Factors Influencing the Honeydew Production of *Ultracoelostoma* Scale Insects in New Zealand Beech Forests

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Table of Contents

List of Figures

List of Tables

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Abstract

In New Zealand *Nothofagus* forests *Ultracoelostoma* spp. scale insects produce abundant honeydew which is an important food for native birds, invertebrates, sooty mould, and invasive wasps. Previous models have underestimated honeydew production, potentially because they do not allow for the flow rate of honeydew to vary between insects based on characteristics such as insect size. This research focused on honeydew production rates at the level of the individual insect, how insect characteristics influence production, and whether the strongest predictor of production, ambient air temperature, acts directly on insects or indirectly via effects on trees. Finding out how temperature acts to increase honeydew production will better reveal the physiological processes involved. The study site was Mt. Richardson, Canterbury.

In the first part of this study, during March-April 2012, daily mean ambient temperature (range 7.2 – 15.1 °C) had a positive relationship with honeydew production. Insect size positively influenced production at high temperatures, with the largest insects producing 0.296 µg insect^{-1} h^{-1} and the smallest insects 0.115 µg insect^{-1} h^{-1} at the highest temperature, 15.1 °C.

In the second part of this study, during October 2012-January 2013, I manipulated temperature on areas of tree trunk using reflective or clear plastic covers, creating a mean temperature difference of 1.1 °C. However, the effects of tree and insect temperature could not be separated as there was no relationship between either manipulated or ambient temperature and honeydew production.

These results show that honeydew production is influenced by individual insect characteristics. This will be important for future models of production. The results also show contradictory effects of temperature on honeydew production, perhaps because of interactions with other unknown factors, which bears further investigation.

Chapter 1: Introduction to Phloem-Feeders, *Ultracoelostoma* Scale Insects and Their Place in New Zealand Beech Forests.

1.1 Honeydew Producing Insects

Honeydew is a sugary liquid produced primarily by phloem-feeding insects from the order Hemiptera. These insects secrete it as a waste product from feeding on phloem sap. Phloem sap is a high sugar, low nutrient diet and these insects have a number of adaptations to aid in processing it (Ashford et al. 2000, Douglas 2006, Dinant et al. 2010). Nitrogen in phloem sap (like other plant tissues) is at low concentrations compared to the insects that feed from it (Ayres et al. 2000, Sandström et al. 2000, Douglas 2006). More specifically phloem sap has a low concentration of amino acids, and of these there is a low ratio of essential amino acids, making phloem sap a nitrogen poor diet for the insects that feed on it (Ayres et al. 2000, Sandström et al. 2000, Douglas 2006). The large amount of honeydew produced by phloem-feeding insects may be due to the quantity of sap that they have to process for the insect to obtain adequate nitrogen (Henneberry et al. 2000, Wardhaugh et al. 2006, Wool et al. 2006). The bacteria are vertically transmitted and differ in species and abundance depending on the host species and the amino acids available (Douglas 2006, Chandler et al. 2008). Furthermore, some phloem-feeding insects have been found to manipulate host plants to alter amino acid concentrations to help meet nutritional needs (Thompson and Goggin 2006, Giordanengo et al. 2010, Leroy et al. 2011). Obligate phloem-feeders have symbiotic bacteria that produce essential amino acids compensate for the lack in the phloem (Gullan and Kosztarab 1997, Chandler et al. 2008). These bacteria may also be important for disease resistance and processing plant secondary compounds that may be toxic to the insect (Gullan and Kosztarab 1997). The high sugar concentration in the ingested sap also means that high osmotic pressure is created in the gut, drawing water out of the haemolymph and potentially dehydrating the insect (Shakesby et al. 2009). To avoid dehydration the simple sugars in the sap are converted into oligosaccharides in the gut. This makes the ingested sugars isosmotic with haemolymph (Ashford et al. 2000). The ability

to produce oligosaccharides for osmoregulation and the presence of symbiotic bacteria that produce essential amino acids are the essential to a diet of phloem sap (Douglas 2006). These traits have evolved independently multiple times within the order Hemiptera, which contain the only phloem dependent animals (Douglas 2006). These include insects of the suborders Sterorrhyncha (such as scales, aphids and whiteflies) and Auchenorrhyncha (such as leafhoppers and planthoppers) (Gullan and Kosztarab 1997, Douglas 2006). Some honeydew producing insects have other adaptations, such as a waxy anal filament to remove honeydew from their vicinity to decrease adverse effects, such as the growth of sooty mould (Gullan and Kosztarab 1997).

Sap-feeders are herbivores, so they create a drain on host plant resources (primarily carbohydrate) that either reduces host fitness or is compensated for by increasing photosynthesis (Meyer and Whitlow 1992, Maron and Crone 2006, Dungan et al. 2007b). Plants have defences against phloem feeding to reduce losses of carbohydrates and nutrients that insects must overcome, such as sieve plate occlusion and plant secondary compounds (Thompson and Goggin 2006, Will et al. 2007, Giordanengo et al. 2010). Phloem sap is typically poorly defended chemically (Douglas 2006, Zvereva et al. 2010). Many species of honeydew producing insects are regarded as agricultural, horticultural or ecological pests worldwide due to damage to plants that they, or the sooty mould associated with them, creates (Dewet and Botha 2007, Culik et al. 2008, Brück et al. 2009, Giordanengo et al. 2010, Zvereva et al. 2010). For example aphids (family: Aphididae), are the most common honeydew-producing insects (Leroy et al. 2011). They have a worldwide distribution and commonly cause damage to garden plants and crops directly from feeding or indirectly by spreading diseases or sooty mould (Giordanengo et al. 2010, Leroy et al. 2011). They are therefore often controlled using pesticides, biological control or genetically engineered plants to reduce their impacts (Dewet and Botha 2007, Stark et al. 2007, Brück et al. 2009). In Turkey and Greece the scale insect *Marchalina hellenica* has been propagated for honey production, however the insects damage the host pine trees and are associated with the general decline of pines in these countries (Bacandritsos et al.

2004, Petrakis et al. 2010). In a meta-analysis of herbivory studies (Zvereva et al. 2010) concluded that sap-feeders caused greater damage to woody plants than folivores. Honeydew production tends to be highly variable (Henneberry et al. 1999, Michalzik et al. 1999) but is often produced in very large quantities, enough to be used as a food resource by a number of different species (Michalzik et al. 1999, Douglas 2006). Consequently, phloem-feeding insects can have the ability to influence whole communities through the production of honeydew (Bach 1991, O'Dowd et al. 2003).

After excretion, honeydew becomes available for other organisms in the community as an easily available and energy rich food source that may be present year-round (Gaze and Clout 1983, Didham 1993, Völkl et al. 1999, Beggs 2001, Wäckers et al. 2008, Gardner-Gee and Beggs 2010). Large amounts of sooty mould typically grow from feeding on the sugar; this is one of the most obvious signs of infestation by honeydew producing insects (Bach 1991, McKenzie et al. 2000, Wardhaugh et al. 2006). A number of invertebrates use honeydew indirectly by utilizing the mould as habitat and food (Didham 1993, Ewers 2002). Honeydew falling to the ground also becomes available for soil fauna which may then influence key ecosystem processes such as nutrient cycling and decomposition (Grier and Vogt 1990, Stadler and Michalzik 1998, Michalzik et al. 1999). In particular, levels of available nitrogen seem to become depressed in soil exposed to honeydew (Grier and Vogt 1990, Michalzik et al. 1999). Some vertebrates such as birds and geckos consume honeydew as part of their diet (Gaze and Clout 1983, Beggs 2001, Fölling et al. 2001, Gardner-Gee and Beggs 2010). Social hymenopterans are well known for exploiting honeydew resources (Beggs 2001, Douglas 2006). Bees make honeydew honey (also known as bush or forest honey) from honeydew, which in some countries accounts for the majority of honey produced (Crozier 1981, Markwell et al. 1993, Astwood et al. 1998). Social wasps and ants can reach extremely high densities exploiting honeydew; they can then become pests, threatening human well-being and economic interests as well as doing major damage to ecosystems and threatening endangered species (Beggs 2001, O'Dowd et al. 2003).

Ants monopolize honeydew resources in many countries and form a mutualisitic relationship with honeydew producers, though they may also prey on them if some foods are scarce (Bach 1991, Silveira et al. 2010). This relationship increases ant aggressiveness and abundance leading to largely negative impacts on other invertebrate species (Styrsky and Eubanks 2007). Honeydew may be used as an alternate food source for predators and parasitoid invertebrates, increasing their fecundity relative to those that do not use honeydew, which has particular implications for biological control (Wäckers et al. 2008). Honeydew production may therefore have a large impact on the ecosystems where it occurs, directly and indirectly affecting individual species and communities as a whole. For this reason species that produce large amounts of honeydew have often been recognized as 'keystone species' (Beggs et al. 2005, Styrsky and Eubanks 2007). Keystone species are species that have a higher impact in their community than would be expected from their biomass, and the loss of which would have widespread consequences for the community (Beggs et al. 2005, Beggs and Wardle 2006).

1.2 *Ultracoelostoma* scale insects

There are a number of endemic coelostomidiid scale insects in New Zealand (with various host species) that produce honeydew and result in the growth of sooty mould (Crozier 1981, Gardner-Gee and Beggs 2009). The most researched are *Ultracoelostoma assimile* and *U. brittini,* as these are the most widespread and produce the most honeydew (Morales 1991, Gardner-Gee and Beggs 2009). Both sooty beech scale insects were previously regarded as a single species, *U. assimile*, but (Morales 1991) revised this and described *U. brittini*. *Ultracoelostoma assimile* and *brittini* are sap-sucking scale insects endemic to New Zealand, that feed from the phloem of several native *Nothofagus* beech tree species (Morales et al. 1988, Wardhaugh et al. 2006). Another species is also

in this genus, *U. dracophylli*, however this feeds on *Dracopyllum* spp. and henceforth '*Ultracoelostoma*' will refer to *U. assimile and U. brittini*. *Ultracoelostoma* live much of their lives sedentary in woody cases on the trunk, branches and root buttresses of *Nothofagus* (Morales et al. 1988, Wardhaugh et al. 2006). The host species are *Nothofagus solandri* (black beech)*, N. solandri* var. *cliffortioides (mountain beech), N. truncata* (hard beech) and *N. fusca* (red beech)*,* however the insects are rarely found on *N. menzieii* (silver beech) (Kelly 1990, Beggs 2001). These insects are largely sedentary but have a dispersing juvenile stage (crawlers) as well as mobile male prepupae and adults. The crawlers settle on suitable areas of host trees and insert their stylet to suck phloem sap. They form an anal tube and become covered in a waxy substance secreted by epidermal glands; this then hardens to form a case. These cases may serve to protect against predators, water loss, and honeydew as well as help attach the insects to the tree (Morales et al. 1988, Gullan and Kosztarab 1997). After forming a case the female remains sedentary until death and the male until after the third instar when it emerges to forms a prepupae and later a winged adult male (Morales et al. 1988). The sedentary, cased insects, have a globular shape, a pink or orange colour and reduced appendages (Crozier 1981, Morales et al. 1988). *Ultracoelostoma* do not appear to be highly seasonal, honeydew is produced year round, all instars are present year-round (overlapping generations), apart from adult males, and eggs counts show no seasonal patterns (Morales et al. 1988). Generation times for *Ultracoelostoma* are unknown, however other scale insects usually have one to four generations per year (Crozier 1981, Morales et al. 1988). *Ultracoelostoma* are typically found at much higher densities on black and mountain beech than on red and hard beech, though this is not the case in all regions (Kelly 1990). Distribution of *Ultracoelostoma* is very patchy across a number of spatial scales, within trees, between trees and between regions (Kelly 1990). The area of beech forest with major infestation in New Zealand is about one million hectares, particularly in the north of the South Island (Morales et al. 1988, Beggs 2001). *Ultracoelostoma* reach high densities in these beech forests; Beggs et al. (2005) found that mean late summer densities of

actively feeding *Ultracoelostoma* on *Nothofagus fusca* ranged from approximately 40 to 700 scale insects m^{-2} of trunk. Another study found scale insect densities up to 1535 m^{-2} (Dungan et al. 2007a). Like other phloem-feeders *Ultracoelostoma* must process large amounts of phloem sap to get adequate nitrogen (Wardhaugh et al. 2006). The insect excretes excess sugars as honeydew from a waxy anal tube (~2-3 cm long) that projects out from the tree (Crozier 1981, Morales et al. 1988). This anal tube is the most conspicuous part of the insect, since the case is often covered in sooty mould (Crozier 1981). Grant and Beggs (1989) analysed honeydew sugar composition and found the mean proportional composition of such samples were 42 ± 5 % fructose, 23 ± 8 % sucrose, 1 ± 0.4 % glucose, and 33 ± 6% oligosaccharides, Beggs et al. (2005) found similar compositions. The level of protein in this honeydew was low (<50 mg g^{-1}) (Grant and Beggs 1989). Due to the high amount of sugar each insect processes and the high density of insects, overall honeydew production is very high (Beggs et al. 2005). It is estimated that *Ultracoelostoma* produce 3500–4500 kg dry weight honeydew per hectare annually in *Nothofagus* forests (Beggs et al. 2005).

This honeydew is an important resource for many organisms including native birds, invertebrates and sooty mould, and influences ecosystem processes such as litter breakdown and nutrient cycling (Gaze and Clout 1983, Beggs 2001, Wardle et al. 2010). Bellbirds (*Anthornis melanura*), tui (*Prosthemadera novaeseelandiae*), silvereyes (*Zosterops lateralis*) and kaka (*Nestor meridionalis*) are among the native birds that feed on honeydew (Gaze and Clout 1983, Beggs and Wilson 1991, Murphy and Kelly 2003). Honeydew may account for most of the energy these birds consume in beech forests, this is supported by forests with more honeydew hosting more of these birds (Gaze and Clout 1983, Beggs and Wilson 1991). Beech forests often have low plant diversity and few species produce fruits or large amounts of nectar, therefore honeydew may be the most abundant sugar resource (Grant and Beggs 1989, Murphy and Kelly 2003). The sugar loss through *Ultracoelostoma* also stimulates photosynthesis in the beech trees through compensating processes, increasing the productivity of the whole beech community (Dungan et al. 2007b). Some studies have

suggested that honeydew production may have consequences for the host tree, due to the drain of insects on host resources, though recent work suggests any impact may be small (Kelly et al. 1992, Dungan et al. 2007b).

Honeydew is utilised by honeybees to produce honeydew honey. Honeydew honey has been an economically lucrative export to Europe since 1970 (Crozier 1981, Astwood et al. 1998). A number of species of fungus (consisting of at least ten genera) grow on the honeydew produced by *Ultracoelostoma*. These form the characteristic black sooty mould that covers trees infected with *Ultracoelostoma* giving black beech trees (*N. solandri*) their name (McKenzie et al. 2000). This mould also covers the ground and plants underneath trees, feeding on honeydew that has fallen or been washed off by rain (McKenzie et al. 2000). This mould provides habitat and food to a number of invertebrates and can also be used as a reasonably accurate way of estimating insect population size on the tree (Didham 1993, Ewers 2002, Wardhaugh et al. 2006). The fallen honeydew can have a negative effect on ecosystem services, decreasing litter decomposition rates and therefore decreased nutrient cycling (particularly decreasing available soil nitrogen), causing a negative impact on beech trees. The honeydew that falls to the forest floor becomes available to soil microbes, altering the balance between fungi and bacteria in favour of fungi (Wardhaugh and Didham 2006, Wardle et al. 2010). The shift to fungi may be responsible for the decrease in litter breakdown (Wardle et al. 2010). However it also increases the amount of carbon sequestered in the soil (Wardhaugh and Didham 2006, Wardle et al. 2010). Ants commonly form a mutualism with honeydew producing insects. Honeydew has allowed exotic ants in other countries to establish and become invasive, threatening native species (Beggs 2001, O'Dowd et al. 2003, Gardner-Gee and Beggs 2009). Ants in New Zealand do not seem to exploit honeydew frequently, however they do consume honeydew opportunistically, particularly Argentine ants (Beggs 2001).

In New Zealand beech forests, another social hymenopteran, the common wasp (*Vespula vulgaris*) dominates honeydew resources. The high energy density of honeydew allows wasps to reach very high densities in beech forest infested with *Ultracoelostoma* (Sandlant and Moller 1989, Beggs 2001). The wasps are active January-May, after which workers die and queens hibernate over winter until September-October when they re-emerge and form new nests (Moller and Tilley 1989, Barlow et al. 2002). *Vespula vulgaris* originally invaded in the late 1970s, and then spread throughout New Zealand during the 1980s, reaching extreme densities in areas with honeydew (Beggs 2001, Beggs et al. 2011). They appear to have largely displaced a previous invader, *V. germanica*, in honeydew beech forests, likely due to their more efficient foraging of honeydew (Beggs et al. 2011). The biomass and nest density of *V. vulgaris* in honeydew beech forests is among the highest in the world (Barlow et al. 2002, Beggs et al. 2011). A study that tracked wasp density across seven beech forest sites for ten years found a mean nest density of 11.3 ha^{-1} (Beggs 2001). Moller and Tilley (1989) found up to 283 wasps m^{-2} recorded on trees trunks with honeydew with an mean of 58 m^{-2}.

These wasps are highly efficient at utilising honeydew, they can reduce the standing crop by more than 99% for four months of the year (Moller et al. 1991). They compete with other organisms for honeydew, which has been noted as a concern for conservationists as some endangered species, such as kaka, feed on honeydew (Beggs and Wilson 1991). This exploitation competition changes the feeding behaviour of the birds and may mean they cannot persist in beech forests (Beggs and Wilson 1991). A further impact of the wasps is that they may be able to reduce the amount of honey that bees produce through competition for honeydew and cause economic impacts (Moller and Tilley 1989). The overall however may be low, as wasps only dominate honeydew production for part of the year (Markwell et al. 1993). Before human settlement much of the honeydew may have been consumed by native birds but most is now consumed primarily by wasps (Beggs et al. 2005). As the wasps are seasonal the amount of honeydew reaching the forest floor is much higher when the wasps are inactive with subsequent consequences for soil fauna (Wardle et al. 2010). The wasps are

generalist predators, that heavily predate on many invertebrates for protein to supplement the high carbohydrate honeydew (Beggs 2001, Brockerhoff et al. 2010). For example Toft and Rees (1998) found that the chance of an orb-web spider (*Eriphora pustulosa*) surviving until the end of the wasp season was 1×10^{-18}, effectively zero. Nelson beech forests were also found to have wasps consuming 1.4 - 8.1 kg ha $^{-1}$ season $^{-1}$ of invertebrates (Harris 1991). This likely leads them to compete with other insectivorous species such as native bellbirds (Toft and Rees 1998, Beggs 2001, Murphy and Kelly 2003). However the wasps don't appear to predate on the mobile stages of *Ultracoelostoma,* or at least not enough to influence population dynamics (Wardhaugh and Didham 2004). Predation on mobile males may also not have significant consequences, as the insects are likely facultatively parthenogenic (Morales et al. 1988, Wardhaugh and Didham 2004), which is common among scale insects (Gullan and Kosztarab 1997). This is perhaps to maintain reproductive assurance in the face of difficulty finding mates (Gullan and Kosztarab 1997). Wasps also chew on the end of *Ultracoelostoma* anal tubes, this allows them to gain more honeydew, but may eventually lead to the death of the insect. However it is unknown how frequently this occurs (Moller and Tilley 1989). The wasps pose a nuisance to humans that use the beech forests, such as hunters and trampers (Beggs 2001). Some attempts at control of these wasps have been made, though results are mixed and the percentage reduction in wasp density required to reduce their impacts on other invertebrate species is likely over 90% (Beggs et al. 1998, Toft and Rees 1998, Beggs et al. 2011). Poison baiting has been effective at small scale management, although there have been difficulties with developing commercial products and biocontrol efforts using parasitoid wasps have been ineffective (Beggs et al. 2011). Since its introduction *V. vulgaris* has become one of the most ecologically damaging invasive invertebrates in New Zealand (Brockerhoff et al. 2010, Beggs et al. 2011).

1.3 Previous *Ultracoelostoma* studies

Due to the important role that *Ultracoelostoma* honeydew plays in beech forest communities a number of studies have attempted to estimate population densities (Wardhaugh and Didham 2005, Wardhaugh et al. 2006) and honeydew production rates (Kelly 1990, Kelly et al. 1992, Beggs et al. 2005, Dungan et al. 2007a, Dungan et al. 2007b). Both have been noted to be highly variable due potentially to a large number of factors (Kelly et al. 1992). Tree size, sun exposure, temperature, altitude, insect density and host species are among the factors that have been noted as potentially impacting honeydew production or insect densities (Beggs et al. 2005). Beggs et al. (2005) estimated production of between 3500 and 4500 kg dry weight ha^{-1} $year^{-1}$. Honeydew production as a proportion of tree net primary production has been estimated to be between 1.8 % (Dungan and Kelly 2003) and 80 % (Kelly et al. 1992). These large differences have been attributed errors in earlier estimates, with the most current estimate at around 6-8% (Beggs et al. 2005). The honeydew produced by *Ultracoelostoma* is remarkable globally in terms of the sheer quantity produced per unit area, which is why it is such an important resource to so many species (Beggs et al. 2005). The amount of honeydew produced by *Ultracoelostoma* scale insects in New Zealand beech forests has an influence on a number of conservation, economic and recreational factors. Investigating what controls honeydew production by individual insects, is therefore important for understanding the dynamics of populations and communities in beech forests including the population dynamics of a prolific invader (James et al. 2007). Previous studies have focussed on broad scale patterns of production, but this study focuses on insect-level factors. Tying production to insect-level variables should allow more accurate quantification of, and better reveal the physiological influences on, honeydew production in *Ultracoelostoma*.

Due to the importance of this production, there have been some models that attempt to predict individual insect production (James et al. 2007, James and Kelly 2011). However these models have underestimated honeydew production. They make the assumption that honeydew production fits a

Poisson distribution, where switches between producing and non-producing states are equally likely to happen at any time. An aim of this study is to explain this difference in predicted and actual production. James et al. (2007) used a dynamical model of honeydew droplet production based on simple fluid dynamics. Their model assumes that the insect does not regulate the flow rate and that the pressure gradient from phloem to atmosphere controls honeydew production in a continuous process (they also assumed that production follows a Poisson distribution). However to account for the actual honeydew production seen the sap flow rate would have to be 10-60 times higher than predicted by the Poiseuille flow model used. The Poiseuille flow model used is based on the Hagen-Poiseuille equation of fluid dynamics which is used to determine the pressure drop of liquid flowing through a cylinder and has previously been applied to insects with sucking mouthparts (Loudon and McCulloh 1999). James et al. concluded that the insects may be regulating the honeydew production due to this difference. Honeydew production rate would therefore be decoupled from feeding rate; the insect would feed at discrete times, process the phloem sap, and then actively excrete it as honeydew at discrete times. James and Kelly (2011) expanded on this, finding an episodic pattern of insect feeding and honeydew production (with a relatively small amount of time spent on each). They concluded that production is likely limited by the time required for gut processing (low metabolic rate), which may be limited by the low nutrient content of phloem sap, as nitrogen is needed to produce the required enzymes. The insect must process the sap to absorb nutrients and convert simple sugars to oligosaccharides to aid in osmoregulation. Their model suggested that insects spend 2 hours of any 12 hour period processing phloem sap and 12 minutes excreting honeydew. Their model provided predictions that were qualitatively similar to field data but again underestimated production. The 90 % confidence interval of the model only encompassed about 60% of an independent data set. The relatively low amount of time the insects spend on feeding and digesting may be attributable to its life history. Mobile animals may be vulnerable during excretion or may foul their living areas and typically have large, infrequent excretion events. *Ultracoelostoma* is sedentary, has a tube to remove excrement, and excretes long before being filled

with sugar so it does not face the same selection pressures (James and Kelly 2011). Despite their large numbers, *Ultracoelostoma* also appears to have few natural enemies so may suffer little for having a slow growth rate (Morales et al. 1988). These models suggested that temperature may influence insect gut enzymes and increase honeydew production. They also suggested that insect size could account for the model underestimation of honeydew production and honeydew weights.

1.4 Present Study

The present study investigated the role that as well as temperature (both ambient and manipulated) has on honeydew production. The purpose of this research is to investigate what controls honeydew production at the level of the individual of *Ultracoelostoma* insects in New Zealand beech forests. As so many species rely on this resource in beech forests, honeydew production is an important factor in the community dynamics of this system as well as the population dynamics of single species. These species include those that are commercially valuable, those that are invasive pests and those that are conservation priorities for New Zealand. This is the first study to investigate how characteristics of *Ultracoelostoma* individuals may influence honeydew production.

Chapter 2: The Influence of Insect Size and Instar on Individual Insect Honeydew Production

2.1 Introduction

A large number of factors influence the production of honeydew such as host species and quality, environmental variables, and characteristics of the honeydew producing insect itself. In many phloem feeding species (e.g. aphids, whiteflies, mealybugs) instar, age, size and weight influence honeydew production (Henneberry et al. 1999, Fischer et al. 2002, Zhou et al. 2013). For instance Fischer et al. (2002) found that in the tansy aphid (*Metopeurum fuscoviride*), honeydew production varied significantly among instars with adults producing twice as much honeydew as early instars. This may influence individual insect populations as well as wider production due to variations in age structure between populations. The metabolic changes between instars change how energy is managed and what resources are required by the insect (Lorenz and Gade 2009, Zhou et al. 2013). Discrete differences in feeding and subsequent honeydew production may then occur to match needs (Fischer et al. 2002). This may vary between insect species due to different life histories and metabolisms (Zhou et al. 2013). A species that experiences large amounts of growth in a particular instar may feed more to fuel this growth and consequently produce more honeydew during that stage (Fischer et al. 2002, Zhou et al. 2013). Also at larger sizes and weights, the insects may have an increased capacity for honeydew production or requirement for nutrients. This would likely lead to more continuous changes in honeydew production than changes due to instar, with size and weight proportional to production (Fischer et al. 2002, Zhou et al. 2013).

In *Ultracoelostoma* most previous studies have focussed on environmental and host tree effects on honeydew production (Dungan and Kelly 2003). Individual insect characteristics have not been

investigated as a predictor of honeydew production. However previous papers have noted that insect size or instar may be an important part of predicting aspects of honeydew production. James and Kelly (2011) suggested that adding an insect size variable to their model could account for their underestimation of honeydew production. Beggs et al. (2005) suggested that different instars could produce honeydew with different sugar compositions. Current knowledge about *Ultracoelostoma* life cycle, population dynamics and generation times however is however limited.

The life cycle of *Ultracoelostoma* consists of four female instars and five male instars (Morales et al. 1988). First instar juveniles of both sexes are mobile, and disperse into preferred habitat (usually bark fissures). Next they then settle, insert their mouthparts into the beech tree phloem and begin to feed. Next they moult, form a case and an anal tube from which they excrete honeydew. They then become second instar, losing many of their appendages and become sedentary. After the second instar the sexes split up. Males leave their cases as pre-pupae (third instar) and pupate (fourth instar) on the forest floor to emerge as winged, fifth instar adults that may mate with females. Females however remain sedentary and continue to feed and produce honeydew into the third instar. Adult, fourth instar females stop feeding, lose their anal tube, lay eggs, and die (Wardhaugh and Didham 2005). The eggs then hatch as first instar juveniles, exit the mother's case through the anal pore and disperse. Honeydew is produced by the first and second instars of both sexes and third instar females (Morales et al. 1988). Morales (1988) investigated the size distribution of honeydew producing instars and found the the first instar 1 mm long and 0.5 mm wide, second 1 - 1.6 mm diameter, third instar 2.5 - 4.5 mm diameter (female). Studies have found conflicting findings on seasonal variation in the abundance of *Ultracoelostoma* instars. Morales found overlapping generations and no differences in instar abundance, however (Moller and Tilley 1989) found declining numbers of anal tubes from spring to summer and inferred a drop in second and third instar insects. They suggested that the non-random, low sample size of Morales may have been responsible for the lack of seasonality seen in that study. Generation times are currently unknown

though estimates vary from one to four generations a year (Crozier 1981, Morales et al. 1988). Little research has been done on the insect characteristics since Morales (1991).

To study the effect of insect characteristics on honeydew production I measured production across a number of insects and then measured these insects for case size, insect size, instar, and dry weight. Other factors such as temperature, humidity and tree effects may mask the effect of insect instar or size on honeydew production (Dungan et al. 2007a) and also need to be accounted for. I predict that larger, later instar insects will exhibit higher production due to their higher metabolic requirements for energy and nutrients. In particular, if large third instar insects (which are exclusively female) produce larger drops this may explain the periodic large drops that occur. This unexplained production of infrequent larger drops is partly responsible for the underestimation of production by current models (James and Kelly 2011).

This study may also serve to answer another question related to insect size. Dungan et al. (2007a) suggested that the relationship they found between insect density and tree diameter may be partly related to insect size. Larger trees appear to have low insect densities, they suggested that this may be because only the largest insects are able to penetrate the thick bark to reach the phloem. This means that I should find larger insects on larger trees.

2.2 Methods

Study Site

Sampling took place at Mt. Richardson (43.194° S, 172.254° E) within the Mt. Thomas Forest in the foothills of north-west Canterbury (Figure 2.1) at approximately 400 m a.s.l. Mt. Richardson is located north-east of Oxford township, accessed via Glentui Bush Road and is part of the DOC Waimakariri conservancy. The area contains two public walking tracks and a picnic area. The forest composition is primarily black beech canopy (*Nothofagus solandri*) (forest composition shifts in many places from almost pure beech to highly mixed stands). Other tree species present include tree fuchsia (*Fuchsia excorticata*), broadleaf (*Griselinia littoralis*), mahoe (*Melicytus ramiflorus*), and lancewood (*Pseudopanax crassifolius*). Undergrowth is dominated by crown fern (*Blechnum discolour)*, with a number of other fern species in lesser abundance. Other understory species include *Coprosma* spp., climbing rata (*Metrosideros* sp.) horopito (*Pseudowintera colorata*), bush lawyer (*Rubus* sp.), black beech juveniles and juveniles of other tree species. As in most beech forests infested with *Ultracoelostoma* spp., wasps (*Vespula vulgaris*) are abundant in the summer months harvesting honeydew. Since *U. assimile* is found primarily on braches and *U. brittini* exclusively on trunks it is likely that the species examined in this study is *U. brittini* (Morales 1991).

In accordance with DOC guidelines all sampling was done away from the track to avoid any interference with the research, and to avoid encouraging others to leave the track.

Tree selection

A general area on the Mt. Richardson loop track was randomly selected by using a random number generator. From this area three sites were chosen to sample in, two downhill of the track (see map of sites) based on subjective assessments of distance and visibility from track, ease of access from track, safety, and presence of scale insect (*Ultracoelostoma* spp.) infected live beech trees. Within

each of these sites five *N. solandri* trees were randomly selected to give a total number of 15 trees to attach sampling frames to. Sampling frames were flat rectangular 33 x 23 cm white plastic sheets with two 25 x 5 cm rectangular holes (henceforth referred to as panels) which were the areas on the tree used for sampling. Each of these trees was selected by first defining an area within the site as the centre or starting point. Individual trees were selected by starting from this point then moving in a randomly selected distance and direction. From this point the closest live beech tree with DBH >7.5 cm was selected and a sampling frame nailed to it (randomised aspect) which was repeated to get five trees per site. Some smaller trees (those 7.5< x <13 cm DBH) could only fit one panel on the trunk so only had one sampling area. Positions of insects that appeared to be producing honeydew from each frame were then mapped onto scale diagrams of the frames. As sampling continued, insects that started to produce honeydew were added to the maps. This allowed tracking of live, honeydew producing insects, so that honeydew production of individuals could be recorded. At the end of sampling all insects that had produced a drop at some point were assumed to have been alive throughout the sampling period. Therefore for instance if an insect produced drops only on the last day of sampling, on all previous sampling days it was retrospectively recorded as being present and producing no honeydew. It was included in mean honeydew production per insect calculations and subsequent test and zero counts of drop weight for these times were included in drop weight calculations. High wasp density meant that nylon mesh had to be used to cover sampling frames and prevent harvesting, which would have caused underestimation of honeydew production. This netting may have influenced factors such as air movement and evaporation, this may have be an issue since refractometer readings were taken from honeydew that was not under netting. The netting was placed over the frame and nailed to the tree, the netting was either held away from the frames by the nails attaching the frame to the tree or left loose. This was necessary so that the netting would not come into contact with the anal tubes and absorb honeydew drops. During sampling therefore it was necessary to open up the netting and sample under it. Insect density on each tree and tree DBH were measured. Insect density was measured by counting the number of

apparently active anal threads within one panel (26 x 5 cm) of sampling frames placed on the four cardinal aspects of each tree (as in Wardhaugh and Didham 2006) then using this to calculate insects per m^2 of trunk.

Measuring Honeydew Production and Temperature

Honeydew drops were cleared from all the anal tubes within the frames in the morning, moving from site 1 through to 3 (starting from 9 am) then at three, 3-hour intervals (approximately 12 pm, 3 pm, 6 pm) new drops were removed and drop volumes measured. Honeydew volume and sugar concentration were measured using micro-capillary tubes (10 µl capacity, 41 mm long) and a refractometer respectively. Volume was measured by sucking up honeydew drops into microcapillary tubes, measuring the length of microcap taken up by honeydew, recording this, then later converting it to volume. A refractometer reading (honeydew taken from unframed trees) was taken at each site per three hours (along with honeydew measurement) to give a mean honeydew concentration from each site for that time. This was required because it was difficult to get the drops out of microcaps onto the refractometer and a large number of drops were necessary for a reading. Measurement of volume and or sugar concentration using microcapillary tubes and refractometer has been used in previous studies (Kelly et al. 1992, Dungan and Kelly 2003) and is explained in detail in (Corbet 2003) (in relation to flower nectar). The amount of time between sampling days was chosen to allow a weather change to compare production under varying temperatures and humidities. Sampling was not undertaken in wet weather, as rainfall knocks off honeydew drops from anal tubes. Temperature and humidity data were measured at Mt. Richardson at every sampling period at each site with HOBO® sensors (Onset Computer Corporation, Massachusetts, USA). These sensors were attached to stands and partially covered to keep them off the ground and keep them dry and out of direct sunlight. They were set up to run continuously from 9 am 13 March 2012, recording temperature and humidity every 15 minutes, until the end of sampling. (Dungan et

al. 2004) suggested a gravimetric method for sampling honeydew as it was easier and faster than the refractometer and microcapillary method. However the gravimetric method provides no information on droplet volume, which was required in this study to relate to previous models of production (James et al. 2007), hence I used the microcapillary method.

Measuring Insect Characteristics

After measuring was complete across four sampling days all active insects from the frames were removed, to gather insect instar, size, and weight data. All 304 active-insect cases were removed (trying to keep the case intact) from the trees using a knife, cutting a small amount of bark away from the tree with the case to keep it intact, and placed into labeled plastic tubes. If other, non-producing cases were taken along with the producing case, the anal tubes of the non-producing cases were removed to make sure that the producing case was easy to identify. Some insects were punctured during removal; these were placed onto dried, weighed filter paper so that fluids were not lost. All insects were then taken back to the lab and stored in a freezer. The cases were measured externally (diameter and length) using digital callipers, then removed from the insects using a binocular microscope, fine tweezers, and a scalpel taking care not to damage the insect. The insect instar was then determined by looking at sclerotisation patterns on the insect body (Figure 2.2) (Morales 1988). Mainly the second and third instars produce honeydew, and the third instar has a distinctive band of black dots around the anal region. A few of the smallest insects had unformed cases yet had produced honeydew and these were assumed to be first instars. If the insect was undamaged then the insect itself was measured for both diameter and length. Both damaged and undamaged insects were then weighed. Filter paper was cut up into small triangles (one for each insect) that were then numbered and then dried in an oven for two hours and cooled with silica gel for 15 minutes, and weighed to 0.00001 g. An insect was then placed on the paper, burst, and spread over the paper (to ensure proper drying). Each piece of filter paper was then folded over and

placed into an individual manila folder and dried in the oven overnight at 60 ^{o}C. After this they were again cooled (with silica gel), weighed and the previous value of paper weight subtracted from this to give the weight of each individual insect. Some insects had died and decomposed or produced eggs before being collected, so could not be weighed or measured and were excluded from further analysis.

Statistical Analysis

The gravimetric refractometry values (g/100 g) were then converted to volumetric values (g/100 ml) using the quadratic equation:

$y = 1.123 + 0.994x + 0.0049x^2$ Obtained from (Dungan et al. 2004).

All honeydew values used in analysis were therefore in µg dry weight of sugar.

Regression analysis was used to test the relationship between temperature and honeydew production. ANOVAs were used to test the effect of Julian day and temperature on the number of active insects and mean drop weight. The order of Julian day and temperature were reversed in each ANOVA to control for correlations between these. Repeated measures nested ANOVAs were used to test the effect of insect size on per-insect honeydew production at two levels: mean honeydew production per insect per site at each sampling day (µg h^{-1} $insect^{-1}$ $site^{-1}$) and individual insects (µg h^{-1} $insect^{-1}$). The nested ANOVA was required to account for non-independence of honeydew production within site, within trees and within insects. The repeated measures ANOVA was required to account for similarities due to sampling the same insects across multiple sampling events. Testing the effect of insect instar on individual drops (µg) required a quasipoisson GLM with a nested structure as drop weight did not adhere to a normal distribution. Instances where a drop was not formed (i.e. drop weight = 0 µg) were not used in analysis. Honeydew production and drop weight was logged (Log_{10}+1) when used in analysis. Insect size and insect instar were the primary variables

of interest but other variables were included to account for other likely sources of variability. These variables included air temperature, humidity, tree insect density, and tree DBH (to explain between tree variability). Temperature and humidity were averaged over the preceding 24 hours before sampling for use in the test to ensure that the variation that would influence production was accounted for, consistent with Dungan and Kelly (2003). Humidity however was not used in analysis, as its primary effect on honeydew production is to alter drop volumes, and honeydew production was measured in sugar weight. Temperature was then used in analysis with insect instar and size to explain variation and to test interactions. Insect size was calculated as elliptical area (in mm^2) using the formula: insect size $= \pi \times \frac{diamter}{2} \times \frac{length}{2}$, to be used in analysis. Linear and quadratic regressions were used to test the relationship between insect density and tree DBH and insect size and tree DBH respectively. All statistics were conducted using R (v. 2.14.1; R Development Core, 2011).

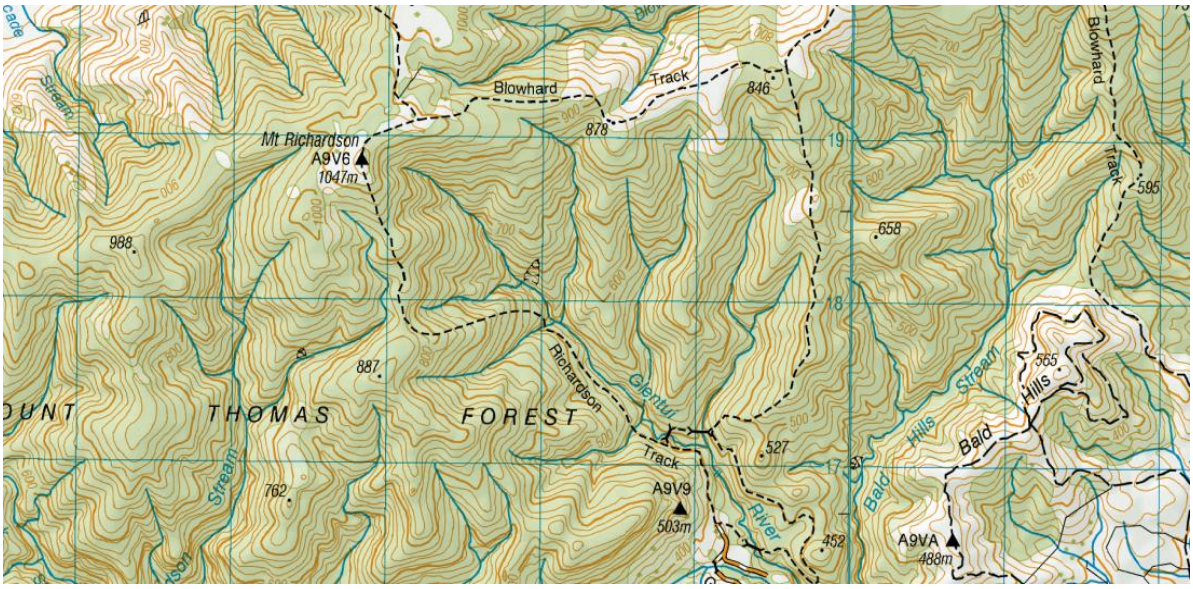

Figure 2.1: Map showing Mt. Richardson, Mt. Richardson track and Loop track. Scale lines are 1km apart (http://www.topomap.co.nz/).

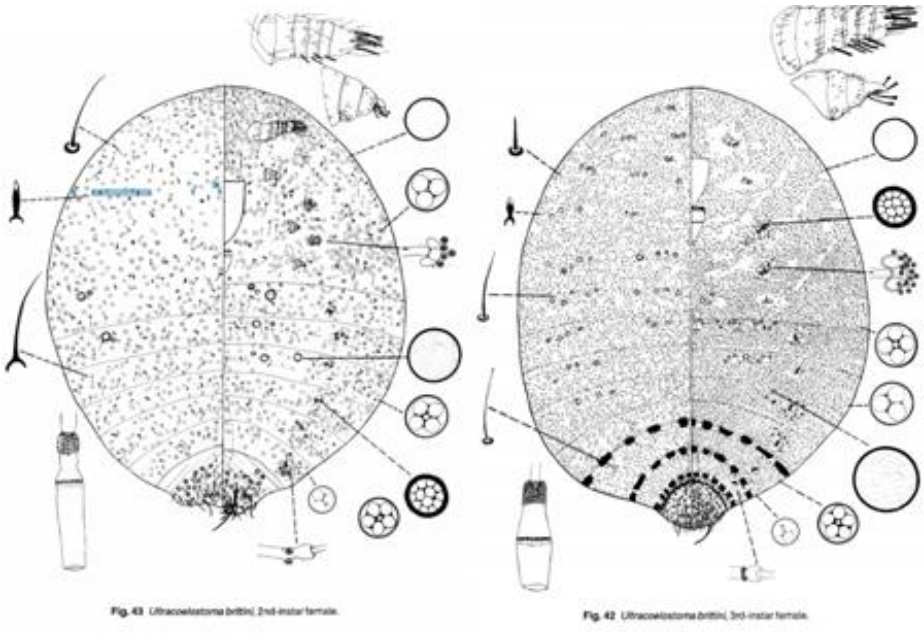

Figure 2.2: Diagrams of second (left) and third (right) instar insects showing the black pattern of dots used to identify 3rd instar insects. Reproduced from Morales (1988).

2.3 Results

Honeydew Production and Temperature

The total number of honeydew producing insects was 276, however some were damaged and only 255 had their size measured. The number per tree ranged from 2 - 53. Honeydew production measured per insect per hour (mean over 9 hours) ranged from 0 (62% of all data) to 1.15 µg with a mean of 0.0461 µg. A total of 603 drops were produced. The mean drop weight when a drop was produced was 0.232 µg and the maximum was 2.46 µg. Tree DBH ranged from 7.48 cm - 42.29 cm with a mean of 24.24 cm. Insect densities (at breast height) per tree varied between 380 and 1440 insects m^{-2} with a mean of 851.2 insects m^{-2}.

Temperature was found to have a strong positive relationship with honeydew production (Fig 2.3). Honeydew production (µg $insect^{-1}$ h^{-1}) was positively correlated with ambient temperature averaged over the 24 hours preceeding sampling ($F_{(1,2)} = 41.97$, $R^2 = 0.712$, $p = 0.023$) (Table 2.1). This strong positive relationship is due to both increasing quantities of honeydew produced by each insect and an increasing quantity of active insects at higher temperatures (Fig 2.4 and 2.5, Table 2.2). The highest mean rate of honeydew production was 0.131 µg h^{-1} from site 3 on 18 March 2012 which was at the highest temperature, 15.1 ^{o}C. Temperature decreased steadily across the four sampling days, ranging from 15.1 ^{o}C on the first sampling day to 7.2 ^{o}C on the last. There was no significant difference between trees in mean honeydew production per insect ($F_{(2,2)} = 3.022$, $p = 0.249$) (Table 2.1). There was no significant difference in honeydew production between sampling days when controlling for temperature changes (Table 2.1).

Honeydew ~ Temperature + Tree + Sampling day

Error: Sampling day/Site

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
Temp	1	0.09201	0.09201	41.968	0.023	*
Tree	2	0.01325	0.00663	3.022	0.249	
Sampling day	3	0.01957	0.00652	2.976	0.262	
Residuals	2	0.00438	0.00219			

Error: Within

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
Temp	1	0.0433	0.04326	27.652	<0.00001	***
Tree	14	0.0353	0.00252	1.611	0.07	.
Residuals	996	1.5583	0.00156			

Table 2.1: ANOVA table of relationship between honeydew production and temperature, with added factors of tree and sample day.

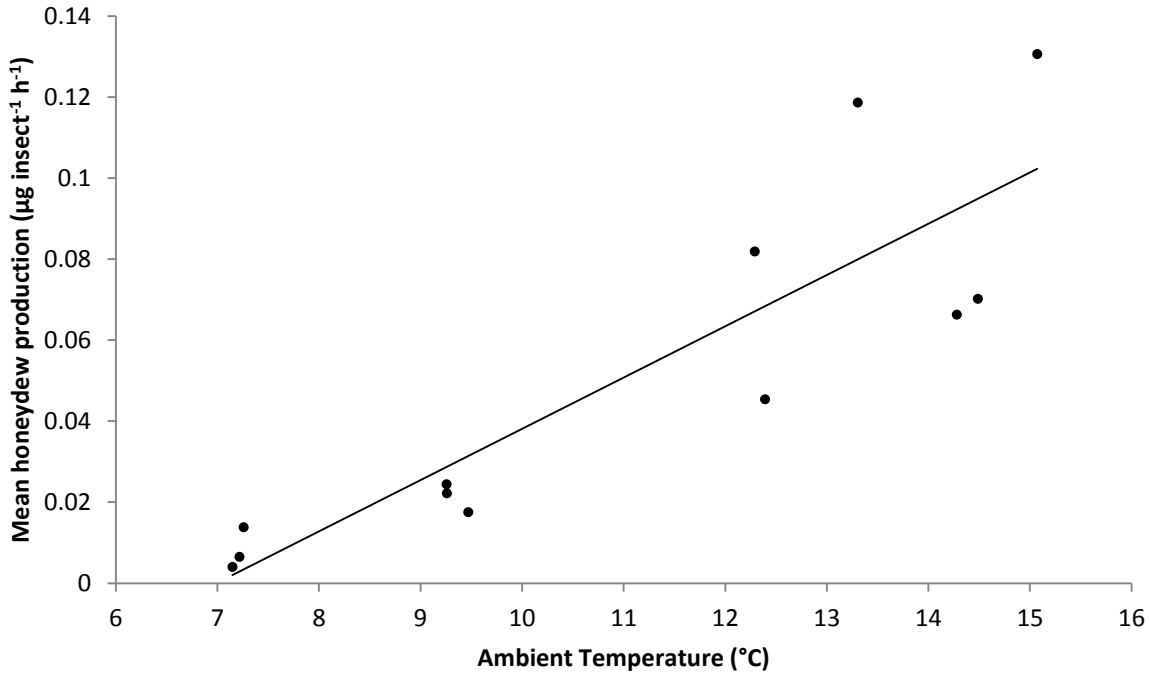

Figure 2.3: Mean honeydew production per insect per site (µg insect^{-1} h^{-1}) against temperature (^{o}C average air temp for preceding 24 hours). Across four sampling days at Mt. Richardson with three sites and one point per site per day between 18 March and 12 April 2012.

There was a significant positive relationship between temperature and mean drop weight (Table 2.2). At higher temperatures the weight of produced drops was much higher, 0.125 µg at the lowest temperature compared to 1.85 µg at the highest (Fig. 2.5a). There was a significant positive relationship between the number of active insects and temperature (Fig. 2.5b). The relationship between temperature and the number of active insects was not as strong with a mean of 41 insects active at the lowest temperature and 61 at the highest. There was also a significant negative relationship between Julian day and mean drop weight (Fig. 2.4a) and between Julian day and number of active insects (Fig. 2.4b). However when controlling for temperature in the analysis there was no effect of Julian day on either the number of active insects or the drop weight (Table 2.2).

Drop weight ~ Day * Temp

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Day	1	6.627	6.627	70.679	<0.0001***
Temp	1	0.065	0.065	0.689	0.430
Day : Temp	1	0.087	0.087	0.929	0.363
Residuals	8	0.750	0.094		

Drop weight ~ Temp * Day

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Temp	1	6.609	6.609	70.489	<0.0001***
Day	1	0.082	0.082	0.879	0.376
Temp : Day	1	0.087	0.087	0.929	0.363
Residuals	8	0.750	0.094		

No. of active insects ~ Day * Temp

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Day	1	732.5	732.5	6.960	0.0298*
Temp	1	22.0	22.0	0.209	0.6595
Day : Temp	1	46.3	46.3	0.440	0.5256
Residuals	8	842.0	105.3		

No. of active insects ~ Temp * Day)

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Temp	1	650.0	650.0	6.176	0.0378*
Day	1	104.5	104.5	0.993	0.3482
Temp : Day	1	46.3	46.3	0.440	0.5256
Residuals	8	842.0	105.3		

Table 2.2: Tables of ANOVAs for the relationships of the number of active insects and mean drop weight against Julian day and temperature (tried with each of the predictors entered first).

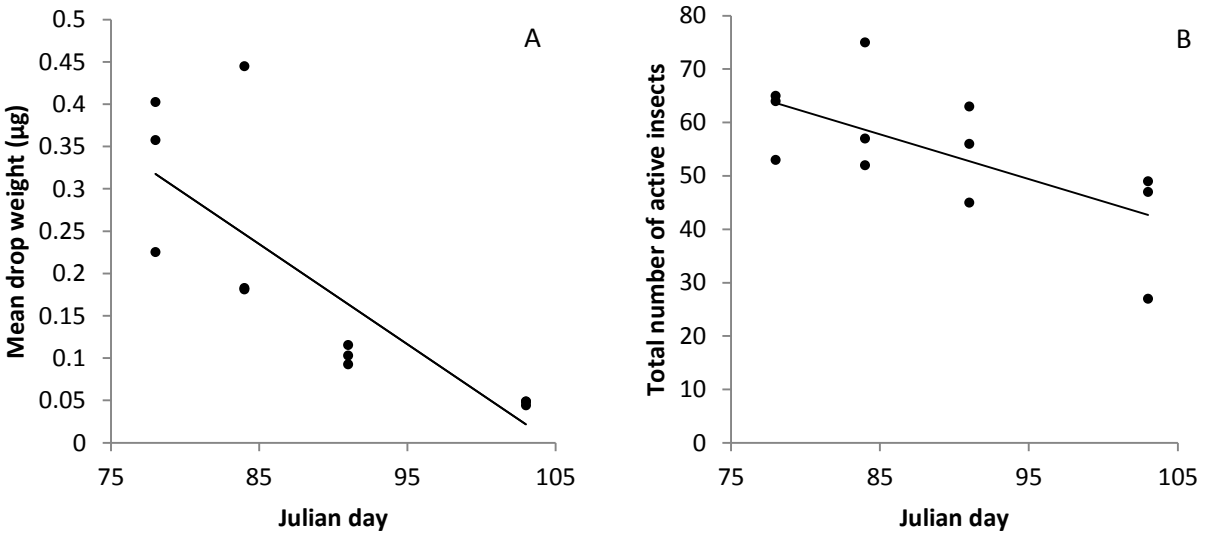

Figure 2.4: (a) Mean drop weight and (b) number of active insects, against Julian day with one point per site per day.

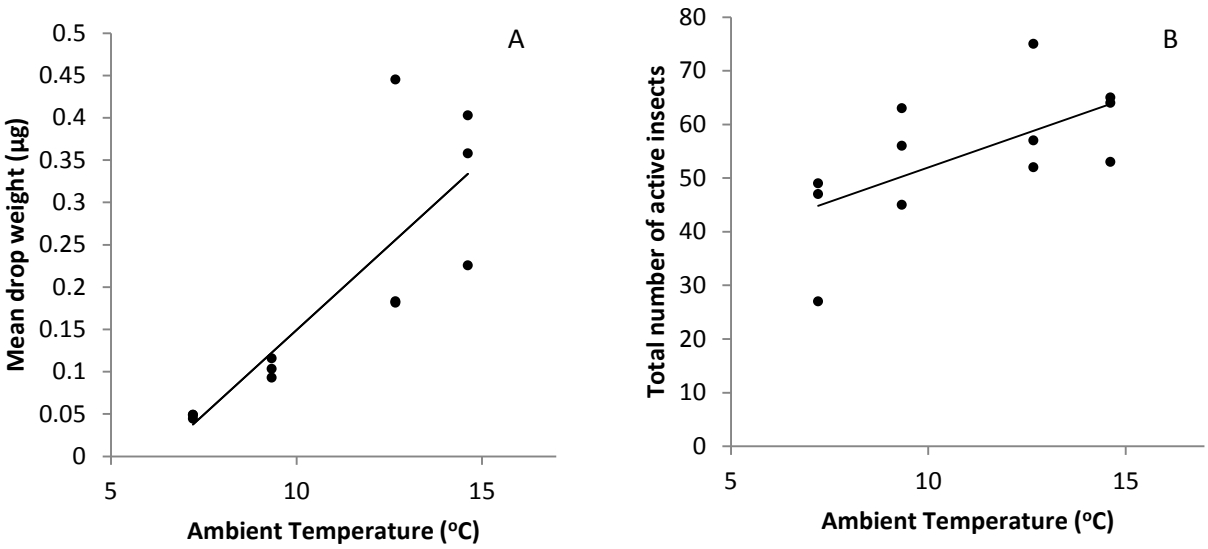

Figure 2.5: (a) Mean drop weight and (b) number of active insects, against temperature with one point per site per day.

Insect Characteristics and Honeydew Production

The insects sampled in this study varied in size from 0.1005 mm^2 to 16.41 mm^2 cross sectional eliptical area with a mean of 4.215 mm^2 (Fig. 2.6). There were 3 insects in the 1st instar, 118 in the second instar and 127 in the third instar. Later instar insects were significantly larger than earlier

instars though there was some overlap (Table 2.3). The mean size (±SD) of first instar insects was 0.2309 ± 0.1635 mm^2, second instars 2.073 ± 0.9948 mm^2 and third instars 6.410 ± 3.342 mm^2. The distributions of first and second instar sizes were unimodal while third instar insects have an approximately bimodal distribution. Honeydew production varied with insect characteristics. Insect instar had a stronger effect on the weight of individual drops than to production over time.

Insect size ~ Instar

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Instar	2	1993	996.3	100.7	<0.0001 ***
Residuals	245	2423	9.9		

Table 2.3: ANOVA table of the relationship between insect instar and insect size.

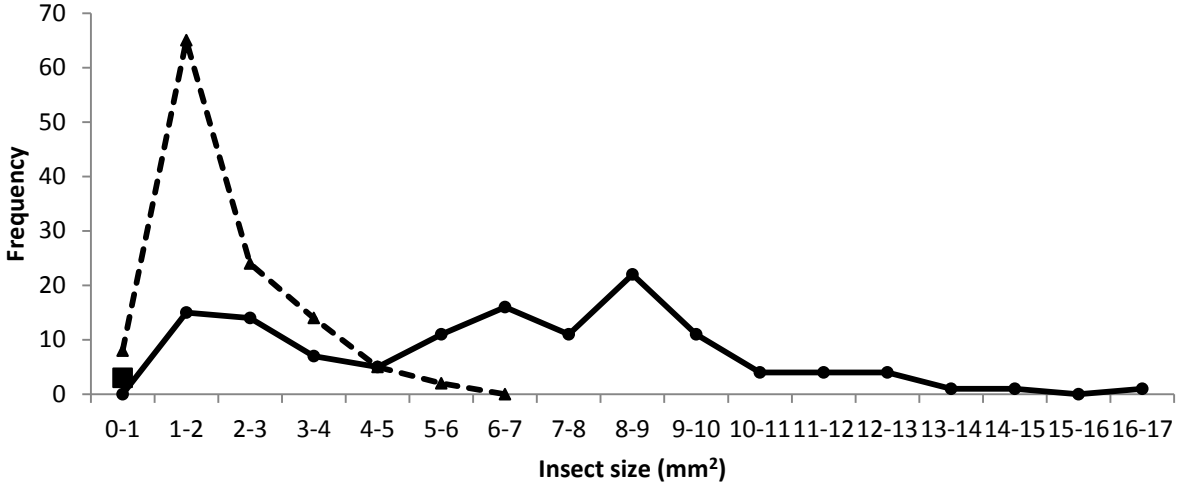

Figure 2.6: Insect size distributions with 1mm bins for seperate instars. Square markers and dotted line for first instar, triangle markers and dashed line for second instar, circle markers and solid line for third instar.

A significant positive relationship was found between insect instar and honeydew drop weight (Table 2.4) though the R^2 is low, likely due to the large overlap between instars (Fig. 2.8). Later instar insects produced significantly more sugar per drop than earlier instars (Fig. 2.7). First instar insects produced a mean of 0.106 ± 0.0855 µg, second instars produced a mean of 0.187 ± 0.192 µg and third instars 0.276 ± 0.371 µg per drop.

First instar insects produced only smaller drops (though there was a low sample size for first instars), second instar and third instar produced a similar quantity of smaller drops. However third instar insects also produced a number of larger drops.

Drop weight ~ Temperature+ Tree/Instar

	Df	Deviance	Resid. Df	Resid. Dev	F	Pr(>F)
NULL			602	40.981		
Temp	1	0.00228	601	40.978	0.0304	0.861727
Tree	14	1.09563	587	39.883	1.0433	0.407901
Tree : Instar	17	3.04181	570	36.841	2.3854	0.001433 **

Table 2.4: Table of deviance for quasipoisson GLM of the relationship between drop weight and insect instar.

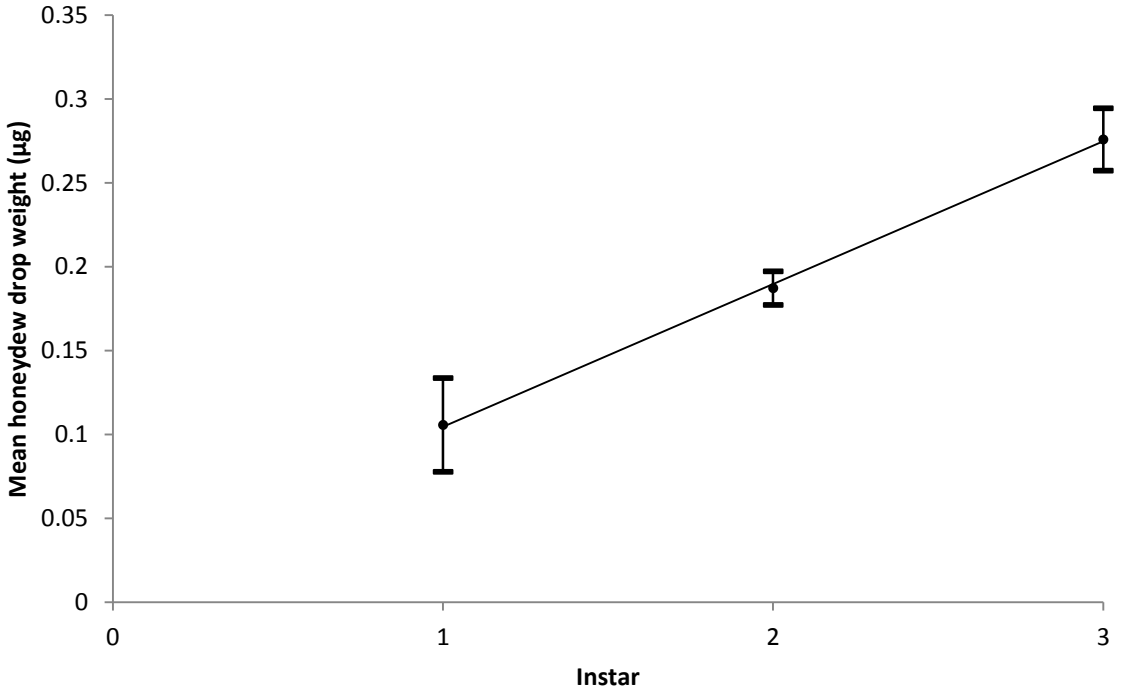

Figure 2.7: Mean honeydew drop weight (µg) of each instar with 95% confidence interval error bars.

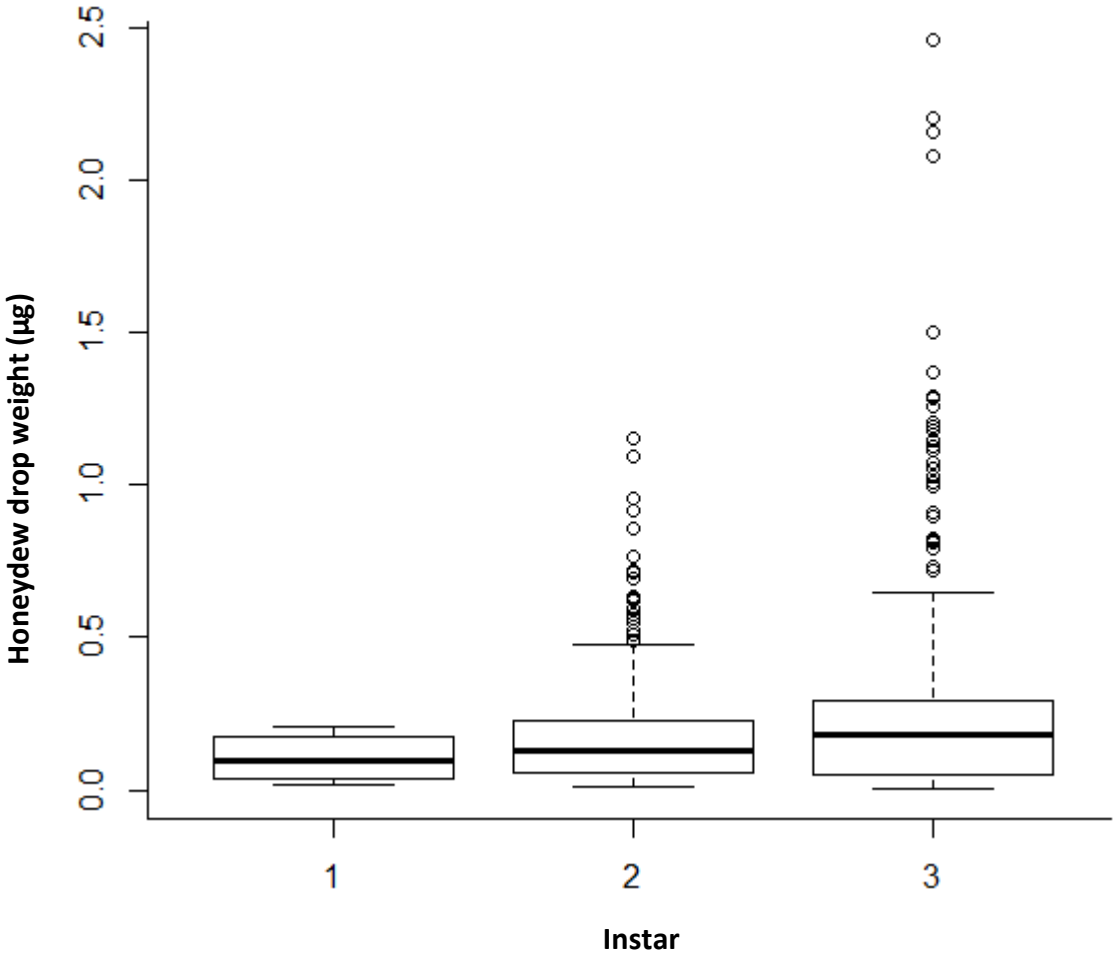

Figure 2.8: Box plot of honeydew drop weight (µg) of different instars.

Later instar insects were able to produce larger honeydew drops, with only third instar insects producing drops with >1.15 µg (0.332 logged) of sugar (Fig. 2.9). There was an absolute limit to the drop weight produced by each instar. First instars did not produce more than 0.211 µg (0.0831 logged), third instars were the only ones to produce more than 1.15 µg drops, with the maximum, third instar, drop being 2.46 µg (0.539 logged). Most drops produced by any instar were in the smallest category with the frequency of drops decreasing with increasing weight.

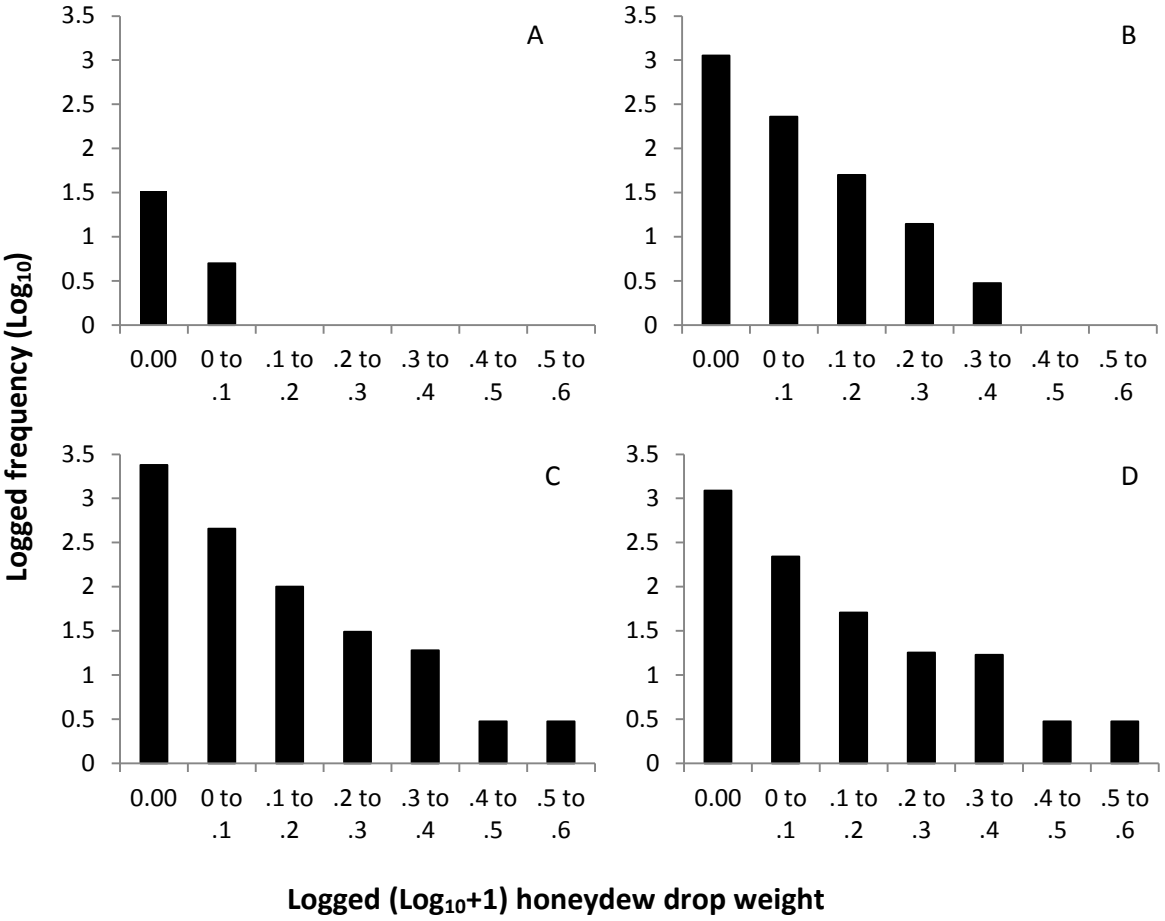

Figure 2.9: Frequency (log_{10}) distribution of honeydew drops (log_{10}+1 µg) separated by insect instar. (a.) First instar (b.) second instar (c.) third instar (d.) all instars combined.

Insect size (elliptical area) had a significant interaction effect with temperature on mean honeydew production ($p < 0.0001$) (Table 2.5). A quasipoisson GLM was also used to test this relationship but didn't change the conclusions, therefore the ANOVA in table 2.5 was used. No relationship was found between honeydew production and insect weight or case size ($F_{(1,242)} = 0.399$, $p = 0.528$). The

insect size variable used was a cross sectional elliptical area as this had the best relationship with production. All size classes have a positive relationship between temperature and honeydew production. At high temperatures the differences in honeydew production between insect sizes are most pronounced (Fig. 2.10). Honeydew production at the highest temperature is lowest in medium sized insects and highest (over four times higher) in the largest group of insects. At 15.1 ^{o}C, mean production by the smallest insects was 0.115 µg h^{-1}, the largest 0.296 µg h^{-1} and medium insects 0.0572 µg h^{-1}. The honeydew production of medium insects was on average consistently higher than that of small insects (Fig. 2.10). The highest rate of production 1.15 µg h^{-1} was attained by a larger insect (10.80 mm^2) at 15.1 ^{o}C. At 7.2 ^{o}C differences among insect size classes were negligible and overall production was much lower (~0.005 µg h^{-1}).

Honeydew ~ Insect size * Temperature

Error: Site/Tree/Insect)

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Size	1	0.0019	0.001938	1.331	0.24976
Temp	1	0.0150	0.015000	10.304	0.00151 **
Size : Temp	1	0.0249	0.024944	17.135	<0.0001 ***
Residuals	242	0.3523	0.001456		

Error: Within

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Size	1	0.0000	0.00001	0.006	0.9373
Temp	1	0.1298	0.12985	80.955	<0.0001 ***
Size : Temp	1	0.0054	0.00538	3.353	0.0675 .
Residuals	771	1.2366	0.00160		

Table 2.5: ANOVA table of the relationship between honeydew production and insect size (elliptical area).

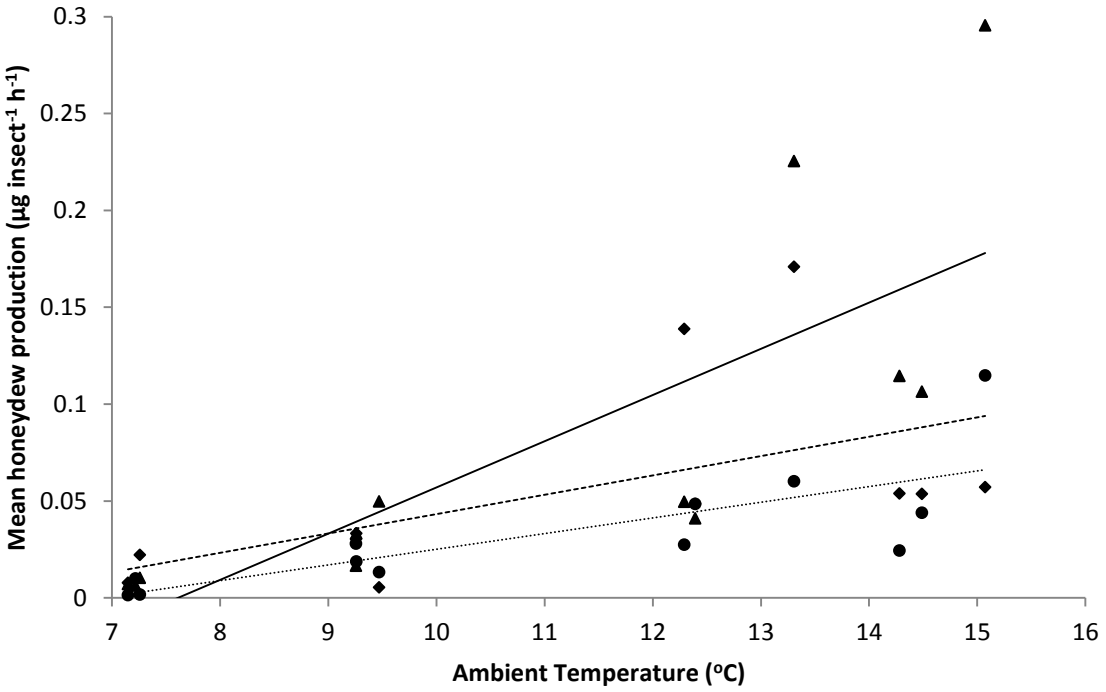

Figure 2.10: The effect of temperature (°C) on honeydew production (µg insect^{-1} h^{-1}) at different insect size classes. Insect size was analysed as a variate but is grouped as a 3 level factor here for better visual presentation. Size classes are organised so that equal numbers of insects fall into each category. Small class range is 0 – 1.876 mm^2 medium class range is 1.876 – 5.254 mm^2 large class range is 5.254 – 16.39 mm^2. Circles and dotted line for small class, diamonds and dashed line for medium class, triangles and solid line for large class.

Insect Characteristics and Tree DBH

There was no relationship between insect size and tree DBH (Table 2.6). Only one tree had a DBH >40cm and this had the lowest insect density, of 380 insects m^{-2} (Fig. 2.11). The lowest DBH tree, at 7.48cm, had 1440 insects m^{-2} which was the highest density of insects.

Density ~ Dbh + Dbh^2

Residuals:

Min	1Q	Median	3Q	Max
-400.61	-293.94	29.16	145.26	714.55

Coefficients:

	Estimate	Std. Error	t value	Pr(>|t|)
(Intercept)	596.8294	442.5197	1.349	0.202
Dbh	20.2192	41.3353	0.489	0.634
Dbh^2	-0.4043	0.8613	-0.469	0.647

Residual standard error: 346.7 on 12 degrees of freedom

Multiple R-squared: 0.01966, Adjusted R-squared: -0.1437

F-statistic: 0.1203 on 2 and 12 DF, p-value: 0.8877

Table 2.6: Quadratic regression analysis of insect density against tree DBH.

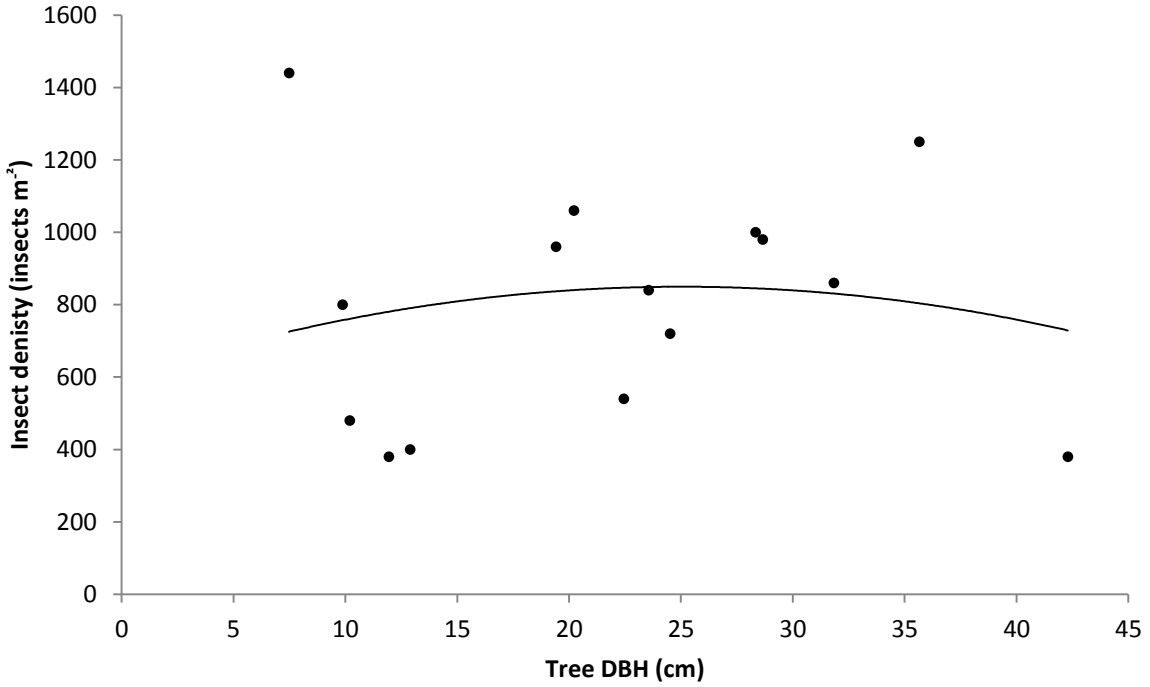

Figure 2.11: Tree DBH in cm (diameter at breast height) against insect density (insects m^{-2}), a point for each tree.

A significant positive, but modest, relationship was found between tree DBH and insect size (R^2 = 0.295, p = 0.0364) (Table 2.7). The highest DBH tree had a mean insect size of 5.56 mm^2 and the lowest DBH tree 4.41mm^2 (Fig. 2.12). The largest insect was present on the highest DBH tree and trees that were DBH <25 cm did not hold any insects >12 mm^2.

Dbh ~ Size

Error: Tree

	Df	Sum Sq	Mean Sq	F value	Pr(>F)	
Size	1	5026	5026	5.441	0.0364	*
Residuals	13	12008	924			

Error: Within

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Size	1	1.000e-29	7.400e-30	0.01	0.92
Residuals	239	1.738e-25	7.273e-28		

Table 2.7: ANOVA table for the relationship between tree DBH and insect size.

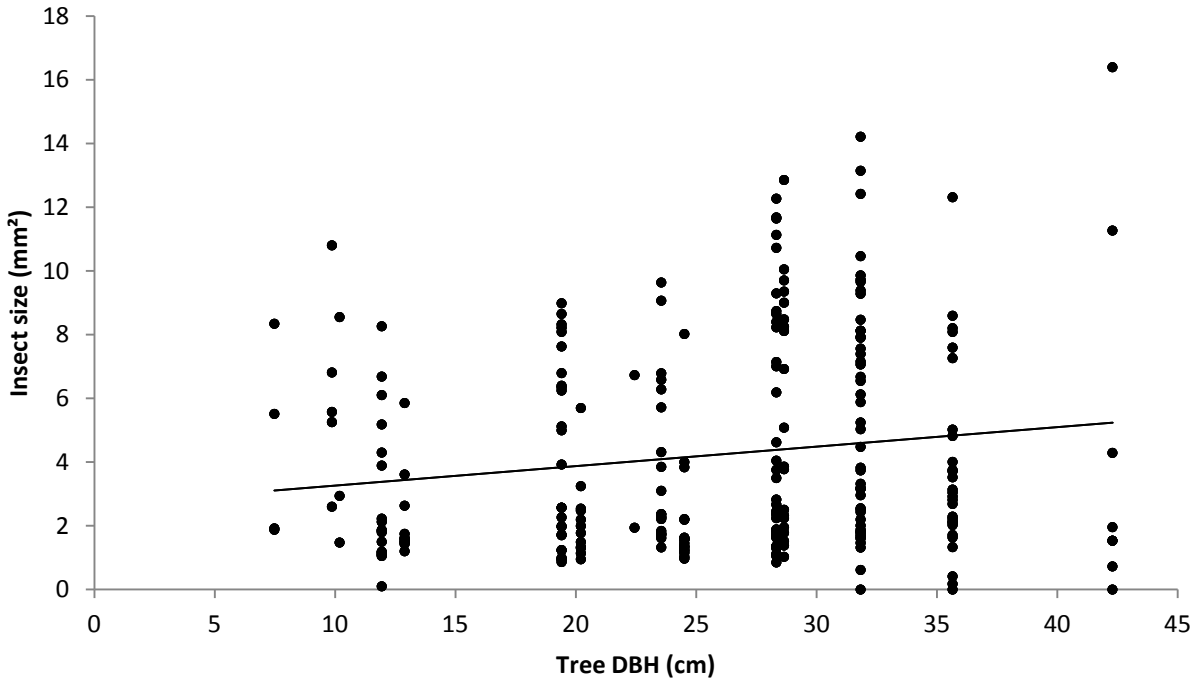

Figure 2.12: Tree DBH in cm (diameter at breast height) against insect size (mm^2).

2.4 Discussion

Honeydew Production and Temperature Relationship

The strong positive relationship between temperature and honeydew production found in this study is consistent with previous research and with the idea that honeydew production may be limited by gut processing speed (Dungan and Kelly 2003, Dungan et al. 2007a, James and Kelly 2011). With increasing temperature, gut processing enzymes may increase in activity and therefore increase honeydew production (James and Kelly 2011). However it is unknown whether this is the case and whether it is an effect at the level of the insect or the level of the tree, which will be addressed in Chapter 3. I found that insects on different trees did not differ significantly in their rate of production of honeydew. This was unexpected as there are many variables that may differ between trees, such as photosynthetic rates and sap nitrogen content as well local microclimate and the density and characteristics of the insects themselves (Douglas 2006, Dungan et al. 2007a). Dungan et al. (2007a) however found in their study that manipulations of tree photosynthesis and nitrogen did not influence honeydew production. I also found that there was no difference in mean production per insect between sampling days when controlling for temperature. This is not surprising as the majority of variation in production between years, between seasons and within seasons is attributable to environmental variables (Beggs et al. 2005, Dungan et al. 2007a). Especially since, with sampling for this study taking place over a single month, demographic seasonal changes in insect populations may not have occurred. Temperature had a positive relationship with both mean drop weight and the number of active insects. So temperature may not only act to increase the drop weight of already producing insects but also increase the number of productive insects i.e. increasing the rate of production events.

Insect Characteristics

The insects in this study had a similar mean diameter to those in Morales (1988). However there was large amounts of overlap in insect sizes between second and third instars in this study which was not evident in Morales. The third instar size distribution was bimodal and had a much larger range than the other instars. This bimodality may be evidence of some seasonality in *Ultracoelostoma* with the peak at lower size representing newly third instar insects and the peak at higher size an earlier cohort. An unexpected result was that more third instar insects were identified than second instars. This is unexpected as the second instar contains both males and female insects whereas the third instar consists of only females. There are two, non-mutually exclusive, explanations. Firstly, as larger (typically third instar) insects have higher honeydew production rates over part of the study, more may have produced a drop during the sampling time and been identified as active. It may also be that the third instar lasts longer in time than the second so that there are many insects across a wide range of ages. This is supported by the wide range of sizes seen in the third instar. This wide range of sizes also suggests that a large amount of growth occurs in the third instar. As in Morales (1988) I found that the second and third instars produce the majority of honeydew with only a small contribution from the first instars.

Insect Characteristics and Production

In this study I did find that larger insects produced more honeydew than smaller insects but only at higher temperatures. It may be that at lower temperatures there were still real differences in honeydew production between insect sizes, but this was not detectable. This may be due to relatively larger errors in measurement of honeydew compared to the low means of honeydew production at low temperatures. No effect was found of insect weight, despite finding an effect of insect size (mm^2), this may be due to the fact that insect elliptical area instead of volume had the best relationship with production. Size seems to influence honeydew means (through its interaction

with temperature) and instar seems to influence honeydew drop weights (through a main effect). This suggests that larger insects will produce more honeydew overall but that only third instar insects will produce particularly large drops. It may be that discrete physiological changes (perhaps to do with gut enzymes, size, stylet or anal tube width) after moulting and becoming third instar allows production of larger drops but do not influence nutritional need alone so do not result in increased honeydew production unless the insect is also larger. The lack of a direct effect of instar on honeydew production rates may be due to the bimodality of third instar insects, with many at a similar size to second instar insects. The higher amount of production seen in larger third instar insects may be due to the large amount of growth seen in this stage and the preparation of large third instar females to become adults and produce eggs.

There seem to be few examples of studies that have investigated the relationship between insect size or instar and honeydew production in other species. Those that are published primarily relate to various aphid species. One study from Germany found that tansy aphids (*Metopeurum fuscoviride*) produced more honeydew in older groups of aphids (Fischer et al. 2002). They attributed increased ant attendance of these groups to the increased production. There were also age-specific changes in the amino acids present in the honeydew. *Marchalina hellenica* is a European scale insect of pine that produces honeydew used for a large proportion of honey in some countries such as Greece and Turkey (Gullan and Kosztarab 1997, Bacandritsos et al. 2004). Honeydew production in the insect has been found to be mainly related to insect age and size (Hatjina and Bouga 2009). A whitefly species (*Bemisia argentifoli*) also appears to produce more honeydew in later instars, larger insects also produce more honeydew, with females being more productive than males likely for this reason. Age specific changes in sugar composition was also found(Henneberry et al. 1999). In *Aphis fabae*, later instars also produce more honeydew (Fischer et al. 2005). Other aphid species, *Tuberolachnus salignus, Megoura viciae* and *Myzus persicae* have also shown a pattern of increasing honeydew

production with increasing age and instar (Fischer et al. 2002). In another aphid species (*Aphis gossypi*) however, it was found that smaller, one day old, nymphs produced more drops and overall honeydew than four day old nymphs (mean age until adulthood was 4.1 days) (Henneberry et al. 2000). This suggests that increasing honeydew production with size or developmental stage does not hold as a general pattern. This may be due to very high resource requirements for early growth in some species that outweighs the increased resource intake that would be expected to maintain the larger biomass. Fischer et al. (2002) found that the third instar aphids studied produced the most honeydew. They suggested that this may be due to the large amount of growth that aphids typically go through in the third instar.

Tree DBH and Insect Characteristics

Previous studies have found a relationship between DBH and insect density, with the highest density of insects on medium DBH trees (Kelly 1990, Wardhaugh et al. 2006). This relationship is likely due to bark thickness and fissuring. Scale insects often settle in bark fissures for easier access to the phloem. Small trees have few fissures to settle in, intermediate trees have some fissures, and larger trees have fissures but also thicker bark that is difficult to penetrate (Wardhaugh et al. 2006). Larger trees also have many old insect cases, which remain on the tree after the insect has left or died, that may take up space and prevent other insects from settling (Wardhaugh and Didham 2005). In this study however such a relationship was not found. It may be that my low sample of trees and low number of large trees in particular influenced the relationship. In (Kelly 1990) the DBH 10-40 cm was considered an intermediate range with a maximum tree size of ~100 cm whereas in this study only one tree had a >40 cm DBH.

Dungan et al. (2007a) suggested that insect size may be one reason why honeydew production of individual insects could vary between trees if larger insects were more likely to be found on larger trees due to a better ability to penetrate bark. In this study there was a positive relationship

between insect size and tree DBH; only trees >25 cm DBH held insects >12 mm^2. This lends some support to the idea proposed by Dungan that larger insects settle on larger trees. However smaller insects still colonise larger trees. It may be that larger trees have particular characteristics, for instance increased nutrient content in phloem sap, that allow insects to grow larger. This is supported by Wardhaugh and Didham (2005), who found that female *Ultracoelostoma* on the highest DBH red beech trees produced more eggs on average than those on lower DBH trees. They suggested that this may be due to an increased ability of larger trees to acquire resources, particularly nitrogen. My result suggests that differences between trees, influences the age structure of their insect populations. As larger insects produce more honeydew (at least at certain temperatures) this should then lead to a relationship between tree DBH and honeydew production. However no difference between trees in honeydew production per insect was found, likely because of the only moderate strength of the relationship between insect size and tree DBH.

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2.5 Conclusions

These results show that insect size and instar are associated with aspects of increased honeydew production. It was also found that ambient temperature had a strong relationship with overall production. The results found seem to at least partially answer the question arising from James and Kelly (2011). They found that their model of episodic insect feeding and honeydew production qualitatively matched that of real data but underestimated production. This was especially apparent due to the long tail of the data; larger drops were being produced than the model could account for. A possible way to explain this was to extend their model to allow honeydew flow rate to vary between individual insects, possibly explained by an insect characteristic such as size. My results, measuring characteristics of individual insects (size, instar) seem to confirm the idea that these can influence honeydew production, in particular the production of large drop by third instar insects.

In conclusion the hypothesised effect was found: the insect characteristics of size and instar had an effect on aspects of honeydew production. This suggests that the insects gain an increased capacity to produce and/or an increased need to produce honeydew to gain sustenance at larger size or higher instars that is only evident at higher temperatures. My results suggest that future models of production, especially those that focus on production at the level of individual insects, should include considerations of insect characteristics. My results also suggest that shifts in insect population age structures, as well as factors that influence insect size and development, will have impacts on subsequent honeydew production during the summer months when honeydew production is highest and when wasps are active.

I also showed that larger trees support larger insects. This suggests that knowledge of insect age structures and how these relate to tree age structures may be important for predicting honeydew production during times of peak production when temperatures are high. Though larger trees support larger insects, and larger insects produce more honeydew, the ability of tree age structure alone to predict production is not apparent. This may be because the moderate strength of the relationship between tree DBH and insect size dilutes this effect, and other factors that influence insect size need to be accounted for.

In the next chapter I explore the relationship, found here and in other studies, between honeydew production and temperature. So far this relationship has been based on ambient temperature, which may influence honeydew production directly through its effects on the insect metabolism, indirectly through its effect on the tree or some combination of both. I attempt to separate out these effects by manipulating temperatures on small areas of *Nothofagus* trunk and measuring honeydew production to see if differences occur.

Chapter 3: The Influence of Temperature Manipulations on Individual Insect Honeydew Production

3.1 Introduction

Honeydew production in many phloem-feeding species is strongly related to environmental conditions (Stadler et al. 1998, Dungan et al. 2007a, VanLaerhoven and Stephen 2008). In particular, temperature has been noted as an important predictor, presumably due to its impact on insect metabolism (Fischer et al. 2002, Zhu and Cheng 2002). This has been investigated both in field and laboratory conditions (Stadler et al. 1998, Henneberry et al. 2000). Temperature may influence honeydew production by increasing the activity of enzymes in the insect's gut, therefore decreasing gut processing time (James et al. 2007). In *Ultracoelostoma*, previous studies (as well as Chapter 2) have noted that honeydew production increases with ambient temperature (Dungan and Kelly 2003, Dungan et al. 2007a). For example Dungan et al. (2007a) found that air temperature and moisture deficit explained 60 % of the variability in honeydew production. In an early study, Crozier (1981) suggested that the more sun-exposed, thus warmer, north aspect of trunks were more favourable for the insects, supporting higher population densities and abundant honeydew. At the level of individual trees or individual insects, more sun exposure relative to other trees or insects may act to increase temperature and perhaps production (Crozier 1981). However other studies have failed to find an effect of aspect on production, perhaps in some cases because of sampling on cloudy days (Kelly 1990, Kelly et al. 1992, Wardhaugh et al. 2006). Other effects of temperature are also apparent, for example highly evaporated drops may also block the anal tube and stop production (Kelly et al. 1992).

However ambient temperature changes also influence the host plants of phloem feeding insects, making it difficult to separate out the effect of temperature directly on the insect from indirect effects via the host (Stadler et al. 1998, Zvereva et al. 2010). Moller and Tilley (1989) suggested that

temperature may influence soil moisture and evapotranspiration, alter tree turgor pressure and cause changes to honeydew drop production. The concentration of sugars and nutrients in phloem sap is influenced by abiotic factors, including temperature and temperature may also influence phloem circulation (Douglas 2006, Dungan et al. 2007a, Dinant et al. 2010). In a previous study, Dungan et al. (2007a) attempted to separate out the relationship between insect level and tree level environmental conditions on honeydew production. They did this indirectly by manipulating sugar and nitrogen content of the host plant phloem, variables that may change due to environmental conditions. They then tested the relationship of these changes and environmental conditions with honeydew production. They found that environmental conditions and not phloem manipulation had an influence on production, concluding that environmental conditions act primarily on the insect rather than the tree to increase production.

In this experiment I will attempt to separate out insect effects from other effects by manipulating temperature at a much smaller level than that of the whole forest or tree. A more direct method of lab study is not possible, as removing the insects from their host tree is fatal. A pair of enclosures was added to a number of trees; one was cooled and one was heated. Differences in production between heated and cooled enclosures will serve to show whether small area changes in temperature can influence production. The hypothesis is that insects in heated enclosures will increase the rate of honeydew production through a temperature effect on gut enzyme activity. If this is not found then it would suggest that tree temperature is influencing insect honeydew production indirectly. I hope to gain a better insight into the role that temperature plays in honeydew production, influencing insect physiology or host qualities.

3.2 Methods

Temperature Manipulation

This part of the study was also conducted at Mt. Richardson (Fig. 2.1) along the loop track (43.203°S, 172.257°E). Three study sites were identified that had enough trees meeting certain criteria for successful temperature manipulation and sampling. From these, one site was picked at random to be used in the experiment. The criteria were that trees were >20 cm DBH (diameter at breast height) to accommodate frames and enclosures, that trees were close enough together (within 5 m) to run temperature probes out to multiple trees from each datalogger, to have moderate to dense infestations of insects on the trunk producing honeydew, and to not have the trunk shaded out by other canopy. Five trees within the site were then selected for use in the experiment based on these criteria. As in Chapter 2, sampling was done away from the walking track.

To manipulate temperature on the trees, areas of the trunk either had solar radiation input blocked with reflective foil (mylar), or had local air temperatures increased by enclosing areas under clear plastic (polythene) (Fig. 3.1). Each tree had two adjacent enclosures, one warmed and one cooled, and the treatments were reversed between sampling days. Treatments were reversed to ensure that an effect of position on the tree (top vs bottom) on honeydew production would not be confused for a treatment effect (heated vs cooled).

This was done by first nailing 31 x 21 cm white plastic sampling frames (identical to those used in Chapter 2) to the trees. Unlike in Chapter 2 however there were two frames per tree (one at 1.5 m and the other at 1.3 m) with both nailed to the north side of the trunk to maximize sun exposure. A rectangular polystyrene spacer (29 x 16 cm, 5 cm deep with sides 2 cm wide) was then attached to each sampling frame with Velcro, enclosing the panels. The combination of sampling frame and polystyrene spacer is henceforth referred to as an enclosure. These enclosures were required to

insulate the area, as well as to house a probe for measuring air temperature and to avoid the cover making contact with insect anal tubes that may displace honeydew drops. The sides of each enclosure were covered by nylon netting (~1 mm mesh) as in Chapter 2 (but not covering the front of the enclosure) to prevent harvesting of honeydew by wasps. To manipulate temperature, the open face of each enclosure was covered with either a reflective mylar, or clear plastic sheet. The mylar cover reflected any incoming solar radiation, cooling the enclosed area. The plastic cover exposed the enclosed area to solar radiation as well as creating a greenhouse effect, trapping outgoing longwave radiation and heating the enclosed area. Henceforth mylar covered enclosures are referred to as a cooled treatment and plastic covered enclosures as a heated treatment. The covers were attached to the front of the enclosures using map pins so that they were easily removable for sampling and for reversing treatments.

Figure 3.1: Setup of enclosures on one of the sampled trees (before netting was added). Wires are attached to temperature probes at the top of each enclosure and run down to the datalogger at the bottom right of the photo.

I measured the temperature inside of each frame using Campbell dataloggers (one CR21X and one CR10; Campbell Scientific Inc, Utah, USA) with Campbell 107 thermistor temperature probes and HMP50 thermistor temperature probes. At the top of each enclosure was an extended area of polystyrene with a hole at the top, 2 cm in diameter, which opened into the enclosure. This housed the probe to keep it out of direct sunlight and out of the way of sampling while still measuring temperature of the enclosure. The dataloggers were programmed to take a temperature reading once per minute then store the mean of these every 15 minutes. To record ambient air temperature at the site, a HOBO® temperature and humidity sensor (Onset Computer Corporation, Massachusetts, USA) was set up near each datalogger in a metal radiation shield 1 m off the ground. The shield was open sided, allowing air flow, and was placed to avoid direct sunlight hitting the sensor which would have interfered with temperature readings. The sensors recorded temperature and relative humidity at 15 minute intervals.

Measuring Honeydew Production

On each sampling day, honeydew drops were cleared off all insect anal tubes within the enclosures at 10 am. At two hour intervals after this, the honeydew was collected and measured (i.e. 12 pm and again at 2 pm). This was to ensure that the sampling times coincided with the midday sun to maximize temperature differences between treatments. The microcapillary method was used to sample honeydew and honeydew concentration was measured the same way as in Chapter 2. As in Chapter 2, each insect that was recorded as producing a drop was mapped to track production to it. At the end of sampling all insects that had produced a drop at some point were assumed to have been alive throughout the sampling period. Therefore for instance if an insect produced drops only on the last day of sampling, on all previous sampling days it was retrospectively recorded as being present and producing no honeydew. It was included in mean honeydew production per insect calculations and subsequent test and zero counts of drop weight for these times were included in

drop weight calculations. The exact time of sampling for each frame was recorded for the purpose of 24 hour temperature averages, so that temperature readings were not included when the enclosure had been opened for sampling. This sampling was done over December 2012 and January 2013 with 3 sampling days in each month. Enclosure covers were kept in place between sampling dates. Sampling was done on sunny days with little or no cloud cover so that temperature differences were maximized by the treatment. This also helped avoid interference from dew or raindrops on honeydew drops. Honeydew production was also sampled on three previous days, in late September and October, however due to problems with the dataloggers manipulated temperatures values were not available. However these data were able to be used in tests of ambient temperature.

Statistical Analysis

As in Chapter 2, the gravimetric refractometry values (g/100 g) were converted to volumetric values (g/100 ml) using the quadratic equation:

$y = 1.123 + 0.994\ x + 0.0049\ x^2$ Obtained from (Dungan et al. 2004).

All honeydew values used in analysis were therefore in µg dry weight of sugar.

The two sampling times per day (12 pm and 2 pm) were averaged in analysis to give one value per day. A paired t-test was used to test for temperature differences between mylar and plastic covered enclosures within trees. Honeydew production was tested at the level of the individual insect (µg $insect^{-1}\ h^{-1}$) which was calculated by taking the mean production per hour over the four hour sampling day. Honeydew production and honeydew drop weight was logged ($Log_{10}+1$) when used in analysis. Temperature was averaged over the previous 24 hours (from 12pm and from 2pm) for the ambient temperature from the HOBO® sensor and manipulated temperature from the dataloggers. I tested the effects of manipulated and ambient temperature on honeydew production using nested, repeated measures ANOVAs. Differences in honeydew production due to sampling day, tree,

enclosure position on the tree and treatment were also tested in these ANOVAs. As in Chapter 2, ANOVAs were used to test the effect of Julian day and temperature on the number of active insects and mean drop weight. They were then reversed in order in the model to account for correlation between the predictors. This study ran over from 2012 to 2013, this posed a problem for using Julian day, as Julian day only runs up to 366 (on a leap year). To deal with this, days in 2013 were added to the maximum Julian day value (e.g. 1st of January would be Julian day 367). All statistics were conducted using R (v. 2.14.1; R Development Core, 2011).

3.3 Results

Effect of frames on temperature

There was a significant temperature difference between mylar covered and clear plastic covered enclosures ($t_{(1,29)} = 3.207$, $p = 0.00326$) (Fig. 3.2). The mean temperature of the heated treatment was 17.2 ± 2.7 °C, the mean temperature of cooled treatment was 16.1 ± 2.4 °C and the mean difference was 1.1 °C ± 1.8 (±SD). The variation in temperature between sample days was much larger (Table 3.1). Mean temperature per day varied between 14.0 °C and 20.2 °C, but the heated treatment always had a higher mean temperature than the cooled one (Fig 3.2).

Temperature ~ Treatment +Tree + Sample

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Treatment	1	11.52	11.52	9.396	0.00353 **
Tree	4	7.94	1.98	1.619	0.18436
Sample	5	266.06	53.21	43.418	<0.0001 ***
Residuals	49	60.05	1.23		

Table 3.1: ANOVA table of the relationship between temperature and treatment including factors for tree and sampling day.

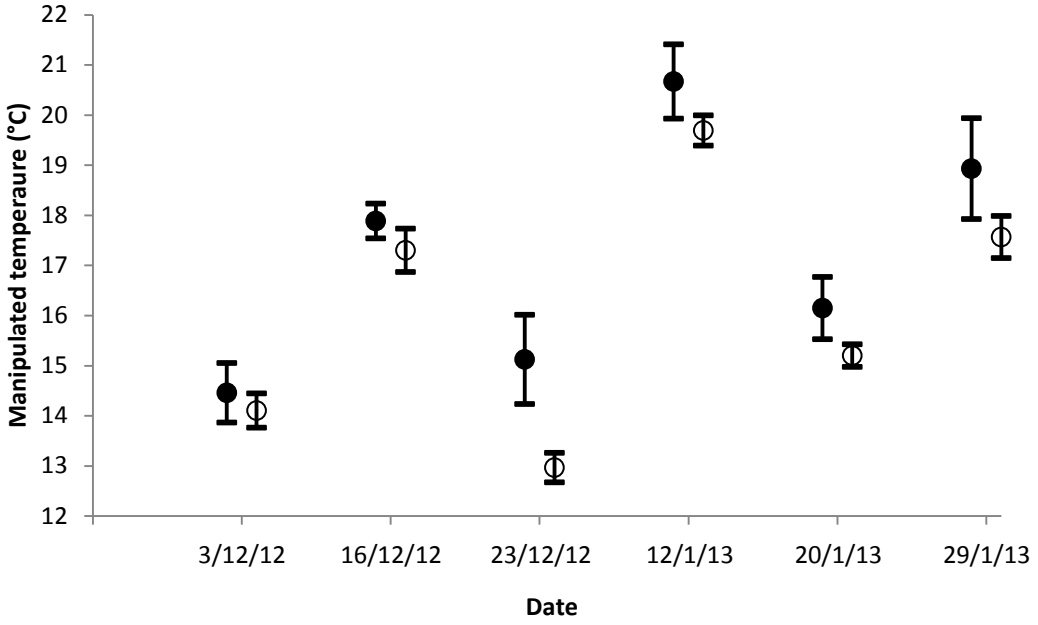

Figure 3.2: Mean difference in temperature (°C) (±SEM) between mylar covered (unfilled circles) and clear plastic covered (solid circles) enclosures, over sampling days. From December 3rd to January 29th.

Effect of manipulated temperature on production

The total number of honeydew producing insects, including September and October data, was 452. The number measured per enclosure ranged from 5 - 42. The mean honeydew production per insect per hour (mean over 4 hours) was 0.01706 µg insect^{-1} h^{-1} and the maximum was 0.1242 µg insect^{-1} h^{-1}. This was approximately three-fold lower than the production in Chapter 2 from March - April (0.0461 µg insect^{-1} h^{-1}). The mean drop weight when a drop was produced was 0.2540 µg, the maximum was 0.8913 µg and a total of 1478 drops were produced.

A relationship was not found between manipulated temperature and honeydew production (Table 3.2) and the overall trend was negative though the cooled treatment had a slightly positive trend (Fig. 3.3). No relationship was found between treatment and honeydew production (Table 3.2). However a significant relationship was found between honeydew production and enclosure position,

the insects in the higher enclosures produced more honeydew on average ($t_{(1,29)} = 2.6883$, $p = 0.01177$). The mean production of insects in top enclosures was 0.01932 ± 0.01674 μg $insect^{-1}$ h^{-1} and in the bottom enclosures was 0.01325 ± 0.009672 (±SD). Mean honeydew production per insect varied between trees (Table 3.2).

Honeydew ~ Temperature + Treatment + Position + Tree

Error: Sampling day/Tree

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Temp	1	0.0000008	7.500e-07	0.014	0.908
Tree	4	0.0002387	5.969e-05	1.096	0.394
Residuals	15	0.0008165	5.444e-05		

Error: Within

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Temp	1	0.0000487	4.873e-05	2.781	0.1051
Treatment	1	0.0000404	4.036e-05	2.303	0.1389
Position	1	0.0000868	8.682e-05	4.955	0.0332 *
Tree	4	0.0002447	6.118e-05	3.492	0.0178 *
Residuals	32	0.0005607	1.752e-05		

Table 3.2: ANOVA table of the relationship between manipulated temperature and honeydew production with factors for frame position, treatment and tree.

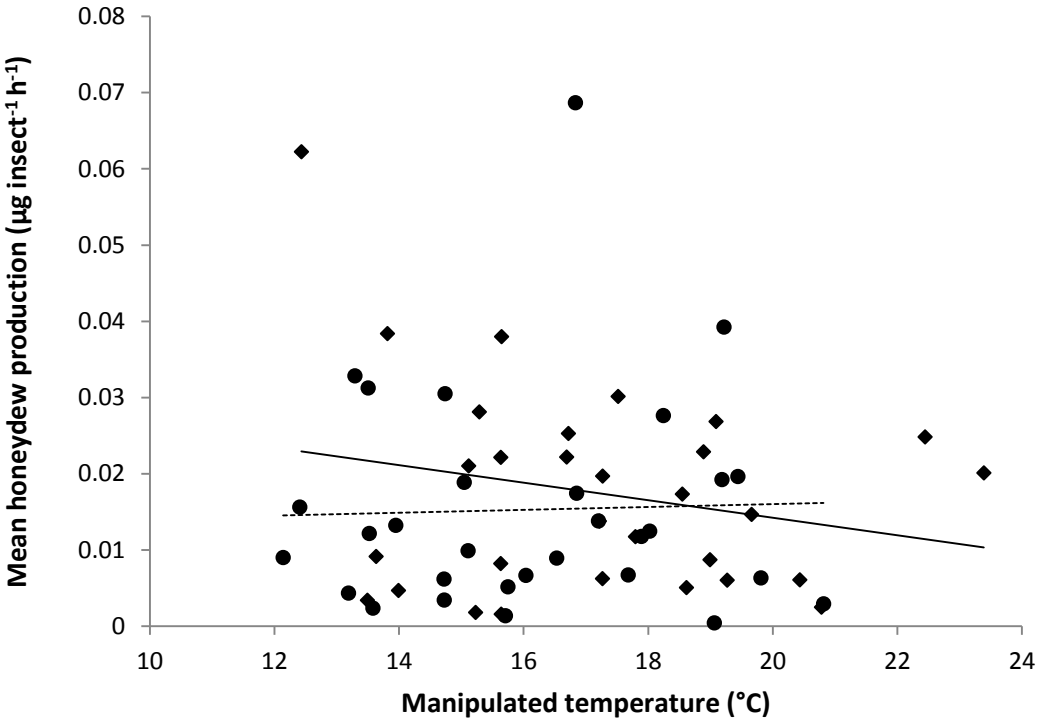

Figure 3.3: Mean honeydew production per insect per site (µg h^{-1}) against manipulated temperature (°C) separated by treatment. Dashed line for cooled trend and circles for points. Solid line for heated trend diamonds for points. Dot per enclosure per day. The lines are fitted in non-significant regressions to show the direction of the weak trends.

Effect of ambient temperature on honeydew production

Because manipulated temperatures showed no relationship to honeydew production, ambient temperatures were then used to test a tree level effect of temperature. This also allows for the inclusion of September and October honeydew data that had been gathered but for which, due to technical difficulties, there was no manipulated (within-enclosure) temperature data for.

Ambient temperature showed no relationship to honeydew production (Table 3.3) and had an overall negative trend (Fig. 3.4). The range of ambient temperatures was 3.7 – 25.3 °C. With ambient temperature there was also no effect of treatment (Table 3.3). The mean (±SD) insect honeydew production of the heated treatment was 0.01813 ± 0.01177 µg $insect^{-1}$ h^{-1} and the cooled was

0.01610 ± 0.01231 µg insect^{-1} h^{-1}. As in Chapter 2 this was then split into its constituent parts and analysed as mean drop weight and active insect number.

Honeydew ~ Ambient temperature * Treatment

Error: Sampling day/Tree

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Ambient temp	1	0.000118	0.0001179	0.615	0.439
Residuals	31	0.005942	0.0001917		

Error: Within

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Ambient temp	1	0.000017	1.653e-05	0.135	0.715
Treatment	1	0.000092	9.249e-05	0.753	0.389
Ambient temp : Treatment	1	0.000057	5.653e-05	0.460	0.500
Residuals	54	0.006631	1.228e-04		

Table 3.3: ANOVA table of the relationship between honeydew production and ambient temperature including a factor for treatment.

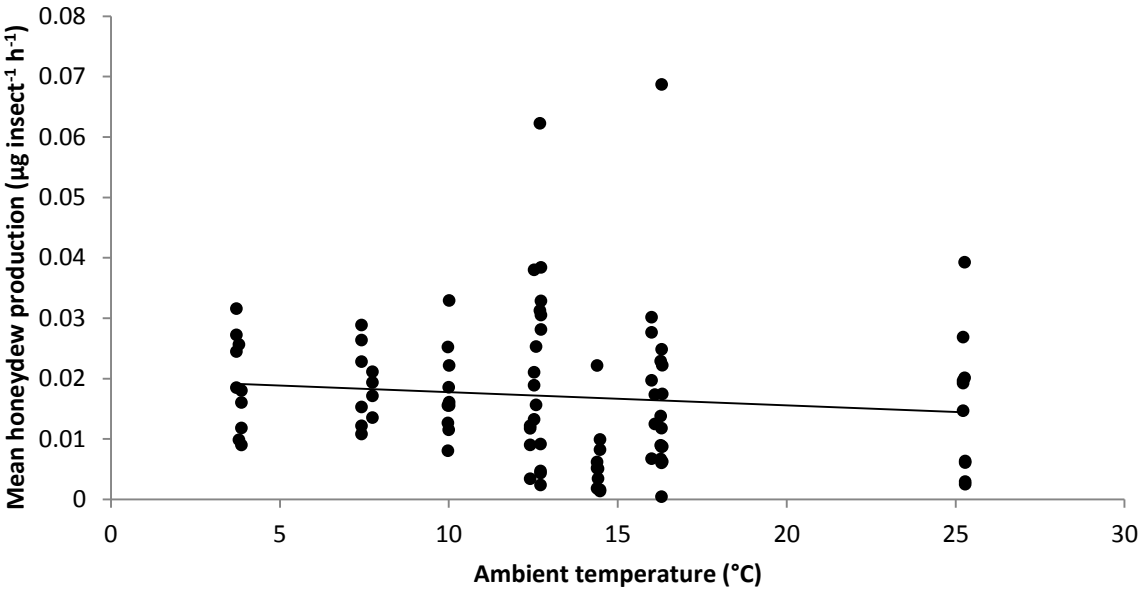

Figure 3.4: Mean honeydew production per insect per site (µg h^{-1}) against ambient temperature (°C) from 29th September 2012 to 29th January 2013.

There was a significant negative relationship between temperature and the number of active insects (Fig. 3.5b, Table 3.4). There was also a significant relationship between the number of active insects and Julian day (Fig. 3.6b). When controlling for sampling day the effect of temperature on the number of active insects is not significant and when controlling for temperature the effect of Julian day on the number of active insects is significant (Table 3.4). This means that the observed relationship between temperature and the number of active insects is purely due to the effect of Julian day. There was no relationship between mean drop weight and temperature (Fig. 3.5a) or Julian day (Fig. 3.6a).

No. of active insects ~ Temperature * Day

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Temp	1	6067	6067	11.315	0.00463**
Day	1	3601	3601	6.715	0.02133*
Temp : Day	1	62	62	0.116	0.73815
Residuals	14	7507	536		

No. of active insects ~ Day * Temperature

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Day	1	9668	9668	18.029	<0.001***
Temp	1	1	1	0.002	0.969437
Day : Temp	1	62	62	0.116	0.738149
Residuals	14	7507	536		

Drop weight ~ Temperature * Day)

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Temp	1	0.0946	0.09459	1.210	0.290
Day	1	0.0358	0.03580	0.458	0.510
Temp : Day	1	0.0902	0.09019	1.154	0.301
Residuals	14	1.0945	0.07818		

Drop weight ~ Day * Temperature

	Df	Sum Sq	Mean Sq	F value	Pr(>F)
Day	1	0.0156	0.01564	0.200	0.661
Temp	1	0.1148	0.11475	1.468	0.246
Day : Temp	1	0.0902	0.09019	1.154	0.301
Residuals	14	1.0945	0.07818		

Table 3.4: Tables of ANOVAs for the relationships of the number of active insects and mean drop weight against Julian day and temperature (tried with each of the predictors entered first).

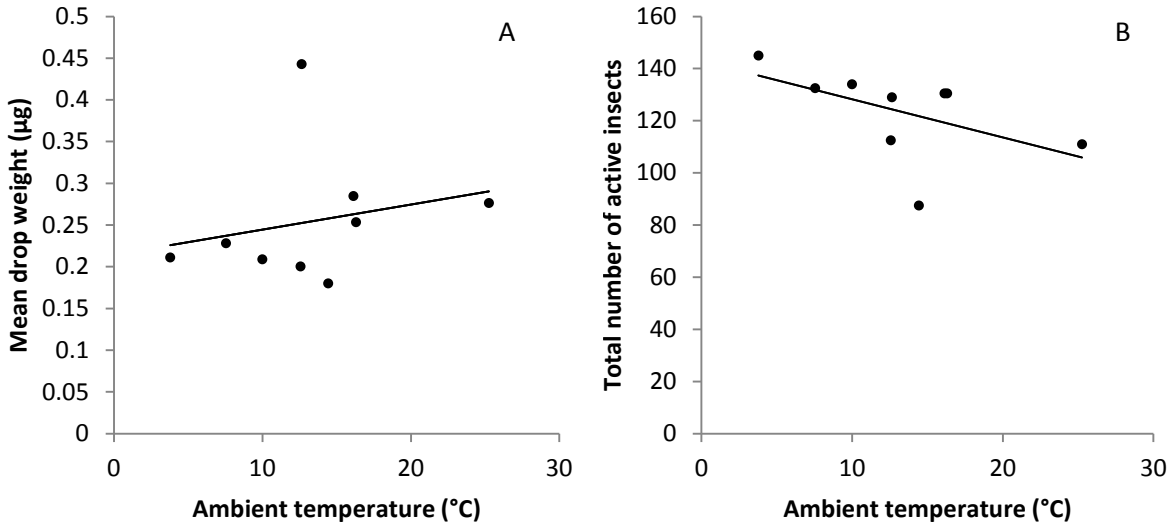

Figure 3.5: (a.) Mean honeydew drop weight (µg) against ambient temperature (°C). (b.) Number of productive insects against ambient temperature (°C). Dot per sampling day.

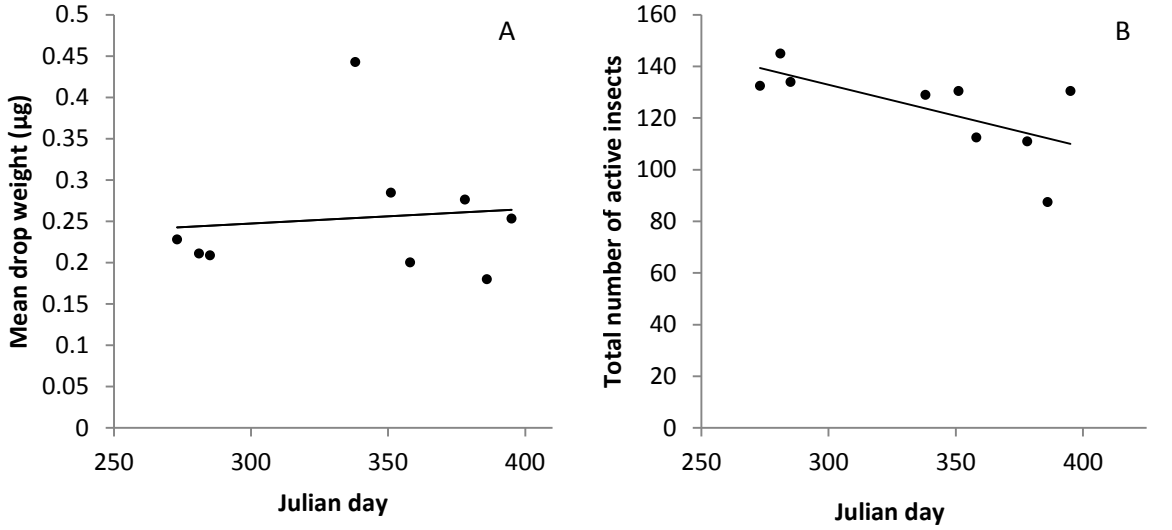

Figure 3.6: (a.) Mean drop weight (µg) against Julian day. (b.) Number of honeydew producing (active) insects against Julian day. Dot per sampling day.

3.4 Discussion

Manipulated Temperature and Honeydew Production

The results clearly show that the experiment was successful at manipulating temperature within the enclosures. Clear plastic covers heated enclosures up over the mylar covered enclosures. This difference was quite small on average (1.1 °C) compared to the variation in temperature between sampling days, which was much higher. This is likely because temperature was averaged over the preceding 24 hours which would include much time where, due to the lack of sun, there were no differences between enclosures in temperature.

Unexpectedly, a relationship was not found between manipulated temperature and honeydew production. Increasing the temperature of small areas of trunk over others did not result in changes to honeydew production. This suggests that the relationship between honeydew production and temperature is not acting at the level of the individual insect but at the level of the tree. This is contrary to previous studies on both *Ultracoelostoma* and other species that suggest a positive effect of temperature on insect gut enzyme activity and subsequent honeydew production. This is discussed further below with the relationship between ambient temperature and production. There was also no relationship found of the treatment, independently of manipulated temperature, on honeydew production.

There was however an effect of enclosure position on honeydew production. The insects in the higher enclosures on the trees had a higher mean production regardless of what treatment it was under, which was unexpected as the enclosures were so close to one another. It may be that larger, more productive insects settle further up the tree. It may also be that phloem conditions are different further up the tree. The phloem may be at different pressure or have a lower nutrient concentration so that it is easier to suck more sap or that more must be processed. Beggs et al. (2005) found an effect of position on production but that was found between the trunk and

subcanopy. In my study there was also a tree effect on honeydew production; some trees had a mean honeydew production per insect per hour that was higher than others. This may have been due to a variety of factors including variation between trees in microclimate, sun exposure, age structure of insects, genetic differences of trees in susceptibility to infestation, or variation in available soil nutrients.

Ambient Temperature and Honeydew Production

Ambient temperature, like manipulated temperature, also had no relationship with honeydew production, and an overall negative trend even when including September and October sampling data. This is unlike previous studies, including Chapter 2, where strong positive relationships were found. This suggests that perhaps a seasonal effect is occurring that is overriding the temperature effect. This is likely a seasonal demographic effect, as changing seasons would be primarily expected to influence honeydew production through temperature changes which would be accounted for in the study. Dungan et al. (2007a) suggested that the variation in honeydew production seen in their study were primarily influenced by temperature changes (influencing production at the level of the individual insect by, for instance, increasing feeding rates) as well as changes in scale insect densities. The factor of sampling day showed an effect on production independent of temperature, which suggests a change in insect demographics. Previous studies have shown decreases in honeydew production from December to January and attributed these to seasonal changes in the presence of productive insects. Beggs et al. (2005) found a decrease of about 50 % in the Nelson Lakes National Park and Dungan et al. (2007a) of 25 % in the Canterbury foothills.

Breaking down the relationship between temperature and honeydew production shows that the number of productive insects decreases over time. There are a number of possible reasons that this

occurred. There may be demographic factors that are causing insects to stop producing honeydew, such as death or instar changes. This may represent a normal seasonal shift in the presence of honeydew producing instars as inferred by previous studies (Beggs et al. 2005, Dungan et al. 2007a), or some unintended effect of the manipulation. It may be that the enclosures, in place from late September 2012 to the end of sampling on 29 January 2013 (122 days), interfered with the normal demographic processes of the insects. January – December is when Morales (1988) found peak numbers of adult males and first instar crawlers. First instar crawlers may have been unable to settle into the enclosure, as the covers were kept in place between sampling dates, and start producing honeydew and some insects in the enclosures may have died or changed instar. Any insects that had hatched within the enclosures would likely have settled there however, as most newly hatched crawlers settle near the mother (Morales et al. 1988). It may also be that the enclosures block adult males from entering (males pupate on the forest floor to become adults so males from within the enclosure would be unlikely to become adults) and breeding with females. *Ultracoelostoma* has been inferred to be facultatively parthenogenic due to the presence of this trait in other, related, species. Initially it was assumed to be obligately parthenogenic until the discovery of males of the species (Morales et al. 1988). *Ultracoelostoma* may not be facultatively parthenogenic however, and if so, it could be obligately sexual. This may mean that the females are not mated with and cannot produce eggs and renew the population within the enclosure. The lack of studies on the generation times, duration of instars and breeding system of *Ultracoelostoma* make it difficult to tell what the impact of enclosures on the insect demographics might be. It may also be that some insects simply stopped producing, while remaining alive and in a honeydew producing instar.

There was no relationship between honeydew drop weight and temperature or sampling day, which contrasts with Chapter 2 where there was a very strong relationship. This is the main cause of the non-significant overall relationship between honeydew production and temperature, and contradicts previous studies. It also contradicts the idea that temperature influences production though increasing insect gut enzyme activity (James et al. 2007). Possible reasons for this are

discussed below. There was also no effect of the treatment on mean drop weights or the number of productive insects. Treatment was tested to ensure that it was truly having no influence on any part of honeydew production.

There are many potential reasons for the lack of a relationship between honeydew drop weight and temperature; it may be that in this situation temperature was not the limiting factor on honeydew production, or again may be some unintended consequence of the manipulation. Even with the netting, some small insects such as ants may have gotten into the enclosures and consumed the honeydew, leading to an underestimation of production and a change to the relationship between drop weight and temperature, although I saw no evidence for this on sampling visits. There may have been some effect of humidity, which was not measured within the enclosures, though would presumably be higher than ambient. This may also have caused some insects deaths for instance from fungal diseases. The previous chapter had a 24 hour average temperature range of about 7 - 15 °C and this chapter had a range of about 4 - 25 °C. At higher temperatures (and therefore lower humidity) honeydew drops dry out and become more concentrated potentially slowing the flow of honeydew (Dungan et al. 2007a, James et al. 2007). However in this study, drops were likely removed too quickly for this to occur. The relationship seen may also be a true reflection of reality, that on this occasion the honeydew production of *Ultracoelostoma* was insensitive to temperature. It may be that some tree level factor such as nitrogen or sugar levels was limiting production instead of temperature. For example if the trees in this study had very high phloem nitrogen levels then production may have been low, as the insects would have fulfilled their nutritional needs quickly. The mean honeydew production per insect per hour in Chapter 2 was about three-fold higher than in this part of the study. It may be that this unknown limiting factor is constraining honeydew to this low level. This difference in honeydew production between chapters may be related to a number of factors. The trees in this chapter and Chapter 2 were both along the Mt. Richardson loop track at approximately the same altitude. The trees in this chapter were on a north facing slope and the sites of Chapter 2 were on a south facing slope. The sampling in this chapter took place over December -

January (2012/2013) and Chapter 2 took place in March-April 2012. However the enclosures may have had some unknown, potentially metabolic, negative effect on the insects that caused them to produce less honeydew. This may be because the sampling all occurred within a small area, which was necessary due to the limited reach of the datalogger temperature probes.

3.5 Conclusions

In conclusion I found an unexpected result, neither ambient nor manipulated temperature had a relationship with honeydew production. This means that the initial question of the experiment, whether the relationship between temperature and honeydew production is due to an effect of temperature on the tree or the insect, could not be answered. However I found no support for the idea that insect temperature changes could influence production. Consistent with other studies, a drop in the number of productive insects from December to January was observed. However across time and temperatures the productivity per insect did not change. This result was not explainable by any measured variables but may have been due to a problem with methodology or an unknown variable that was limiting honeydew production.

Chapter 4: General Discussion and Future Directions

The aim of this thesis was to explain variation in the production of honeydew at the level of the individual insect. To do this I investigated the role of insect characteristics in honeydew production as well as the role of temperature, both ambient and manipulated. In investigating insect characteristics I found that later instars can produce drops with higher sugar content, that at high temperatures the largest insects produce the most honeydew and that at higher temperatures overall production was higher. I also found that higher diameter trees tended to host larger insects. In James and Kelly (2011) their model was unable to explain some of the particularly large drops, >0.88 µl, that were produced. They suggested that allowing flow rates (of honeydew through the insect) in their model to differ between insects could explain this. These accounted for about 1.2 % and 0.7 % of the total number of drops of each of their datasets, which is including zero-counts of drops. To compare these to the drop sugar weights in my study I assumed a mean honeydew refractometer reading of 60 g/100 g for their data (a roughly typical value in my study), using this 0.88 µl gives a value of 0.69 µg. Comparing this value of 'large drop' weight to my data shows that 1.4 % of my total drop data exceeds this value, this suggests my data is at least qualitatively similar to that of James and Kelly (2011). Also 2.0 % of the drops produced by third instar insects exceed this value. This suggests that third instar insects are producing a majority of these large drops, and that future models of individual insect production should vary the flow rate between instars and account for the prevalence of third instar insects (51.2 % of honeydew producing insects in this study).

In the second part of this thesis, temperature was manipulated on small areas of bark to attempt to separate out the effects of tree temperature (ambient) and insect temperature (manipulated) on honeydew production. This was to explain the strong positive relationship between temperature and production. However neither manipulated nor ambient temperature showed a relationship with production. This was an unexpected result, was contrary to previous studies (including my Chapter 2) and meant that the effect of tree and insect level temperature on honeydew production could not

be separated. However it did show not support for insect temperature changes increasing honeydew production. It is unclear whether this is an as-yet unknown real effect or some artefact of the experimental manipulation. There was a steady decrease in the number of productive insects from the start to the end of sampling which may represent seasonal shifts in productive instars. However even when accounting for these changes, mean insect honeydew production was not influenced by temperature. This result may have been an unintended consequence of the enclosures, these were in place for 122 days, and may have interfered with insect demographic processes or harmed the insects in some way, perhaps promoting pathogens. It may have been due to an equipment problem, for instance the netting failing to keep out other insects. It may also represent a previously unseen insensitivity of *Ultracoelostoma* to temperature. Due to the studies, at various times of the year, that have shown a positive relationship between honeydew production and temperature (Beggs et al. 2005, Dungan et al. 2007a), it seems most likely that some artefact of the manipulation caused the lack of a relationship.

Consistent with studies on other species such as *Cinara* spp. in a German coniferous forest (Stadler et al. 1998) and *Bemisia argentifolii* in the USA (Henneberry et al. 1999) I found a positive effect of temperature and insect size on honeydew production in Chapter 2. Zhu and Cheng (2002) also found a positive effect of temperature on honeydew production, however at temperatures >30 °C production decreased. VanLaerhoven and Stephen (2008) found a negative effect of temperature on honeydew production in a southern USA pine and hardwood forest. In a Chinese study on *Phenacoccus solenopsis* mealybugs, Zhou et al. (2013) found a strong positive relationship between honeydew production and insect weight unlike this study, where an effect of insect size was found but not of weight. Another finding from Chapter 2, that larger insects are typically found on higher DBH trees, may be relevant and important to scale insect species in other countries. Only if, however, this relationship is due to some effect of increased tree size (higher phloem nitrogen for example) that may generalise to other tree species. Honeydew production by the scale insect *Marchalina hellenica* is highly dependent on insect size (Hatjina and Bouga 2009). If larger trees

support larger insects in Greece as they did in this study, then honeydew production could be increased by preserving large trees and mature pine forests. The majority of honey production in Greece is derived from the honeydew of this scale insect so this could provide economic benefits (Bacandritsos et al. 2004). The relationship would be less important for highly mobile species such as aphids that may simply move between hosts, so are not tied to the quality of a single plant (Fischer et al. 2005). The lack of a relationship between honeydew production and temperature in Chapter 3 may demonstrate some of the difficulties in performing manipulations on sedentary honeydew-producing insects on large hosts. The significant effect of enclosure position on honeydew production seen in Chapter 3 also suggests that there can be a change in phloem conditions over even relatively small (20cm in this case) distances on the host. However in a study on galling-inducing aphids, Wool et al. (2006) found that aphids from different areas of the trees did not show differences in honeydew composition. Overall, the findings of this study would be best generalised to other sedentary, honeydew-producing species.

The first priority for further investigation in *Ultracoelostoma* should be the unexpected result of the temperature manipulation. Future studies should sample from trees across a larger area, to gain a potentially wider range of variable such as tree nutrients. They should also measure variables such as humidity inside enclosures, and phloem nitrogen and sugar composition to account for possible limiting factors on honeydew production. Using other methodologies for the manipulation may help to determine whether the current result was an artefact of my methodology. Alternate methods of heating enclosures such as battery-run Peltier effect heating/cooling pads may be more effective at changing temperatures compared to the passive, solar method used in the current study. In this study the insects were kept covered between sampling days for convenience, which may have interfered with demographic processes or caused some harm to the insects. Future studies, if using covered enclosures, should remove these covers after sampling and replace them a day or two before the next sampling takes place to ensure that the insects inside the enclosures are exposed to normal conditions are much as possible. Covers and enclosures should also be designed with

materials that allow easy air flow past the insect to avoid moisture build-up. It may be most revealing to have some covers on over the whole study period (as in my study) then some that are removed after sampling in the way previously described. Any differences between these treatments in honeydew production may then clarify the results of the current study. Future studies should attempt to test the patterns seen here on different areas of the tree as insect densities and production rates can vary across different areas of the tree (Beggs et al. 2005, Wardhaugh et al. 2006). In this study I only sampled from the lower trunk for the sake of simplicity, which may limit the ability to generalise these results. In particular, do the larger insects that I found to inhabit larger trees also live on canopy branches or only on the lower trunk? This may provide some idea of whether the relationship was due to an effect of bark characteristics (as Dungan et al. (2007a) suggested) or something else such as phloem pressure of sap composition. Further research into how insect age structures vary among trees and sites and across time may allow better prediction of honeydew production. In particular, trees >40 cm DBH (of which there was only one in this study) should be sampled to see if there is a consistent increase in the size of insects in line with the relationship found in this study. Knowledge of insect age structures may also provide an insight into differences in honeydew composition across space and time, composition may vary with insect characteristics such as instar and attract different consumers (Beggs et al. 2005, Dhami et al. 2011).

Future studies may also attempt to follow a group of insects from their settled crawler stage through to adulthood. This could serve to address a pattern found in this study that there were a larger number of third instar, than second instar insects. This is despite the fact that cased third instars consist entirely of females whereas the second instar consists of both males and females. This suggests that the third instar may be longer in duration. This could be accomplished by mapping insect positions as in this study. This would be difficult for male insects, as they leave the case after the second instar to pupate, but females could be tracked as they stay in the case until death. At certain intervals (e.g. three-monthly) a proportion of these could be removed at random and the instar identified. This could serve to estimate the insect generational time and instar durations.

However it would need to be balanced in terms of the number of insects initially sampled, the proportion removed at each interval, and the interval length, to ensure that all the insects were not removed before the generation ended (i.e. the insects started to die off). This could be balanced to previous estimates of generational times e.g. 1 year (Morales et al. 1988). Another effect found in this study that bears further investigation is the position effect on honeydew production in Chapter 3. Insects within the top enclosures at 1.5 m produced significantly more than those in the bottom enclosures at 1.3 m. The honeydew production of insects could be sampled at regular intervals from the low trunk, to the upper trunk and canopy branches, to see if this pattern continues above and below where my enclosures were. This may demonstrate some quality of the phloem sap, such as pressure or composition, which is changing steadily down the tree. The density of insects at each interval should be taken and compared between trees to see if this influences the relationship between sampling height and honeydew production. If so, then this would suggest that this relationship is driven by insects; they may influence the phloem sap composition as it flows down to the insects below them, altering honeydew production of the lower insects. To further test this, a manipulation could be added, where certain trees could have insects removed at different height intervals. However a previous study on *Ultracoelostoma*, suggested that the insects exert a whole tree, rather than local area, effect on phloem sap nutrients (Wardhaugh and Didham 2005).

This current study helps to explain the quantity of honeydew produced by *Ultracoelostoma*. These results highlight importance of individual insect characteristics in honeydew production as well as a surprising result of temperature manipulation, showing that in some cases honeydew production may not be limited by temperature. It contributes to the previous work of James et al. who have put forward and developed models for individual insect honeydew production. This is important from an ecological perspective as honeydew is a large energy source for many species in beech forest communities, and has both direct and indirect effects on species across trophic levels. These results provide some of the first insights into what controls the honeydew production of *Ultracoelostoma* individuals in New Zealand beech forests. Future models of honeydew production should

incorporate the fact that aspects of honeydew production vary with insect size and instar, and that insect sizes vary with tree DBH.

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