | |
| | | quantile | | 0.8th | 0.7th | 0.7th | | |
| | | p | | **0.0099** | **0.0407** | **0.0125** | | |
| | | slope | | +0.5379 | +0.3625 | +0.3848 | | |
| | | constant | | 11.1516 | 11.7047 | 11.6765 | | |
| | "Minute" wasps ($\sqrt{w}$) | r | | | | *0.4977* | | |
| | | quantile | | | | *0.6th* | | |
| | | p | | | | ***0.0801*** | | |
| | | slope | | | | *+0.3972* | | |
| | | constant | | | | *10.5830* | | |
| | 2009 SEPHs ($\sqrt[3]{S}$) | r | | | | | 0.5208 | |
| | | quantile | | | | | 0.6th | |
| | | p | | | | | **0.0119** | |
| | | slope | | | | | -0.5040 | |
| | | constant | | | | | 5.8564 | |
| | 2009 *Sextius* ($\sqrt[3]{S}$) | r | | | | | 0.5912 | |
| | | quantile | | | | | 0.7th | |
| | | p | | | | | **0.0465** | |
| | | slope | | | | | -0.5288 | |
| | | constant | | | | | 6.1895 | |
| | 2011 SEPHs ($\sqrt[3]{S}$) | r | 0.5257 | | | | | 0.8766 |
| | | quantile | 0.65th | | | | | 0.9th |
| | | p | **0.0458** | | | | | **0.0510** |
| | | slope | -0.1733 | | | | | -0.9481 |
| | | constant | 3.8151 | | | | | 8.0448 |

Table 22: Correlations of densities of single arthropod taxa on survey shrubs in January 2010. (Pearson correlation, √a) (Only significant results are presented. )

| n = 55 minus outliers | | Mirid/ lygaeids | Predators | Thrips | "Other" Auchenorrhyncha | Psyllids | "Other" herbivores |
|---|---|---|---|---|---|---|---|
| 2009 SEPH | r | +0.350 | +0.350 | | | | *-0.254* |
| | p | **0.008** | **0.008** | | | | ***0.059*** |
| 2009 *Sextius* | r | | +0.357 | | | | |
| | p | | **0.007** | | | | |
| "Minute" wasps | r | +0.622 | | +0.422 | +0.534 | +0.375 | +0.471 |
| | p | **<0.001** | | **0.001** | **<0.001** | 0.004 | **<0.001** |
| Mirid/ lygaeids | r | | | +0.398 | +0.572 | +0.265 | +0.388 |
| | p | | | **0.002** | **<0.001** | 0.048 | **0.003** |
| Thrips | r | | +0.265 | | +0.306 | +0.273 | +0.431 |
| | p | | **0.049** | | **0.022** | 0.042 | **0.001** |
| "Other" Auchenorrhyncha | r | | +0.288 | | | | +0.414 |
| | p | | **0.031** | | | | **0.002** |
| Psyllids | r | | | | | | +0.301 |
| | p | | | | | | **0.024** |

Table 23: Correlations of densities of pooled versus single arthropod taxa on survey shrubs in January 2010. (Pearson correlation, √a) (Only significant results are presented. )
(Number in parentheses beside taxon or group indicates number of outliers removed. )

| n = 55 | | 2009 SEPH | "Minute" wasps | Mirid/ lygaeids | Thrips | "Other" herbivores |
|---|---|---|---|---|---|---|
| All arthropods except "minute" wasps or SEPHs (1) | r | | +0.631 | | | |
| | p | | **<0.001** | | | |
| All herbivores | r | *-0.262* | +0.606 | +0.537 | | |
| | p | ***0.051*** | **<0.001** | **<0.001** | | |
| Phloem-feeders | r | *-0.257* | +0.546 | +0.478 | +0.379 | +0.439 |
| | p | ***0.055*** | **<0.001** | **<0.001** | **0.004** | **0.001** |

## SBI effects on *Sextius*

Four days after the predation experiment started, rainfall greater than twice the mean monthly occurred over two consecutive days. During the experiment, daily maximum temperatures ranged from 24 to 38°C and average maximum temperature was 30-31°C, which was about 2 to 3°C cooler than average over years.

During the project, SBIs were observed attacking and killing larvae and sometimes transported dead termites along trails. No evidence of SBIs preying on *Sextius* was seen.

During the predation experiment, SBIs maintained trails onto branches without Tangle-Trap. Although within the territory where SBIs were dominant other ants species were present on some shrubs with low *Sextius* numbers, on shrubs with high *Sextius* densities, SBIs were the only ants observed. In territories dominated by *Iridomyrmex lividus* colonies, they attended *Sextius*. Interestingly, numbers of nymphs on some branches were higher at the end than at the start of the experiment (figure 24). There must have been a few new *Sextius* emergences, since a small proportion of nymphs were first instar at the end of the experiment (pers. obs.), but there were not enough to discern any pattern. A few days after Tangle-Trap was applied, a substantial amount of honeydew was observed near nymphs, on many twigs and on the cage below just as on Tangle-Trap stems on high-*Sextius* shrubs in the large field experiment. Some nymphs appeared to be unable to get away from the honeydew even when they were trying to move. At the end of the experiment, some nymphs that had died were stuck to leaves with honeydew. Honeydew droplets were rare on branches with no Tangle-Trap.

On Shrub 2 in the branch with Tangle-Trap and closed cages, a flower spider (Family Thomisidae, Sub-family Misumeninae, *Diaea* sp.) which eats soft-bodied insects had been discovered at the end of the experiment and produced outlying data, so this branch was removed from analyses. Several of these spiders were seen on the shrubs, but not in other closed cages. After observation of thousands of *Sextius* with SBIs, there was never any evidence of SBI aggression towards *Sextius* such as biting. No live predator larger than 1mm in width was found in any other closed cage. Including both open and closed cages combined, Tangle-Trap made no difference to nymph survival (two-factor, n = 4 except outlier removed, p = 0.8929) (figure 25) but closed cages made a difference including both Tangle-Trap and no Tangle-Trap combined (two-factor, n = 4 except outlier removed, p = 0.0192). On branches with cages closed, Tangle-Trap had no significant effect (two-factor, n = 4 except outlier removed, p = 0.5229) even though it reduced the proportion of nymphs surviving to 0.88 on average. By contrast, on branches with Tangle-Trap, the insignificant effect of closed cages (two-factor, n = 4 except outlier removed, p = 0.6578) appeared to increase nymph survival by a factor of 1.09. On branches with cages open, Tangle-Trap increased nymph survival (two-factor, n = 4 except outlier removed, p = 0.0299) on average by a factor of 1.47, and on branches with no Tangle-Trap, closed cages strongly increased nymph survival (two-factor, n = 4 except outlier removed, p = 0.0287) on average by a factor of 1.84.

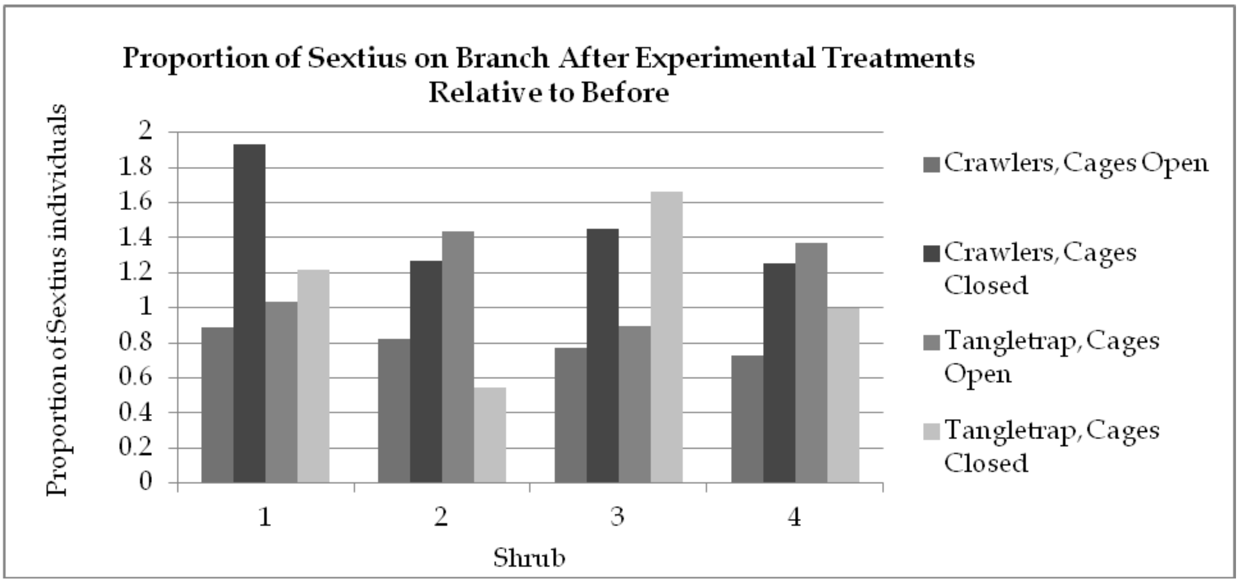


Figure 24: *Sextius* nymphs remaining on branch at end of experiment as proportion of those counted at beginning, for each replicate shrub

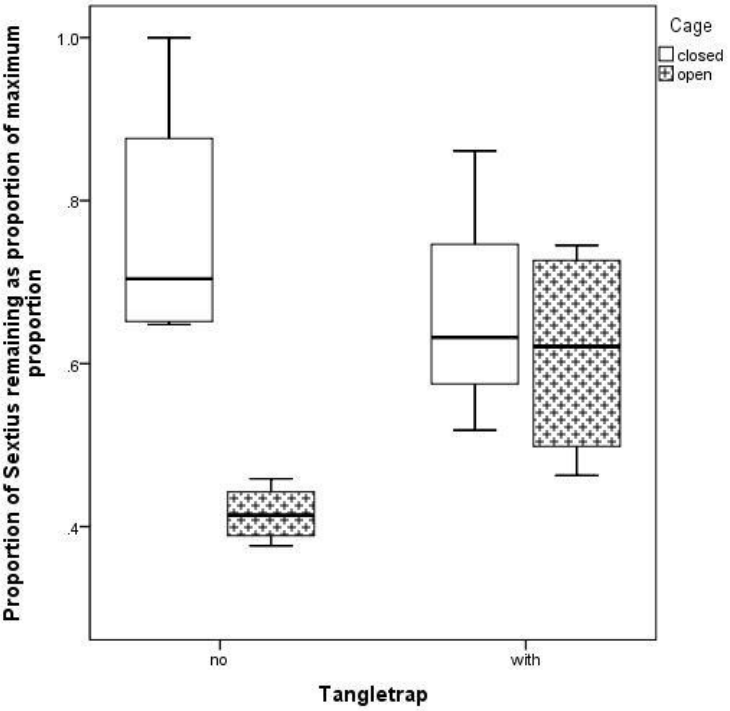


Figure 25: *Sextius* nymphs remaining on branch at the end of experiment as proportion of those counted at beginning, by treatment

# Discussion

This study was subject to the variability that is characteristic of arid environments, particularly those in Australia (Stafford Smith and Morton 1990). The second year of the study took place during a time of unusually high rainfall, which would represent conditions of relatively high resource abundance. By contrast, in the first year of the study, rainfall was near the average, which might have resulted in more typical conditions. Water is generally the most important limiting resource in arid systems (Whitford 2002), but in times of sufficiently high rainfall, soil nutrients might instead become the primary limiting factors to growth of shrubs and higher trophic levels interacting with them. In this study, many of the variables considered could be affected directly or indirectly by rainfall and soil nutrients (figure 26). Because of the complexity of interconnections between the variables measured, some correlations found might be artefacts of other causative correlations, but nevertheless they provide hypotheses to test. In several instances there are discrepancies between results for the experiment and survey sites, and these may be due to differences in their design that introduce variation in these interconnected factors, which can create slightly different ecological patterns. The survey sites involved much greater environmental variation, since shrubs were located in four places (rather than two) and rather than being flat they included different slope-aspects, which affected some important parameters (Chapter 5). Also, in comparison to experiment sites, survey sub-sites were sampled at a different scale, there was greater replication in the survey, survey shrubs included a greater range of sizes, and non-*Sextius* SEPHs remained on survey shrubs. In this way, while the effects of *Sextius* level were tested in the relatively controlled conditions of the main field experiment, the strength of effects of *Sextius* and other SEPHs amongst a greater degree of this high natural variation was also examined in the field survey, providing a much broader ecological context to the questions investigated.

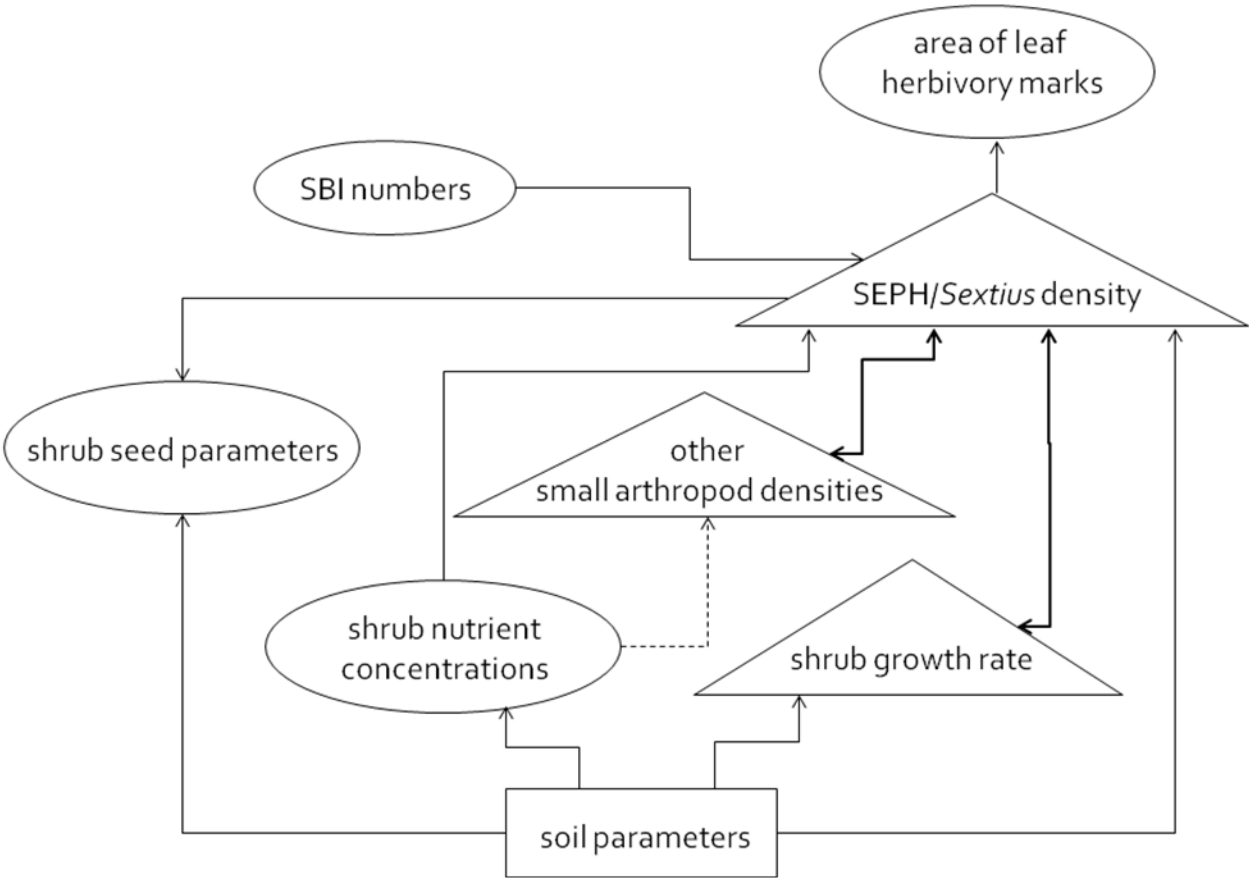


Figure 26: Interactions of variables that have been considered in this study

This study has found that distribution of SEPHs associated with SBIs on shrubs is influenced by soil parameters, leaf nutrient concentrations, shrub growth rate, and SBI presence. On experiment shrubs, high *Sextius* levels occurred on soils with relatively lower electric conductivity and sulphur concentration. However, survey sub-site means of SEPH/*Sextius* densities were negatively correlated with mostly different soil parameters from those in the experiment. As alluded to, survey soil samples were obtained from a larger area over more sites and included different slope-aspects, representing a larger sampling scale.

Interestingly, at both scales, high soil sulphur concentration, directly or indirectly, may have been limiting to SEPHs/*Sextius*. This result could point to the importance for *Sextius* of soil sulphur, which was important in both experiment and survey. Soil sulphur and electrical conductivity both correlated positively with leaf concentrations of magnesium, and soil conductivity also correlated positively with leaf iron and negatively with zinc. Concentrations of these nutrients might influence *Sextius* densities, probably indirectly through plant defence or through physiological processes such as sap flow. Concentrations of some other leaf nutrients were different for different *Sextius* levels, which might have been related to intrinsic characteristics of the shrub or to a suite of soil parameters. Importantly, SEPH/*Sextius* density is constrained by shrub growth, which appears to support the PVH. The density of SEPHs/*Sextius* associated with a particular shrub growth rate is probably regulated by soil nutrients and densities of other arthropods as described in the paragraph below. Effect of shrub growth on SEPHs/*Sextius* appeared to be stronger than the opposite effect of SEPH density on shrub growth. In addition, SBI populations are of crucial importance to the *Sextius* sp. association, because SBI access to stems appears to be required for adult *Sextius* sp. populations to be present on *A. victoriae*, and nymph populations largely disappeared in the absence of SBIs. This shows that variation in both shrub and SBI partners of SEPHs can create variation in SEPH populations.

When comparing shrubs of low and high *Sextius* densities, there are differences in levels of folivory, shrub growth rate, reproductive success of shrubs, and arthropod assemblages. Shrubs with naturally higher *Sextius* densities grow faster, but the extent to which this pattern is influenced by populations of *Sextius* on shrubs that are already growing faster was difficult to determine. However, removal of *Sextius* from shrub stems with Tangle-Trap reduced shrub stem growth rate. There may be a two-way interaction between shrub growth and *Sextius* density, or unmeasured factors may link them. Low-*Sextius* shrub leaves have higher rates of herbivory, suggesting that the mechanism of higher growth of high-*Sextius* shrubs or stems might be through reduced non-SEPH herbivory rates. However, plant chemistry might also influence differences in general herbivory across natural *Sextius* densities. Shrub reproductive output on experiment and survey sites was much higher for shrubs with high SEPH/*Sextius* densities primarily because of lower seed destruction rates by a weevil larva found inside developing seeds. Level of seed consumption was strongly positively correlated with soil sulphur concentration and other soil parameters, probably through changes to plant physiology, as discussed above with respect to leaf nutrient concentrations. Soil parameters were important to other small non-SEPH herbivore taxa, higher densities of which occurred with higher soil conductivity, pH, total nitrogen, sulphur, organic carbon, or lower potassium. However, competition between non-SEPH herbivorous taxa on shrubs was not detected. Densities of parasitoid wasps and predators correlated positively with densities of these other insects, probably as they share a relationship that is density dependent. By contrast, there was a complex non-linear relationship between parasitoid wasps and SEPHs where each might limit densities of the other. This might be driven by a combination of density-dependent SBI deterrence of some arthropods, delayed density-dependent parasitisation of SEPH eggs, and that not all parasitoids used SEPHs as hosts, likely in combination with competition release for other phloem-feeders. This hypothesis relies on density dependent capability of SBIs to deter insects from twigs, which was observed in the chrysomelid trials under partially controlled conditions, but it is not known which insects are susceptible or under which conditions it occurs.

Little evidence of SBI protection of *Sextius* from predation was found during the study, even though some evidence was found for deterrence of small arthropods by SBIs.  This could easily have been because the predators that are susceptible to SBIs were not found during the predation experiment.  Some evidence for SBI prevention of *Sextius* nymph "drowning" (in accumulated honeydew) and fungal growth were observed but were not detected as significant to nymph survival, which could have been due to low replication of the relevant experiments.

## SEPH/*Sextius* sp. occurrence on *A. victoriae*

### Soil effects on shrub nutrient concentrations and on SEPH densities

Neither nitrogen, which is often the limiting nutrient for herbivores and for sap-suckers in particular (Mattson 1980), nor phosphorous, which can be a limiting nutrient for arthropods (Elser et al 2000), were limiting for *Sextius* during a year of high rainfall.  During times of lower rainfall, the shrubs might undergo intermittent stress, which elevates nitrogen levels that favour sap-suckers (Huberty and Denno 2004), but this was not tested.  Since higher *A. victoriae* leaf phosphorous did not correlate with soil phosphorous, but was strongly preferred by *Sextius*, the level of mycorrhizal activity might influence the shrub-*Sextius* association and might be affected by other soil parameters such as E.C. or sulphur, which was also not tested.  In arid areas, soil nitrogen and phosphorous concentrations due to active soil microbes can be high enough such that plant growth is not limited, and the high moisture levels in particular months and in the second year may have facilitated this. Also, acacias are usually fixers of nitrogen. Other soil nutrients might have been limiting during this study, particularly for arthropods that are not very mobile and do not disperse easily between shrubs to find better resources. The most important soil parameter for *Sextius* could be either conductivity or sulphur, since both together appear to limit *Sextius* density on individual shrubs at high concentrations. Also, both soil parameters have positive regression relationships with leaf magnesium, of which high *Sextius* density required low levels. Additionally, soil conductivity showed a positive regression relationship with leaf iron, which was lower with high *Sextius* density, and a negative regression relationship with zinc, which was higher with high *Sextius* density. It is not generally known which nutrient concentrations are favoured by particular phloem-feeders, but magnesium is required for production of chlorophyll and may be limiting, which could affect shrub growth and sugar levels in sap. However, phloem-feeders can induces changes to nutritional content of sap (Nowak and Komor 2010), so determination of causation requires manipulative experiments. Soil and leaf nutrients pinpointed here can provide starting points for further study to understand relationships among them with *Sextius* densities.

At the larger spatial scale of survey sub-sites, higher mean *Sextius* densities occurred with lower soil concentrations of sulphur and organic carbon. In the case of total SEPHs, higher densities occurred with lower concentrations of these nutrients and also total nitrogen. Although the higher number of total SEPHs may have enabled detection of the weak pattern with nitrogen whereas it was not apparent with *Sextius*, it might reflect different ecological dynamics of different SEPH species. Since all these soil parameters are strongly correlated with each other and with conductivity, it is possible that only one nutrient is limiting to *Sextius*. In this study, potassium is the only soil parameter correlated with shrub growth (for means of survey sub-sites). However, SEPHs/*Sextius* were not correlated with potassium as would be expected, although densities of other phloem-feeders (particularly psyllids) were, so relationships between insects and soil in this study were apparently not confounded by any effects of higher growth rate on insects. The positive regressions of leaf magnesium and boron with soil organic carbon might be important, since higher *Sextius* densities occurred with lower quantities of organic carbon in the survey. Leaf magnesium seems to be more likely to be important since data for experiment shrubs also reflected this. Organic carbon in soil is the result of deposition by the plant community (and death of soil organisms or other organisms), so this relationship could reflect vegetation history. Both organic carbon and sulphur were positively correlated with shrub volume. Therefore, the strong positive correlation of soil organic carbon with total nitrogen may be from both being linked to higher overall plant biomass, not just shrub biomass, where abundant invasives such as *C. annua* and *A. fistulosis* probably use top-soil nitrogen quickly. The difference between experiment and survey shrub relationships with soil parameters and SEPH densities may reflect greater environmental variation in the survey sites and larger scale on which samples were taken (as discussed above).

This study has found a link between soil characteristics and densities of SEPH/*Sextius*, which might partially explain SEPH distribution with respect to environmental variables, to produce testable hypotheses. Effects of soil on leaf nutrient concentrations probably affect spatial distribution of *Sextius* since different *Sextius* levels occur with different levels of particular leaf nutrients and with different soil matrix parameter levels. Correlations of *A. victoriae* leaf nutrient concentrations with soil parameters are weak, but they confirm that soil parameters in the matrix outside the zone of influence of shrub roots can affect shrub dynamics. Importantly, in this system parameters of soil from two canopy diameters away from a shrub also correlate with some parameters of soil directly under the canopy of shrubs, which has probably been altered by shrub presence (data not presented), confirming the link between them. Total nitrogen in soil on the south side of the canopy was particularly strongly correlated with total soil nitrogen in the matrix outside the zone of influence of shrub roots, and potassium and pH under both the north and south sides were correlated moderately strongly with the same parameter in the soil matrix (data not presented). In the Sonoran Desert in North America, mesquite leaf P:C ratio was weakly positively correlated to soil phosphorous concentration, but it fluctuated from month to month, and it was also weakly positively correlated to abundance of a non-dispersing weevil consuming it (Schade et al 2003). The current study provides another example of patterns of soil parameters linking to insect distributions through plant nutrient concentrations. These results suggest that the link between soil and *Sextius* can be mainly through soil parameter influence on leaf trace elements rather than on macro-nutrients.

### SEPH/*Sextius* occurrence on shrubs with higher growth rate

Higher shrub growth rate was associated with higher maximum SEPH/*Sextius* densities on survey shrubs. Similarly, *Sextius* density was positively correlated with shrub growth rate on experiment shrubs. Survey results indicate that shrub growth rate, or more likely fitness factors linked to it, pose a limit to SEPH/*Sextius* densities. The similarity of results for total SEPHs and *Sextius* indicates that there is little difference in the relationship of *Sextius* and non-*Sextius* SEPHs with shrub growth. When including data for all shrubs regardless of whether they changed in SEPH density between years, SEPH numbers might be more strongly influenced by reproductive success of SEPHs already on shrubs in the previous season, rather than by dispersal to preferred shrub individuals. Any increases in shrub growth which might be due to SEPHs could confound the relationship. However, when using data only from shrubs on which SEPH levels changed from the beginning to the end of the year when the growth was measured, the error in the relationship was similar to when using all shrubs, relative to the reduced replication. On these shrubs, higher growth rate would not be caused by higher previous SEPH density. Also, the similar slopes of the shrub growth-SEPH density regressions for both shrub datasets indicate a similar influence of growth rate on SEPHs for both groups. Therefore, the positive regression relationship of growth with SEPH density is probably not increased by pre-existing high SEPH density. This tendency for higher SEPH densities on shrubs that have grown faster would be due to host preference during dispersal and perhaps partly to greater reproductive rates on faster growers. These results support the PVH.

Consistent with results of the current study, a meta-analysis found that sap-suckers prefer faster growing plants more than do all other taxa studied, and this tends to also cause higher survivorship for insects living on stems and leaves (Cornelissen et al 2008). The reason for sap-sucker preference for faster growth rate is not known. Softness of new growth tissue may substantially reduce energy required for oviposition and new growth may enclose eggs more rapidly. In the current study, *Sextius* populations are low from autumn until emergence in spring, so most dispersal and oviposition must occur in late summer when adult *Sextius* populations are large while shrubs are growing very little and have few new shoots. This means *Sextius* can not generally be dispersing more to hosts that are already growing faster due to oviposition into the new softer shoots requiring less energy. Abscission rates can be lower on longer shoots, perhaps due to greater shrub fitness (Cornelissen et al 2008), and this might be particularly important in arid environments where abscission due to unpredictable moisture levels is common. *Sextius* probably takes around 40-50 days to develop to maturity from emergence if it is similar to other membracids (Linares et al 2010). Growth might correlate with other shrub characteristics such as particular aspects of plant chemistry (although it did not correlate with most soil nutrient concentrations) and this may be why higher growth rate is preferred by sap-suckers. Plant chemistry related to herbivore induced plant volatiles (HIPVs) may play a part since there can be a trade-off between growth and production of plant defense chemicals generally (Orians and Milwewski 2007). Increased nutrient concentrations in new growth tissue and higher osmotic potential are also potential reasons for herbivores to choose fast growing plants (Cornelissen et al 2008). These factors could be linked to performance/preference of SEPHs/*Sextius*. Whether higher occurrence of SEPHs/*Sextius* on faster growing shrubs results in higher survival and reproduction of SEPHs/*Sextius* is not known. More investigation isolating individual factors is needed to understand reasons for SEPH/*Sextius* preference for faster growers and the link between this and SEPH/*Sextius* performance.

## *Sextius* phenology and effects of the tritrophic association

Timing of SEPH/*Sextius* dispersal, oviposition, emergence, and development influence length of time for which a particular SEPH/*Sextius* population can affect a shrub, and the time over which environmental conditions and other taxa can potentially have an impact on the tritrophic association being studied. There were few *Sextius* nymphs on Tangle-Trap stems in the spring following Tangle-Trap application, so little oviposition would have occurred before Tangle-Trap application in mid-January 2010. During mid-January to mid-February some shrubs showed moderate increases and some substantial increases or decreases in *Sextius* density (pers. obs.), which indicates dispersal events had occurred. Emergence of *S. virescens*, a closely related species in mesic south-eastern Australia, occurred through spring to early autumn but was concentrated to late spring and early summer (Cookson and New 1980, Buckley 1983). Most emergence of *Sextius* sp. in the current study occurred in spring, and might differ according to local weather and perhaps climate. *Sextius* emerges around the same time as *A. victoriae* growth peaks and soon before shrubs flower, and both occur earlier in warmer years. *Sextius* populations appear to be relatively stable from the emergence period, during which large populations of juveniles cannot disperse, approximately to the end of the peak growth phase of shrubs in mid-summer. This “flush phase” is likely to be when the highest arthropod activity occurs on shrubs (Palmer 2010) and the time when SBI deterrent effects on herbivores would most affect shrub growth. Total benefits and costs to shrubs would depend on timing of emergence of local insects (and their susceptibility to SBI deterrence) and shrub growth in the particular year. The highly variable activity of arid zone insects is strongly dependent on both temperature and rainfall (Palmer 2010), so both would affect the length of time over which effects of the association on herbivory take place and their strength of effects. This could affect the outcome of the tritrophic association from year to year, either qualitatively or quantitatively, by changing the balance of its costs and benefits for shrubs.

### SBI presence and *Sextius* densities

Numbers of attending ants can be limiting for membracids (Cushman and Whitham 1991). However, on the sites studied here, numbers of foraging SBIs in trails did not appear to limit *Sextius*. There were always SBI trails near shrubs that were connected to the entire SBI colony and there were relatively large numbers of SBIs in all survey pitfall traps. Moreover, all adult *Sextius* quickly dispersed from experiment stems after Tangle-Trap application prevented SBI attendance. Clearly, *Sextius* has strong preference for shrub stems with SBIs present, and numbers of SBIs might limit numbers of *Sextius* in some ecosystems, particularly where SBI numbers are very low. Strong preference of *Sextius* for SBI presence seems to contrast with gray scale insects, which seemed to be present on stems to which SBI shad access. If so, the relationship between SBIs and different SEPHs may be significantly different, particularly with respect to their relative effects on each others population dynamics.

## SBI-SEPH/*Sextius* effects on Shrubs

### *Sextius* effects on growth and seed production

There is a strong tendency for shrubs with naturally high *Sextius* densities to grow substantially more in the following growth period than those with naturally low *Sextius* densities, which the experiment revealed. The lack of correlation of shrub growth and pre-existing SEPH/*Sextius* density for shrubs on survey sub-sites may be due to presence of other SEPHs that might have different effects on shrubs, higher variation in soil affecting both variables separately, the non-SEPH arthropod community, microclimate effects such as slope-aspect and shade, and other microsite conditions. Also, the shrub stems with naturally high *Sextius* densities which ants were prevented from accessing via Tangle-Trap, resulting in mature *Sextius* dispersing, grew substantially less until the end of the following peak growth period than stems on the same shrub where SBIs and *Sextius* remained. Both these results point to positive effects of *Sextius* density on shrub growth, but there might be different reasons for each. Indeed, without isolating the effect of growth rate on *Sextius* density, it is difficult to determine the effect of *Sextius* density on growth rate. Replication of shrubs for which *Sextius* level changed between years was insufficient to isolate effects of Tangle-Trap on shrub growth without potential bias from pre-existing *Sextius* levels. Growth may be higher on shrubs with high SEPH/*Sextius* densities because they already tend to have a pre-existing fast growth rate. Growth rate of experiment shrubs whose *Sextius* density changed between low and high in successive years was not related to *Sextius* density at the beginning of the growth period. Correlation of *Sextius* density of experiment shrubs in different years was higher than on survey shrubs, possibly due to reduced variation in the experiment compared to the survey. The lower variation may have created more similarity among experiment shrubs than in survey shrubs in relationships between growth rate and both pre-existing and resulting *Sextius* density. Therefore, after growth rate partially determines SEPH/*Sextius* density through host preference in one year, it might then continue at a similar rate so that SEPHs/*Sextius* maintains similar densities on the shrub in the following year.

SEPH/*Sextius* selection of shrubs in response to plant chemistry or changes in the arthropod assemblage which affect growth may be driving the relationship between their density and shrub growth. The effect of a particular SEPH/*Sextius* density on shrub growth would be likely to depend on phenotypic characteristics of the individual shrub, so there may be an optimal SEPH/*Sextius* density above which shrub growth starts to decrease and this density may be quite different for each shrub. Once SEPH/*Sextius* density becomes too high, not only might growth decrease from stress, but stress might induce changes to plant chemistry which repel SEPHs/*Sextius*. There may be a temporary trade-off of this plant chemistry with growth rate. Even for shrubs with low SEPH/*Sextius* density and low growth rate, growth rate would suddenly increase in response to increased nutrient uptake due to increased moisture levels, as occurred between years in this study, or possibly due to some unknown factor. As to the effect of Tangle-Trap, even if growth rate is not increased by higher *Sextius* density, for shrubs with naturally high *Sextius* densities, preventing SBIs from accessing a stem might allow more non-SEPH herbivory damage which explains the reduction to the naturally high growth rate. The insignificantly higher growth rate of high-*Sextius* Tangle-Trap stems than both control and Tangle-Trap low-*Sextius* shrub stems was consistent with this explanation. In any case, the effect of shrub growth on SEPH/*Sextius* density was stronger than the positive effect of SEPH/*Sextius* density on shrub growth, since the relationships found for the former were stronger.

Photosynthate losses due to non-SEPH herbivory damage might be substantially higher than losses to SEPHs which induce protection from other herbivores. In addition to reduction or reallocation of photosynthate which might otherwise be used for growth, the main cost of SEPHs/*Sextius* on a shrub would possibly be damage to phloem cells, which depends on how the phloem-feeder species accesses phloem with the stylet (Walling 2000). However, phloem cell herbivory damage can significantly decrease photosynthesis (Nabity et al 2009). Carbon lost to sap-suckers in a mesic system without attendant ants represented 4% of gross annual photosynthesis and the sap-suckers may have increased photosynthetic rate (Dungan et al 2007). Other sap-suckers such as cicadellids may damage phloem cells more than SEPHs. Therefore, benefits of reduced non-SEPH herbivory could outweigh costs of carbon lost to sap-suckers, not only because of reduced costs of leaf edge and phloem cell repair and replacement of plant biomass, but because reduced photosynthesis due to non-SEPH herbivory damage could outweigh costs of photosynthate and small quantities of nutrients lost to SEPHs. Different types of herbivory have different effects on plant responses and fitness (Strauss and Agrawal 1999), so the balance of costs and benefits might depend on types of herbivory. Therefore, herbivores of different guilds on different shrubs might potentially bring about different SBI-SEPH association outcomes.

Seed productivity is another measure of plant fitness. With regard to abiotic influences on reproductive success of shrubs, there were moderately strong regression relationships of some soil parameters with most seed parameters. These reflect soil influences on seed development and viability, although they could be artefacts of auto-correlations and interactions such as that between soil sulphur and SEPH density. Negative regression relationships of number of initiated and viable seeds per pod with the same soil parameters which are all auto-correlated, reflect influence of at least one soil parameter on seed production. Positive correlation of number of seeds eaten per pod and strong correlation with seeds eaten per initiated seeds per pod with soil parameters might indicate that sulphur was limiting for the seed weevils which were responsible for the damage to large numbers of seeds. Although the lower rate of seed destruction on shrubs with high SEPH densities and the negative correlation of SEPH densities with sulphur suggest correlations between soil sulphur and seed parameters might be an artefact, the stronger correlation of sulphur with seed destruction than with SEPH density suggests that this is not the case. However, the much stronger relationship of sulphur with number of seeds eaten per initiated than with number of viable seeds per initiated suggests there are factors linked to soil other than seed weevil consumption affecting seed viability. This points to soil parameters contributing to reproductive success of the shrub, as would be expected.

The proportion of initiated seeds eaten was much higher on low- SEPH/*Sextius* shrubs than on high- SEPH/*Sextius* shrubs, indicating that reproductive success (fitness) of shrubs was substantially higher on shrubs with high SEPH/*Sextius* densities. The survey results indicate that the much larger number of viable seeds per pod on high- SEPH/*Sextius* shrubs was due to reduced seed damage by a seed-feeding weevil. The cause may have been that attendant SBIs deterred the weevil since high- SEPH/*Sextius* survey shrubs also had lower numbers of "other" herbivores, but it may also be due to other indirect effects such as induction of HIPV emissions. The SEPH/*Sextius* association with shrubs may have affected seed development from initiation to maturity because seed development started after *Sextius* had emerged. Therefore, the greater seed initiation of high- SEPH/*Sextius* shrub seed pods may be due to better conditions for seed set, either because of the association or because of intrinsic factors such as soil and plant nutrient levels which SEPH/*Sextius* may have preferred or which affected abundances of herbivores on the shrub. The strongest effect on fitness would be if seed viability influenced shrub population density. However, seed loss can have little effect on plant abundance if plant populations are more limited by availability of safe sites than seeds (Maron and Crone 2006). This is likely to be the case in dense populations of *A. victoriae* with large seed banks and abundant ant and avian seed harvesters.

### Soil effects on non-SEPH small arthropods

The weevil-mesquite study mentioned above demonstrates that spatial variation in soil parameters can be linked to spatial distribution of herbivore arthropods on individual plants. It shows that this can be mediated by moisture levels (Schade et al 2003), which can vary both spatially and temporally and particularly so in arid systems. Manipulation of soil nitrogen and phosphorous lead to elevated concentration of nutrients in the host plant and in two different species of phloem-feeding plant-hoppers feeding on it which showed two different responses to nutrient deficiency: compensatory feeding and dispersal to better quality resources (Huberty and Denno 2006). These are examples of bottom-up effects of soil nutrients on arthropod community structure through soil effects on plant nutrient content. In the current study, there was moderately to strongly positive regression of soil parameters with "minute" wasps, predators, thrips, mirid/lygaeids, "other" Auchenorrhyncha, and "other" herbivores, suggesting some degree of indirect bottom-up regulation of the association from soil through the shrubs and the degree to which this occurs is likely to depend on rainfall. Moreover, these soil effects on different arthropods might affect the SEPH/*Sextius*-SBI association by altering the balance of processes such as competition, apparent competition, predation, and parasitism between species.

Insects tend to prefer resources of higher quality, so regression relationships for arthropod taxon densities with soil parameters are likely to represent preference or performance proportional to shrub nutritional value. Predator and parasitoid populations in general tend to be larger on shrubs on which herbivore populations are larger because of higher nutrient concentrations, through density dependent effects. Also, predators and parasitoids might perform better when their prey and hosts have sufficient concentrations of a potentially limiting nutrient. Total nitrogen may be the most important soil parameter for arthropod assemblages as a whole, and in the current study this may also be true because it has a positive regression relationship with densities of "minute" wasps. These parasitoids probably have a strong influence on SEPH population dynamics since some are probably egg parasitoids of SEPHs (and possibly their predators), so soil nitrogen concentrations might have important effects on the tritrophic interaction through these wasps. Moreover, total soil nitrogen also showed a regression relationship with combined non-wasp non-SEPH arthropod densities, with SEPHs, and with all the auto-correlated soil parameters that are also correlated with arthropod densities. This suggests that total soil nitrogen is important to arthropods in this system. Aside from nitrogen, positive regression of arthropod densities with organic carbon concentration might mean some herbivores prefer shrubs that are larger or that have a higher photosynthetic rate. This tendency might be influenced by suites of other soil parameters that correlate with carbon. Effects of at least one of these soil nutrients are probably important to arthropod densities and further study should ascertain this.

Semi-arid systems have high spatial and temporal environmental variation that makes adequately recording the full range of conditions relevant to the association difficult, since studies may not be of sufficient length. Indeed, this is a common short-coming of data obtained by many field-based studies. Likely effects of soil parameters on some arthropods have been found in the current study. Experiment shrubs on average have lower mean soil phosphorous, potassium, and pH and higher sulphur and conductivity than shrubs from survey sub-sites, which might explain other differences between experiment and survey. Theory predicts that growth of herbivores can be limited by more than one nutrient simultaneously (Sperfeld et al 2012). Also, the nutrient limiting an arthropod, and the time of year this occurs, could change among years. High rainfall over the period of the study allowing shrubs to obtain more nutrients from soil than during previous dry periods could have given nutrients a stronger effect on successive trophic levels or released them from limitation, although time lags are possible. This may have also enhanced beneficial mycorrhizal and rhizobial interactions, so that nitrogen and phosphorous are not limiting. If so, these effects may be substantially reduced or absent in dry years. Clearly, manipulative experiments are needed to find which soil parameters, including moisture levels, affect particular insect densities, the relative degree of effect, and how these effects interact for different nutrients and insects.

### SEPH/*Sextius* density in interaction with folivory and densities of other small arthropods

More folivory damage in the form of both TAD and LAL occurred on the large majority of leaves of experiment low-*Sextius* shrubs than on high- *Sextius* shrubs. There was also a different distribution of amounts of herbivory marks for leaves on shrubs of the two *Sextius* levels. Leaf-miners produced more damage on low-*Sextius* shrubs than high-*Sextius* for all damage level categories. High levels of coccoid damage are less frequent and an absence of them more frequent on high-*Sextius* shrubs, suggesting that any SBI consumption of SEs these Sternorrhyncha produce is not a signficant influence on herbivory levels. There was no difference in galling between *Sextius* levels. Gall-makers are well protected and likely to not be susceptible to SBIs at most life-stages and they may occur at a different time of the year from *Sextius*. Coccoids are sessile for much of their life cycle and would not venture onto traps; gall-makers and leaf miners were rarely encountered. This made determining meaningful patterns of herbivory by individual taxonomic or functional groups difficult. Here, it was not possible to identify to a high taxonomic level most types of herbivores responsible for various types of damage, sticky traps generally only catch small taxa, and folivory marks represent accumulated damage, depending on edge repair or abscission rates of leaves. For these reasons, herbivore marks could not be linked to specific groups of small arthropods on sticky traps. Interestingly, since gall-makers, miners, and chewers tend to prefer faster growing plants (Cornelissen et al 2008) as sap-suckers do, these might be expected to show some preference for high-*Sextius* shrubs, but this was not seen. Damage by insects that occur less on high-*Sextius* than low-*Sextius* shrubs might indicate deterrence of the responsible insects by SBIs or by plant chemistry.

For the majority of leaves for which there were differences in LAL and TAD between *Sextius* levels, area of leaf damaged is only up to 10%. Most damage occurred in small points scattered across the leaf rather than in few large spots (pers. obs.). Insect herbivory removes substantial amounts of plant biomass, but total costs to the plant may be far greater than the biomass removed, due to reduced photosynthesis in the remaining tissue surrounding the damage (Nabity et al 2009). Three quarters of the reduction in photosynthesis caused by caterpillar consumption of leaves of wild parsnip (*Pastinaca sativa*) occurred in remaining leaf tissue, the area of which was six times as large as the area lost, and which was impaired for at least three days after the loss (Zangerl et a 2002). Also, the total effect of herbivory damage on a plant depends on the type of damage and the mode of defence employed by the plant (Nabity et al 2009) and nothing is known of the latter on *A. victoriae*. This means total folivory damage could be substantial enough to affect allocation of resources to functions such as defense compounds or phloem sap, affecting how benefits to shrubs balance with costs of SEPHs.

Since numbers of lycaenid caterpillars (which are also SEPHs attended by SBIs) did not correlate with *Sextius* numbers, effects of caterpillar folivory that occurred on shrubs before *Sextius* were removed from experiment shrubs do not need to be taken into consideration with respect to *Sextius* levels on shrubs. As mentioned, non-*Sextius* SEPHs are likely to have different population dynamics from *Sextius* and may substantially increase folivory on shrubs where they were not removed, such as those on survey sites. This may have had a substantial effect on shrub growth and might have contributed large amounts of noise in terms of confounding the correlation of pre-existing SEPH/*Sextius* density with shrub growth. However, lycaenids did not constitute a large proportion of SEPHs on survey shrubs, which would have limited their effects.

In the behavioural trials of the chrysomelid with SBIs, SBIs successfully deterred the beetles. Interestingly, distance of SBIs from *Sextius* produced different SBI aggression levels and corresponding rates of deterrence. SEPHs bring more SBIs onto the shrubs where they can deter some arthropods, including herbivores of the shrub. When further from *Sextius* on shrubs where *Sextius* is present, some SBIs are more likely to be transporting honeydew back to the nest as efficiently as possible. This might explain the different SBI deterrence levels, which vary with *Sextius* density. SBIs might react similarly with other SEPH species since the benefits they confer may be similar.

Given that SBI deterrence of small arthropods can occur, the patterns of folivory marks suggest that SBIs may reduce the time that small herbivorous arthropods spend damaging shrubs, possibly through herbivores spending less time on shrubs, reducing their densities. However, small (<1%) proportions of total leaf area damaged or removed by herbivores were more common on high-*Sextius* shrubs, whereas larger proportions of leaf area damage were much more common on low-*Sextius* shrubs. This suggests that reduced folivory may not have been only through reduced numbers of herbivores on shrubs. It is likely that larger numbers of SBIs interrupt herbivore feeding such that insects spend more time searching for safe sites on the same shrub and less time at each feeding site or less time damaging shrubs with many SBIs. It is easy for SBIs to venture onto leaves since their activity rates are very high relative to other ants, but also some herbivores which SBIs might deter, such as cicadellids, feed on branches and twigs, where SBIs spend most time. SBIs may have a deterrent effect on most areas of the shrub simply because they can potentially venture onto leaves where other herbivores may feed. In this case, they are likely to alter arthropod assemblages on shrubs relative to SEPH density since flying insects can quickly disperse between different plants.

During peak activity in summer 2009, assemblages of small arthropods caught on sticky traps on shrubs with high SEPH/*Sextius* densities were different from those on shrubs with low SEPH/*Sextius* densities. For herbivores susceptible to SBI attack, the lack of a detectable effect of Tangle-Trap on most insect taxa in 2011 February could have been due to the generally very low insect activity at the time. However, SBI presence on shrubs may have an important effect only when activity level of small arthropods is higher, making interactions between them more frequent. Also, if the shrub-*Sextius*-SBI association strongly affects "other" herbivores such as chrysomelid beetles, seed weevils, and predators, lack of detection of effects may have been due to small numbers of these taxa on traps, reducing the statistical power of the analysis. Commonly observed herbivores such as shield bugs (Pentatomidae), alydids, harlequin bugs (Pyrrhocoridae), and crusader bugs (Coreidae) were absent on sticky traps (pers. obs.), probably because of their larger size, and some of these can be deterred by SBIs (pers. obs.). Almost no nymphs or larvae were present on sticky traps. Non-SEPH herbivory probably has most impact on shrubs in spring, due to peak levels of shrub growth and the insects that feed on the new growth. Most non-SEPH herbivory may be produced by nymphs and larvae (particularly for Lepidoptera), at least some of which SBIs attack. Emergence of these insects is likely to be unpredictable, varying with weather and other variables such as biotic interactions. Therefore the very different insect assemblages present at one particular time of the year or during the flush of new shrub growth make experimental detection of ecological interactions more difficult. This illustrates the importance of ecological studies that run over sufficient time to encompass variability in local weather conditions. Any arthropods which are susceptible to SBI deterrence, which might be responsible for a large proportion of non-SEPH shrub herbivory or predation on *Sextius*, and which might be present at the same time as *Sextius* nymphs, might not be caught by sticky traps, particularly if they are unable to fly. Nevertheless, the chrysomelid behavioural trials provide evidence that SBIs can deter at least some small arthropods, whether *Sextius* is present or not.

On experiment shrubs, the positive relationship between total abundances of "minute" wasps and *Sextius* density was the biggest detected difference in arthropod communities. This was true for differences both between naturally occurring high- and low-*Sextius* densities and between Tangle-Trap treatment and control stems on high *Sextius* shrubs. "Minute" wasps represented total abundances of wasp parasitoids of eggs and thrips larvae, which were in very high densities relative to other taxa, and their continued presence on shrubs would depend on densities of suitable hosts (mostly eggs and thrips larvae) to parasitize. Host-parasitoid relationships are poorly defined among the fauna in this ecosystem, so interactions cannot be predicted for these data. There may be regression relationships between particular subsets of "minute" wasps and other taxa which are not detectable due to the taxonomic level of classification. Also, some parasitoid wasps can lay multiple eggs in one host egg or larva, altering populations dynamics. Therefore it is impossible to understand ecological interactions with these parasitoids in detail. Although in the survey the dipped envelope relationship of "minute" wasps with SEPHs/*Sextius* seems to contradict experiment findings, low numbers of "minute" wasps were more common in the presence of very low SEPH/*Sextius* densities. Also, for average densities of low and high *Sextius* on experiment shrubs and the same densities of SEPHs/*Sextius* on survey shrubs, a similar average density of "minute" wasps occurred in both experiment and survey. Perhaps high numbers of these "minute" wasps found on some shrubs with very low SEPH/*Sextius* density in the survey, were caused by greater numbers of other taxa that also provide hosts for them.

It is possible that the most important negative effect on SEPH/*Sextius* abundance occurs at the egg stage, through parasitoids which SBIs might deter as described in the above paragraph. Potential parasitoids of *Sextius* eggs include wasps from the families Mymaridae and Trichogrammatidae which probably oviposit into eggs before winter. Ants prey on some hyperparasitoids (Delabie 2001) and small ants of only a few millimetres in length protect lycaenid larvae from parasitoids at a rate that might regulate mutualistic outcomes (Pierce and Mead 1981). In South African vineyards, parasitoids introduced as biological control agents for hemiptera are substantially reduced in efficacy when ants foraging for the hemipteran SE are present (Mgocheki and Addison 2010). Substantial rates of egg parasitism were found in a mesic system with similar species where 39% of eggs of *S. virescens* on *A. decurrens* had been parasitized (Cookson and New 1980), so parasitoids can have strong effects in such systems. SBIs may be effective in protecting SEPHs/*Sextius* in this way, since there is a high abundance of these wasps on the sampled shrubs. Since it is not possible to identify particular parasitoid-host wasp relationships or to identify the taxonomic level of insects, if these species occurred on sticky traps, specific patterns would most likely have been masked. However, temporal synchronicity between parasitoids and their prey is crucial for parasitoids to regulate their numbers, so in the highly variable Australian arid environment, levels of SBI protection from from parasitoids that is required of adult SEPHs/*Sextius* might vary substantially between years.

All correlations between non-SEPH herbivores were positive, so competition between them did not appear to be a substantial process regulating their populations, at least not in years of average or higher rainfall. Very heavy rainfall in November 2009 near the beginning of the peak growth period of *A. victoriae* would have contributed to an abundance of resources and reduced competition. These correlations probably reflect increases in herbivore insect densities with increases in shrub resource quality. Explanations for specific patterns are not known since little is known of the taxa, so only speculation is possible as to the biological causes. On the next trophic level, the positive correlation of total abundances of "minute" wasps versus total abundances of all other arthropods summed, along with numerous positive correlations of "minute" wasps with individual taxa, point to density dependence on hosts. Since summed phloem-feeders correlate almost as strongly as all herbivores with "minute" wasps (more strongly than thrips do) and both were abundant, these phloem-feeders might be the most important host taxon for "minute" wasps. However, thrips densities form a strong envelope for "minute" wasps, so thrips are probably also important to total abundance of "minute" wasps.  These parasitoids tend to be common on Australian foliage.

There are three likely non-mutually exclusive explanations for the patterns found for arthropod assemblages on shrubs of different SEPH/*Sextius* density.  First of all, shrub nutrient concentrations, influenced by soil parameters, probably contribute to SEPH/*Sextius* density and to other herbivore populations.  Second, non-SEPH herbivore populations generally have a positive relationship with parasitoid populations, with which SEPH/*Sextius* density had a quadratic relationship.  The reason these two relationships are stronger than that of other herbivores with SEPH/*Sextius* must be because parasitoid densities depend on densities of SEPHs and other herbivores.  Third, although SEPH density probably both facilitates and is regulated by parasitoid populations, SBIs attending SEPHs may regulate parasitoid populations through deterring foraging parasitoids.  The two mechanisms that may be responsible for the weak negative correlation between SEPH/*Sextius* densities and densities of other herbivores are resource competition, including changes to plant chemistry, and apparent competition regulated partly by egg parasitoids.

The quadratic relationship of densities of SEPHs/*Sextius* with abundances of "minute" wasps occurring two months after SEPHs were counted may reflect a combination of effects.  Higher plant nutritional quality appears to increase densities of all herbivores including SEPHs, and as a result, there are higher densities of "minute" wasps and, probably, predators.  In particular, it is likely that large numbers of relatively sessile SEPHs on a shrub mean there are large numbers of parasitoids associated with them.

Alternatively, HIPVs which attract parasitoids might be emitted in response to high densities of either SEPHs or folivores. There can be different emissions depending on which insects have attacked the plant and the type and amount of damage (Dicke 2009). Phloem-feeders trigger release of HIPVs which attract particular predators and parasitoids of the phloem-feeders (Gosset et al 2009), and furthermore, HIPV emissions can be density dependent. At low SEPH densities, different dynamics might regulate "minute" wasp numbers than at high SEPH densities. For example, facilitated by reductions in competition or SBI deterrence, there may be higher densities of other phloem-feeders with eggs of similar size to those of SEPHs that the same parasitoids could exploit. Alternatively, since number of SBIs on a shrub was relatied to number of SEPHs (chapter 3), SEPH densities might produce a density-dependent inverted-hump shaped relationship with "minute" wasps. There might be a combination of such effects which augments the variation in insect densities depending on the relative strength of each effect, but without knowledge of host-parasitoid relationships or at least knowledge of densities to a lower taxonomic level, these dynamics cannot be confirmed.

Ants can deter parasitoid wasps of phloem-feeders they attend (Völkl 1992), but their behaviour can differ with wasp species. Looking at figure 23, the minimum number of "minute" wasps or "other" Auchenorrhyncha occurs with around 45 SEPHs $m^{-3}$ (or around 30 *Sextius* $m^{-3}$). This represents one SEPH per volume of a cube with sides of about 30 cm each side. This may seem like a low SBI density for deterring other arthropods, but most of this volume is empty and *Sextius* inhabits only twigs and not major branches. Multiple SBIs often attend one SEPH (pers. obs.) and the average number may change with SEPH density or species. If SEPHs are dispersed throughout the shrub near the ends of twigs, SBIs must necessarily traverse the rest of the connected foliage, potentially deterring other insects. Various factors might have caused the higher densities of "minute" wasps noted above SEPH densities of 45 $m^{-3}$. Protection of ants to hemipterans they attend for honeydew can be density dependent (Cushman and Whitham 1991). There may be fewer SBIs per SEPH on *A. victoriae* at higher SEPH densities (Chapter 3), in which case SBIs may be more focussed on feeding from SEPHs/*Sextius* so they deter fewer arthropods. Additionally, SBIs might deter different arthropods depending on SBI proximity to SEPHs/*Sextius*. In the chrysomelid trials (at low SEPH density), SBIs were more aggressive near *Sextius* than far from them. Therefore, when near *Sextius* or perhaps SEPHs generally, SBIs might deter more parasites and predators which venture closer because SBIs are very near them by contrast to plant resources, whereas when further away SBIs might deter more herbivores located further from the attended SEPHs/*Sextius*. Also, herbivores might be easier to deter and/or more noticeable to SBIs than predators. However at higher densities of SEPHs, wasp oviposition into more eggs before disturbance by SBIs may be possible because egg batches are closer together. Understanding mechanisms of insect deterrence on *A. victoriae* and their density dependence would require further experiments as well as investigation into host-parasitoid relationships.

The peaked envelope relationship for 2011 SEPH densities with "minute" wasp densities of the previous season suggests that some of these "minute" wasps limit SEPH densities, which peak at a density which is regulated by variables such as food quality and enemy free space. SEPH densities in the following year might increase with higher parasitoid density on a shrub in the year before because both indicate increased shrub resources that filter up to higher trophic levels. Also, higher SEPH densities, potentially producing higher densities of eggs, may increase the proportion of eggs parasitized, as discussed in the paragraph above. At a density where increased egg parasitism due to higher existing wasp densities starts to reduce egg survival percentage more than high quality shrub resources increase reproductive output and survival of insects, populations of host insects will decrease with further increases in parasitoid densities. Also, natural enemies can deter phloem-feeders from selecting a plant (Inbar and Gerling 2008), a process which may be density dependent. Thus the results of the current study suggest a strong two-way interaction between "minute" wasps and SEPHs/*Sextius*, either direct or indirect. As mention, patterns are somewhat confounded by lack of taxonomic knowledge of individual species. Egg parasitoids are generally less specific than parasitoids of larval, pupal, and mature insects and sometimes attack insects from multiple families. Species composition of parasitoid wasp assemblages would correspond to the most abundant hosts. Populations of "minute" wasps would be influenced by other phloem-feeders they use as hosts, and some of these wasps would probably use SEPHs as hosts also. If densities of "minute" wasps reach a certain threshold, they might dramatically reduce populations of SEPHs/*Sextius* on shrubs. Interconnectedness of parasitoid wasps with SEPHs points to them having a key role in this system through likely effects of both groups of Hymenoptera (i.e. SBIs and parasitoids) on other arthropods. Parasitoid wasp densities might even be the main reason for numbers of SEPHs/*Sextius* on shrubs changing from one season to the next, although, once again, knowledge of host-parasitoid relationships is crucial to understanding this.

Parasitization of *S. virescens* eggs occurs primarily in partly exposed eggs in observed cases of a mesic south-eastern Australian system (Cookson and New 1980). SBIs might deter parasitoid wasps from ovipositing into partially exposed *Sextius* eggs or perhaps deterrence occurs while the eggs are being laid or during the period the shrub closes the slit made by the ovipositor. For *Sextius*, oviposition probably occurs mostly in late summer when adult *Sextius* populations are still substantial. *Sextius* densities on some shrubs change dramatically at this time, which suggests that adult dispersal was occurring, which probably precedes oviposition. Other SEPH eggs might be parasitized at different times. Throughout spring and summer, the lycaenid *Jalmenus icilius* lays eggs in batches on twigs and scale insects tend to lay and then sit above large numbers of eggs on leaves and twigs (pers. obs.). These eggs or the resulting larvae might be parasitized at different rates from *Sextius* eggs and perhaps by different wasp species. Not all SEPHs host the same parasitoids, which would add further statistical noise to correlations.

Densities of late spring 2009 survey shrub SEPH/*Sextius* form a similar quadratic envelope for "other" Auchenorrhyncha densities as for "minute" wasp densities. Furthermore, "other" Auchenorrhyncha densities form a similar envelope relationship for summer 2011 SEPH/*Sextius* densities to that formed for 2011 SEPH/*Sextius* densities with "minute" wasp densities. This suggests an important relationship between these three abundant taxa. Experiment high-*Sextius* shrubs had lower "other" Auchenorrhyncha densities than low-*Sextius* shrubs, which seems to partly contradict the pattern found in the survey shrubs. This might be for the same reasons as the similar trend found for "minute" wasps. It is unlikely that the quadratic pattern was caused by total "minute" wasp numbers closely tracking those of "other" Auchenorrhyncha since there may be other taxa on the shrub providing resources for them. Variation in "minute" wasps or phloem-feeder density is high when SEPH/*Sextius* density is low. On low- SEPH/*Sextius* shrubs, this may be through different competitive effects, including plant-mediated ones, possibly due to higher variation in resource quality. Another possibility is that at low preceding SEPH/*Sextius* densities, "other" Auchenorrhyncha densities may increase through reduced competition or by reduced apparent competition through there being lower densities of shared egg parasitoids. Alternatively, SBI deterrence of both "other" Auchenorrhyncha and "minute" wasps may have caused the similar patterns, with different deterrence rates on each at different SEPH/*Sextius* densities as described above.

Since thrips and psyllids are in higher numbers on traps than "other" Auchenorrhyncha, the pattern of "other" Auchenorrhyncha densities with SEPH/*Sextius* densities is not simply because of greater densities that enable detection of the pattern. "Other" Auchenorrhyncha are very similar to *Sextius* in morphology and feeding behaviour, and for this reason may be particularly affected by the SBI-SEPH association. By contrast with SEPHs, they were never seen aggregating on *A. victoriae* (pers. obs.), so are unlikely to be important to SBIs. Since sap-suckers cannot withdraw stylets from plant tissue quickly, they are more susceptible to attack by predators and parasitoids than other insects are (Delabie 2001), which might contribute to this pattern by making them more susceptible to SBIs. Phloem-feeders, feeding on twigs, would likely be encountered by SBIs more readily than thrips, which feed on flowers and sometimes leaves and whose smaller size might help them to escape detection or might make them unworthy of predation effort.

Since “other” phloem-feeders correlated positively with “minute” wasps and both groups within “other” phloem-feeders are abundant, the correlation probably means many “minute” wasps kill them at some developmental stage. Phenologies of different insect species in semi-arid Australia tend to differ within seasons in different years (Palmer 2010), so eggs of different species would be laid at different times and could potentially sustain egg parasitoids throughout much of the season. Since SEPHs can be common, are relatively sessile, and are of a similar size to “other” Auchenorrhyncha, egg size of “other” Auchenorrhyncha may be near the most common size, so parasitoids using them as hosts may be the most common.

Negative correlations of densities of total “other” phloem-feeders or “other” herbivores with 2009 SEPH/*Sextius* densities may be due to SBI deterrence. Alternatively, the correlation might reflect competition between two large populations using the same resource. It could be a combination of the two. The feeding of one insect herbivore on a plant commonly induces HIPVs which might have negative effects on densities of another insect herbivore. This type of plant-mediated competition is complex; competition is common among herbivorous insects regardless of feeding guild, and usually occurs indirectly through feeding-induced changes to plant chemistry, sometimes even between insects at low density feeding on different plant parts at different times (Kaplan and Denno 2007). Competitive effects of SEPHs/*Sextius* against herbivores caught on sticky traps are likely to be asymmetric when they are at high densities, perhaps augmented by the lower chance of their nymphs dispersing to another plant. Competition between herbivorous insects is usually asymmetrical (Kaplan and Denno 2007). Competition between SEPHs/*Sextius* and other herbivores is difficult to test since taxa can easily move to other sites on the shrub or elsewhere and shrub resources might be transported to sites where competition would otherwise occur.

Both predator densities and mirid/lygaeid densities, some of which may be predators, correlated positively with 2009 survey SEPH/*Sextius* densities. The small numbers of these groups on traps and the functional diversity of the mirid/lygaeids makes these patterns tentative. These two correlations might be caused by SBI deterrence of parasitoids of the taxa or other factors such as correlation with resource quality and consequent herbivore densities. Positive correlation of predator densities with thrips and "other" Auchenorrhyncha densities probably reflects the latter, but there is a lack of information on predator-prey relationships of these species in the literature to inform this.

## SBI effects on predation of *Sextius*

Closed cages had a positive effect on *Sextius* nymph survival which was on average greater than the effect of Tangle-Trap, although there was considerable variation in the effectiveness of each across individual shrubs. Despite the difficulties of counting nymphs in the predation experiment, a significant pattern amongst treatments emerged, suggesting that a relatively constant proportion of nymphs were counted. Since cages lead to only slightly higher nymph survival than Tangle-Trap, suggesting that Tangle-Trap stopped most of the predators, the majority of predation on *Sextius* nymphs at this time would have been by crawling arthropods that could not get through the 1.5 mm mesh holes or past Tangle-Trap, such as spiders. A low proportion of the predation would have been effected by predators that could either fly or jump over Tangle-Trap. This proportion might be substantially higher at times when their activity levels are higher. The effect on nymph survival of Tangle-Trap without closed cages was positive and this was consistent across replicates. That Tangle-Trap increased nymph survival means that SBIs had no detectable positive effect on nymph survival. However, since honeydew accumulates when SBIs are removed and some nymphs both dead and alive at the end of the experiment were stuck to twigs by it, SBIs probably increase nymph survival by removing it. The insignificant negative effect on average of Tangle-Trap with closed cages may have been detectable with more replication: in ecological studies where conditions are highly variable and difficult to control, and often little is known of the biology of taxa or multi-species interactions, lack of sufficient replication is a common problem.

If SBIs do not protect *Sextius* from predators, they may not deter herbivores either. Relationship between size and type of arthropod and SBI predation or deterrence rate is not known. Since predators are generally larger and more mobile than herbivore arthropods, have superior vision, and are more capable of defending themselves, they may be less susceptible to SBIs. However, other species of *Iridomyrmex* are known to successfully attack prey much larger than themselves (Shattuck 1999), and the type or specific identity of the predator may be most important. At different times, SBIs might protect *Sextius* from various insects and spiders including predatory nymphs and larvae and from parasites which attack *Sextius* adults and eggs. Lizards are rarely observed on *A. victoriae* and birds sometimes perch on their branches, so predation on *Sextius* by vertebrates is probably incidental and SBIs may deter many of them. Beetles in the family Coccinellidae and larvae of flies in the family Syrphidae are common predators of sap-sucking Hemiptera (Delabie 2001). Adult mantids and spiders such as Thomisidae prey on adult *Sextius* and mantid nymphs, adult earwigs, and lacewing larvae prey on *Sextius* nymphs, while pipunculid flies sometimes parasitize *Sextius* adults (Cookson and New 1980). Few of these were detected on sticky traps, but those traps might not be a suitable method for catching them. No parasitoids of *Sextius* nymphs are known, although it is likely that pipunculids attack late stage nymphs, but such parasitoids would take longer to emerge after oviposition than the duration of the experiment, so their effect would not be detected directly. Relative immobility and smaller size of many nymphs and larvae probably make them more susceptible to ants than other arthropods although they might not be parasitized (Cookson and New 1980). No larvae or predatory nymphs were seen during the predation experiment, but in a mesic Australian system involving a different *Acacia* and *Sextius* species, *Iridomyrmex* sp. attacked any larva less than 5mm long on the plant (Buckley 1983) and this behaviour was also observed from time to time during the present study. Predators of 4-5mm would be likely to be able to kill *Sextius* nymphs. Abundance of larvae and nymphs (including predators) would depend on varying local weather conditions. Perhaps due to the weather during the experiment, few predators small enough for SBIs to deter were active. The erratic movement within trails and fast locomotion of SBIs, particularly those consuming SE, might enable them to cover large areas of the shrub, which could make them efficient deterrents. The chrysomelid trials prove that SBIs can deter insects, whether or not *Sextius* is present, so it is unlikely that they have no effect on the arthropod assemblage. If different crawling predators had been abundant on shrubs of high *Sextius* level during the experiment, Tangle-Trap may have had a negative effect on nymph survival through preventing SBI deterrence of these predators.

Predators present while *Sextius* nymphs are abundant would be likely to be the most important ones for SBI protection to benefit *Sextius*. Due to their smaller size, limited mobility, and longer periods spent with stylets inserted into plant tissue, *Sextius* nymphs would be likely to be easier prey than adults. Also, since adult *Sextius* often are not feeding, at least during the day, nymphs are likely to produce more honeydew and therefore attract more attending SBIs. However, turnover of insect species present in the Australian semi-arid zone can depend on minimum temperature and rainfall in the current and preceding month (Palmer 2010). In different years, different insect populations are probably present at the same time as *Sextius* nymphs. Effects of ants on membracids they attend can vary substantially among years (Billick and Tonkel 2003). The large amount of rain in the middle of the experiment probably reduced insect activity while it was occurring. Also, the year in which this experiment was conducted was exceptionally wet and cool, so nymph emergence would have occurred later than usual. Therefore, it is possible that predators susceptible to SBIs were not present while nymph emergence was occurring, due to the unusual conditions prevailing in that year. Timing of *Sextius* nymph and predator nymph or larval emergences are likely to be crucial in determining the amount of population overlap and the resulting outcome of the tritrophic interaction. The variability of the Australian arid zone makes study over several years necessary to discern average tendencies.

## Conclusion

This study has found that SEPH/*Sextius* densities are constrained by growth rate of shrubs. This appears to be a stronger tendency, particularly in the uncontrolled environment of the survey, than the correlation of growth rate found on experiment shrubs with pre-existing high *Sextius* densities, which was probably linked to the lower folivory on those shrubs. Densities of SEPH/*Sextius* on shrubs with different growth rates may be mediated through a mixture of host preference and performance, but this tendency supports the plant vigour hypothesis. More detailed examination of dispersal and oviposition behaviour and performance conditions could determine causative factors. Spatial distribution of SEPH populations is further regulated by soil parameter distribution, since densities were limited by high concentrations of soil sulphur and/or conductivity. Manipulative studies determining the important soil parameters based on the associations they had with SEPH densities that have been found in this study could assist in prediction of spatial distribution of SEPHs.

For *A. victoriae* individuals, the association with SEPHs/*Sextius* and SBIs may be beneficial, because seed productivity is greatly increased on high-*Sextius* shrubs due to lower rates of destruction by a seed-feeding weevil. It is likely that the observed SBI deterrent effects are the reason for reductions in non-SEPH herbivory on high-*Sextius* shrubs, and SBIs may reduce parasitoid wasp oviposition on a range of hemipteran eggs. The patterns found between "minute" wasps (presumed to be mostly egg parasitoids) and SEPHs/*Sextius* suggest a strong two-way interaction, whether directly through SBI deterrence and parasitisation or indirectly through plant chemistry. This interconnectedness with SEPHs and other phloem-feeders points to parasitoid wasps having a key role in this system through regulating numbers of abundant taxa.  Populations of parasitoid wasps depend on populations of their hosts, which most likely include SEPHs, but large decreases in SEPH/*Sextius* densities could result from increases in parasitoid wasp densities.  Soil parameters appeared to affect arthropod assemblages on shrubs, which differed strongly with respect to densities of SEPHs/*Sextius*.  Thus, shrubs with high resource quality might be involved in cycles of fluctuating SEPHs/*Sextius* populations driven from the bottom up. The only important effect of competition between shrub herbivores may be that between SEPHs/*Sextius* and other herbivores, which is likely to be asymmetrical in favour of SEPHs. However, although it is likely that SBIs deter small crawling predators and prevent *Sextius* nymphs from "drowning" in honeydew, more studies need to be conducted in the presence of different arthropod assemblages and with higher replication.  Host-parasitoid relationships need to be determined to understand their importance in this system, in particular on the tritrophic association.  SBI deterrence of seed weevils, shrub folivores, predators of *Sextius*, and parasitoid wasps should be investigated in further detail to understand the dynamics of this association and effects of aridity on its dynamics.  Future experiments with *Sextius* could use low, medium, and high densities based on these results, possibly including greenhouse-grown shrubs with low intrinsic variability to remove variation due to plant chemistry.

This system exhibits different dynamics from that described for *A. decurrens*, *S. virescens*, and *Iridomyrmex* sp. in mesic Australia (Buckley 1983).  These differences may be due merely to differences in weather or study species, but they may illustrate how SBI-sap-sucker-shrub associations tend to function in different biome types. How influences of climate and other environmental variables such as soil parameters drive these dynamics requires comparison of system dynamics under different sets of controlled conditions. It is possible that parasitoids are strongly affected by the low humidity typical of arid biomes due to their size, which might strongly affect SEPH/*Sextius* densities and dynamics of the association. Temperature and moisture effects on photosynthesis, with respect to sugar concentration in sap (in contrast to replacement of vegetation biomass due to herbivory), might regulate the balance of costs and benefits of densities of SEPHs/*Sextius* on shrubs. Study of moisture gradient correlations might determine effects of different rainfall on the interaction. Plant chemistry, such as HIPVs and feeding deterrents for defence against herbivory, can also alter according to different environmental conditions and might be a factor in regulating arthropod communities on shrubs. As well as temporal variability, high arid zone spatial variability in soil parameters and rainfall may lend different tendencies to association dynamics at a landscape scale.

# Chapter 5:

## Slope-aspect affects acacias, sugary-exudate producing herbivores in an association with attending ants, and assemblages of other arthropods in semi-arid Australia

### Abstract

Topographic variation in landscapes, mainly combinations of slope and aspect (slope-aspect, henceforth), produce abiotic differences including different levels of water limitation and insolation in arid zones which can strongly affect community dynamics and structure. In the southern hemisphere, north-facing slopes (NFS) receive the most insolation and south-facing slopes (SFS) receive the least, while rainfall, particularly in large events, runs off slopes onto flats. In a semi-arid Australian location, slope-aspect effects on an association between sugary exudate producing herbivores (SEPHs) and small dominant trailing ants on shrubs were investigated. A survey examined slope-aspect differences in soil parameters, *Acacia victoriae* growth and reproductive success, density on shrubs of SEPHs, and densities of other small arthropods on shrubs through factor analyses. Differences among slope-aspects were found for most parameters, with some possible interactions with SEPH densities on shrubs. SEPH densities were higher on south-facing slopes (SFS) than on north-facing slopes (NFS) and flats after a year of average rainfall, whereas after a year of unusually high rainfall they were higher on NFS than on SFS and flats. An earlier study found correlations of soil parameters, shrub growth rate, and other small arthropods with SEPH densities. Results of the present study are consistent with favourability for SEPHs of particular levels of moisture and temperature or soil nutrient concentrations typical of semi-arid Australia which warrant further investigation including manipulative experiments. The present study is the first to consider possible effects of aridity on SEPH populations involved in a tritrophic relationship with their attendant ants and host shrubs.

# Introduction

Arid zone ecosystems (those in areas where annually rainfall represents half or less of the potential evapotranspiration) possess distinctive structure and function, yet little is known of fundamental processes that shape their ecology, relative to other biome types. Literature on the extensive Australian arid zone with its unique suite of conditions is scant. Along with high insolation and low rainfall, the defining characteristics of the arid zone are high spatial and temporal variability in quantity and periodicity of rainfall and high spatial heterogeneity in soil characteristics such as nutrient concentration and soil type. Nutrient concentrations in soil are typically low in arid zones (Crawford and Gosz 1982), particularly in Australia, of which this is characteristic of the continent as a whole (Stafford Smith and Morton 1990). Slope and topographic aspect (referred to as 'aspect' hereafter), which means directional orientation of a slope, contribute to this spatial heterogeneity, while also representing a climatic gradient of aridity for some of these factors. In the southern hemisphere, south-facing slopes (SFS) receive less solar radiation than north-facing slopes (NFS), resulting in generally lower soil temperatures, higher humidity, and less resultant heat and water stress on shrubs. Low flat areas (flats) at the base of slopes should retain more soil moisture due to receiving run-off in large rainfall events, reduced drainage, and insolation should be intermediate between that of NFS and SFS. Slope-aspect can differentiate soil nutrient concentration (Rech et al 2001, Yimer et al 2006), vegetation species composition, biomass, and density (Sternberg and Shoshany 2001), and insect species richness (Auslander et al 2003, Pryke and Samways 2009). Most studies of slope-aspect have been conducted in the northern hemisphere where $NFS_{northern}$ and $SFS_{northern}$ have the opposite characteristics to those in the southern hemisphere.

In semi-arid to arid areas where water is limiting, slope-aspect differences are important determinants of plant community structure and function. On $SFS_{northern}$ (SFS in the northern hemisphere) or $NFS_{southern}$, there tend to be higher temperatures, lower moisture levels, different soil nutrients and processes, and greater heterogeneity due to higher solar radiation, which create substantial differences in the plant community compared to $NFS_{northern}$ (Nevo 1997, Sternberg and Shoshany 2001). Contrast between slopes and flats is created by drainage effects on the distribution of organic matter, soil nutrients, and water, which can limit plant fitness on slopes relative to flats. Soil composition is also affected by differential mineral weathering on different slope-aspects (reviewed in Rech et al 2001). Further contrast is caused by greater evapotranspiration in plants when insolation is increased because of slope-aspect. For slope-aspect to affect plants these differences must be sufficient to differentiate growth and reproductive success. Slope-aspect differences can filter up to affect the arthropod community (Auslander et al 2003, Pryke and Samways 2009). Insects are relatively highly sensitive to temperature and when lower temperatures occur, such as at night and in the cooler seasons, it limits their activity and developmental rate. Therefore, NFS might be generally more favourable through increased feeding rates from increased activity or faster development. However, this effect would occur up to a threshold at which temperature becomes limiting as insects are susceptible to dehydration, which may lead to relatively lower feeding rates or lower reproductive success, on NFS in mid-summer. However, since insects can move relatively quickly to sites of higher resource quality, these differences may be mitigated more than direct slope-aspect effects on the plant community.

How different environmental conditions affect outcomes of ant-hemipteran mutualisms on plants is not well understood (Styrsky and Eubanks 2007). Variability in net outcomes for partners in facultative food-for-protection interactions involving ants and sugary-exudate producing herbivorous insects (SEPHs) on plants may be particularly high in arid zones due to their high abiotic and biotic heterogeneity. However, studies of outcomes across environmental gradients are rare and have not included many factors relevant to aridity. Ant abundance and species can alter effects of ants on partners in these tritrophic associations (Bronstein 1998), so differences in these factors between biome types might cause differences between biome types in outcome of the associations for partner species. A recent meta-analysis of the role of ants in facultative protection of plants that provide food for them found that ant removal resulted in increases in herbivory damage and decreases in plant reproduction, phenomena that were stronger on tropical than temperate plants (Rosumek et al 2009), suggesting strong effects of biome type. However, dry systems were not represented in that analysis which means that a large proportion of the Earth's surface was not included. Strength of effects of these associations might arise from soil nutrient influences on plant herbivore defence strategies. Another recent meta-analysis found that positive effects of increases in plant vigour on sap-sucker abundance were 20% higher in tropical than temperate systems (Cornelissen et al 2008), but the reason was not determined. Temporal variability of temperature and rainfall over a one year period were implicated in number of ant-plant and ant-SEPH-plant food-for-protection associations in a descriptive survey in central America (Rico-Gray et al 1998). These changes appeared to have different effects in different biome types ranging from montane to tropical to semi-arid, with the highest and most constant number of these associations occurring in tropical habitat, the least in the montane, and intermediate and variable numbers in the two semi-arid locations. Overall, minimum temperature had the strongest effect. A further study found that phloem-feeders benefited from water stress of their host plants that was only intermittent (Huberty and Denno 2004). Also, few studies have investigated variation of these interactions at landscape scale. This is important because within the different landscapes, depending on environmental drivers, the same organisms may be assembled in different functional communities.

Associations between ants, sap-sucking SEPHs, and *Acacia* or *Eucalyptus*, are evidently common and widespread in the Australian arid zone. In fact, unpredictable water availability and low nutrient levels in arid Australian soils are suspected to make ant food-for-protection interactions on plants particularly common there (Stafford Smith and Morton 1990) and may even contribute to fire regimes by decreasing herbivory, increasing available fuel, and raising intensity of fires (Orians and Milewski 2007) hence altering the whole ecosystem function. Despite abundance of species involved in these interactions together and their possible ecological importance, studies on partner outcomes of this interaction type in Australia arid areas are virtually unknown.

Few studies have considered bottom-up effects on ant-SEPH-plant associations, with regard to abiotic factors such as insolation, soil moisture, and nutrient levels (Styrsky and Eubanks 2007). Since most studies span only a few months, they cannot capture much of the high temporal variability which occurs annually although they may capture important events within that variability. This study compares effects of differing aridity (levels of soil moisture and nutrients and in insolation on north- and south-facing slopes and flats) on shrub growth, reproduction, and herbivory, on SEPH populations, and on arthropod communities on shrubs. Gains in understanding of effects of variation in soil water availability and temperature on arid land communities may provide insights into how these factors will be affected by climate change to inform conservation management of these systems.

*Acacia victoriae* is a widespread shrub in Australia, existing in areas with annual rainfall from 100mm to 1000mm (Whibley and Symon 1992). One genus of membracid that frequently colonizes it is *Sextius* which is widely distributed across Australia, as are various Margarodidae and Lycaenidae which are attended by ants, often of the genus *Iridomyrmex*. These shrub-SEPH-ant associations are postulated to be beneficial to *A. victoriae* growth and reproductive success and in the case of *Sextius*, are likely to contribute to survival (chapter 4). This study aimed to examine effects of slope-aspect in a semi-arid environment on this shrub-SEPH-ant association and other abiotic and biotic factors that are important to association outcomes:

1. i. How does slope-aspect affect soil composition, growth, and seed production of reproductive *A. victoriae*? ii. Does slope-aspect interact with SEPH density in its effects on shrub growth?

2. How does slope-aspect affect abundance of shrub and SEPH partners in the tritrophic association?
3. i. How does slope-aspect affect small herbivore arthropod assemblages on reproductive *A. victoriae* shrubs? ii. Does slope-aspect interact with SEPH density in its effects on arthropod assemblages?

## Methods

Details of the site and species have been presented elsewhere in this thesis (Chapter 4). In addition, it is important to include information about the ants on survey sites. SBIs are dominant *Iridomyrmex* ants, and dominance means ants are both abundant and displace or limit abundance of other species of ants (Greenslade 1976). Different species of dominant *Iridomyrmex* can be mutually exclusive in the same area and they can form a competitive hierarchy where more dominant species exclude less dominant ones (Greenslade and Halliday 1983). On and around survey sites, SBI colonies can extend over at least 50m in any one direction (pers. obs.), which could potentially span any survey site in the present study. *Iridomyrmex lividus*, which is around 8mm long and therefore much larger than SBIs, which are around 4mm long, was dominant in some areas surrounding survey sites, and over the period of three years, their territory boundaries differed somewhat (pers. obs.). However, *I. lividus* appears to have substantially lower aggression and activity rates than SBIs (pers. obs.), seems to occur in much lower densities than SBIs (pers. obs.), and was seen attending SEPHs only on shrubs with low SEPH levels (<20m$^{-3}$), although it was extensively searched for on at least 20 shrubs during the period of peak SEPH activity (unpublished data). Some smaller species of ant can occupy a higher place inthe competitive hierarchy than larger ones, at least in some circumstances (Greenslade 1976, Fox et al 1985). On sites of the current study, other *Iridomyrmex* species did not form trails in the same territory as SBIs (pers. obs.). SBIs are extremely aggressive towards other ant species, and shrubs with SBIs attending SEPHs lacked other species of ants (pers. obs.). A study at nearby sites on the same station found that one species in the *I. rufoniger* complex dominated attending of *Sextius* sp. on *A. victoriae* (Clarke 2006). Shrubs with SEPHs within SBI territories are connected by SBI trails, which would include only one species. Therefore, it was improbable that mixed ant species were present amongst SBIs attending survey shrubs.

*I. lividus* is likely to have different effects from SBIs on the tritrophic association, since different ant species can have different effects on parasites and predators of SEPHs (Buckley and Gullan 1991, Del Claro and Oliveira 2000). To reduce different effects of ant species, only shrubs within colonies of SBIs were used in the survey in 2009. More aggressive ants provide better protection in tritrophic associations with SEPHs and plants (Buckley and Gullan 1991), so eliminating from this study shrubs on which the less aggressive and less numerous *I. lividus* occurred probably eliminated much more variation than isolating species of SBIs, even if different SBIs occurred on survey shrubs. Where survey shrubs were associated with ant populations not dominated by SBIs in 2011, the site on which they occurred was removed from appropriate analyses to reduce variation in dynamics of the tritrophic association caused by the different ant species, since on site 3 (see below), NFS and SFS had become part of colonies of different species of ants.

Rainfall during the study period was extremely variable (table 1) and included 2010, which was the second highest in records dating back to 1985 (Bureau of Meteorology). Rainfall in the twelve month period December 2008 to November 2009 was 325mm, approximately equal to the mean of 337mm, while in the next 12 month period it was 564mm, over two standard deviations above the mean. Rainfall and solar exposure are recorded at Oraparinna (31.37° S, 138.72°: elevation 550 m), but not temperature. Temperatures at Oraparinna are similar to those at Leigh Creek (30.60° S, 138.42°, elevation: 259 m), the nearest weather station (91 km away) where temperature is recorded. Monthly averages of daily solar exposure were more variable within one season than among years (table 1). Monthly averages of mean daily insolation from September 2009 to February 2010 were above the mean and monthly temperatures were above the mean except in February. Monthly averages of mean daily insolation from October 2010 to February 2011 were 0.8 to 2.0 $MJ.m^{-2}$ $day^{-1}$ lower than in the previous spring to summer (Bureau of Meteorology) and temperatures were below the mean, except in January.

Table 1: Oraparinna records of rainfall in the month and 6 month-period before SEPH/*Sextius* counts and solar exposure in the month before SEPH/*Sextius* counts (Australian Bureau of Meteorology)

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For this field survey, three sites were selected that were distributed along a curve of approximately 7km at elevations of approximately 500 to 560 m. They were selected to cover the widest area practicable with robustness of biological patterns in mind since there were few suitable slopes within a reasonable distance of each other. For this reason since suitable sites were limited, the NFS and SFS of site 2 were in different locations, meaning they were not paired. Flats selected were the lowest areas to which water would drain from the surface of the slope of the same site, mainly creek beds and the area beside them, and soil properties may have been different from other low areas as this information was not used to select flats. A fourth site was added in late September/October 2010 after results indicated that the level of observed variability required further replication. Each slope-aspect-site combination was referred to as a "sub-site".

Shrubs for the survey were selected that:

- were within a territory dominated by an SBI colony
- were of sufficient size to reproduce i.e. at least 30cm tall;
- looked healthy enough to survive for the duration of the study;
- for slopes: were at least two average shrub heights above flats on slopes and at least one average shrub height below the hilltop on SFS;
- for slopes: were within the compass points N and NW on NFS and within the compass points S and SE on SFS;
- for flats: were within one average shrub height of the lowest point at the foot of the surveyed slope and where water would drain onto them from surveyed slopes on flats;

Individual shrubs for the survey were selected randomly. Survey SEPH densities were not manipulated; *Sextius* comprised 82% of survey SEPH individuals in November 2009, 61% in

January 2011, and 91% in February 2011. Since other SEPH densities comprising a large proportion of total SEPHs were high on some shrubs with no *Sextius*, have similar interactions with SBIs, and shrubs with high SEPH levels were limited in number, SEPH levels are used in the study rather than *Sextius* levels. Additional shrubs with SEPHs on them were selected so that the total number was at least five on each sub-site since many seemed to have none. These are referred to as SEPH shrubs. Because attributes of flats beneath north and south slopes might be different, there were twice as many replicates on the flats to attempt to account for this potential variation.

To assess the effects of slope-aspect on soil parameters, soil was sampled by choosing a point near the centre of each sub-site such that four points 5m apart along each of four rows 5m apart were centred upon it. No point was within two canopy diameters of any shrub. From each point, 50g was taken from the top 5cm of soil using a 5cm long core. Soil from one row at the same height of each sub-site was pooled, giving four pooled samples per slope or flat. Soil samples were oven dried at 70°C for 3-4 days before being sifted through a 1mm mesh and sent to CSBP Laboratories for analysis of % total nitrogen ($N_{total}$), mg/kg available phosphorous ($P_{available}$), mg/kg potassium (K), mg/kg sulphur (S), % organic carbon ($C_{organic}$), dS/m conductivity (EC), and pH (using the method involving $CaCl_2$).

Shrub volume was important for calculating SEPH density and shrub relative growth rate, and was approximated by a cone with oval base (Chapters 2, 4). During the first SEPH survey in November 2009, shrub volume was calculated from height, H, maximum canopy width, $W_{max}$, and canopy width perpendicular to that, $W_{perp}$: $1/3 \times \pi \times H \times W_{max}/2 \times W_{perp}/2$.

Dimensions were re-measured and the volume recalculated at the beginning of the second summer SEPH survey in January 2011.

*Acacia victoriae* growth peaks in spring to early summer even in variable arid conditions (Maconochie 1973, Friedel et al 1994, pers. obs.), so planning timing of experiments for this shrub is easier than responding to growth following rainfall as occurs in many arid zone plants. To measure shrub growth rate, in January 2010 10 – 30 green shoots (depending on shrub size) with no branching off-shoots on the outside of the shrub at a height easy to reach were randomly selected, half being pruned and half not pruned. A dot of acrylic paint was put 5cm from the end of each shoot. All shoots from 5cm from the paint dot to their apex were measured and lengths of branching off-shoots from the same shoot were summed. There was no difference in length of shoots chosen for pruning versus un-pruned shoots. Mean growth of shoots on each shrub was used in analyses. Growth was measured in mid-January 2011. This was the same method as used in the experiment described in Chapter 4.

To record reproductive success of each shrub, seed pod bunches were collected as soon as they were ripe but not yet dehisced, to prevent seed dispersal. Numbers of seed pods vary greatly on different shrub individuals and some did not have the seed pods on the ten small branches intended for collection, so only shrubs with a minimum of ten pods were included in analyses. When developing seed pods were observed on shrubs in November to December 2009, bags made from organza were tied around 10 bunches on each shrub on sites 1 and 2. This was to not only prevent seeds from dispersing, but to record mass of seeds excluding the effects of seed-eating insects. On site 3, seed pods were not placed into bags. After harvesting in January 2010, numbers of initiated seeds in pods were counted, seeds were examined for weevil larvae or exit holes under a 10x magnification dissecting microscope, tested for viability by squeezing as hard as possible with tweezers, and seeds deemed viable were pooled and weighed for each shrub.

SEPH density relative to shrub volume was recorded. SEPHs were counted on whole shrubs as soon as the main emergence appeared to be underway in spring 2009 (7th – 14th November), and then in summer 2010 (4th - 28th February). In the next season, they were counted during peak densities in mid-summer 2011 (3rd – 7th January) and then again in summer 2011 (13th – 15th February). Since SBIs do not feed at first instar *Sextius* sp. nymphs (pers. obs.), which are subject to high mortality, these nymphs were not included in *Sextius* densities. Since many factors which can influence insect populations are highly variable even at the same time of year, it is difficult to compare results for populations from one year to the next since the study was not able to continue for a long enough period to account for the high seasonal variability in this system. Indeed, many studies are afflicted by this shortcoming.

To record densities of arthropods on shrubs, half a yellow sticky trap was hung from a small branch and a twig placed through the middle (to increase contact of crawling insects), on as similar a position as possible at one time on each stem with respect to solar exposure. During the expected peak insect activity period in mid-summer 2010 (mid-January), traps were left for seven days.

Small arthropods were assigned to the lowest taxonomic group possible without dissection. For analyses, wasps were divided among three classes: "minute" wasps which were 1mm in length or less, the majority of which are egg parasitoids or parasitoids of thrips larvae/pupae; those over 2mm were included in the class "predators" since most of the wasp species of this size caught on traps are predatory or are parasitoids of larvae, which also kill their hosts, and are therefore equivalent to predators for the purposes of this study; the very small proportion that were between these lengths were put into the category "other" to reduce noise since there was not sufficient information to determine to which of the other size classes they belonged. "Predators" included all non-SBI arthropods whose diet is thought to consist of at least 50% faunal matter, except mirids or lygaeids, which were allocated their own class since individual species were not identifiable and have a range of different diets. The vast majority of "other" Auchenorrhyncha were cicadellids, along with other rarely caught derbids which do not appear to aggregate on *A. victoriae*. Thrips and psyllids were counted in their own separate classes. When psyllids and "other" Auchenorrhyncha are grouped together they are referred to as "other" phloem-feeders. Any other herbivore was assigned the class "other" herbivores, since the lack of taxonomic knowledge meant that feeding behaviour was not known. The very few other arthropods caught that did not fall within these groups were placed in the category "other".

## Analyses

The statistical program Primer-E 6.0 with PERMANOVA+ (Anderson 2001) with Euclidean distances was used for all factor analyses.  For factor analyses, 9999 permutations were used of the PERMANOVA test with Type III partial squares.  One-factor analyses were used for slope-aspect investigations to find overall average tendencies to account for as much natural variation as possible and because one-factor analyses are stronger.  To correct for effects of site, these data were standardized by site, taking into account the distribution of the data.  With only one factor, unrestricted permutation of raw data was used, while with two or more crossed factors permutation of residuals under the reduced model was used.  Where effects of site and interacting effects of site with slope-aspect were investigated, two-factor analyses of slope-aspect paired by site were used with non-standardized transformed data.  PermDisp was used to test for homogeneity of dispersion in factor analyses for pairs of factor levels, which is very important for two-factor analyses.  Number of permutations used was always 999 and dispersion of medians was used.  For all linear correlations and linear and non-linear non-envelope regressions SPSS software (Versions 17.0 – 19.0, SPSS Inc.) was used.  The Pearson correlation method was applied since parameters were transformed to maximize linearity.  For regressions, normal residual frequency was optimized by considering histograms of residuals and homogeneous variance was optimized by considering the graph of residuals versus predicted values.  For all envelope regression, least absolute deviation (LAD) quantile regression in the Blossom software (Cade and Richards 2005) was used and asymptotic p-value was reported.

Soil parameters, s, transformed to square roots had a near normal frequency distribution, so the square roots, $\sqrt{s}$, were standardized to $(\sqrt{s} - \mu_{site})/\sigma_{site}$ before doing one-factor univariate PERMANOVAs to examine slope-aspect differences. Analyses of site differences and interacting effects of site and slope-aspect were also done. Samples from all four sites were used, of which there were four replicates per slope-aspect.

Since there was a regression relationship between growth, g, and shrub volume, v, but there was no pattern between square root of growth, $\sqrt{g}$, and cubic root of shrub volume, $\sqrt[3]{v}$, (chapter 4), the former was standardized by the latter before analyses (i.e. $\sqrt{g} / \sqrt[3]{v}$ was used) to give relative growth rate (RGR). Distributions of shrub shoot growth measurements, both raw and RGR, g, were closest to exponential frequency distributions, so they were standardized to $(g - \mu_{site})/ \mu_{site}$ before doing one-factor PERMANOVAs to examine slope-aspect differences. Analyses of site differences and interacting effects of site and slope-aspect were also done. RGR was used to examine effects of slope-aspect or effects of SEPH density on shrub growth, whereas raw growth was used to examine any possible effect on SEPH densities. When examining effects of SEPHs on shrub growth, November 2009 SEPH densities or levels were used and only randomly chosen shrubs were used, whereas when examining effects of shrub growth on SEPH host preference, January 2011 SEPH densities or levels were used and all shrubs including extra ones selected for higher SEPH levels were used. Analyses of data for differences between sites and interacting effects of site and slope-aspect were also done. When 2011 SEPH/*Sextius* densities are mentioned without reference to a month, the first count in January is being referred to.

Replicates used for seed data were very uneven, so means unweighted by slope-aspect were used during standardization. Distribution of numbers of viable seeds per pod formed a Poisson distribution and numbers of seeds eaten per pod was closest to an exponential frequency distribution, so they were standardized to $(s - \mu_{site})/ \mu_{site}$ before doing one-factor PERMANOVAs to examine slope-aspect differences. Average seed mass per pod and average numbers of seeds initiated per pod formed normal distributions, so they were standardized to $(s - \mu_{site})/\sigma_{site}$ before doing one-factor PERMANOVAs. Since site 2 NFS and SFS were not paired with each other, uneven numbers of low replicates for unpaired analyses combining sites 1 and 2 were used.

Frequencies of SEPH and *Sextius* densities, S, were closest to exponential frequency distributions, but their cubic roots, $\sqrt[3]{S}$, approximated the exponential distribution more closely. Therefore, SEPH and *Sextius* densities were transformed to cubic roots and standardized to $(g - \mu_{site})/ \mu_{site}$ before doing one-factor PERMANOVAs to examine slope-aspect differences. Because of the difficulty of comparing slope-aspect effects on SEPHs for different sets of sites in different years, various analyses were done using different sets of sites to enable all important comparisons. Direct comparison of years using sites 1-3 in both years was inadequate due to the change of ant species at some sub-sites. Analyses using all sites (sites 1-4) were included, but for 2011 data, analyses using data from sites 1, 2, and 4 were thought to be the most reliable. For analyses of change in SEPH and *Sextius* densities between years, SEPH-shrubs were used since the change in densities was not as sensitive to non-random selection of shrubs as the absolute density. These data were transformed to cubic roots before calculating the change in density, to correspond with the data for individual years, but not standardized, since the inclusion of negative values meant that positive or negative site means might change whether the average value on a particular slope-aspect was positive or negative. Analyses of data for differences between sites and interacting effects of site and slope-aspect were also done.

Small arthropod densities on sticky traps were analyzed in stronger one-factor analyses separately for slope-aspect effects for low-SEPH shrubs to avoid any influence of the SEPH-SBI association on assemblages and also of high-SEPH shrubs for direct comparison with the stronger analysis. SEPH density of 20m$^{-3}$ or less was considered low and density of 60m$^{-3}$ or more was high. Site differences were analyzed on low-SEPH shrubs and for the mixed analyses of low- and high-SEPH shrubs for effects of site without SEPHs and effects of site in the presence of the interactions, respectively. For two-factor PERMANOVAs on site differences and interacting site and slope-aspect effects, data transformed to square roots were used and contained uneven replication. Distributions of counts of most arthropod groups, a, were closest to exponential frequency distributions, except predators which formed a Poisson distribution and were transformed to $(a - \mu_{site})/\mu_{site}$ before doing one-factor PERMANOVAs to examine slope-aspect differences. The exception was thrips, a, which formed a normal distribution and was transformed using $(a - \mu_{site})/\sigma_{site}$ before doing one-factor PERMANOVAs. For the standardized two-factor analyses for slope-aspect differences using both low and high SEPH density shrubs, a subsample with as even replicate numbers as possible for low and high SEPH density shrubs within each site slope-aspect pair were used.

Although values of 0.05 or less indicate clear significance of statistical tests given appropriate data, since the 95% confidence parameter is essentially arbitrary, p-values of up to 0.08 are considered marginally significant.

# Results

## Soil and slope-aspect

Soil characteristics differed with slope-aspect (tables 2, 3). Concentrations of $N_{total}$ were higher on SFS than on NFS and flats and concentrations of $C_{organic}$ and S were higher on SFS than on flats. These soil nutrients all auto-correlated (Chapter 4). By contrast, K was less concentrated in soil on SFS than on NFS or flats. $P_{available}$ levels were similar on NFS and SFS where they were higher than on flats. There were no differences in pH or EC among slope-aspects except that pH values were marginally statistically differently dispersed on flats compared with NFS ($p = 0.070$). $N_{total}$ and $C_{organic}$ soil concentrations were statistically differently dispersed on flats compared with on NFS or SFS and S concentration was statistically differently dispersed on flats compared with NFS.

Table 2: Soil differences for different slope-aspects (site-μ/σ-standardized logarithm-transformed, one-factor permanova)

| n = 4 | Highest Slope-Aspect Mean over Lowest | Difference between Slope-Aspect of Highest and Lowest Values | Slope-Aspect of Highest Values | | | | Slope-Aspect of Lowest Values | Different Dispersion |
|---|---|---|---|---|---|---|---|---|
| $N_{total}$ (%) | 1.24 | | SFS | > | NFS | ≈ | Flats | |
| p-value | | **0.0116** | | **0.0025** | | 0.8608 | | N, F 0.006<br>S, F 0.001 |
| $C_{organic}$ (%) | 1.31 | | SFS | > | NFS | ≈ | Flats | |
| p-value | | **0.0178** | | 0.0845 | | 0.1972 | | N, F 0.004<br>S, F 0.045 |
| S (mg/kg) | 1.22 | | SFS | > | NFS | ≈ | Flats | |
| p-value | | ***0.0695*** | | 0.1167 | | 0.4035 | | N, F 0.020 |
| K (mg/kg) | 1.13 | | NFS | ≈ | Flats | > | SFS | |
| p-value | | **0.0064** | | 0.5614 | | ***0.0574*** | | |
| $P_{available}$ (mg/kg) | 1.34 | | NFS | ≈ | SFS | **>** | Flats | |
| p-value | | **0.0035** | | 0.3436 | | **0.0502** | | |

(For all tables, values in **bold** are statistically significant, and values in ***bold italics*** are marginally significant. )

Table 3: Mean soil parameter values (non-standardized, untransformed) on different slope-aspects and sites

| | Slope-aspect | | | Site | | | | Overall |
|---|---|---|---|---|---|---|---|---|
| | NFS | SFS | Flats | 1 | 2 | 3 | 4 | |
| $N_{total}$ (%) | 0.13 | **0.16** | 0.13 | 0.14 | 0.15 | 0.17 | 0.11 | 0.14 |
| $P_{available}$ (mg/kg) | **21.56** | **20.31** | 16.13 | 17.75 | 17.25 | 22.25 | 20.08 | 19 |
| K (mg/kg) | **651** | 573 | ***633*** | 520 | 653 | 631 | 672 | 619 |
| S (mg/kg) | 6.2 | ***7.3*** | 6.0 | 5.9 | 6.3 | 8.6 | 5.3 | 6.5 |
| $C_{organic}$ (%) | 1.10 | **1.27** | 0.97 | 1.09 | 1.08 | 1.39 | 0.89 | 1.11 |
| EC (dS/m) | 0.165 | 0.161 | 0.144 | 0.167 | 0.130 | 0.198 | 0.132 | 0.157 |
| pH | 7.4 | 7.3 | 7.3 | 7.5 | 7.2 | 7.4 | 7.1 | 7.3 |

There were larger differences in soil parameters among the four survey sites than among slope-aspects (table 4) whether including unmatched site 2 or not. There was no site-slope interaction for $N_{total}$, but there was for $P_{available}$, K, S, and pH. The pairs of sites that differed most in slope-aspect pattern for soil parameters were 1 and 4 or 3 and 4 for which only S and K respectively had a similar pattern (table 3). The site pairs that differed least were 1 and 2 or 1 and 3 for which only K was different for both pairs. There were substantially different nutrient concentrations on some survey sites compared to other survey sites. Site 1 had the lowest K concentration, site 3 had the highest S concentration, and site 4 had the lowest $N_{total}$ and $C_{organic}$ concentrations. Statistical dispersion of values of some soil parameters differed among different sites.

Table 4: Soil differences on different sites (non-standardized, logarithm-transformed, two-factor permanova)

| Soil parameter (n = 4) | Overall interaction term | | | Site pair comparisons | | | | | | Different Dispersion |
|---|---|---|---|---|---|---|---|---|---|---|
| | Slope | Site | Site X Slope | 1 and 2 | 1 and 3 | 1 and 4 | 2 and 3 | 2 and 4 | 3 and 4 | n = 4 |
| $N_{total}$ (%) | | | | | | | | | | |
| p-value | *0.0812* | **0.0058** | 0.3473 | 0.6671 | 0.1626 | **0.0140** | 0.2665 | **0.0035** | **0.0024** | 1, 4 *0.0558*<br>2, 4 0.0007<br>3, 4 *0.0691* |
| $P_{available}$ (mg/kg) | | | | | | | | | | |
| p-value | 0.2419 | **0.0246** | **0.0033** | 0.6892 | **0.0360** | 0.1774 | **0.0080** | ***0.0584*** | 0.2801 | |
| K (mg/kg) | | | | | | | | | | |
| p-value | 0.2358 | **0.0002** | **0.0343** | **0.0004** | **0.0007** | **0.0005** | 0.6673 | 0.4938 | 0.2595 | NS *0.0610* |
| S (mg/kg) | | | | | | | | | | |
| p-value | 0.3450 | **0.0008** | ***0.0730*** | 0.7199 | **0.0094** | 0.3348 | **0.0105** | 0.1441 | **0.0003** | |
| $C_{organic}$ (%) | | | | | | | | | | |
| p-value | 0.1794 | **0.0028** | 0.1121 | 0.9720 | ***0.0799*** | ***0.0647*** | **0.0464** | **0.0259** | **0.0031** | NF 0.0491 |
| EC (dS/m) | | | | | | | | | | |
| p-value | 0.5616 | **0.0041** | 0.2342 | **0.0014** | 0.2705 | **0.0312** | **0.0094** | 0.9204 | **0.0208** | 1, 2 *0.0740* |
| pH | | | | | | | | | | |
| p-value | 0.7933 | **0.0004** | **0.0334** | **0.0331** | 0.1567 | **0.0002** | **0.0298** | 0.3175 | **0.0001** | 1, 2 0.0342<br>2, 3 0.0011<br>2, 4 0.0147 |

## *Acacia victoriae* growth and slope-aspect

When growth was recorded 12 months after the growth shoots were tagged, care was taken to find every marked shoot, but approximately 5% could not be found. There was no difference in length of pruned and un-pruned shoots.

Shrub shoot growth, g, in the 12 months from January2010 to January 2011 on randomly selected shrubs on sites 1, 2, and 3 was higher on SFS than on flats or NFS. This was true whether ignoring possible effects of 2009 SEPHs/*Sextius* and their densities by using shrubs of all *Sextius* densities for existing effects of slope-aspect or using only low-SEPH shrubs (table 5, table 6). Growth on SFS was statistically differently dispersed from on flats in analyses of mean-standardized data from all shrubs. One outlier which corresponded to a shrub that was substantially larger than all others was removed. When including all shrubs, mean shrub shoot growth on SFS was 0.58cm, on flats 0.35cm, and on NFS 0.29, so shrubs grew on average 2.0 times as much on SFS as on NFS and 1.7 times as much on SFS as on flats. Including low-SEPH shrubs only, mean shrub shoot growth on SFS was 0.57 cm.m$^{-3}$, on flats 0.31 cm.m$^{-}$3, and on NFS 0.29 cm.m$^{-3}$, so low-SEPH shrubs grew on average 2.0 times as much on SFS as on NFS.

Table 5: Relative growth rate differences on different slope-aspects (site-$\mu/\sigma$-standardized, square root transformed, one-factor permanova)

| n: see table 6 below | Highest Raw Data Slope-Aspect Mean over Lowest | Slope-Aspect of Highest Values | | | | Slope-Aspect of Lowest Values |
|---|---|---|---|---|---|---|
| All randomly selected shrubs, site-$\mu/\sigma$-standardized, $\sqrt{g}/v^{1/3}$ paired by site | | SFS | > | Flats | ≈ | NFS |
| p-value | **0.0001** | | **0.0006** | | 0.6097 | S, F 0.023 |
| All randomly selected shrubs, site-$\mu/\sigma$-standardized, $\sqrt{g}/v^{1/3}$ paired by 2009 SEPH level | | | | | | |
| p-value | **0.0007** | | **0.0100** | | 0.3561 | |
| All randomly selected shrubs, $\sqrt{g}/v^{1/3}$ paired by site | 2.0 | | 1.7 | | | |
| p-value | **0.0076** | | **0.0258** | | 0.3818 | |
| All low-SEPH shrubs, site-$\mu/\sigma$-standardized, $\sqrt{g}/v^{1/3}$ paired by site | | | | | | |
| p-value | **0.0151** | | **0.0029** | | 0.9460 | |
| All low-SEPH shrubs, $\sqrt{g}/v^{1/3}$ paired by site | 2.0 | | | | | |
| p-value | **0.0164** | | **0.0033** | | 0.9530 | |

Table 6: Number of shrubs of each 2009 SEPH level on each site by slope-aspect sub-site (not including SEPH shrubs)

| n | Site 1 | | | | Site 2 | | | | Site 3 | | | | Total | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $N_{slope}$ | $F_N$ | $S_{slope}$ | $F_S$ | $N_{slope}$ | $F_N$ | $S_{slope}$ | $F_S$ | $N_{slope}$ | $F_N$ | $S_{slope}$ | $F_S$ | $N_{slope}$ | $F_N$ | $S_{slope}$ | $F_S$ |
| Low SEPH | 7 | 6 | 9 | 9 | 2 | 6 | 3 | 6 | 10 | 10 | 10 | 6 | 19 | 22 | 22 | 21 |
| High SEPH | 0 | 4 | 1 | 1 | 8 | 0 | 7 | 4 | 0 | 0 | 0 | 4 | 8 | 4 | 8 | 9 |

($N_{slope}$ or $S_{slope}$ refer to slopes of NFS or SFS explicitly, wheras $F_N$ and $F_S$ refer to flats at the bottom of NFS and SFS, respectively, which are combined into one treatment. )

There were differences in relative growth rate among sites but there was no interaction found between slope-aspect and site (table 7, 8). There were no statistically differently dispersed values when comparing pairs of sites. When including data for all shrubs, mean shrub shoot growth on site 1 was 0.55 cm.m$^{-3}$, on site 2 0.40 cm.m$^{-3}$, and on site 3 0.25 cm.m$^{-3}$. Therefore, for the highest growth rate, which occurred at site 1, shrub shoots grew 2.2 times as much on average as they did on site 3, which had the lowest growth rate. For low-SEPH shrubs only, mean shrub shoot growth on site 1 was 0.53 cm.m$^{-3}$, site 2 0.32 cm.m$^{-3}$, and site 3 0.25 cm.m$^{-3}$.

Table 7: Relative growth rate differences on different sites (unstandardized, square root transformed, two-factor permanovas)

| n: see table 8 below | Overall interaction term | | | Difference between sites | | |
|---|---|---|---|---|---|---|
| | Slope | Site | Slope X Site | 1 and 2 | 1 and 3 | 2 and 3 |
| All randomly selected shrubs $\surd g/v^{1/3}$ paired | | | | | | |
| p-value of difference | **0.0040** | **0.0002** | 0.4781 | **0.0240** | **0.0005** | **0.0281** |
| All low-SEPH shrubs $\surd g/v^{1/3}$ paired (n = as above) | | | | | | |
| p-value of difference | **0.0001** | **0.0083** | 0.8163 | **0.0208** | **0.0001** | 0.4210 |

Table 8: Number of shrubs of each 2009 SEPH level on each site by slope-aspect sub-site (including SEPH shrubs)

| n | Site 1 | | | | Site 2 | | | | Site 3 | | | | Total | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | N$_{slope}$ | F$_N$ | S$_{slope}$ | F$_S$ | N$_{slope}$ | F$_N$ | S$_{slope}$ | F$_S$ | N$_{slope}$ | F$_N$ | S$_{slope}$ | F$_S$ | N$_{slope}$ | F$_N$ | S$_{slope}$ | F$_S$ |
| Low SEPH | 10 | 6 | 10 | 12 | 2 | 7 | 3 | 6 | 11 | 11 | 12 | 6 | 23 | 24 | 25 | 24 |
| High SEPH | 2 | 4 | 3 | 1 | 8 | 2 | 7 | 4 | 1 | 4 | 1 | 6 | 11 | 10 | 11 | 11 |

(N$_{slope}$ or S$_{slope}$ refer to slopes of NFS or SFS explicitly, wheras F$_N$ and F$_S$ refer to flats at the bottom of NFS and SFS, respectively, which are combined into one treatment. )

Only on flats was the growth level found to be different among low and high SEPH shrubs, although of marginal significance ($p = 0.0750$). Within low-SEPH shrubs, there was a difference in growth rate of shrubs between SFS and flats ($p = 0.0004$), whereas within high-SEPH shrubs there was no difference ($p = 0.3323$) across slope-aspects. Average RGR of low-SEPH shrubs was 0.38 $cm.m^{-3}$ and of high-SEPH shrubs was 0.48, so high-SEPH shrubs on average grew 1.3 times more than low-SEPH shrubs. On flats, average RGR of low-SEPH shrubs was 0.321 and high-SEPH shrubs was 0.61, so high-SEPH shrubs grew on average 1.9 times more than low-SEPH.

## Shrub seeds and slope-aspect

On average, 4.2 seeds were initiated in pods and seeds had a mean final mass of 0.037 g over all sites. On sites 1 and 2 where seed pods were bagged (before the majority of insects had had access to the seedpods), when including all shrubs that bore seeds, there were few seeds eaten per pod and there was no difference by slope (table 9, table 10). There were 54% more viable seeds on south-facing slopes than on flats with an average of 3.10 viable seeds per pod over these two sites ($p = 0.009$, unpaired). When considering only shrubs with low SEPH densities, there were 63% more viable seeds on SFS than on NFS ($p = 0.0327$, unpaired) and 62% more than on flats ($p = 0.021$, unpaired).

Table 9: Seed parameter differences on different slope-aspects (site-μ/σ-standardized, one-factor permanova, unpaired)

| n: see table 10 below | | | Low-SEPH shrubs | | | | | | All shrubs | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Highest Slope-Aspect Mean over Lowest | | | | | | Highest Slope-Aspect Mean over Lowest | | | | | |
| Sites 1, 2, and 3 | Initiated seeds | | 1.06 | SFS | = | Flats | = | NFS | 1.17 | SFS | = | NFS | = | Flats |
| | 3 sites | p-val. | 0.5816 | | 0.5781 | | 0.9693 | | 0.2297 | | 0.9480 | | 0.4022 | |
| | | | | N: n = 7; S: n = 7; F: n = 9 | | | | | | N: n = 12; S: n = 14; F: n = 15 | | | | |
| Sites 1 and 2 only | Seed mass | | | NFS | = | Flats | = | SFS | | NFS | = | Flats | = | SFS |
| | 2 sites | p-val. | 0.5543 | | 0.7740 | | 0.6580 | | 0.1929 | | 0.5318 | | 0.3957 | |
| | Eaten seeds | | 2.71 | Flats | = | NFS | = | SFS | 1.40 | Flats | > | NFS | = | SFS |
| | 2 sites | p-val. | 0.9819 | | 0.5525 | | 0.9147 | | 0.9819 | | 0.5525 | | 0.9147 | |
| | Viable seeds | | 1.62 | SFS | > | NFS | = | Flats | 1.54 | SFS | > | NFS | > | Flats |
| | 2 sites | p-val. | **0.0327** | | 0.1020 | | 0.6762 | | **0.0128** | | 0.4811 | | 0.3032 | |

Table 10: Number of seed pod shrub replicates on each site by slope-aspect sub-site

| n | Site 1 | | | | Site 2 | | | | Site 3 | | | | Total | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $N_{slope}$ | $F_N$ | $S_{slope}$ | $F_S$ | $N_{slope}$ | $F_N$ | $S_{slope}$ | $F_S$ | $N_{slope}$ | $F_N$ | $S_{slope}$ | $F_S$ | $N_{slope}$ | $F_N$ | $S_{slope}$ | $F_S$ |
| Low SEPH | 7 | 3 | 4 | 4 | 0 | 0 | 3 | 2 | 4 | 2 | 8 | 2 | 11 | 5 | 15 | 8 |
| High SEPH | 2 | 2 | 2 | 1 | 3 | 0 | 5 | 3 | 1 | 1 | 1 | 2 | 6 | 3 | 8 | 6 |

($N_{slope}$ or $S_{slope}$ refer to slopes of NFS or SFS explicitly, wheras $F_N$ and $F_S$ refer to flats at the bottom of NFS and SFS, respectively, which are combined into one treatment. )

There were no differences in number of seeds initiated per pod by slope-aspect. On site 3 where seed pods did not receive bags, on the SFS there were over three times more seeds eaten per pod than on the flat (table 11) and twice as many as on the NFS. There was a mean 1.7 seeds per pod eaten and 1.8 viable on low-SEPH shrubs and 1.2 eaten and 2.5 viable on all shrubs on site 3. On site 3, there were approximately twice as many viable seeds on the flat than on the SFS (table 11) and more than twice as many on the NFS as on the SFS. Seed mass was higher on NFS than on flats only on site 3.

Table 11: Seed parameter differences on different slope-aspects on site 3 (site-μ/σ-standardized, two-factor permanova, unpaired)

| | | Low-SEPH shrubs | S: n = 8<br>N: n = 4<br>F: n = 4 | | | | | All shrubs | S: n = 9<br>N: n = 5<br>F: n = 7 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Highest Slope-Aspect Mean over Lowest | | | | | | Highest Slope-Aspect Mean over Lowest | | | | | |
| Seed mass | | 1.12 | NFS | > | SFS | = | Flats | 1.12 | NFS | > | SFS | = | Flats |
| | | | 0.038 | | 0.033 | | 0.034 | | 0.038 | | 0.033 | | 0.032 |
| | p-value | ***0.0554*** | | 0.2152 | | 0.5957 | | **0.0130** | | *0.0935* | | 0.6849 | |
| Initiated seeds | | | NFS | = | Flats | = | SFS | | NFS | **=** | Flats | = | SFS |
| | | | 3.325 | | 3.517 | | 4.316 | | 5.098 | | 4.690 | | 4.798 |
| | p-value | 0.6039 | | 0.9403 | | 0.5581 | | 0.6113 | | 0.7171 | | 0.8519 | |
| Eaten seeds | | 3.56 | SFS | > | NFS | = | Flats | 3.60 | SFS | > | NFS | = | Flats |
| | | | 2.455 | | 1.111 | | 0.690 | | 2.204 | | 0.889 | | 0.612 |
| | p-value | **0.0212** | | **0.0831** | | 0.5714 | | **0.0089** | | *0.0823* | | 0.5877 | |
| Viable seeds | | 5.53 | Flats | ≈ | NFS | > | SFS | 2.72 | Flats | ≈ | NFS | > | SFS |
| | | | 3.175 | | 2.904 | | 0.574 | | 3.138 | | 3.095 | | 1.155 |
| | p-value | **0.0046** | | 0.8007 | | **0.0145** | | **0.0309** | | 0.9746 | | 0.1153 | |

## SEPH/*Sextius* density and slope-aspect

Large numbers of *Sextius* nymphs, mostly of 1st instar, were first observed on *A. victoriae* shrubs in 2009, during counting which began on 5th November. In 2010 on the 2nd November, at the same time of year, there were also some 1st instar *Sextius* nymphs present, although counting in this season did not occur until January 2011. By the end of February 2010, there were only negligible densities of *Sextius* on most survey shrubs (with very few scale insects), whereas in February 2011 there were still many shrubs with high densities of SEPHs, comprised of 81% *Sextius* and 19% scale insects. In February 2011, only 24% of *Sextius* were mature, indicating that substantial emergence had occurred later than the spring emergence.

There was no relationship between shrub size and SEPH or *Sextius* densities. Survey SEPH densities in November 2009 and January 2011 correlated weakly but positively, as did *Sextius* densities for the same times (Chapter 4).

In January 2011, some SFS and most shrubs on flats beneath SFS on site 3 were no longer associated with SBI foraging, and the few SEPHs remaining were attended by *I. lividus* or *Rhytidoponera* sp. By February 2011, only negligible numbers of SEPHs were present on shrubs on these sub-sites. This appears to have made replication in analyses of 2011 SEPH densities and 2009 SEPH densities on sites 1-3 insufficient for statistical purposes (table 14).

For other analyses of 2011 SEPH densities, site 3 data was not included because of this change to the site, as explained in the methods.

In 2009, SEPH densities were higher on SFS than NFS, but no significant difference in *Sextius* densities was detected (figure 1; table 14). In January 2011, SEPH densities were highest on NFS, lower on SFS, and lowest on flats, whereas *Sextius* densities were higher on NFS than on SFS or flats (figure 2; table 14). In February 2011, SEPH densities were higher on NFS than on SFS and flats and *Sextius* densities followed the same pattern (figure 3; table 14). From the drier summer of 2009/2010 to the wetter summer of 2010/2011, changes in total SEPH and *Sextius* densities on survey sites 1 to 3 differed substantially between slope-aspects (tables 12, 13, 14). From December 2009 to January 2011, SEPH and *Sextius* densities changed significantly differently from zero only on NFS (SEPHs: p = 0.0014; *Sextius*: p = 0.0220) where they changed differently from on SFS or flats and were larger in January 2011 than in December 2009. In the year of high rainfall, SEPH populations were present for longer.

Table 12: Mean additive changes in SEPH/*Sextius* densities between years on shrubs for different slope-aspects (randomly selected shrubs only) (January 2011 density minus December 2009 density)

| Slope-Aspect | 2009 Nov to 2011 Jan – 3 sites (0 d.p.) | | 2011 Jan to Feb – 4 sites (0 d.p.) | |
|---|---|---|---|---|
| | [1]Total SEPHs | [1]*Sextius* | Total SEPHs | *Sextius* |
| **N** | +119 | +44 | +172 | +116 |
| **S** | -24 | -22 | +73 | +55 |
| **F** | +55 | +26 | +1 | +18 |
| Site | | | | |
| **1** | +146 | +92 | | |
| **2** | +22 | -17 | | |
| **3** | -7 | -12 | | |

Table 13: Mean slope-aspect and site raw SEPH and *Sextius* densities (m$^{-3}$) for December 2009 and January 2011 survey

| | **2009** | | **2011** | |
|---|---|---|---|---|
| Slope-Aspect: | SEPH | *Sextius* | SEPH | *Sextius* |
| **N** | 25.5 | 15.3 | 151.5 | 80.9 |
| **S** | 43.9 | 33.9 | 62.7 | 32.8 |
| **F** | 25.8 | 21.9 | 71.8 | 43.2 |
| Site: | | | | |
| **1** | 14.1 | 10.5 | 160.2 | 102.0 |
| **2** | 62.2 | 47.2 | 76.4 | 24.4 |
| **3** | 16.5 | 13.7 | 9.8 | 1.9 |
| **4** | | | 128.4 | 86.5 |

Table 14: SEPH and *Sextius* count differences for different slope-aspects (site-μ/σ-standardized (unless otherwise stated), one-factor permanova, unpaired) between years or months (randomly selected shrubs only)

(this table is continued on the next page ... )

| n: see table 15 below | Sites | Largest Difference | Slope-Aspect of Highest Values | | | | Slope-Aspect of Lowest Values | Different Dispersion |
|---|---|---|---|---|---|---|---|---|
| Nov. 2009 SEPH (no SEPH shrubs) standardized, $\sqrt[3]{S}$ | 1,2,3 | | SFS | > | Flats | ≈ | NFS | |
| p-val. of difference | | **0.0474** | | 0.7071 | | 0.0937 | | |
| Nov. 2009 *Sextius* (no SEPH shrubs) standardized, $\sqrt[3]{S}$ | 1,2,3 | | SFS | = | Flats | > | NFS | |
| p-val. of difference | | 0.1961 | | 0.3192 | | ***0.0823*** | | N, F<br>0.082 |
| Jan. 2011 SEPH (no SEPH shrubs) standardized, $\sqrt[3]{S}$ | 1, 2, 3 | | NFS | = | SFS | = | Flats | |
| p-val. of difference | | 0.5959 | | 0.5091 | | 0.9672 | | |
| Jan. 2011 *Sextius* (no SEPH shrubs) standardized, $\sqrt[3]{S}$ | 1, 2, 3 | | NFS | = | SFS | = | Flats | |
| p-val. of difference | | 0.8799 | | 0.6341 | | 0.7417 | | |
| Jan. 2011 SEPH (no SEPH shrubs) standardized, $\sqrt[3]{S}$ | 1, 2, 3, 4 | | NFS | = | Flats | = | SFS | |
| p-val. of difference | | 0.5157 | | 0.3779 | | 0.7324 | | |
| Jan. 2011 *Sextius* (no SEPH shrubs) standardized, $\sqrt[3]{S}$ | 1, 2, 3, 4 | | NFS | = | Flats | > | SFS | |
| p-val. of difference | | 0.6718 | | 0.3855 | | | 0.6419 | |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Jan. 2011 SEPH (no SEPH shrubs) standardized, $^3\sqrt{S}$ | 1,2,4 | | NFS | > | SFS | > | Flats | |
| p-val. of difference | | **0.0006** | | **0.0381** | | **0.0460** | | N, F<br>0.063<br>N, S<br>0.020 |
| Jan. 2011 *Sextius* (no SEPH shrubs) standardized, $^3\sqrt{S}$ | 1,2,4 | | NFS | > | SFS | = | Flats | |
| p-val. of difference | | **0.0031** | | **0.0361** | | 0.3463 | | N, S<br>0.049 |
| Feb. 2011 SEPH (no SEPH shrubs) standardized, $^3\sqrt{S}$ | 1,2,4 | | NFS | > | SFS | = | Flats | |
| p-val. of difference | | **0.0001** | | **0.0002** | | 0.3994 | | N, F<br>0.016<br>N, S<br>0.0008 |
| Feb. 2011 *Sextius* (no SEPH shrubs) standardized, $^3\sqrt{S}$ | 1,2,4 | | NFS | > | SFS | = | Flats | |
| p-val. of difference | | **0.0001** | | **0.0001** | | 0.9963 | | N, F<br>0.024<br>N, S<br>0.001 |
| Change in SEPH from Nov. 2009 to Jan. 2011 (with SEPH-shrubs) non-standardized, $^3\sqrt{S}$ | 1,2,3 | Table 12 | NFS | > | Flats | = | SFS | |
| p-val. of difference | | **0.0050** | | **0.0041** | | 0.6499 | | |
| Change in *Sextius* from Nov. 2009 to Jan. 2011 (with SEPH-shrubs) non-standardized, $^3\sqrt{S}$ | 1,2,3 | | NFS | > | Flats | = | SFS | |
| p-val. of difference | | **0.0207** | | **0.0095** | | 0.9404 | | |

( … this table is continued from the previous page. )

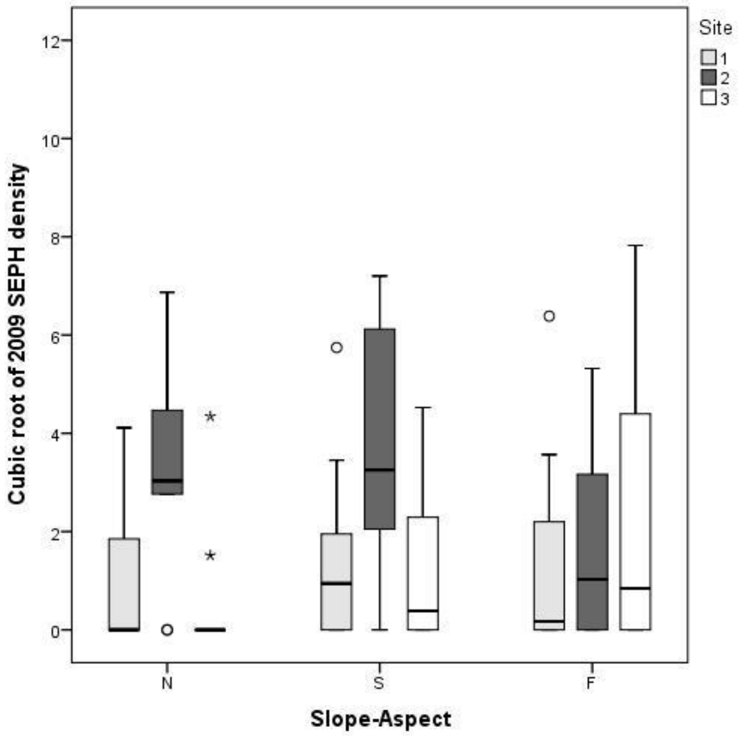

Figure 1: 2009 SEPH densities on sites by slope-aspect ($^3\sqrt{S}$)

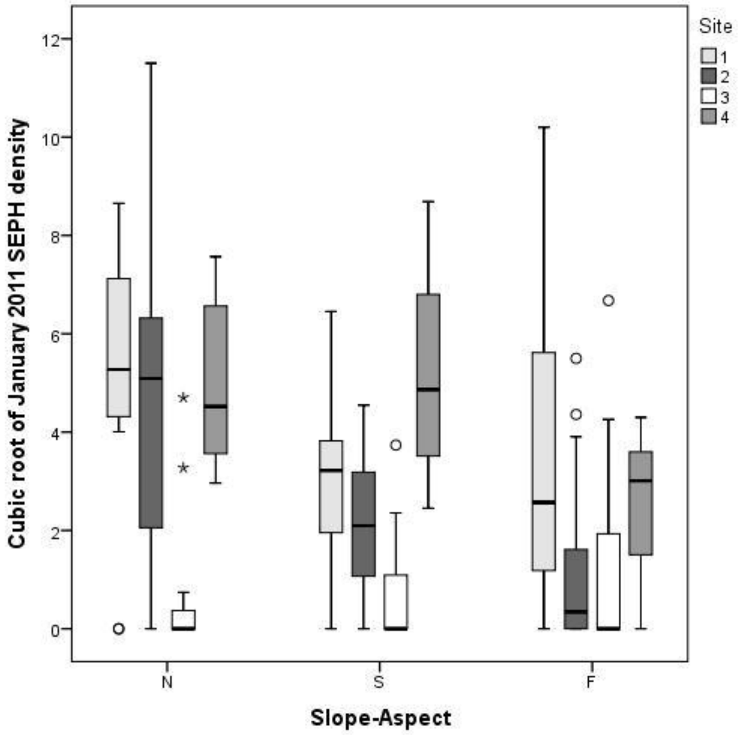

Figure 2: January 2011 SEPH densities on sites by slope-aspect ($^3\sqrt{S}$)

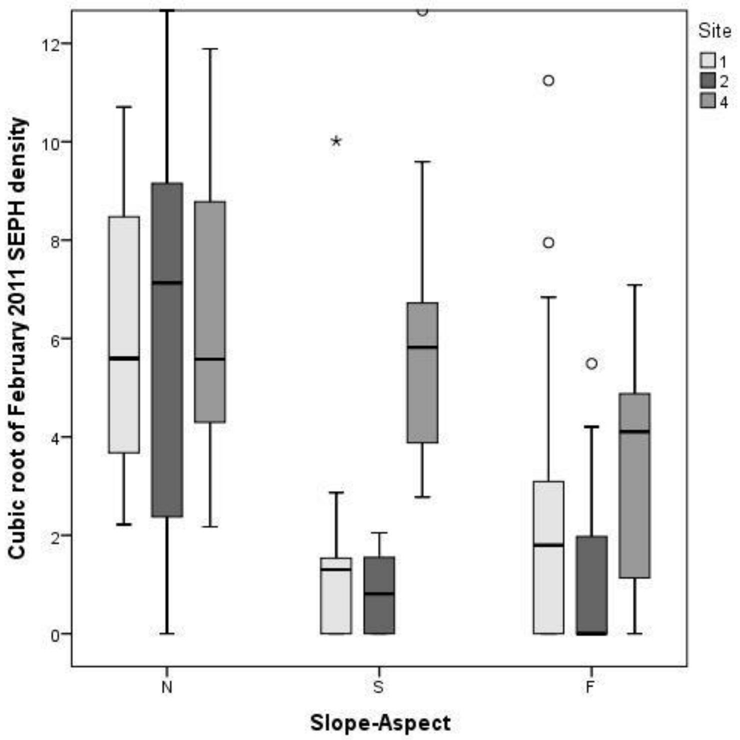

Figure 3: February 2011 SEPH densities on sites by slope-aspect ($^3\sqrt{S}$)

Table 15: SEPH/*Sextius* replicates on each site by slope-aspect sub-site

| n | Site 1 | | | | Site 2 | | | | Site 3 | | | | Site 4 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $N_{slope}$ | $F_N$ | $S_{slope}$ | $F_S$ | $N_{slope}$ | $F_N$ | $S_{slope}$ | $F_S$ | $N_{slope}$ | $F_N$ | $S_{slope}$ | $F_S$ | $N_{slope}$ | $F_N$ | $S_{slope}$ | $F_S$ |
| no SEPH-shrubs | 7 | 9 | 9 | 10 | 10 | 11 | 10 | 10 | 10 | 10 | 10 | 10 | 10 | 5 | 10 | 5 |
| with SEPH-shrubs | 12 | 9 | 13 | 13 | 10 | 14 | 10 | 10 | 12 | 15 | 14 | 12 | 10 | 5 | 10 | 5 |

($N_{slope}$ or $S_{slope}$ refer to slopes of NFS or SFS explicitly, wheras $F_N$ and $F_S$ refer to flats at the bottom of NFS and SFS, respectively, which are combined into one treatment. )

SEPH/*Sextius* densities differed between sites, were differently dispersed among some slope-aspects across sites, and there were interactions between slope-aspect and site in effects on their densities (table 16). SEPH/*Sextius* densities in December 2009 were higher on site 2 than on sites 1 and 3. In January 2011, the rank order of sites with regard to *Sextius* densities was 1 > 4 > 2 > 3, but although SEPH densities followed a similar pattern, differences between some pairs of sites were not significant.

Table 16: Shrub volume, SEPH,density and *Sextius* density differences for different sites ($^3\sqrt{S}$ transformed, two-factor permanova) between years or months (randomly selected shrubs only)

| n: see table 15 above | Slope | Site | Slope X Site | 1 and 2 | 1 and 3 | 1 and 4 | 2 and 3 | 2 and 4 | 3 and 4 | Different Dispersion |
|---|---|---|---|---|---|---|---|---|---|---|
| 2009 SEPH densities, $^3\sqrt{S}$ | | | | | | | | | | 1, 2 0.042 |
| p-val. | 0.4282 | **0.0001** | **0.0029** | **0.0002** | 0.7847 | | **0.0001** | | | |
| 2009 *Sextius* densities, $^3\sqrt{S}$ | | | | | | | | | | 1, 2 0.047 |
| p-val. | 0.7815 | **0.0001** | **0.0152** | **0.0001** | 0.8198 | | **0.0002** | | | |
| January 2011 SEPH densities, $^3\sqrt{S}$ | | | | | | | | | | N, F 0.003<br>N, S 0.003<br>1, 3 0.005<br>2, 3 0.018 |
| sites 1 to 3 p-val | | | | | | | | | | |
| sites 1, 2, and 4 p-val | **0.0021** | **0.0029** | 0.1355 | 0.2865 | | ***0.0539*** | | **0.0006** | | |
| all four sites p-val. | **0.0026** | **0.0001** | **0.0011** | 0.1516 | **0.0001** | 0.2295 | **0.0001** | **0.0016** | **0.0001** | |
| January 2011 *Sextius* densities, $^3\sqrt{S}$ | | | | | | | | | | N, F 0.001<br>N, S 0.001<br>1, 2 0.025<br>1, 3 0.001<br>1, 4 0.062<br>2, 3 0.002<br>3, 4 0.001 |
| sites 1 to 3 p-val | | | | | | | | | | |
| sites 1, 2, and 4 p-val | **0.0004** | **0.0174** | ***0.0758*** | 0.1578 | | 0.2233 | | **0.0018** | | |
| all four sites p-val. | **0.0034** | **0.0001** | **0.0172** | **0.0296** | **0.0001** | ***0.0553*** | **0.0001** | **0.0003** | **0.0001** | |

Overall, 2nd instar to adult *Sextius* comprised 89.5% of SEPH densities in the 2009/2010 survey, only 56% in the 2010/2011 January survey, and 81.2% in the 2011 February survey (chapter 4).

### Shrub growth with respect to slope-aspect and SEPH/*Sextius* density

Raw growth rate, which limits densities of SEPH/*Sextius* (chapter 4) was 1.9 times higher on SFS than on NFS and higher on SFS than on flats (table 17, 20). Including all shrubs, mean shrub shoot growth on SFS was 52.2cm , on flats 35.7cm, and on NFS 27.3cm. Including low-SEPH shrubs only, mean shrub shoot growth was similar, giving 50.1cm on SFS, 29.6cm on flats, and 27.9cm on NFS. Thus low-SEPH shrubs grew on average 1.8 times as much on SFS as on NFS.

Table 17: Raw growth differences for different slope-aspects (square-root transformed, all shrubs)

| n: see table 20 below | Highest Raw Data Slope-Aspect Mean over Lowest | Slope-Aspect of Highest Values | | | | Slope-Aspect of Lowest Values |
|---|---|---|---|---|---|---|
| All shrubs, site μ/σ-standardized raw, √g paired by site | | SFS | > | Flats | ≈ | NFS |
| p-val. | **0.0002** | | **0.0015** | | 0.5668 | |
| All shrubs, √g paired by site | 1.9 | | | | | |
| p-val. | 0.1618 | | ***0.0737*** | | 0.8330 | |
| All low-SEPH shrubs, site μ/σ-standardized raw √g paired | | | | | | |
| p-val. | **0.0038** | | **0.0013** | | 0.6539 | |
| All low-SEPH shrubs, √g paired | 1.8 | | | | | |
| p-val. | **0.0082** | | **0.0021** | | 0.9350 | |

Site and slope-aspect did not interact in their effects on raw growth (tables 18, 20). Including all shrubs, mean shrub shoot growth on site 1 was 48.7cm, on site 2 39.5cm, and on site 3 26.8cm, so site 1 shrub shoots grew 1.8 times as much on average as those on site 3. Including only low-SEPH shrubs, mean shrub shoot growth on site 1 was 50.5cm, on site 2 31.7cm, and on site 3 26.4cm.

Table 18: Raw growth differences for different sites (all shrubs)

| n: see table 20 below | Interaction term | | | Difference between sites | | | Different Dispersion |
|---|---|---|---|---|---|---|---|
| | Slope | Site | Slope X Site | 1 and 2 | 1 and 3 | 2 and 3 | |
| All shrubs,<br>√g paired | | | | | | | NS 0.0031<br>NF 0.0001 |
| p-value of difference | *0.0930* | **0.0001** | 0.1995 | **0.0167** | **0.0001** | ***0.0515*** | |
| All low-SEPH shrubs<br>√g paired | | | | | | | NS *0.0525*<br>SF 0.0354 |
| p-value of difference | **0.0014** | **0.0030** | 0.3218 | ***0.0753*** | **0.0004** | 0.6240 | |

(N refers to SFS, S refers to SFS, and F refers to flats. Pairs of these, e.g. NS, refer to differences between the two. )

2011 SEPH levels and slope-aspect interacted in their effects on raw shrub growth (p = 0.028) (table 19, 20). Growth on low-SEPH shrubs was different from on high-SEPH shrubs only on flats, where it was less. On low-SEPH shrubs only, growth was different on SFS compared to NFS and flats. Over all shrubs, mean raw growth on high-SEPH shrubs was 50.5cm and on low-SEPH shrubs was 32.4cm, so high-SEPH shrubs grew on average 1.6 times more. On flats only, mean raw growth on high-SEPH shrubs was 73.3cm and on low-SEPH shrubs was 31.2cm, so high-SEPH shrubs grew on average 2.3 times as much.

Table 19: Survey raw growth rate slope-SEPH interactions site-μ/σ-standardized square root values

| n: see table 20 below | | | | | | Different Dispersion |
|---|---|---|---|---|---|---|
| | N | S | F | Low-SEPH | High-SEPH | |
| Low-SEPH versus high | *0.0926* | 0.1881 | **0.0082** | | | 0.0020 |
| NS | | | | **0.0003** | 0.2261 | |
| NF | | | | 0.5736 | 0.2162 | |
| SF | | | | **0.0001** | 0.8424 | 0.0060 |

Table 20: Number of shrubs of each 2011 SEPH level on each site by slope-aspect sub-site, including SEPH shrubs

| n | Site 1 | | | | Site 2 | | | | Site 3 | | | | Total | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $N_{slope}$ | $F_N$ | $S_{slope}$ | $F_S$ | $N_{slope}$ | $F_N$ | $S_{slope}$ | $F_S$ | $N_{slope}$ | $F_N$ | $S_{slope}$ | $F_S$ | $N_{slope}$ | $F_N$ | $S_{slope}$ | $F_S$ |
| Low SEPH | 2 | 1 | 4 | 10 | 4 | 12 | 6 | 8 | 10 | 14 | 12 | 8 | 16 | 27 | 22 | 26 |
| High SEPH | 10 | 9 | 9 | 3 | 6 | 2 | 4 | 2 | 2 | 1 | 1 | 4 | 18 | 12 | 14 | 9 |

($N_{slope}$ or $S_{slope}$ refer to slopes of NFS or SFS explicitly, wheras $F_N$ and $F_S$ refer to flats at the bottom of NFS and SFS, respectively, which are combined into one treatment. )

## Small arthropods on shrubs and slope-aspect

There were differences among slope-aspects in assemblages of small arthropods caught on sticky traps in 2010 January on low-SEPH shrubs and some were statistically differently dispersed among slope-aspects (table 21). "Minute" wasp densities were higher on SFS than on flats. Predators, mirid/lygaeids, thrips, and "other" Auchenorrhyncha were in higher densities on SFS than on NFS. Occurrences of other taxa were low and when included in analyses, the matrix degraded due to excessive zeroes, so they were left out.

Table 21: Small arthropod assemblage differences on different slope-aspects on low-SEPH shrubs in 2010 January on sites 1 to 3 (site-μ/σ-standardized, square root transformed, one-factor permanovas)

| n = 13, (except S: n= 11) | Highest Slope-Aspect Mean over Lowest | Slope-Aspect of Highest Values | | | | Slope-Aspect of Lowest Values | Different dispersion |
|---|---|---|---|---|---|---|---|
| "Minute" wasps | 2.0 | SFS | ≈ | NFS | > | Flats | |
| Mean count | | 196.5 | | 148.5 | | 97.8 | |
| p-value | **0.0370** | | 0.4167 | | 0.2313 | | N, F 0.080, S, F 0.031 |
| Predator | 2.2 | SFS | = | Flats | > | NFS | |
| Mean count | | 3.3 | | 2.8 | | 1.5 | |
| p-value | 0.1457 | | 0.6194 | | 0.1064 | | N, S 0.023, S, F 0.016 |
| Mirid/ Lygaeid | 2.1 | SFS | > | Flats | ≈ | NFS | |
| Mean count | | 7.3 | | 4.3 | | 3.4 | |
| p-value | ***0.0770*** | | *0.0865* | | 0.6355 | | |
| Mirid/lygaeids and predators | | SFS | > | Flats | = | NFS | |
| p-value | ***0.0669*** | | 0.2001 | | 0.3187 | | |
| Thrips | 1.7 | SFS | > | Flats | > | NFS | |
| Mean count | | 44.4 | | 34.2 | | 26.3 | |
| p-value | ***0.0656*** | | 0.2769 | | 0.2545 | | |
| Psyllid | 1.2 | SFS | = | NFS | > | Flats | |
| Mean count | | 39.5 | | 38.6 | | 21.3 | |
| p-value | 0.1409 | | 0.3593 | | 0.6315 | | N, S 0.020 |
| "Other" Auchenorrhyncha | 1.6 | SFS | = | Flats | > | NFS | |
| Mean count | | 12.9 | | 14.9 | | 8.2 | |
| p-value | **0.0297** | | 0.9056 | | **0.0461** | | |
| Herbivore, "other" | 1.9 | SFS | > | Flats | > | NFS | |
| Mean count | | 2.6 | | 2.1 | | 1.4 | |
| p-value | 0.3169 | | 0.6924 | | 0.6505 | | |

On low-SEPH shrubs there was an interaction of site and slope-aspect only for "minute" wasp densities (table 22). There were differences in density of "minute" wasps, psyllids, "other" Auchenorrhyncha, and "other" herbivores by site.

Table 22: Small arthropod assemblage differences on different sites on low-SEPH shrubs in 2010 January on sites 1 to 3 (unstandardized, square root transformed, two-factor permanovas)

| n = 13, (except S: n= 11) | Interaction Term | | | Difference between sites: | | | Different Dispersion |
|---|---|---|---|---|---|---|---|
| | Slope | Site | Slope X Site | 1 and 2 | 1 and 3 | 2 and 3 | |
| "Minute" wasp | | | | | | | |
| p-val. | 0.5560 | **0.0002** | **0.0054** | 0.2955 | **0.0045** | **0.0002** | SF *0.0608* |
| Predator | | | | | | | |
| p-val. | 0.1863 | 0.1141 | 0.7600 | 0.5974 | **0.0497** | 0.1405 | |
| Mirid/ Lygaeid | | | | | | | |
| p-val. | 0.2935 | *0.0931* | 0.2805 | 0.3229 | 0.2218 | **0.0433** | |
| Thrips | | | | | | | |
| p-val. | 0.3129 | 0.3333 | 0.1658 | 0.9619 | 0.1507 | 0.2407 | |
| Psyllid | | | | | | | |
| p-val. | 0.6867 | **0.0292** | 0.1029 | **0.0012** | 0.3012 | 0.1402 | 2,3 0.0078 |
| "Other" Auchenorrhyncha | | | | | | | |
| p-val. | 0.1425 | **0.0009** | 0.3942 | 0.2000 | **0.0139** | **0.0005** | |
| Herbivore, "other" | | | | | | | |
| p-val. | 0.3639 | **0.0292** | 0.4682 | 0.2064 | 0.1937 | **0.0043** | |

High-SEPH shrubs were analyzed in a similar way to low-SEPH shrubs in one-factor analyses of site mean standardized data and there were differences from patterns on low-SEPH shrubs (table 23). For predators, on high-SEPH shrubs densities on both flats and SFS were higher than on NFS, whereas they were all the same on low-SEPH shrubs. For mirid/lygaeids, on high-SEPH shrubs densities were similar on all slope-aspects, whereas on low-SEPH shrubs they were higher on SFS than on flats or NFS. On high-SEPH shrubs, thrips densities were similar on all slope-aspects, whereas on low-SEPH shrubs they were higher on SFS than on NFS. Psyllid densities, on high-SEPH shrubs densities were higher on flats and SFS than on NFS, whereas on low-SEPH shrubs they were all similar. On high-SEPH shrubs, "other" Auchenorrhyncha were not more abundant on flats than on NFS, whereas they were on low-SEPH shrubs.

Table 23: Small arthropod assemblage differences on different slope-aspects on high-SEPH shrubs in 2010 January on sites 1 to 3 (site-μ/σ-standardized, square root transformed, one-factor permanova, even numbers of shrubs on each sub-site)

| n = 4 (N), 5 (S), 9 (F) | Highest Slope-Aspect Mean over Lowest | Slope-Aspect of Highest Values | | | | Slope-Aspect of Lowest Values |
|---|---|---|---|---|---|---|
| "Minute" wasp | 2.7 | SFS | > | NFS | ≈ | Flats |
| Mean count | | 191.4 | | 118.3 | | 71.0 |
| p-value | **0.0089** | | 0.1861 | | 0.1797 | |
| Predator | 4.7 | Flats | = | SFS | > | NFS |
| Mean count | | 7.0 | | 3.8 | | 1.5 |
| p-value | **0.0338** | | 0.9386 | | ***0.0667*** | |
| Mirid/ Lygaeid | | SFS | > | Flats | > | NFS |
| Mean count | | 4.4 | | 3.4 | | 1.7 |
| p-value | 0.2541 | | 0.6208 | | 0.2348 | |
| Thrips | | Flats | = | SFS | > | NFS |
| Mean count | | 31.6 | | 38.6 | | 16.3 |
| p-value | 0.1111 | | 0.7780 | | 0.2077 | |
| Psyllid | 3.4 | Flats | ≈ | SFS | > | NFS |
| Mean count | | 27.5 | | 13.8 | | 8.0 |
| p-value | **0.0074** | | 0.1698 | | ***0.0527*** | |
| "Other" auchenorrhyncha | 3.6 | SFS | > | Flats | > | NFS |
| Mean count | | 17.0 | | 10.0 | | 4.7 |
| p-value | **0.0111** | | 0.1193 | | 0.1480 | |
| Herbivore, "other" | | SFS | > | NFS | = | Flats |
| Mean count | | 1.4 | | 0.8 | | 0.6 |
| p-value | 0.1494 | | 0.4028 | | 0.6927 | |

When considering both high and low SEPH densities, there was an interaction of psyllids densities with slope-aspect by SEPH level (table 24), confirming the difference between densities on shrubs with low and high SEPH levels noted above. Within low-SEPH shrubs, NFS shrubs did not have significantly different numbers of psyllids compared to those on flats (p = 0.1220) or on SFS (p = 0.9916), but within high-SEPH shrubs, NFS shrubs had lower densities of psyllids compared to shrubs on flats (p = 0.0620) and lower than shrubs on SFS (p = 0.07010), both of marginal significance. Within flats, psyllids densities were not different among low- and high-SEPH shrubs (p = 0.5908) whereas on NFS and SFS, there were higher densities on low-SEPH shrubs (p = 0.0273 and p = 0.0310, respectively). As for low-SEPH shrubs, densities of "minute" wasps were higher on SFS than on flats. Densities of thrips and "other" Auchenorrhyncha were higher on shrubs on SFS than on NFS and densities of "other" Auchenorrhyncha and "other" herbivores were higher on shrubs on SFS than on flats. On high-SEPH shrubs higher densities of "minute" wasps occurred on shrubs on SFS than on NFS.

Table 24: Small arthropod assemblage differences on different slope-aspects on low- and high-SEPH shrubs in 2010 January on sites 1 to 3 (site-μ/σ-standardized, square root transformed, one-factor permanovas, even numbers of low and high SEPH shrubs on each sub-site, paired by SEPH-level)

| n = 4 (N), 5 (S), 9 (F) | Highest Slope-Aspect Mean over Lowest | Slope-Aspect of Highest Values | | | | Slope-Aspect of Lowest Values | Different Dispersion | Slope X SEPH |
|---|---|---|---|---|---|---|---|---|
| "Minute" wasp | 1.6 | SFS | > | NFS | = | Flats | | |
| p-val of difference | **0.0042**<br>(unpaired: )<br>**0.0038** | | ***0.0712***<br>***0.0697*** | | 0.8491<br>0.8574 | | NS *0.0720*<br>SF 0.0030 | 0.1831 |
| Predators | 3.5 | Flats | ≈ | SFS | > | NFS | | |
| p-val of difference | 0.1107 | | 0.8737 | | 0.1728 | | | 0.3507 |
| Mirid/ Lygaeid | 1.2 | SFS | > | NFS | ≈ | Flats | | |
| p-val of difference | ***0.0603*** | | 0.2434 | | 0.7721 | | | 0.4764 |
| Thrips | 1.8 | SFS | > | Flats | > | NFS | | |
| p-val of difference | **0.0260** | | 0.1525 | | 0.2548 | | | 0.2776 |
| "Other" auchenorrhyncha | 2.0 | SFS | > | Flats | > | NFS | | |
| p-val of difference | **0.0463** | | **0.0496** | | 0.1737 | | | 0.1817 |
| Psyllid | | Flats | = | NFS | = | SFS | | |
| p-val of difference | 0.3625 | | 0.9304 | | 0.3694 | | | **0.0121** |
| "Other" herbivores | 1.1 | SFS | > | NFS | ≈ | Flats | | |
| p-val of difference | **0.0009** | | 0.2659 | | 0.5592 | | | 0.3203 |

Considering shrubs of low and high SEPH-levels, some arthropod taxa densities differed by site (table 25), these being "minute" wasps, predators, thrips, psyllids, and "other" Auchenorrhyncha. Slope-aspect and site interacted in their effects on thrips. Site and SEPH level interacted in their effects on "minute" wasps. There was no interaction between slope-aspect and SEPH density in their effects on assemblages of other small arthropods. There were no three-way interactions of site X slope-aspect X SEPH level.

Table 25: Small arthropod assemblage differences on different sites on low- and high-SEPH shrubs in 2010 January on sites 1 to 3 (unstandardized, square root transformed, two-factor permanova)

| Low-SEPH: (N: n = 10, S: n= 10, F: n=13)<br>High-SEPH: n = 6 (N), 7 (S), 9 (F) | Interaction Term | | | | | Difference between sites | | | Different Dispersion |
|---|---|---|---|---|---|---|---|---|---|
| | Slope | Site | SEPH | Slope X Site | Site X SEPH | 1 and 2 | 1 and 3 | 2 and 3 | |
| "Minute" wasp | | | | | | | | | SF 0.0001 |
| p-value | 0.2711 | ***0.0644*** | 0.6200 | 0.1206 | **0.0045** | 0.1217 | **0.0424** | 0.2256 | |
| Predator | | | | | | | | | |
| p-value | 0.1636 | ***0.0800*** | **0.0046** | 0.1319 | 0.9590 | 0.9919 | ***0.0557*** | **0.0425** | |
| Mirid/ Lygaeid | | | | | | | | | |
| p-value | 0.2287 | 0.4828 | 0.2430 | 0.7692 | 0.5096 | 0.8921 | 0.3183 | 0.2948 | N, S 0.0250<br>S, F 0.0340<br>1, 3 0.0430 |
| Thrips | | | | | | | | | |
| p-value | 0.2262 | ***0.0518*** | 0.4523 | ***0.0673*** | *0.0881* | **0.0389** | **0.0284** | 0.7204 | |
| Psyllid | | | | | | | | | |
| p-value | 0.4266 | **0.0362** | 0.1534 | 0.5542 | 0.8501 | **0.0027** | 0.3420 | 0.1707 | 2, 3 0.0170<br>lo/hi 0.0230 |
| "Other" auchenorrhyncha | | | | | | | | | |
| p-value | 0.1371 | ***0.0780*** | 0.5316 | 0.1406 | *0.0971* | 0.6801 | ***0.0512*** | ***0.0682*** | |
| Herbivore, "other" | | | | | | | | | |
| p-value | 0.3365 | 0.3818 | 0.4558 | 0.3198 | 0.1343 | 0.6476 | 0.4389 | 0.1201 | |

## Discussion

This study has found that slope-aspect affects the growth and reproductive success of *A. victoriae* shrubs and assemblages of insects on them. Both effects are likely to be determined by both microclimate and soil differences. Some of these factors interacted, bi-directionally in some cases (figure 4), and interacting effects of both slope-aspect and site on other factors suggest a complex model of ecological dynamics. This study has confirmed that soil nutrient concentrations can differ among slope-aspects in semi-arid Australia, even though effects of site differences were stronger. Slope-aspect effects on shrub growth did not interact with effects of site differences, suggesting that moisture had primary importance in regulating growth. During a year of very high rainfall *A. victoriae* shoot growth was higher on the cooler SFS than on flats or NFS, which was probably due to higher soil moisture and associated nutrient uptake. Since effects on shrub growth were observed during a 12-month period of unusually high rainfall when moisture was likely not to be limiting, soil nutrient differences are likely to have contributed to both slope-aspect and site differences in plant growth. Slope-aspect also affected shrub reproductive success, which was an important variable affected by the SEPH-SBI association on shrubs (Chapter 4). On sites where insects that destroy seeds were excluded, during a year of average rainfall there were 54-63% more viable seeds on SFS than on flats. However, where insects could consume seeds, there were over three times more seeds destroyed on SFS than on flats and more on SFS than on NFS. This resulted in much higher numbers of viable seeds per pod on flats and on NFS than on SFS, which suggests important indirect slope-aspect effects on shrubs through small arthropods.

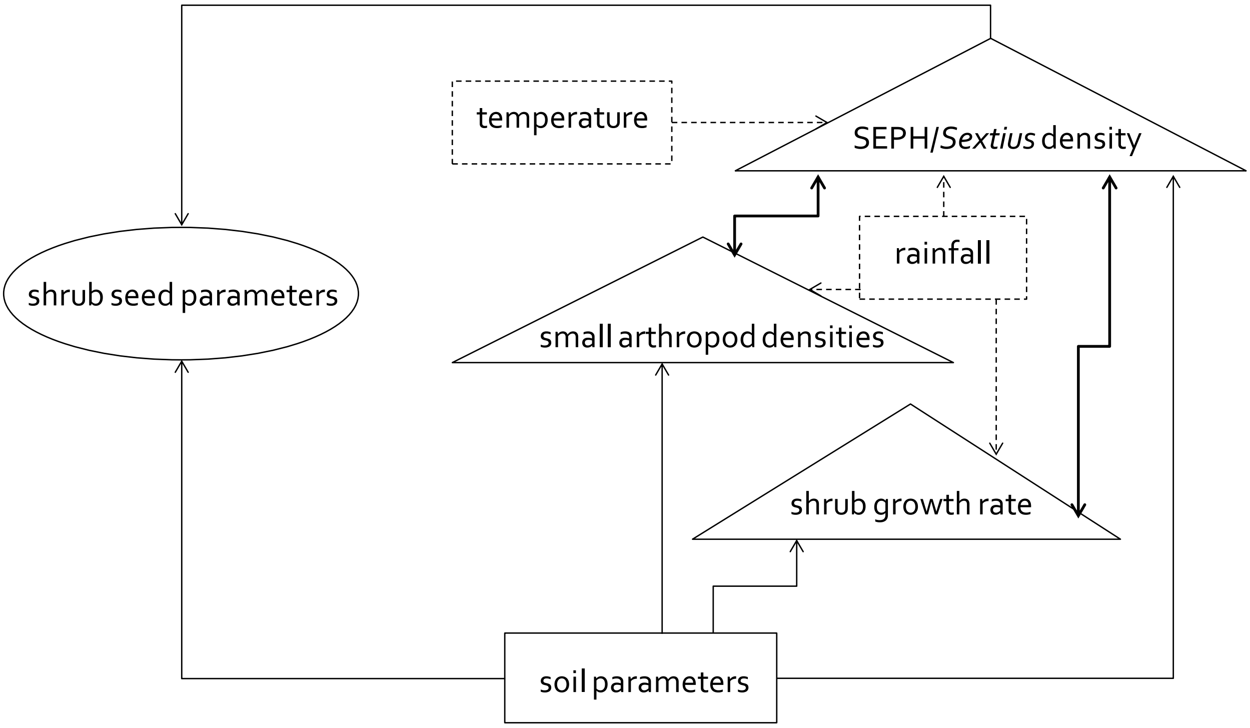

Figure 4: Interactions between factors considered in this study which slope-aspect might affect

Changes among years in densities of SEPHs among slope-aspects are consistent with the possibility of there being an optimum moisture level /humidity and temperature for SEPHs in semi-arid conditions. In the year of average rainfall, there were higher SEPH densities on SFS than on NFS, but in the year of high rainfall, there were higher densities of total SEPHs and of *Sextius* on NFS than on flats and also higher densities of *Sextius* than on SFS. Strong interactions of slope-aspect and site effects and large differences between sites point to substantial influences of other important factors which may be related to typical arid-zone concentrations of some soil nutrients such as low soil nitrogen or sulphur. During a year of relatively low rainfall, differences in SEPH densities between slope-aspects and sites might vary quantitatively or qualitatively due to moisture limitation overriding the importance of nutrient uptake. Also, reduced photosynthesis caused by moisture limitation might lead to reductions in recruitment to rhizobia, resulting in reduced nitrogen availability to the shrub. The higher densities of many insect taxa on shrubs on SFS than on NFS in the year of mean rainfall would be consistent with preference for cooler damper conditions. However total densities of parasitoid ("minute") wasps were more abundant on SFS than on flats on low-SEPH shrubs and more abundant on SFS than on NFS or flats on high-SEPH shrubs. This points to parasitoid populations being potentially regulated by interactions of slope-aspect and SEPH density and since parasitoid wasps and SEPHs might regulate populations of each other, the interaction might be important to SEPH populations on different slope-aspects. Site differences in arthropod assemblages were in some cases stronger than slope-aspect differences, which might be caused by soil nutrients with which many arthropod groups correlated. This might even be the mechanism through which site differences affect SEPH densities. Thus slope-aspect differences in the abiotic environment appear to affect dynamics of arthropod assemblages and probably interact with both site differences and local weather, forming a complex network of effects.

## Slope-aspect effects on soil

Soil macronutrient concentrations on the sites in this study differ with slope-aspect, as they do in environments on other continents (Rech et al 2001, Yimer et al 2006). That soil parameters on different unmatched slope-aspects on site 2 were no more different than on the matched sites lends support for effects of slope. Different dispersion of total nitrogen, organic carbon, and pH on flats could be due to flats being sampled at the bottom of two different slopes. Alternatively, it might be caused by some different process on flats such as different topography causing variability in spatial dynamics with regard to water drainage and plant litter accumulation. These differences might affect these three soil parameters directly or subsequently through different distribution of vegetation. Increased weathering of rock from higher insolation on NFS or higher soil moisture levels on SFS (Rech et al 2001) might contribute to different soil nutrient concentrations on slope-aspects, as might hill-slope stability as a result of vegetation differences (Rech et al 2001), and deposition by different plant communities on contrasting slopes. Microbial activity is likely to be higher at times on the moister cooler SFS when NFS temperatures are excessive but higher on NFS when high temperatures are not limiting and when SFS temperatures are low. This might result in higher sulphur mineralization rates and rhizobial and mycorrhizal activity on SFS, which could contribute to increased shrub growth. Higher soil concentrations of total nitrogen and organic carbon on SFS could result from accumulation of successive generations of plants of which there is typically higher total biomass on $\mathrm{SFS}_{\mathrm{southern}}$ similar to $\mathrm{NFS}_{\mathrm{northern}}$ (Noy-Meir 1973). Fallen organic matter would on average remain moist for longer on SFS, which might allow more time for decomposition and resultant organic carbon deposition. Higher soil sulphur on SFS might be due to increased accumulation by microbes in the higher density organic matter. Lower potassium levels on SFS than on NFS or flats might be from higher uptake by vegetation, facilitated by higher soil moisture and probably greater plant biomass from which it is quickly recycled. Also, lower potassium on SFS may be due to lower weathering rates of cooler rocks, slowing the release of potassium into the surrounding soil. Higher available phosphorous levels on slopes than flats might be from greater rock weathering on slopes that are more exposed to wind and rain. Since total nitrogen, which is the most commonly limiting nutrient with respect to vegetative growth and tends to be particularly low in arid zone soils (Crawford and Gosz 1982), was in higher concentrations on SFS, these data provide some reason to associate NFS with soil characteristics more typical of aridity than SFS. The different tendencies of soil nutrients with respect to slope-aspect such as the patterns found for total nitrogen, available phosphorous, potassium, and organic carbon probably alter plant community composition and structure.

### Site and soil

Despite significant patterns in soil nutrient concentrations between slope-aspects, site differences were larger and caused substantial interactions with slope-aspect. This means the effect of slope-aspect on soil does not seem to be as important as site variation. For example, the largest difference in slope-aspect mean concentrations was 134% for available phosphorous, whereas the smallest difference in site means was 129% for available phosphorous and potassium and the largest 156% for organic carbon. The largest difference in slope-aspect pattern was among sites 1 and 4 which were adjacent to each other (Chapter 4), so strong heterogeneity can occur at a relatively small spatial scale. Different statistical dispersion of soil parameters including total nitrogen among some pairs of sites were possibly the result of different factors dominating soil sorting on soils that reflected differences in topography by slope-aspect mentioned above. However, slope-aspect contributes to spatial heterogeneity, and possibly biodiversity as a result, in arid lands.

## Weather and slope-aspect effects on the system

Unusually high rainfall at the study site in 2010 was likely to have caused unusual ecological dynamics. High temporal variability of moisture levels in arid lands means studies conducted at the same location in different years might obtain vastly different results. In a year of such unusual conditions, results may not represent average tendencies of the ecosystem. However, it has been argued that unusually high rainfall events are the predominant influences on ecosystem function in arid lands (Noy Meir 1973), so ecological patterns found during such a year may be particularly important. Also, in a year of higher rainfall, plants can take up more nutrients by which growth might be limited. Nutrients then could be more closely correlated with shrub growth and densities of SEPHs and other small arthropods on shrubs. Reduced photosynthesis due to water limitation would mean less photosynthate available to rhizobia in roots, which could result in reduced nitrogen uptake. This in turn might reduce SEPH populations on shrubs. Although this is not consistent with the hypothesis that arid conditions may favour SEPHs, there are other effects of aridity which may impact more strongly in favouring SEPHs. Temperature variation also contributes to population dynamics and timing of life cycle events, particularly in the case of ectotherms such as insects. Monthly abundances of many taxa at another arid Australian site were strongly correlated with rainfall in the same month along with temperature, and some phenology variables were also correlated with rainfall of the previous month (Palmer 2010). *Sextius* appears to emerge earlier in years with a warmer spring, and the beginning of growth and like most plants, flowering of *A. victoriae* occurs sooner due to some combination of higher temperatures and moisture levels (per. obs.). This means that variation due to combination of weather factors occurring within a season during which SEPHs/*Sextius* are present and when shrubs are most affected by their association with SBIs would partially regulate strength of effects of the association. Such effects of moisture and temperature might interact with slope-aspect in their effects on organisms when they are likely to be limiting. A predominating wind direction, which might ameliorate effects on a particular slope, is not known for the survey sites. In addition, low insolation is most likely to limit photosynthesis on SFS in spring when insects are emerging and the angle of light is lower than during summer. Photosynthesis restriction at times when insolation is low, such as in the morning (Noy-Meir 1973), is most likely on $SFS_{southern}$ during summer. The resulting lower sugar availaility to SEPHs might affect the rate of SBI attendance on them, altering dynamics of interactions between SBIs and other insects on shrubs. This might even change the rate of SBI attendance within a season. Weather conditions that change how favourable for a given organism each slope-aspect is relative to other organisms might create predictable changes in ecological dynamics among slopes.

## Slope-aspect effects on shrub growth

Shrub shoot growth during a year of high rainfall on SFS was on average nearly twice that on NFS and was greater on SFS than on flats. Rates of seed production and growth of *A. victoriae* in various locations strongly positively correlated with rainfall (Davies 1976, Friedel et al 1994, Grice et al 1994, Reid and Stafford Smith 2000). The effect of slope-aspect on shrub growth is consistent with higher shrub growth rate occurring with higher humidity, since temperatures will be lower on SFS than on both NFS and flats, even though flats receive more moisture from run-off. Soil moisture levels would usually be higher on flats than on slopes due to run-off/on, although in years of very low rainfall with only small rain events, reduced insolation on SFS may exceed importance of drainage patterns. Organic carbon usually indicates levels of organic matter and higher soil microbe activity, so higher levels on SFS point to increased water holding capacity, which might explain higher shrub growth rates there. Higher availability of nutrients due to higher moisture and higher organic matter might also increase shrub growth on SFS. Also, average shoot growth on sub-sites was correlated negatively with potassium (chapter 4) of which there was less on SFS. Potassium is relatively mobile in soils and its negative correlation with growth may be a result of lower soil concentration due to higher plant uptake from higher plant biomass while it is recycled quickly in soil. Alternatively, this correlation is an artefact of some other process such as its high solubility in water which means it is easily lost in run-off, which might be higher on SFS for some unknown reason. Also, lower temperatures and higher moisture levels would encourage activity of micro-organisms during periods of excessive heat, making nutrients such as nitrogen and phosphorous more available to plant growth. One year of high growth may not translate to higher overall shrub growth, since in years of low rainfall biomass is lost (Reid and Stafford Smith 2000). Mean shrub volume of shrubs correlated positively with sulphur moderately strongly and with organic carbon weakly (chapter 4), suggesting long term effects related to sulphur, which was higher on SFS than on flats, although this could also be an artefact of other correlations.

Substantial differences in relative growth rate by site were found which were likely to be caused by soil differences and probably by strength and amount of run-off/run-on. However, average sub-site shrub growth only correlated with potassium, yet potassium levels on sites do not correlate with growth rate on sites. Moisture accumulation due to topography or water holding capacity of different soil characteristics would be important. Although there were considerable differences in growth rate on different sites, no slope-aspect interaction with site was detected, which suggests that soil differences were not as important as moisture. Different statistical dispersion of growth rates on SFS from flats might be for the same reasons suggested for soil parameters.

Although raw shrub growth rate appears to limit SEPH density, both are probably limited by shrub fitness, and there are other strong factors affecting SEPH density on shrubs (Chapter 4). In the survey, no effect on growth rate of SEPH density was detected (Chapter 4). The similarity in growth rate between low- and high-SEPH shrubs on slopes by contrast with flats may have been caused by the same effect as the similarity in growth rate between slope-aspects on high-SEPH shrubs (for both raw growth and RGR, since the pattern was almost the same). This would produce less growth on high-SEPH shrubs on SFS than on flats, which might occur if there were higher rates of herbivory on SFS on high-SEPH shrubs than on flats. The different statistical dispersion of raw growth on shrubs of low and high 2011 SEPH level also points to something differently affecting growth of these two sets of shrubs. This might relate to SEPHs having lower net benefits for shrub growth on slopes through some as-yet unknown factors. It may be that SEPHs are less likely to select or perform well on some shrubs of higher growth rate on slopes because of some other factors. This could be for example, more frequent or intense disturbance by wind or higher numbers of a given arthropod. “Minute” wasps were in insignificantly higher densities on slopes than flats, although those consuming SEPH eggs would constitute only a subset of total densities. These interacting effects of slope-aspect with SEPH level probably explain the overall lack of pattern between RGR and 2009 SEPH density found in the survey in a previous study and much of the variation in pattern between raw growth and 2011 SEPH density (Chapter 4). However, this effect may just be an artefact of the much lower number of replicates of high-SEPH shrubs, particularly on slopes, and high variation in shoot growth.

## Slope-aspect effects on seed production

Many more seeds were destroyed by weevil larvae on SFS than on NFS or flats when the insect was not experimentally excluded, particularly when including only low-SEPH shrubs. Due to insufficient replication in the natural conditions, an interacting effect between slope-aspect and SEPH level could not be tested. High SEPH density on some shrubs on site 3 would have decreased the number of seeds per pod eaten on SFS since at other sites, higher SEPH/*Sextius* density on a shrub was correlated with fewer seeds per pod being damaged by the seed weevil, meaning more are viable (chapter 4). Higher seed consumption on SFS could be because of moisture and temperature effects of slope-aspect on the seed weevil, either directly or indirectly through their effects on the shrub and nutrient uptake, since most small arthropods preferred SFS. Higher sulphur levels on SFS might increase consumption rates also, since consumed seeds per number initiated were strongly positively correlated with sulphur and densities of some herbivores, which might have similar nutritional requirements, were positively correlated with sulphur (chapter 4). This link between sulphur and herbivorous insects is likely to result from some physiological effect of sulphur on plants that in turn affects the insects, but the precise mechanism is unclear. On sites where seed pods were enclosed in bags that excluded insects, there were more viable seeds per pod on SFS than on flats. This suggests there may be interaction in effects of slope-aspect and SEPH level, since the two would produce competing effects on seeds in years when higher SEPH densities on NFS (meaning higher SBI densities) cause fewer seeds to be destroyed there. Number of seeds initiated was the same for all slope-aspects, so differences in other seed parameters are not due to differences in number of initiated seeds. Seed mass was similar on all slope-aspects when seed pod bags were used, and seed mass tends to be intrinsic to the plant, but it was higher on NFS than flats on site 3 where there was lower replication. This could be because shrubs with larger seeds are preferred by the weevil for oviposition as providing better resources for resultant larvae and fewer seeds are eaten on NFS, although, it may merely be due to insufficient replication. Even if seed productivity is higher on flats and NFS due to higher weevil damage on SFS, it might not be sufficient to create differences in the soil seed bank that affect recruitment. Seeds probably remain viable for enough years that recruitment on SFS is not limited by the infrequent high rainfall required for successful recruitment (chapter 2). This could mean that the interaction of SEPHs and slope might not have a substantial effect on shrub populations.

## Slope-aspect effects on SEPHs

Slope-aspect affected SEPH and *Sextius* densities and it appeared to do so differently in the year of average rainfall from the year of very high rainfall. In mid-summer in the year when the preceding two, six, or twelve months of rainfall were close to or less than the long term mean for the same period (table 1), there were greater SEPH densities on SFS than on NFS.

By contrast, when the preceding two, six, or twelve months of rainfall were high (table 1), there were higher SEPH densities on NFS than on SFS and higher SEPH densities on SFS than on flats. There were also higher *Sextius* densities on NFS than on SFS or flats. Therefore, according to the available data, it appears that SEPH/*Sextius* densities from the year of average rainfall to the year of very high rainfall increased on NFS. However, the inclusion of site 3 in this data means differences between slope-aspects in changes to SEPH/*Sextius* density between years and reasons for them cannot be fully understood until it is known why the dominant ant species on SFS and flats beneath it on site 3 changed. Since direct comparisons of exactly the same sites were not possible due to the omission of site 3 and the addition of site 4 between years, these results must be viewed with caution. It is possible that low SEPH/*Sextius* densities on site 3 in 2009 caused SBIs to leave the territory or vice versa, but it was deemed better to leave out the added variation due to the change at site 3 when causes were not known. Although time lags can occur between rainfall and its effects on herbivores in arid environments (Ayal 1994), the very high number of nymphs present in 2011 February suggests that populations may respond quickly to rainfall under suitable conditions. This might be an adaptation to aridity. Although SEPH densities on different slopes were statistically differently dispersed, the univariate analyses were robust. However different statistical dispersion indicates that there are important unmeasured factors influencing insect densities. This different statistical dispersion may have been caused by different densities of shrubs on different sub-sites. Higher density may mean soil parameters include less heterogeneity and distance between shrubs may affect frequency of SEPH dispersal among shrubs or dispersal of other insects important to the tritrophic association such as parasitoid wasps.

These results described above suggest that there may be an interacting effect of rainfall and slope-aspect on SEPH densities. Thus it appears possible that humidity around shrubs in the drier year was below optimum for SEPHs on NFS, but humidity may have been as good as possible on SFS in both years. This may simply be a result of SEPHs performing better in conditions of higher moisture where it had been insufficient. Low temperatures may have limited insects in the cooler wetter year by reducing activity, particularly feeding rates, when moisture and therefore photosynthesis was probably not limiting. This might have limited SEPH densities on SFS. That intermediate SEPH densities occurred on SFS, with the lowest light levels, during the year of high rainfall, means light levels are not important to this pattern, so moisture must be the driving factor. By contrast, in the year of mean rainfall, SFS had the highest SEPH densities, which may be due to superior water holding capacity of soil or lower insolation to dehydrate soil. Flats would receive run-off from slopes during larger rainfall events, which would have contributed substantially to soil moisture in a year of high rainfall but possibly not in a year of average rainfall. This would be consistent with the slope-aspect patterns of SEPH densities in both years. Thus, the relationship of SEPH density with different slope-aspects suggests that not only drainage patterns but insolation and soil moisture holding capacity are probably important, as for shrub growth. SEPH densities might be higher where there is sufficient moisture available, which explains the increase on NFS in the wetter year. However, since densities were higher on NFS, representing more arid conditions, in the wetter year, results are consistent with the possibility that SEPHs might perform best in semi-arid conditions.

Indirect effects on SEPH densities through other arthropods on shrubs might either enhance or compete with other effects of slope-aspect or aridity. Higher moisture and improved nutritional quality of shrubs or greater photosynthate production in years with higher rainfall might increase densities of other small herbivorous arthropods on the shrubs. It is possible that parasitoid wasp preference for slope-aspect is a strong driver of SEPH densities. Effects of parasitoid ("minute") wasps on SEPHs might confound slope-aspect patterns if wasps are favoured by different slope-aspect conditions to SEPHs. Also, SBIs appear to have higher activity rates in reasonably high temperatures and they may provide better protection for nymphs on NFS overall, while SEPHs might require a particular moisture level for feeding. This might equalize SEPH densities on the slope-aspects. However, when temperatures are too high, SBI activity ceases, which might decrease SEPH densities more on NFS in years of lower humidity. The various factors which might be the most important ones driving different SEPH densities on different slope-aspects deserve further investigation.

The interaction of site and effects of slope-aspect on SEPH/*Sextius* densities was strong. This means that site differences are very important and appear to be of comparable strength to slope-aspect. This along with the different dispersions of SEPH densities among sites and slopes points to unmeasured factors having a strong influence. This could explain the high variation found in the relationship between shrub growth and SEPH density in a previous study (Chapter 4). Since there are slope-aspect-site interactions with soil parameters and not with shrub growth, these might depend on soil effects on induced plant defence compunds or on other arthropods on the shrubs that influence the SBI-SEPH association. SEPHs occur in higher densities, at least after a year of very high rainfall, in the presence of higher soil concentrations of organic carbon, sulphur, and total nitrogen (Chapter 4) which differed among sites. This might be an artefact of higher levels of these nutrients on particular slope-aspects and sites where SEPH densities are higher, greater nutrient uptake with higher rainfall, or it might indicate more favourable conditions for SEPHs with different levels of a nutrient.

Although in the summer 2010/2011 survey SEPH count occurred later after emergence than the summer 2009/2010 count, it is impossible to know whether time of counting affected relative numbers of SEPHs on different slope-aspects in different years, since there is so much variability in the system. However, there was no difference among slope-aspects in proportions of 1st instar nymphs as a proportion of all nymphs or nymphs as a proportion of total *Sextius* numbers among slope-aspects in November 2009 during the initial main emergence. This suggests that timing of emergence was not earlier on any particular slope-aspect (Chapter 4) due to temperature and moisture conditions there and therefore SEPH/*Sextius* densities at that time were likely to not be biased due to being counted earlier in the year.

## Slope-aspect effects on non-SEPH arthropods

SBI behavioural patterns may differ with slope-aspect and this might affect densities of SEPHs and other small arthropods. On NFS in spring or summer after SEPH emergence, warmer soil on days of higher temperatures allows SBI activity to start earlier in the day and continue for longer after sunset (pers. obs.). On sufficiently hot days this would be interrupted by ground temperatures exceeding 42°C, around which temperature SBI activity in trails cease (Chapter 3). Ground temperatures can easily reach 65°C by 3pm (pers. obs.), and the more intense the insolation, the earlier and longer SBI activity would cease. Only on the hottest days are temperatures sufficient for SBI activity to continue throughout the night. Therefore whether higher temperatures on NFS or lower temperatures on SFS would increase or decrease total SBI activity would depend on the specific temperatures. Increases on NFS would be likely during cooler periods, but data relating temperature to SBI activity would be required to determine how temperature affects SBI activity at specific times of the year. Effects of temperature on sap flow of *A. victoriae* or effects of this on SE production and on SBI attending are not known. High temperatures would reduce activity of SBIs more than that of other arthropods on shrubs since temperatures are higher on the ground than on shrubs where insects can take refuge in shaded more humid sites. Higher SBI activity might occur with increasing sap sugar concentration when there are higher photosynthetic rates. Since photosynthesis is limited by moisture, when it is not limited by rainfall, SBI attendance might increase, strengthening any deterrent effect on other arthropods without incurring extra costs to the shrub. This would be more likely on NFS on most days.

At the end of a year of average rainfall in January 2010, substantially higher densities of thrips, “other” Auchenorrhyncha, and mirid/lygaeids on SFS than on NFS were on low-SEPH shrubs. As well as lower humidity increases the potential for dessication of insects on NFS, greater stress of shrubs on NFS due to high temperatures could be expected to stress at least some herbivores.  "Other" Auchenorrhyncha numbers were also higher on flats than on NFS, suggesting that their numbers may have depended on higher shrub moisture levels or lower temperature.  Shrubs in the following 12-month period of high rainfall grew faster on SFS and are likely do so in general due to moisture limitation on NFS.  Higher growth rate of the dominant canopy on SFS is likely to encourage higher abundances of herbivorous insects that prefer faster growers, such as sap-suckers.  Herbivores could have benefitted from more (or better quality) sap, better plant nutrition from increased nutrient uptake, and possibly higher photosynthetic rate on hot mid-summer days when midday temperatures might reduce photosynthesis on NFS.

On high-SEPH shrubs, "other" Auchenorrhyncha densities were higher on SFS than on flats, unlike for low-SEPH shrubs.  The pattern of their densities on different slope-aspects is similar to that of SEPHs in December 2009, possibly through similar requirements since most are phloem-feeders, or alternatively through level of protection by SBIs.  On high-SEPH shrubs, psyllid numbers were higher on flats or SFS than on NFS, unlike on low-SEPH shrubs where there were no slope-aspect differences.  Numbers of these three other categories of sap-suckers may have reduced due to reduced SBI deterrence of their parasitoids or predators and might be relatively lower on NFS from lower SEPH densities there.  Weak competitive effects, probably asymmetric, might occur between SEPH densities and other herbivores, particularly other phloem-feeders (Chapter 4).  These may have reduced numbers of psyllids and thrips on SFS relative to other slope-aspects.  A significant interaction of SEPH level and slope-aspect was found only for psyllids.  Their lower numbers on NFS only on high-SEPH shrubs may have been related to the sedentary habit of the nymphs, receiving less protection from parasitoid wasps from the lower density of SBIs there or alternatively increased reliance on favourable sites and competition with SEPHs. Higher densities of "other" herbivores and mirid/lygaeids on SFS than on flats when considering both low- and high-SEPH shrubs support the general patterns found for more numerous taxa. Relatively lower densities of mirid/lygaeids on SFS and on NFS are hard to explain, but this is not surprising since there are different functional groups within them, and there were low numbers of them on traps. Lower numbers of predators on both low- and high-SEPH shrubs on NFS than on flats or SFS are likely to be a response to lower prey densities there along with lower humidity. It is possible that higher densities of SEPHs and SBIs on SFS than on flats decreased predator numbers on SFS relative to flats. Numbers of predators, mirid/lygaeids, and "other" herbivores were very low on some traps and some may not be specifically associated with *A. victoriae*, so conclusions drawn about them must be tentative. Slope-aspect might affect herbivory on NFS through reductions in herbivore densities on shrubs due to low moisture levels, although some important insect herbivores of *A. victoriae* may not be present on sticky traps (Chapter 4). Although shrubs probably grow faster on SFS as a general rule, during a year of high rainfall higher SEPH densities on NFS than on SFS might deter many of the herbivores that prefer the faster growers.

"Minute" wasp densities were higher on SFS and NFS than on flats on low-SEPH shrubs, but on high-SEPH shrubs, densities decreased on NFS relative to other slope-aspects. This suggests an interaction which the level of replication may not have been sufficient to detect. Higher densities of SEPHs and SBIs on SFS would be likely due to the cooler moister conditions on SFS, since such small flying insects would be prone to dehydration. Higher shrub nutrient levels would have increased densities of "minute" wasps via their hosts. Relatively reduced densities of "minute" wasps on NFS in the presence of high SEPH densities are difficult to explain. The interaction of slope-aspect and site for "minute" wasps on low-SEPH shrubs might be related to complex indirect factors involving differences in the arthropod community such as soil nutrients regulated by interactions such as slope-aspect and site. The strong interaction of site and SEPH level for "minute" wasp densities is also likely to be the result of complex interacting factors. Site differences in densities on shrubs of all small arthropods caught on sticky traps either on low-SEPH shrubs or on all shrubs and interactions in effects of slope-aspect and site on thrips on both high and low SEPH shrubs could have been influenced by soil differences as mentioned for SEPH densities. Higher numbers of parasitoid wasps on SFS than on other slope-aspects might affect dynamics of SEPHs on shrubs such as by limiting their numbers more on SFS relative to NFS in the following year. Population dynamics of parasitoids would be a substantial influence on SEPH densities, but their effects might interact substantially with other factors affected by slope-aspect.

## Conclusion

Densities of SEPHs on different slope-aspects in years of different rainfall are consistent with the hypothesis of semi-arid conditions being favourable for this association. SEPH densities may be affected by moisture levels and possibly by temperature, which vary with slope-aspect and rainfall. Site effects on SEPH densities appeared to be stronger than slope-aspect effects, with which they interact strongly, at least in a year of high rainfall. Site effects on SEPH densities on shrubs may be more likely to occur in areas with contrasting levels of soil nitrogen, sulphur, or organic carbon (which each correlated negatively with SEPH densities in the year of high rainfall). Differences in soil parameters may be an important factor in SEPH densities varying with slope-aspect. The limiting soil parameter might change between years or between locations with different rainfall, since different soil moisture levels would make nutrients more or less available to plants and the insects feeding on them. For example, in years of low rainfall, moisture limitation on photosynthesis might limit SBI attendance of SEPHs on SFS, whereas in years of high rainfall, uptake of a nutrient might limit SEPH densities on NFS. Low nutrient concentrations are characteristic of arid lands, particularly in Australia, but also of the entire continent. Soil sulphur may be particularly low on degraded rangelands where this tritrophic association is reported to be common, which might explain its reported abundance in such environments. More knowledge of soil parameters at sites with *A. victoriae* is needed to understand distribution of this association as it relates to soil variability. Manipulative experiments using gradients of moisture and soil nutrients should be done to better understand the nature of the interactions and relative importance of individual or combined soil variables.

Increased abundances of parasitoid wasps of <1mm on SFS and to a lesser extent NFS might alter dynamics of the shrub-SEPH/ *Sextius*-SBI association among slope-aspects. Most herbivores preferred SFS to either NFS or flats, probably due to higher humidity levels and the higher concentrations of soil nutrients with which they are positively correlated. This means herbivory levels are likely to be higher on SFS, although growth rate is also higher. Slope-aspect effects on soil moisture and nutrient uptake probably contribute to SEPH/ *Sextius* densities. Manipulations of relevant moisture and nitrogen concentrations identified here on different slope-aspects might reveal the importance of each to arthropod assemblages.

This study has found slope-aspect effects which would contribute to soil heterogeneity in arid Australia. Further studies could investigate the range of soil differences that can occur and the resulting effects of temporal and spatial variation in soil nutrients and moisture on associations between shrubs, SEPHs, and trailing dominant ants. These differences probably contribute to *A. victoriae* growth rate in years of high growth and they would influence abundance of insects, including those that perform better with or have preferences for shrubs with faster growth rate such as sap-suckers. Slope-aspect affects *A. victoriae* seed production both directly through influence on shrubs and indirectly through the SEPH-SBI-association, each factor potentially increasing viable seed production on different slope-aspects. Direct effects on seed production of different soil nutrient concentrations on different slope-aspects might be stronger in seasons of high rainfall.

With a large area of arid lands containing diverse biota, Australia provides much opportunity to gain understanding of effects of aridity factors on this type of interaction and the widespread distribution of both *A. victoriae* and *Sextius* makes them convenient study taxa.

# Chapter 6:

# Conclusion

This study has investigated effects of a tritrophic association between shrubs, SEPHs, and SBIs to determine whether the association might be beneficial to shrub and SEPH partners and it has explored factors involved in distribution of the SEPHs on the shrubs. It has examined effects of slope-aspect on soil, on the shrubs, on the SEPHs, and on populations of other arthropods on the shrubs, to reveal how outcomes of the association for shrubs and SEPHs might vary with abiotic heterogeneity. Spatial distribution of the partner shrub *Acacia victoriae*, the second most widespread acacia in Australia, was investigated as it is important to structure of vegetation in semi-arid Australian ecosystems. Spatial association between the shrubs, populations of SEPHs that colonize them, and SBIs that feed off the sugary exudates produced by the SEPHs was assessed to reveal how the tritrophic association is structured spatially and to provide clues to both how this is affected by the abiotic environment and how it might affect the ant community.

Like in the case of most tritrophic associations between ants, SEPHs, and plants in other locations, *A. victoriae* with high densities of *Sextius* sp. tends to grow more and stems grow less when SBIs and *Sextius* sp. are excluded from them. Lower herbivory levels on the large majority of leaves on high-*Sextius* shrubs points to herbivory as the likely causative factor. Lower folivory on high-*Sextius* shrubs may be due to SBI deterrence or to plant chemistry, since plant hormones that induce deterrence of folivorous insects are different from those that deter sap-sucking insects (Walling 2000). Shrub growth rate appears to limit SEPH/*Sextius* densities in a positive correlation, and no soil parameter correlating to both shrub growth rate and SEPH/*Sextius* density was found. It is not known how much the presence of high densities of *Sextius* sp. on the shrubs is the result of pre-existing plant chemistry that also correlates with high growth rate. Between spring growing seasons, *Sextius* density on some shrubs changes dramatically, and whether this occurs due to plant chemistry or some process in the arthropod assemblage is unknown. Shrub growth appears to have a stronger impact on SEPH/*Sextius* populations than these insects have on shrub growth, but this was not correlated with soil parameters.

The much higher seed production rate of shrubs with high SEPH/*Sextius* densities may be the result of an indirect interaction or it may be due to SBI deterrence of the weevil that destroys the seeds. It may be the result of correlating factors, such as preference of both SEPHs and the weevil for shrubs in low sulphur soil. Correlations of SEPH density with soil nutrients (Chapter 4), of *Sextius* density with leaf nutrients that correlated with soil parameters and with soil parameters (Chapter 4), of shrub neighbour group SEPH density with soil parameters (Chapter 3), and possibly of shrub neighbour group SEPH count with shrub volume per area (Chapter 3) point to bottom-up influences from soil on SEPH populations on shrubs. The correlations of other arthropods with soil parameters might also indicate bottom-up influence which might further influence populations of SEPHs on shrubs. There are contradictions in the negative association of *Sextius* density with soil sulphur and with electrical conductivity on individual shrubs (Chapter 4) and of the negative regression relationships of SEPH/*Sextius* density with soil nitrogen, sulphur, and organic carbon on survey subsites (Chapter 4) by contrast with the positive correlation of neighbour group average SEPH density with soil nitrogen, sulphur, electrical conductivity, and organic carbon (Chapter 3). This might be related to the different years in which the SEPHs were counted: for the neighbour group study, SEPHs on shrubs were counted in December 2008 after twelve months of low rainfall, whereas for the field experiment, SEPHs were counted in December 2010 and in the main field survey SEPHs were counted in January 2011, after twelve months of very high rainfall. In the low rainfall year, SEPHs might have preferred shrubs with high soil moisture, which might be positively correlated with organic carbon and organic matter, while in the high rainfall year, SEPHs may have preferred individual shrubs with low salinity (and soil electrical conductivity of survey sub-sites correlated positively and strongly with soil nitrogen, sulphur, and organic carbon), indicated by electrical conductivity, or with low quantities of defensive compounds, which may have been those individuals with low soil sulphur. Interestingly, the site where the dominant ant species changed from SBIs to *I. lividus* (site 3) between years was higher in soil sulphur than the others. In any case, these bottom-up influences could play an important part in determining distribution of SEPHs across the landscape and possibly their distribution among different biomes.

SEPH densities were different on different slope-aspects. The influence of slope-aspect appeared to interact with rainfall, with SEPH densities increasing on the drier southern hemisphere NFS from the year of average rainfall to the year of high rainfall. Since the largest SEPH densities occurred on NFS in the year of high rainfall, these results were consistent with the possibility of these tritrophic associations being the most common in semi-arid environmental conditions. However, the effects of slope-aspect interacted with site, which had a stronger effect than slope-aspect. This might be the result of variation in soil parameters. Therefore, the influence of slope-aspect on soil and on shrub growth that was found in this study might be important to slope-aspect differences in SEPH densities in addition to affecting other aspects of the community.

The relationships found between total densities of parasitoid wasps (which were mainly parasitoids of eggs an d possibly larvae) and total SEPH densities suggested that these groups may have a strong influence on densities of each other. This might even be the reason for sudden reductions in SEPH density on shrubs. Large increases in SEPHs might lead to large increases in species of egg parasitoid wasps that they host, which may comprise the majority of parasitoid wasps on shrubs with high SEPH densities. This might heavily reduce the number of SEPHs on the shrub in the next season. Conversely, if densities of SEPHs and the other herbivores with which they share egg parasitoids are both low on a shrub, a large proportion of eggs of SEPHs of either the small pre-existing populations or of newly dispersed individuals might emerge, particularly of SEPHs with protected eggs such as *Sextius* sp. The influence of slope-aspect on these parasitoid wasps might further influence SEPH populations on different slope-aspects.

Soil parameters were different near dead *A. victoriae* from in the surrounding soil matrix, so spatial distribution of *A. victoriae* as the dominant canopy in arid lands is likely to influence distribution of soil nutrients even after the plant dies. The influence of slope-aspect on soil that was found also contributes to heterogeneity of soil resources as might the slope-aspect effects on shrub growth that were found.

The positive spatial association found between SBIs and SEPHs on shrubs points to the importance of spatial distribution of woody perennials and the SEPHs inhabiting them to spatial distribution of SBIs. Since SEs can make dominant ants more aggressive (Grover et al 2008) and appears to enable them to invade new locations and compete with other species of ants (Helms and Vinson 2002), their dominance on sites with SEPHs might depend on SEPH populations providing sufficient SE to enable them to defend their territory against other dominant ants (Davidson 1997). Thus, it is possible that high rainfall in the second year of the main field survey changed availability of a nutrient to shrubs which consequently changed chemistry of the shrubs in a way that made them unfavourable to SEPHs, causing their populations to dwindle there. The site from which they became absent on most shrubs in January 2011 (site 3) had the highest average levels of soil nitrogen, sulphur, and organic carbon, with which sub-site SEPH densities were negatively correlated, as well as the highest soil conductivity and phosphorous levels. It is also possible that plant chemistry changed in response to increased availability of these nutrients, causing shrubs to deter SEPHs. However, since *Sextius* sp. nymphs disappeared from stems from which SBIs were excluded, and since different ant species confer different benefits to the same SEPH species (Buckley and Gullan 1991, Del Claro and Oliveira 2000), presence of substantial populations of this SEPH and possibly others might depend on SBI presence. If some other factor caused the different ant species to occupy the shrubs and this species does not provide sufficient benefits to their fitness which SBIs do provide, then many if not all of the SEPHs may have become absent as a result. The dependence of SBI dominance on adequate SEPH populations and the dependence of substantial SEPH populations on sufficient SBI attendance may not be mutually exclusive.

Overall, this study has substantially increased the understanding of a very common and almost certainly important interaction type in arid Australia. While causality could not always be determined, the study provided very valuable information on important factors in spatial heterogeneity of abiotic and biotic factors in the highly variable Australian arid zone. This contributes to more general understanding of arid ecosystems. This information can inform land management practises. From this work, many questions and hypotheses have arisen that indicate important directions of future research.

**Further Research Stemming from this Study**

More work on the taxonomy and species biology of taxa important to the tritrophic shrub-SEPH-ant association would allow better understanding of the ecological interactions between them. Key questions have been identified on which further research would inform and extend the aims of the present study. These are listed below:

Questions relating to plant dynamics, more generally:

- What is the physical structure of the root system of *A. victoriae* shrubs? Does its phenotype vary with biome? For example, shrubs growing in arid areas might have broader shallow roots which capture moisture in small rain events. Are roots of the shrub in the top 5cm of soil which uptake nutrients where they are at the highest concentrations? Do their roots reach underground water reserves? Information relating to these questions would increase understanding of spatial distribution of this species and its influences on arid zone plant communities, including its effects in rehabilitating degraded rangelands.
- How do woody perennials such as *A. victoriae* in arid Australia, particularly in degraded rangelands, affect sub-canopy plant species? Do they directly or indirectly through these sub-canopy species change parameters of the top 5cm of soil? Do

they change the plant community through hydraulic lift? Information on these points would inform conservation management of the degraded areas which it inhabits.

- Does removal of arid zone woody perennials such as *A. victoriae* affect growth rate of neighbours? This could increase knowledge of structuring of arid zone plant communities through intraspecific and interspecific competition among these species. It would also provide a baseline with which to compare effects of other interactions such the SBI-SEPH-shrub association.
- Why do dead *A. victoriae* shrubs appear to facilitate conspecific seedlings more than live shrubs do?
- How does distribution of soil resources affect distribution of *A. victoriae*?

Questions relating to the tritrophic shrub-SEPH-SBI association, specifically:

- How does spatial distribution of soil resources affect distribution of SEPHs on woody perennials? Do degraded areas of semi-arid Australia have particularly low soil sulphur or salinity and if so is this the soil parameter that causes large SEPH or SBI populations?
- Does spatial distribution of host plants of SEPHs such as *A. victoriae* and intraspecific competition between these plants affect spatial distribution of SEPH populations on them?

- How does spatial distribution of SE/SEPHs affect spatial distribution of SBIs? Is it required for SBIs to gain dominance over other *Iridomyrmex* species? What other food resources interact with effects of SE to regulate SBI spatial distribution?

- Does SBI attendance increase survival of *Sextius* sp. eggs, nymphs, or adults through deterrence of predators or does it increase survival or reproduction in another way such as by increasing feeding rates or drowning in honeydew? If so, do SBIs confer more fitness benefits on *Sextius* sp. than other *Iridomyrmex* species such as *I. lividus*? Is this different for other SEPH species?

- Which arthropods do SBIs deter from *A. victoriae* and when do their populations coincide from year to year, taking into account annual variation in environmental conditions?

- What is the mechanism of effects on arthropods and on herbivory on *A. victoriae* for different SEPH levels? Is it partly plant chemistry, or deterrent effects of SBIs on herbivores of vegetative parts, or deterrent effects of or the parasitoid-host relationship with parasitoid wasps?

- How do benefits to *A. victoriae* of protection by SBIs change with the density of SBIs and with densities of different SEPHs they attend?

- Do SEPH species other than *Sextius* sp. require SBI attendance to maintain populations on shrubs?

- How do arthropod communities in ecosystems with abundant SE differ from those in ecosystems with negligible quantities of SE?

- How do different SEPHs such as *Sextius* sp., grey and red scale insects, and lycaenid larvae differ in their effects on *A. victoriae* and on the arthropod communities on the shrubs? What proportion of parasitoid wasps do they co-host? Do some SEPH species have asymmetrical competitive effects on some other herbivores on the shrubs?

- Which SEPH species host which parasitoid wasp species?

- Is there apparent competition through parasitoid wasps of *Sextius* sp. or another SEPH species with non-tended herbivores such as cicadellids? How does this affect populations of each herbivore group?

- How much effect do soil moisture and particular soil parameters such as nutrient concentrations have on densities of different SEPH species? Through what mechanisms does this occur?

- Do SEPHs exert strong competition through the plant on other herbivores such as cicadellids? If so, is this competition strongly asymmetrical?

- How much does folivory by lycaenid caterpillars contribute to the variation in the regression relationship of shrub growth per SEPH density?

- Is this type of tritrophic association more abundant on woody perennials in the arid zone than for mesic ecosystems, in particular in Australia?

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# Appendix: Photographs of the Site and Species

Figure 1: *Acacia victoriae* in flower on site 2 of the SBI spatial distribution study, 2008. Photo N. Weichel.

Figure 2: *Acacia victoriae* on site 2 of the SBI spatial distribution study, 2009. Photo N. Weichel.

Figure 3: Adult *Sextius* sp. mating on *Acacia victoriae* in February, 2011. Photo N. Weichel.

Figure 4: SBIs attend juvenile *Sextius* sp. on *Acacia victoriae*, 2010. Photo N. Weichel.

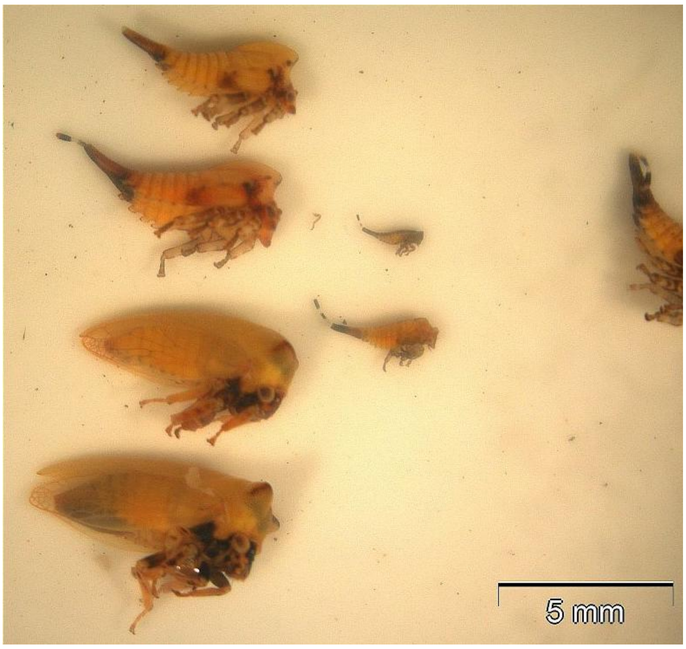

Figure 5: *Sextius* sp. instars (from left to right) (from top to bottom) adult, adult, fifth instar, fourth instar, (right column:) second instar, first instar, 2011. Photo R. Glatz.

Figure 6: SBIs attend larval *Jalmenus icilius* on *A. victoriae*, 2010. Photo N. Weichel.

Figure 7: Margarodidae: grey scale on *A. victoriae*, 2010. Photo N. Weichel.

Figure 8: Margarodidae: red scale on *A. victoriae*, 2009. Photo N. Weichel.

Figure 9: SBIs dragging larval prey along an *A. victoriae* twig, 2010. Photo N. Weichel.

Figure 10: *Monolepta* nr *divisa* on *A. victoriae* flower, 2011. Photo N. Weichel.