Interactions between aphids and their host plants under drought stress

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Thesis submitted to
The University of Edinburgh
for the degree of
Doctor of Philosophy

School of GeoSciences
2013

Declaration

I declare that this thesis has been composed solely by myself and that it has not been submitted, either in whole or in part, in any previous application for a degree. Except where otherwise acknowledged, the work presented is entirely my own.

Katrina L S Simpson
2012

Acknowledgements

This research was funded by The Natural Environment Research Council under a UK research studentship, NE-F00879-1.

Without the advice and support of my family this work would not have been possible, and without my supervisors Gail Jackson and John Grace it would not have been achieved.

Katie
2012

Abstract

Interactions between host plants and aphids under water stress conditions were examined. Two controlled environment room studies, a field experiment and phenological modelling analysis were used to investigate three different plant-aphid relationships.

The aim of these studies was to determine the results of water shortage on plant and pest populations, which are likely to result under climate change scenarios. The central hypothesis was that mild drought stress would lead to greater aphid populations through a beneficial increase of small nitrogenous molecules in the phloem sap providing improved diet quality — the Plant Stress Hypothesis (White, 1969).

Myzus persicae (the peach–potato aphid) is a broad-range feeder with a preference for senescent foliage. Contrary to expectations, on young cabbage plants, *Brassica oleracea* var *capitata*, it was found to increase faster and to a greater extent on well-watered hosts. Aphids were also found to prevent osmoregulation in droughted plants. Despite this, the aphid infestation led to a drought-like response in all plants irrespective of watering regime, causing significantly reduced growth.

Metopolophium dirhodum (the rose–grain aphid) overwinters as eggs on *Rosa* spp. but most of its life-cycle is spent on grasses and cereal crops. As with *M. persicae*, aphid infestation caused drought-like symptoms even in well-watered plants. The mechanism of reduction in plant health is most likely through aphid damage to the efficiency of Photosystem II within 7 days of infestation.

On young barley plants, *Hordeum vulgare*, the rose–grain aphid was found to increase faster and to greater extent on droughted hosts. In contrast to *M. persicae*, the study strongly supported the Plant Stress Hypothesis, finding that even mild water stress of approximately −0.3 MPa could lead to conditions favourable to rapid aphid population increase. The difference in the response of different species of aphids may be associated more with the levels of soluble nitrogen in their diet than specifically host plant water status.

Drepanosiphum platanoidis is an aphid specific to sycamore, *Acer pseudoplatanus* and spends its entire life-cycle on that species. In the field, the total number of aphids supported by a single tree was found to correlate most strongly with soluble leaf nitrogen. In addition, the

number of aphids on individual leaves was found to correlate strongly and negatively with stomatal conductance and leaf water potential.

Finally, analysis of historical aphid suction-trap data was carried out. UK Rothamsted suction-traps are 12.2 m tall and sample air at 0.75 m^3 s^{-1}, with daily aphid sampling between April and November. The traps are designed to catch the largest proportion of aphids in the total insect sample and to reduce the effect of surrounding land-use on the sample. The area represented by a trap may be as much as 700 km^2 (Harrington and Woiwod, 2007). The dataset is the longest and most geographically extensive for aphid populations, comprising 46 sites across Europe as of 2011. In this analysis, aphid and meteorological data from the Edinburgh East Craigs site were used. This also enabled comparison with *D. platanoidis* numbers collected in the field, which showed a strongly significant positive relationship between the two. Since the data collected in suction-traps are correlated with numbers in the field, aphid population analysis can be improved with use of these longer datasets, without reliance on shorter-term field surveys.

The various population characteristics examined were found to be strongly correlated with each other, but were unconnected to data from previous years. The date of first catch was found to be significantly advancing over time, with strong influences from daily minimum temperatures and vapour pressure deficits of the air within the year. Higher temperatures with lower humidity tend to bring forward the date of first catch. Lower VPD tends to depress the population in the summer.

The overall hypothesis that mild drought would benefit aphid populations cannot be accepted, but in two cases of the experiment sections of the work there were strong relationships between aphid numbers and both leaf temperature and soil moisture. The meteorological data analysis indicates that climatic changes towards a hotter, drier climate could substantially alter population dynamics.

Harrington, R. and Woiwod, I. (2007). Foresight from hindsight: The Rothamsted Insect Survey. *Outlooks on Pest Management*, **18**, 9–14.

White, T. (1969). An index to measure weather-induced stress of trees associated with outbreaks of psyllids in Australia. *Ecology*, **50**, 905–909.

Lay Summary

Interactions between host plants and aphids under water stress conditions were examined. Two controlled environment room studies, a field experiment and population analysis were used to investigate three different plant-aphid relationships.

The aim of these studies was to determine the results of water shortage on plant and pest populations, which are likely to result under climate change scenarios. The central hypothesis was that mild drought stress would lead to changes in the quality of phloem sap that could benefit aphids.

The peach–potato aphid (*Myzus persicae*) is a broad-range feeder with a preference for senescent foliage. Contrary to expectations, on young cabbage plants, *Brassica oleracea* var *capitata*, it was found to increase faster and to a greater extent on well-watered hosts. Aphids were also found to limit a droughted plant's ability to respond to drought. The infestation led to a drought-like response in all plants irrespective of watering regime, causing significantly reduced growth.

The rose–grain aphid (*Metopolophium dirhodum*) overwinters as eggs on *Rosa* spp. but most of its life-cycle is spent on grasses and cereal crops. As with the peach–potato aphid, infestation caused drought-like symptoms even in well-watered plants. The mechanism of reduction in plant health is most likely through aphid damage to photosynthesis.

On young barley plants, *Hordeum vulgare*, the rose–grain aphid was found to increase faster and to greater extent on droughted hosts. In contrast to the peach–potato aphid, even mild water stress could lead to conditions favourable to rapid aphid population increase. The difference in the response of different species of aphids may be associated more with the levels of soluble nitrogen in their diet than specifically host plant water status.

The sycamore aphid (*Drepanosiphum platanoidis*) is specific to sycamore, *Acer pseudoplatanus* and spends its entire life-cycle on that species. In the field, the total number of aphids supported by a single tree was found to correlate most strongly with soluble leaf nitrogen. In addition, the number of aphids on individual leaves was found to correlate strongly and negatively with drought indicators.

Finally, analysis of historical aphid suction-trap data was carried out. UK Rothamsted suction-traps are 12.2 m tall and sample air with daily aphid sampling between April and November. The traps are designed to catch the largest proportion of aphids in the total insect sample and to reduce the effect of surrounding land-use on the sample. The dataset is the longest and most geographically extensive for aphid populations, comprising 46 sites across Europe as of 2011. In this analysis, aphid and meteorological data from the Edinburgh East Craigs site were used. This also enabled comparison with sycamore aphid numbers collected in the field, which showed a strongly significant positive relationship between the two. Since the data collected in suction-traps are correlated with numbers in the field, aphid population analysis can be improved with use of these longer datasets, without reliance on shorter-term field surveys.

The various population characteristics examined were found to be strongly correlated with each other, but were unconnected to data from previous years. The date of first catch was found to be significantly advancing over time, with strong influences from daily minimum temperatures and estimates of the drying power of the air within the year. Higher temperatures with lower humidity tend to bring forward the date of first catch. Wetter air is associated with reduced populations in the summer.

The overall hypothesis that mild drought would benefit aphid populations cannot be accepted, but in two cases of the experiment sections of the work there were strong relationships between aphid numbers and both leaf temperature and soil moisture. The meteorological data analysis indicates that climatic changes towards a hotter, drier climate could substantially alter population dynamics.

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Nomenclature

Acronyms:	
ABA	Abscisic acid
ANCOVA	Analysis of covariance
ANOVA	Analysis of variance
IPCC	Intergovernmental Panel on Climate Change
JPEG	'Joint photographic experts group' (image file type)
PAR	Photosynthetically active radiation
PSH	Plant Stress Hypothesis
SEM	Standard error of the mean
SPAD	'Soil plant analysis development' (Minolta chlorophyll measurement unit)
VPD	Vapour pressure deficit
VWC	Volumetric water content
Phenology responses:	
ENDS1	Ordinal date of the end of the spring season
ENDS2	Ordinal date of the end of the summer season
ENDS3	Ordinal date of the end of the autumn season
FC	Ordinal date of first catch
S1C	*ln* (cumulative sum of aphids during the spring season)
S1	Spring rate of increase
S2C	*ln* (cumulative sum of aphids during the summer season)
S2	Summer rate of increase / decrease

S3C	*ln* (cumulative sum of aphids during the autumn season)
S3	Autumnal rate of increase
TC	*ln* (total catch)
TS	Total length of aphid season
Statistical:	
F	F-test statistic
p	Probability value
t	t-test statistic
d.f.	Degrees of freedom
n	Total sample size per day
R^2	Cross-validated square of the product-moment correlation
r^2	Square of the product-moment correlation
Terms:	
F_v/F_m	Ratio of variable to maximum fluorescence — a measure of the quantum efficiency of open Photosystem II centres
$\Delta F/F_{m'}$	Change in steady state fluorescence yield divided by maximum fluorescence yield (in the light)
A_{max}	Maximum photosynthetic rate
g_s	Stomatal conductance rate
r_m	Intrinsic rate of increase
Ψ	Total leaf water potential
Ψ_π	Leaf osmotic / solute potential
Ψ_ρ	Leaf turgor potential
PSII	Photosystem II
Treatments:	
A	Aphid infested
D	Droughted / water excluded
N	No aphids / aphids removed
W	Well watered / water permitted
Non-S.I. Units:	
L	litre

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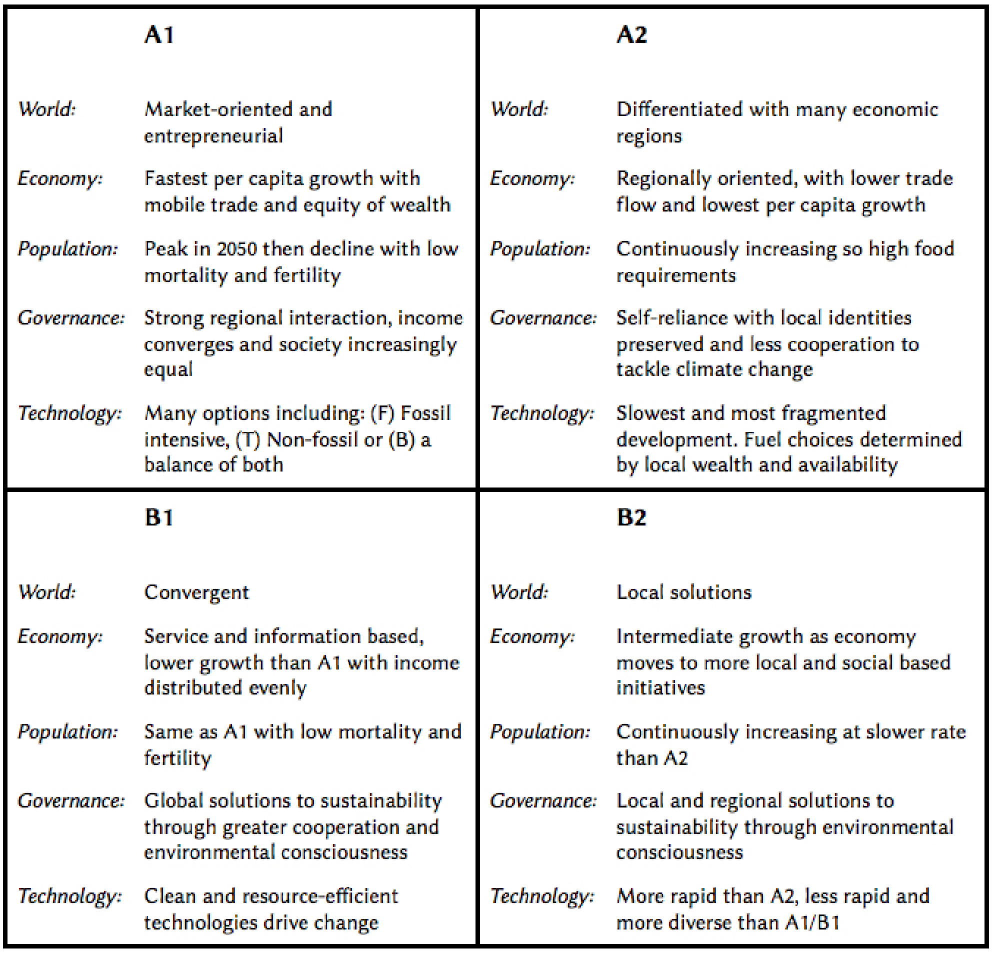
Introduction

1.1 Changing climate

One of the greatest challenges facing ecologists is the uncertainty of population responses to expected rapid changes in climate. Since 1750, global atmospheric concentrations of gases that lead to Earth warming (CO_2, methane and nitrous oxide) have increased as a result of human activity. Globally, climate changes are diverse and affect, for example, arctic temperatures, precipitation, ocean salinity, wind patterns, sea levels and extreme weather events (Solomon et al., 2007). This work focusses on aphid-plant interactions in a changing temperate climate – the United Kingdom.

Analysis of the current situation by the Intergovernmental Panel on Climate Change (IPCC, Alcamo et al., 2007b) finds that overall, Europe has warmed by at least 0.90 °C in the last 100 years and in the past 30 years alone by 1.23 °C. This has an effect on plant and animal phenology. Temperatures are becoming more variable and prone to extremes. Precipitation trends vary across the continent, and the seasonal patterns of rainfall are changing. Europe is currently more likely to experience extreme seasons, increased risk of storms and long-term pressures on the coast through rising sea-levels. The rapidity of these changes is likely to pose a significant challenge to species, particularly in coastal and mountain regions that see their possible habitat heavily reduced by rising sea-levels and tree-lines.

To help model possible climate developments, the IPCC have published four emissions scenarios, A1, A2, B1 and B2. This framework is described in Figure 1.1 and features different levels of world cooperation, economy, population growth, governance and technology (Nakićenović et al., 2000). These models can be used to estimate the differing effects of these global paths on the future production of greenhouse gases (that increase warming effects) and aerosol emissions (that increase cooling effects). Whilst no one of the scenarios is expected to be more likely than another, and each presumes that no global change policy is implemented, they prove useful in estimating challenges that will need to be overcome. The different potential paths are broad strokes that require greater spatial resolution (Carter et al., 2007).



	A1		**A2**
World:	Market-oriented and entrepreneurial	*World:*	Differentiated with many economic regions
Economy:	Fastest per capita growth with mobile trade and equity of wealth	*Economy:*	Regionally oriented, with lower trade flow and lowest per capita growth
Population:	Peak in 2050 then decline with low mortality and fertility	*Population:*	Continuously increasing so high food requirements
Governance:	Strong regional interaction, income converges and society increasingly equal	*Governance:*	Self-reliance with local identities preserved and less cooperation to tackle climate change
Technology:	Many options including: (F) Fossil intensive, (T) Non-fossil or (B) a balance of both	*Technology:*	Slowest and most fragmented development. Fuel choices determined by local wealth and availability
	B1		**B2**
World:	Convergent	*World:*	Local solutions
Economy:	Service and information based, lower growth than A1 with income distributed evenly	*Economy:*	Intermediate growth as economy moves to more local and social based initiatives
Population:	Same as A1 with low mortality and fertility	*Population:*	Continuously increasing at slower rate than A2
Governance:	Global solutions to sustainability through greater cooperation and environmental consciousness	*Governance:*	Local and regional solutions to sustainability through environmental consciousness
Technology:	Clean and resource-efficient technologies drive change	*Technology:*	More rapid than A2, less rapid and more diverse than A1/B1

FIGURE 1.1: Adapted from (Nakićenović et al., 2000; Carter et al., 2007): The four proposed IPCC scenarios and their definitions.

Europe and the United Kingdom offer an existing scenario of differentiated, wealthy countries with relatively low population growth that are self-reliant with diverse fuel systems and a growing environmental awareness (Alcamo et al., 2007b; Central Intelligence Agency, 2013). Economic growth is also currently low. From Figure 1.1 the most moderate matching scenarios are either A1B or A2.

The UK Climate Projections centre has developed models based on low (B1), medium (A1B) and high (A1F) scenarios of carbon emissions. The greatest probability for the UK was found to be increased temperatures across all seasons and all scenarios, with the extent of these increases growing rapidly over the next 70 years (DEFRA, 2012). Whilst increasing atmospheric CO_2 may improve plant productivity (e.g. Robredo et al., 2007; Leakey et al., 2009), this may be countered by changes to water availability. Currently the model suggests increased rainfall across all seasons and all scenarios. Despite this overall trend, precipitation is the most unpredictable factor in the models and the likelihood of extreme events such as severe drought and flood are also high (Duan et al., 2013). An example of these extremes could be seen in England and Wales over the period 2010–2012, where the lowest 18 month rainfall for 100 years was closely followed by the highest spring rainfall on record for 230 years (Marsh and Parry, 2012), although there is no evidence that these events were climate change related.

Under all scenarios, drought intensity and duration may increase in England and Wales, and contiguous areas affected by moderate drought may increase, particularly in Scotland (Rahiz and New, 2013). Closer examination of the interaction between climate change and land-use capability, particularly soil moisture values, indicates that water deficits may become a significant agricultural risk to Eastern and Southern Scotland (Brown et al., 2008, 2011). Crop water requirements and the ability of the soil type to hold water are key factors in determining the drought risk.

Greater relative aridity can be seen across all scenarios, with lower flows in river basin regions reducing the quantity of water available for industry and agriculture leading to the greatest supply-demand deficits in Southern England (Wade et al., 2013). Particularly in England, drought effects are increased where groundwater is the major source of water supply and demand from the population, agriculture and industry are at their highest (Marsh and Parry, 2012).

Models based on the other moderate, but ideologically distinct, scenario A2 (Alcamo et al., 2007b) also indicate that potentially warmer conditions in the summer and overall decreases in mean annual precipitation could lead to increased drought in central and southern Europe. Winter warming in northern Europe could lead to greater mean annual precipitation, where the risk of winter floods increases. The drought risk for western Europe is also increasing, with growing irrigation requirements. Under these conditions, Alcamo et al. (2007b) project moderate to severe water stress for southern Britain, with increasing water shortages worldwide.

There is no certainty in any model, with presumptions and generalisations that may not come to pass. They are a tool necessary to spur action on greenhouse gas emissions and investment in research. Climate change is predicted but its direction and subsequent impact on organisms is difficult to gauge. In this work, the focus is on phloem-feeding insect herbivores: aphids. The flexibility of aphid and plant populations and their ability to adapt to a changing environment is still to be determined.

1.2 A brief history of aphids

Aphids are insects belonging to the superfamily Aphidoidea within the suborder Sternorrhyncha of the order Hemiptera.

Life-cycles vary from species to species, but there are key groups. Aphids may be holocyclic (i.e. alternating parthenogenetic and a sexual reproduction stage) or anholocyclic (i.e. only parthenogenetic reproduction). In some species, such as *Myzus persicae* (Sulzer) the life-cycle is driven by climatic conditions, with anholocyclic strains occurring in particularly warm temperate and tropical regions (van Emden et al., 1969). Aphids with a holocyclic life-cycle either live on a single host species (monoecious) or alternate hosts in winter and summer (heteroecious).

Ancestors of modern aphids are believed to have reproduced sexually on a single woody host plant, with parthenogenesis and heteroecy evolving later (Moran, 1992). The rapid radiation of aphids is believed to have occurred 80–150 million years ago as they moved from gymnosperm to angiosperm hosts (von Dohlen and Moran, 2000). Most aphid species are monoecious (Eastop, 1973).

Polyphenism, where there are multiple morphs within a population of genetically identical individuals is characteristic of aphid species. The production of different morphs is associated with seasonal fluctuations in diet quality, but they can be triggered by factors such as host plant stress or temperature (Moran, 1992).

Aphids elect to remain on a host following a succession of suitability tests, reviewed by Powell et al. (2006). The sequence involves a cursory identification by winged forms before landing; first contact and assessment of the plant surface; an epidermal probe; then prolonged stylet penetration followed by puncture of the sieve element and ingestion of phloem sap. Tjallingii (2006) describes the stylet sheath exuded between the stylet and cell walls, blocking plant defences. This area becomes the site of salivation as a preliminary tasting of cells confirms host quality and suitability. Plant metabolites detected during stylet penetration appear to be the most important factor in host selection. Once established, phloem pressures of 1.5–3.0 MPa force sap up through the stylet (Dixon and Kindlmann, 1998), although aphids still require to swallow actively (Mittler, 1958).

Phloem sap is characterised by high sugar concentrations and low soluble nitrogen concentrations. Insects of the order Hemiptera have two key adaptations to phloem sap feeding. Aphids have bacterial symbionts (*Buchnera* sp.), which modify the quality of sap ingested by upgrading nonessential amino acids to essential amino acids. This enables aphids to utilise phloem sap and maintain higher reproductive rates on poor diets, overcoming the barrier of low nitrogen quality. The profile of amino acids provided by the symbiont, and its versatility may vary between aphid higher taxa or between aphid species (Wilkinson et al., 1997; Dixon and Kindlmann, 1998; Douglas, 1998, 2006).

To overcome high sugar concentrations, hemipterans contain sucrase-transglucosidase enzymes in their gut. These polymerise the incoming sugar, transforming ingested sucrose into long chain oligosaccharides to be excreted via honeydew (Douglas, 2006; Pritchard et al., 2007). The difference in water potential between aphid and diet creates a large driving force, which may dehydrate aphids. The polymerisation raises the water potential of the diet, combating the water potential difference.

Aphids secrete two kinds of saliva: a gelling material that forms the stylet sheath; and non-gelling watery saliva that carries salivary enzymes. This topic was reviewed in depth by Miles (1999). The stylet sheath is composed mainly of protein, with active sulphydryl groups and oxidases that are able to absorb and immobilise potentially harmful phytochemicals. Watery saliva contains free amino acids and sulphydryl groups, with several enzymes for breaking down plant defences: pectinase, cellulase, polyphenol oxidase and peroxidase. Secreting watery saliva into sieve tubes keeps sieve pores open, and prevents coagulation of defence proteins (Miles, 1999; Tjallingii, 2006). Loss of sieve element turgor pressure caused by insertion of the stylet is minimised by its tiny volume. There may also be a collective suppression of plant defences and communication between aphid individuals via watery saliva (Will and van Bel, 2006).

Aphids exploit a niche diet with highly specialised adaptations. Their success depends on the ability to ingest and digest phloem sap. Unpredictable periods of plant stress are to be expected in temperate climates, but these are typically balanced out over time. The odds of climate-led plant stress are increasing and this may change the quality of the aphid diet.

1.3 Plants under stress

Structural and physiological changes to the plant caused by drought may affect aphids directly through a change in diet quality and ability to feed, or indirectly through a change in cues and host selection.

Decreased growth by cell enlargement is the first factor to be affected by moderate water stress (Hsiao et al., 1976). Therefore, decreased tissue is available for infestation in droughted plants; leaves are smaller, thicker and waxier, potentially making it more difficult for the aphid to penetrate and feed (Pritchard et al., 2007).

Lowering plant osmotic potential by accumulating solutes is known as osmotic adjustment. In this way, plants can maintain cell turgor, enlargement and growth for a short period (Hsiao et al., 1976; Taiz and Zeiger, 2006). The types of solute typically accumulated include stress metabolites, osmolytes, soluble nitrogen and sugars, abscisic acid (ABA) and other secondary compounds (Wearing, 1967; English-Loeb et al., 1997; Meyer et al., 2006; Kitao et al., 2007).

ABA promotes root growth by inhibiting ethylene production during drought as well as inducing stomatal closure. Sending roots further into the ground in search of water is a key plant defence against water stress. An increase in below ground biomass or root:shoot ratio is observed (Khalil and Grace, 1992; Kang et al., 2000; Taiz and Zeiger, 2006; Wu et al., 2007). Increasing ABA concentration in the guard cells decreases water potential, resulting in decreased turgor, closing stomata to prevent further water loss. This could diminish aphid ability to feed, since they require phloem pressures of 1.5–3.0 MPa to force sap up through the stylet (Dixon, 1998). However, the reduction in transpirational cooling also means that water stressed plants are warmer (Mattson and Haack, 1987a; Brodribb, 1996; Taiz and Zeiger, 2006). This could allow insects to live closer to their optimum temperature, enabling them to develop and reproduce at maximal rates (Mattson and Haack, 1987b).

Protein hydrolysis also increases with water stress, liberating small nitrogenous compounds to the phloem sap. For example, as leaf water potential in alfalfa (*Medicago sativa*, L.) decreases, total amino acid concentration increases, with proline concentration up to 60 times the normal level in the phloem sap (Girousse et al., 1996). Proline increase appears to be a typical response to water deficit (Showler, 2002). Plant metabolites detected during stylet penetration appear to be the most important factor in host selection. Aphid species that cause visible chlorotic lesions on the leaf surface, for example, *Schizaphis graminum* (Rondani) and Russian wheat aphid (RWA, *Diuraphis noxia* (Mordvilko)), are modifying the proportion of essential amino acids, reducing their dependency on bacterial symbionts (Sandstrom et al., 2000). If the proportion of amino-nitrogen in the phloem sap changes with plant stress, this may also reduce the number of processing stages in an aphid's metabolism.

The osmotic pressure of the sieve element increases under drought stress, suggesting solute concentrations rise, creating a more nutritious diet (Hale et al., 2003). However, this could mean the aphid digestive system needs to work harder to reduce the already large difference in osmotic pressure between aphid and phloem. Previous research has found increasing sucrose concentration decreases the percentage of dietary sucrose absorbed by aphids (Mittler and Meikle, 1991).

Under mild to moderate water stress leaf ageing may temporarily halt; leaves are physiologically younger for longer than normal. As the severity of drought continues, leaves begin to senesce earlier and drop (Mattson and Haack, 1987b). The spectral quality of a leaf changes with increasing water stress. The 'water band' of 970 nm; the 'xanthophyll' band of 531 nm; and possibly the 'oxidised brown compound' far-red band are of interest in drought response reflectance (Penuelas et al., 2004). The spectral changes due to leaf yellowing and greater infrared reflectance may cue insect herbivores and attract them to land (Mattson and Haack, 1987a,b). Severe stress leads to metabolic changes equivalent to those in senescing tissues, with the breakdown and mobilisation of nitrogen in soluble forms away from tissues affected. The aphid *Therioaphis maculata* (Buckton) causes a senescence type response in alfalfa, selecting older leaves for feeding (Dillwith et al., 1991). Barley strains resistant to RWA are less sensitive to water stress than susceptible lines (Oswald and Brewer, 1997).

Alterations effected in a plant by drought may favour aphids (e.g. accumulating solutes, increasing soluble nitrogen, warmer leaf temperatures), whilst others (e.g. loss of turgor, reducing leaf area, leaf thickening) may be detrimental to their survival. Experimental evidence is required to elucidate which of these factors has the greatest effect on aphids.

1.4 Potential effects of host stress on aphids

Different species of aphid have been found to be affected by host-plant stress in different ways. Host plant quality is a key determinant in insect fecundity, previously reviewed by Awmack and Leather (2002). Carbon, nitrogen and defensive metabolites directly affect potential and achieved fecundity; influencing egg size, quality, resource allocation, and oviposition sites. Fecundity of sap-feeding and chewing herbivores is often affected differently by the same hosts.

The response to water shortage varies with aphid species. *Myzus persicae* is less dependent on food supply under pressure than *Aphis fabae* Scopoli or *Brevicoryne brassicae* (L.) on the same host (Wearing, 1967; Wearing and van Emden, 1967). *Macrosiphum euphorbiae* (Thomas) survival, growth and reproduction are negatively affected by water deficit of its host, potato (*Solanum tuberosum*, L.), due to decreased plant cell water content and phloem turgor pressure (Nguyen et al., 2007). These differences between species may be attributable to the dietary scope of the aphid, whether it is polyphagous like *M. persicae* or oligophagous like *B. brassicae*.

Crop plants infested by aphids can lead to significant economic losses, and much research focuses on aphids of the world's crops. Within crop sub-groups there are mixed responses of aphids to host stress. Oswald and Brewer (1997) found that *Rhopalosiphum maidis* (Fitch) performed better on unstressed barley (*Hordeum vulgare*, L.). Other studies find that *Metopolophium dirhodum* (Walker) and *Rhopalosiphum padi* (L.) decrease in abundance with decreasing plant quality, whilst *Sitobion avenae*

(Fabricius) is unaffected (Honěk, 1991; Pons and Tatchell, 1995; Hale et al., 2003). *Schizaphis graminum* feeding on droughted and previously infested barley had lower developmental and mean relative growth rates (Cabrera et al., 1995). However, more RWA are found on plants grown under water stress (Archer et al., 1995; Oswald and Brewer, 1997). Therefore, species response varies between cereal aphids and within the same feeding guild.

The abundance of aphids is highly seasonal and varies annually (Wellings and Dixon, 1987). For example: green spruce aphids (*Elatobium abietinum* (Walker)) and sycamore aphids (*Drepanosiphum platanoidis* (Schrank)) track seasonal changes in sap nutrient availability (Dixon, 1970; Day et al., 2004). To model herbivore outbreak accurately, we need to consider active defensive participation of plants (Rhoades, 1983, 1985) and the pattern of nutritional quality in host species.

The increase in the phloem amino-nitrogen levels of droughted plants may be analogous to that in young and senescent leaves, which are richer in amino acids. Higher levels of amino nitrogen allow the development of large adult aphids with high reproductive rates and heavy young (Dixon, 1970). In the short term, stress can therefore increase the concentration of previously limiting amino acids. Herbivores feeding on such tissue have a more accessible supply of nitrogen, so can increase in abundance, with particular benefits for larvae (White, 1984; Brodbeck and Strong, 1987; Hale et al., 2003).

This dependance on nitrogen levels gave rise to the Plant Stress Hypothesis (PSH).

1.5 The Plant Stress Hypothesis & other proposals

White (1969) initially proposed the PSH suggesting that plant stress led to increased nitrogen availability for phytophagous insects and therefore increased reproduction rates and survival of young. However, continuous water stress tends to affect sap-feeders adversely, despite increased foliar nitrogen. A simultaneous decrease in plant turgor and water content interferes with the herbivore's ability to access and utilise the nitrogen (Wearing, 1972; Speight, 1986; Louda and Collinge, 1992; Huberty and Denno, 2004). Intermittent water stress tends to be beneficial, giving rise to the 'Pulsed-Stress Hypothesis' where bouts of stress and recovery of turgor allow sap feeders to benefit from stress-induced increases in nitrogen (Wearing, 1972; Speight, 1986; Louda and Collinge, 1992; Huberty and Denno, 2004).

Since species vary in their requirements (van Emden and Bashford, 1969), the original PSH has been modified to take into account species preferences for different developmental stages of plant tissue (White, 2009). It is hypothesised that flush-feeding aphids will prefer plants in good health, whilst senescence-feeding aphids will prefer plants under mild stress. The first part is akin to the 'Plant Vigour Hypothesis' put forward by Price (1991) where it is suggested that some herbivore species may prefer to feed on rapidly growing, larger than average plants as opposed to plants with reduced growth and size.

Closely linked to the PSH, is 'Climatic Release'. Here, climatic anomalies may permit insect herbivores to be released from otherwise strict climatic controls (Martinat, 1987). Most of the evidence linking weather to insect outbreak is inferred from correlative study, so it is difficult to establish a causal relationship. It may be better to examine catastrophic weather events of shorter duration, than to test climatic anomalies over time (Martinat, 1987; Mattson and Haack, 1987b) due to the difficulties of testing hypotheses using random weather variables.

It is possible that the PSH is more relevant to long-lived trees, capable of adaptation, rather than short-lived herbaceous plants (English-Loeb et al., 1997). Insect performance on experimentally stressed woody plants has previously been reviewed (Koricheva and Larsson, 1998), finding that plant stress had no significant effect on insect growth rate, fecundity, survival or colonisation density. In general, boring and sucking insects performed better on stressed plants, whilst gall makers and chewing insects were negatively affected. The reproductive potential of sucking insects was reduced by water stress.

It is valuable to know how broadly applicable the PSH could be; most evidence suggests the main variation is between feeding guilds (Mattson and Haack, 1987a; Larsson, 1989; Koricheva and Larsson, 1998). The apparent lack of a single common explanation does not mean there is no general system response (Price, 1991; Hoover and Newman, 2004), particularly since experimental methodologies are so widely different.

Ultimately, no one of these hypotheses can be expected to explain all patterns of herbivore behaviour. We need to discover whether the relationship between stress intensity and plant response is linear or non-linear. This makes a difference when designing experiments, since many studies use one level of drought, unlikely to capture the full plant/insect response. In tomato (*Solanum lycopersicum*, L.), drought increases plant phenolics in a monotonic but not strictly linear pattern. Drought stressed plants showed an increased concentration of total nitrogen (English-Loeb et al., 1997), which should positively impact phloem sap feeders (Louda and Collinge, 1992).

Changes in plant physiology such as water potential, photosynthetic capacity and stomatal conductance are appropriate for measuring plant stress level, but need to be related to insect performance through measures of survival, growth rate and fecundity (Larsson, 1989).

1.6 The effect of aphids on stressed hosts

If the PSH proves generally true, then under climate change, aphid populations are likely to become greater and pose a more significant threat to plant populations. Alongside the scientific value of understanding aphid-plant interaction, there is significant economic value in understanding plant pests. Crops depend heavily on irrigation to maintain plant health. In a climate with increasing drought risk, higher irrigation requirements may not always be met (Alcamo et al., 2007b). Agriculturalists are being encouraged to reduce pesticide use (European Parliament, 2009) and may be restricted in methods to reduce the aphid threat.

So, what effects do aphids have on host plants?

A sink for soluble nitrogen

Feeding on the phloem sap removes soluble nitrogen in the form of amino acids and amides. This leads to a significant decrease in leaf crude protein as estimated from total leaf nitrogen (Shannag, 2007). This estimate will, however, include nitrogen not sourced from protein.

The aphid *Uroleucon caligatum* (Richards) creates an additional sink when feeding on goldenrod (*Solidago altissima*, L.) (Meyer and Whitlow, 1992). Similarly, short-term infestation of alfalfa by pea aphid (*Acyrthosiphon pisum* (Harris)) allows the insects to redirect assimilates, intercepting normal flow and redirecting part of the phloem stream towards themselves (Girousse et al., 2003). *Acyrthosiphon pisum* infestation has a systemic effect on plant tissue, causing uninfested tissue to shift from nitrogen-sink (leaves usually use nitrogen for protein synthesis and growth) to nitrogen-sources exporting nitrogen to the infested zone (Girousse et al., 2005). Some aphids appear to stimulate photosynthesis but act as such a large sink that plants cannot compensate and become deficient in soluble carbon (Collins et al., 2001).

As a result of the alteration in a plant's source-sink metabolism under aphid infestation, a significant reduction in plant performance is expected. The size of this reduction may be affected by the initial health of the plant. Healthier plants should be more tolerant, whilst stressed plants already show physiological changes. These effects reported in the literature also vary widely.

A reduction in overall plant success

Rhopalosiphum padi decreased the dry weight, yield, leaf area, number of tillers and number of leaves of barley. Infestation decreased carbon fixation by reducing biomass increase per unit leaf area per day (unit leaf rate). Plants compensated for this drop in assimilation rate by allocating dry weight to production of leaf laminae, but overall growth decreased due to assimilate loss (Mallott and Davy, 1978).

Aphis craccivora Koch infestation reduced cowpea (*Vigna unguiculata*, L.) shoot biomass and seed yield (Agele et al., 2006). *Schizaphis graminum* infestation negatively impacted photosynthetic rate and leaf chlorophyll content in barley. The aphid decreased CO_2 assimilation by decreasing photosynthetic capacity in the mesophyll rather than by decreasing leaf conductance to water vapour (Cabrera et al., 1994).

In willow (*Salix viminalis*, L.), infestation by the giant willow aphid (*Tuberolachnus salignus* (Gmelin)) decreased growth above and below ground; altering previously developed tissue, the proportion of solid matter of roots and decreasing tree survival (Collins et al., 2001).

Altered gas exchange

Contrastingly, a twelve-day feeding period by *U. caligatum* did not decrease photosynthetic rates per unit leaf area of damaged goldenrod leaves, with no effect on plant relative growth rates or height (Meyer and Whitlow, 1992).

Leaf water potential, chlorophyll content, CO_2 assimilation rate and total soluble carbohydrate decreased in barley infested with *S. graminum* (Cabrera et al., 1995). Increased water use was observed in aphid-infested willow hydroponic culture, possibly due to the stimulation of photosynthesis or aphid feeding and sap removal, but it is unknown whether that translates to the field (Collins et al., 2001).

Accumulation of solutes

Riedell (1989) found that the concentration of the amino acid proline increased up to twenty-fold, and sugar concentration decreased five-fold with respect to control plants. Changes caused were similar to those of drought stress, even in the presence of sufficient soil moisture. Rwa also induce drought stress symptoms in leaves of infested plants, and prevented barley recovery after stress was removed.

Barley aba concentration increased after *S. graminum* infestation (Cabrera et al., 1994, 1995). Similarly, *A. fabae* increases bean plant (*Vicia fabae*, L.) transpiration rate, leading to greater water losses (Shannag, 2007).

Colonies increase in size and extent during summer months (Collins et al., 2001); since the risk of water shortage is higher during summer, it is likely that a concurrent aphid infestation would exacerbate drought impact on host plants.

In summary, aphids have been found to act as a sink for soluble nitrogen, to decrease plant yield and alter the balance of gas exchange and to trigger solute accumulation. Were periods of plant stress to overlap with aphid infestation, the damage caused by aphids on host plants may increase. Under climate change, exogenous forces may cause new risk periods.

1.7 Timing of stress

Aphid phenology and its connections to climate, land use and location has been extensively researched (e.g. Cocu et al., 2005; Harrington et al., 2007; Lima et al., 2008; Estay et al., 2009; Klueken et al., 2009). Combining projections from the United Kingdom Hadley Centre rm03 climate change model for Southern Britain with a general mechanistic model of the interaction between climate, temperate grass physiology and cereal aphid population dynamics predicts an increasingly dramatic decline in cereal aphid abundance and pest status. Across low, medium and high CO_2 emission scenarios, increases in temperature and decreases in rainfall were most important to the forecast reduction (Newman, 2005). However, temperature and rainfall may not fully identify drought periods or other weather conditions that can affect aphid populations such as wind speed (Dixon, 1979) or vapour pressure deficit (Agele et al., 2006).

There is little published on the direct effects of changing precipitation patterns, but it is likely that rainfall enhances insect mortality (Bale et al., 2002). The phenology of stress also requires consideration. The Frampton et al. (2000) study on the effect of spring drought and irrigation on farmland arthropods in Southern Britain found counts for all taxa were highest under irrigation and in most cases reduced under drought. Aphids specifically seemed to peak at an intermediate stress level, but there was an overall negative impact.

Long term datasets tracking aphid abundance in the field are limited in availability and scope. Broader coverage would assist analysis of aphid populations and meteorology. The suction-trap dataset is the longest and most geographically extensive network of data collection for aphid populations, comprising 46 sites across Europe as of 2011. UK Rothamsted suction-traps are 12.2 m tall and sample air at 0.75 $m^3\ s^{-1}$, with daily aphid sampling between April and November. The traps are designed to catch the largest proportion of aphids in the total insect sample and to reduce the effect of surrounding land-use on the sample. The area represented by a trap may be as much as 700 km^2 (Harrington and Woiwod, 2007). The date of first record of aphid species in suction-traps is expected to be earlier in the year in the United Kingdom; the rate varying with location and species (Harrington et al., 2007).

Meteorological data are usually aggregated to monthly or seasonal periods and analysis is focused on two population parameters: date of first catch and total aphid numbers. Insect outbreak models require more research into variables such as rate of increase and life-cycle timings to improve our understanding. Aphids have short generation times and some species adopt generation specific strategies (Dixon, 2005, pp.11, 28). Definitions of 'months' and 'seasons' are imposed by the researcher and average values lead to a loss of information. Where biological-based models are unavailable and datasets are small, penalised signal regression utilising daily data can offer an alternative to stepwise regression (Roberts, 2012). Aphid suction-trap data have not yet been correlated with daily weather values and therefore may help develop a better understanding of how aphid populations interact with the climate. Site-specific analyses are likely to fit aphid data better than generalised models for wide geographical areas (Harrington et al., 2007), perhaps because of local genotype adaptation.

In addition to natural patterns of rainfall or water shortage, intervals in agricultural irrigation may create pulsed stress, more suited to aphids than continuous drought. With agricultural water usage likely to increase with global change (Alcamo et al., 2007a,b), the examination of how to reduce consumption without increasing vulnerability to insect outbreak and attack is necessary.

1.8 The hypotheses and experimental design

The following three hypotheses were formulated:

1. Plants under stress offer an altered diet to aphids, these changes are hypothesised to be favourable to aphid survival (PSH).
2. As aphids feeding on plants are an additional potential obstacle to a stressed plant's survival, it is hypothesised that aphid infestation will cause the greatest damage to already stressed host plants. Well-watered plants are more likely to have the ability to defend against aphid infestation or mitigate its effects.
3. It is also hypothesised that daily climate data may be of more benefit to studies of aphid population data, given the short individual aphid life-cycle may respond to short term extremes rather than monthly or weekly trends.

The following three sub-topics are discussed as further possible sources of variation in plant-aphid interaction:

- The response of aphids to the changes in phloem sap may vary among those that have a single, narrow or wide host plant range. It is suggested that polyphagous feeders can 'afford' to be more selective in their choice of individual plants given their potentially broader range and less specific adaptations. Oligo- and monophagous feeders may be more tolerant of poorer quality diets due to specific adaptations allowing them to overcome a particular hosts' defences or variation in quality due to stress.

- The preference for flushing or senescent tissues is a more subjective matter, especially with host plants of varying ages. In these studies an aphid preference is hypothesised to have no effect, rather that aphids have a preference for stressed tissues with higher soluble nitrogen content.
- The stress responses of plant species are likely to vary depending on life-cycle and water requirements, and it is hypothesised that annual herbaceous plants with high water requirements will respond more quickly to aphid infestation and drought than perennial woody plants with lower water requirements.

To investigate the effect of host plant water stress on aphids, three distinct relationships were selected to test whether aphid numbers would increase rapidly and to greater extent on stressed host plants. Each investigation represented a different combination of insect-host interaction.

Myzus persicae [Aphididae: Macrosiphini] is known as the peach–potato aphid or the green peach aphid. It is highly variable in morphology, colour, life-cycle and host plant preference (van Emden et al., 1969; Takada, 1979). In temperate areas *M. persicae*'s life-cycle may be holocyclic or anholocyclic, and where the life-cycle is complete it is heteroecious. Its primary hosts are members of the *Prunus* genus with secondary hosts in over forty plant families, it is also a vector for several viruses (Blackman and Eastop, 1985, pp. 308–309). It is a polyphagous feeder with a preference for senescent foliage. In this work, cabbage *Brassica oleracea* var *capitata*, L. [Brassicaceae] was selected as the host; it is an annual herbaceous plant with high water requirements and also a significant UK crop.

Metopolophium dirhodum [Aphididae: Macrosiphini] is known as the rose–grain aphid. Its life-cycle may be holocyclic or anholocyclic, and where the life-cycle is complete it is heteroecious. *Metopolophium dirhodum*'s primary hosts are members of the *Rosa* genus with secondary hosts typically members of the Poaceae family: *Bromus* spp., wheat (*Triticum aestivum*), barley (*Hordeum vulgare*) and oats (*Avenae sativa*). It is an oligophagous feeder with a preference for flushing foliage. In this work barley was selected as the host; it is an annual grass with medium water requirements and is the main cereal crop in Scotland.

Drepanosiphum platanoidis [Aphididae: Drepanosiphinae] is known as the sycamore aphid. Its life-cycle is holocyclic and monoecious, spending the entire period on sycamore (*Acer pseudoplatanus*, L.) [Aceraceae]. *Drepanosiphum platanoidis* is a monophagous feeder, and it shifts from flush- to senescence-feeder with the seasons. Sycamore is a common tree in the landscape with drought avoidance and shows potential in the wood-production industry.

The final element used local meteorological and aphid suction-trap data for *D. platanoidis* over a 32 year period. The aim was to test the relationships of population rates of increase, significant dates such as first catch and seasonal timings affecting the life-cycle with each other and with the meteorological data. Climate variables selected were minimum temperature, rainfall and atmospheric vapour pressure deficit in monthly stepwise regression, compared to daily penalised signal (*P*- spline) regression.

1.9 Summary

Aphid-plant interactions under drought stress are unpredictable. Plants under stress offer an altered diet to aphids, but are these changes favourable to aphid survival? The key differences amongst aphid species are dietary scope (mono–, oligo–, polyphagous), feeding guild (e.g. roots, leaves, fruits, buds) and success on developmental stages (flushing or senescent tissues). Responses appear to vary between aphid and plant species, depending partly on plant resistance or tolerance and partly on experimental method. Aphid inter-specific variation is complex, encompassing feeding site, ability to cope with poor diet quality or loss of phloem sap pressure and an aphid clone's genetic adaptation to its local environment. Aphids feeding on plants are an additional potential obstacle to a stressed plant's survival, but the significance of this obstacle is unclear. The information gathered in pursuit of these answers could ultimately inform climate change models of aphid and plant populations.

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2

The case of peach–potato aphid, *Myzus persicae* (Sulzer) and cabbage, *Brassica oleracea* var *capitata* L.

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Published in *Entomologia Experimentalis et Applicata* (2012) **142**:191–202

2.1 Abstract

The possible repercussions of decreasing water availability in plant-insect interactions largely remain unclear. To study this, we designed two factorial studies applying two levels of water stress to cabbage *Brassica oleracea* var *capitata*, L. (Brassicaceae) plants inoculated with the aphid *Myzus persicae* (Sulzer) (Hemiptera: Aphididae: Macrosiphini). One study investigated the effect of aphids and water stress on leaf water potential components, aphid population size, aphid intrinsic rate of population increase (r_m) and fecundity. The other investigated the effect of aphids and water stress on gas exchange, leaf fluorescence yield, estimation of leaf chlorophyll and above ground dry weight.

Drought treatment reduced the size of the *M. persicae* population. Aphid numbers were strongly and positively correlated with soil moisture and negatively correlated with leaf temperature. Aphids on droughted plants had lower r_m and lower fecundity per aphid. Under aphid stress, plants showed reduced maximum photosynthetic rate (A_{max}), lower leaf chlorophyll as estimated by ‘soil plant analysis development’

(SPAD) values, higher leaf osmotic potential (Ψ_π), and reduced final above-ground dry weight compared with control plants.

Aphid infestation also led to significantly higher leaf water potentials and reduced light-adapted fluorescence yield. Compared with control plants, water-stressed plants showed reduced A_{max}, lower SPAD values, reduced leaf water potential (Ψ), lower leaf turgor potential (Ψ_ρ), and reduced final above-ground dry weight. Ψ_π was lower in droughted plants when aphids were not present.

Plants under combined aphid and water stress showed significantly reduced SPAD and final above-ground dry weight, with comparatively higher Ψ.

These results show that aphid infestation limits solute accumulation in the vacuole of drought stressed cabbage. Aphid infestation reduced the host's ability to photosynthesise optimally and led to reduced growth. Plant stress hypotheses propose that a senescence feeder such as *M. persicae* would show higher rates of growth in response to the increasing 'quality' of the phloem sap under water shortage. In the case of *M. persicae*, our results do not support the hypothesis that drought leads to improved aphid population growth and survival.

2.2 Introduction

The Intergovernmental Panel on Climate Change has suggested that there will be an increased incidence of drought in central, southern and western Europe, with higher irrigation requirements. Projections for the next 50–70 years indicate moderate to severe water stress for southern Britain, with increasing water shortages worldwide (Alcamo et al., 2007a,b). If this happens, the patterns of pests and diseases are likely to alter. Newman (2005) predicted dramatic declines in cereal aphid abundance and pest status in response to global warming. Across all CO_2 emission scenarios, increases in temperature and decreases in rainfall were the most important factors in the forecast reduction of cereal aphid numbers.

However, further experimental data are needed to inform models of the effects of climate change in a range of aphid/host combinations. As a result of decreased water availability under climate change, agricultural water requirements are likely to be difficult to fulfil (Alcamo et al., 2007a,b), and water shortage may increase crop vulnerability to insect outbreak and attack. To that end, this work investigated the interaction between the aphid, *Myzus persicae* (Sulzer) (Hemiptera; Aphididae: Macrosiphini) and water stress in cabbage (Brassicaceae). *Myzus persicae* is a generalist feeder with multiple suitable hosts. *Brassica* species account for ca. 21 % of the field vegetable area in the UK, and contributed 25 % of the home vegetable production market in 2009/10 (DEFRA, 2010).

Water shortage is a well-known plant stress factor, but the impact of the resulting physiological changes in the plant on phytophagous insects remains less-well known. The original starting point for our work was the 'Plant Stress Hypothesis' (White, 1969), which states that host stress can lead to an increased availability of small nitrogenous molecules for consumption by phytophagous insects, resulting in their greater reproduction and survival. As the stress on a plant increases, the value as an insect food source increases to a maximum point before falling off as the plant begins to senesce. Field observations originally supported the idea, showing that insect populations sometimes significantly increased after periods of drought (Mattson and Haack, 1987). White (2009) modified the original plant stress hypothesis to specify that it applies to insects with a preference for senescent foliage and that stress levels studied must be intermediate to moderate. Aphids as a family contain both flush and senescence feeders. On plants of the Brassicaceae family, *M. persicae* appears to prefer leaves with the greatest concentrations of mobile nutrients, primarily immature and older leaves (Kennedy, 1958; van Emden et al., 1969; Wearing, 1972; Harrington and Taylor, 1990).

The modified plant stress hypothesis (White, 2009) is related to the 'Pulse-Stress Hypothesis' (Huberty and Denno, 2004). This proposes that periods of stress and recovery are more beneficial to the quality of aphids' diet than constant stress. In a drought scenario, pulses of water availability within periods of water shortage might permit increases in stress-induced nitrogen without the plant wilting due to reduced leaf turgor and water content. The resulting maintenance of turgor pressure would allow aphids to continue feeding from the phloem. This may be because the early reduction in protein synthesis leaves spare amino acids (readily converted to essential amino acids by aphid gut symbionts), whereas the sap remains dilute enough (in comparison to severe drought) for the aphid enzymes to function (Auclair, 1963; Mittler, 1967; Douglas, 2006). For example, higher levels of essential amino acids in the phloem lead to higher survival, higher rates of increase and enhanced fecundity with shorter development times in *M. persicae* on potato (*Solanum tuberosum*, L.). (Karley et al., 2002). Alternatively, Khan et al. (2010), suggested that the increased sugar and decreased glucosinolate levels in water-stressed *Brassica oleracea* L. var. *italica* (Plenck) led to increased aphid numbers.

Increasing day length and air temperature positively affects *M. persicae* population characteristics (Mittler, 1967; Wyatt and Brown, 1977). As leaf stomata close in response to water stress, transpirational cooling declines and leaf temperature rises. This may influence rates of growth and fecundity, irrespective of the expected response to changing diet quality.

Another intriguing aspect of the aphid-plant interaction is the similarity of the drought stress response to the aphid infestation response. For example, Riedell (1989) found that Russian wheat aphid (RWA, *Diuraphis noxia* (Mordvilko)) caused drought stress symptoms in barley (*Hordeum vulgare*, L.). Cabrera et al. (1994) found significant reductions in CO_2 assimilation rate and leaf chlorophyll content in barley infested with *Schizaphis graminum* (Rondani). Given these findings, a factorial design and measures of plant physiology could elucidate the areas where aphid and drought response overlap and/or interact.

In the following studies, we test firstly whether the *M. persicae* population increases more quickly and to larger numbers on intermittently water-stressed host plants compared to well-watered plants. This is the expected response to the increasing quality of the plant sap under water shortage (measured by changes in concentration of the sap vacuole). Secondly, we test whether the increasing temperature of droughted leaves is related to increased growth and fecundity in the aphids. Thirdly, we test whether aphid infestation will reduce the yield and performance of host plants in a manner similar to drought stress.

2.3 Materials & Methods

2.3.1 Experimental design

Cabbage, ‘Greyhound’ variety (commercially available seed), was grown in a controlled environment room. Air temperature was 21 °C ± 0.2 with photosynthetically active radiation (PAR) levels of 100 µmol photons m^{-2} s^{-1} provided by two 36 W T8 Triphosphor 830, 3000 K Warm White fluorescent tubes (Philips Electronics, Guildford, UK) timed to give a 16-h photoperiod. The average relative humidity was 50 %, giving an approximate vapour pressure deficit (VPD) of 1.24 kPa.

Many response variables were involved in this experiment and the sampling processes may have interfered with each other, so that two separate studies prevented over-sampling of the young plants used. In addition, to ensure that plants were all at the same optimum point in their diurnal cycle, a time-window of 10:00 to 13:00 hours was selected. It was not always possible to sample all plants and all response variables within that time window. This gives a broader variation in sample numbers than the ideal. Study I used 24 plants, and ran from November to December 2009; study II used 40 plants and ran from February to March 2010. Throughout the method, the total sample size per day (n) was divided as evenly as possible amongst the four treatment groups.

Plants were grown in 2 L pots containing 80 % Scotts ‘Shamrock’ medium general purpose sphagnum peat (Everris, Ipswich, UK) and 20 % horticultural sand; 0.3 % ground limestone and 0.12 % controlled release fertiliser were included. The fertiliser was Scotts ‘Osmocote 3–4 months’ (Everris), with NPK 14:14:14 that comprises 14 % N as 7.2 % nitrate nitrogen and 6.8 % ammonical nitrogen; 14 % phosphorus pentoxide equivalent to 6.1 % P; 14 % potassium oxide equivalent to 11.6 % K.

At 5 weeks, plants were at the six-leaf stage and were randomly assigned to one of four treatments in a fully factorial design: twice weekly watered (W), once weekly watered (D), aphid infested (A) and no aphids (N). This description of treatments introduces the abbreviations used throughout. In this experiment, small quantities of water, applied weekly, emulate a rationing of water supply or occasional rainfall. Each plant pot had a dish reservoir at the base to take the water dosage. ‘D’ plants received

125 mL on watering days, 'W' plants were watered *ad libitum* and could receive up to 500 mL on watering days. By the end of each study (day 35), all the plants were still immature. The timescale for each study was identical, Figure 2.1. The *M. persicae* strain was descended from an insecticide-susceptible colony, provided by the James Hutton Institute (Dundee, UK).

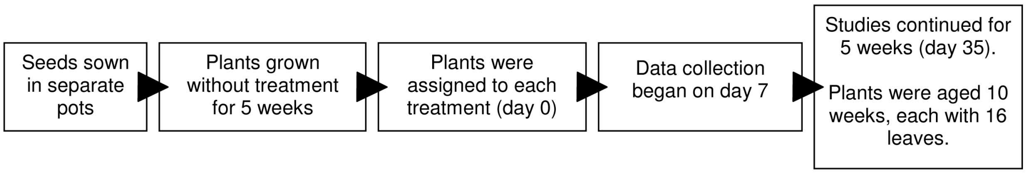

Figure 2.1: Timeline of studies I and II.

2.3.2 Water deficiency monitoring

Soil moisture was measured with a Delta-T Theta Probe ML2 (Delta-T Devices, Cambridge, UK) connected to a Delta-T HH2 Moisture Meter. The unit of measurement was volumetric water content (VWC) m^3 m^{-3}. Measurements were made on 24 plant pots on days 7, 14, 21, and 35 of study I. For study II, measurements were made on days 7, 14, 21, 28, and 35 with $n = 19–40$. This measure was used to confirm that the same soil moisture conditions were created during both studies.

2.3.3 Aphid population study

Population size: Five adult aphids were placed on each plant at the start of the watering treatments (day 0), and were replenished if necessary until day 7. Aphids were counted on 20 plants in study II (days 21, 28, 35) using multiple photographs taken with a digital camera of 6.0 mega pixel resolution saved in high quality resolution JPEG format. Pictures of the upper and lower surface of each leaf were taken, as well as the still unfurled young leaves at the cabbage centre. Aphids were counted in Adobe Photoshop Elements (San Jose, CA, USA). Individuals were marked on the photograph as they were counted to ensure no accidental repetition.

Intrinsic rate of increase (r_m): Clip cages were constructed (Kennedy et al., 1958) for the population study. Two adult aphids were caged separately on each experimental plant at the beginning of the water or drought treatment. The next day, each adult was removed from its clip cage and one of the newly produced nymphs was left in the cage. Afterwards, the cages were checked daily, with progeny removed if produced. Twenty-three cages were studied to completion in study II.

All aphids were followed to the end of their life — beyond the period required for the intrinsic rate of increase calculation. This allowed measurement of total fecundity. The method of Wyatt and White (1977) was used to calculate r_m as in Equation 2.1

$$\mathrm{r_m} = 0.738(log_{\mathrm{e}}M_{\mathrm{d}}/d) \tag{2.1}$$

where d is the pre-reproductive period in days and M_d is the number of progeny produced in a period equal to d.

2.3.4 Plant physiology study

Leaf temperature: Individual leaf temperature was measured with a non-contact Infra-Red Thermometer (Fluke, Norfolk, UK), positioned 20 mm from the surface of the youngest fully expanded leaf. In study I, n = 20–24 and readings were made on days 14, 21, and 35. For study II, n = 19–40 with data from days 14, 21, 28, and 35.

Gas exchange measurements: Maximum photosynthetic rate (A_{max}) and stomatal conductance (g_s) were recorded using an Li-6400 Photosynthesis System (Li-cor Biosciences, Lincoln, NE, USA). These measurements of gas exchange enabled an assessment of drought impact. All measurements were made between 10:00 and 13:00 hours. An initial light response curve was determined on day 0 to define a suitable chamber PAR level, allowing comparison of the A_{max} between stressed and unstressed plants. The chosen light level was 1200 µmol photons m^{-2} s^{-1}, this being the point where CO_2 uptake levelled with respect to PAR on the light response curve. Chamber temperature was controlled at 21 °C, CO_2 was controlled at 370 µmol CO_2 mol^{-1} and flow rate to the sample cell was set to 500 µmol air s^{-1} (as Li-cor Biosciences, 2001). The youngest fully expanded leaf of the 5-week-old plant was tagged on day 1 and used for all gas exchange measurements in study I on days 7, 14, and 21 with n = 9–23.

Fluorescence measurements: The chlorophyll fluorescence yield parameters $\Delta F/F_{m'}$ and F_v/F_m were measured using a Walz Mini-PAM system (Heinz-Walz, Effeltrich, Germany). These parameters were defined by Genty et al. (1989) to investigate the yield of electron transport in Photosystem II, which in turn affects the efficiency of CO_2 uptake. All measurements were made between 10:00 and 13:00 hours using the same leaves sampled for gas exchange on days 7, 14, 21, and 28 with n = 9–23.

Measurements were conducted on both light- and dark-adapted leaves. 'In the light' measures give the Yield- parameter (based on the Genty parameter $\Delta F/F_{m'}$). 'Dark-adapted' measures give F_v/F_m (after at least 30 minutes dark adapt time). The system was operated on 'Burst-mode' for measurements, and all other settings were default. The Mini-PAM leaf measurement head was used for light-adapted measures, and the proprietary leaf-clips were used to hold the fibre optic unit for dark-adapted measures. Background elements of the fluorescence signal were eliminated with the 'Auto-Zero' function before readings were taken.

Leaf chlorophyll estimation: Several studies show that leaf chlorophyll content is strongly correlated with leaf nitrogen content (e.g. van den Berg and Perkins, 2004), and so, a non-destructive estimation of chlorophyll content in the same leaves sampled for gas exchange and fluorescence was made using a Minolta SPAD meter (Konica Minolta Sensing, Basildon, UK). Each leaf was sampled three times to generate a mean value in study I on days 7, 14, 21, and 28 with n = 10–20.

Above ground dry weight: Plant matter from above ground only, was harvested at the end of study I to determine any impact of drought and aphids on plant growth and yield. Plant material was oven dried to constant weight at 80 ° C, n = 24.

Water potential components: The components of plant water potential are related as in Equation 2.2

$$\text{Total }\Psi = \Psi_\rho + \Psi_\pi \tag{2.2}$$

where Ψ is the total leaf water potential, Ψ_π is the leaf osmotic potential and Ψ_ρ is the leaf turgor potential.Ψ gives an immediate reading for water stress; Ψ_π gives the concentration of vacuolar and phloem constituents. Ψ_ρ is expected to have a strong relationship with aphid performance.

Ψ was measured using a Scholander-type chamber (Plant Moisture System SKPM 1400, Skye Instruments Ltd., Powys, UK) between the hours of 10:00 and 13:00. Fully expanded leaves were excised and immediately transferred to the chamber. Readings were taken in study II on days 7, 14, 21, 28, and 35 with n = 6–21.

After the leaf was removed from the pressure chamber, it was sealed in a 10 mL plastic syringe and immediately snap-frozen in liquid nitrogen. Leaves were stored in liquid nitrogen and defrosted for 30 minutes prior to measurement. At least 10 µL of cellular sap was expressed by depressing the syringe plunger. The expressed sap was collected in a sterile Petri dish and immediately sampled with a 10 µL pipette, transferred to a filter paper disc and analysed using a Wescor Vapro5520 Vapour Pressure Osmometer (Wescor, Logan, UT, USA). The osmometer output in mmol kg^{-1} was converted to Ψ_π in MPa according to van't Hoff, Equation 2.3

$$\Psi_\pi\ (\text{MPa}) = -\text{R}\cdot\text{T}\cdot(\text{OSM}/1000) \tag{2.3}$$

where R = universal gas constant, 0.00831 MPa kg mol^{-1} K^{-1}, T = room temperature, 293 K and OSM = osmometer output. The error component from dilution of symplasmic water by apoplasmic water is acknowledged from Callister et al. (2006), but was judged to be small and not corrected for. Ψ_π readings are therefore a bulk estimation of leaf osmotic potential, relevant for water stress studies, and field sample collection (Bannister, 1986; Pullman and Johnson, 2009). Ψ_ρ was derived from the relationship between the two measured potentials.

2.3.5 Data analysis

All analyses and figures were generated using Minitab 15 Statistical Software (Minitab, Coventry, UK). All data were normally distributed and showed equal variance, and therefore, two-way repeated-measures analysis of variance (ANOVA) were performed on the gas exchange, water potential, fluorescence, and SPAD data using the General Linear Model function of the programme. The factors were 'Day' (random factor, within groups), 'Aphids' (fixed factor, between groups), 'Watering' (fixed factor, between groups), and 'Aphids*watering' (interaction) (Minitab, 2007). All means are given with standard error of the mean (SEM). Aphid photographic count data were log transformed for linear regression with soil moisture and leaf temperature. These count data were also left untransformed and analysed in their original Poisson distribution using the appropriate *t*-test. The fecundity data were also Poisson distributed, and analysed using a *t*-test with this distribution. $\mathrm{r_m}$ and nymph per adult per day data were analysed using ANOVA as they were normally distributed with equal variance.

2.4 Results

2.4.1 Drought treatment

Soil moisture levels for the two watering treatments were the same in the two separate studies, with no significant difference (two-sample *t*-test: $t = -1.24$, d.f. = 66, $p = 0.220$). Plants that were infrequently watered experienced significantly drier soil (study I: $F_{1,67} = 48.02$ & study II: $F_{1,123} = 446.80$, both $p < 0.001$; Figure 2.2C, D). Their leaf temperatures were consistently higher than well-watered plants (Fig. 2.2A, B; Table 2.1).

TABLE 2.1: ANOVA repeated measures summary table for leaf temperature

Leaf temperature (study I)					
	Day	Aphids	Water	Aphids*Water	Error
d.f.	2	1	1	1	62
SS	17.18	2.24	73.46	1.05	94.84
F	5.62	1.47	48.02	0.69	
p	**0.006**	0.230	**<0.001**	0.411	

Leaf temperature (study II)					
	Day	Aphids	Water	Aphids*Water	Error
d.f.	3	1	1	1	113
SS	147.99	2.83	17.26	0.33	80.81
F	68.98	3.96	24.13	0.497	
p	**<0.001**	0.049	**<0.001**	0.497	

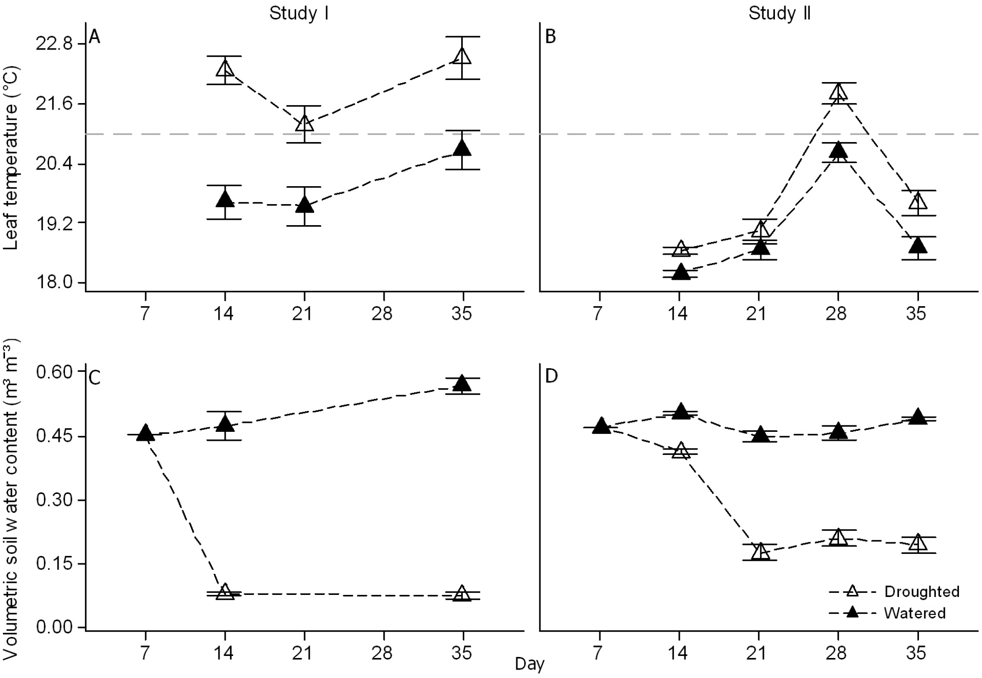

Figure 2.2: Mean (± sem) leaf temperature and vwc during both studies. (A, B) Mean leaf temperatures are shown with the reference line at 21 °C marking mean ambient room temperature. There is a significant increase in the mean leaf temperature of droughted plants that increases with time (anova, see Table 2.1). (C, D) Vwc is shown, with a significant reduction in the drought treatment. There is no significant difference between the studies in the moisture levels for each treatment.

2.4.2 Correlation of aphid numbers

The number of aphids on each plant was strongly and negatively related to the leaf temperature ($r^2 = 0.644$, $p = 0.003$) and less strongly, but positively related to the soil moisture content ($r^2 = 0.374$, $p = 0.047$; Fig. 2.3A, B). Water stress significantly ($p < 0.001$) raised leaf temperature (Table 2.1). In combination with the regression data, the data showed that increasing leaf temperature associated with water stress did not result in increased aphid population size.

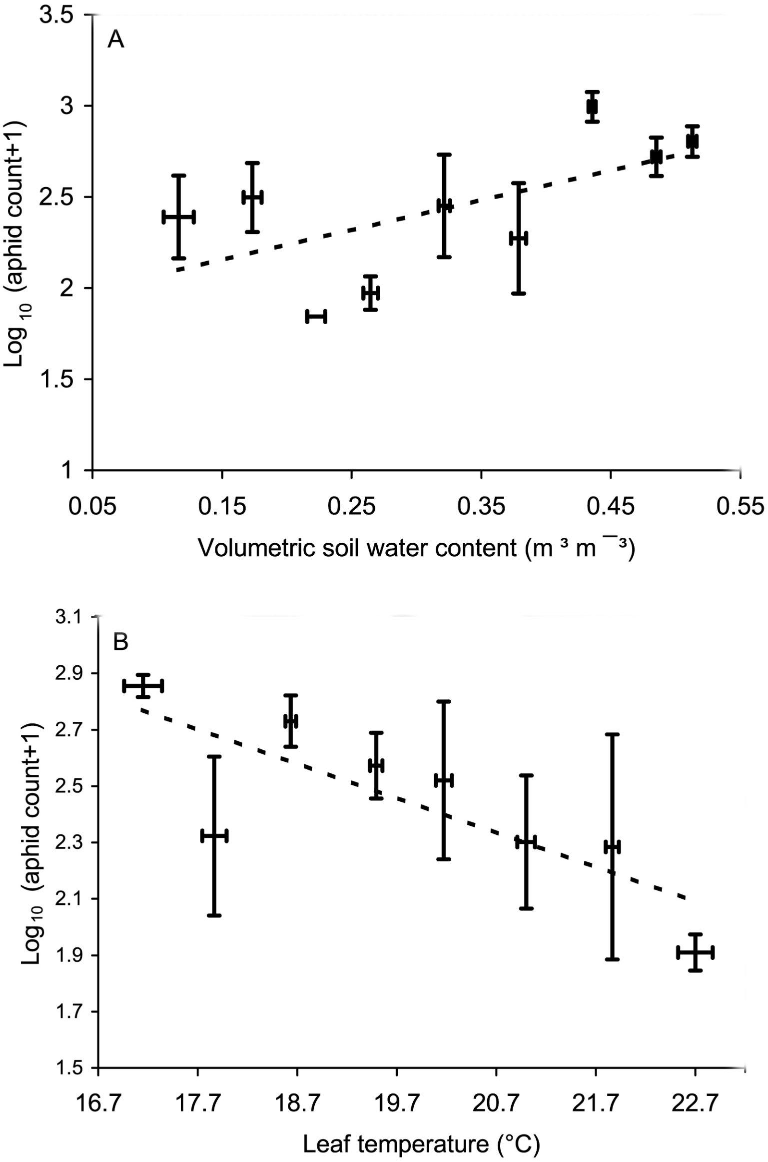

Figure 2.3: This figure has been simplified by grouping the data from study II. (A) The regression equation for aphid numbers against soil water content was $\log_{10}$(aphid count+1) =1.9 + 1.64 (volumetric soil water content), r^2 = 0.374, $p = 0.047$. There was a strong positive correlation showing more aphids per plant as volumetric soil water increased. (B) The regression equation for aphid numbers against leaf temperature was $\log_{10}$(aphid count+1) = 4.9 − 0.12 (leaf temperature), r^2 = 0.644, $p = 0.003$. There was a strong negative correlation, showing fewer aphids per plant as leaf temperature increases.

2.4.3 Aphid population analysis

Poisson distributed *t*-test analysis of the raw count data (Figure 2.4A) showed significantly more aphids on watered plants ($z = 76.2$, $p < 0.001$). In the drought treatment, the overall mean number of aphids was 212 ± 48 aphids, but on well-watered plants, 821 ± 81 aphids were observed. The mean r_m (Figure 2.4B) was reduced by 42 % in the drought treatment ($F_{1,21}$=6.05, $p = 0.023$). The total fecundity, measured by number of nymphs per adult aphid (Figure 2.4C) was reduced by 32 % in the drought treatment ($z = 2.80$, $p < 0.001$). The fecundity measured by number of nymphs per adult per day (Figure 2.4D) was reduced by 53 % in the drought treatment ($F_{1,20}$=6.59, $p = 0.018$).

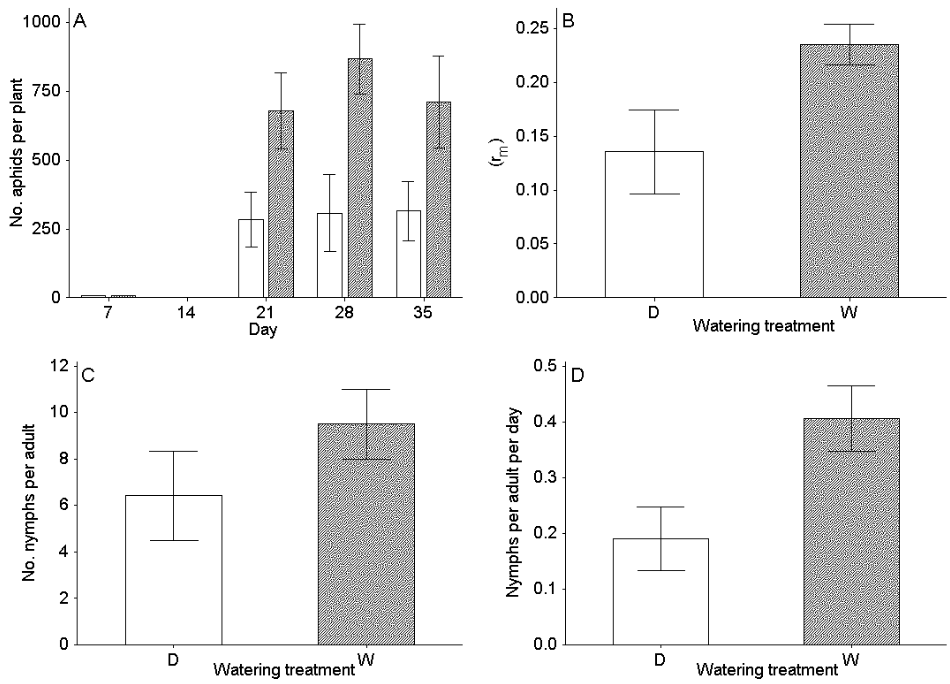

FIGURE 2.4: Mean (± SEM) aphid population data in study II. White bars represent the drought treatment, and filled bars represent the watered treatment. (A) There were significantly more aphids per plant in the well-watered treatment ($p < 0.001$). (B) The r_m rate was significantly higher in aphids on well-watered plants ($p = 0.023$). (C) Total fecundity, the number of nymphs per adult, was significantly greater for aphids on watered plants ($p < 0.001$). (D) Fecundity per adult aphid per day was significantly greater for aphids on well-watered plants ($p = 0.018$).

2.4.4 Effect of treatments on leaf gas exchange

Aphids had a strong physiological effect on the plants. A summary of the repeated-measures ANOVA performed on the A_{max}, g_s, water potential components, chlorophyll fluorescence, and SPAD values is presented in Table 2.2.

There was a significant effect of aphid infestation and water stress on A_{max}, with a weak interaction (Figure 2.5A, B). The drought treatment led to a decline in A_{max} over time, and aphid infestation in combination with drought led to the lowest rates of A_{max}. At least part of this decline in A_{max} is presumed to have been caused by the reduced g_s (Figure 2.5C, D), which was significantly decreased by the drought treatment.

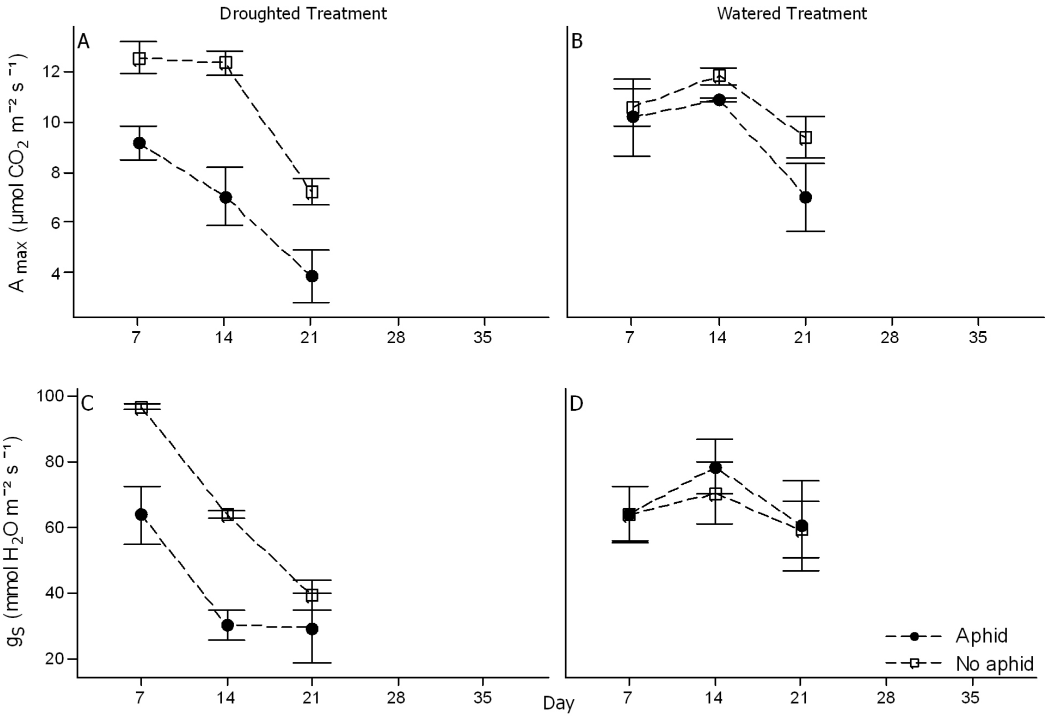

FIGURE 2.5: Mean (± SEM) A_{max} and corresponding g_s in study I.(A, B) A_{max} was reduced with aphid treatment ($p = 0.009$), drought treatment ($p < 0.001$), and over time ($p < 0.001$). (C, D) g_s was reduced with drought treatment ($p = 0.022$) and over time ($p = 0.011$).

Table 2.2: Anova repeated measures summary table for physiological plant responses

A_{max}	Day	Aphids	Water	Aphids*Water	Error
d.f.	2	1	1	1	39
SS	147.22	77.79	29.46	13.39	153.12
F	18.75	19.81	7.50	3.41	
p	**<0.001**	**0.009**	**<0.001**	0.072	
g_s	Day	Aphids	Water	Aphids*Water	Error
d.f.	2	1	1	1	36
SS	4262.9	661.2	2402.6	1071.4	15113.5
F	5.08	1.58	5.72	2.55	
p	**0.011**	0.218	**0.022**	0.119	
Ψ	Day	Aphids	Water	Aphids*Water	Error
d.f.	4	1	1	1	65
SS	0.15	0.042	0.198	0.014	0.28
F	6.86	4.21	34.73	7.12	
p	**<0.001**	**0.048**	**<0.001**	**<0.001**	
Ψ_π	Day	Aphids	Water	Aphids*Water	Error
d.f.	4	1	1	1	63
SS	0.021	0.078	0.02	0.011	0.207
F	1.62	23.6	6.00	3.32	
p	0.180	**<0.001**	**0.018**	0.078	
Ψ_ρ	Day	Aphids	Water	Aphids*Water	Error
d.f.	4	1	1	1	52
SS	0.163	0.027	0.112	0.002	0.348
F	6.25	4.09	17.21	0.30	
p	**<0.001**	**0.045**	**<0.001**	0.586	
$\Delta F/F_{m'}$	Day	Aphids	Water	Aphids*Water	Error
d.f.	3	1	1	1	77
SS	0.47	0.058	0.029	0.018	0.74
F	16.27	6.01	3.03	1.89	
p	**<0.001**	**0.016**	0.086	0.173	
SPAD	Day	Aphids	Water	Aphids*Water	Error
d.f.	2	1	1	1	43
SS	98.08	220.22	345.52	232.35	1206.04
F	1.75	7.85	12.32	8.28	
p	0.186	**<0.001**	**0.008**	**0.006**	

2.4.5 Effect of treatments on leaf water potential components

As expected, Ψ was reduced by the drought treatment (Figure 2.6A, B), but when leaves were infested with aphids, this reduction was less acute, particularly between days 20 and 35.

Ψ_π (Figure 2.6C, D) showed the expected decrease with drought treatment ($p = 0.018$), but there was a higher Ψ_π when aphids were present ($p < 0.001$). The data illustrated clear osmotic regulation in the droughted plants, which was prevented in plants that were aphid-infested.

Turgor potential (Figure 2.6E, F) was significantly higher in the watered treatment ($p < 0.001$), rising over time ($p < 0.001$). Turgor potential was reduced in aphid-infested plants ($p = 0.045$) although, over a period of several weeks, this began to rise as Ψ unexpectedly increased, suggesting that the plants acclimated to the treatment.

2.4.6 Effect of treatments on chlorophyll fluorescence and SPAD chlorophyll estimation

Fluorescence signals were a useful method of demonstrating stress on plants. The chlorophyll fluorescence yield in the light ($\Delta F/F_{m'}$) tended to decrease with both water stress and aphid infestation (Figure 2.7A, B). There was a significant aphid effect on the $\Delta F/F_{m'}$ ratio ($p = 0.016$), but no significant effect of any treatment on dark-adapted measures. The fluorescence yield of watered and aphid-infested plants appeared similar to that of droughted plants. The SPAD readings (Figure 2.7C, D) strongly suggest that aphids and water stress reduced chlorophyll concentrations ($p = 0.006$).

Aphid and water stress individually reduced the SPAD meter readings. The combination of aphid and water stress significantly ($p = 0.006$) interacted to give lower SPAD readings, implying reduced chlorophyll and potentially reduced leaf nitrogen concentration (Figure 2.7C, D).

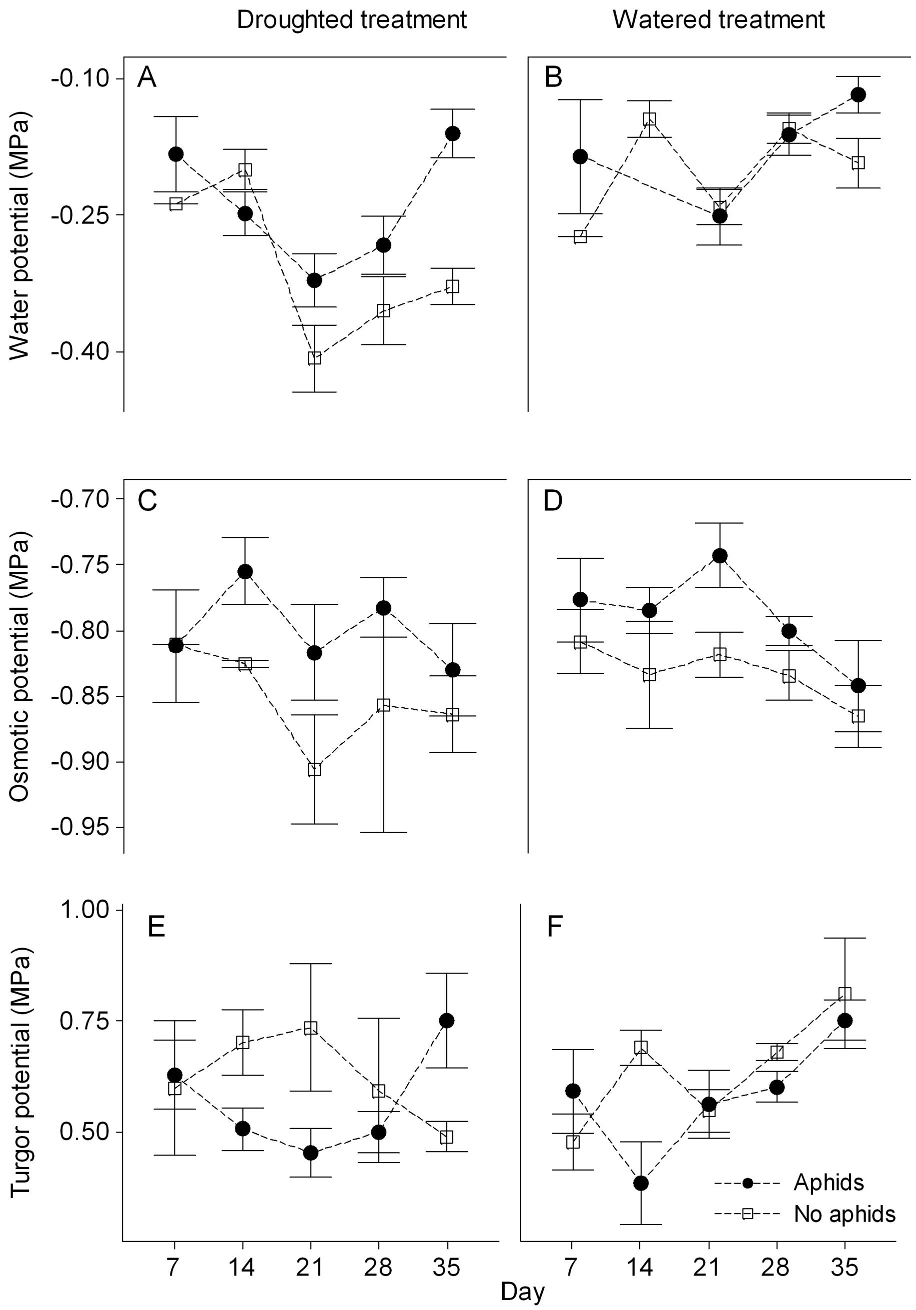

Figure 2.6: Trends in mean (± sem) Ψ and its components in study II. (A, B) Ψ was reduced by water stress ($p < 0.001$) and aphid infestation ($p = 0.048$). (C, D) Ψ_π was more negative with drought treatment ($p = 0.018$) and less negative with aphid infestation ($p < 0.001$). (E, F) Ψ_p was significantly reduced by drought treatment ($p < 0.001$).

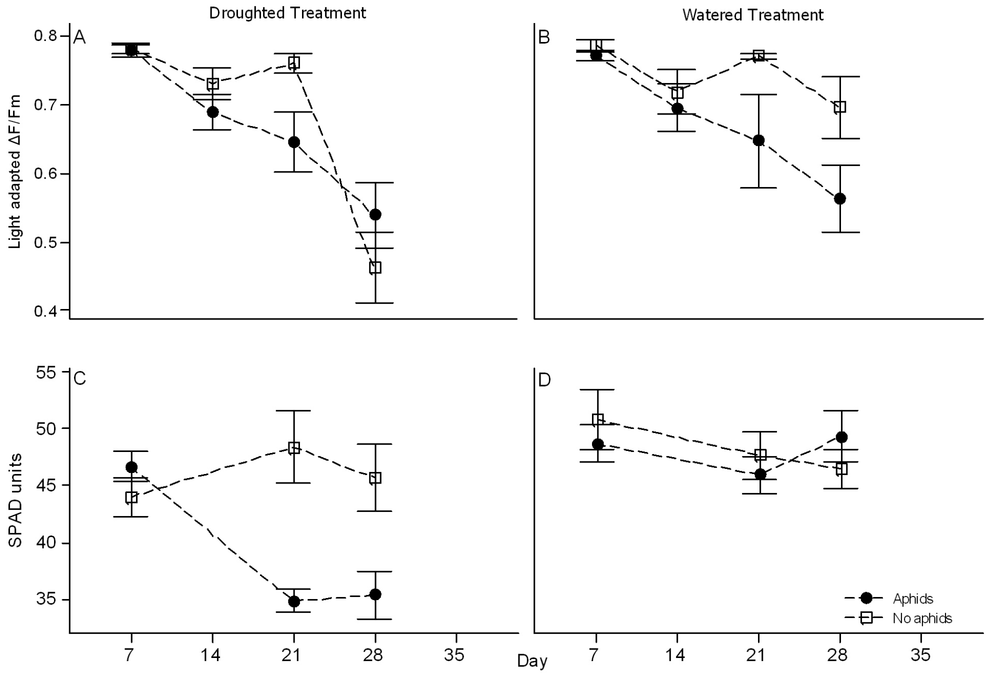

Figure 2.7: Trends in mean (± SEM) chlorophyll fluorescence and leaf chlorophyll content (SPAD readings)in study I.(A, B) The chlorophyll fluorescence data showed a significant reduction with aphid infestation ($p = 0.016$) and an overall reduction over time ($p < 0.001$). (C, D) SPAD values were significantly reduced by drought treatment ($p = 0.001$) and aphid infestation ($p = 0.008$), with an interaction effect ($p = 0.006$).

2.4.7 Effect of treatments on plant weight

Above-ground dry weight (Figure 2.8) was significantly decreased by both water stress ($F_{1,20}$=51.21, $p < 0.001$) and aphid infestation ($F_{1,20}$=40.8, $p < 0.001$). There was a significant interaction effect ($F_{1,20}$=5.38, $p = 0.031$). Water stress alone led to a decrease of 52.5 % in the mass of droughted plants compared with watered plants, but when accompanied by aphid infestation, the decrease was as much as 75 %.

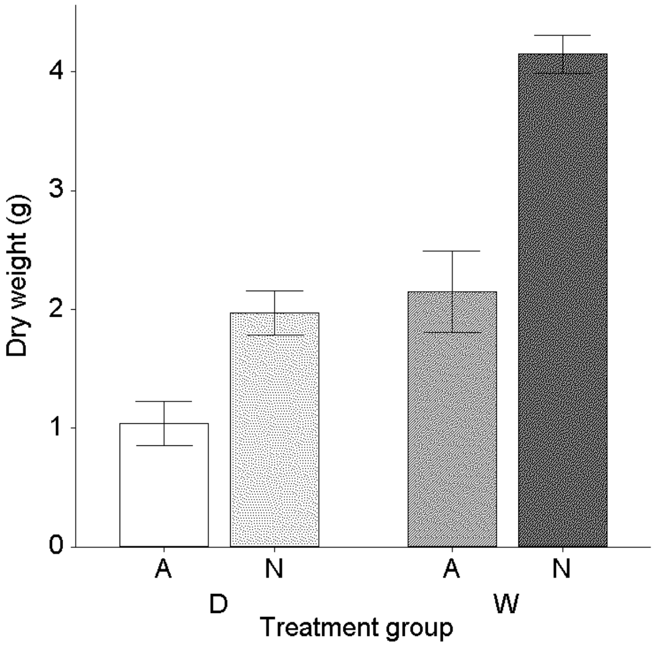

Figure 2.8: The effect of treatments on mean (± sem) final above-ground dry weight of plants in study I. Axis letters: drought 'D', watered 'W', aphid 'A', and no aphid 'N'. Above-ground dry weight values were significantly ($p < 0.001$) reduced by aphid and water stress with significant interaction ($p = 0.031$).

2.5 Discussion

The experimental method produced a satisfactory level of soil water shortage in the droughted treatment. This water shortage led to reduced Ψ, reduced gas exchange and partial closing of stomata. The closure of stomata restricted transpirational cooling and led to higher leaf temperatures. The two studies followed the same protocol of soil water shortage and the two-sample *t*-test result confirmed the overall similarity of the studies and allows us to consider them as components of one experiment.

All the measures of aphid population performance showed a significant negative impact of water stress, with lower aphid numbers, r_m and fecundity. Aphid numbers were significantly correlated with soil moisture and leaf temperature. It is perhaps surprising that higher temperatures were associated with fewer aphids, as other work has found that a rise in air temperature positively affects *M. persicae* populations (Mittler, 1967; Wyatt and Brown, 1977). However, these readings did not distinguish between air temperature and leaf temperature. Under outdoor conditions, the two can be substantially different. It may be that continuously higher leaf temperatures without a nocturnal fall in temperature may have a negative impact on *M. persicae*.

Several studies concur with the findings here, showing that water stress led to a reduced population size (Mody et al., 2009) and reduced growth and development rates (Cabrera et al., 1995; Pons and Tatchell, 1995; Hale, 2002). However, Fereres et al. (1988) found that *Sitobion avenae* (Fabricius) significantly increased in population density under a severe water stress of −2.0 to −2.4 MPa. In that study, the combination of aphids and water shortage caused losses to crop yields of over a third, and our work also shows an exacerbation of water stress by aphid infestation.

Aphid infestation resulted in a fast-acting stress that was similar in magnitude and style to water shortage. Despite receiving more than double the water ration of a droughted plant, watered plants infested with aphids showed significant reductions in A_{max} and final weight with significantly higher Ψ_π. This effect of aphid infestation on watered plants is similar to the effects shown here in droughted plants. Other research has found similar trends (Riedell, 1989; Cabrera et al., 1994; Burd and Elliot, 1996; Macedo et al., 2003), but the Ψ_π component is new information.

The maintenance of a higher than expected Ψ_π in droughted and aphid-infested plants in this study could be due to limitation of the typical drought response. Osmotic potential is typically lowered by the accumulation of proline and other amino acids (Riedell, 1989). Similarly, Cabrera et al. (1994) found a rise in proline accumulation in droughted and aphid-infested plants, but this was lower than the rise in solely droughted plants. They concluded that aphids induced a water-stress-like response in plants, which could have made an impact on local Ψ_ρ. With the results from our study, it is reasonable to say that although some aphids cause drought-like symptoms in their host, their presence acts to suppress osmoregulation in the vacuole leading to reduced Ψ_ρ over days 7–21 of the experimental period. This is surprising, given the assumption that aphids require relatively high Ψ_ρ to maintain effective feeding. However, rising Ψ after day 21 counteracted the lack of osmoregulation, and Ψ_ρ began to rise. This suggests that aphids affect the regulation of water potential components, possibly associated with levels of drought-induced hormones (e.g., abscisic acid) or amino acids (e.g., proline). Divol et al. (2005) also found that *M. persicae* infestation on celery (*Apium graveolens*, L.)) led to gene upregulation. These genes were associated with cell wall modification and phloem dilution, suggesting that aphids induced some control on Ψ_ρ.

What other dietary changes occur when aphids attack a host? Plant responses to aphid attack are more similar to reactions under pathogen attack rather than wounding responses (Walling, 2000). Aphid infestation induces genes usually expressed under oxidative stress, as well as calcium dependent signalling and pathogen responses (Moran et al., 2002). Genes encoding production of the classic defence compounds salicylic and jasmonic acid are also upregulated under aphid attack (Walling, 2000; Moran and Thompson, 2001; Moran et al., 2002; Ferry et al., 2004; Smith and Boyko, 2007).

Reduction in above-ground dry weight with aphid infestation, found here, was similar to the reduction in above-ground dry weight caused by drought alone. There are further similarities between water and aphid stress as types of stress effect, as found by Warrington and Whittaker (1990) on Sitka spruce (*Picea sitchensis* (Bong.) Carr.) infested with green spruce aphids (*Elatobium abietinum* (Walker)). Aphid-infested plants show increased production of abscisic acid, a compound that causes stomatal closure, which is typical in drought stressed plants (Smith and Boyko, 2007). In addition, Riedell (1989) concluded that the aphids had induced drought-like symptoms in well-watered aphid-infested barley, causing reduced g_s and relative water content, lower Ψ and lower chlorophyll levels. Cabrera et al. (1995) similarly found lower sucrose, soluble sugar, total chlorophyll levels and A_{max} in well-watered aphid-infested barley seedlings.

The significant reduction in A_{max} and lower fluorescence yield on aphid-infested plants implies damage to the efficiency of Photosystem II electron transport. These results are similar to those found with RWA by Burd and Elliot (1996) and Macedo et al. (2003). It was expected that measurements of dark-adapted F_v/F_m would have shown clear reductions under aphid stress as found in Blanco et al. (1992), where the method is suggested as a rapid identifier of aphid stress. No significant effect of drought treatment on dark-adapted F_v/F_m was found in this research. The effects of drought treatment were restricted to light-adapted $\Delta F/F_{m'}$, possibly due to the low light levels in the controlled environment room compared with outdoors.

Most of the responses reported here only became apparent after prolonged exposure to the stress treatments. This indicates that short-term experiments could easily miss some of the stress responses (Koricheva and Larsson, 1998). Many studies cover only periods of infestation of about 7 days (e.g. Riedell, 1989; Cabrera et al., 1995; Divol et al., 2005) and could miss some of the effects seen in this study.

In this study, the pulse stress hypotheses (Huberty and Denno, 2004; White, 2009) do not hold for *M. persicae*. Aphid adaptations to phloem can buffer some variability in diet composition and quality (Douglas, 2006). However, long-term drought, even with 'pulses' of watering does not lead to dietary changes that can provide a direct benefit to the aphid population. Leaf turgor needs to be maintained, so that aphids can insert their stylets and successfully access the contents of the phloem. Some aphid species have been found to multiply on stressed hosts (Wearing, 1972; Fereres et al., 1988; Cabrera et al., 1995; Xing et al., 2003; Khan et al., 2010), but the level of experimentation required to find the exact patterns and combinations that would cause increased aphid performance on stressed plants would be impracticable. It seems more reasonable to conclude that aphids perform better on a secure diet provided by a well-watered host. Although the host is negatively affected with reduced physiological efficiency and growth, the supply of food will last longer and be more predictable than on a stressed plant.

In summary, the hypothesis that the *M. persicae* population would increase faster and to larger numbers on pulse-water-stressed host plants was rejected. The aphids did not reproduce at a faster rate or to greater numbers on droughted plants. The increased temperature of droughted leaves did not lead to increased growth and fecundity in the aphids. However, aphid stress did reduce the yield and performance of host plants in a manner similar to drought stress. Under future drought scenarios, *M. persicae* is more likely to become a significant threat to well-irrigated crops rather than crops under water stress.

2.6 Acknowledgements

The research was funded by the Natural Environment Research Council through a UK research studentship (NE/F008791/1). Our thanks are also due to the Scottish Crop Research Institute, Dundee, UK for kindly supplying the aphids for this research.

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Synopsis I

In the previous chapter, it was found that *M. persicae* was more successful on healthy host plants and that aphid infestation is associated with an increase in osmotic potential. Additionally, aphids caused a drought-like response in well-watered host plants and exacerbated the impact of water shortage on stressed hosts. In the next chapter, an experiment with identical methods tests whether there is a different response to water stress in *M. dirhodum* on barley.

3

The case of rose–grain aphid, *Metopolophium dirhodum* (Walker) and winter barley, *Hordeum vulgare* L.

K.L.S. Simpson, G.E. Jackson & J. Grace

Under submission to *Entomologia Experimentalis et Applicata*

3.1 Abstract

Water stressed winter barley *Hordeum vulgare* (L.) plants were infested with the rose–grain aphid, *Metopolophium dirhodum* (Walker) in two fully-factorial experiments. Under controlled environment conditions the combination of aphids and water stress led to a significant reduction of maximum photosynthetic rate (A_{max}), dark-adapted fluorescence signals (F_v/F_m), leaf chlorophyll content and plant growth.

The aphid population on droughted plants increased faster and to greater extent than on well watered plants. This was followed by a slowing of the increase rate on droughted plants as the aphid infestation damaged the host plant. The mechanism of damage to the host plant was investigated by analysis of changes to the gas exchange efficiency. Stomatal conductance (g_s) showed the expected decrease in response to limited water supply, and was lower when aphids were present. A_{max} however, was reduced by the effect of aphids alone, not drought. This is likely to be a result of aphid damage to the efficiency of Photosystem II (PSII) electron transport, since F_v/F_m

was significantly reduced by aphids, not by the watering treatment. A reduction in leaf chlorophyll content was also associated with aphid infestation rather than drought treatment. Aphid infestation led to a significant decrease in the size of plants, particularly causing a reduction in the root and shoot dry weight in watered plants. This reduction produced watered plants with the same root and shoot weight as droughted plants.

In conclusion, the rose–grain aphid population increased faster on droughted plants, but the size of the population was limited by two processes. Firstly, damage to PSII leads to stomatal closure and wilting that may slow population increase. Secondly, the infestation causes damage to the host plant productivity resulting in smaller plants that cannot support a larger population.

3.2 Introduction

The Plant Stress Hypothesis (PSH) was proposed by White (1969), who suggested that stressed plants begin to senesce earlier, releasing additional soluble nitrogen into the phloem that improves insect survival rates. It has been much debated since. Some aphid species have been found to multiply more quickly on stressed hosts compared with controls (Wearing, 1972; Fereres et al., 1988; Xing et al., 2003; Khan et al., 2010). Other studies have found reduced population size, growth and development rates in other aphid species on drought stressed cereals (Cabrera et al., 1995; Pons and Tatchell, 1995; Hale et al., 2003).

It is further hypothesised that long-term or severe stress may be too detrimental to both host and insect success (Huberty and Denno, 2004). In addition, White (2009) proposed that the insect's feeding preference for senescing or flushing plant material may have a significant bearing on their response to plant stress. These authors suggest that intermittent stress will lead to greater insect fecundity and survival due to a balance of stress-induced increased soluble nitrogen availability and turgor maintenance through osmotic regulation and periodic watering (Huberty and Denno, 2004).

Either way, the risks of both prolonged and intermittent water shortage are increasing. Climate projections for the next 50–70 years indicate moderate to severe water stress for Southern Britain, with increasing water shortages worldwide (Alcamo et al., 2007a,b). This is likely to impact negatively on the ability of industry to maintain sufficient levels of irrigation to support adequate agricultural production.

In general, cereal crops under aphid attack can suffer from the aphids directly and from aphid-introduced viruses. *Rhopalosiphum padi* (L.) has been found to decrease the dry weight, yield, leaf area, number of tillers and number of leaves of barley. Infestation is associated with related decreases in carbon fixation and biomass. Plants compensated for this drop in assimilation rate by allocating dry weight to production of leaf laminae, but overall growth decreased due to assimilate loss (Mallott and Davy, 1978). *Schizaphis graminum* (Rondani) infestation was shown to impact photosynthetic rate and leaf chlorophyll content in barley (*Hordeum vulgare*, L.) negatively. The aphid decreased CO_2 assimilation by decreasing photosynthetic capacity in the mesophyll rather than by decreasing leaf conductance to water vapour (Cabrera et al., 1994). Leaf water potential, chlorophyll content, CO_2 assimilation rate and total soluble carbohydrate decreased in barley infested with *S. graminum.* Concentration of the amino acid proline increased up to twenty-fold, and sugar concentration decreased five-fold with respect to control plants. Changes caused were similar to those of drought stress, even in the presence of sufficient soil moisture (Cabrera et al., 1995). Russian wheat aphid (RWA, *Diuraphis noxia* (Mordvilko)) also induce drought stress symptoms in leaves of infested plants, and prevented barley recovery after stress was removed (Riedell, 1989). Barley yellow dwarf virus is spread by aphids, and a greater proportion of plants are infected under drought stress and increased temperatures (Smyrnioudis et al., 2000; Harrington, 2002).

Robredo et al. (2007) found that barley plants were less susceptible to drought under increased atmospheric CO_2 concentrations. However, the defence mechanism they propose is based on reduced stomatal conductance, which maintains elevated leaf water and osmotic potential. These characteristics may coincide with the environmental conditions aphids prefer and/or instigate, as seen with *Myzus persicae* (Sulzer) that acts on the host to increase osmotic potential (Simpson et al., 2012). Could aphids be an unforeseen threat to barley arising from its own mechanism to adapt to climate change?

This has not currently been determined, although Newman (2005) predicted dramatic declines in cereal aphid abundance and pest status in response to all climate scenarios as a result of increases in temperature and decreases in rainfall: a severe, prolonged drought pattern. The model predicts population reductions for all cereal aphids based on a generalisation from *Rhopalosiphum padi* (L.). The aphid we study here, *Metopolophium dirhodum* (Walker), has been found to reproduce maximally at 20 °C, which is 5 °C below *R. padi* (Dean, 1974).

Newman's model (2005) assumes that the variability in response to climate scenarios amongst species of cereal aphid is a result of the density-dependent response in alate reproduction and the species' own nitrogen requirements. Previous work with *M. dirhodum* and *R. padi* has found that the two aphids' success is largely determined by plant vigour (Honěk, 1991, 1994; Honěk and Martinkova, 2002; Hale et al., 2003). In particular, aphid numbers and impact on the host plant tend to decrease with reduced chlorophyll content (Honěk and Martinkova, 2002), increased root:shoot ratio and decreased plant size (Honěk, 1991). In this work, the original PSH (White, 1969) is tested, but if *M. dirhodum* is a flush feeder as the work referenced above suggests, it may be adversely affected by plant stress (White, 2009).

Some previous work has identified similarities between water-stress effects and insect-stress effects on plants (Riedell, 1989; Cabrera et al., 1995; Simpson et al., 2012). We explore this similarity using several plant physiological factors, examining whether aphid stress is likely to exacerbate and/or mimic the impacts of drought.

This work tests whether the *M. dirhodum* population will increase more quickly and to larger numbers on droughted host plants. This is hypothesised to be a result of increasing phloem sap quality in senescing tissues and faster population development and fecundity in the aphids as a result of increasing temperatures in droughted leaves. The impact of aphid infestation on the growth and performance of host plants will be tested to determine if the aphid stress in the host plant compounds the water-stress damage.

3.3 Materials & Methods

3.3.1 Experimental design

Hordeum vulgare cultivar 'Sequel' (six-row feed variety; Syngenta, Guildford, UK), was grown in a commercial walk-in controlled environment growth room. Air temperature was 21 °C ± 0.2. On each shelf, photosynthetically active radiation (PAR) levels of

100 µmol photons m^{-2} s^{-1} was provided by two 36 W T8 Triphosphor 830, 3000 K Warm White fluorescent tubes (Philips Electronics, Guildford, UK) timed to give a 16-h photoperiod. The experiment occupied two adjacent shelves and plants were rotated to different shelves each week. The average relative humidity was 50 %, giving an approximate vapour pressure deficit (VPD) of 1.24 kPa.

Three experiments were designed to prevent over-sampling of the young plants and interference of the sampling processes. The primary (study I) focused on the impact of aphids on plant physiology with aphid population counts; a parallel study was run afterwards to monitor leaf water potential components and intrinsic rate of aphid population increase. The remaining experiment (study II) focused on the effect of drought on aphid numbers. Study I used 24 plants, and ran from November to December 2009; the parallel ran from January to February 2010; study II used 20 plants and ran from November to December 2010. The details of these studies and the data collected are presented in Figure 3.1. Throughout the method, the total sample size per day (n) was divided as evenly as possible amongst the four treatment groups. In addition, to ensure that plants were all at the same optimum point in their diurnal cycle, a time-window of 10:00 to 13:00 hours was selected. It was not always possible to sample all plants and all response variables within that time window. This gives a broader variation in sample numbers than the ideal.

Plants were grown in 2 L pots containing 80 % Scotts 'Shamrock' medium general purpose sphagnum peat (Everris, Ipswich, UK) and 20 % horticultural sand; 0.3 % ground limestone and 0.12 % controlled release fertiliser were included. The fertiliser was Scotts 'Osmocote 3–4 months' (Everris), with NPK 14:14:14 that comprises 14 % N as 7.2 % nitrate nitrogen and 6.8 % ammonical nitrogen; 14 % phosphorus pentoxide equivalent to 6.1 % P; 14 % potassium oxide equivalent to 11.6 % K.

At 5 weeks, plants were at Zadoks stage 14, 22 (4th leaf more than half visible, main shoot and two tillers, (Zadoks et al., 1974)). Plants were randomly assigned to one of four treatments in a fully factorial design: watered twice per week (W), watered once per week (D), aphid infested (A) and no aphids (N). This description of treatments introduces the abbreviations used throughout. In this experiment, small quantities of water, applied weekly, emulate a rationing of water supply or occasional rainfall. Each plant pot had a dish reservoir at the base to take the water dosage. 'D' plants received 125 mL on watering days, 'W' plants were watered *ad libitum* and could receive up to 500 mL on watering days. Any water that initially passed through the compost was re-absorbed within 2 hours. It is therefore assumed that minimal nutrients were lost by leaching. By the end of each study, all plants were still immature, with no stem elongation. The *M. dirhodum* strain was provided by the James Hutton Institute (Dundee, UK).

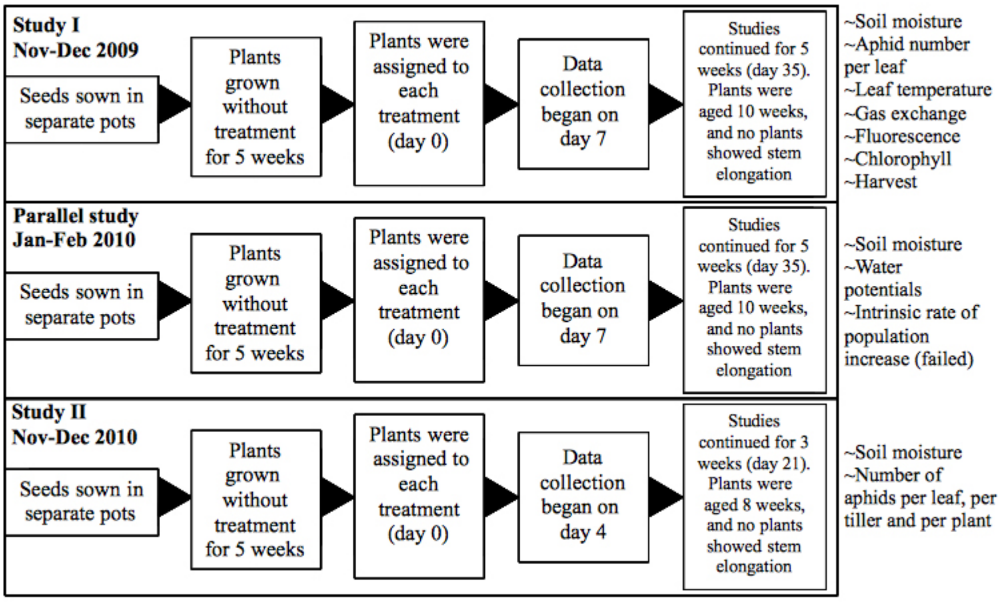

FIGURE 3.1: Timeline of all studies with readings taken in each case.

3.3.2 All studies

Water Deficiency Monitoring: A Delta-T Theta Probe ML2 (Delta-T Devices, Cambridge, UK) connected to a Delta-T HH2 Moisture Meter measured soil moisture as volumetric water content (vwc) in $m^3\ m^{-3}$. Measurements were made on 24 plant pots on days 1, 14, 21, 28 & 35 of study I and the parallel study. For study II, measurements were made on days 4, 8, 10, 15 & 18 with n = 20.

Aphid Inoculation: In all studies, five adult aphids were placed on each plant at the start of the watering treatments (day 0), and maintained at five aphids until day 7 when measurements began. This ensured the starting population size was the same across watering treatments.

3.3.3 Study I: Aphid counts and plant physiology

Aphid Population: Aphids were counted on the leaves selected for gas exchange and fluorescence measurements. Aphids were counted per leaf and by upper and lower surface in study I (days 14, 28 with n = 24). A 6 cm^2 window held over the centre of the leaf was used to guide the counting area in a position matching that of the gas exchange and fluorescence measurements.

Leaf Temperature: Individual leaf temperature was measured with a non-contact Infra-Red Thermometer (Fluke, Norfolk, UK), positioned 20 mm from the upper surface of the youngest fully expanded leaf. In study I, n = 20–24 and readings were made on days 14 and 21.

Gas Exchange Measurements: The maximum photosynthetic rate (A_{max}) and stomatal conductance (g_s) were recorded using a Li-6400 Photosynthesis System (Li-cor Biosciences, Lincoln, NE, USA). All measurements were made between 10:00 and 13:00 hours. An initial light response curve was determined on day 0 to define a suitable chamber PAR level, allowing comparison of A_{max} between stressed and unstressed plants. The chosen light level was 1200 µmol photons m^{-2} s^{-1}, this being the point where CO_2 uptake levelled with respect to PAR on the light response curve. Chamber temperature was controlled at 21 °C, CO_2 was controlled at 370 µmol CO_2 mol^{-1} and flow rate to the sample cell was set to 500 µmol air s^{-1} (as Li-cor Biosciences, 2001). Leaf width was measured and entered into the Li-6400 settings for accurate calculation of gas exchange. The leaf widths were also used as a measure of treatment effects.

The youngest fully expanded leaf of the 5-week-old plant was tagged on day 1 and used for all gas exchange measurements in study I on days 7, 14, and 21 with n = 9–23.

Fluorescence Measurements: The chlorophyll fluorescence yield parameters $\Delta F/F_{m'}$ and F_v/F_m were measured using a Walz Mini-Pam system (Heinz-Walz, Effeltrich, Germany). These parameters were defined by Genty et al. (1989) to investigate the yield of electron transport in PSII, which in turn affects the efficiency of CO_2 uptake. Specifically, $\Delta F/F_{m'}$ represents the proportion of the light absorbed by chlorophyll and used in PSII, it is directly related to the efficiency of carbon fixation; F_v/F_m is a measure of the intrinsic efficiency of PSII, and measurements using dark-adapted leaves offer an estimate with all PSII centres open (Genty et al., 1989; Maxwell and Johnson, 2000).

All measurements were made between 10:00 and 13:00 hours using the same leaves as those sampled for gas exchange on days 7, 14, and 21 with n = 11–20.

Measurements were conducted on both light- and dark-adapted leaves. 'In the light' measures give the Yield-parameter (based on the Genty parameter $\Delta F/F_{m'}$). 'Dark-adapted' measures give F_v/F_m (after at least 30 minutes dark adapt time). The system was operated on 'Burst-mode' for measurements, and all other settings were default. The Mini-PAM leaf measurement head 2030-B was used for light-adapted measures, and the proprietary Walz DLC-8 dark leaf-clips were used to hold the fibre optic unit for dark-adapted measures. Background elements of the fluorescence signal were eliminated with the 'Auto-Zero' function before readings were taken.

Leaf Chlorophyll Estimation: Several studies show that leaf chlorophyll content is strongly correlated with leaf nitrogen content (e.g. van den Berg and Perkins, 2004). A non-destructive estimation of chlorophyll content in the same leaves sampled for gas exchange and fluorescence was made using a Minolta 'soil plant analysis development' (SPAD) meter (Konica Minolta Sensing, Basildon, UK). Each leaf was sampled three times to generate a mean value in study I on day 21 with n = 21.

Total dry weight: Root and shoot plant biomass was harvested and separated at the end of study I to determine any impact of drought and aphids on plant growth and yield. Plant material was oven dried to constant weight at 80 °C, n = 17 (seven plants had died during week 4).

3.3.4 Study II: Aphid counts by leaf, tiller and plant

In study II, counts were made by observations per leaf, tiller and whole plant (days 4, 8, 10, 15 and 18 with n = 20).

3.3.5 Parallel study: Leaf water potentials and aphid study

Water Potential Components: A parallel study, identical to study I was carried out. The aphid population in this experiment failed, so the results cannot be fully integrated. However, the water potential components determined in the two watering treatments allow us to estimate the impact of the drought process. The components of plant water potential: leaf water potential (Ψ), leaf turgor potential (Ψ_ρ) and leaf osmotic potential (Ψ_π) are related as in Equation 3.1

$$\text{Total } \Psi = \Psi_\rho + \Psi_\pi \tag{3.1}$$

Ψ gives an immediate reading for water stress; Ψ_π gives the concentration of vacuolar and phloem constituents. Ψ_ρ is expected to have a strong relationship with aphid performance. Ψ was measured using a Scholander-type chamber (Plant Moisture System SKPM 1400, Skye Instruments Ltd., Powys, UK) between the hours of 10:00 and 13:00. Fully expanded leaves were excised and immediately transferred to the chamber. Readings were taken on days 14, 21, 28, and 35 of the parallel study with n = 16.

After the leaf was removed from the pressure chamber, it was sealed in a 10 mL plastic syringe and immediately snap-frozen in liquid nitrogen. Leaves were stored in liquid nitrogen and defrosted for 30 minutes prior to measurement. At least 10 µL of cellular sap was expressed by depressing the syringe plunger. The expressed sap was collected in a sterile Petri dish and immediately sampled with a 10 µL pipette, transferred to a filter paper disc and analysed using a Wescor Vapro5520 Vapour Pressure Osmometer (Wescor, Logan, UT, USA). The osmometer output in mmol kg^{-1} was converted to Ψ_π in MPa using the van't Hoff, Equation 3.2

$$\Psi_\pi \text{ (MPa)} = -R \cdot T \cdot (\text{OSM}/1000) \tag{3.2}$$

where R = universal gas constant, 0.00831 MPa kg mol^{-1} K^{-1}, T = room temperature, 293 K and OSM = osmometer output. The error component from dilution of symplasmic water by apoplasmic water is acknowledged from Callister et al. (2006), but was judged to be small and not corrected for. Ψ_π readings are therefore a bulk estimation of leaf osmotic potential, relevant for water stress studies, and field sample collection (Bannister, 1986; Pullman and Johnson, 2009). Ψ_ρ was derived from the relationship between the two measured potentials.

The intrinsic rate of population increase (r_m) was studied using clip cages constructed as in Kennedy et al. (1958) and the method of Wyatt and White (1977) was used to calculate r_m. During the parallel study, the r_m experiment failed to yield any results suitable for analysis. Of the 20 plants studied, only 5 aphids survived to the end of the required period. One of the caged aphids was on a droughted plant, yielding an r_m value of 0.12, the remaining four caged aphids on the watered treatment yielded r_m of 0.13 ± 0.05. Leaves from the parallel study were sampled for estimation of soluble nitrogen, but with the failure of the aphid population, it was not valid to proceed.

3.3.6 Data analysis

All analyses and figures were generated using Minitab 15 Statistical Software (Minitab, Coventry, UK). **Study I**: All data were normally distributed and showed equal variance, and therefore, two-way repeated-measures ANOVA were performed on the VWC, aphid count, leaf temperature, gas exchange and fluorescence data using the General Linear Model function of the programme. The factors were 'Day' (random factor, within groups), 'Aphids' (fixed factor, between groups), 'Watering' (fixed factor, between groups), and 'Aphids*watering' (interaction) (Minitab, 2007). Two-way ANOVA were used for SPAD and all growth variables with factors as above without the 'Day' element. **Parallel study**: The water potentials data were tested with repeated measures ANOVA, as above, but the failure of the aphid population prevented full interpretation of the data collected. **Study II**: The aphid count data were square root transformed because they showed Poisson distribution, they were then normally distributed with equal variance and analysed by two-way repeated-measures ANOVA. The effect of aphids on the number of leaves and tillers was tested with ANCOVA, the data did not violate homogeneity of regression, so analysis could proceed. All means are given with standard error of the mean (SEM).

3.4 Results

3.4.1 Drought treatment

Figure 3.2 shows that the VWC values for plants that were infrequently watered were significantly drier in all studies. A three-way ANOVA comparing study I and its parallel confirmed that there was no significant difference between the two: $F_{1,284}=0.02$, $p = 0.900$ whilst there was a significant difference by treatment: $F_{1,284}=227.50$, $p < 0.001$. The soil moisture values for study II were not comparable to the others, but still showed a significant soil drying ($F_{1,92}$=130.30, $p < 0.001$). These higher soil moistures values in study II may have been a result of the shorter droughting period, but the scale of difference in the soil water content between the treatments was still 50 % less in the drought treatment. Since the drought treatment was similar, the measures of Ψ and its components from the parallel study are assumed to give an indication of the likely water potentials in study I, and mean values are presented in Table 3.1.

There was a mildly significant drought effect on Ψ with $F_{1,43}$=6.56, $p = 0.014$. There was no significant effect of drought on Ψ_π. Ψ_ρ was slightly higher in the watered treatment ($F_{1,41}$=3.92, $p = 0.054$). Although the drought stress measured by water potentials appears to be mild, the droughted plants in study I were visibly less healthy than the watered plants with wilted, yellowed leaves and premature senescence.

Table 3.1: Overall mean (±SEM) summary table for Ψ (MPa) and its components in the parallel study

	Droughted	Watered
Ψ	−0.273 ± 0.012	−0.239 ± 0.014
Ψ_π	−0.888 ± 0.021	−0.931 ± 0.015
Ψ_ρ	0.603 ± 0.026	0.690 ± 0.018

3.4.2 Aphid population in study I

Table 3.2 provides a summary of the repeated measures ANOVA results for aphids in study I. The aphid population counts for study I are presented in Figure 3.3A. In the absence of the r_m readings from the parallel study, an approximate linear estimation of the rate of population increase between day 7 and day 14 gives significantly higher ($F_{1,10}$=23.28, $p = 0.001$) population increase rates on the 'D' treatment than the 'W' treatment. Over the next two weeks, however, the 'D' and 'W' treatments had the same estimated rate ($F_{1,10}$=3.72, p = ns). Figure 3.3B and Table 3.4 show that the aphid population was significantly greater on droughted plants, but the scale of the difference between treatments decreased over time. There were significantly more aphids on the lower surface of the leaf, but the proportion on the upper surface increased as time passed. Surface preference was unaffected by watering treatment, so the move to the upper surface may have been a result of crowding on the lower surface.

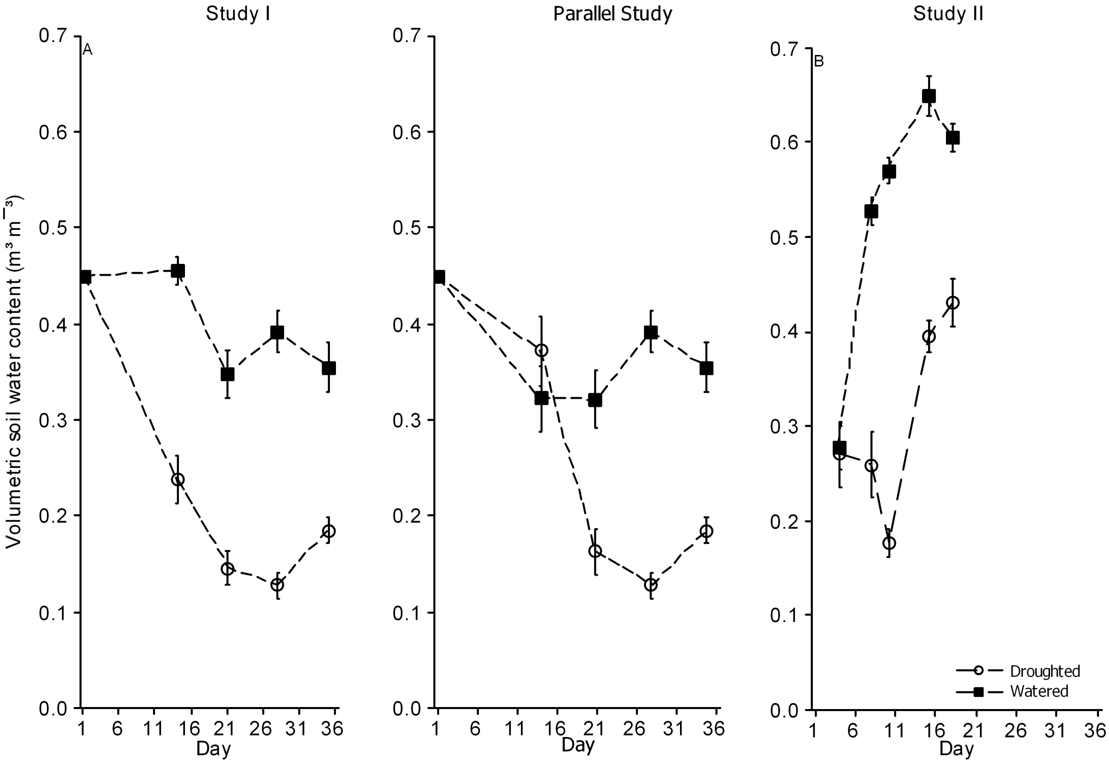

Figure 3.2: Mean (± sem) volumetric water content for all three studies is shown, with a significant reduction in the drought treatment for all cases.

Table 3.2: Anova summary table for aphid counts in study I

Aphid count	Day	Surface	Water	Surface*Water	Error
d.f.	1	1	1	1	35
SS	1.194	0.830	0.406	0.064	1.208
F	34.58	11.76	24.04	1.85	
p	**<0.001**	**0.001**	**0.002**	0.182	

3.4.3 Aphid population in study II

In Figure 3.3 it is clear that there are more aphids per leaf on the droughted treatment at day 14 of study I. In study II, the separation of aphids per plant, tiller and leaf (Figure 3.4) found that there was an equal number of total aphids on both droughted and watered plants, but the distribution was altered due to a reduced number of tillers and leaves in droughted plants.

Ancova analysis of the number of tillers and leaves is presented in Table 3.3, where the aphid infestation had no effect on the number of leaves or tillers, but the drought treatment reduced them both. Only the number of leaves per tiller showed a significant effect of drought treatment ($F_{1,66}$=7.37, p = 0.008). There is a slowing in the rate of increase after day 8 and a levelling of the drought treatment after day 15. The tenfold difference in aphids per leaf between studies I and II may have been associated with younger plants or the higher vwc in study II for both treatments.

Table 3.3: Ancova summary table for tillers and leaves in study II

Tiller number	√(aphids)	Day	Water	Error
d.f.	1	4	1	83
SS	0.01	42.01	36.39	172.48
F	0.00	5.05	17.51	
p	0.980	**0.001**	**<0.001**	
Leaf number	√(aphids)	Day	Water	Error
d.f.	1	4	1	83
SS	39.81	214.64	275.82	2174.67
F	1.52	2.05	19.53	
p	0.221	0.095	**0.002**	

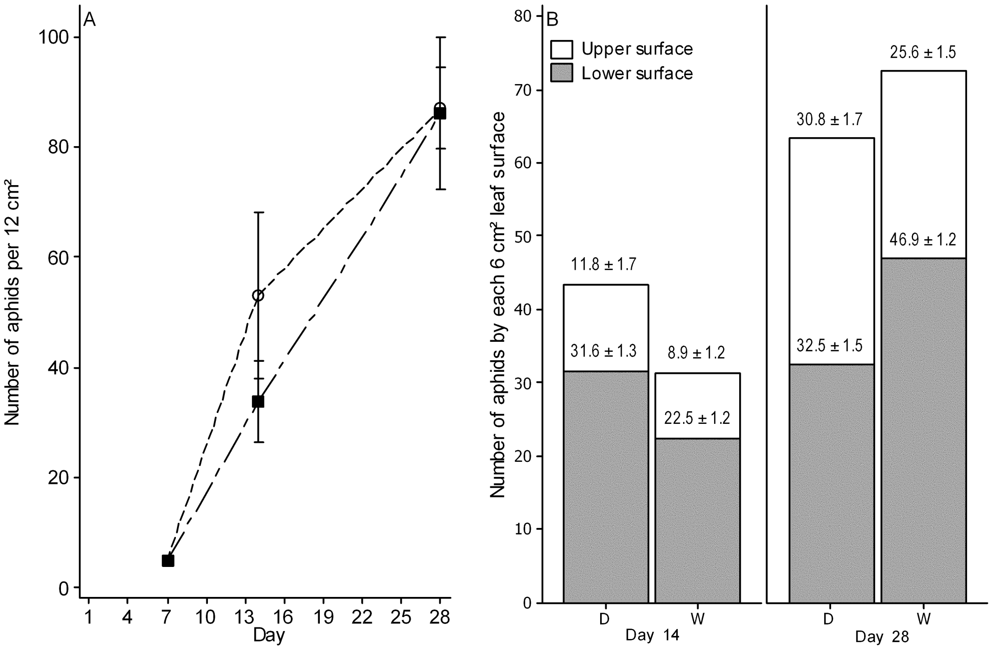

Figure 3.3: (A) Mean (± SEM) aphids per treatment in study I. Day 7 to 14: Estimated population increase rates: D = 3.8 ± 0.3 aphids leaf^{-1} day^{-1} and 'W' = 2.1 ± 0.2 aphids leaf^{-1} day^{-1}. Day 14 to 28: D = 2.4 ± 0.5 aphids leaf^{-1} day^{-1} and 'W' = 3.8 ± 0.5 aphids leaf^{-1} day^{-1}. (B) The mean (± SEM) aphid numbers from (A) divided by leaf surface and watering treatment. 'D' = drought treatment, 'W' = watered treatment.

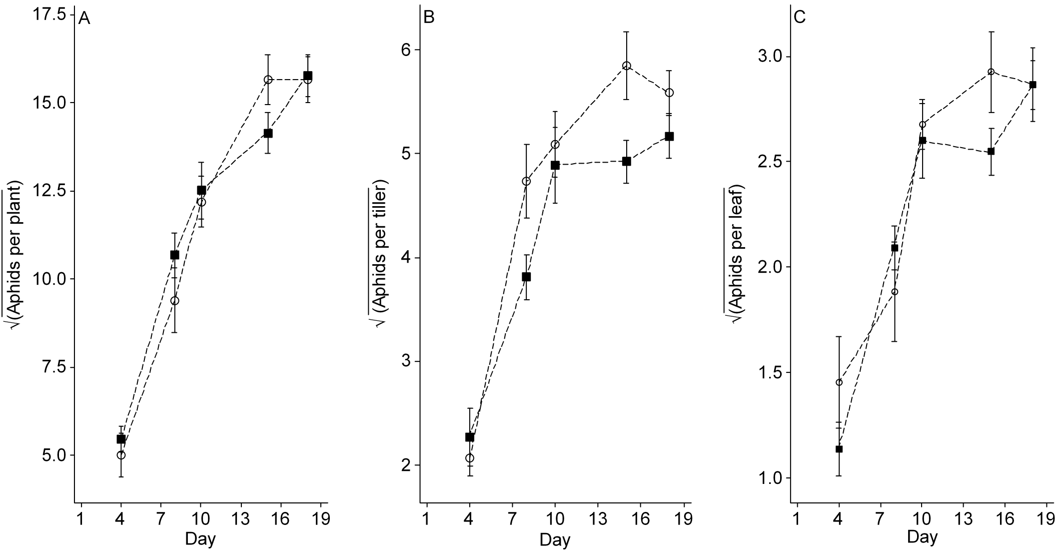

Figure 3.4: Mean (± SEM) transformed aphid numbers in study II by plant (A), tiller (B) and leaf (C)

Table 3.4: Anova summary table for physiological plant responses measured in study I

Leaf temperature	Day	Aphids	Water	Aphids*Water	Error
d.f.	1	1	1	1	36
SS	5.54	11.51	7.76	2.30	18.47
F	12.88	26.78	18.06	5.35	
p	**<0.001**	**<0.001**	**<0.001**	**0.026**	
A_{max}	Day	Aphids	Water	Aphids*Water	Error
d.f.	2	1	1	1	37
SS	395.12	41.31	10.52	8.45	195.85
F	37.32	7.80	1.99	1.60	
p	**<0.001**	**0.008**	0.167	0.214	
g_s	Day	Aphids	Water	Aphids*Water	Error
d.f.	2	1	1	1	39
SS	2250.7	3115.4	7951.4	341.8	35618.4
F	1.23	3.41	8.71	0.37	
p	0.303	0.072	**0.005**	0.544	
F_v/F_m	Day	Aphids	Water	Aphids*Water	Error
d.f.	2	1	1	1	37
SS	0.001	0.001	0.001	<0.000	0.005
F	6.33	5.24	8.73	0.35	
p	**0.004**	**0.028**	**0.005**	0.556	
$\Delta F/F_{m'}$	Day	Aphids	Water	Aphids*Water	Error
d.f.	2	1	1	1	42
SS	0.267	0.008	0.008	0.011	0.241
F	23.29	1.44	0.14	2.03	
p	**<0.001**	0.236	0.708	0.161	
SPAD		Aphids	Water	Aphids*Water	Error
d.f.		1	1	1	18
SS		879.61	61.34	66.31	202.27
F		78.28	5.46	5.90	
p		**<0.001**	**0.031**	**0.026**	

3.4.4 Effect of treatments on leaf temperature and gas exchange

Table 3.4 provides a summary of the repeated measures ANOVA results for study I. Leaf temperature tended to be higher for droughted compared to well-watered plants ($p < 0.001$). The aphid treatment tended to have lower temperatures in study I. In study I the overall means for each treatment were: DA = 18.5 °C ±0.25, DN = 19.0 °C ±0.30, WA = 17.2 °C ±0.13, WN = 18.6 °C ±0.11.

Aphids had an effect on plant gas exchange (Table 3.4). Aphid infestation led to a significant reduction in A_{max}. Aphid infestation in combination with drought led to the lowest rates of A_{max} (Figure 3.5A, B). At least part of this decline in photosynthesis is presumed to have been caused by the reduced g_s (Figure 3.5C, D), which was significantly decreased by the drought treatment.

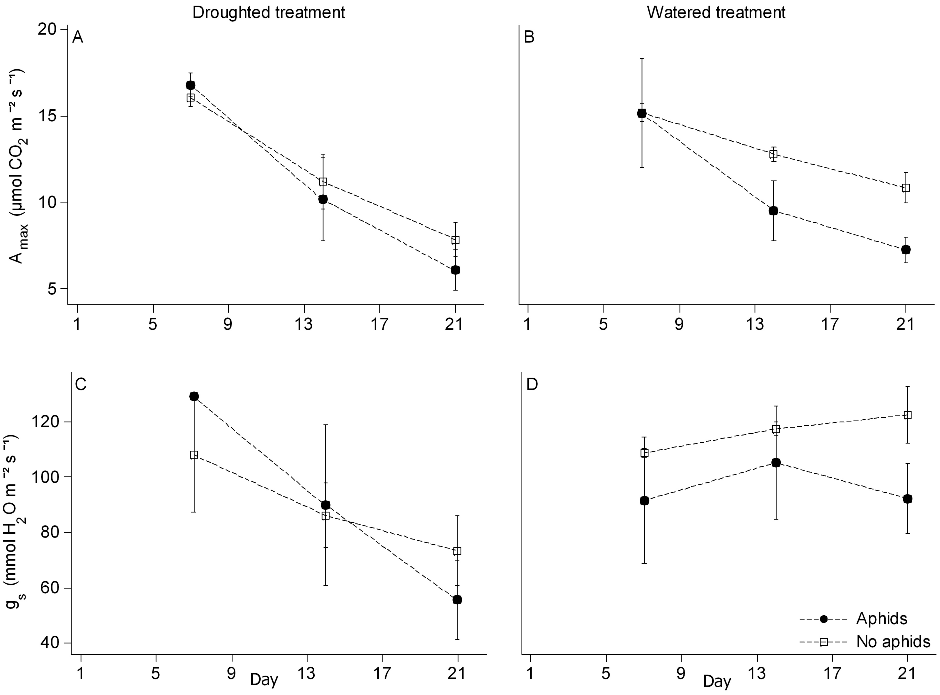

FIGURE 3.5: Mean (± SEM) A_{max} and corresponding g_s in study I. (A, B) A_{max} was reduced with aphid treatment and over time, but not by drought treatment (C, D) g_s was reduced more by drought treatment than by aphids or over time.

3.4.5 Effect of treatments on chlorophyll fluorescence and SPAD estimates

The measures of chlorophyll fluorescence were differently affected by the treatments (Table 3.4). F_v/F_m, a measure of the maximum quantum yield of PSII, was significantly reduced with aphid treatment and drought treatment (Figure 3.6). The F_v/F_m of watered and aphid-infested plants showed a similar gradient of reduction to that of droughted plants. Fluorescence signals are potentially useful for demonstrating aphid stress on plants. There was no significant effect on $\Delta F/F_{m'}$ by any treatment. Aphid infestation reduced the SPAD meter readings to a greater extent than water stress (Table 3.4). This suggests that aphid stress leads to reduced chlorophyll. The drought treatment did not lead to as significant a reduction in SPAD readings.

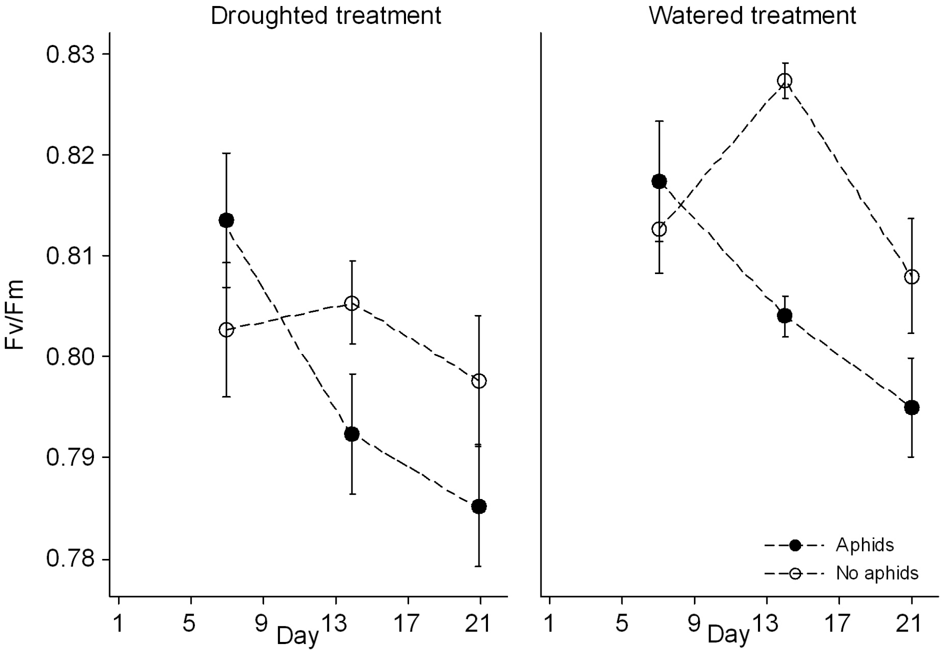

FIGURE 3.6: Trends in mean (± SEM) F_v/F_m in study I. There was a significant reduction with both aphid infestation and drought with an overall reduction over time.

3.4.6 Effect of treatments on plant growth

All growth variables were significantly affected by both of the treatments (Figure 3.7, Table 3.5). Aphid infestation led to a significant decrease in dry shoot weight, as did drought stress. The factors interacted to reduce shoot dry weight significantly. The aphid treatment alone led to a significant decrease in root dry weight for watered and droughted plants. The ratio of root:shoot dry weight was significantly increased by water stress, but unaffected by aphid treatment. The ratio in droughted plants was a balance of close to 1:2 in contrast to watered plants that showed a split nearer 1:3. Leaf width was reduced in the droughted plants, but was not affected by aphid treatment.

Table 3.5: Anova summary table for plant growth variables in study I

Dry shoot weight	Aphids	Water	Aphids*Water	Error
d.f.	1	1	1	13
SS	0.226	0.041	0.016	0.016
F	184.27	33.25	13.27	
p	**<0.001**	**<0.001**	**0.003**	
Dry root weight	Aphids	Water	Aphids*Water	Error
d.f.	1	1	1	13
SS	0.061	<0.001	<0.001	0.006
F	124.42	1.83	1.39	
p	**<0.001**	0.199	0.259	
Root:shoot	Aphids	Water	Aphids*Water	Error
d.f.	1	1	1	13
SS	<0.001	0.079	<0.001	0.058
F	0.04	14.93	0.10	
p	0.845	**0.002**	0.757	
Leaf width	Aphids	Water	Aphids*Water	Error
d.f.	1	1	1	38
SS	0.009	0.077	0.006	0.360
F	0.98	8.07	0.65	
p	0.325	**0.007**	0.425	

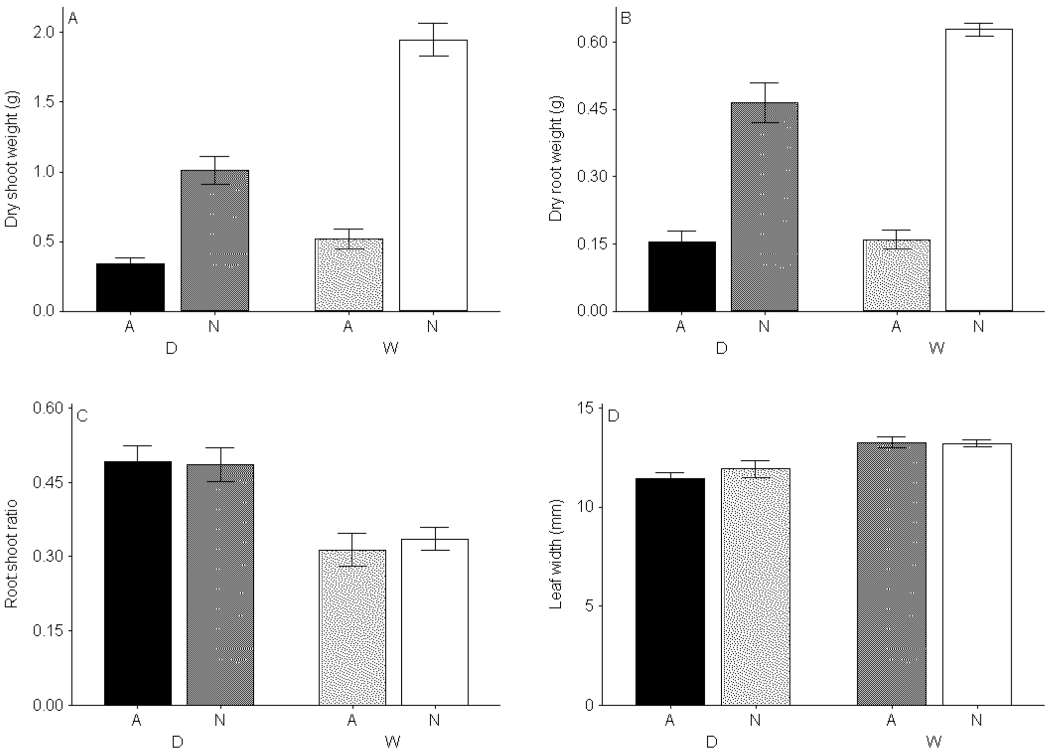

Figure 3.7: Mean (± sem) values for plant growth variables at the end of study I by treatment. Axis letters: 'D' = droughted, 'W' = watered, 'A' = aphid infested, 'N' = no aphids. (A) Dry shoot weight (B) Dry root weight (C) Root:shoot ratio (D) Leaf width

3.5 Discussion

The experimental method produced a significant difference in soil moisture content between watering treatments, $p < 0.001$. Although the plants in study I are estimated to have experienced a general reduction in Ψ to −0.3 MPa, the values used for estimation (from the parallel study) were not as low as would be expected, and did not show a very large range. The vwc fell below half of the well-watered treatment, but stomatal closure in the droughted treatment is likely to have prevented a significant reduction in Ψ. This partial closing of stomata restricted transpirational cooling (Table 3.4). This led to higher leaf temperatures in the droughted treatment, raising leaf temperatures during week 2 to a more optimal environment for aphid reproduction (Dean, 1974).

The findings here show that water stress led to an increased aphid population size. The aphid colony was greater on droughted plants, particularly during the first two weeks of the experiment. As time passed the number of aphids on the watered plants caught up with the droughted plants. This may be because the aphid numbers on droughted plants had reached a maximum sustainable density sooner than the watered plants. The speed of population increase can be seen in Figure 3.3, where the aphids increase faster and to greater numbers on droughted plants.

The unsustainable speed of aphid population increase may have been a result of the benefits to aphids suggested by the Plant Stress Hypothesis (White, 1969). Plants under water stress reallocated resources to the root system, so may have temporarily had greater quantities of small mobile nitrogenous molecules in the phloem than the healthier watered plants. This may permit the aphids to take advantage of the higher leaf temperatures by maximal reproduction. Honěk (1991) found that *M. dirhodum* numbers on droughted *Triticum aestivum* fell as plant size decreased and root:shoot ratio increased. However, our results for plant physiology and growth indicate that aphid infestation can cause significant, lasting damage to the plants before the aphid population becomes limited by poor plant health. Our results support plant stress hypotheses, with optimal aphid reproductive conditions at a mild stress level. Further study to include specific soluble nitrogen levels would be valuable (White, 1993).

The faster aphid population growth on droughted plants resulted in lower rates of A_{max}, g_s, F_v/F_m and leaf chlorophyll which led to significantly smaller, poorer quality plants. In time the impact of aphid infestation was significantly detectable in the watered treatment as well. It was disappointing to be unable to fulfil the study of r_m, and future work to analyse the changes in population development over time and under stress could confirm the patterns seen here.

Aphid infestation led to plant stress that was similar in style to water shortage, but effected greater damage to the plant. Despite receiving more than double the water ration of a droughted plant, watered plants infested with aphids had significantly reduced A_{max} and g_s. This is similar to previous work with barley by Riedell (1989); Cabrera et al. (1995), and with other aphid species (Simpson et al., 2012).

This study adds fluorescence signal data to elucidate the effects on the photosystem. The effect of aphid infestation on watered plants is similar to the effects shown here in droughted plants. The significant reduction in A_{max} and lower F_v/F_m on aphid-infested plants implies damage to the efficiency of PSII electron transport. These results are similar to those found with Russian wheat aphids (*D. noxia* Mord.) by Burd and Elliot (1996) and Macedo et al. (2003). This is also supported by the significant reduction in estimated leaf chlorophyll in aphid infested plants, irrespective of watering treatment. In this study, measurements of dark-adapted F_v/F_m were reduced under aphid stress as also found in Blanco et al. (1992), where the method is suggested as a rapid identifier of aphid stress. The results of our study concur, with mean values in the aphid treatment below the 'healthy' value of 0.832 ± 0.004 (Björkman and Demmig, 1987).

Fereres et al. (1988) found that *Sitobion avenae* Fab., another grain aphid species, significantly increased in population density under a severe water stress of −2.0 to −2.4 MPa. In that study, the combination of aphids and water shortage caused losses to crop yields of over a third. Our work also shows an exacerbation of water stress biomass reduction by aphid infestation under the estimated milder water stress of −0.3 MPa. Drought and aphids reduced the size of plant shoots; DA plants were the smallest. Aphids reduced the weight of roots produced irrespective of watering treatment. The impact of *M. dirhodum* infestation on a field grown barley crop could be severe.

It would be worthwhile to follow the same response variables through subsequent life-stages of cereals (e.g. Zhou and Carter, 1992), and to test the pulse stress hypothesis on a long-term basis. In this way, we could identify whether the significant impacts of aphid infestation last through the lifetime of the plant and whether bouts of re-watering would trigger the aphid population to increase at a greater rate.

In summary, the hypothesis that the *M. dirhodum* population would increase faster on droughted host plants was supported. The aphids reproduced to greater numbers on droughted plants, but this became limited by plant health. Aphids reduced the A_{max}, F_v/F_m and biomass of host plants more than drought stress. Under future drought scenarios, *M. dirhodum* may become a significant threat to crops under even mild water stress.

3.6 Acknowledgements

The research was funded by the Natural Environment Research Council through a UK research studentship (NE/F008791/1). Our thanks are also due to the Rothamsted Insect Survey, Harpenden, UK for kindly supplying the aphids for this research.

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Synopsis II

In the previous chapter, it was found that *M. dirhodum* was more successful on stressed host plants and aphids caused a drought-like response in well-watered host plants as well as increasing the impact of water shortage on stressed hosts. In the next chapter, a field experiment is used to investigate the response to water stress in *D. platanoidis* on sycamore.

4

The case of sycamore aphid, *Drepanosiphum platanoidis* (Schrank) and sycamore saplings, *Acer pseudoplatanus* L.

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Under submission to *Canadian Journal of Forest Research*

4.1 Abstract

A field investigation into the effects of decreased water availability on the relationship between aphids and host plants was designed. Three year old sycamore (*Acer pseudoplatanus*, L.) saplings were inoculated with the sycamore aphid *Drepanosiphum platanoidis* (Schrank). The trees were subject to two watering treatments: water-excluded and well-watered over a period of two years prior to the work. The aphid population was counted and correlated with plant physiological measurements. Each tree's aphid population over the season was used to analyse the impact of aphid load and water treatment on sycamore size, growth, leaf area and stem weight.

Increased aphid numbers correlated with reduced leaf stomatal conductance, decreased total leaf water potential and increased leaf soluble nitrogen (N) in mildly water-stressed trees. Aphids were more numerous on the largest saplings with greatest

leaf area and investment in woody growth. Trees with a faster growth rate and greatest increases in height and girth had a greater aphid load over the season.

Although mild drought symptoms led to an increase in aphid numbers, this was outweighed by a greater aphid population number on larger trees. It is suggested that in an exposed site aphids flourish best on larger trees that potentially offer the greatest protection from wind. In this experiment neither water shortage nor aphid infestation decreased sycamore productivity.

4.2 Introduction

Our work aimed to test the Plant Stress Hypothesis (PSH) initially proposed by White (1969), that aimed to explain how host plant stress may lead to increased nitrogen availability for phytophagous insects, resulting in greater reproduction and survival of young. It is especially relevant today, as it may be applied to many cases of climatic stress, brought about by the changing and irregular climate (Alcamo et al., 2007b). Severe water stress may be expected to lead to significant negative effects on phloem-feeding insects, caused by reducing leaf turgor and water content (see the meta-analysis by Huberty and Denno, 2004). In contrast, many plants are adapted to coping with mild or intermittent stress, using osmotic adjustment to accumulate solutes and maintain cell turgor and plant growth during times of water shortage (Hsiao, 1973; Taiz and Zeiger, 2006). It is hypothesised that this turgor maintenance mechanism would enable aphids to continue to feed on phloem sap through the stylet whilst benefiting from a stress-induced nitrogen increase.

The role of nitrogen in the diet is key to aphid success (Dixon et al., 1993; White, 1993), and it is the changes in plant nitrogen content, as a result of environmental perturbation, that determine our expectations for aphid response to host plant stress. White (2009) adapted the original PSH in response to comparisons with the plant vigour hypothesis of Price (1991). The adapted PSH suggests that aphids which prefer to feed on senescing tissue will benefit most from mild or intermittent drought stress. Aphids which prefer to feed on flush tissues will benefit most from a host plant with vigorous growth and rich resources. These adjustments mean that our experimental hypotheses relating to the PSH may depend upon knowledge of whether aphids flourish better on senescing material or flush growth.

Most experimental studies into aphid-host interactions under water-stress are based on crop plants and annuals, with comparatively few studies of aphids on deciduous trees (Koricheva and Larsson, 1998; Jactel et al., 2012). There is variation in results across aphid and tree species, life-cycles and locations (Huberty and Denno, 2004; Harrington et al., 2007; White, 2009). For example, whilst the meta-analysis by Rouault et al. (2006) found a tendency for aphid outbreak after climatic drought, Mody et al. (2009) observed a negative response to experimental drought stress, finding greater numbers of *Aphis pomi* de Geer on unstressed trees. Perennial, woody plants would be expected to respond and interact with phytophagous aphids in a different way to annual plants, as a result of the differences in lifespan and partitioning of nutrients. The length of time required to initiate the stress and to produce a measurable physiological effect is also expected to be longer than for annual plants. The use of saplings as experimental material might reveal more pronounced stress responses in a single season than for the case of mature trees (Morecroft and Roberts, 1999).

We chose the perennial plant sycamore and the sycamore aphid *D. platanoidis* to study the effects of host plant water stress on their relationship. The interaction between this plant and aphid species has been extensively studied (Dixon, 2005), but not in the context of the plant stress hypotheses.

The origin of the UK sycamore population is unclear (Leslie, 2005; Alexander et al., 2006; Townsend, 2008), but native or exotic, this cannot define the value of a species to the ecosystem (Brown, 1997). Sycamore plays an important role in the forest ecosystem, supporting significant numbers of epiphytes (including at least 170 lichen taxa) and foliage invertebrates (at least 43 species) whilst providing large quantities of leaf litter and pollen/nectar sources (Leslie, 2005; Alexander et al., 2006). Research indicates that sycamore's speed of growth, timber quality and wide tolerance of soil conditions also offer high value returns (Crockford et al., 1987). It is a potential timber crop for farm forestry sites and is encouraged by UK governmental policy (Cundall et al., 1998; Forest Research, Roslin, UK, 2001). Across Europe its economic and ecological values are being increasingly considered worthy of investment (Hein et al., 2009). There are no currently available studies on the likely response of sycamore to climate change in the UK, but a recent report focused on Germany indicates that the species may be susceptible to elevated temperatures and reduced precipitation (Kölling and Zimmermann, 2007). Models by Alcamo et al. (2007a,b) find that an increased incidence of drought is likely in central, southern, and western Europe over the next 50–70 years; moderate to severe water stress is likely for southern Britain. Without the presence of *D. platanoidis*, sycamore has the potential to produce significantly more stem wood (Dixon, 1971). Aphid infestation may also reduce the efficiency of establishment of new sycamore stands. It is worthwhile to determine both the plant's response to climate change and the interactive effects this may have on its pests and diseases.

Sycamore aphids complete their life-cycle on their host plant, overwintering as eggs and hatching into a first generation of alates in spring. Short generation times enable aphids to respond quickly to changes in available resources. This allows aphids to adopt seasonal strategies (Dixon, 2005). *Drepanosiphum platanoidis* moves from being distributed throughout the canopy in spring to groups of clustered adults in the lower canopy during summer (Dixon, 1969). These aphids move from leaf to leaf and are found on both new and mature leaves (Dixon and McKay, 1970), therefore *D. platanoidis* is a senescence and a flush-feeding aphid depending on the season. For the purpose of testing the PSH we hypothesise that *D. platanoidis* will respond positively to drought stress in the host plant, according to White (1969). Plant stress will increase the proportion of small nitrogenous compounds in phloem sap. Dixon et al. (1993) found that the total amino acid content in host leaf tissue led to increases in the *D. platanoidis* population. We expected that the increased soluble N in mildly stressed host tissue would benefit the aphid. The work of Dixon and Wellings (1975; 1987) has indicated that aphids themselves have no effect on the amino acid content of leaves. However, the effect of external factors influencing amino acid concentrations has not yet been investigated in the case of sycamore aphids.

It is also necessary to investigate whether aphid infestation impacts an already suffering host. Aphids may have no effect on typical sycamore saplings, but with each adult aphid processing at least its own weight of phloem sap per day (Dixon and Logan, 1973) the drain could be significant in smaller plants in poorer health. It has previously been found that other aphid species can induce drought stress symptoms in leaves of infested plants, and prevent recovery even after the stress has been removed (e.g. Riedell, 1989; Simpson et al., 2012). Collins et al. (2001) investigated the effects of infestation of willow (*Salix viminalis*, L.) by giant willow aphid (*Tuberolachnus salignus* (Gmelin)) and found that although aphid presence appeared to stimulate photosynthesis and increased leaf nitrogen content, the aphid-infested trees used more water and showed lower root and shoot production. The potentially aphid-benefitting changes to plant physiology may have also caused the increased water use that would be most detrimental to plants undergoing water stress.

The hypothesis to be tested in this experiment was that reduced soil water content would cause a drought response in the host plant resulting in physiological and biochemical changes beneficial to feeding aphids. Our first aim was to simulate a drought in young sycamore trees by excluding rain whilst keeping other climatological variables constant. The effect of aphid infestation on plant physiological variables associated with drought was examined. These effects were measured through estimates of gas exchange, water potential components, soluble N and leaf temperature. The aphid population was followed over 42 days. The impact of all treatments on tree biomass was finally evaluated with a destructive harvest.

4.3 Materials & Methods

4.3.1 Field site

Experiments were conducted at the King's Buildings Campus, Edinburgh University, Latitude = 55.92°, Longitude = −03.18°, 78 m above sea level. The location has a typical maritime temperate climate with uniform precipitation distribution and warmest month averaging below 22 °C but at least 4 months above 10 °C.

After the coldest winter since 1985/6 and a milder February, the measurements were begun in May 2011. This month was 0.7 °C warmer than the 1971–2000 average and was the wettest May in Scotland since 1910. Gales with winds of 27–38 m s^{-1} were recorded near the field site towards the end of the month. June 2011 was cooler than the 30 year average but had greater than average rainfall (Met Office, Devon, UK, 2012).

The soil was a dense homogenous bed to 500 mm depth of brown-grey poorly graded gravelly clay. This meant that when dry the soil was hard and impenetrable to water from above. The ground was not artificially fertilised and had not been cultivated prior to the experiment. The soil's field water capacity (the point after water drainage has stopped, where large soil pores are filled with both air and water while smaller pores are full of water (Brouwer et al., 1985)) was calculated as part of the calibration for the soil moisture meter (Campbell Scientific Ltd., Shepshed, UK) and was 0.33 m^3 m^{-3}, which is typical of the area (European Commission — Joint Research Centre Institute for Environment and Sustainability, 2012).

4.3.2 Experimental design

All work used sycamore; 400–500 mm tall potted-saplings were supplied by Alba Trees (Gladsmuir, UK) and planted at the field site during September 2009. By the start of work in 2011, the trees had grown to an average of 1500 mm tall.

The specially constructed system to exclude rainfall (Figure 4.1) was built over spring 2008 and involved a grid of drainage trenches at least 300 mm deep by 150 mm wide, filled with plastic perforated land drainage pipe surrounded by pea-gravel. There was some variation in depth of trench due to a high proportion of stones and rubble. The earth moved out of the trenches was use to create raised mounds of improved drainage, where the trees were planted. Nine months after planting, the saplings were randomly assigned to either a water excluded square (D) or a well-watered treatment square (W) distributed randomly across the site's grid. A layer of PVC sheeting was laid over the mounds, raised above the ground surface by a tripod of tent pegs for increased ventilation. The sheeting was secured to the ground with heavy duty tent pegs. The well-watered trees protruded through cross-cut holes, which allowed stem-flow of water and the trees were given supplemental watering if there had been no rain for 5 days. The water excluded trees protruded through cross-cut holes which were then covered with a cap of PVC sheet to prevent stem-flow of water. A rabbit exclusion fence was built around the site perimeter. A windbreak was erected in April 2010. This 2 m high, steel cage was built with a lightweight net across the top and dual purpose shade and wind break fabric around the two most exposed edges. This protected the trees and aphids from direct sunlight and prevailing winds.

Fifty-six trees formed the initial sample population planted in 2009, providing 28 replicates per treatment. There were losses of some trees through reduction in site area; suspected edge effects; animal and snow damage; wind breakages and root damage from a colonisation of common wasps (*Vespula vulgaris*). By the end of the experiment 33 trees remained, leaving 16 trees per treatment, with an additional one in the D group. Sample dates and the total sample sizes per day (n) are detailed for

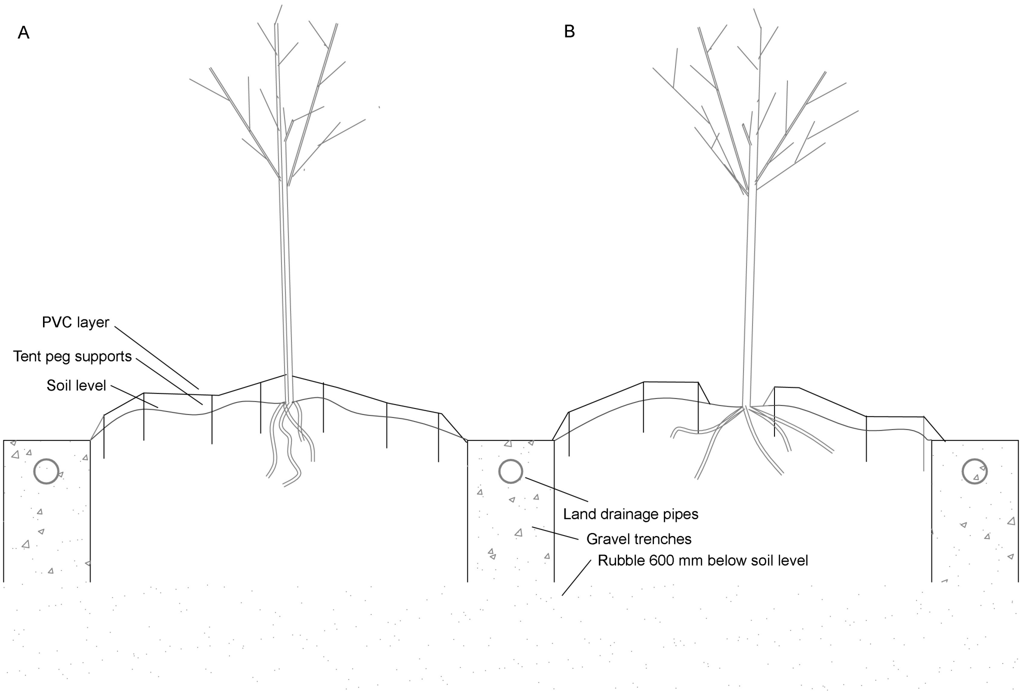

Figure 4.1: Sectioned elevation of the field site. Each plot was 1200 mm square, with the PVC sheet supported with tent pegs above ground level. (A) 'Droughted' treatment indicating the sheet extending up to the stem to prevent stem-flow. (B) 'Watered' treatment indicating the sheet peeled back from the stem to permit water. The drainage channels of gravel and pipe separated each square unit. The diagram of root spread is an estimate of primary roots *only* and is based upon general observation at the excavation after the final harvest. The gravel channels and bed of miscellaneous rubble discouraged root spread beyond the square.

each variable. Not all trees could be sampled on each occasion and the number of samples were distributed as evenly as possible across the treatment groups. In all plant physiological measurements, fully expanded leaves at a height 1 m from the base were selected for uniformity.

4.3.3 Water deficiency monitoring

Soil moisture was measured with a Campbell CS615 water content reflectometer (Campbell Scientific Ltd., Shepshed, UK) connected to an Agilent digital multimeter (Agilent Technologies Ltd., Edinburgh, UK) using the wiring system described in O'Brien and Oberbauer (2001). The 300 mm long probes were inserted into the soil approximately 100 mm from the base of the tree at a 90 °angle to the ground and left for at least 1 minute to stabilise. The unit of measurement was volumetric water content (vwc) in m^3 m^{-3}. Six sample dates with n = 31–33 were recorded between May and June.

4.3.4 Plant physiological measurements

Gas exchange measurements: The stomatal conductance (g_s) measurements were made between 10:00 and 13:00 using an Li-1600 Steady State Porometer (Li-cor Biosciences Inc., NE, USA). Light levels during measurements were the ambient photosynthetically active radiation (par), which varied from approximately 360–1700 µmol photons m^{-2} s^{-1} over the course of the morning.

Leaf temperature was recorded from the leaf thermocouple integral to the porometer.

In random order, one fully expanded leaf was chosen per tree and any aphids were removed before measurement. The data were recorded on 6 sample dates with n = 10–26.

Water potential components: The components of plant water potential: leaf water potential (Ψ), leaf turgor potential (Ψ_ρ) and leaf osmotic potential (Ψ_π) are related as in Equation 4.1

$$\text{Total } \Psi = \Psi_\rho + \Psi_\pi \tag{4.1}$$

Ψ gives an immediate reading for water stress; Ψ_π gives the concentration of vacuolar and phloem sap constituents. Ψ_ρ is expected to have a strong relationship with aphid performance since aphids require a positive pressure in order to insert their stylets and extract phloem sap. For sycamore saplings, where the average sampling height is less than 1000 mm, gravitational potential can be assumed to have no effect.

Ψ was measured using a Scholander-Type Plant Moisture System (Plant Moisture System skpm 1400, Skye Instruments Ltd., Powys, UK). Measurements were made between the hours of 10:00 and 13:00. One fully expanded leaf was excised per tree and immediately transferred to the pressure chamber. Three sample dates with n = 19–38 were recorded.

After measurement of Ψ, the leaf was removed from the pressure chamber, sealed in a 10 mL plastic syringe and immediately snap-frozen in liquid nitrogen for later estimation of Ψ_π. Leaves were stored in liquid nitrogen and defrosted for 30 minutes prior to measurement. At least 10 µL of cellular sap was expressed into a sterile Petri dish by depressing the syringe plunger and immediately sampled with a 10 µL pipette, transferred to a filter paper disc and analysed with a Wescor Vapro5520 Vapour Pressure Osmometer (Wescor Inc., UT, USA). The osmometer output in mmol/kg was converted to Ψ_π in MPa according to van't Hoff, Equation 4.2

$$\Psi_\pi\ (\mathrm{MPa}) = -\mathrm{R}\cdot\mathrm{T}\cdot(\mathrm{OSM}/1000) \tag{4.2}$$

where R = universal gas constant, 0.00831 MPa kg mol^{-1} K^{-1}, T = room temperature, 293 K and OSM = osmometer output. The error component from dilution of symplasmic water by apoplasmic water is acknowledged (Callister et al., 2006), but was judged to be small and not corrected for. Ψ_π readings are therefore a bulk estimation of leaf osmotic potential, relevant for water stress studies and field sample collection (Bannister, 1986; Pullman and Johnson, 2009). Ψ_ρ was derived by difference, from the relationship between the two measured potentials.

Soluble leaf nitrogen: One leaf per tree was sampled at the end of June and snap frozen for individual analysis (n = 15). Analysis was conducted at the University of Edinburgh, Crew Laboratories, Edinburgh, UK with the following method (van Emden and Bashford, 1969; Jackson, 1991). Approximately 200 mg of dry ground material was weighed into a test tube with 4 mL of 0.02 % phenol in a solution of 2.5 % trichloroacetic acid. 1 mL of the supernatant was added to 1 mL of concentrated H_2SO_4 and 0.75 mL of 30 % H_2O_2 and heated for 1 hour at 315 °C. Blanks were run through the same process and subtracted from the sample results. Nitrogen was measured as NH_4 mg L^{-1} by auto-analyser and converted into soluble N mg g^{-1} by molarity.

4.3.5 Aphid population measurements

Primary aphid infestation: During the lead up season in 2010 it was ensured that the trees had an aphid population in place, which were able to overwinter as eggs so that a natural life-cycle could begin with bud-burst in 2011. Due to consistently low numbers in 2010, supplementary aphid infestations were made. Two individual leaves from mature, well-infested sycamore trees were removed and placed in a paper bag. Then two individual leaves on the young sycamore saplings were sealed inside a bag with infested leaves and left for 24 hours. The bags were then removed and the number of aphids that had transferred onto the sapling leaves counted. The aphids were counted again 24 hours later to determine the success of this artificial infestation. The inoculation of the trees in 2010 was successful and the aphid population cycle for 2011 was entirely natural.

Aphid population size: Aphids were counted weekly on all trees using multiple photographs taken with a digital camera of 6.0 megapixel resolution saved in high quality resolution JPEG format. Pictures of the lower surface of each leaf were taken. Aphids were counted in Adobe Photoshop Elements (San Jose, CA, USA). Individuals were marked on the photograph as they were counted to ensure no accidental repetition. In total 7 sample dates with $n = 32–33$ were recorded. The suction-trap data from Science & Advice for Scottish Agriculture (SASA, Edinburgh, UK) data for Edinburgh Gogarbank (Figure 4.2) were used as a reference for data collection. Their data illustrate that our work was carried out during the peak of the aphid season.

4.3.6 Plant growth

Final survey & harvest: The stem heights and basal diameters of the remaining trees were recorded on 22 June 2011. These data were paired with the $\log_{10}$ total aphid numbers recorded on that tree for analysis of covariance (ANCOVA). Stems and leaves were harvested separately to determine any impact of drought and aphids on woody growth and photosynthetic capability. Plant material was oven dried to constant weight at 80 °C, $n = 29$. Leaf area was estimated by photographing 20 fresh leaves against a grid of 20 mm squares allowing calculation of leaf area in Adobe Photoshop Elements (San Jose, CA, USA). The dry weight of these leaves was recorded separately, and used to estimate total leaf area per tree (in cm^2) from the total dry leaf weight. For growth rate estimation, survey data for the heights and basal diameters were compared with the original values when the saplings were planted in 2009.

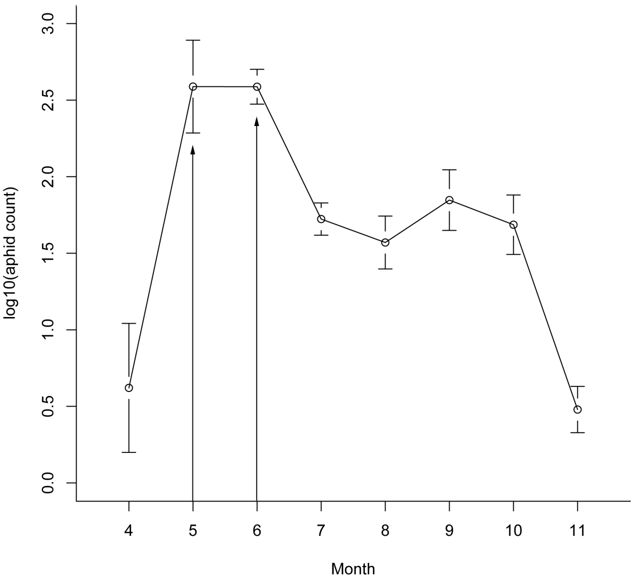

Figure 4.2: The mean (± SEM) monthly log transformed aphid counts over a whole year for Edinburgh Gogarbank 1967-2008 are presented. The population cycle peaks in May and June, with a second smaller peak in September. The arrows show the period that our study corresponds to. Data kindly provided by SASA, Edinburgh, UK.

4.3.7 Data analysis

All analyses and figures were generated using Minitab 15 Statistical Software (Minitab Ltd., Coventry, UK). Repeated measures ANCOVA were performed on the g_s, water potential and leaf temperature data, using the General Linear Model function of the program. The factors were 'Day' (random factor, within groups), '$\log_{10}$ aphid count' (covariate) and 'Watering' (fixed factor, between groups) as defined by Minitab (2007). These data were normally distributed, had equal variance and did not violate homogeneity of regression, so analysis could proceed. Ancova were also performed on the soluble N, final survey and harvest data, with the method as above, but without the repeated measures factor. Aphid count data were analysed with repeated measures analysis of variance (ANOVA) using 'Day' and 'Watering' factors. Aphid photographic count data, and dry weights were $\log_{10}$ transformed to achieve normal distribution and equal variance before applying analysis. Survey measurements of height and basal diameter were transformed to natural logarithms for analysis of growth rates. Means are given ± standard error of the mean (SEM).

4.4 Results

4.4.1 Drought treatment

Soil moisture was significantly lower in the droughted treatment (Figure 4.3). The method stabilised the mean VWC in both treatments, with a mean of the droughted treatment less than 50 % of field water capacity at 0.148 m^3 m^{-3} ± 0.003. The watered treatment mean was 0.194 m^3 m^{-3} ± 0.003. This was a significant ($F_{1,\,181} = 107.75$, $p < 0.01$) average difference between treatments of 33 %, with no significant difference over time. The water exclusion method buffered the system against influxes from high rainfall events and evaporation during dry periods. It is reasonable to conclude that a satisfactory difference in treatments was maintained through the experiment.

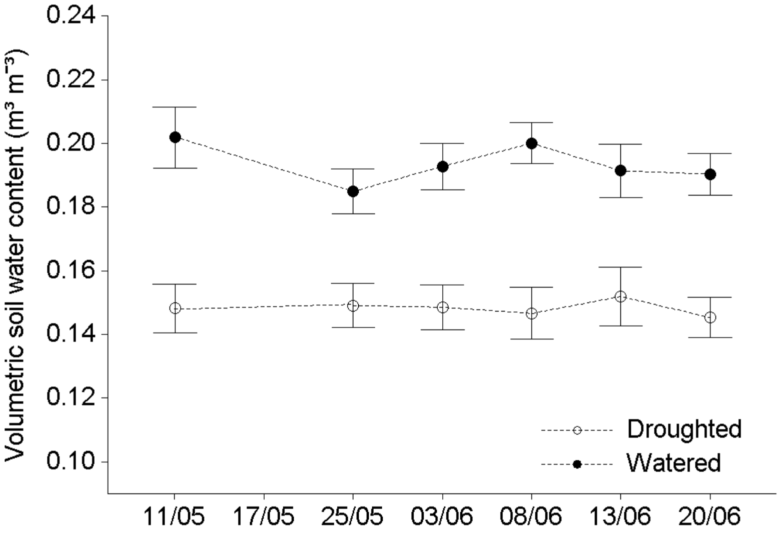

FIGURE 4.3: Volumetric soil water content, values are mean ± SEM. The data illustrate that a stable water content had been established by the start of work.

4.4.2 Covariance of aphid numbers and plant physiology

Despite the difference in soil moisture, the 'watering' treatment had no significant effects in most of the ANCOVA analyses, Table 4.1. However, aphid numbers per leaf were significantly negatively correlated with g_s (Figure 4.4A). The r^2 values are less than 0.5, but highly significant. Trees with a greater aphid load were those with lower g_s. Moreover, aphid numbers were highest when Ψ was most negative (Figure 4.4B).

Aphid numbers were also significantly correlated with leaf temperature as measured by the porometer thermocouple (Table 4.1), with a negative trend of fewer aphids per leaf as leaf temperature rose. The key impact occurred when leaf temperatures rose above ambient air temperature, when significantly fewer aphids were found.

Table 4.1: ANCOVA repeated measures summary table for plant variables, 'Day' = the separate days of measurement, '$\log_{10}$ aphid count' = covariate in the analysis, 'Watering' = the two levels of watering treatment.

g_s	Day	$\log_{10}$ aphid count	Water	Error
d.f.	5	1	1	69
SS	22534.3	6454.5	684.2	95255.2
F	5.25	7.52	0.80	
p	**<0.001**	**0.007**	0.374	
Leaf temperature	Day	$\log_{10}$ aphid count	Watering	Error
d.f.	5	1	1	139
SS	210.73	6.64	0.085	101.60
F	57.66	9.09	0.12	
p	**<0.001**	**0.003**	0.733	
Ψ	Day	$\log_{10}$ aphid count	Watering	Error
d.f.	2	1	1	66
SS	0.016	0.145	0.009	0.245
F	2.15	39.17	2.54	
p	0.125	**<0.001**	0.116	
Ψ_π	Day	$\log_{10}$ aphid count	Watering	Error
d.f.	2	1	1	87
SS	0.237	0.054	0.149	2.10
F	4.92	2.25	6.18	
p	0.137	**0.009**	**0.015**	
Ψ_ρ	Day	$\log_{10}$ aphid count	Watering	Error
d.f.	2	1	1	69
SS	0.106	0.224	0.145	2.990
F	1.23	5.18	3.35	
p	0.300	**0.026**	0.072	

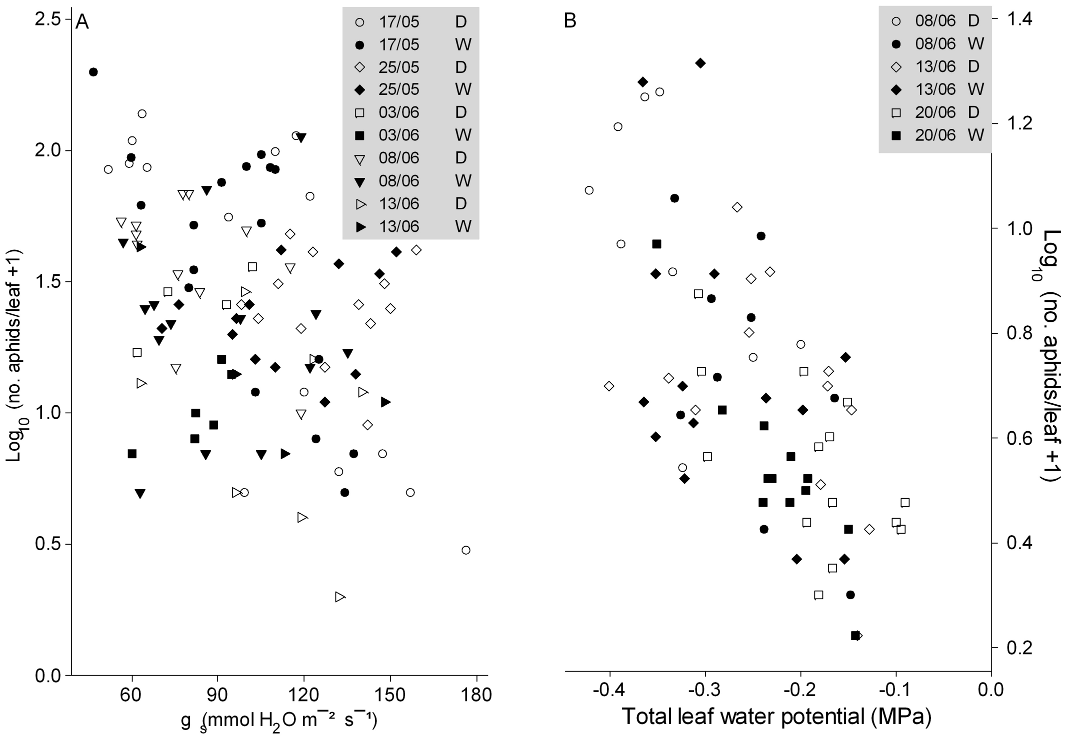

Figure 4.4: (A) Aphid numbers against g_s, $r^2 = 0.33$, $p = 0.007$. Each data point per day of study represents one tree. (B) Aphid numbers against Ψ, $r^2 = 0.44$, $p < 0.001$.

Overall, Ψ was not significantly affected by watering treatment and had no trend towards decreasing over the period of the study. The relationship between aphid numbers and Ψ appears to be more dynamic than the categories of treatment, responding to short term changes. The Ψ_π and Ψ_ρ components were not significantly correlated and were not affected by any treatment.

Soluble leaf nitrogen correlated strongly and positively with mean aphid numbers per leaf (Figure 4.5). Increased leaf nitrogen may be a result of the mild drought response. Soluble nitrogen was slightly statistically significantly different according to watering treatment (Table 4.2, $p = 0.033$)

Table 4.2: ANCOVA summary table for soluble nitrogen, ‘$\log_{10}$ aphid count’ = covariate in the analysis, ‘Watering’ = the two levels of watering treatment.

soluble N	$\log_{10}$ aphid count	Watering	Error
d.f.	1	1	12
SS	0.222	0.032	0.067
F	40.05	5.78	
p	**<0.001**	**0.033**	

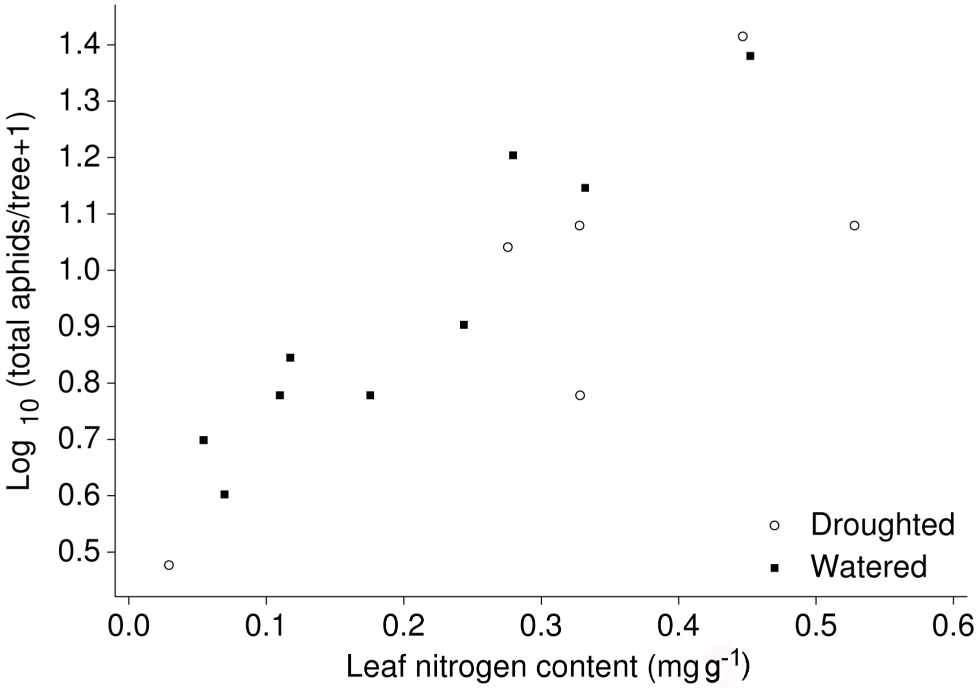

Figure 4.5: Soluble leaf nitrogen was significantly positively correlated with total aphid numbers per tree, $r^2 = 0.71$, $p < 0.001$.

4.4.3 Effect of drought on the aphid population

There were significantly more aphids on the droughted plants (Figure 4.6, Table 4.3). The aphid numbers clearly decreased over time, which is probably a feature of the natural life-cycle of *D. platanoidis* corresponding with the summer diapause.

TABLE 4.3: ANOVA repeated measures summary table for aphid numbers, 'Day' = the separate days of measurement, 'Watering' = the two levels of watering treatment.

$\log_{10}$ aphid count	Day	Watering	Error
d.f.	6	1	221
SS	26.18	0.76	19.47
F	49.52	8.65	
p	**<0.001**	**0.004**	

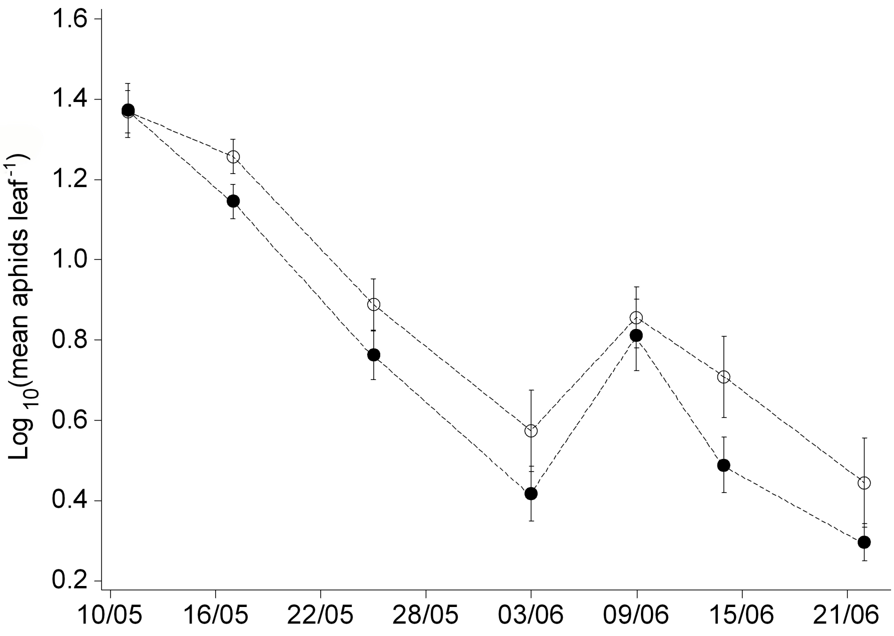

FIGURE 4.6: The mean (± SEM) aphid counts declined overall from early May to late June. Filled circles = 'Watered', Open circles = 'Droughted'. In ANOVA there were significantly more aphids per leaf on the droughted trees ($p = 0.004$), with more aphids on droughted trees on six out of seven occasions.

4.4.4 Covariation of aphids with plant growth

Trees with the greatest numbers of aphids per leaf were significantly larger in height, basal diameter, dry stem weight, total leaf area and growth (Figure 4.7). In the ANCOVA, Table 4.4, watering treatment had no significant effect on any growth measure, indicating that aphid numbers are determined by both physiological characteristics and tree size.

Table 4.4: Ancova repeated measures summary table for plant growth

Height	$\log_{10}$ aphid count	Water	Error
d.f.	1	1	26
SS	11080	4951	42646
F	6.76	3.02	
p	**0.015**	0.094	
Basal diameter	$\log_{10}$ aphid count	Water	Error
d.f.	1	1	26
SS	177.02	11.72	618.42
F	7.44	0.490	
p	**0.011**	0.489	
Stem weight	$\log_{10}$ aphid count	Water	Error
d.f.	1	1	26
SS	0.988	0.211	3.411
F	7.53	1.61	
p	**0.011**	0.216	
Leaf area	$\log_{10}$ aphid count	Water	Error
d.f.	1	1	26
SS	$4.54\text{x}10^{7}$	$6.71\text{x}10^{6}$	$2.39\text{x}10^{8}$
F	4.95	0.73	
p	**0.035**	0.400	
Growth in diameter	$\log_{10}$ aphid count	Water	Error
d.f.	1	1	25
SS	94.28	0.42	383.44
F	6.15	0.03	
p	**0.020**	0.870	
Growth in height	$\log_{10}$ aphid count	Water	Error
d.f.	1	1	26
SS	1.025	0.365	4.127
F	6.46	2.29	
p	**0.017**	0.143	

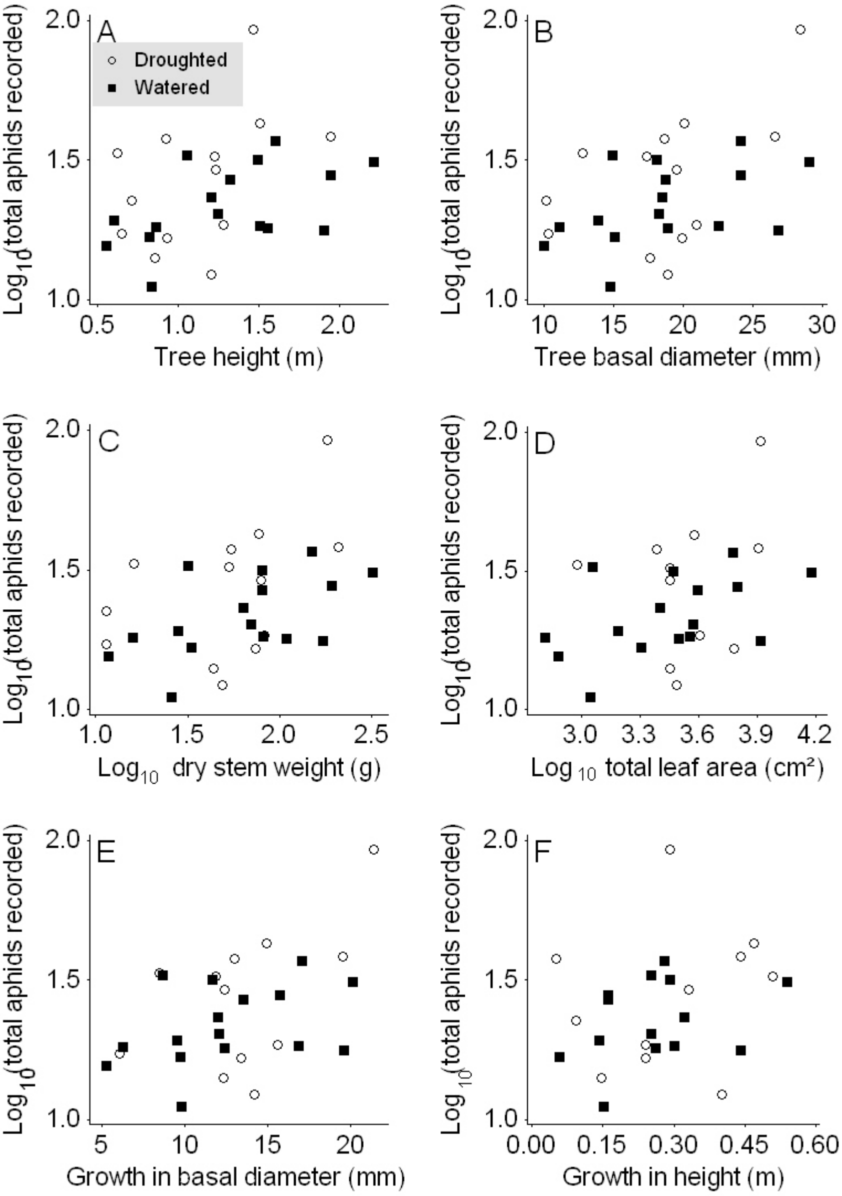

FIGURE 4.7: Correlations of sycamore growth characteristics by aphid load and watering treatment. (A) Tree height, (B) Basal diameter, (C) Dry stem weight, (D) Total leaf area, (E) Diameter increase and (F) Height increase.

4.5 Discussion

The results indicate that there were more *D. platanoidis* on trees from which water had been withheld. Trees and leaves that had lower Ψ, lower g_s and increased soluble N content carried more aphids. Aphids also preferred larger trees that had a faster growth rate.

In this work, aphid numbers were more strongly correlated with g_s, Ψ and soluble N than with categories of watering treatment. Our study therefore finds that the short generation times in *D. platanoidis* permit the aphid population to respond rapidly to fluctuations in the host, as suggested by Dixon (2005).

Leaves may be selected for ease of feeding, with greater aphid numbers on leaves with lower g_s and lower Ψ (both implying water shortage and some loss of turgor). This could make it easier for the aphids to insert their stylets, especially since significant loss of turgor was not found. Turgor pressure is required to direct the phloem sap through the stylet to the aphids (Auclair, 1963; Mittler, 1967; Douglas, 2006). Although direct effects of drought on trees or aphid numbers were not confirmed in ANOVA, there is strong evidence in the covariate data that aphids were detecting and responding positively to drought-associated variables.

The quantity of soluble N in the leaves was another significant factor correlated with aphid numbers. An increased soluble N content reflects a greater mobility of small nitrogenous molecules which are more easily assimilated by feeding aphids (Auclair, 1963; Mittler, 1967; Douglas, 2006). Although in our study the increased quantity of soluble N was not found to be a result of the drought treatment, it was directly correlated with aphid numbers. Therefore, the nomadic winged aphids may be actively selecting the highest quality leaves. The presence of some aphids attracts others, so the populations tend to aggregate on favourable leaves (Dixon, 1966; Dixon and McKay, 1970). Regarding the PSH, the sycamore aphid appears to rely on the availability of nitrogenous molecules to nourish the nymphs best, and aphid numbers were correlated to stress indicators. This provides support for the hypothesis that nitrogen may be the most limiting factor in insect population increase (White, 1993).

Physiologically, the plants in this experiment underwent a mild drought as described above, but no further effect was found on overall plant growth or production of leaves.

Reduction in plant growth was expected as a typical response to water shortage (Hsiao, 1973). Although young sycamore plants can be affected by severe drought (Khalil and Grace, 1992), it is considered a drought-avoiding species by horticulturalists. Morecroft and Roberts (1999) found that mature sycamore trees (50+ years) showed no gas exchange reductions in response to lower soil moisture, yet Scherrer et al. (2011) ranked sycamore as the most drought sensitive deciduous tree species in their comparison of typical temperate tree species. Their data were based on remotely sensed canopy foliage temperature and sap flow data, which are not directly comparable to our study. The 3 year old sycamores in our study fall into an age bracket that, to our knowledge, has not previously been studied. Our data would suggest that the level of drought likely to occur in the field would have little effect on sycamore productivity. Our experimental study with trees planted in the ground rather than pots finds, as in Khalil and Grace (1992), that sycamore is a drought-avoiding species that closes its stomata early in a water shortage period, thus preventing a decline in Ψ. Significant reductions in g_s were not observed in the drought treatment, this may have been the result of sycamore adaptation to the long term mild drought.

The measurements of Ψ_π were expected to find osmotic regulation in droughted plants, but were unaffected by drought or aphid treatments and did not correlate with aphid numbers. It has previously been found by Simpson et al. (2012) that aphid infestation can lead to a prevention of osmotic regulation in droughted cabbage plants (*Brassica oleraceae* var *capitata*), but a similar response was not found here in sycamore, a perennial. In cabbage leaves, the Ψ_π ranged from −0.70 to −0.95 MPa, whereas in sycamore leaves the range was −1.45 to −1.65 MPa. These results are typical of sycamore (Khalil and Grace, 1992), so the aphid action of preventing osmotic regulation may only manifest in annual plants with a more dilute cell sap.

Our plant growth results would also appear to be contrary to those of Dixon (1971) and Wellings and Dixon (1987), who found that aphid infestation led to reduced leaf size and stem wood production. The average number of aphids per leaf in this study (see Figure 4.6) is close to the mean of 10 per leaf in Dixon (1971), and a similar result of greater nitrogen content and increased ‘greenness’ is found to be connected with greater aphid numbers. However, we did not find a change in the partitioning of leaf and stem production. The saplings used in the 1971 paper were two years younger than those in our work and their observed partitioning may be due to a greater impact of aphid infestation on very young plants. The plants used in this work may have had more resources for defence or adaptability. It would therefore be worthwhile to consider the entire lifespan of a tree and its relationship with aphid infestation. Insect attack may have a significant impact on plants under 1 year old, as they establish, but at the 2–3 year old stage, any impact on growth may be negligible.

The field site in this study was exposed to strong winds and unshaded by buildings or large trees. A larger aphid population on larger trees with more leaves could therefore be due to a beneficial shading effect provided by the greater number of leaves and stability of a sturdy trunk that did not whip in the wind. More vigorous trees also represent a more nutritionally adequate resource. Population size of some aphid species, such as *A. pomi* and *Myzus persicae* (Sulzer), positively correlate to tree growth (Grechi et al., 2008; Stoeckli et al., 2008). In this study, aphids were found equally on leaves of all ages and sizes, so we cannot classify whether *D. platanoidis* prefers flushing or senescent growth. It would be valuable to investigate further aphid population characteristics, such as the intrinsic rate of increase, fecundity and survival rates.

Personal observations suggest that there is a further interaction between other aphid species colonising sycamore, specifically the Aceraceae-specific apterous *Periphyllus testudinaceus* (Fernie) (Hemiptera; Aphididae, Chaitophorini). An initial, brief colonisation of some sycamore trees after bud-burst was by *P. testudinaceus*, forming immobile dense clumps on leaf petioles and new shoots (Warrington and Whittaker, 1985). This population was ant-tended, leading to potential predation of the *D. platanoidis* by ants (Skinner and Whittaker, 1981) in the spring. The ants (*Formica rufa*) were possibly attracted by the high concentration of sycamores and the undisturbed soil mounds in which the trees were planted (Skinner, 1980).

The hypothesis that reduced soil water content would cause a drought response in the host plant resulting in physiological changes beneficial to feeding aphids was not fully supported. The correlation of aphid numbers with Ψ, g_s and soluble N was consistent with drought and gives some support to the plant stress hypotheses. However, more aphids were found on comparatively larger trees that bore more leaves and grew more over the season. This does not fit with a drought response and we can conclude that larger trees are more attractive to aphids. Additionally, at the fine scale of host plant selection, aphids select leaves that are of a higher quality and easier to feed from (short term changes in soluble N, lower g_s), but on a coarser scale they may be selecting these leaves from larger trees based on shade and trunk stability. It is also likely that the alate forms of *D. platanoidis* are prepared to move when necessary to obtain the most beneficial environment (Dixon and McKay, 1970).

The direct threat of water shortage to young sycamore trees does not appear to be strong, as there was no connection between reduced vwc and any of the plant physiological measures in this study. The hidden threat of drought was an increase in suitability of host plants to aphid populations and it is a factor worth future consideration. In this study, the aphid numbers were increased by mild drought responses in the sycamore, but they imposed no significant reductions on plant growth in the field. More longer term investigative work would determine whether the increasing pest load on growing and/or droughted trees, would result in a permanent effect on sycamore productivity.

4.6 Acknowledgements

The research was funded by the Natural Environment Research Council through a UK research studentship (NE/F008791/1). Our thanks are also due to W.T. Salter (University of Edinburgh, Edinburgh, UK) for field measurement of the water potential components and J. Pickup (Science & Advice for Scottish Agriculture, Edinburgh, UK) who provided the historical suction-trap data for Gogarbank, Edinburgh, UK.

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Synopsis III

In the previous chapter, it was found that *D. platanoidis* numbers were significantly correlated to plant stress indicators - low g_s, low Ψ and increased soluble N. In the next chapter, meteorological data are tested with calculated population rates of increase and key dates to investigate whether aphid responses to stress can be detected in historical data sets.

5

Analysis of sycamore aphid, *Drepanosiphum platanoidis* (Schrank) data, using meteorological variables and smoothing methods

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Under submission to *Bulletin of Entomological Research*

5.1 Abstract

Organisms with shorter life-cycles may be more closely affected by short term weather patterns and local extremes. Our research focuses on the sycamore aphid, *Drepanosiphum platanoidis* and new methods of analysis using daily meteorological data. Estimation of aphid population scenarios requires well-informed models, based on the most appropriate covariate. We utilise meteorological data for temperature, rainfall and vapour pressure deficit to investigate which has the greatest influence on aphids. Historical aphid suction-trap data is the longest dataset available for the study of aphid populations, and despite some limitations, it is a useful tool in the study of aphid distribution, phenology and activity.

This work first investigates the relationship between key population parameters and then describes a new approach to the modelling of aphid populations in relation to our selected covariates.

In order to investigate the key times of year affecting aphid populations, suction-trap data were regressed against measured meteorological variables using traditional stepwise regression and penalised signal regression. In the latter method, neighbouring data are first fitted with individual overlapping polynomial functions that fit the data closely. Where each individual polynomial crosses its neighbour this point forms a 'knot'. Over the outside curve formed by the multiple functions, a single polynomial is drawn and controlled by a smoothing parameter that balances how closely the line follows the individual splines or roughly interpolates between the knots. This permits the analyst to use daily data that are normally too closely correlated to be permitted in stepwise regression by penalising the differences in slopes between neighbouring regressors.

We find that increased minimum spring temperatures are associated with advanced dates of first catch and that increased vapour pressure deficit values are associated with longer periods of aphid presence in the traps. Since the period of aphid presence in a year is also positively correlated to the date of first catch, increasing temperatures and drier summers may combine to extend the reproductive phases of the *Drepanosiphum* life-cycle.

5.2 Introduction

Aphid phenology and its connections to climate, land use and location has been extensively researched (e.g. Cocu et al., 2005; Harrington et al., 2007; Lima et al., 2008; Estay et al., 2009; Klueken et al., 2009). Typically, weather data are aggregated to monthly or seasonal periods and analysis is focused on two population parameters: date of first catch and total aphid numbers. In this work we explore new approaches to correlation of weather data with aphid data and investigate new population parameters that may be of ecological interest.

Taking a cue from methods that are currently applied to plant phenology (Roberts, 2010, 2012), penalised signal regression is a method used to overcome the problems due to the multicollinearity of daily data using penalties. An example of how the process works is shown in Figure 5.1, which has been taken from (Eilers and Marx, 1996) where the method was first defined. Neighbouring data (for example, in a time series) are first fitted with individual overlapping polynomial functions that fit the data closely.

Where each individual polynomial crosses its neighbour this point forms a 'knot'. Over the outside curve formed by the multiple functions, a single polynomial is drawn and controlled by a smoothing parameter that balances how closely the line follows the individual splines or roughly interpolates between the knots. These techniques permit the use of complete daily datasets and will output a response curve based on the regression coefficients between daily data and neighbouring days. Sections of the curve above the zero correspond to periods of positive correlation between regressor and response, lines below indicate a negative correlation.

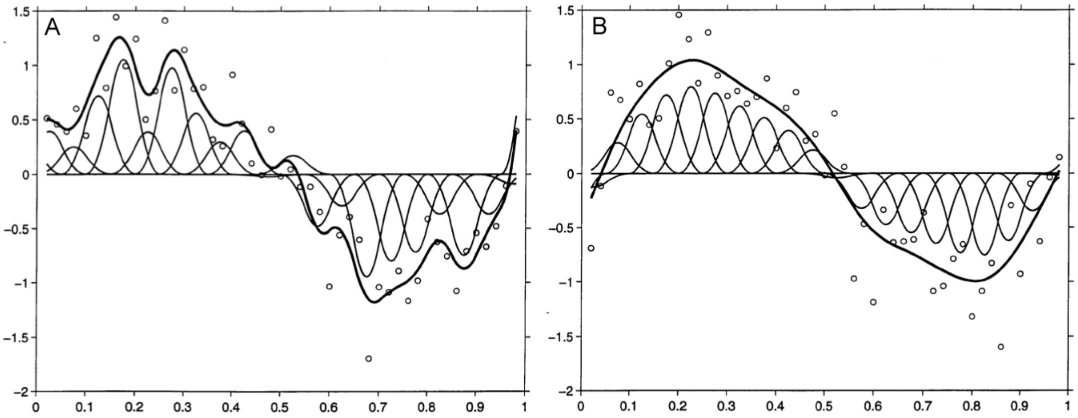

FIGURE 5.1: A graphical definition of P-splines; figures taken from Eilers and Marx (1996) utilising simulated data (open circles). (A) Individual splines are fitted to neighbouring data points. These can be close or far neighbours depending on the relationship of the points. The thick line shows the final fitted curve, which in this case has no penalty for the multicollinearity of the points. (B) In this case, individual splines have been fitted as usual, but the final fitted curve (thick line) has been calculated with a penalty and smoothing factor.

This work applies and compares two different methods of multiple regression to historical aphid suction-trap data. By not aggregating the weather data into months it is hoped that this will allow aphid models to be more accurately targeted and illuminate the key times of year where the aphid population is affected by weather events. This has not been possible when using multiple regression (Cocu et al., 2005).

In this work, the sycamore aphid, *Drepanosiphum platanoidis* (Schrank) was chosen because: (a) it spends the greater part of its life-cycle in the alate form, so is most likely to correlate with suction-trap data and (b) it is not a specific crop pest and not controlled by pesticides, therefore the data are more likely to show natural patterns.

Completing its entire life-cycle on sycamore (*Acer pseudoplatanus*, L.), *D. platanoidis* is a monoecious, holocyclic aphid. Eggs are laid in late October/November and hatch in late March. The first aphids feed on sycamore buds and spring growth, producing clones that develop into alate adults. This adult population enters an aestivation period usually between July and September. Parthenogenetic reproduction resumes in September and this generation produces the 'sexuales' (small alate males and egg-laying females). There are three distinct life-cycle 'seasons' described by Dixon (2005, p. 96). Spring can be defined as the period where population growth is exponential, summer is where the growth is more sigmoidal, whilst autumn is a return to exponential increase. We investigate whether these seasons can be defined in the suction-trap data as well as in the field and may be a starting point for more sensitive analysis of the potential effects of climate change on aphid populations.

Suction-traps of the Rothamsted network are 12.2 m tall and sample air at 0.75 m^3 s^{-1}, with daily aphid sampling between April and November. The traps are designed to catch the largest proportion of aphids in the total insect sample and to reduce the effect of surrounding land-use on the sample. The area represented by a trap may be as much as 700 km^2 (Harrington and Woiwod, 2007). As a representation of aphids in the field, suction-trap data for *Rhopalosiphum padi* (L.) have been shown to have a strong positive correlation to numbers within a crop stand (Harrington, 2002; Klueken et al., 2009). Similarly, two further cereal aphids, *Metopolophium dirhodum* (Walker) and *Sitobion avenae* (Fabricius), show strong correlations between field numbers and suction-trap data (Klueken et al., 2009). Dixon (2005, ch. 7) describes highly variable results from two different suction-traps, finding that one trap only collected sycamore aphids late in the season. We verify that the suction-trap data for *D. platanoidis* used in this study correspond with local field measurements.

The primary focus of our research is to test the potential impact of host plant water stress on aphid populations. Many of the studies referred to so far investigate measures of temperature and rainfall. A measure to combine these meteorological variables could improve relevancy as an indication of water availability to the host plant. Ideally, an implementation of a Penman-Monteith or equivalent equation would provide information on host-plant health, but the input variables required were outside the scope of this study. Atmospheric vapour pressure deficit (VPD) is a measure of the difference between the level of moisture in the air and the potential level of moisture the air can hold when saturated. It is based on a relationship between air temperature and relative humidity and is a measure of the evaporative power of the air (Allen et al., 1998; Prenger and Ling, 2001). To our knowledge this potentially simple method of estimating environmental 'dryness' has not been tested in computer-based analysis of long-term aphid population data. High atmospheric VPD values in the field represent periods of high temperature and solar radiation, with low humidity (Agele et al., 2006; Belko et al., 2012) leading to a decline in plant water status that may dehydrate aphids. Our data are of course for Scotland, therefore very high VPD values are not frequently observed. Nevertheless, the method is still applicable to any dataset.

In summary, this work investigated four questions:

1. Is there a significant relationship between *D. platanoidis* counts in the field and in the suction-trap?
2. Can alternative population parameters, such as spring rate of increase, add value to phenological analysis?
3. What effect does VPD have on aphids, and can it help explain patterns in suction-trap data?
4. Do new methods of regression analysis utilising daily data help better identify the periods of the year that determine aphid populations?

5.3 Method

5.3.1 Aphid data

Suction-trap data for *D. platanoidis* in Edinburgh, UK were kindly provided by J. Pickup (Science and Advice for Scottish Agriculture, Edinburgh, UK). Data collection for the aphid suction-trap was moved from Edinburgh East Craigs (latitude = 55.95°, longitude = −03.31°, 61 m above sea level) to Edinburgh Gogarbank (lat. = 55.93°, long. = −03.34°, 57 m above sea level) at the end of 2005. Data were available from 1969 to the present day.

Quality control of the dataset found no missing values or duplication.

In previous work, described in Simpson et al. (2012), counts were made on a stand of planted sycamore at a site on lat. = 55.92°, long. = −03.18°, 78 m above sea level. In 2010 counts were made on days in May–October; in 2011 counts were made in May–June. To determine whether the suction-trap data were representative of aphid numbers in the field, a linear regression was made for 18 matching days of record.

5.3.2 Population data

The calculation of population parameters was carried out as follows, utilising the plot shown in Figure 5.2 as an example showing sample daily cumulative totals of the aphids caught in the traps.

The date that the first *D. platanoidis* was recorded in the trap sample was the ordinal date of first catch (FC), marked on Figure 5.2 as point **a**. Daily population growth rates were calculated as in Equation 5.1

$$\text{Daily growth rate} = Ln\left(\frac{\text{aphids}_x - \text{aphids}_{\text{FC}}}{\text{date}_x - \text{date}_{\text{FC}}}\right) \tag{5.1}$$

where *aphids*$_x$ is the cumulative number of aphids on day x minus the number of aphids on the date of FC divided by the time passed since FC. The daily rate of increase rose to a peak level during spring then began to drop, signalling the entry into summer diapause; when the daily rate of increase began to rise again this signalled the end of summer and the beginning of the autumn second peak.

The end of the initial period of exponential growth was the ordinal date of the end of the spring season (ENDS1), marked on Figure 5.2 as point **b**. The spring rate of increase (S1), was estimated linearly from Equation 5.2

$$\text{S1} = Ln\left(\frac{\text{aphids}_b - \text{aphids}_a}{\text{date}_b - \text{date}_a}\right) \tag{5.2}$$

where *aphids* is the cumulative number of aphids and *date* is the ordinal day of the year at points *a* or *b* on Figure 5.2 respectively. The size of the peak population in the spring was the accumulated number of aphids caught by the end of spring, natural log transformed to give *ln* (cumulative sum of aphids during the spring season, S1C).

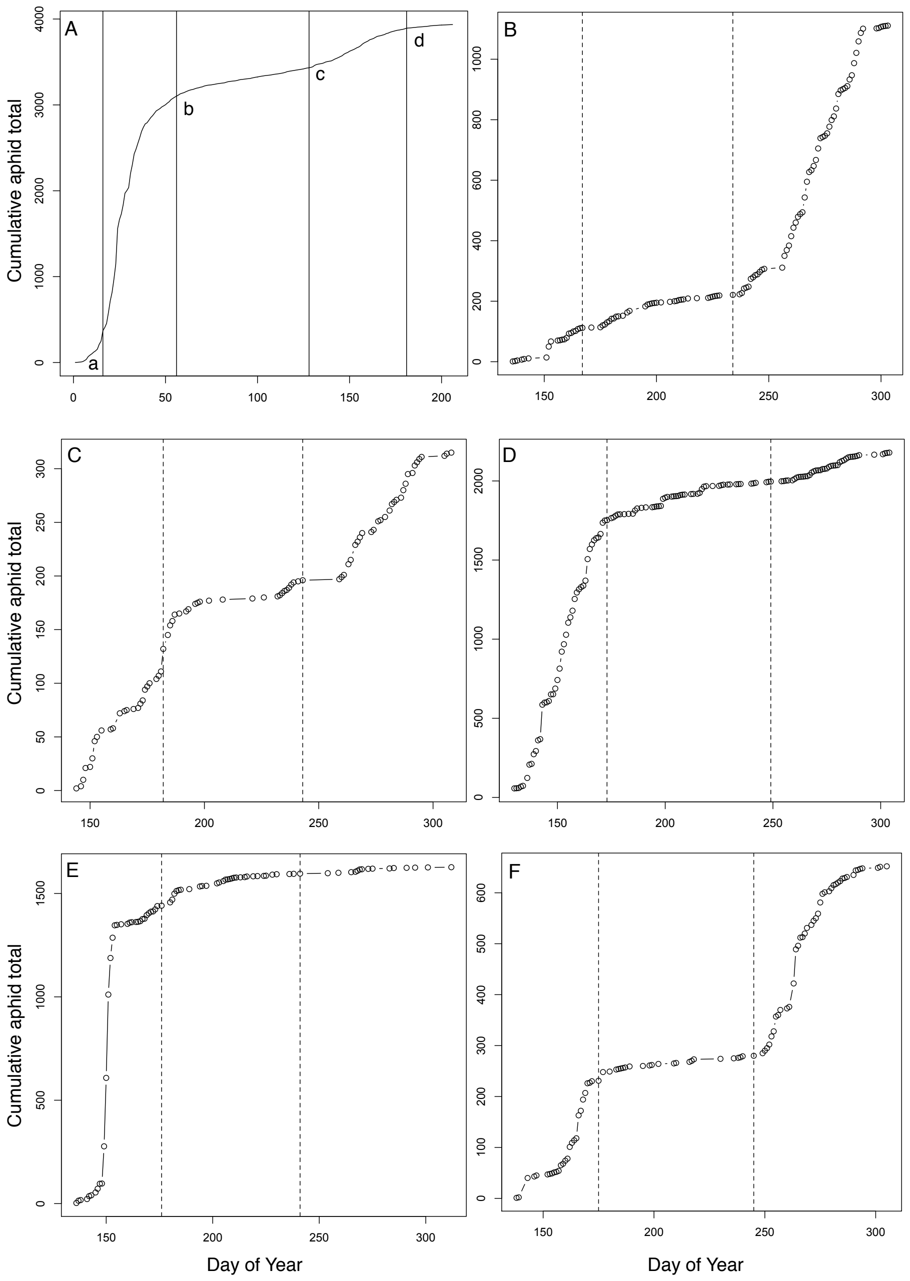

FIGURE 5.2: Example plots of aphids caught in the Edinburgh suction-trap against the day of the year showing the variety amongst years. Reference points mark **(a)** the date of first catch, **(b)** the end of aphid spring, **(c)** the end of aphid summer, **(d)** the end of aphid autumn. (A) Plot of mean cumulative sums over 32 year period shows a typical pattern (fits 69 % of years). (B) Data for 1972 with large autumn increase (C) Data for 1977 with evenly stepped periods. (D) Data for 1982 with typical pattern. (E) Data for 1985 with very steep increase in spring. (F) Data for 1989 with stepped pattern and reference points marked.

As in Figure 5.2, ordinal date of the end of the summer season, ENDS2, was the turning point where population growth was no longer approximately static (Figure 5.2, point **c**). The summer rate of increase/decrease, S2, was estimated linearly from Equation 5.3

$$\text{s2} = Ln\left(\frac{\text{aphids}_c - \text{aphids}_b}{\text{date}_c - \text{date}_b}\right) \tag{5.3}$$

where *aphids* is the cumulative number of aphids and *date* is the ordinal day of the year at points *b* or *c* on Figure 5.2 respectively. Similar to the spring catch, the summer catch was the *ln* (cumulative sum of aphids during the summer season), S2C. The last date before the aphid catch dwindled to single aphids per day with greater than three days between catches was defined as the ordinal date of the end of the autumn season (ENDS3) (Figure 5.2, point **d**). The total number of aphids caught in the trap over a single year (Figure 5.2, point **d**) was transformed by natural logarithms, for comparison with work by Dixon (2005, chapter 5). This gives *ln* (total catch, TC). The total length of aphid season (TS) was simply (date_d–date_a), where *date* is the ordinal day of the year at points *d* or *a* on Figure 5.2 respectively. The autumnal rate of increase (S3) was therefore estimated linearly from Equation 5.4

$$\text{s3} = Ln\left(\frac{\text{aphids}_d - \text{aphids}_c}{\text{date}_d - \text{date}_c}\right) \tag{5.4}$$

where *aphids* is the cumulative number of aphids and *date* is the ordinal day of the year at points *c* or *d* on Figure 5.2 respectively. The *ln* (cumulative sum of aphids during the autumn season), S3C was also calculated.

5.3.3 Weather data

Daily maximum and minimum air temperature, rainfall, wind speed and relative humidity data were obtained from UK Meteorological Office (2006). Since the aphid suction-trap was moved from Edinburgh East Craigs to Edinburgh Gogarbank at the end of 2005, corresponding weather data for the two locations were sourced. The East Craigs dataset runs from 1958–2005, and the Gogarbank dataset from 1998 to the present day. Quality control on the weather data found that after 2003 the data were not supplied daily and the rainfall values were aggregates of several days. In addition, the logging of wind speed and humidity began in 1972. This limited the analysis period to 01/01/1972–31/12/2003, so the available number of years was 32. There were no missing values over that period, but due to errors at the weather station there was some duplication of dates with faulty, followed by corrected readings. The inbuilt MIDAS quality control system (Met Office, Devon, UK, 2012b) was used to select only the data points with confirmed values.

Daily VPD values were calculated from the dry-bulb temperature values combined with the relative humidity values according to the Arrhenius equation (Table 5.1). The relationship is not linear, but in general increased humidity and reduced temperatures give low VPD, whilst lower humidity and warmer temperatures give higher VPD.

Table 5.1: The Arrhenius equation: $e^{(A/T + B + C + DT^2 + ET^3 + F\ln T)}$, where T = temperature of the air in Rankine, and the constants A–F as listed below. The result is VPD in psi, which is then converted to kPa.

Constant values			
A	-1.044×10^{4}	D	1.289×10^{-5}
B	-1.129×10^{1}	E	-2.478×10^{-9}
C	-2.702×10^{-2}	F	6.456

5.3.4 Regression modelling

All analyses utilised the R statistical computing program, version 2.13.0 (R Development Core Team, 2011). Correlograms, which determine the relationship between observations separated by time, were run using the *acf* and *pacf* functions (R Development Core Team, 2011) to test whether any apparent trends for a serial correlation between years were significant. Results indicating density-dependence or moving average terms would alter the model parameters.

Stepwise regression

A stepwise regression using a linear model of the population terms against monthly data were run using the *step* function (R Development Core Team, 2011). The stepwise search was set to allow both backwards and forwards term selection from a null model. The Bayesian Information Criterion (BIC) was used to select the model. For each response variable, the monthly mean minimum temperature, mean VPD and total logarithm to base 10 ($\log_{10}$ rainfall) were analysed together in the same stepwise model. The data for the six months previous to the selected key dates were used, e.g. January–June meteorological data and 'Year' matched to FC, S1, S1C and ENDS1.

Penalised signal regression (*P*-spline)

In penalised signal regression, the penalty is on the squared differences between the regression estimates for consecutive pairs of days (Equation 5.5). This approach results in a smooth profile of regression coefficients and the degree of smoothness is controlled by the smoothing parameter, λ. Here a particular variation on penalised signal regression is employed, using *P*-splines (Marx and Eilers, 1999; Roberts, 2008). This facilitates the use of many covariates. The *mgcv* (Multiple Smoothing Parameter Estimation) package (Wood, 2003, 2004, 2006, 2008) in R was used to implement this method following the advice of Roberts (2012) in setting parameters.

$$\sum_i (y_i - \widehat{y}_i)^2 + \lambda \sum (\widehat{\beta}_i - \widehat{\beta}_{i-1})^2 \tag{5.5}$$

where y_i is the response for observation i, $\widehat{y}_i$ are the prediction values for observation i, λ is the difference penalty, β_i is the regression coefficient for the observation i and $\widehat{\beta}_{i-1}$ is the regression coefficient for the prediction values for observation $i - 1$.

For each response variable, the latest recorded relevant date, e.g. latest date of first catch, was used as the final date in the penalised signal (*P*-spline) dataset. Three separate analyses of periods 365-, 180- and 90-days-previous were carried out using minimum temperature, VPD and logarithm to base 10 ($\log_{10}$ rainfall) singly and in all combinations.

Cross-validated R^2 values were calculated using generalised cross validation (GCV) criterion according to Equation 5.6

$$1 - \left(\frac{\mathrm{n{\cdot}D/(n - d.f.)^2}}{\mathrm{var}} \right) \tag{5.6}$$

where *n* = number of data, *D* = deviance, *d.f.* = effective degrees of freedom of the model and *var* = variance of the data. The GCV is also used to select the optimal value of λ in the regression.

5.4 Results

5.4.1 Comparison with field data

During field work in 2010 and 2011 (as described in Chapter 4), aphid counts were made on sycamore saplings. These data were paired by observation day with the Gogarbank suction-trap data. The range of dates could be improved with more counts from July–September, but there is reasonable correspondence between flight and field data (Figure 5.3). As representative samples of the proportion of aphids present in the area, both flight and field data can be regarded as fair measures of population, both in spring, when aphids are abundant and in summer when the aphids are less so.

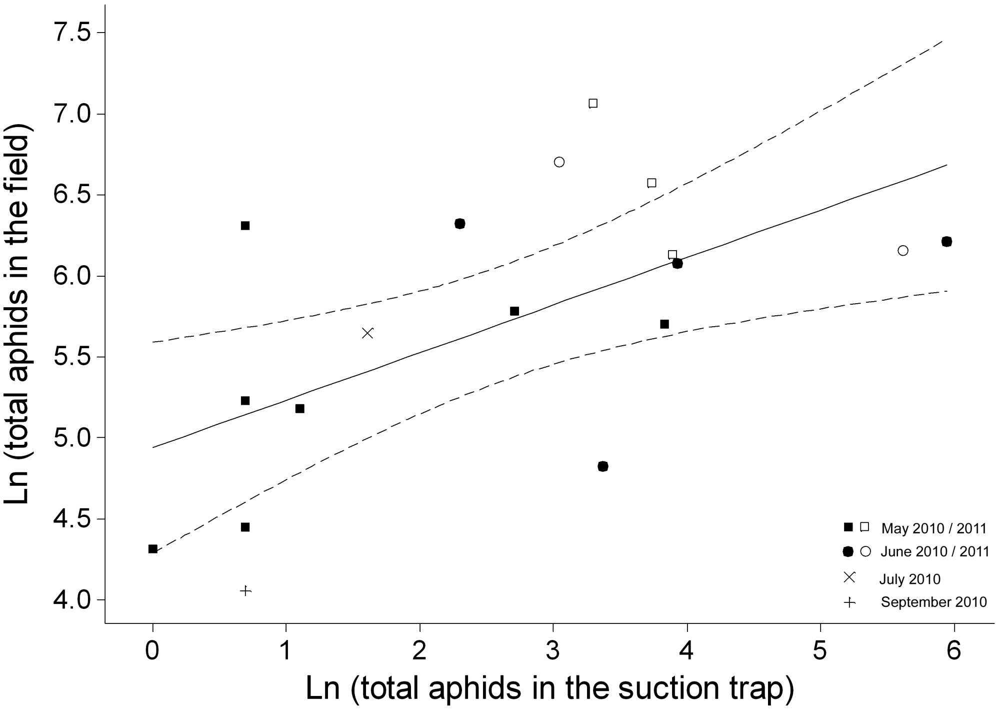

Figure 5.3: During May–September 2010 (filled dots) and May–June 2011 (unfilled dots) data were paired by observation day with the Gogarbank suction-trap data. Each point on the graph represents a unique day within this time-frame. The linear relationship: *Ln* (field count) = 4.942 + 0.2934 *Ln* (trap count) with $r^2 = 0.32$ was significant, $p = 0.005$.

5.4.2 Response variables over time

Calculated dates for the aphid seasons were relatively stable over time, Figure 5.4. FC was trending to become earlier with time ($r^2 = 0.40$, $p < 0.001$), implicating an exogenous effect. ENDS1, ENDS2 and ENDS3 were approximately stable over the 32 year period. As can be seen in Figure 5.5, the TC was relatively stable over time. S1C showed a very similar pattern to S1, large numbers of aphids were combined with fast population increases. S2 showed no pattern with low variation. S3 had a broad range, but was essentially static over time. TS was trending to become later ($r^2 = 0.19$, $p < 0.001$), with one significant outlier. For context, full leaf flush for sycamore in southern England is on average around day 91 (Thompson and Clark, 2008, ref: Marsham series).

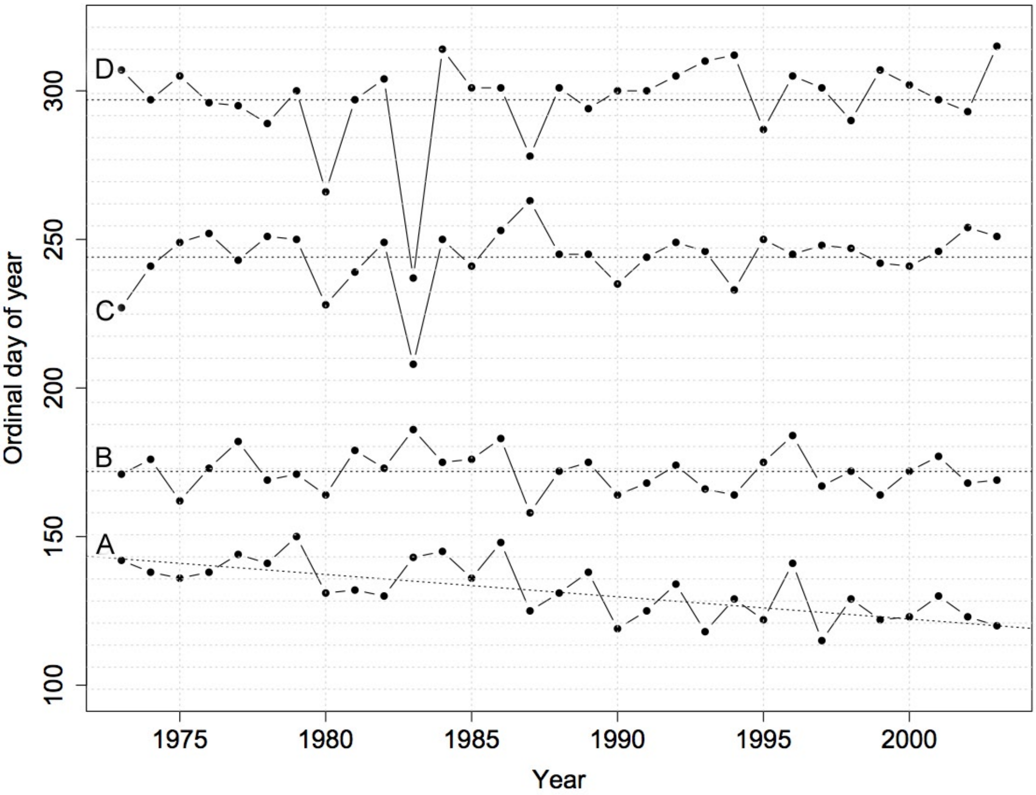

FIGURE 5.4: Calculated dates of aphid seasons by year with trends if present. If there was no significant trend, then the 32-year average date was marked with a horizontal black dotted line. (A) Date of first catch, $r^2 = 0.41$, $p < 0.001$. (B) Date of the end of the spring season. (C) Date of the end of the summer season. (D) Date of the end of the autumn season or date of last catch.

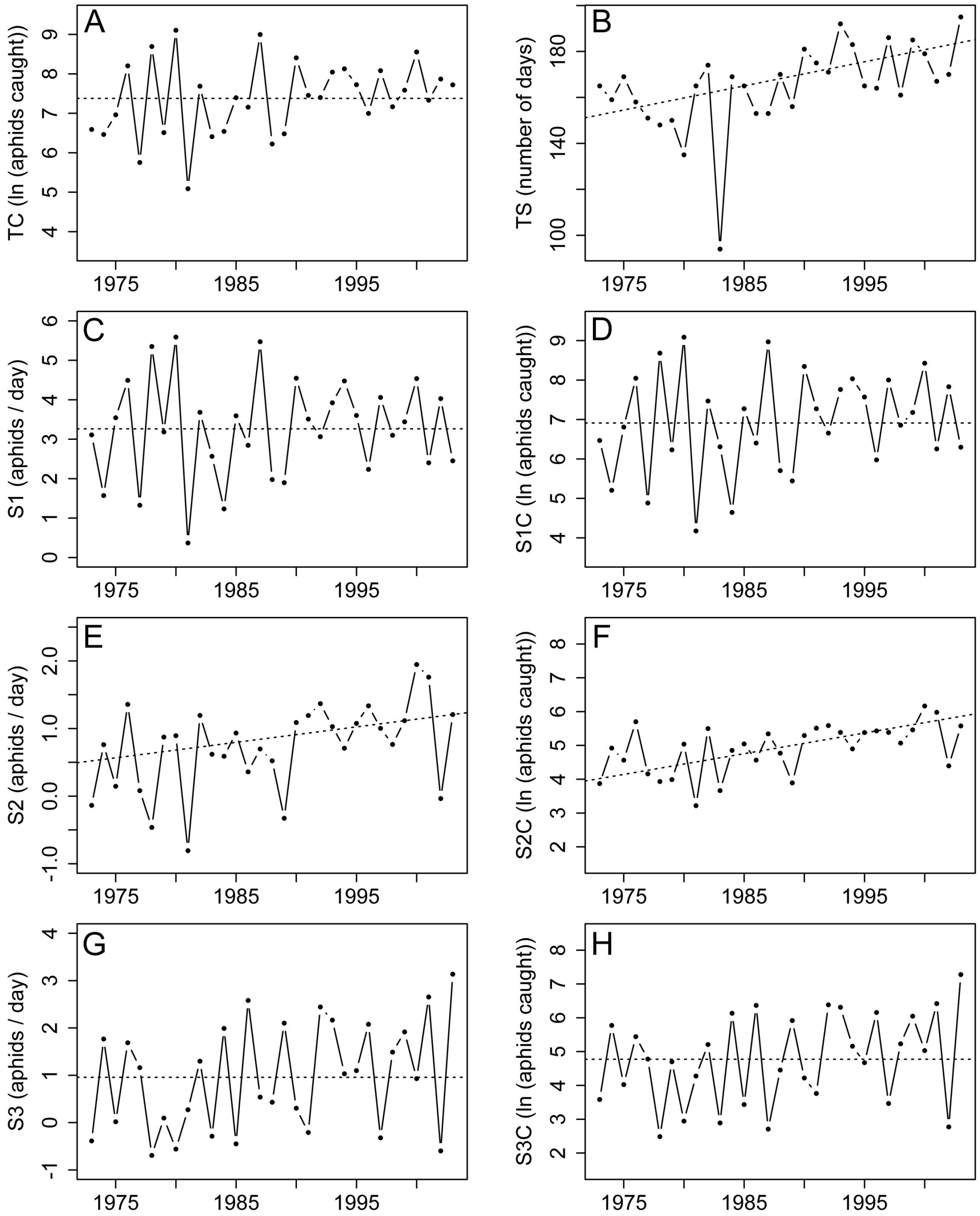

Figure 5.5: Population sizes, rates of increase and length of season by year with significant linear trends if present (A) *Ln* (total catch), TC. (B) Total length of season, TS: $r^2 = 0.19$, $p < 0.001$. Removal of the outlier would improve the r^2 to 0.29. (C) Spring rate of increase, S1. (D) *Ln* (cumulative total of aphids in spring), S1C. (E) Summer rate of increase/decrease, S2: $r^2 = 0.20$, $p = 0.008$. (F) *Ln* (cumulative total of aphids in summer), S2C: $r^2 = 0.27$, $p = 0.002$. (G) Autumnal rate of increase, S3. (H) *Ln* (cumulative total of aphids in autumn), S3C.

Correlogram analysis was inconclusive. Some patterns were suggestive of autoregressive functions, Figure 5.6, but the significances are slight and further data would be required for certainty. Partial auto correlation functions for population sizes, rates of increase and length of season by year, indicate that there were no moving average terms. The most likely pattern is for the spring values, which show a consistent trend for negative correlation from year to year.

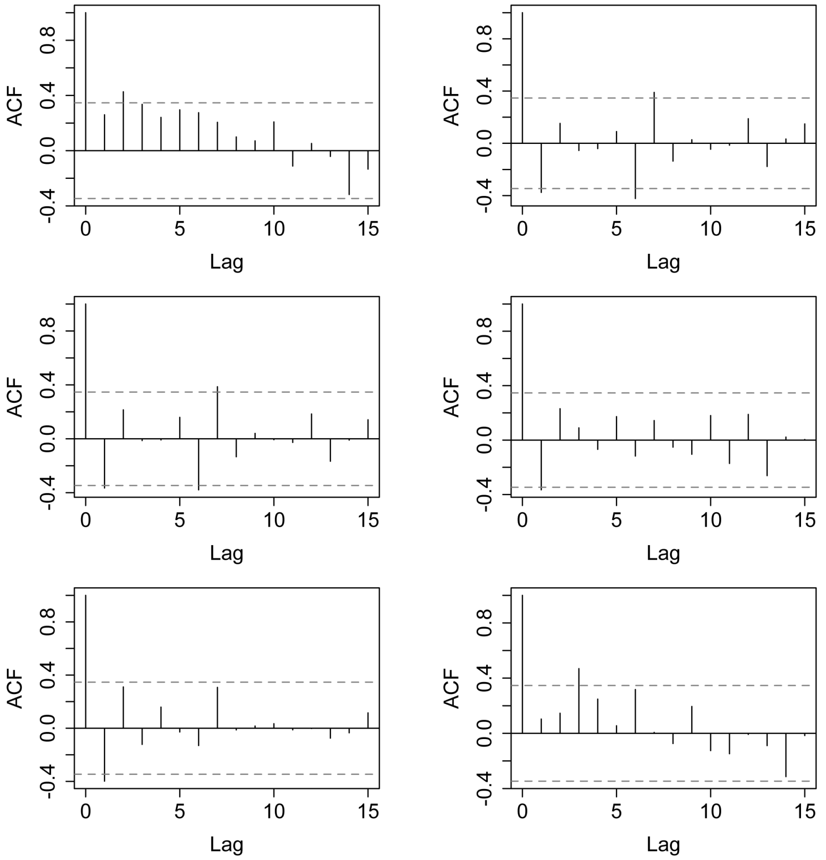

Figure 5.6: Auto correlation functions for the aphid response variables showing potential correlations. Bars exceeding the dotted line indicate correlation with previous year values. (A) There was a potential short term correlation in fc. (B) There was an erratic negative correlation for s1. (C) The pattern for s1c was similar to s1. (D) A potential negative relationship with previous years for s3. (E) A possible correlation for tc showed a consistent but not fully significant negative correlation. (F) Short term correlation was possible for ts.

5.4.3 Correlation of response variables

Given the patterns in Figures 5.4 and 5.5, all population parameters were plotted against each other in Figure 5.7 to identify significant relationships as a basis for further analysis. The clearest correlations of **tc** are with s1, fc, s1c, ends1 and s2c. The spring catch of aphids contributes most to the total catch of aphids in the year. Where the population has increased faster in the spring, there are more aphids in total. Earlier dates of first catch and earlier endings to the spring season tend to be associated with greater total catches of aphids. Notable correlations of **fc** are with s1, s1c, ends1 and ts. Large initial aphid abundances increase the likelihood of an aphid being caught in the suction-trap, leading to some automatic relationship between catch size and early first catch. However, correlations also indicate that earlier fc are associated with an earlier end to spring and longer flight seasons. The **s1** is correlated with tc, fc, ends1 and s1c. Faster rates of increase are associated with larger total and spring catches, earlier first catch and later entry into the summer diapause. **S1c** is correlated to tc, fc, s1 and ends1. **Ends1** is correlated to tc, fc, s1 and s1c. **Ends2** has no correlations, whilst **s2** and **s3** are correlated only to their respective s2c and s3c values. **Ends3** is correlated to ts, while **ts** is correlated to fc and ends3. The **s3c** of one year is correlated to the **s1c** of the next year ($r^2 = 0.34$, $p<0.001$).

5.4.4 Results of the stepwise regression

The appropriate aggregated monthly values for minimum temperature, vpd and $\log_{10}$ rainfall were run through the stepwise regression together. The results are summarised in Table 5.2. None of the models selected have an r^2 greater than 0.7, so there is room for improvement in the method and parameter selection. The bic selection of terms reduces the number of terms selected, but cross-validation is not used in the stepwise regression, so the results are likely to be overly optimistic.

For **tc** the stepwise regression technique found the mean minimum temperature in April to be the only relevant term. There was a significant effect of time with additional effects of mean minimum temperatures in January–April and the vpd in January and March for **fc**. The regression for **s1** with monthly data selected no terms, whilst **s1c** showed a slightly effect of March vpd. The **ends1** regression contained effects of time,

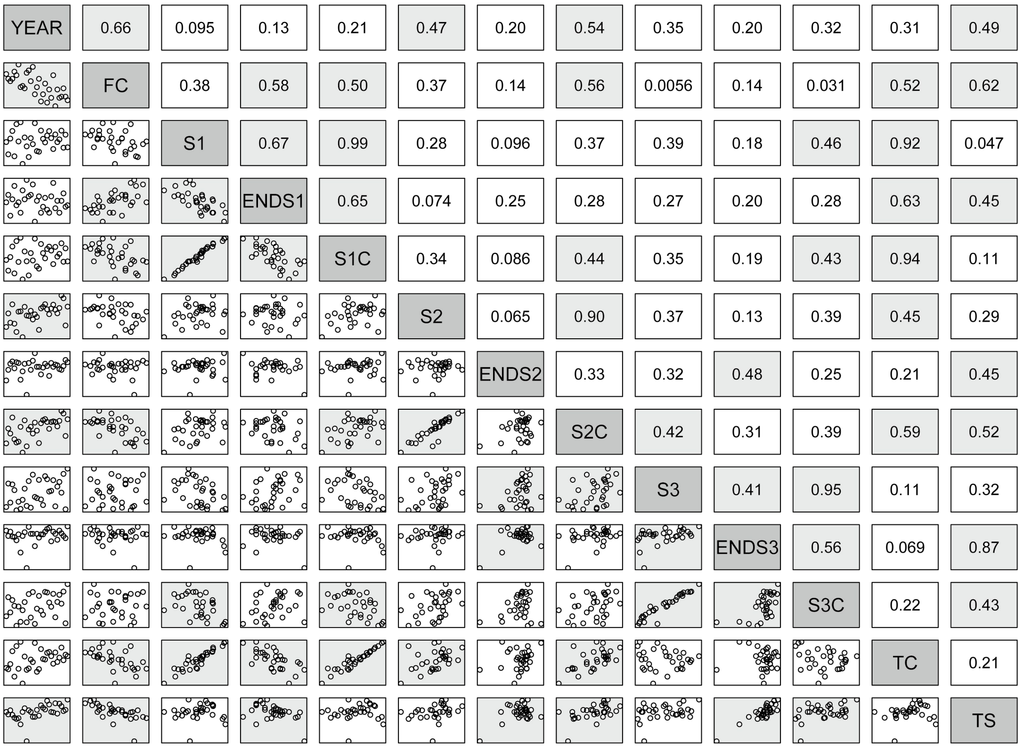

FIGURE 5.7: The paired correlation table matched each measured factor against the other and provides Pearson correlation values for each relationship with the strongest correlations on a greyed background.

April minimum temperatures and March VPD. The mean minimum temperature in April contributed to S2. For ENDS2, the mean minimum temperature in September and the mean VPD in June and August were selected. The S3 monthly regression found that the mean minimum temperature in June, July and September with the mean VPD in September and October and total $\log_{10}$ rainfall in July contributed. The monthly regression found that the mean minimum temperature in August and September with the mean VPD and total $\log_{10}$ rainfall in June, August and September contributed to ENDS3. The consistency of monthly effects is an indicator that August and September meteorology may have an effect on the aphid population. There was a consistent August and September effect on TS.

Table 5.2: Results of the stepwise regression for the aphid population parameters.

	Covariate p-values				Model			
Variable	Year	Min. temp.	VPD	$\log_{10}$ rainfall	d.f.	F	R^2	p
FC	0.04	JAN = 0.05 FEB = 0.08 MAR = 0.02 APR = 0.10	JAN = 0.07 MAR = 0.04		6, 25	9.63	0.63	<0.001
S1								
ENDS1	0.02	APR = 0.01	MAR = 0.04		3, 28	3.72	0.21	0.023
S1C			MAR = 0.05		1, 30	4.13	0.09	0.051
S2		APR = 0.01			1, 30	8.07	0.19	0.003
ENDS2		SEP = 0.02	JUN = 0.01 AUG = 0.01		3, 28	4.99	0.28	0.007
S2C		APR = 0.01	MAY = 0.05	APR = 0.07	3, 28	7.56	0.39	0.001
S3		JUN = 0.12 JUL = 0.04 SEP = 0.01	SEP = 0.09 OCT = 0.02	JUL = 0.02	6, 25	5.42	0.46	0.001
ENDS3		AUG = 0.01 SEP = 0.01	JUN = 0.03 AUG = 0.01 SEP = 0.02	JUN = 0.01 AUG = 0.02 SEP = 0.08	8, 23	4.89	0.50	0.001
S3C	0.01	JUN = 0.01 JUL = 0.03 AUG = 0.15 SEP = 0.01 NOV = 0.01	JUL = 0.01 SEP = 0.11 OCT = 0.01 NOV = 0.13	JUN = 0.01 AUG = 0.05	12, 19	6.89	0.70	<0.001
TC		APR = 0.06			1, 30	3.88	0.08	0.058
TS	0.15	AUG = 0.03 SEP = 0.01	JUN = 0.01 AUG = 0.02 SEP = 0.10	JUL = 0.04 AUG = 0.01 SEP = 0.08	8, 23	5.74	0.55	<0.001

5.4.5 Results of the *P*-spline regression

A summary table of the cross-validated R^2 for the *P*-spline regressions is presented in Table 5.3. No results are shown for S1, S1C or S2, since no significant terms were selected under any combination of meteorological variables.

The results for FC show that the *P*-spline regression with the greatest cross-validated R^2 was the 365 day period previous to the date of first catch combining minimum temperature, $\log_{10}$ rainfall and VPD. The most important component was minimum temperature (▲ in Table 5.3) and increasing the smoothing factor improved the cross-validated R^2 from 55.4 to 57.0, with results presented in Figure 5.8. Higher minimum temperatures in February through to April were correlated to earlier FC.

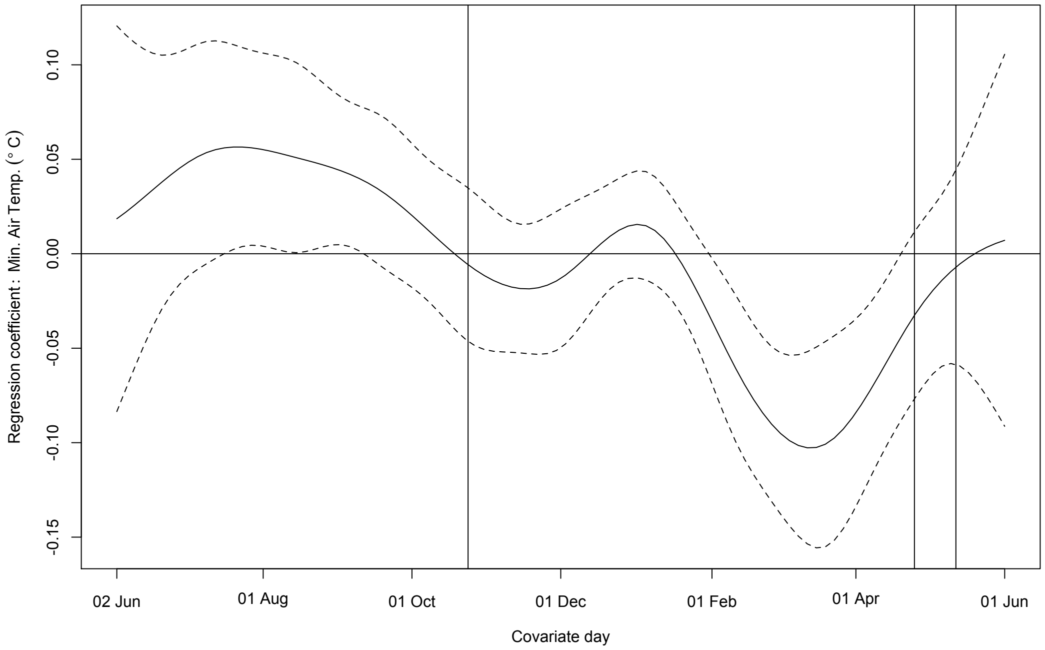

FIGURE 5.8: The 365 day *P*-spline model for FC. Dotted lines indicate 2 standard errors around the coefficient, and the vertical lines mark the mean date of ENDS3, earliest FC and mean date of FC. Minimum temperature showed a clear dip in the fit profile from February–April.

Table 5.3: Cross-validated R^2 of the *P*-spline regression for the aphid population parameters. Three separate analyses were run for 90-, 180- and 365-day periods using a smoothing factor of *number of years −2*. (*) values are the 'best' value for the response variable. S1, s1c and s2 showed no significant association with any of the variable over any time period. (▲, ♦, ■) values are for the component contributing most to the fit and are detailed in the text.

90 day period

Response	Temp	VPD	Rain	Temp +VPD	Temp +Rain	Rain +VPD	Temp+VPD +Rain
FC	50.8	28.8	-9.8	55.5	55.3	6.2	35.0
ENDS1	0.2	-7.8	9.0	-10.6	-6.2	-5.5	0.8
ENDS2	-2.5	7.4	-8.5	-1.0	-12.8	-1.2	6.3
S2C	-3.8	-9.7	-3.7	-11.3	14.8	-9.3	19.7
S3	1.0	-7.3	-6.9	-6.2	9.5	-8.4	4.1
ENDS3	-0.2	-4.3	-2.8	-5.3	14.9	7.2	*17.9
S3C	3.0	-9.3	4.9	-4.0	32.1	7.6	29.1
TC	-8.3	-10.3	*♦20.3	-16.2	14.4	15.0	-4.52
TS	17.2	-2.9	-10.6	11.9	9.7	-8.8	9.7

180 day period

Response	Temp	VPD	Rain	Temp +VPD	Temp +Rain	Rain +VPD	Temp+VPD +Rain
FC	38.9	30.1	-10.5	38.2	35.0	25.5	33.9
ENDS1	-4.5	-8.8	19.3	-11.0	*28.1	20.0	23.4
ENDS2	-5.4	-10.6	*18.2	-13.2	13.1	9.5	-6.8
S2C	18	3.2	-8.2	16.8	18.8	7.2	15.4
S3	-4.9	-9.1	-6.3	-12.5	-6.4	-10.0	-13.5
ENDS3	-3.4	-5.6	3.4	-10.2	-2.7	-5.5	-9.1
S3C	-3.4	-9.7	-7.3	-10.8	-7.1	-13.9	-14.3
TC	-10.4	-7.0	-10.5	-14.3	-18.3	-14.9	-22.8
TS	8.0	18.9	-6.2	13.5	5.2	14.7	9.7

365 day period

Response	Temp	VPD	Rain	Temp +VPD	Temp +Rain	Rain +VPD	Temp+VPD +Rain
FC	▲55.4	12.2	-10.2	67.8	53.0	6.1	*68.2
ENDS1	8.0	-10.2	-9.2	1.6	6.9	-17.2	0.6
ENDS2	-10.5	-9.8	-8.0	-21.5	-15.7	-15.6	-24.2
S2C	6.1	-8.5	-9.1	1.3	18.7	-15.1	17.3
S3	-7.1	-8.3	16.6	-18.7	*21.1	7.1	20.0
ENDS3	-8.7	-5.0	-1.4	-6.1	-9.7	-12.8	-6.1
S3C	-6.4	-8.2	27.5	-12.3	24.5	6.4	22.8
TC	-9.9	-10.6	-0.6	-17.9	-13.1	-7.6	-15.6
TS	9.3	*■19.6	-7.0	17.3	6.1	18.1	15.6

The TC data showed weak evidence for a relationship with $\log_{10}$ rainfall. The *P*-spline regression with the greatest cross-validated R^2 was the 90 day period previous to the date of last catch utilising $\log_{10}$ rainfall (♦ in Table 5.3). The original model utilised a low smoothing factor; increasing the smoothing factor improved the cross-validated R^2 from 20.3 to 25.1, the result is presented in Figure 5.9. Based on the stepwise regression, there is a case for finding a way to combine temperature effects from earlier in the year with rainfall and VPD effects from later in the year. Greater rainfall in August and September was correlated to greater TC.

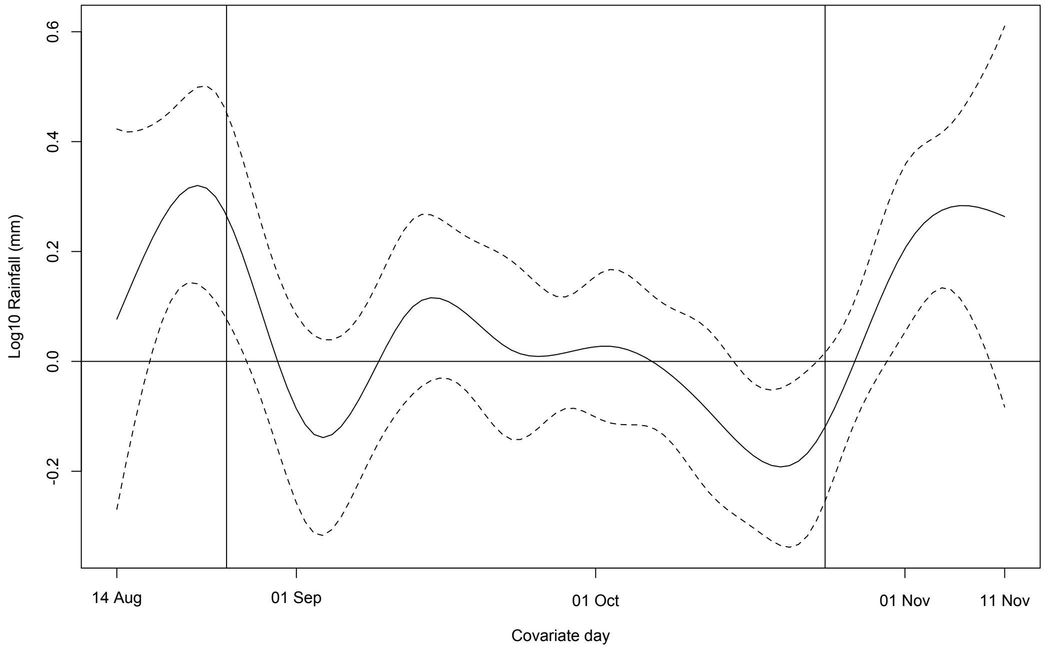

FIGURE 5.9: The 90 day *P*-spline model for TC, with vertical line marking the earliest and the mean ENDS3. The $\log_{10}$ rainfall component was significant around the end of summer and just before the dates of last catch.

The TS *P*-spline regression with the greatest cross-validated R^2 was the 365 day period of VPD prior to the latest date of ENDS3 (■ in Table 5.3). Increasing the smoothing level improved the cross-validated R^2 from 19.6 to 22.7 and is presented in Figure 5.10. This figure indicates that the most important impacts on TS are VPD in March–May (positive) and August–September (negative).

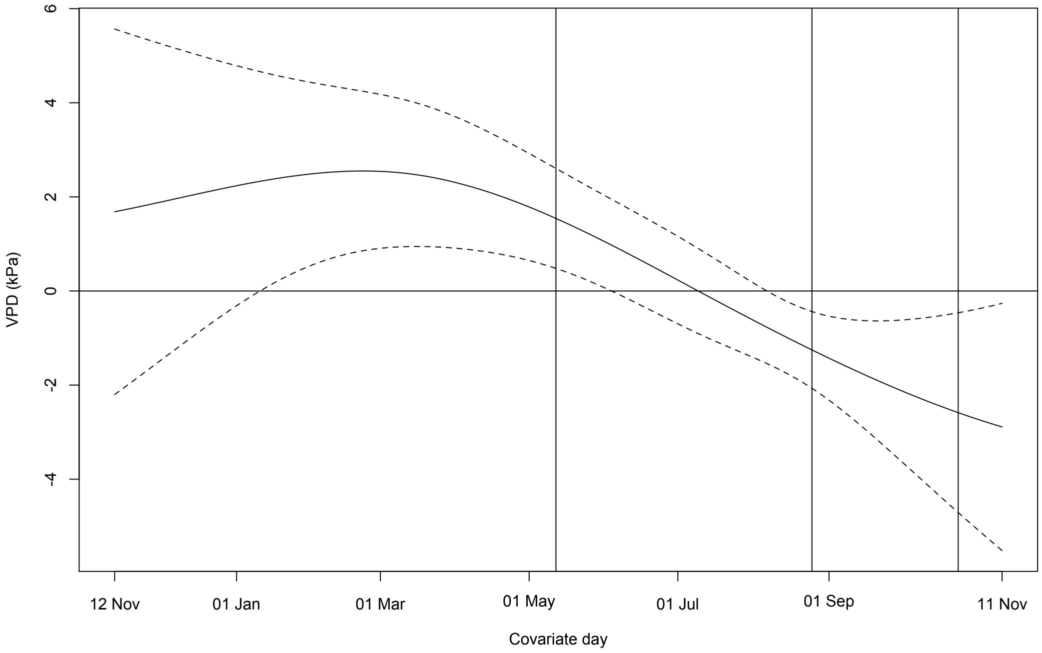

Figure 5.10: The 365 day regression results for TS. Dotted lines indicate 2 standard errors around the coefficient, and the vertical line marks the mean FC, earliest ENDS3 and mean ENDS3 respectively. The pattern for VPD has a positive relationship in March–May with a negative relationship in August–September.

5.5 Discussion

To analyse long term aphid datasets, it is useful to be able to correlate suction-trap data to numbers in the field. Whilst some cereal aphids have been shown to correlate (Harrington, 2002; Klueken et al., 2009), our work suggests that *D. platanoidis* field and suction-trap data correlate as well.

Most of the population parameters were closely correlated to each other, with TC closely associated with the spring rate of increase and the date of first catch. The *P*-spline analysis found that spring temperatures combined with autumn rainfall and VPD also contribute to TC. The close relationship between population parameters indicate that if only one of the factors is influenced by the climate, there is the potential for a domino effect into the other factors. Of particular note are the data for FC. It must be taken into consideration that FC is closely associated to abundance, in that the probability of catching an aphid in the suction-trap is increased when initial spring populations are high. If the temperatures and vapour pressure were to change in the key months, leading to earlier FC, this could be associated with an increased total catch of aphids, faster rates of increase in the spring, a greater number of aphids in the spring and a longer length of aphid season. The summer diapause would likely begin earlier. Thus, the risk of asynchrony developing (Dixon, 2003) between the phenology of aphid life-cycles and the host plant appear to be low.

With the relatively short series of 32 years, it was not possible to be certain from the correlogram data that a single significant correlation over time had not occurred by chance (Chatfield, 2003, p. 24). However, experimental research could elucidate the relationships between population factors in consecutive years or examine on a seasonal rather than annual scale.

Another point to note is the annual advancing trend of FC. Some data are available for the study of tree phenology (Woodland Trust, UK, 2012), but these are averaged over the whole of the United Kingdom and the dataset has large sections of missing information that prevent a full comparison with the aphid or climate data. Alternatively there are very long datasets such as the Marsham series (Stratton, Norwich, lat. = 52.43°, long. = 1.16°) with sycamore data spanning 143 years (Thompson and Clark, 2008). Although not specific to Edinburgh, the biological response of sycamore to climate warming will hold, and it is clearly shown by Thompson and Clark (2008) that sycamore flushing will advance at least 10 days with a 1.5 °C increase in February and March.

There is evidence that whilst the autumnal population of aphids is closely associated to the size and health of the spring population (Dixon, 2005, ch. 7), there is also a meteorological effect on the aphids in August to November. This effect is associated with rainfall and VPD levels that can trigger the end of the parthenogenetic season. This could result in knock-on effects the following year as the S3C of one year is correlated to the S1C of the next year.

The length of season was intriguingly associated with FC and ENDS3. If FC was later, the total season shortened and this was realised through an earlier end to the autumn season. The year with the shortest season, 1983, had a generally average weather pattern. The year with the longest season, 2003, was also one of the worst droughts for Scotland (CIWEM 2012). It had a much more extreme weather pattern with the VPD measure detecting significant atmospheric 'dryness' in the June of 2003 when rainfall was approximately 50 % of average (Met Office, Devon, UK, 2012a) set between two months of lower temperature, moister conditions. This pattern coincides with the largest and fastest autumn population increase of aphids as well as the longest aphid season recorded. There is potentially some support for the further investigation into 'Climatic Release' (Martinat, 1987; Mattson and Haack, 1987), particularly considering significant extreme weather events of short duration that may not appear in monthly weather summaries.

Observationally, years with extremes of VPD and lengthy warm periods are associated with larger TC, earlier FC and greater S1, S2, S3. An empirical validation of these apparent trends would be worthwhile.

The regression methods fitted the aphid data best with a blend of the meteorological regression covariates. Minimum temperature was often selected in the stepwise regression, but had fewer distinctive correlations in the P- spline analysis. Monthly $\log_{10}$ rainfall was selected least often in the stepwise regression, but worked well in combination with minimum temperature and VPD under P- spline analysis. The VPD values were selected regularly in the stepwise regression and often in the P- spline results. An estimate of the canopy temperature would greatly improve the accuracy of the VPD calculation, but this was not available from the MIDAS dataset. Alternatively, calculated drought indices (Palmer, 1965; Phillips and McGregor, 1998; Lloyd-Hughes and Saunders, 2002; Niemeyer, 2008) may be more suitable regression covariates in determining effects of water shortage.

Overall, the analysis showed that stepwise regression using aggregated monthly data was an adequate method for aphid population analysis, but may have been more optimistic than justifiable. The definition picked up several of the same effects as the P- spline regression. The daily data took into account the relationship from day to day, but there was no rule for selecting the lengths of period to cover. All periods from 60–365 days had validity for different response variables.

This research has found that daily data can illuminate effects on aphid populations occurring in a greater variety of time-frames than those traditionally studied. Different annual patterns of meteorology can be assimilated to identify seasonal triggers for the *D. platanoidis* population. There is also a basis for investigation of the impact of extreme weather events on the population, that could be studied further. VPD was found to be a potentially useful combination of climate variables that could be more relevant to insects that are unable to regulate body moisture and rely on their host and the environment for balance. As spring advances, so too does the date of *D. platanoidis* first catch, bringing longer seasons and increased total catches. If this were to hold true for aphid species with similar life-cycles, climate change could significantly alter pest population dynamics.

5.6 Acknowledgements

The research was funded by the Natural Environment Research Council through a UK research studentship (NE/F008791/1). Our thanks are also due to J. Pickup (Science & Advice for Scottish Agriculture, Edinburgh, UK) who provided the historical suction-trap data for Gogarbank, Edinburgh, UK.

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Synopsis IV

In the previous chapter, it was found that *D. platanoidis* population response variables were related to each other and could also be affected by climate. The testing of penalised spline regression on daily values for temperature, rainfall and vapour pressure deficit proved interesting and provided a basis for further development. The following conclusions and discussion section will bring together the body of work presented so far.

6

Discussion and conclusions

6.1 Summary

Using both controlled environment and field experiments in combination with long-term data analysis, the hypotheses tested by this work were:

1. Plants under stress offer an altered diet to aphids, these changes were hypothesised to be favourable to aphid survival (Plant Stress Hypothesis (PSH)).
2. As aphids feeding on plants are an additional potential obstacle to a stressed plant's survival, it was hypothesised that aphid infestation would cause greater damage to already stressed host plants. Well-watered plants may have the ability to defend against aphid infestation or mitigate its effects.
3. It was hypothesised that daily climate data may be of more benefit to studies of aphid population data than aggregated data, given shorter individual aphid life-cycles may respond to short term rather than monthly patterns.

6.2 Effect of changing diet quality

In two out of three experiments, the PSH had some support, with greater aphid populations on water-stressed host plants. *Metopolophium dirhodum* (Walker) and *Drepanosiphum platanoidis* (Schrank) populations were larger and increased faster on stressed host plants whilst *Myzus persicae* (Sulzer) populations were larger and more fecund on well-watered than droughted hosts, **Figures 2.4, 3.3 & 4.6**.

The success of *M. persicae* on well-watered host plants does not support the PSH. The host plants were physically affected by the drought treatment with reduced leaf area and growth above ground, **Figure 2.8**. However, with no specific measure of phloem sap quality, we cannot be certain that diet quality was associated with aphid performance. The leaf osmotic potential (Ψ_π) represents an increase in the concentration of soluble compounds released to the phloem sap, but offers no estimate of leaf soluble nitrogen (N). Tests to establish whether there is a correlation of soluble N and leaf chlorophyll content with portable chlorophyll meter readings, such as those in van den Berg and Perkins (2004), would be valuable, establishing further non-destructive methods of plant analysis.

Concentrating on the cases of *M. dirhodum* and *D. platanoidis*, changes in diet quality under mild water stress led to an increased number of aphids. A combination of mechanical and physiological advantages enabled the aphid populations to survive better in these instances. Aphid numbers were correlated with reduced stomatal conductance (g_s), leaf water potential (Ψ) and increased soluble N, **Figures 4.4 & 4.5**. Reduced g_s can be viewed either as a symptom of drought stress or of aphid infestation, and may be the result of accumulating increases in abscisic acid (ABA) production. Although too great a decrease in leaf turgor would be undesirable for aphids, limiting their ability to access the phloem sap, slight wilting may have made it easier for aphids to penetrate the leaf surface. This would be of particular importance to aphids that move and resettle frequently. Increases in soluble N have already been determined to improve the survival of aphid offspring, and it seems likely to be the most important determining factor in population success (White, 1993). In this work, however, an increase in soluble N in sycamore (*Acer pseudoplatanus*, L.) leaves was not associated with the drought treatment, providing little support for that aspect of the PSH.

Emphasis must be placed on the severity of drought. Too severe or prolonged drought conditions do not lead to aphid success, and a number of alternative growing methods are being considered to help reduce agricultural water-usage. For example, regulated deficit irrigation is the practice of maintaining a crop with less water than calculated to be optimal, a strategy trading-off increased water use efficiency against reduced yield (Zegbe-Dominguez et al., 2003; Gorantiwar and Smout, 2003; Dodd, 2007). Partial root drying (PRD) is an irrigation technique where half of the root zone is irrigated while the other half is allowed to dry out (De Souza et al., 2003; Stikic et al., 2003; Zegbe-Dominguez et al., 2003). The benefits of PRD to plant-growers are that a continuous root-shoot signal for ABA production is maintained, reducing shoot vigour without loss of yield. However, ABA expression has been found to increase under aphid infestation (Cabrera et al., 1995). Whilst an overproduction of ABA may improve plant efficiency under well-watered conditions, there is no significant improvement in plant efficiency under droughted conditions (Thompson et al., 2007).

Since water saving techniques must create some level of water stress, it is surprising that there has been no investigation for insect interaction. Studies tend to find that the methods reduce plant size, maximum photosynthetic rate (A_{max}), g_s, and Ψ, but increase water use efficiency. De Souza et al. (2003) found that PRD reduced g_s to maintain Ψ close to fully irrigated levels, whilst using half as much water. Since the procedure alternates the halves of the root-zone being watered, a key question for further work is whether these methods produce an aphid-beneficial mild drought with bouts of re-watering or an aphid-detrimental prolonged drought.

Whilst the *M. dirhodum* and *D. platanoidis* studies broadly supported the PSH, we cannot definitively state that mild to moderate host plant stress provides an enriched phloem sap diet that increases aphid survival and growth.

6.3 Importance of host plant range

Myzus persicae, a polyphagous feeder was the most sensitive to water stress and the population was much smaller on stressed hosts. The species may be able to ‘afford’ to be selective of hosts since a wide range is available to them. This is of advantage when the stress is local. Observations made in the controlled environment suggest that the aphids are very mobile and tend to move amongst host plants. The method of suitable host detection is unknown. Alternatively, as a generalist aphid, *M. persicae* may be less well-adapted to specific plant volatiles or chemicals that tend to increase under stress. A study of a broader range of plant hosts similar to that of Heathcote (1962), but under drought conditions would help define a response profile for *M. persicae*. An experiment of that design was planned for this thesis, but parasitic infestation of the aphid colony prevented its fulfilment.

The brassicaceae-specific glucosinolates (GS) may be associated with *M. persicae* population development. Khan et al. (2010) showed no effect of GS on *M. persicae*; Mewis et al. (2005) found both generalist and specialist aphids reduced under higher GS; and Kim et al. (2008) found that increased GS negatively affected *M. persicae*. There are three key classes of GS: aliphatic derived from methionine, indolyl derived from tryptophan, and aromatic derived from phenylalanine. It is difficult to generalise when glucosinolates have over 120 forms in nature (reviewed: Fahey et al., 2002).

Metopolophium dirhodum on the other hand has a restricted host range, and may be forced to accept varying host plant quality, this is of advantage if the stress is prolonged. In contrast to our work, much of the previous research by Honěk (1991, 1994); Honěk and Martinkova (2002) indicated that *M. dirhodum* was significantly more successful on healthy and vigorous than stressed host plants. The difference may be in the contrast between controlled environment conditions and field experiments.

Only apterous morphs of *M. persicae* and *M. dirhodum* were investigated. The alate *D. platanoidis*, studied here, were therefore the most mobile over long distances of the species examined and tended to move to the most suitable host available at the time. This behaviour is of advantage when the weather is fair and flight is possible. There is a difference in the activity level of adults and nymphs moving from leaf to leaf throughout the year (Dixon, 2005, p.27), indicating that resource tracking is most important to developing aphids rather than adults. The field study presented here could be improved with an assessment of aphid ages from the photographic data. This would assess any changes in population demographics as found by Aslam et al. (2012) working with *Rhopalosiphum padi* (L.) on barley (*Hordeum vulgare*, L.).

Locally adapted genotypes develop in response to the prevailing host species, plant quality and micro-climate (Heathcote, 1962; Takada, 1979; Pons and Tatchell, 1995; Ruiz-Montoya et al., 2005), with some inter-mixing from migration (Llewellyn et al., 2003). This may be another source for the variation amongst results seen, leaving little hope of finding a universally applicable theory. Climate change may alter the environment at a speed too great for larger organisms to adapt to, but the short generation times of aphids gives them an advantage (Dixon, 1973; Llewellyn et al., 2003). A potential hindrance to aphid success could arise from host plants lagging behind in their adaptation to climate change – phenological asynchrony (Dixon, 2003). This issue is addressed in **section 6.7**.

There is potential evidence from our studies that the aphid response to host plant stress may be associated with adaptations to available hosts. The polyphagous feeder was most sensitive to host drought stress whereas the limited range feeders increased to greater numbers on droughted hosts. These responses may be complicated by specific local adaptations to host species and prevailing conditions.

6.4 Preferences for flushing or senescing leaves

The effect of an aphid preference for flushing or senescent leaves as defined by White (2009) requires further study. The definition of 'senescence' in tissue is debatable (White, pers. comm., 2012). The same aphid species produce different results across the literature.

In our work, *M. persicae* was most successful on well-watered host plants, but Tariq et al. (2012) found that mild to medium drought stress had the most positive effect on *M. persicae*. Although preferring older tissue in cabbage *Brassica oleracea* var *capitata*, L. and other brassicas (Wearing, 1972; Harrington and Taylor, 1990), changes in behaviour of *M. persicae* on different host plants (Karley et al., 2002) point to an alternative common factor. A possible candidate is soluble N (van Emden, 1966), but glucosinolates (Cole, 1997; Khan et al., 2010) or the amino acid profile (Karley et al., 2002) have been proposed.

The definition and estimation of 'drought stress' is highly variable in the literature. Soil water content, percentage watering related to a control and maintenance of defined Ψ are all valid methods that will vary based on soil composition and host plant characteristics. As shown in this body of work, water stress of approximately the same scale had widely differing effects on the range of host plants studied.

As with Larsson and Bjorkman (1993), our experiment with sycamore detected no specific stress effect, but the aphids were strongly associated with trees expressing mild drought symptoms. In *D. platanoidis*, soluble N was found to be significantly correlated with aphid numbers, **Figure 4.5**. This is consistent with White (1993, 2009), suggesting that any process that increases the concentration of soluble N could benefit phytophagous insects. This would include mild water stress and supplemental fertilisation. Ideally, we would have studied the amino acid composition of leaves in our work, but this was not possible at the time. Wellings and Dixon (1987) found that *D. platanoidis* showed higher growth rates and greater survival on young sycamore leaves, but this was not associated with changes in available amino acids.

The leaf stage preferences of aphid species and their relationship to plant age still require to be defined before they can be used consistently in hypothesis development.

6.5 Host plant resilience and life-cycle

In the case of annual plants and grass crops, aphids are a significant threat to plant survival causing reduced yields and performance. Many annuals fall in to the bracket of ‘drought escapers’, completing their life-cycle before the water deficit takes hold (Bacelar et al., 2012), but aphid infestation may interfere with their strategy of storing reserves in stems and roots (Girousse et al., 2005). The work with barley found that *M. dirhodum* alone had no influence on the root:shoot ratio (**Figure 3.7**) but other systems may differ. In some cases it has been found that cabbage can exhibit ‘drought avoidance’ by recovering turgor without additional water uptake (Levitt, 1986; Weisz et al., 1989), were this to occur it may explain how *M. persicae* success varies in different drought experiments.

Contrastingly, more *D. platanoidis* were found on trees with lower g_s, lower Ψ and higher soluble N. Trees with higher growth rates carried a greater aphid load over the season (**Table 4.4**). When the complete life-cycle occurs in one season, checks to growth and productivity are more difficult to recover from. On the other hand, older trees tend to have lower photosynthetic and stomatal conductance rates (Kolb et al., 1997; Bond, 2000), but are less resistant to drought (Martínez-Vilalta et al., 2012).

Whilst annual plants exhibited drought symptoms more clearly than sycamore, this cannot be attributed to differences in life-cycle. *Metopolophium dirhodum* and *D. platanoidis* numbers tended to increase in relation to decreasing g_s, Ψ and increasing leaf temperatures and soluble N.

6.6 Aphid-induced changes in the host plant

Myzus persicae and *M. dirhodum* had similar effects, altering the ratio of root:shoot nutrient allocation, damaging the efficiency of Photosystem II (PSII) and reducing growth. Infestation by these aphids leads to smaller plants with low gas exchange efficiency, irrespective of watering treatment.

The effects detected in the root:shoot ratio (**Figure 3.7**) could be a result of aphid-induced drought, but galling insects have been found to increase Ψ through altering the root:shoot ratio (Fay et al., 1993). Such a mechanism may benefit aphids, and there is potential evidence for this in our work with *M. persicae*, **Figure 2.6**. Other aphid species have been found to alter the allocation of nutrients in the host plant, redirecting resources to the site of aphid feeding (Girousse et al., 2003, 2005).

Damage to the PSII, found in our work with *M. dirhodum* (**Figure 3.6**) has been found with *Diuraphis noxia* (Mordvilko) on cereals (Burd and Elliot, 1996), but not with *Aphis glycines*, Matsumara (Macedo et al., 1996). These species cause different levels of damage to the host plant: *D. noxia* causes extensive structural damage to host plants (Saheed et al., 2007), *A. glycines* does not cause chlorosis or other visible damage.

In the work with both *M. dirhodum* and *M. persicae*, it was found that the estimation of chlorophyll was significantly reduced by aphid treatment, **Figures 2.7 & Table 3.4**. Although chlorophyll concentrations are linked to the capacity of PSII, a decrease in chlorophyll is not necessarily the cause of photosynthetic reduction. A very similar effect of reduced PSII coinciding with reduced chlorophyll has been found in spider mites (Iatrou et al., 1996), where reduced photosynthesis is attributed to changes in stomatal conductance, Ψ and mite-induced water stress, feeding back to photosynthetic processes. In our work, the pattern of reduction in chlorophyll fluorescence in aphid stressed plants showed a similar pattern to the reduction in droughted plants.

Under drought and aphid stress, ABA levels rise. The pathways for ABA production activated by aphid feeding can lead to up-regulation of pathways producing reactive oxygen species that must be carefully regulated by the plant to prevent oxidative stress and damage to the photosystem (Divol et al., 2005; Goggin, 2007; Smith and Boyko, 2007; Erb et al., 2012). In the experiment studying the effect of *M. dirhodum* infestation on chlorophyll fluorescence, damage to PSII was detected. This indicates that drought and insect stress cause PSII damage in the same way, and some aphids can damage chlorophyll levels through a separate mechanism.

The reduction in growth parameters is consistent with reductions in nutrient allocation and photosynthesis, with less energy directed into above-ground growth. Overall, the effects of drought stress are exacerbated and effects similar to drought stress are induced in well-watered plants, **Figure 2.8**. The induction of drought-like symptoms implies a link to drought-related phloem constituents such as ABA or proline. ABA is likely, considering that aphid infested plants tended to be warmer as a result of decreased transpirational cooling and above ground growth was also inhibited.

In woody perennials, aphids had no effect. More *D. platanoidis* were present on trees with lower g_s, lower Ψ and higher soluble N. Trees with higher growth rates carried a greater aphid load over the season. It is conceivable that there is a threshold aphid quantity that can cause the changes to g_s, Ψ and soluble N, but we have no evidence that the aphids induced any physiological changes in the sycamore saplings.

In previous work with saplings (Dixon, 1971; Wellings and Dixon, 1987), aphid infestation caused a reduction in leaf size, growth and soluble N; no such reduction in growth was found in the field (Simpson et al., 2012). Observations in the field note that large numbers of aphids would be found on leaves with severe premature senescence (leaf roll, wilting, fungal mildew and eventual leaf drop). The aphids were either causing these effects or relocating to the leaf after the symptoms developed. Clip cages or leaf enclosures were impractical due to the wind levels on site, but could be of value in further work tracking the aphid-leaf interaction.

Aphid infestation caused the greatest impact on already stressed host plants, but also triggered a drought-like response in well-watered hosts.

6.7 A dynamic interaction with climate

Using the data available, the results of the penalised signal (*P*-spline) daily analysis and a combination of minimum temperature, rainfall and vapour pressure deficit (VPD) data offer a promising method for interpreting aphid suction-trap data.

Whilst ordinal date of first catch (FC) is advancing to earlier in the year (**Figure 5.4**) it is also the aphid response variable with the closest connection to meteorological variables. Monthly and daily analyses agreed that higher minimum temperatures in March were correlated to earlier catches of aphids, **Figure 5.8**. In addition, years with earlier FC are correlated to greater *ln* (total catch, TC) and longer total length of aphid season (TS).

The TC regressions were less conclusive, with a potential correlation to rainfall in autumn, **Figure 5.9**. A wetter August and a drier September correlates to greater total catches of aphids. However, this effect may be less important to determining TC, given a potential autocorrelation from year to year, **Figure 5.6**. Longer datasets are required to assess the relationship of data between years. Years with a greater TC are years in which the population has increased fastest in the spring, and the cumulative number of aphids in the spring is the largest contributor to the total catch. The summer and autumn populations of aphids were more associated with weather conditions than the spring populations.

Drepanosiphum platanoidis is also staying parthenogenetic until later in the year (**Figure 5.5**) and the length of season is closely associated with VPD in spring and late summer (**Figure 5.10**). Warm, dry spring seasons and a cooler, wetter September would combine to delay the aphid winter cycle.

Improvements can be made by matching the suction-trap database to higher quality meteorological data, particularly regarding the need to fill in the gaps from 2004–2012. Databases with finer resolution and additional features are available (e.g. Mitchell and Jones, 2005). Their Climate Research Unit Time Series has a refined quality control system and provides information for normal values, anomalies and frequencies that could be very useful to phenological modelling. Alternatively, databases generated with re-analysis (e.g. Uppala et al., 2005) eliminate missing data, provide vertical data resolution and account for changes in data acquisition methods over time.

All meteorological covariates were shown to have validity. It was hypothesised that VPD would have a direct influence on aphid population fluctuations, being a combination of temperature and humidity, but this was not supported by the analysis. The strongest and most consistent correlations to weather variables were found in FC. The dates for *D. platanoidis* FC are also advancing to earlier in the year, and this is likely to be associated with climate change effects on the host plant (Thompson and Clark, 2008). Daily data are potentially more useful than aggregated monthly data, but require more detailed analysis and possibly greater computing power.

6.8 Further issues

In many studies testing drought responses or changing CO_2 levels, pesticide spraying has been a standard practice to remove aphids. This is valid as a method to reduce external effects on an experiment, but the impact of aphid infestation in the field cannot be ignored. According to Directive 2009/128/EC (European Parliament, 2009) covering sustainable pesticide use and its promotion of 'integrated pest management' to reduce the use of pesticides, we may not be able to rely on these tools to reduce aphid infestation. The body of evidence that finds aphid infestations increasing under drought conditions shows that aphids are a threat to areas suffering water shortage. There is also evidence that finds aphid infestations increasing on vigorous and well-watered plants; aphids are therefore also a threat to areas experiencing greater rainfall under climate change. Increasing atmospheric CO_2 concentrations tend to improve plant efficiency (Robredo et al., 2007), but the effect on aphids requires further investigation (Xing et al., 2003; Newman, 2004; Pritchard et al., 2007; Himanen et al., 2008).

Changes to interactions with natural predators of aphids under drought conditions (tritrophic interactions) are particularly important to study in the field. Hoover and Newman (2004) considered the effects of increasing temperatures and atmospheric CO_2 but not drought. Aslam et al. (2012) investigated the impact of host plant drought on *R. padi* and a parasitoid wasp, finding that the change in population balance between nymphs and adults altered the levels of parasitisation. Climate change may disturb the external factors acting on aphid-plant interactions, particularly if one or more of the participants is sensitive to water stress.

Similar to predator-prey interactions, host plant pathogens may be affected by drought. Barley yellow dwarf virus is spread by aphids, and a greater proportion of plants are infected under drought stress and increased temperatures (Smyrnioudis et al., 2000; Harrington, 2002). Host plant infection by aphid-transmitted viruses can attract and alter the behaviour of aphids (Alvarez et al., 2007). In the case of endophytic fungi associating with host plant, there is evidence for beneficial effects on aphids in sycamore and the tarspot fungus (Gange, 1996) and for negative to neutral effects on *R. padi* (Harri et al., 2008; Zust et al., 2008). Research on this area is growing and it appears that there may be changes in photosynthetic rates and water use efficiency (reviewed: Arnold, 2007) and in some cases increased soluble N (Gange, 1996). Future work could address these broader networks of predators and prey under changing climate conditions.

6.9 Conclusions and future directions

Although water-stress was found to induce physiological changes to the plant that could benefit aphid populations, none of the hypotheses can be accepted based on the results found by this work alone.

A wider range of water-stress levels would provide a clearer indication of the shape of any plant-stress based aphid success relationship. From the results in **Chapter 4**, the response of sycamore aphids appears to be monotonic in the field, but controlled conditions would enable full analysis of aphid intrinsic growth rates and population variables. Adding interactions involving changing atmospheric CO_2 and temperatures would complicate experimental design but possibly illuminate the climate change element in aphid-plant interactions.

To develop a greater understanding of the contribution of single, narrow or wide host plant ranges to the interaction, larger scale testing is required. Comparing the same aphid on different host plants would be of value: for example an experiment using *M. persicae* on plants of four separate plant families (Brassicaceae, Amaranthaceae, Asteraceae and Fabaceae) was designed to investigate the response of the same aphid to a range of species. This project did not come to fruition, but could be a useful model for future work.

More definition needs to be provided for flushing and senescent tissues: for example whether these terms are based on leaf age, plant age, nitrogen levels or photosynthesis levels and how these tissues are affected by changes in plant water status.

Aphids feeding on plants posed an additional threat to a stressed plant's survival, and caused the greatest damage to already stressed host plants. Well-watered plants were able to defend against aphid infestation in the short term, but experienced drought-like symptoms. Drought conditions were most difficult to achieve in the field. Whilst the system we adopted for the sycamores was practical and created a relatively natural environment, there were difficulties caused by site exposure to wind. There is a need to determine whether data resulting from experiments under controlled conditions can be applied to the field — in the case of this work I was unable to confirm the *M. persicae* and *M. dirhodum* results in the field due to lack of drought and aphid success. However, the design and building of effective, economical, outdoor water exclusion sites are an essential bridge between laboratory and field.

In this work, annual herbaceous plants with high water requirements exhibited drought symptoms more quickly and affected the aphid population faster than perennial woody plants with lower water requirements. However, further work is required to compare a greater range of host plant life-cycles under stress and also comparisons of a single aphid species across a range of hosts with varying drought tolerance.

Daily climate data were found to be a potentially useful tool in the analysis of aphid population fluctuations in the suction-trap data, since they provide a continuum of data without losses to monthly averaging. More testing is needed to determine the most appropriate model terms for all data used in daily regressions. This is particularly relevant to rainfall data through the year, which are more randomly distributed in our temperate climate than temperature data. The population rates calculated from suction-trap data may provide useful bases for research into other aphid species.

In conclusion, aphids pose a challenge to both plants and the researcher, with a large range of questions still remaining to be answered. Key difficulties lie in both the theory (selecting and narrowing down research targets) and the practicality (managing aphid populations under experimental conditions) of plant-aphid interactions. Focus for the future may be in generating experimental data that can inform models projecting plant and aphid responses to climate change.

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Appendices

A

Project Evaluation

This thesis addresses a small proportion of the larger topic of interactions between aphids and their host plants under stress. Whilst each chapter follows a logical progression and is individually successful, the scope of the entire project was initially much broader. A number of other experiments were designed to fulfil those goals, and are briefly described here, with a summary in Figure A.1.

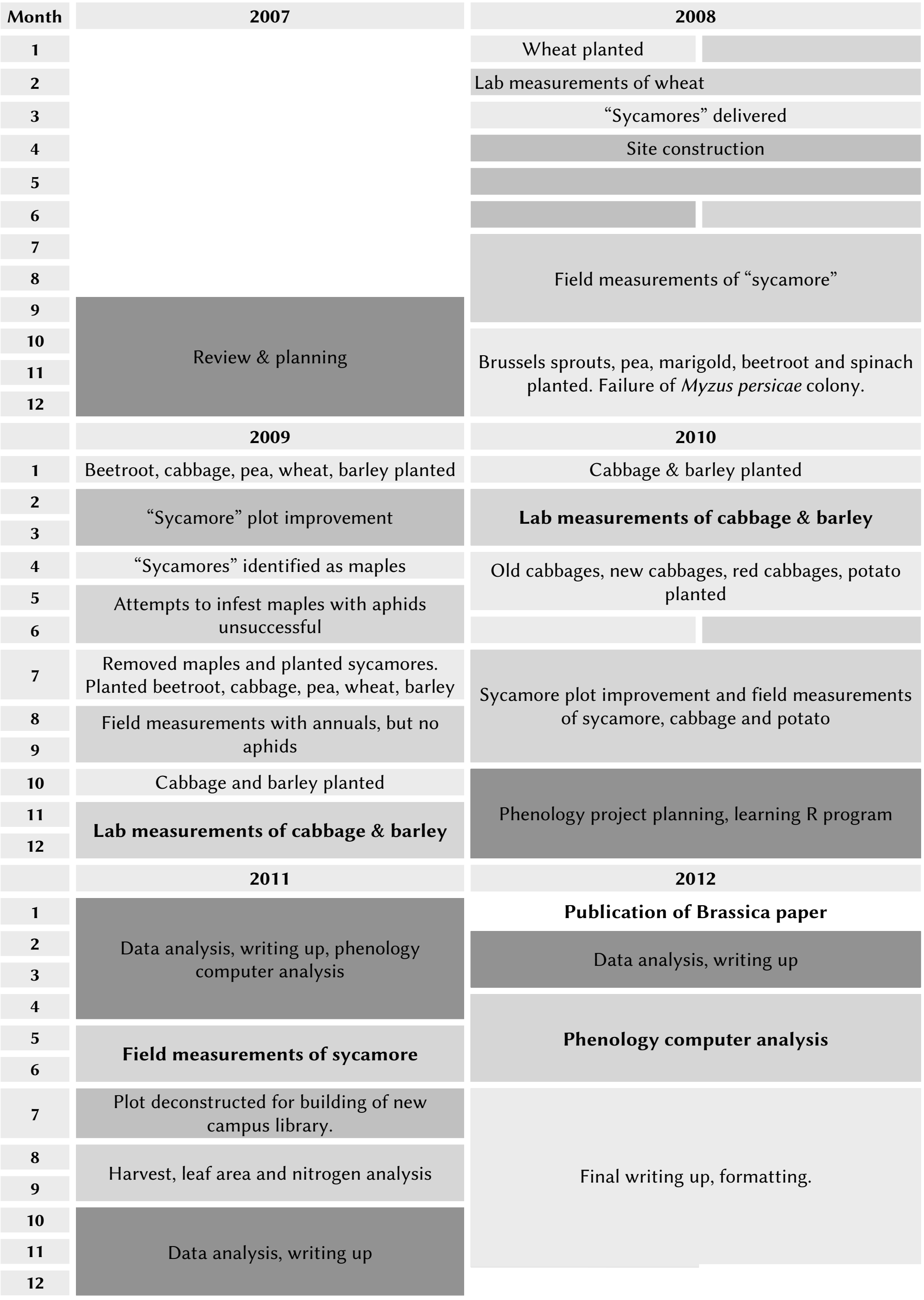
An initial experiment utilising *Metopolophium dirhodum* (Walker) and winter wheat (*Triticum aestivum*, L.) was designed to investigate a range of watering levels and their effect on aphid survival. The aphid population struggled under conditions with too low humidity and the results of this project were inconclusive. Different watering levels had little effect on potted plants in the controlled environment rooms.

The sycamore (*Acer pseudoplatanus*, L.) field project from Chapter 4 was designed and built in 2008, with the ambition to simulate different severities of water stress. The method of rainfall exclusion was untried, but successful, using incremental layers of weed-control fabric. Unfortunately a supplier error meant that *Acer platanoides* was sold as *Acer pseudoplatanus* and the *Drepanosiphum platanoidis* (Schrank) would not establish on these trees. The maples were more resistant to aphid infestation and were unsuitable for the project, This work was therefore scrapped in 2009 and restarted with the correct host plants and an alternative two-level rainfall exclusion method.

As *Myzus persicae* (Sulzer) is a polyphagous feeder, there are a number of different host plants to study. A study using plants of four separate plant families (Brassicaceae, Amaranthaceae, Asteraceae and Fabaceae) was designed to investigate the response of the same aphid to a range of species. Each species was anticipated to have a unique response to water stress and it was hoped that the aphid responses would help clarify the PSH. The *M. persicae* population in this case was eliminated by parasite infection, and the experiment had to be abandoned. This work was attempted again as a field experiment with a parallel study using *M. dirhodum* on wheat and barley. The aphids did not establish. As a pilot study though, it indicated that the *M. persicae* were most successful on Brassicaceae, leading to the work in Chapter 2.

Cabbage, *Brassica olereacea* var *capitata*, L. plants from Chapter 2 were planted outside to follow the plants to maturity and investigate whether there were any differences between infestation histories. New, young plants of the same variety were planted alongside a different variety with red leaves. The aim of this experiment was to compare field and controlled environment conditions and possible effects of leaf pigmentation. In all cases, *M. persicae* did not survive the colder night conditions at the field site, despite protection with plant fleece. A parallel study using *M. persicae* on potato (*Solanum tuberosum*, L.) was also carried out. Although the aphid population was moderately large, causing reductions in A_{max} and g_s, there was no significant evidence that a drought had been achieved. That field research also found a significantly higher leaf water potential under aphid stress.

Finally, the phenology computer analysis was originally intended to encompass *D. platanoidis*, *M. persicae* and *M. dirhodum* at two locations: Edinburgh and Rothamsted. This would have shown species and geographic variability. However, the quality of meteorological data was too variable and the quantity of analysis was too involved for a single chapter. It was decided to reduce the breadth of the study and focus on a specific topic in detail.



Month	2007	2008
1		Wheat planted
2		Lab measurements of wheat
3		"Sycamores" delivered
4		Site construction
5		
6		
7		Field measurements of "sycamore"
8		
9	Review & planning	
10		Brussels sprouts, pea, marigold, beetroot and spinach planted. Failure of *Myzus persicae* colony.
11		
12		
	2009	**2010**
1	Beetroot, cabbage, pea, wheat, barley planted	Cabbage & barley planted
2	"Sycamore" plot improvement	**Lab measurements of cabbage & barley**
3		
4	"Sycamores" identified as maples	Old cabbages, new cabbages, red cabbages, potato planted
5	Attempts to infest maples with aphids unsuccessful	
6		
7	Removed maples and planted sycamores. Planted beetroot, cabbage, pea, wheat, barley	Sycamore plot improvement and field measurements of sycamore, cabbage and potato
8	Field measurements with annuals, but no aphids	
9		
10	Cabbage and barley planted	Phenology project planning, learning R program
11	**Lab measurements of cabbage & barley**	
12		
	2011	**2012**
1	Data analysis, writing up, phenology computer analysis	**Publication of Brassica paper**
2		Data analysis, writing up
3		
4		**Phenology computer analysis**
5	**Field measurements of sycamore**	
6		
7	Plot deconstructed for building of new campus library.	Final writing up, formatting.
8	Harvest, leaf area and nitrogen analysis	
9		
10	Data analysis, writing up	
11		
12		

FIGURE A.1: Timeline of the entire project

B

Chapter 2: Published version

Entomologia
Experimentalis et Applicata

DOI: 10.1111/j.1570-7458.2011.01216.x

The response of aphids to plant water stress – the case of *Myzus persicae* and *Brassica oleracea* var. *capitata*

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Accepted: 28 November 2011

Key words: osmoregulation, insect performance, global climate change, pulse stress, water-deficit, drought, plant–insect interactions, Hemiptera, Aphididae, Brassicaceae

Abstract

The possible repercussions of decreasing water availability in plant–insect interactions largely remain unclear. To study this, we designed two factorial studies applying two levels of water stress to *Brassica oleraceae* var. *capitata* L. (Brassicaceae) plants inoculated with the aphid *Myzus persicae* Sulzer (Hemiptera: Aphididae: Macrosiphini). One study investigated the effect of aphids and water stress on leaf water potential components, aphid population size, aphid intrinsic rate of increase (r_m), and fecundity. The other investigated the effect of aphids and water stress on gas exchange, leaf fluorescence yield, estimation of leaf chlorophyll, and above-ground dry weight. Drought treatment reduced the size of the *M. persicae* population. Aphid numbers were strongly and positively correlated with soil moisture and negatively correlated with leaf temperature. Aphids on droughted plants had lower r_m and lower fecundity per aphid. Under aphid stress, plants showed reduced maximum photosynthetic rate (A_{max}), lower leaf chlorophyll as estimated by soil plant analysis development (SPAD) values, higher osmotic potential, and reduced final above-ground dry weight compared with control plants. Aphid infestation also led to significantly higher leaf water potentials and reduced light-adapted fluorescence yield. Compared with control plants, water-stressed plants showed reduced A_{max}, lower SPAD values, reduced leaf water potential, lower turgor, and reduced final above-ground dry weight. Osmotic potential was lower in droughted plants when aphids were not present. Plants under combined aphid and water stress showed significantly reduced SPAD and final above-ground dry weight, with comparatively higher leaf water potentials. These results show that aphid infestation limits solute accumulation in the vacuole of drought stressed cabbage. Aphid infestation reduced the host's ability to photosynthesise optimally and led to reduced growth. Plant stress hypotheses propose that a senescence feeder such as *M. persicae* would show higher rates of growth in response to the increasing 'quality' of the phloem sap under water shortage. In the case of *M. persicae*, our results do not support the hypothesis that drought leads to improved aphid population growth and survival.

Introduction

The Intergovernmental Panel on Climate Change has suggested that there will be an increased incidence of drought in central, southern and western Europe, with higher irrigation requirements. Projections for the next 50–70 years indicate moderate to severe water stress for southern Britain, with increasing water shortages worldwide (Alcamo et al., 2007a,b). If this happens, the patterns of pests and diseases are likely to alter. Newman (2005) predicted dramatic declines in cereal aphid abundance and pest status in response to global warming. Across all CO_2 emission scenarios, increases in temperature and decreases in rainfall were the most important factors in the forecast reduction of cereal aphid numbers.

However, further experimental data are needed to inform models of the effects of climate change in a range of aphid/host combinations. As a result of decreased water availability under climate change, agricultural water requirements are likely to be difficult to fulfil (Alcamo et al., 2007a,b), and water shortage may increase crop

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vulnerability to insect outbreak and attack. To that end, this work investigated the interaction between the aphid, *Myzus persicae* Sulzer (Hemiptera; Aphididae: Macrosiphini) and water stress in cabbage [*Brassica oleraceae* var. *capitata* L. (Brassicaceae)]. *Myzus persicae* is a generalist feeder with multiple suitable hosts. *Brassica* spec. account for ca. 21% of the field vegetable area in the UK, and contributed 25% of the home vegetable production market in 2009/10 (DEFRA, 2010).

Water shortage is a well-known plant stress factor, but the impact of the resulting physiological changes in the plant on phytophagous insects remains less-well known. The original starting point for our work was the 'plant stress hypothesis' (White, 1969), which states that host stress can lead to an increased availability of small nitrogenous molecules for consumption by phytophagous insects, resulting in their greater reproduction and survival. As the stress on a plant increases, the value as an insect food source increases to a maximum point before falling off as the plant begins to senesce. Field observations originally supported the idea, showing that insect populations sometimes significantly increased after periods of drought (Mattson & Haack, 1987). White (2009) modified the original plant stress hypothesis to specify that it applies to insects with a preference for senescent foliage and that stress levels studied must be intermediate to moderate. Aphids as a family contain both flush and senescence feeders. On plants of the Brassicaceae family, *M. persicae* appears to prefer leaves with the greatest concentrations of mobile nutrients, primarily immature and older leaves (Kennedy et al., 1958; van Emden et al., 1969; Wearing, 1972; Harrington & Taylor, 1990).

The modified plant stress hypothesis (White, 2009) is related to the 'pulse stress hypothesis' (Huberty & Denno, 2004). This proposes that periods of stress and recovery are more beneficial to the quality of aphids' diet than constant stress. In a drought scenario, pulses of water availability within periods of water shortage might permit increases in stress-induced nitrogen without the plant wilting due to reduced leaf turgor and water content. The resulting maintenance of turgor pressure would allow aphids to continue feeding from the phloem. This may be because the early reduction in protein synthesis leaves spare amino acids (readily converted to essential amino acids by aphid gut symbionts), whereas the sap remains dilute enough (in comparison to severe drought) for the aphid enzymes to function (Auclair, 1963; Mittler, 1967; Douglas, 2006). For example, higher levels of essential amino acids in the phloem lead to higher survival, higher rates of increase and enhanced fecundity with shorter development times in *M. persicae* on *Solanum tuberosum* L. (Karley et al., 2002). Alternatively, Khan et al. (2010), suggested that the increased sugar and decreased glucosinolate levels in water-stressed *Brassica oleracea* L. var. *italica* (Plenck) led to increased aphid numbers.

Increasing day length and air temperature positively affects *M. persicae* population characteristics (Mittler, 1967; Wyatt & Brown, 1977). As leaf stomata close in response to water stress, transpirational cooling declines and leaf temperature rises. This may influence rates of growth and fecundity, irrespective of the expected response to changing diet quality.

Another intriguing aspect of the aphid–plant interaction is the similarity of the drought stress response to the aphid infestation response. For example, Riedell (1989) found that Russian wheat aphids (*Diuraphis noxia* Mordvilko) caused drought stress symptoms in barley (*Hordeum vulgare* L.). Cabrera et al. (1994) found significant reductions in CO_2 assimilation rate and leaf chlorophyll content in barley infested with *Schizaphis graminum* (Rondani). Given these findings, a factorial design and measures of plant physiology could elucidate the areas where aphid and drought response overlap and/or interact.

In the following studies, we test firstly whether the *M. persicae* population increases more quickly and to larger numbers on intermittently water-stressed host plants. This is the expected response to the increasing quality of the plant sap under water shortage (measured by changes in concentration of the sap vacuole). Secondly, we test whether the increasing temperature of droughted leaves is related to increased growth and fecundity in the aphids. Thirdly, we test whether aphid infestation will reduce the yield and performance of host plants in a manner similar to drought stress.

Materials and methods

Experimental design

Brassica oleraceae var. *capitata*, 'Greyhound' variety (commercially available seed), was grown in a controlled environment room. Air temperature was 21 ± 0.2 °C. Photosynthetically active radiation (PAR) levels of 100 μmol photons m^{-2} s^{-1} was provided by two 36-W T8 Triphosphor 830, 3 000 K Warm White fluorescent tubes (Philips Electronics, Guildford, UK) timed to give a 16-h photoperiod. The average relative humidity was 50%, giving an approximate vapour pressure deficit of 1.24 kPa.

Many response variables were involved in this experiment, and the sampling processes may have interfered with each other, so that two separate studies prevented oversampling of the young plants used. Study I used 24 plants, and ran from November to December 2009; study II used 40 plants and ran from February to March 2010.

Plants were grown in 2-l pots containing 80% Scotts 'Shamrock' medium general purpose sphagnum peat (Everris, Ipswich, UK) and 20% horticultural sand; 0.3% ground limestone and 0.12% controlled release fertiliser were included. The fertiliser was Scotts 'Osmocote 3–4 months' (Everris), with NPK 14:14:14 that comprises 14% N as 7.2% nitrate nitrogen and 6.8% ammonical nitrogen, 14% phosphorus pentoxide equivalent to 6.1% P, and 14% potassium oxide equivalent to 11.6% K.

At 5 weeks, plants were at the six-leaf stage and were randomly assigned to one of four treatments in a fully factorial design: twice weekly watered (W), once weekly watered (D), aphid-infested (A), and no aphids (N). This description of treatments introduces the abbreviations used throughout. In this experiment, small quantities of water, applied weekly, emulate a rationing of water supply or occasional rainfall. Each plant pot had a dish reservoir at the base to take the water dosage. 'D' plants received 125 ml on watering days, and 'W' plants were watered ad libitum and could receive up to 500 ml on watering days. By the end of each study (day 35), all the plants were still immature. The timescale for each study was identical (Figure 1). The *M. persicae* strain was descended from an insecticide-susceptible colony, provided by the Scottish Crop Research Institute (Dundee, UK).

Water deficiency monitoring

Soil moisture was measured with a Delta-T Theta Probe ML2 (Delta-T Devices, Cambridge, UK) connected to a Delta-T HH2 Moisture Meter. Measurements were made on 24 plant pots on days 7, 14, 21, and 35 of study I. For study II, measurements were made on days 7, 14, 21, 28, and 35 with $n = 19–40$. This measure was used to confirm that the same soil moisture conditions were created during both studies.

Aphid population study

Population size. Five adult aphids were placed on each plant at the start of the watering treatments (day 0), and were replenished if necessary until day 7. Aphids were counted on 20 plants in study II (days 21, 28, 35) using multiple photographs taken with a digital camera of 6.0 mega pixel resolution saved in high quality resolution JPEG format. Pictures of the upper and lower surface of each leaf were taken, as well as the still unfurled young leaves at the cabbage centre. Aphids were counted in Adobe Photoshop Elements (San Jose, CA, USA). Individuals were marked on the photograph as they were counted to ensure no accidental repetition.

Intrinsic rate of increase (r_m). Clip cages were constructed (Kennedy et al., 1958) for the population study. Two adult aphids were caged separately on each experimental plant at the beginning of the water or drought treatment. The next day, each adult was removed from its clip cage and one of the newly produced nymphs was left in the cage. Afterwards, the cages were checked daily, with progeny removed if produced. Twenty-three cages were studied to completion in study II.

All aphids were followed to the end of their life – beyond the period required for the intrinsic rate of increase calculation. This allowed measurement of total fecundity.

The method of Wyatt & White (1977) was used to calculate r_m:

$$r_m = 0.738(\ln M_d/d),$$

where d is the pre-reproductive period in days and M_d is the number of progeny produced in a period equal to d.

Plant physiology study

Leaf temperature. Individual leaf temperature was measured with a non-contact Infra–Red Thermometer (Fluke, Norfolk, UK), positioned 20 mm from the surface of the youngest fully expanded leaf. In study I, $n = 20–24$ and readings were made on days 14, 21, and 35. For study II, $n = 19–40$ with data from days 14, 21, 28, and 35.

Gas exchange measurements. Maximum photosynthetic rate (A_{max}) and stomatal conductance (g_s) were recorded using a LI-6400 Photosynthesis System (LI-COR Biosciences, Lincoln, NE, USA). These measurements of gas exchange enabled an assessment of drought impact. All measurements were made between 10:00 and 13:00 hours. An initial light response curve was determined on day 0 to define a suitable chamber PAR level, allowing comparison

Figure 1 Timeline of studies I and II.

of the maximum photosynthetic ability between stressed and unstressed plants. The chosen light level was 1 200 μmol photons m^{-2} s^{-1}, this being the point where CO_2 uptake levelled with respect to PAR on the light response curve. Chamber temperature was controlled at 21 °C, CO_2 was controlled at 370 μmol CO_2 mol^{-1} and flow rate to the sample cell was set to 500 μmol air s^{-1}.

The youngest fully expanded leaf of the 5-week-old plant was tagged on day 1 and used for all gas exchange measurements in study I on days 7, 14, and 21 with n = 9–23.

Fluorescence measurements. The chlorophyll fluorescence yield parameters $\Delta F/F_m$ and F_v/F_m were measured using a Walz MiniPAM system (Heinz-Walz, Effeltrich, Germany). These parameters were defined by Genty et al. (1989) to investigate the yield of electron transport in photosystem II, which in turn affects the efficiency of CO_2 uptake. All measurements were made between 10:00 and 13:00 hours using the same leaves sampled for gas exchange on days 7, 14, 21, and 28 with n = 9–23.

Measurements were conducted on both light- and dark-adapted leaves. 'In the light' measures give the Yield-parameter (based on the Genty parameter $\Delta F/F_m$). 'Dark-adapted' measures give F_v/F_m (after at least 30 min dark adapt time). The system was operated on 'Burst-mode' for measurements, and all other settings were default. The Mini-PAM leaf measurement head was used for light-adapted measures, and the proprietary leaf-clips were used to hold the fibre optic unit for dark-adapted measures. Background elements of the fluorescence signal were eliminated with the 'Auto-Zero' function before readings were taken.

Leaf chlorophyll estimation. Several studies show that leaf chlorophyll content is strongly correlated with leaf nitrogen content (e.g., van den Berg & Perkins, 2004), and so, a non-destructive estimation of chlorophyll content in the same leaves sampled for gas exchange and fluorescence was made using a Minolta SPAD meter (Konica Minolta Sensing, Basildon, UK). Each leaf was sampled three times to generate a mean value in study I on days 7, 14, 21, and 28 with n = 10–20.

Above-ground dry weight. Plant matter from above ground only was harvested at the end of study I to determine any impact of drought and aphids on plant growth and yield. Plant material was oven dried to constant weight at 80 °C, n = 24.

Water potential components. The components of plant water potential are related as: total leaf water potential = turgor potential + osmotic potential. Total leaf water potential gives an immediate reading for water stress; osmotic potential gives the concentration of vacuolar and phloem constituents. Turgor pressure is expected to have a strong relationship with aphid performance.

Leaf water potential was measured using a Scholander-type chamber (Plant Moisture System; Skye Instruments, Powys, UK) between the hours of 10:00 and 13:00. Fully expanded leaves were excised and immediately transferred to the chamber. Readings were taken in study II on days 7, 14, 21, 28, and 35 with n = 6–21.

After the leaf was removed from the pressure chamber, it was sealed in a 10-ml plastic syringe and immediately snap-frozen in liquid nitrogen. Leaves were stored in liquid nitrogen and defrosted for 30 min prior to measurement. At least 10 μl of cellular sap was expressed by depressing the syringe plunger. The expressed sap was collected in a sterile Petri dish and immediately sampled with a 10 μl pipette, transferred to a filter paper disc and analysed using a Wescor Vapro5520 Vapour Pressure Osmometer (Wescor, Logan, UT, USA). The osmometer output in mmol kg^{-1} was converted to osmotic potential in MPa using the van't Hoff Equation:

$$\text{Osmotic potential (MPa)} = -RT(\text{mol} \cdot \text{kg}^{-1}),$$

where R = universal gas constant, 0.00831 MPa kg mol^{-1} K^{-1} and T = room temperature, 293 K.

The error component from dilution of symplasmic water by apoplasmic water is acknowledged from Callister et al. (2006), but was judged to be small and not corrected for. Osmotic potential readings are therefore a bulk estimation of leaf osmotic potential, relevant for water stress studies, and field sample collection (Bannister, 1986; Pullman & Johnson, 2009).

Turgor potential was derived from the relationship between the two measured potentials.

Data analysis

All analyses and figures were generated using Minitab 15 Statistical Software (Minitab, Coventry, UK). All data were normally distributed and showed equal variance, and therefore, two-way repeated-measures ANOVA were performed on the gas exchange, water potential, fluorescence, and SPAD data using the General Linear Model function of the programme. The factors were 'Day' (random factor, within groups), 'Aphids' (fixed factor, between groups), 'Watering' (fixed factor, between groups), and 'Aphids*watering' (interaction) (Minitab, 2007).

All means are given with standard error of the mean (SEM). Aphid photographic count data were log transformed for linear regression with soil moisture and leaf temperature. These count data were also left

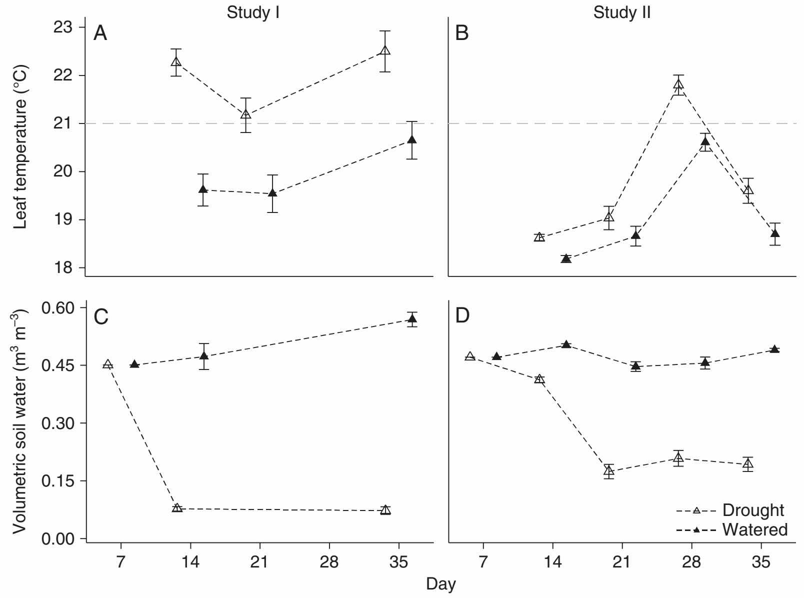

Figure 2 Mean (±SEM) leaf temperature and soil moisture during both studies. (A, B) Mean leaf temperatures are shown with the reference line at 21 °C marking mean ambient room temperature. There is a significant increase in the mean leaf temperature of droughted plants that increases with time (ANOVA, see Table 1). (C, D) Volumetric soil water content is shown, with a significant reduction in the drought treatment. There is no significant difference between the studies in the moisture levels for each treatment.

untransformed and analysed in their original Poisson distribution using the appropriate t-test. The fecundity data were also Poisson distributed, and analysed using a t-test with this distribution. r_m and nymph per adult per day data were analysed using ANOVA as they were normally distributed with equal variance.

Results

Soil moisture levels for the two watering treatments were the same in the two separate studies, with no significant difference (two-sample t-test: $t = -1.24$, d.f. = 66, $P = 0.22$).

Plants that were infrequently watered experienced significantly drier soil (study I: $F_{1,67} = 48.02$ and study II: $F_{1,123} = 446.80$, both $P<0.01$; Figure 2C, D). Their leaf temperatures were consistently higher than well-watered plants ($P<0.01$; Figure 2A, B; Table 1).

The number of aphids on each plant was strongly and negatively related to the leaf temperature (accounting for 64.4% of the variance with $P<0.01$) and less strongly, but positively related to the soil moisture content (accounting for 37.4% of the variance with $P<0.05$) (Figure 3A, B). Water stress significantly ($P<0.01$) raised leaf temperature (Table 1). In combination with the regression data, the data showed that increasing leaf temperature associated with water stress did not result in increased aphid population size.

Poisson analysis of the raw count data (Figure 4A) showed significantly more aphids on watered plants ($z = 76.2$, $P<0.01$). In the drought treatment, the overall mean number of aphids was 212 ± 48 aphids, but on well-watered plants, 821 ± 81 aphids were observed.

The mean rate of increase in the aphid population (r_m, Figure 4B) was reduced by 42% in the drought treatment. The total fecundity, measured by number of nymphs per adult aphid (Figure 4C) was reduced by 32% in the

Table 1 The ANOVA repeated-measures summary table for leaf temperature in studies I and II

	Day	Aphids	ater	Aphids*water	Error
(A) Leaf temperature (study I)					
d.f.	2	1	1	1	62
SS	17.18	2.24	73.46	1.05	94.84
F	5.62	1.47	48.02	0.69	
P	0.01	0.23	0.01	0.41	
(B) Leaf temperature (study II)					
d.f.	3	1	1	1	113
SS	147.99	2.83	17.26	0.33	80.81
F	68.98	3.96	24.13	0.50	
P	0.01	0.05	0.01	0.50	

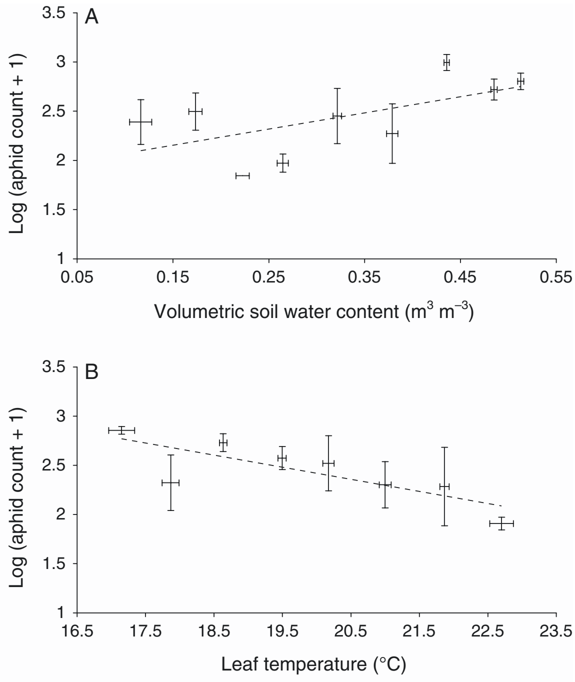

Figure 3 This figure has been simplified by grouping the data from study II. (A) The regression equation for aphid numbers against soil water content was log (aphid count + 1) = 1.9 + 1.64 (volumetric soil water content), $r^2 = 0.374$, $P<0.05$. There was a strong positive correlation showing more aphids per plant as volumetric soil water increased. (B) The regression equation for aphid numbers against leaf temperature was log (aphid count + 1) = 4.9–0.12 (leaf temperature), $r^2 = 0.644$, $P<0.01$. There was a strong negative correlation, showing fewer aphids per plant as leaf temperature increases.

drought treatment ($z = 2.80$, $P<0.01$). The fecundity measured by number of nymphs per adult per day (Figure 4D) was reduced by 53% in the drought treatment.

Aphids had a strong physiological effect on the plants. A summary of the repeated-measures ANOVA performed on the A_{max}, g_s, water potential components, chlorophyll fluorescence, and SPAD values is presented in Table 2. There was a significant effect of aphid infestation and water stress on photosynthetic rate (both $P<0.01$), with a weak interaction (Figure 5A, B). The drought treatment led to a decline in photosynthetic rate over time, and aphid infestation in combination with drought led to the lowest rates of A_{max}. At least part of this decline in photosynthesis is presumed to have been caused by the reduced stomatal conductance (Figure 5C, D), which was significantly decreased by the drought treatment. As expected, leaf water potentials were reduced by the drought treatment (Figure 6A, B), but when leaves were infested with aphids, this reduction was less acute, particularly between days 20 and 35.

The osmotic component of water potential (Figure 6C, D) showed the expected decrease with drought treatment ($P<0.02$), but there was a higher osmotic potential when aphids were present ($P<0.01$). The data illustrated clear osmotic regulation in the droughted plants, which was prevented in plants that were aphid-infested.

Turgor potential (Figure 6E, F) was significantly higher in the watered treatment ($P<0.01$), rising over time ($P<0.01$). Turgor potential was reduced in aphid-infested plants ($P<0.05$) although, over a period of several weeks, this began to rise as leaf water potential unexpectedly

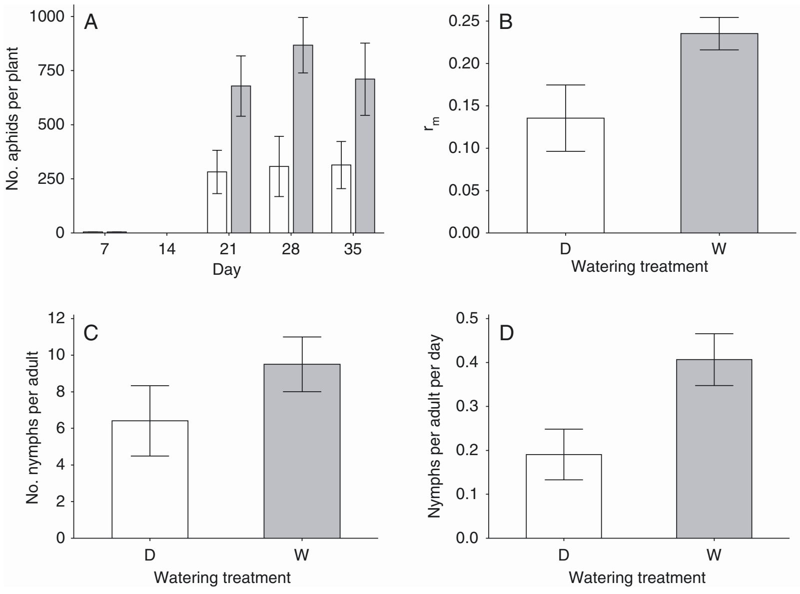

Figure 4 Mean (±SEM) aphid population data in study II. White bars represent the drought treatment, and filled bars represent the watered treatment. (A) There were significantly more aphids per plant in the well-watered treatment ($P<0.01$). (B) The r_m rate was significantly higher in aphids on well-watered plants ($P<0.03$). (C) Total fecundity, the number of aphids per adult, was significantly greater for aphids on watered plants ($P<0.01$). (D) Fecundity per adult aphid per day was significantly greater for aphids on well-watered plants ($P<0.02$).

Table 2 The ANOVA repeated-measures summary table for physiological characters

	Day	Aphids	Watering	Aphids*watering	Error
(A) Rate of maximum photosynthesis					
d.f.	2	1	1	1	39
SS	147.22	77.79	29.46	13.39	153.12
F	18.75	19.81	7.50	3.41	
P	0.01	0.01	0.01	0.07	
(B) Rate of stomatal conductance					
d.f.	2	1	1	1	36
SS	4262.9	661.2	2402.6	1071.4	15 113.5
F	5.08	1.58	5.72	2.55	
P	0.01	0.22	0.02	0.12	
(C) Total leaf water potential					
d.f.	4	1	1	1	65
SS	0.15	0.04	0.12	0.01	0.28
F	6.86	4.21	34.73	7.12	
P	0.01	0.05	0.01	0.01	
(D) Osmotic potential					
d.f.	4	1	1	1	63
SS	0.02	0.08	0.02	0.01	0.21
F	1.62	23.6	6.00	3.32	
P	0.18	0.01	0.02	0.08	
(E) Turgor pressure					
d.f.	4	1	1	1	52
SS	0.16	0.03	0.11	0.002	0.35
F	6.25	4.09	17.21	0.30	
P	0.01	0.05	0.01	0.59	
(F) Chlorophyll fluorescence					
d.f.	3	1	1	1	77
SS	0.47	0.06	0.03	0.02	0.74
F	16.27	6.01	3.03	1.89	
P	0.01	0.02	0.09	0.17	
(G) SPAD estimation of chlorophyll					
d.f.	2	1	1	1	43
SS	98.08	220.22	345.52	232.35	1206.04
F	1.75	7.85	12.32	8.28	
P	0.19	0.01	0.01	0.01	

increased, suggesting that the plants acclimated to the treatment.

Fluorescence signals were a useful method of demonstrating stress on plants. The chlorophyll fluorescence yield in the light ($\Delta F/F_m$) tended to decrease with both water stress and aphid infestation (Figure 7A, B). There was a significant aphid effect on the $\Delta F/F_m$ ratio ($P<0.02$), but no significant effect of any treatment on dark-adapted measures. The fluorescence yield of watered and aphid-infested plants appeared similar to that of droughted plants. The SPAD readings (Figure 7C, D) strongly suggest that aphids and water stress reduced chlorophyll concentrations ($P<0.01$).

Above-ground dry weight (Figure 8) was significantly decreased by both water stress ($F_{1,20} = 51.21$, $P<0.01$) and aphid infestation ($F_{1,20} = 40.8$, $P<0.01$). There was a significant interaction effect ($F_{1,20} = 5.38$, $P<0.05$). Water stress alone led to a decrease of 52.5% in the mass of droughted plants compared with watered plants, but when accompanied by aphid infestation, the decrease was as much as 75%.

Discussion

The experimental method produced a satisfactory level of soil water shortage in the droughted treatment. This water shortage led to reduced leaf water potentials, reduced gas exchange and partial closing of stomata. The closure of stomata restricted transpirational cooling and led to higher leaf temperatures. The two studies followed the same protocol

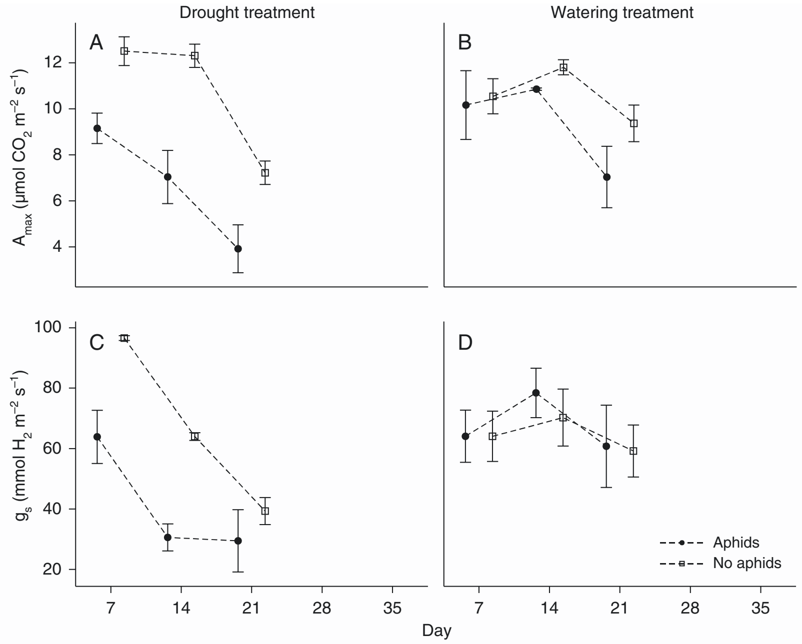

Figure 5 Mean (±SEM) rates of maximum photosynthesis (A_{max}) and corresponding stomatal conductances (g_s) in study I. (A, B) A_{max} was reduced with aphid treatment ($P<0.01$), drought treatment ($P<0.01$), and over time ($P<0.01$). (C, D) g_s was reduced with drought treatment ($P<0.03$) and over time ($P<0.02$).

of soil water shortage and the two-sample t-test result confirmed the overall similarity of the studies and allows us to consider them as components of one experiment.

All the measures of aphid population performance showed a significant negative impact of water stress, with lower aphid numbers, r_m and fecundity. Aphid numbers were significantly correlated with soil moisture and leaf temperature. It is perhaps surprising that higher temperatures were associated with fewer aphids, as other work has found that a rise in air temperature positively affects *M. persicae* populations (Mittler, 1967; Wyatt & Brown, 1977). However, these readings did not distinguish between air temperature and leaf temperature. Under outdoor conditions, the two can be substantially different. It may be that continuously higher leaf temperatures without a nocturnal fall in temperature may have a negative impact on *M. persicae*.

Several studies concur with the findings here, showing that water stress led to a reduced population size (Mody et al., 2009) and reduced growth and development rates (Cabrera et al., 1995; Pons & Tatchell, 1995; Hale, 2002). However, Fereres et al. (1988) found that *Sitobion avenae* Fab. significantly increased in population density under a severe water stress of −2.0 to −2.4 MPa. In that study, the combination of aphids and water shortage caused losses to crop yields of over a third, and our work also shows an exacerbation of water stress by aphid infestation.

Aphid infestation resulted in a fast-acting stress that was similar in magnitude and style to water shortage. Despite receiving more than double the water ration of a droughted plant, watered plants infested with aphids showed significant reductions in photosynthetic rate and final weight with significantly higher osmotic potential. This effect of aphid infestation on watered plants is similar to the effects shown here in droughted plants. Other research has found similar trends (Riedell, 1989; Cabrera et al., 1994; Burd & Elliot, 1996; Macedo et al., 2003), but the osmotic potential component is new information.

The maintenance of a higher than expected osmotic potential in droughted and aphid-infested plants in this study could be due to limitation of the typical drought response. Osmotic potential is typically lowered by the accumulation of proline and other amino acids (Riedell, 1989). Similarly, Cabrera et al. (1994) found a rise in proline accumulation in droughted and aphid-infested plants, but this was lower than the rise in solely droughted plants. They concluded that aphids induced a water-stress-like response in plants, which could have made an impact on local turgor pressure. With the results from our study, it is reasonable to say that although some aphids cause drought-like symptoms in their host, their presence acts to suppress osmoregulation in the vacuole leading to reduced leaf turgor over days 7–21 of the experimental period. This is surprising, given the assumption that aphids require

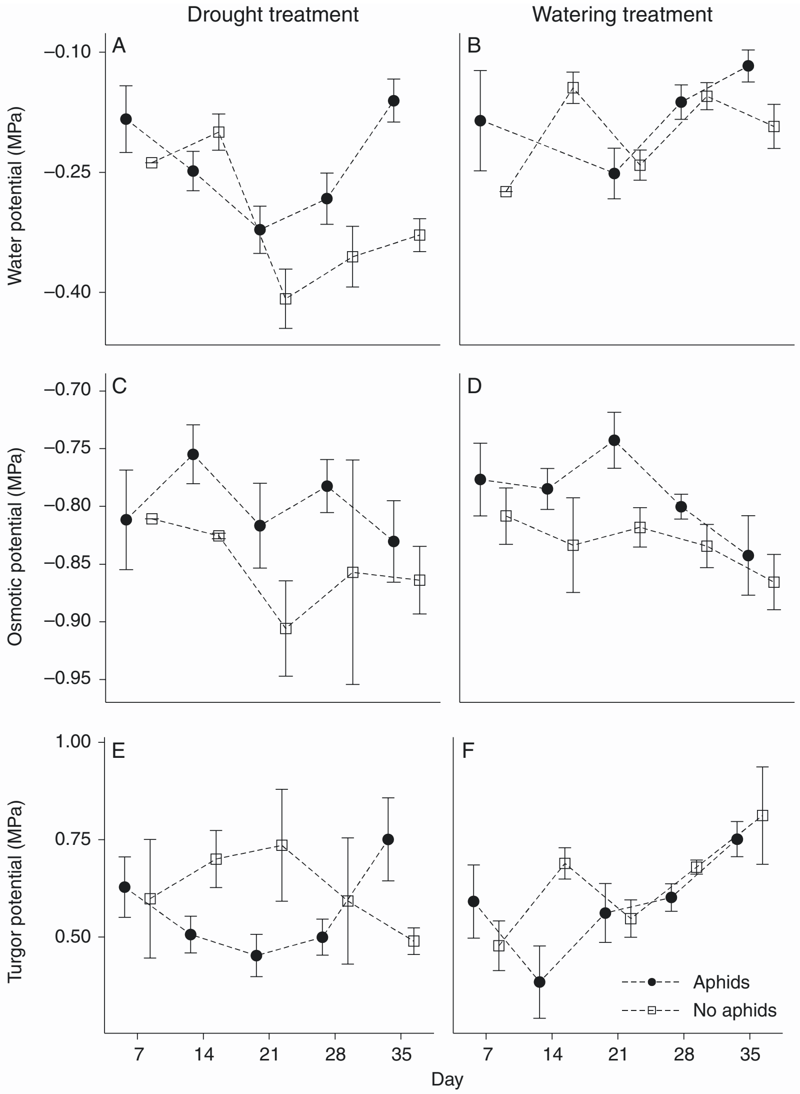

Figure 6 Trends in Mean (±SEM) total leaf water potential and its components in study II. (A, B) Total leaf water potential was reduced by water stress ($P<0.01$) and aphid infestation ($P<0.05$). (C, D) Osmotic potential was more negative with drought treatment ($P<0.02$) and less negative with aphid infestation ($P<0.01$). (E, F) Turgor pressure was significantly reduced by drought treatment ($P<0.01$).

relatively high turgor pressure to maintain effective feeding. However, rising leaf water potential after day 21 counteracted the lack of osmoregulation, and turgor pressure began to rise. This suggests that aphids affect the regulation of water potential components, possibly associated with levels of drought-induced hormones (e.g., abscisic acid) or amino acids (e.g., proline). Divol et al. (2005) also found that *M. persicae* infestation on celery (*Apium graveolens* L.) led to gene upregulation. These genes were associated with cell wall modification and phloem dilution, suggesting that aphids induced some control on turgor pressure.

What other dietary changes occur when aphids attack a host? Plant responses to aphid attack are more similar to reactions under pathogen attack rather than wounding responses (Walling, 2000). Aphid infestation induces genes usually expressed under oxidative stress, as well as calcium dependent signalling and pathogen responses (Moran et al., 2002). Genes encoding production of the classic defence compounds salicylic and jasmonic acid are also upregulated under aphid attack (Walling, 2000; Moran & Thompson, 2001; Moran et al., 2002; Ferry et al., 2004; Smith & Boyko, 2007).

Reduction in above-ground dry weight with aphid infestation, found here, was similar to the reduction in above-ground dry weight caused by drought alone. There are further similarities between water and aphid stress as

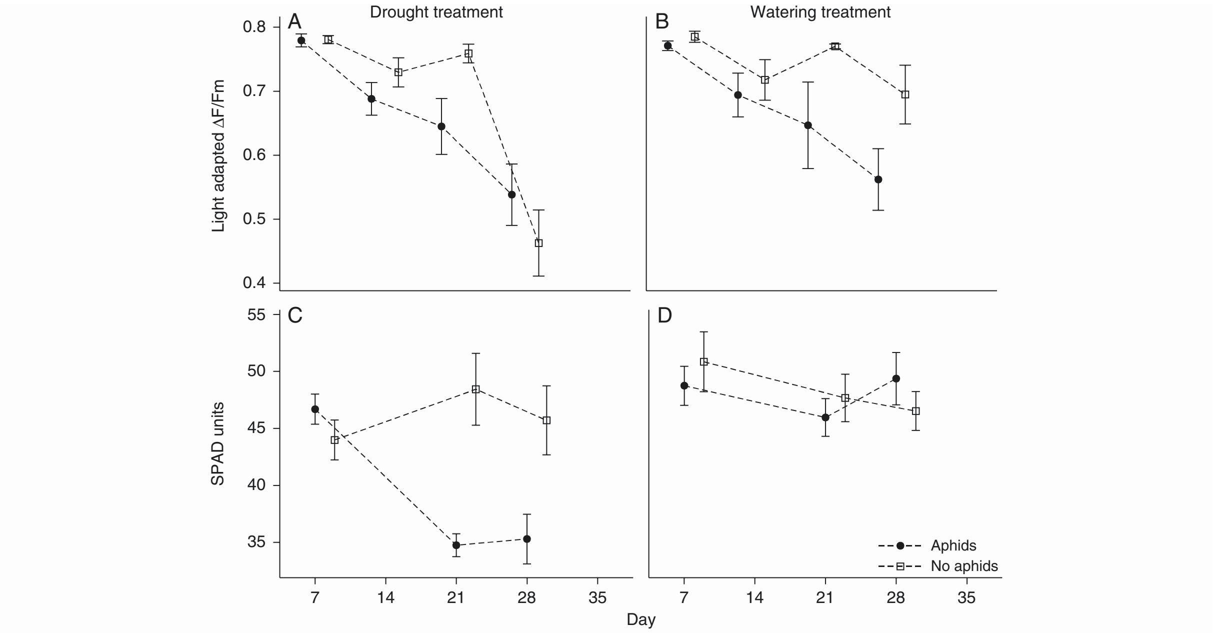

Figure 7 Trends in Mean (±SEM) chlorophyll fluorescence and leaf chlorophyll content (SPAD readings) in study I. (A, B) The chlorophyll fluorescence data showed a significant reduction with aphid infestation ($P<0.02$) and an overall reduction over time ($P<0.01$). (C, D) SPAD values were significantly reduced by drought treatment ($P<0.01$) and aphid infestation ($P<0.01$), with an interaction effect ($P<0.01$).

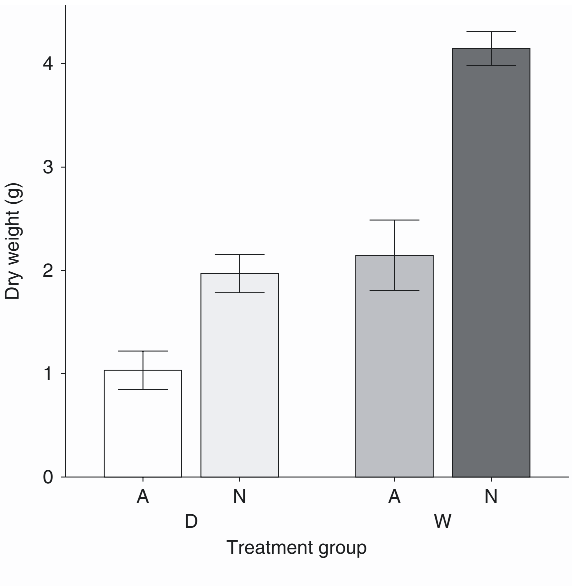

Figure 8 The effect of treatments on Mean (±SEM) final above-ground dry weight of plants in study I. Axis letters: drought 'D', watered 'W', aphid 'A', and no aphid 'N'. Above-ground dry weight values were significantly ($P<0.01$) reduced by aphid and water stress with significant interaction ($P<0.05$).

types of stress effect, as found by Warrington & Whittaker (1990) on Sitka spruce [*Picea sitchensis* (Bong.) Carr.] infested with green spruce aphids (*Elatobium abietinum* Walker). Aphid-infested plants show increased production of abscisic acid, a compound that causes stomatal closure, which is typical in drought stressed plants (Smith & Boyko, 2007). In addition, Riedell (1989) concluded that the aphids had induced drought-like symptoms in well-watered aphid-infested barley, causing reduced stomatal conductance and relative water content, lower leaf water potential and lower chlorophyll levels. Cabrera et al. (1995) similarly found lower sucrose, soluble sugar, total chlorophyll levels and photosynthetic rates in well-watered aphid-infested barley seedlings.

The significant reduction in photosynthesis and lower fluorescence yield on aphid-infested plants implies damage to the efficiency of photosystem II electron transport. These results are similar to those found with Russian wheat aphids (*D. noxia* Mord.) by Burd & Elliot (1996) and Macedo et al. (2003). It was expected that measurements of dark-adapted F_v/F_m would have shown clear reductions under aphid stress as found in Blanco et al. (1992), where the method is suggested as a rapid identifier of aphid stress. No significant effect of drought treatment on dark-adapted F_v/F_m was found in this research. The effects of drought treatment were restricted to light-adapted $\Delta F/F_m$, possibly due to the low light levels in the controlled environment room compared with outdoors.

Most of the responses reported here only became apparent after prolonged exposure to the stress treatments. This indicates that short-term experiments could easily miss some of the stress responses (Koricheva & Larsson, 1998). Many studies cover only periods of infestation of about 7 days (e.g., Riedell, 1989; Cabrera et al., 1995; Divol et al., 2005) and could miss some of the effects seen in this study.

In this study, the pulse stress hypotheses (Huberty & Denno, 2004; White, 2009) do not hold for *M. persicae*. Aphid adaptations to phloem can buffer some variability in diet composition and quality (Douglas, 2006). However, long-term drought, even with 'pulses' of watering does not lead to dietary changes that can provide a direct benefit to the aphid population. Leaf turgor needs to be maintained, so that aphids can insert their stylets and successfully access the contents of the phloem. Some aphid species have been found to multiply on stressed hosts (Wearing, 1972; Fereres et al., 1988; Cabrera et al., 1995; Xing et al., 2003; Khan et al., 2010), but the level of experimentation required to find the exact patterns and combinations that would cause increased aphid performance on stressed plants would be impracticable. It seems more reasonable to conclude that aphids perform better on a secure diet provided by a well-watered host. Although the host is negatively affected with reduced physiological efficiency and growth, the supply of food will last longer and be more predictable than on a stressed plant.

In summary, the hypothesis that the *M. persicae* population would increase faster and to larger numbers on pulse-water-stressed host plants was rejected. The aphids did not reproduce at a faster rate or to greater numbers on droughted plants. The increased temperature of droughted leaves did not lead to increased growth and fecundity in the aphids. However, aphid stress did reduce the yield and performance of host plants in a manner similar to drought stress. Under future drought scenarios, *M. persicae* is more likely to become a significant threat to well irrigated crops rather than crops under water stress.

Acknowledgements

The research was funded by the Natural Environment Research Council through a UK research studentship (NE/F008791/1). Our thanks are also due to the Scottish Crop Research Institute, Dundee, UK for kindly supplying the aphids for this research.

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